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

PATRONES ONTOGENÉTICOS Y POSIBLE VALOR ADAPTATIVO EN LA TRANSFERENCIA DE NUTRIENTES DE LAS MADRES A SUS EMBRIONES EN DESARROLLO EN PECES VIVÍPAROS DE LA FAMILIA POECILIIDAE

TESIS
QUE PARA OPTAR POR EL GRADO DE:
DOCTOR EN CIENCIAS

PRESENTA:
NABILA SALEH SUBAIE

DIRECTOR DE TESIS
DR. JOSÉ JAIME ZÚÑIGA VEGA FACULTAD DE CIENCIAS
COMITÉ TUTOR
DRA. MARIANA BENITEZ KEINRAD
INSTITUTO DE ECOLOGÍA
DR. RAÚL CUEVA DEL CASTILLO MENDOZA
FACULTAD DE ESTUDIOS SUPERIORES IZTACALA

CDMX AGOSTO 2021

AGRADECIMIENTOS ACADÉMICOS

- A la Universidad Nacional Autónoma de México (UNAM) y a la Facultad de Ciencias.
- Al programa de Doctorado en Ciencias Biomédicas de la Universidad Nacional Autónoma de México.
- Al Consejo Nacional de Ciencia y Tecnología (CONACYT) por proporcionarme la beca (596700) para el desarrollo de la presente investigación.
- Al apoyo PAEP por el apoyo para realizar la estancia de investigación en Provo (Utah), E.U.A.
- A todos los miembros del comité Académico tanto del Instituto de Fisiología Celular como el Instituto de Ecología, así como a todo el personal académico y administrativo del Posgrado en Ciencias Biomédicas. Muchas gracias por toda su dedicación, por siempre resolver mis dudas y apoyarme con todos los trámites necesarios para poder concluir con mi doctorado. Gracias por su trabajo y por todas sus atenciones.
- A mi tutor el Dr. Jaime Zúñiga Vega por toda su dedicación y apoyo durante la realización de este proyecto. Por todos sus consejos y enseñanzas. Gracias por todas las revisiones, comentarios y apoyo para poder realizar este proyecto. Gracias por estar siempre al pendiente de todos los pasos de esta investigación.
- A los miembros de mi comité tutor, la Dra. Mariana Benitez Keinrad y el Dr. Raúl Cueva del Castillo Mendoza por todo su apoyo, consejos, revisiones y todo el tiempo dedicado en la realización de esta tesis.
- Al doctor Jerald Johnson por recibirme en su laboratorio y permitirme realizar una estancia de investigación. Gracias, aprendí mucho. Recuerdo con mucho cariño cada uno de los días que pasé en Provo.
- A los miembros de mi jurado: Dr. Alejandro Córdoba Aguilar, José Jaime Zúñiga Vega, Dra. Tatiana Fiordelisio Coll, Dr. Ernesto Maldonado Olvera y la Dra. Laura Roxana Torres Ávila por todo su apoyo y comentarios para la mejora de esta tesis.
- A Israel Solano Savaleta, Pedro Eloy Mendoza Hernández y Mariana Hernández Apolinar por todo su apoyo y asistencia técnica durante el desarrollo de la presente investigación.
- A la Doctora Jhoana Díaz Larrea por su apoyo y sus sabios consejos. Gracias por siempre.
- A la Doctora Tatiana Fiordelisio Coll por permitirme utilizar las instalaciones de su laboratorio.

- A la doctora Ana Luisa Velasco Monroy por todo el apoyo incondicional en mis primeros años de doctorado. Gracias por sus enseñanzas, comentarios y revisiones. Gracias por permitirme la cercanía con los pacientes y con su grupo de trabajo.
- A la doctora Luisa Lilia Rocha Arrieta por todas sus enseñanzas y el tiempo dedicado a mi investigación.

AGRADECIMIENTOS A TÍTULO PERSONAL

Esto tal vez sea largo, así que prepárese para leer dos páginas de mis pensamientos y sentimientos más valiosos. O no, y puede seguir a la introducción de la tesis 😊

- A mi tutor, el doctor Jaime Zúñiga por todo su tiempo y ayuda. Aprendí mucho. Muchas gracias por este tiempo y por trabajar en conjunto para obtener esta tesis. El tiempo de pandemia fue más ameno gracias a todo tu apoyo, eres una gran persona.
- A mi estimado Tino Macías, gracias por las charlas y por compartir todo tú conocimiento conmigo, eres una gran persona, estoy muy agradecida de haberte conocido.
- A mi mamá por estar siempre ahí. Por ser la mamá más bonita del mundo (ya sé que todos dicen eso de sus madres, pero la mía es la más bonita).
- A mis hermanos por ser los mejores hermanos del mundo. Gracias por toda su ayuda y su tiempo, gracias por las risas y todos los juegos de mesa. “La Oso Republic” trascenderá por siempre. Por cierto, me caen mal… Co---, Ca---- y derivados. Fin del comunicado. Nah!! Choro, me faltó decir que los quiero mucho hermanitos mayores.
- Diego, eres una de mis personas favoritas en el mundo. Gracias por todas tus risas y momentos inolvidables. Gracias por recordarme lo bonito que es el mundo y sus

alrededores, así como todo tu apoyo. Gracias a ti existen: blu, el oso, el bonsai y quién sabe cuántas cosas más.

- A Memo, gracias por estar siempre a mi lado. Mire! Ya casi soy doctora!!!! Lo quiero mucho! Quien iba a pensar que ese tiempo en Vertiz daría tantos frutos. Gracias, por tanto.
- A mi Erika querida, muchas, muchas pero muchas gracias por tu cariño y amistad. Gracias por estar a mi lado en los momentos buenos y también en los difíciles. Gracias por las tardes de pelis y las largas noches de pláticas.
- A mis amigas incondicionales, Ley y Ely. Las quiero y adoro con todo mi corazón. No sé qué hubiera sido de estos años sin su compañía. Gracias por cada día y noche juntas. Gracias por sus enseñanzas y toda su comprensión.
- A Mary y a mi Glo Glo por estar siempre conmigo. Las quiero mucho a las dos. Gracias por todo lo que hicieron por mí. Pokge mi Glo Glo.
- Mi querido Gonzi, tendría que dedicarte un libro entero de agradecimientos. Pero el más importante es: gracias por todo tu tiempo. Te quiero mucho amigo. Quien sabe a dónde nos lleve la vida, espero que sea siempre al lugar más deseado.
- Selene, gracias por todas esas risas, por todo ese tiempo, por todo tu cariño. Gracias por ser una persona que siempre se preocupa por los demás.
- Dane, gracias por tus risas, por tu amistad. Hubiese deseado que continuará por más tiempo, que se hiciera cada día más sólida. Agradezco cada uno de los momentos que pasamos juntos y nunca olvidaré aquella frase que cambió mi forma de ver la vida. Algún día iremos todos juntos a Ixtapa, así sea de fantasmas.

- Gracias a todas las personas que conocí mientras estuve en el Cinvestav, el Gea González y el Hospital General. Gracias por su ayuda, por todas las risas y por todo el conocimiento.
- En este punto ya casi se me salen mis lagrimitas. Gracias al Especializado!!! Los mejores días de mi doctorado los pasé en esas paredes rodeada de personas hermosas. Voy a nombrar algunos, no se sientan ofendidos si no escribo el nombre de alguien: Consuelo, Irene, Tere, Pedro, Isra, Marianita, Gonzi, Selene, Bruno, Mariana, Karla, Cinturita, Pole, Luisa, Victor, Natalia, Mariana y ya no se me ocurre nadie más. Gracias a todos!!!!!!
- Gracias a todos esos peces que utilicé para realizar esta investigación. Ahora sabemos un poquito más sobre ustedes, ojalá pudieran hablar…
- Y pues… gracias a mí jajajaja. Estoy contenta de lo que logré y de todo lo que aprendí durante mi doctorado. Fue cansado y estresante algunas veces, pero la mayor parte del tiempo lo disfruté mucho.

I Phantasy Star 1
II Chrono Trigger
III Stardew Valley

Corrí mientras el viento soplaba contra mi rostro…
Corrí mientras caía una fuerte tormenta.
Corrí a trompicones por la vida…
Corrí más aún para escapar de las olas del mar.
Ahora es tiempo de caminar.

Nabila Saleh

A mis hermanos, por la grata fundación de “La Oso Republic” ㅋㅋㅋㅋㅋㅋㅋㅋ.

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RESUMEN

La matrotrofía es un modo de aprovisionamiento materno en la cual madre transfiere nutrientes a sus embriones después de la fecundación y durante todo el desarrollo hasta el nacimiento. La lecitotrofía es otro modo de aprovisionamiento materno, que ocurre antes de la fecundación, en el que la hembra destina recursos en forma de vitelo a sus embriones. La superfetación es un mecanismo por el cual las hembras tienen camadas en distintos estadios de desarrollo de forma simultánea.

Los objetivos de la presente tesis fueron: conocer si los grados de matrotrofía y superfetación cambian dependiendo del tamaño de las especies y si estas estrategias afectan de manera conjunta la fecundidad de las hembras; evaluar si existe un efecto del tamaño (edad) de las hembras sobre los grados de matrotrofía y superfetación; y por último, poner a prueba un modelo de aprovisionamiento materno, en el que se sugiere que el alimento puede ser un factor ecológico que favorezca la presencia de matrotrofía o lecitotrofía.

En el primer capítulo evalué si la matrotrofía y la superfetación funcionan como mecanismos compensatorios debido a las restricciones de espacio impuestas por tener un tamaño corporal pequeño a nivel de especie. Puse a prueba la hipótesis en la que, la matrotrofía y la superfetación deberían ser más prominentes en especies de tamaño pequeño de la familia Poeciliidae. No se encontró evidencia a favor de esta hipótesis. Las especies pequeñas no muestran mayores grados de superfetación y matrotrofía comparado con las especies de tamaño mayor. En conclusión, estas dos estrategias reproductivas no funcionan como mecanismos para compensar la restricción de espacio de las especies de tamaño pequeño.

En el segundo, capítulo puse a prueba la hipótesis en la cual la matrotrofía y la superfetación deberían ser más prominentes en hembras de tamaño pequeño. Fue un estudio en el que comparé hembras de diversas poblaciones de 13 especies. Se encontró el patrón esperado en 3 poblaciones de 2 especies (*Poeciliopsis infans* y *P. turrubarensis*). Este resultado indica que, aunque es un fenómeno raro, está presente en la naturaleza. Las hembras chicas (jóvenes) en estas tres poblaciones fueron más matrotróficas que las de tamaño mayor. Este es el primer estudio que demuestra un cambio ontogenético en el modo de aprovisionamiento materno para embriones en desarrollo en peces vivíparos. No encontré evidencia a favor en la parte de superfetación, en general las hembras de mayor tamaño presentan mayores grados de superfetación.

Por último, en mi tercer capítulo se puso a prueba el modelo de Trexler y DeAngelis de aprovisionamiento materno. Este modelo sugiere que los ambientes en donde haya una gran cantidad de alimento y que se encuentre disponible de forma constante favorecerán la presencia de la matrotrofía. Por el contrario, ambientes con poca o fluctuante disponibilidad de alimento deberían favorecer la lecitotrofía. Se obtuvieron dos medidas de disponibilidad de alimento, el índice de llenado estomacal y la condición corporal. Además, estimé la varianza de ambas medidas. Los resultados indicaron que ninguna de las dos medidas se asocia con tener altos o bajos índices de matrotrofía. Es decir, no se encontró apoyo para el modelo de Trexler y DeAngelis. En las 5 especies se encontró que las hembras con mayores grados de matrotrofía tenían peor condición corporal en comparación con hembras que destinan menos recursos a sus embriones (lecitotrofía). Este hallazgo es consistente con una de las hipótesis planteadas en el modelo de Trexler y DeAngelis. Es decir, las hembras con mayores grados de matrotrofía experimentan una disyuntiva (“trade-off”) entre la cantidad de recursos que destinan a su propio mantenimiento corporal y la que invierten en sus embriones. Este resultado revela que la matrotrofía es una estrategia fisiológicamente costosa.

ABSTRACT

Matrotrophy is the post-fertilization transfer of nutrients to developing embryos by the mother until birth. Lecithotrophy, is the pre-fertilization transfer of nutrients in which mothers deposit nutrients into the mature ova in the form of yolk before fertilization. Superfetation is the ability of females to simultaneously bear multiple broods of embryos at different developmental stages.

The objectives of this thesis were: to evaluate if the degrees of matrotrophy and superfetation change depending on the size of the species and whether these strategies jointly affect the fecundity of the females; to evaluate if there is a size effect of the females (due to age) on the degrees of matrotrophy and superfetation; and finally to test a model of maternal supply, in which it is suggested that food can be an ecological factor that favors the presence of either matrotrophy or lecithotrophy.

In the first chapter it was evaluated whether matrotrophy and superfetation could work as compensatory mechanisms due to space constraints imposed by having a small body size at the species level. The hypothesis was tested that both matrotrophy and superfetation would decrease throughout the reproductive life of the females. That is, both matrotrophy and superfetation should be more prominent in small-sized species in the family Poeciliidae. No evidence was found in favor of this hypothesis. Small species do not show higher degrees of superfetation and matrotrophy compared to larger species. In conclusion, these two reproductive strategies do not function as mechanisms to compensate for the space restriction of small species.

The second chapter focused on testing the previous hypothesis on a smaller scale. Through a population study, the hypothesis that matrotrophy and superfetation should be more prominent in small females was tested. This pattern was partially found in 3 populations of two species (*Poeciliopsis infans* y *P. turrubarensis*), which shows that, although a rare phenomenon, it is present in nature. The smaller young females in these three populations were more matrotrophic than the larger ones. This is the first study to demonstrate an ontogenetic change in the mode of maternal provisioning for developing embryos in viviparous fish. On the other hand, no evidence was found in relation to the surface area: in general larger females have higher levels of superfetation.

The third and final chapter tested the Trexler-DeAngelis model of maternal provisioning. This model indicates that environments where there is a large amount of food in a constant way will favor the presence of the matrotrophy, while environments with little food availability found in a variable way would favor lecithotrophy. Two food availability measures were obtained: the index of fullness and body condition. The variance of both measures was also determined. Results indicate that neither measure is associated with having either high or low matrotrophy rates and as such do not support the Trexler-DeAngelis model. In the five species analyzed, it was found that females with higher degrees of matrotrophy had worse body condition compared to females that devote less resources to their embryos and had good body condition. This finding is consistent with one of the assumptions of the Trexler and DeAngelis model: it was found that females with higher degrees of matrotrophy experience a trade-off between the amount of resources they devote to their own body maintenance and to their embryos. This reveals that matrotrophy is a costly strategy.

I INTRODUCCIÓN GENERAL

I.1 Viviparidad

En los vertebrados se conocen dos tipos principales de estrategias reproductivas; la oviparidad y la viviparidad (Blackburn, 1999). La oviparidad se define como un fenómeno reproductivo en el que las hembras depositan huevos que se desarrollan y eclosionan en el ambiente (Blackburn, 1999). Los huevos depositados en el ambiente contienen suficientes nutrientes para permitir que el organismo se desarrolle antes de la eclosión del huevo. En consecuencia, emerge del huevo un organismo completamente desarrollado (Blackburn, 1999). Algunos ejemplos de animales ovíparos son: aves, cocodrilos, quelonios, la mayoría de los lagartos, camaleones e iguanas y la mayoría de los anamniota (peces y anfibios) (Angelini and Ghiara, 1984; Blackburn, 1999). En contraste, la viviparidad es un modo reproductivo en el que los embriones se desarrollan, después de la fecundación, en estructuras especializadas dentro del vientre de la madre, es decir, las hembras retienen los embriones en desarrollo. Como resultado de la viviparidad, la descendencia nace en un estado avanzado de desarrollo (Blackburn, 1992, 1999; Ostrovsky et al., 2016). La viviparidad se presenta en todos los grupos de vertebrados menos las aves (Angelini and Ghiara, 1984). Dentro de los organismos vivíparos podemos encontrar: tiburones, ranas de África oriental, pocas lagartijas, algunas serpientes, la mayoría de los peces poecílidos entre otros (Blackburn, 1992, 1999; Ostrovsky et al., 2016).

Se considera que la viviparidad ha evolucionado a partir de una condición ancestral de oviparidad y que ha evolucionado de manera independiente al menos 160 veces en vertebrados (Wourms, 1981; Blackburn, 1995,1999; Shine, 1995; Lambert and Wiens, 2013; Roberts et al., 2016; Wu et al., 2017). Debido a sus múltiples orígenes se han estudiado ampliamente las ventajas y desventajas de la viviparidad (Blackburn, 1995; Shine, 1995; Lambert and Wiens, 2013; Roberts et al., 2016; Wu et al., 2017). La ventaja más sobresaliente de la viviparidad es que los embriones se encuentren dentro de las diferentes estructuras maternas. De esta forma los embriones permanecen protegidos de depredadores, agentes microbianos, deshidratación, luz ultravioleta y temperaturas extremas (Wourms, 1981; Blackburn, 1999).

Por otro lado, las desventajas de la viviparidad suelen afectar a la hembra gestante. Durante el tiempo de gestación la habilidad locomotora de las hembras y la capacidad de conseguir alimento se ven afectadas, esto pone en riesgo tanto a la hembra como a los embriones en desarrollo (Blackburn, 1995; Shine, 1995; Lambert and Wiens, 2013; Roberts et al., 2016). Además, es posible que presenten restricciones de espacio en las cavidades reproductoras maternas, por lo que es posible que se limite el tamaño de la camada o de las crías (Wourms, 1981; Blackburn, 1999). Es posible que la viviparidad represente un costo energético más grande para las hembras comparado con la oviparidad. Sin embargo, se considera que la viviparidad debe ser beneficiosa en ambientes extremos (lugares con poca luz solar o bajas temperaturas) debido a sus múltiples orígenes convergentes (Blackburn, 1992, 1999; Ostrovsky et al., 2016).

La evolución de la viviparidad está íntimamente relacionada con la evolución del sistema reproductivo de las hembras. Mientras ocurría la transición evolutiva de oviparidad a la viviparidad, el sistema reproductivo de las hembras desarrolló estructuras más complejas, por ejemplo: modificación del saco vitelino, mirovellosidades, sistemas vesiculares, pseudoplacentas y placentas. La evolución del sistema reproductivo lleva a su vez a la evolución de nuevos mecanismos a través de los cuales las hembras alimentan a sus embriones en desarrollo. (Pollux et al., 2009, 2014; Olivera-Tlahuel et al., 2019).

I.2 Tipos de transferencia de nutrientes en organismos vivíparos

En organismos vivíparos conocen dos modos dos tipos de transferencia de nutrientes de las madres a sus embriones (Blackburn, 1992; Reznick et al., 2002; Marsh-Matthews, 2011). Estos dos modos representan un continuo en la forma en la que las madres pueden transferir nutrientes a sus embriones en desarrollo. El primer tipo de transferencia de nutrientes ocurre antes de que el ovocito sea fecundado y se le denomina lecitotrofía. La hembra realiza un proceso denominado vitelogénesis, el cual consiste en almacenar o empaquetar nutrientes en forma de vitelo. Una vez que el ovocito es fecundado se alimenta del vitelo y no recibe más nutrientes de la madre durante su desarrollo. Es decir, el embrión utiliza los recursos nutricionales que obtiene a través del vitelo anteriormente empaquetado por la hembra para desarrollarse (Blackburn, 1992; Marsh-Matthews, 2011). Algunos ejemplos de organismos vivíparos que presentan lecitotrofía son algunos peces cartilaginosos, ciertos peces óseos, algunos lagartos y muy pocos anfibios (Blackburn, 1992).

La segunda forma de transferir nutrientes de las madres a los embriones en organismos vivíparos es la matrotrofía. La matrotrofía es un modo de aprovisionamiento materno que sucede después de que el ovocito ha sido fecundado. La madre transfiere nutrientes después de la fecundación y durante todo el desarrollo del embrión hasta que el mismo llega al nacimiento (Blackburn, 1992; Reznick et al., 2002; Marsh-Matthews, 2011). Muy pocos peces de esqueleto óseo, algunos peces cartilaginosos, mamíferos y ciertos reptiles son ejemplos de organismos vivíparos cuya transferencia de nutrientes es la matrotrofía.

Es posible que la evolución de la matrotrofía esté ligada a la evolución de la viviparidad. La viviparidad evolucionó a partir de organismos ovíparos en donde no existía una interacción fuerte entre la madre y sus embriones. En cambio, en la viviparidad se pueden observar interacciones materno-embrionarias como lo es el intercambio gaseoso, interacciones inmunológicas, endocrinológicas entre otras. Este proceso evolutivo dio como resultado una relación mayor entre el embrión y la madre y una pérdida de la autonomía metabólica del embrión. Es posible que estas modificaciones mejoraran la supervivencia de la descendencia y por ende confirió una ventaja selectiva. Es posible que la dependencia materna se haya logrado debido a la especialización estructural y funcional de los tejidos maternos. En consecuencia, la viviparidad ya no solo protegía a los embriones del entorno, además los embriones comenzaron a recibir nutrientes que no provenían exclusivamente del vitelo. La evolución de la matrotrofía es posiblemente resultado de la especialización de estructuras maternas que permitieron una mayor interacción entre la madre y sus embriones en desarrollo (Wourms et al., 1988).

Se han descrito cambios en el tamaño del embrión a lo largo de su desarrollo dependiendo del tipo de transferencia de nutrientes de la madre a los embriones. En el caso de la lecitotrofía, el peso seco de los embriones disminuye durante el desarrollo debido a costos metabólicos. En cambio, en la matrotrofía, el peso seco de los embriones incrementa durante el desarrollo debido a que la madre está transfiriendo sustancias nutritivas durante la gestación (Pollux et al., 2009; Ostrovsky et al., 2016)

La transferencia de nutrientes de la madre a los embriones se puede calcular a través del índice de matrotrofía (Reznick et al., 2002; Marsh-Matthews, 2011). Este índice considera el peso seco del embrión al momento de la fecundación y el peso seco del embrión en el último estadio de desarrollo. Así se realiza una división entre el peso del embrión al último estadio de desarrollo entre el peso del embrión al ser fecundado. El resultado de la división se interpreta de la siguiente manera: si el índice es menor o igual a 0.75 corresponde a lecitotrofía. Si el índice es igual o mayor a 1, entonces se considera que es matrotrofía. Si el índice de matrotrofía calculado se encuentra entre 0.76 y 0.99 entonces se considera como matrotrofía incipiente. La matrotrofía incipiente indica que la hembra ha transferido la mayoría de los recursos a través del vitelo (lecitotrofía) y que las hembras tienen la capacidad de transferir algunos nutrientes después de la fecundación durante el desarrollo de los embriones. (Reznick et al., 2002; Marsh-Matthews, 2011).

Uno de los grupos de vertebrados que presenta estos dos tipos de transferencia de nutrientes de las madres a sus embriones en desarrollo es el de los peces de la familia Poeciliidae. La matrotrofía está presente en al menos 11 géneros dentro de la familia Poeciliidae (Pollux et al., 2009; Furness et al., 2019, 2021). Este grupo de vertebrados presenta un continuo en la transferencia de nutrientes entre las madres y sus embriones en desarrollo (Reznick et al., 2002). Es decir, algunas de sus especies pueden ser lectitotróficas, matrotróficas o tener grados intermedios de matrotrofía. Algunas especies son aparentemente estrictamente lecitotróficas (e.g. *Gambusia yucatana*, *Poeciliopsis pleurospilus y Xiphophorus maculatus*), mientras que otras especies exhiben matrotrofía extensa (e.g. *Poeciliopsis retropinna*, *P. turneri* y *Micropoecilia branneri*), con numerosas especies entre estos dos modos extremos de aprovisionamiento materno que combinan distintos grados de nutrición a través de vitelo (aprovisionamiento previo a la fecundación) con transferencia activa de nutrientes a lo largo del desarrollo (aprovisionamiento después de la fecundación) (Reznick et al., 2002; Pollux et al., 2009, 2014; Olivera-Tlahuel et al., 2015).

Algunos estudios han demostrado que el grado de transferencia de nutrientes después de la fertilización (es decir, el grado de matrotrofía), puede variar ampliamente tanto dentro de especies (entre poblaciones) como dentro de poblaciones (entre individuos) (e.g., en *Poecilia parae*, *Poeciliopsis gracilis, P. lucida* y *Xenodexia ctenolepis*; Reznick et al., 2007; Turcotte et al., 2008; Pires et al., 2010; Molina-Moctezuma et al., 2020). Esta gran variación inter e intraespecífica en la forma en que las hembras de especies de poecílidos proporcionan a los embriones los nutrientes necesarios para el desarrollo ha planteado preguntas sobre los posibles beneficios de la matrotrofía sobre la lecitotrofía. En resumen, la gran variabilidad que se observa entre especies y poblaciones de la familia Poeciliidae hacen que esta familia sea un buen modelo de estudio de la matrotrofía, particularmente acerca de cuál es el valor adaptativo y los mecanismos funcionales y fisiológicos de esta estrategia reproductiva (Trexler and DeAngelis, 2003; Pollux et al., 2009, 2014; Saleh-Subaie and Zúñiga-Vega, 2019).

I.3 Modelo de Trexler y De Angelis

Uno de los modelos teóricos que propone un potencial valor adaptativo de la evolución de la matrotrofía a partir de la lecitotrofía es el modelo de aprovisionamiento materno de Trexler y DeAngelis (Trexler and DeAngelis, 2003, 2010). Este modelo sugiere que la disponibilidad de alimento es una condición ecológica que podría favorecer un cambio en el tipo de transferencia de nutrientes de las madres a su decendencia. Es decir, estos autores sugieren que la cantidad de alimento disponible en el ambiente puede favorecer la evolución de adaptaciones como la matrotrofía (Trexler and DeAngelis, 2003, 2010).

Los autores consideraron varios aspectos teóricos y supuestos sobre la matrotrofía y la lecitotrofía para realizar su modelo. 1) La lecitotrofía es favorecida en ambientes fluctuantes debido a que toda la energía necesaria para alimentar a una camada es previamente empaquetada en forma de vitelo (Thibault and Schultz, 1978; Wourms and Lombardi, 1992). 2) Uno de los beneficios de la matrotrofía radica en aumentar la fecundidad de las hembras (Trexler, 1997). Considerando que la cantidad de nutrientes que las hembras destinan a cada embrión (costo) es el mismo tanto en hembras matrotróficas como lecitotróficas, los embriones de las hembras lecitotróficas deberían ser más costosos y grandes que los de las hembras matrotróficas ya que las hembras lecitotróficas destinan todo el recurso para el desarrollo de sus embriones en forma de vitelo.

Por el espacio que ocupa el vitelo, las hembras que presenten matrotrofía serían capaces de tener un mayor número de embriones y aumentar su fecundidad considerando que tanto las hembras matrotróficas como las lecitotróficas tienen embriones del mismo tamaño al final del desarrollo y asumiendo que el costo por embrión es el mismo para ambas estrategias. Esto solo se cumpliría si la hembra matrotrófica tiene los recursos suficientes para llevar a término a todos sus embriones. 3) Debido a que las camadas de las hembras con matrotrofía son más grandes, el costo de la matrotrofía puede ser mayor para las hembras matrotróficas que para las hembras lecitotróficas, ya que están transfiriendo contantemente alimento, el costo a largo plazo es mayor que para las hembras lecitotróficas. 4) Las hembras que presentan matrotrofía podrían utilizar la energía almacenada en forma de lípidos en su cuerpo como amortiguador cuando los recursos disponibles en el ambiente fluctúan. De esta forma pueden continuar transfiriendo recursos a sus embriones en desarrollo (Jonsson, 1997).

El modelo predice que la matrotrofía debería favorecerse en ambientes en donde la cantidad de alimento es alta y se encuentra de manera constante. Esto se debe a que las hembras matrotróficas transfieren de manera constante recursos para mantener a sus embriones durante todo su desarrollo. Debido a esto, debería haber disponibilidad de alimento suficiente para que la hembra se mantenga y para proveer de nutrientes a sus embriones. Por el contrario, sugiere que lugares en donde el alimento es escaso o es variable debería favorecerse la lecitotrofía. En este caso, en aquellos momentos en los que las hembras pueden acceder a alimento, deberían almacenar energía en forma de vitelo, puesto que probablemente después no contarán con los nutrientes necesarios para transferirlos durante la gestación. Por este motivo, la lecitotrofía debería favorecerse en entornos donde la disponibilidad de recursos es baja o muy variable (Trexler and DeAngelis, 2003, 2010).

Varios estudios han puesto a prueba el modelo de Trexler y DeAngelis, tanto en condiciones de laboratorio (Marsh-Matthews and Deaton, 2006; Pires et al., 2007; Banet and Reznick, 2008; Banet et al., 2010; Pollux and Reznick, 2011; Itonaga et al., 2012; Van Dyke et al., 2014; Molina-Moctezuma et al., 2020), como en condiciones naturales (Schrader and Travis, 2008, 2012; Riesch et al., 2013; Molina-Moctezuma et al., 2020), obteniendo resultados contradictorios. Por ejemplo, Marsh-Matthews y Deaton, (2006) pusieron a prueba el modelo de aprovisionamiento materno con la especie *Gambusia geiseri* en condiciones de laboratorio. En este estudio encontraron que altos grados de matrotrofía no fueron acompañados por un tamaño de huevos pequeños al momento de la fecundación.

Además, encontraron que una cantidad de recursos mayor favorece la presencia de matrotrofía. Estos resultados indican que el modelo se cumple al menos de manera parcial, ya que, sí hubo mayor índice de matrotrofía en hembras bajo un régimen de alimento abundante, pero este aumento no estuvo acompañado de la reducción del tamaño de los huevos al momento de la fecundación, uno de los supuestos del modelo.

Por otro lado, Riesch et al. (2013) utilizaron como especie de estudio a *Gambusia hubbsi* en condiciones naturales. Los investigadores encontraron que no hubo influencia de la cantidad de alimento disponible en el ambiente sobre el grado de matrotrofía que presentaron las hembras. Por el contrario, sí encontraron una disminución del tamaño de los embriones asociado con el nivel de transferencia de nutrientes. Por lo tanto, de nueva cuenta, el modelo de Trexler y DeAngelis solo se cumplió de manera parcial.

Dado que no se han encontrado resultados concluyentes, ni en condiciones experimentales y naturales, es importante estudiar la generalidad de este fenómeno para determinar si la matrotrofía ocurre con mayor cantidad y estabilidad de alimento. Por lo tanto, uno de los objetivos de este trabajo fue evaluar el modelo de Trexler y DeAngelis. En particular, abordamos las condiciones específicas que conducirían a un aumento de la matrotrofía al comparar poblaciones naturales de cinco especies del género *Poeciliopsis*.

I.4 Superfetación

La superfetación es la capacidad de las hembras de tener simultáneamente múltiples embriones en diferentes etapas de desarrollo dentro de su tracto reproductivo (Scrimshaw, 1944a; Zúñiga-Vega et al., 2010, 2017; Olivera-Tlahuel et al., 2015). Se considera que la superfetación es la ovulación, fecundación e implantación de un segundo conjunto de ovocitos durante la gestación de un conjunto de embriones previamente fecundados (Scrimshaw, 1944a; Zúñiga-Vega et al., 2010, 2017; Olivera-Tlahuel et al., 2015). Algunos ejemplos de organismos que presentan superfetación son: el visón americano (*Mustela vison*), el cual presenta una segunda ovulación siete días después de la primera, lo cual da lugar a dos camadas provenientes de diferentes ciclos de ovulación; el tejón europeo (*Meles meles*) en el cual se ha encontrado evidencia de superfetación y paternidad múltiple; la liebre europea (*Lepus europaeus*) y la liebre de montaña *(Lepus timidus)* son capaces de tener dos camadas simultáneas en diferentes estadios de desarrollo; los bivalvos de las especies *Sphaerium* y *Musculium*, los cuales tienen camadas secuenciales que coexisten hasta completar su desarrollo (Cooley and Foighil, 2000; Roellig et al., 2011).

La superfetación es una estrategia reproductiva que ocurre en al menos tres familias de peces vivíparos: Clinidae, Poeciliidae y Zenarchopteridae (Scrimshaw, 1944a; Reznick et al., 2007; Pollux et al., 2009). Específicamente en la familia Poeciliidae este rasgo evolutivo ha evolucionado al menos cuatro veces de forma independiente (Furness et al., 2019). La superfetación se observa en algunos géneros como *Heterandria* y *Poeciliopsis,* mientras que está ausente en otros géneros como *Brachyrhaphis* y *Gambusia* (Scrimshaw, 1944b; Thibault and Schultz, 1978; Johnson and Belk, 2001; Riesch et al., 2013). De manera similar a lo que ocurre con la matrotrofía, el grado de superfetación (es decir, el número de crías que tienen las hembras) varia de forma inter e intraespecífica. Por ejemplo, las hembras de *Poeciliopsis turrubarensis* pueden tener entre dos y cuatro camadas simultáneas, mientras que las hembras de *Heterandria formosa* pueden tener hasta ocho camadas de manera simultánea (Scrimshaw, 1944b; Zúñiga-Vega, et al., 2007). También se ha documentado la variación en el número promedio de camadas simultáneas entre poblaciones de algunas especies de poecilíidos como *Poeciliopsis baenschi, P. gracilis* y *Xenodexia ctenolepis* (Reznick et al., 2007; Frías-Alvarez et al., 2014; Zúñiga-Vega, et al., 2017). Como sucede con la matrotrofía, se han realizado estudios para analizar los potenciales beneficios de presencia de la superfetación o de niveles más avanzados de superfetación (Zúñiga-Vega et al., 2007; Zúñiga-Vega et al., 2010; Fleuren et al., 2019).

I.5 La superfetación y la matrotrofía como estrategias conjuntas

Una hipótesis que se ha propuesto para explicar los beneficios de la presencia conjunta de superfetación y matrotrofía sugiere que, asumiendo un número igual de embriones producidos en cualquier momento en particular, la masa y el volumen de las hembras preñadas pueden ser sustancialmente menores en hembras matrotróficas y que presentan superfetación en comparación con especies lecitotróficas y sin superfetación (Reznick and Miles, 1989; Pollux et al., 2009).

En las especies matrotróficas, los ovocitos son pequeños y, después de la fecundación, durante el desarrollo crecen gradualmente en masa y volumen debido a que las hembras les proporcionan nutrientes durante toda la gestación. El tamaño inicial del embrión y los aumentos graduales de masa durante el desarrollo implican que la masa y el volumen de las hembras preñadas aumentan poco durante una gran proporción de la gestación, al menos antes de las fases tardías del desarrollo del embrión (Fleuren et al., 2018). Por el contrario, en las especies lecitotróficas, los ovocitos son de mayor tamaño y contienen cantidades altas de vitelo antes de la fecundación y, por lo tanto, el peso y el volumen de las hembras son considerablemente grandes incluso antes de la fecundación, pudiendo aumentar aún más durante todo el desarrollo debido a la absorción de agua por los embriones en desarrollo (Pollux et al., 2009; Fleuren et al., 2018).

Con respecto a la superfetación, las hembras superfetadoras parecen dividir el esfuerzo reproductivo en dos o más camadas más pequeñas (con menos embriones). Dado que la masa húmeda de embriones aumenta a medida que avanza el desarrollo, las especies superfetadoras en general aumentan menos en masa y volumen durante la gestación debido a que los embriones en las etapas iniciales del desarrollo son más pequeños que los embriones en las etapas tardías (Zúñiga-Vega et al., 2010). Por el contrario, las hembras de especies que no superponen sus camadas producen todos sus embriones en un solo episodio y todos alcanzan las últimas etapas (cuando se vuelven más grandes) al mismo tiempo, lo que da como resultado hembras preñadas con masa y volumen corporal sustancialmente mayores. Esta diferencia esperada entre especies superfetadoras y no superfetadoras en el volumen y la masa corporal de las hembras gestantes también asume que el número total de embriones portados por las hembras es el mismo (Zúñiga-Vega et al., 2010).

La menor masa y volumen de hembras durante el desarrollo embrionario debería ser cierto para la superfetación o la matrotrofía (es decir, considerando cada una de estas estrategias por separado), pero este beneficio es presumiblemente mayor cuando las dos estrategias ocurren juntas en una sola especie (Pollux et al., 2009). La matrotrofía permite que la masa corporal y el volumen de las hembras gestantes permanezcan relativamente sin cambios durante las primeras fases del desarrollo embrionario y, junto con la superfetación, la masa corporal y el volumen aumentan solo un poco cuando avanza la gestación, porque solo un subconjunto de los embriones en desarrollo alcanza el tamaño más grande.

En los peces vivíparos de la familia Poeciliidae existe una amplia variación intra e interespecífica en los grados de superfetación y matrotrofía (Pollux et al., 2014; Furness et al., 2019). Dado que las hembras que presentan superfetación y matrotrofía presentan un menor aumento de masa y volumen corporal cuando producen un número particular de embriones en comparación con las hembras de especies que carecen de estos modos reproductivos, propuse la hipótesis de que su presencia conjunta permite a las hembras tener un número relativamente grande de embriones en cualquier volumen dado del tracto reproductivo. Por lo tanto, la matrotrofía y la superfetación deberían ser más ventajosas para las especies pequeñas, porque su presencia conjunta podría compensar las restricciones de espacio por tener un tamaño pequeño (existe poco espacio disponible para los embriones en desarrollo dentro de las estructuras reproductivas maternas), al maximizar la producción reproductiva de las hembras. Evalué esta hipótesis a nivel de especies realizando análisis filogenéticos y a nivel de poblacional. Además, las especies de peces de la familia Poeciliidae presentan crecimiento indeterminado (Turner y Snelson, 1984; Abney et al., 2005). Es decir, que esta hipótesis también puede ser interpretada desde el punto de vista de la edad de las hembras, en donde las hembras de tamaño pequeño son más jóvenes compradas con las hembras de tamaño mayor. Esto sugiere que las hembras jóvenes podrían ser más matrotróficas para compensar las restricciones de espacio y esta estrategia cambiaría conforme las hembras crecen debido a que, conforme las hembras crecen tienen mayor espacio corporal disponible y tienen menor restricción de espacio en su ovario. De esta forma, las hembras pueden permitirse tener mayores grados de lecitotrofía, sin sacrificar la fecundidad.

I.6 Familia Poeciliidae

Las especies de la familia de peces Poeciliidae son nativas de América y habitan ambientes de agua dulce y salobre. Se encuentran en una amplia variedad de hábitats como zonas templadas, lagos, manantiales y manglares (Miller et al., 2005). Se distribuyen desde Estados Unidos hasta el noroeste de Argentina. Esta familia se conforma de al menos 22 géneros y 273 especies (Miller et al., 2005; recuperado en 2021 de fishbase.org). Suelen encontrarse en lugares con aguas poco profundas y también en zonas amplias como lagos y ríos (Miller et al., 2005). Son peces que pueden alcanzar un tamaño adulto de entre 30 mm y un máximo reportado de 68 mm de longitud estándar (Miller et al., 2005).

La fecundación es interna a través de la inserción del órgano copulador de los machos (gonopodio) en el poro genital de la hembra. El gonopodio es una modificación de la aleta anal (Rosen y Tucker, 1961). En todas las especies de la familia, las hembras siguen creciendo después de alcanzar la madurez sexual, lo que significa que el rango de tamaños de las hembras dentro de las poblaciones puede ser bastante amplio. Estas especies son consideradas con crecimiento indeterminado, por lo que las hembras más grandes son también las más viejas (Turner and Snelson, 1984; Abney et al., 2005).

I.7 Preguntas de investigación y objetivos particulares que componen esta tesis

En los peces de la familia Poeciliidae existe una amplia variación intra e interespecífica en los grados de superfetación y matrotrofía (Pollux et al., 2014; Furness et al., 2019). Esta variación ha sido estudiada en términos de éxito reproductivo (e.g. mayor fecundidad, Trexler, 1997) y supervivencia (e.g. al permitir que las hembras puedan tener formas corporales más hidrodinámicas, Zúñiga-Vega et al., 2010). La mayoría de los estudios que se encuentran en la literatura sobre matrotrofía y superfetación evalúan sus posibles causas y consecuencias de manera separada, sin considerar que son estrategias reproductivas que se encuentran relacionadas (Meredith et al., 2011; Pollux et al., 2014). Es necesario evaluar a ambas estrategias de manera conjunta ya que se considera que estas estrategias evolucionan de forma conjunta o una de ellas puede favorecer la presencia de la otra (Meredith et al., 2011; Pollux et al., 2014). Por otra parte, son pocos los estudios que toman en cuenta el efecto del tamaño o de la forma de las hembras sobre estas estrategias reproductivas, en general estas estrategias se estudian de forma separada (e.g. Zúñiga-Vega et al., 2010).

En resumen, aún existen interrogantes acerca de cómo han evolucionado la matrotrofía y la superfetación y bajo qué condiciones podrían resultar beneficiosas. En esta tesis me enfoqué en contestar tres preguntas: **1)** ¿La matrotrofía y la superfetación son más prominentes en especies de tamaño pequeño?, **2)** ¿Los niveles de matrotrofía y superfetación pueden variar dependiendo del tamaño (edad) de las hembras? y **3)** ¿El alimento es un factor determinante en el grado de matrotrofía que presentan las hembras? (Posible valor adaptativo de la matrotrofía. Modelo de Trexler y DeAngelis de aprovisionamiento materno). Cada una de estas preguntas se aborda en un capítulo diferente de la tesis.

En la presente tesis perseguí tres objetivos principales: **1)** conocer si los grados de matrotrofía y superfetación cambian dependiendo del tamaño de las especies y si estas estrategias de manera conjunta afectan la fecundidad de las hembras, **2)** a nivel intraespecífico evaluar si existe un efecto del tamaño (edad) de las hembras sobre los grados de matrotrofía y superfetación (Este es el primer estudio que considera que pueden existir cambios en el nivel de aprovisionamiento materno y superfetación a lo largo de la vida reproductiva de las hembras.) y por último, **3)** me enfoqué en poner a prueba un modelo de aprovisionamiento materno, en el que se sugiere que el alimento puede ser un factor ecológico que favorezca la presencia de matrotrofía o lecitotrofía. Existen trabajos que reportan resultados contradictorios ya sea en condiciones experimentales y/o naturales. Dado que no se han encontrado resultados concluyentes, es importante estudiar los efectos que podría tener el alimento sobre la matrotrofía. Por lo tanto, uno de los capítulos de esta tesis se enfocó en evaluar el modelo de Trexler y DeAngelis. Cada uno de estos objetivos se aborda en un capítulo diferente de la tesis.

A continuación, presento las hipótesis, predicciones y una descripción general de los métodos de cada uno de los tres capítulos que componen esta tesis. Después de la introducción de cada capítulo se encuentran tres artículos. Cada uno corresponde a un objetivo/capítulo de mi tesis.

II CAPÍTULO 1 ¿La matrotrofía y la superfetación son más prominentes en especies de tamaño pequeño?

Título del artículo: Are superfetation and matrotrophy more prominent in small species?

En este capítulo me enfoqué en responder la siguiente pregunta: ¿La matrotrofía y la superfetación son más prominentes en especies de tamaño pequeño? La hipótesis de este capítulo es que la matrotrofía y la superfetación compensan las restricciones reproductivas impuestas por tener tamaños corporales pequeños. En este capítulo tuve dos predicciones: **1)** las especies con tamaños pequeños serán más matrotróficas y presentarán mayores grados de superfetación, siendo esta una forma de compensar las restricciones de espacio, mientras que las especies más grandes serán más lecitotróficas y tendrán menores grados de superfetación y **2)** La matrotrofía y la superfetación permiten que las hembras de especies de tamaño pequeño alcancen una fecundidad total que sea relativamente similar a la de las especies de gran tamaño a nivel inter-específico. La fecundidad de las especies pequeñas con matrotrofía y superfetación debe ser similar a la de especies de mayor tamaño y que no presenten matrotrofía y superfetación, ya que ambas estrategias compensan las restricciones de espacio corporal.

Descripción general de los métodos

Primero realicé un análisis piloto para conocer si en efecto, la matrotrofía es más prominente en hembras de tamaño pequeño. Realicé un análisis en donde incluí datos de 6 especies: *Poecilia mexicana, Xiphophorus helleri* (ambas son lecitotróficas y no presentan superfetación, *Poecilia butleri* (matrotrófica con superfetación), *Poeciliopsis gracilis* (lecitotrófica con superfetación), *Poeciliopsis infans* (Presenta matrotrofía incipiente con superfetación), y *Poeciliopsis prolífica* (con altos niveles de matrotrofía con superfetación).

Después realicé análisis morfométricos para obtener las variables de forma y tamaño de las hembras. Analicé los efectos de la forma y el tamaño sobre el nivel de matrotrofía y superfetación realizando un set de modelos con sentido biológico. Después realicé una búsqueda bibliográfica del tamaño promedio de las hembras reproductivas (mm), superfetación (número de camadas simultáneas presentes), la fecundidad total y la cantidad de nutrientes transferidos por las hembras después de la fertilización (obteniendo el índice de matrotrofía) para 91 especies de la familia Poeciliidae. Dividí a las especies en cuatro categorías: lecitotróficas sin superfetación, matrotróficas con superfetación, lecitotróficas con superfetación y matrotróficas sin superfetación. Utilicé métodos filogenéticos comparativos para buscar una asociación entre el tamaño de las especies, el nivel de matrotrofía y/o superfetación y la fecundidad total, tomando en cuenta las relaciones filogenéticas entre las especies.

III CAPÍTULO 2: ¿Los niveles de matrotrofía y superfetación pueden variar dependiendo del tamaño (edad) de las hembras?

Título del artículo: Small sizes, big strategies: the relationship between female size, matrotrophy and superfetation throughout the reproductive lives of poeciliid fishes

En este Segundo capítulo mi objetivo fue evaluar si existen diferencias en los grados de matrotrofía y superfetación dependiendo del tamaño (edad) de las hembras. Planteé dos hipótesis: **1)** La cantidad de alimento que las hembras transfieren a sus embriones en desarrollo posterior a la fecundación, así como el grado de superfetación, cambian a lo largo de la vida reproductiva de las hembras y **2)** Altos índices de matrotrofía y superfetación cuando las hembras son de tamaño pequeño permiten a las mismas compensar las restricciones de espacio. Mis predicciones fue la siguiente: se observarán altos niveles de matrotrofía y superfetación en hembras de tamaño pequeño (adultos jóvenes), patrón que cambiará conforme las hembras crecen (envejecen). Esperamos que las hembras sean más lecitotróficas y con menos camadas simultáneas cuando tengan un tamaño corporal más grande.

Descripción general de los métodos

Este estudio lo realicé con hembras de 77 poblaciones pertenecientes a 13 especies de la familia Poeciliidae de la colección del Laboratorio de Ecología Evolutiva y Demografía Animal de la Facultad de Ciencias de la UNAM, así como de la colección de peces de la Universidad Brigham Young en Utah, E.U.A. Las especies fueron: *Gambusia sexradiata*, *Gambusia vittata*, *Pseudoxiphophorus jonesii*, *Poecilia butleri*, *P. mexicana*, *Poeciliopsis baenschi*, *P. gracilis*, *P. infans*, *P. latidens*, *P. prolífica*, *P. turrubarensis*, *Priapella* *intermedia* y *Xiphophorus helleri*. Escogí estas especies ya que todas tienen diferentes grados de matrotrofía y superfetación y que contaban con hembras preservadas provenientes de distintas poblaciones. Las hembras fueron medidas, disectadas y se contaron los embriones presentes, así como el número de camadas (superfetación) y el peso seco de los mismos. Después se obtuvo el índice de matrotrofía para cada población. Una vez que obtuve todos los datos, examiné estadísticamente los cambios en los niveles de matrotrofía y superfetación en función del tamaño corporal de las hembras (que a su vez es un indicador de la edad).

IV CAPÍTULO 3 ¿El alimento es un factor determinante en el grado de matrotrofía que presentan las hembras? (Posible valor adaptativo de la matrotrofía. Modelo de Trexler y DeAngelis de aprovisionamiento materno).

Título del artículo: Examination of the Trexler-DeAngelis model of maternal provisioning reveals that matrotrophy is costly

Para este último capítulo mi hipótesis fue que el alimento abundante y disponible de manera constante en el ambiente son condiciones ecológicas que pueden favorecer el cambio de estrategia de provisionamiento materno, de lecitotrofía a matrotrofía. En este capítulo tuve dos predicciones: **1)** en ambientes con alta disponibilidad de alimento, la estrategia que se verá favorecida será la matrotrofía. Por el contrario, en ambientes donde el alimento es escaso la estrategia que se favorecerá será la lecitotrofía y **2)** las hembras que habiten en ambientes en donde la cantidad de alimento sea constante presentarán mayores grados de matrotrofía. En cambio, en ambientes donde el alimento fluctúa la estrategia predominante será la lecitotrofía.

Descripción general de los métodos

Utilicé datos de 45 poblaciones que corresponden a 5 especies del género *Poeciliopsis* (*P. baenschi, P. gracilis, P. infans, P. latidens* y *P. turrubarensis*) que obtuve a partir de la colección del Laboratorio de Ecología Evolutiva y Demografía Animal de la Facultad de Ciencias de la UNAM, así como de la colección de peces de la Universidad Brigham Young en Utah, E.U.A. Las hembras fueron medidas, disectadas y se contaron los embriones presentes, así como el número de camadas (superfetación). Con esta información se obtuvo el índice de matrotrofía por población (Reznick et al., 2002; Marsh-Matthews, 2011). También calculé un índice de matrotrofía individual para 51 hembras superfetadoras que presentaron camadas simultáneas en estadios 4 (a la fecundación) y 11 (el último estadio de desarrollo). Para estimar la cantidad de alimento disponible en el ambiente, utilicé el índice de llenado estomacal (Hyslop, 1980) y la condición corporal (Labocha et al., 2014). Ambas son medidas indirectas de la cantidad de alimento disponible. Para ambas aproximaciones de la cantidad de alimento disponible obtuve un promedio por población. Además, calculé la varianza del índice de llenado estomacal y de la condición corporal tanto entre individuos como a lo largo del tiempo como una forma de estimar la magnitud de las fluctuaciones en la disponibilidad de alimento para cada de cada una de las poblaciones estudiadas. Después realicé modelos lineales con sentido biológico para poner a prueba el modelo de Trexler y DeAngelis, intentando encontrar asociaciones estadísticas entre mis medidas de disponibilidad de alimento y el nivel de matrotrofía.

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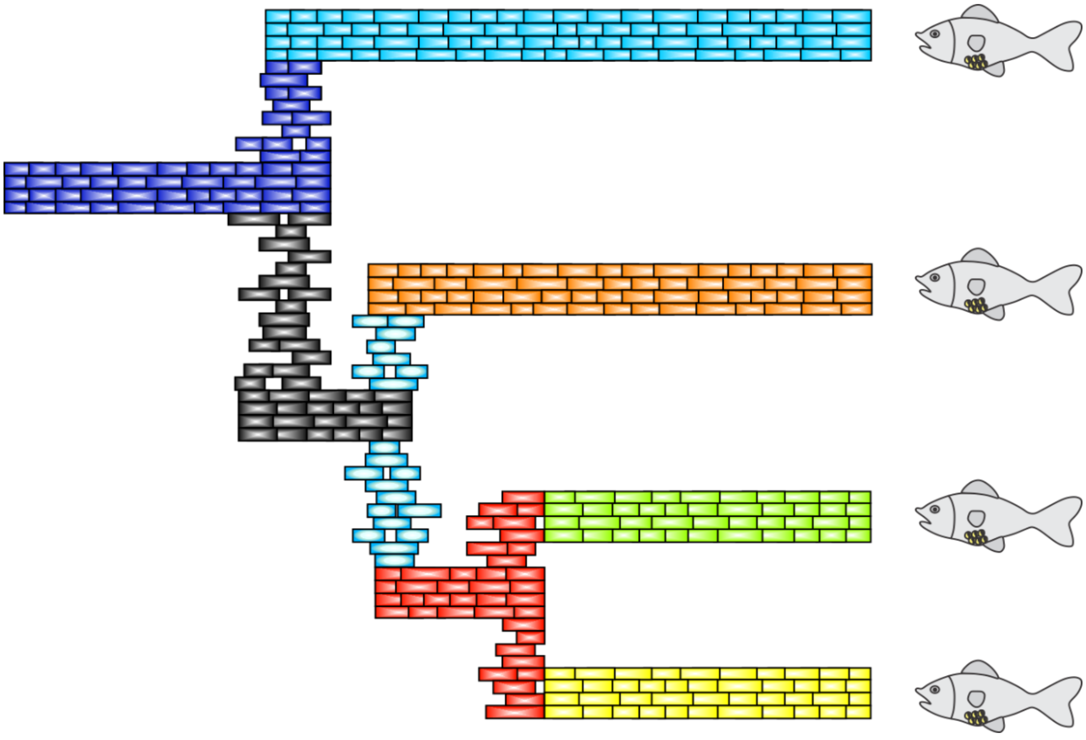
CAPÍTULO 1

Are superfetation and matrotrophy more prominent in small species?

Nabila Saleh-Subaie y J. Jaime Zúñiga-Vega

Artículo publicado en Evolutionary Ecology Research

2019



Evolutionary Ecology Research, 2019, **20:** 403–425

Are superfetation and matrotrophy more prominent in small species?

Nabila Saleh-Subaie[1] and J. Jaime Zúñiga-Vega[2]

[1]Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, México and [2]Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México

ABSTRACT

Background: Superfetation is the ability of females to simultaneously bear multiple broods of embryos at different developmental stages. Matrotrophy is the post-fertilization transfer of nutrients to developing embryos by the mother. In viviparous fishes of the family Poeciliidae there is wide interspecific variation in the degree of these two reproductive modes. Given that superfetating and matrotrophic females increase less in mass and volume when producing a particular number of embryos compared with females of species that lack these reproductive modes, we propose the hypothesis that their joint presence allows females to fit a relatively large number of embryos in any given volume of the reproductive tract. Thus, matrotrophy and superfetation should be more advantageous for small species, because their presence could compensate the space restrictions of a small body size by maximizing their reproductive output.

Question: Do superfetation and matrotrophy compensate the reproductive constraints imposed by a small body size, and thus are more prominent in small species?

Data description: Previously published data on female size, degree of superfetation, matrotrophy index, and total fecundity of 91 poeciliid species.

Search method: We implemented phylogenetic generalized least squares regressions to examine potential effects of female size on superfetation and matrotrophy. In addition, we compared total fecundity among four categories of species that differ in the presence or absence of matrotrophy and superfetation, accounting for both phylogenetic relatedness and differences in female size among species.

Conclusions: We failed to find support for the hypothesis that superfetation and matrotrophy increase the total reproductive output of small species. Small species do not exhibit more pronounced superfetation and matrotrophy than large species. Therefore, these two reproductive strategies do not function as mechanisms to compensate the physical restrictions of a small body size.

Keywords: body size, comparative analysis, reproductive modes, Poeciliidae, viviparous fishes.

Correspondence: J.J. Zúñiga-Vega, Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, 04510, Ciudad de México, México. email: jzuniga@ciencias.unam.mx

INTRODUCTION

Superfetation is the ability of females to simultaneously bear two or more groups of embryos at different developmental stages (Turner, 1937; Scrimshaw, 1944a). This is a rare reproductive strategy that occurs in three families of viviparous fishes [Clinidae, Poeciliidae, and Zenarchopteridae (Gunn and Thresher, 1991; Reznick *et al.*, 2007; Pires *et al.*, 2011)] and in bivalves of the family Sphaeriidae (Cooley and Foighil, 2000). In mammals, superconception and superfertilization are similar phenomena that have been observed in a few species only [European brown hare, American mink, and European badger (Roellig *et al.*, 2011)]. Within the Poeciliidae, a Neotropical family of viviparous fishes, superfetation is relatively frequent and has evolved independently multiple times (Pollux *et al.*, 2009; Meredith *et al.*, 2011; Gorini-Pacheco *et al.*, 2018). In addition, there is wide variation among species as well as among populations within species in the degree of superfetation (i.e. in the number of simultaneous broods that females bear), from only two [for example in *Poeciliopsis catemaco* (Bassar *et al.*, 2014)] up to eight simultaneous broods [for example in *Heterandria formosa* (Scrimshaw, 1944b)].

Matrotrophy is the post-fertilization provisioning of nutrients to developing embryos by the mother (Wourms, 1981; Marsh-Matthews, 2011). In matrotrophic species, nutrient transfer occurs through placental structures (Kwan *et al.*, 2015; Olivera-Tlahuel *et al.*, 2019). Matrotrophy is the opposite of lecithotrophy, in which mothers deposit nutrients into the mature ova in the form of yolk before fertilization. In lecithotrophic species, developing embryos feed on the yolk with no additional source of nutrients from the mother (Wourms, 1981). Poeciliid fishes exhibit wide interspecific variation in the amount of nutrients that females transfer to their developing embryos after fertilization, from strictly lecithotrophic species to species that exhibit extensive matrotrophy, with numerous species in between these extremes that vary in the relative amounts of pre- and post-fertilization maternal provisioning (Reznick *et al.*, 2002; Pollux *et al.*, 2009, 2014). Extensive matrotrophy has evolved independently multiple times within the fish family Poeciliidae and some studies have demonstrated that the amount of matrotrophy may also vary among populations within species (Riesch *et al.*, 2010a; Zúñiga-Vega *et al.*, 2011; Gorini-Pacheco *et al.*, 2018).

Given the multiple independent evolutionary origins of both superfetation and matrotrophy and the wide variation among and within species in the degree of these two reproductive modes, some studies have attempted to show their potential benefits in terms of fitness (Travis *et al.*, 1987; Trexler and DeAngelis, 2003, 2010; Macías-Garcia and González-Zuarth, 2005; Zúñiga-Vega *et al.*, 2007, 2010, 2017; Olivera-Tlahuel *et al.*, 2015). Most of these studies have focused separately on either superfetation or matrotrophy, thereby ignoring the fact that there is an evolutionary correlation between the two strategies (Meredith *et al.*, 2011; Pollux *et al.*, 2014). This means that species with the most pronounced matrotrophy also tend to overlap more broods and vice versa, although there are a few exceptions whereby superfetating species are lecithotrophic or matrotrophic species do not exhibit superfetation (Reznick and Miles, 1989; Arias and Reznick, 2000; Pires and Reznick, 2018). Therefore, it is necessary to analyse the two traits together when attempting to understand their adaptive value.

One hypothesis that has been proposed to explain the benefits of the joint presence of superfetation and matrotrophy suggests that, assuming an equal number of embryos produced at any particular point in time, the body mass and volume of pregnant females may be substantially lower in matrotrophic and superfetating species than in lecithotrophic and non-superfetating species (Reznick and Miles, 1989; Pollux *et al.*, 2009). In matrotrophic species, mature ova are quite small and, after fertilization, they gradually grow in mass and volume during development because females provide them with nutrients throughout gestation. Small initial embryo size and gradual increases in mass during development imply that the mass and volume of pregnant females increase little during a large proportion of pregnancy, at least before the late phases of embryo

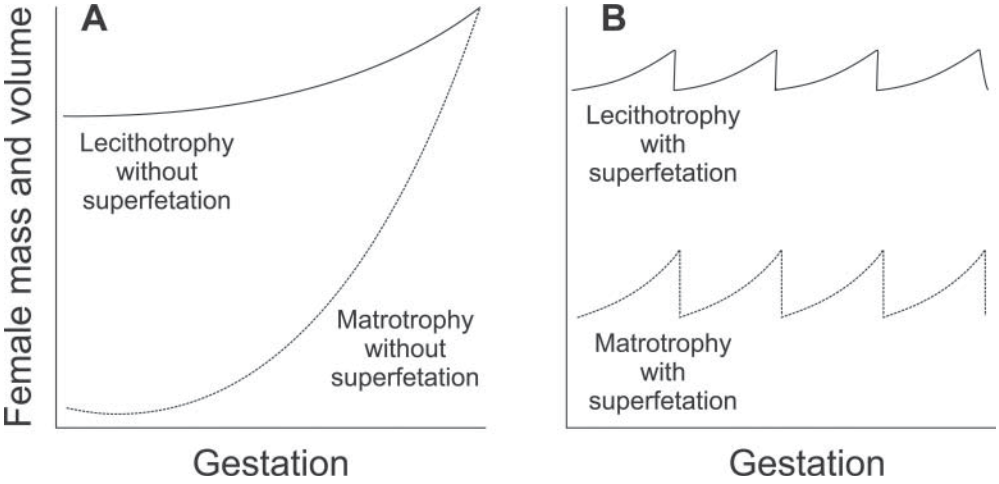


Fig. 1. Predicted changes throughout gestation in the mass and volume of pregnant females for lecithotrophic (solid lines) and matrotrophic (dashed lines) species without (A) and with (B) superfetation. Modified from Pollux *et al.* (2009).

development (Fig. 1A) (Fleuren *et al.*, 2018). In contrast, in lecithotrophic species, mature ova are large and full of yolk before fertilization, and hence the mass and volume of reproductive females are considerably large even before fertilization and they may increase further throughout pregnancy due to water uptake by developing embryos (Fig. 1A).

With respect to superfetation, females that adopt this strategy appear to split a large reproductive bout into two or more smaller broods. Given that wet mass of embryos increases as development progresses, superfetating species overall increase less in mass and volume during pregnancy because embryos in early developmental stages are smaller than embryos in late stages (Zúñiga-Vega *et al.*, 2010). In contrast, non-superfetating species produce all their embryos in a single bout and all of them reach the latest stages (when they become largest) at the same time, which results in pregnant females with substantially larger body mass and volume. This expected difference between superfetating and non-superfetating species also assumes that the total number of embryos carried by females is the same.

Lower female mass and volume during pregnancy should be true for either superfetation or matrotrophy (i.e. considering each of these strategies separately), but this benefit is presumably greatest when the two strategies occur together in a single species (Pollux *et al.*, 2009). Matrotrophy allows the body mass and volume of pregnant females to remain relatively unchanged during the early phases of embryo development and, in concert with superfetation, body mass and volume increase only little when pregnancy progresses, because only a subset of the developing embryos reach the largest sizes (Fig. 1B).

Based on this hypothesis, we wish to go one step further and propose that, given that matrotrophic and superfetating females increase less in mass and volume when producing a particular number of embryos compared with lecithotrophic and non-superfetating females (i.e. a particular number of developing embryos weigh less and occupy a smaller volume of the reproductive tract in matrotrophic and superfetating females), matrotrophic and superfetating females would be able to fit a larger number of embryos in any given volume of the reproductive tract than lecithotrophic and non-superfetating females. Therefore, matrotrophy and superfetation should be more advantageous for small species, because the presence of these two reproductive modes could compensate the space restrictions of a small body size by maximizing their fecundity. In many taxa, smaller species produce fewer offspring than larger species (Hines, 1991; Sokolovska *et al.*, 2000; Prado and Haddad, 2005). Hence, natural selection could promote the evolution of anatomical, behavioural, physiological, or life-history adaptations that allow small species to maximize their total reproductive output (Blanckenhorn, 2000; Bergström and Wiklund, 2002). Therefore, we predict that higher levels of matrotrophy and superfetation will be more frequent in small than larger taxa. In addition, given that the range of female sizes within poeciliid species may be quite wide (Johnson and Bagley, 2011), such as in other vertebrates with indeterminate growth, we also tested the prediction that, within populations of species that exhibit both matrotrophy and superfetation, smaller females should exhibit a higher degree of both reproductive modes than larger females.

According to our hypothesis, matrotrophy and superfetation may allow females from small species to reach total fecundities that are relatively similar to those of large species, because small species with matrotrophy and superfetation would be able to produce a relatively large number of newborns despite the space restrictions that are inherent to a small body size. To test this hypothesis, we compared fecundity among four categories of species that differ in the presence or absence of matrotrophy and superfetation (lecithotrophic species with and without superfetation and matrotrophic species with and without superfetation). In this comparison, we also accounted for differences in average female size among species and predicted that small species with matrotrophy and superfetation should have: (1) higher fecundities than similar-sized species that lack these two reproductive modes, and (2) similar fecundities to those of larger species.

METHODS

Study system and data collection

We focused on the fish family Poeciliidae because of the wide interspecific variation in matrotrophy, superfetation, and female size (Reznick and Miles, 1989; Pollux *et al.*, 2009, 2014). We conducted a literature search to gather data on average standard length (mm) of reproductive females, degree of superfetation (average number of simultaneous broods), and the amount of nutrients transferred to embryos during development (estimated by means of the matrotrophy index) for 91 poeciliid species (see evolutionary-ecology.com/data/3178Appendix.pdf, Table S1). The matrotrophy index (MI) is a standard measure of post-fertilization maternal provisioning and is calculated as the dry mass of the offspring at birth divided by the dry mass of the egg at fertilization (Reznick *et al.*, 2002; Marsh-Matthews, 2011). MI values <0.75 indicate that all nutrients are provided before fertilization in the form of yolk (i.e. strict lecithotrophic species); MI values between 0.75 and 1 indicate that small amounts of nutrients are provided during embryo development (i.e. incipient matrotrophy); MI values >1 indicate that substantial amounts of nutrients are provided after fertilization; and MI values >5 indicate extensive matrotrophy (Reznick *et al.*, 2002; Pollux *et al.*, 2009; Marsh-Matthews, 2011; Pires *et al.*, 2011). Even though developing embryos of some species with lecithotrophy or incipient matrotrophy can absorb radiotracers shortly after injecting pregnant females, which has been interpreted as the presence of true matrotrophy in these species (Marsh-Matthews *et al.*, 2001, 2005), a recent study demonstrated that eggs from oviparous species can also absorb radiolabelled compounds from their surrounding environment (Morrison *et al.*, 2017). Thus, the presence of radioactivity in embryos of lecithotrophic species does not indicate active transfer of nutrients by the mother, but instead represents a general capacity of embryos of both oviparous and viviparous species to absorb organic molecules from their surrounding environment. Hence, our classification of species as having lecithotrophy, incipient matrotrophy, or true matrotrophy based on the matrotrophy index is appropriate.

In addition, for all 91 species we also obtained data on total fecundity (i.e. total number of embryos carried by females at any particular point in time) (see Table S1). For non-superfetating species, this was equivalent to brood size. However, for superfetating species, we calculated total fecundity as brood size multiplied by the number of simultaneous broods (assuming that the number of embryos per brood is on average similar among all the broods within a female). We log-transformed all these variables prior to our comparative analyses.

Morphological comparisons

Our hypothesis that matrotrophy and superfetation should be more prominent in small species – and in small females within species – is based on the assumption that, for any given body length, higher degrees of these two reproductive strategies should result in a lower body volume of reproductive females compared with species and females with less pronounced matrotrophy and superfetation (Fig. 1). We searched for evidence that would support this assumption by comparing the body profile of pregnant females from the following six species: *Poecilia mexicana*, *Xiphophorus hellerii* (both are lecithotrophic and lack superfetation), *Poecilia butleri* (matrotrophic without superfetation), *Poeciliopsis gracilis* (lecithotrophic with superfetation), *P. infans* (has incipient matrotrophy with superfetation), and *P. prolifica* (highly matrotrophic with superfetation). We obtained preserved females of *Poecilia mexicana, X. hellerii*, and *Poeciliopsis prolifica* from the National Collection of Fishes (Instituto de Biología, Universidad Nacional Autónoma de México) and digital photographs of pregnant females of *Poecilia butleri*, *Poeciliopsis gracilis*, and *P. infans* from the archives of Zúñiga-Vega *et al.* (2011) and Frías-Alvarez and Zúñiga-Vega (2016) (sample size per species varied from 16 to 123 females). We digitized 17 landmarks on the photographs of all these pregnant females (as per Zúñiga-Vega *et al.*, 2011) and applied geometric morphometric techniques to obtain shape variables using the programs tpsDig v.2.17 and tpsRelw v.1.69 (Zelditch *et al.*, 2004; Rohlf, 2013, 2017).

We statistically compared the first shape axis (i.e. the first relative warp) among species by comparing the fit of a linear model that considers differences among species against the fit of a model that does not include interspecific differences (an intercept-only model). For this purpose, we used the Akaike Information Criterion adjusted for small sample sizes [AICc (Burnham and Anderson, 2002)]. We visualized shape differences among species by means of deformation grids (Zelditch *et al.*, 2004) and, according to the main assumption underlying our hypothesis, we predicted that pregnant females would have narrower abdomens in *P. infans* and *P. prolifica* because these two species exhibit matrotrophy with superfetation.

In addition, we also analysed body shape within two species of the genus *Poeciliopsis: P. gracilis and P. infans.* We obtained reproductive data and digital photographs of pregnant females for two populations of each species from Frías-Alvarez and Zúñiga-Vega (2016). In both species, the two populations differed in their degree of matrotrophy (see Results) but showed little variation in the number of simultaneous broods. Thus, our comparisons between populations tested only the effect of matrotrophy on body shape. It should be noted here that *P. gracilis* has been considered as lecithotrophic, with an average matrotrophy index of 0.72 (Bassar *et al.*, 2014; Pollux *et al.*, 2014). However, data from Frías-Alvarez and Zúñiga-Vega (2016) indicate that some populations of this species exhibit incipient matrotrophy, which reveals intraspecific variation in the amount of post-fertilization maternal provisioning. Based on digital photographs of pregnant females, we implemented the same protocol that we explained above to obtain shape variables. Then, we compared the first shape axis between populations (separately for each species) by comparing the fit of a model that included inter-population differences against the fit of an intercept-only model. Model fit was assessed using AICc. We expected narrower abdomens in the populations with higher MI values.

Interspecific evaluation of the effect of size

We implemented a phylogenetic comparative method [phylogenetic generalized least squares, PGLS (Martins and Hansen, 1997)] to search for a statistical effect of average female size (independent variable) on both matrotrophy and superfetation (dependent variables) while accounting for phylogenetic relatedness. We based these analyses on a robust phylogeny of the family Poeciliidae that was assembled from 28 different genes (Pollux *et al.*, 2014). We trimmed this phylogeny to our 91 species of interest using the program Mesquite v.3.51 (Maddison and Maddison, 2018) and transformed it to an ultrametric tree using a semi-parametric smoothing method based on penalized likelihood (Sanderson, 2002) in the R package 'ape' (Paradis *et al.*, 2004; R Core Team, 2018) (Fig. 2). PGLS incorporates the phylogenetic information as part of the error term of the regression model (Martins and Hansen, 1997).

We compared the fit of four different evolutionary assumptions when analysing the effect of female size on superfetation and matrotrophy by means of PGLS implemented in the R package 'ape'. First, we assumed that traits accumulate changes steadily through time in a way that is well-described by a Brownian motion model (PGLS-BM), which results in substantial phylogenetic signal in the residuals of the regression model. Second, we assumed stabilizing selection towards a fixed optimum, which corresponds to an Ornstein-Uhlenbeck model of evolution (PGLS-OU). This model estimates alpha as a measure of the strength of stabilizing selection. Hence, when α is close to zero, selection is weak and phenotypes retain substantial phylogenetic signal. As α increases, selection is strong and phenotypes adapt quickly to the local environment, leaving behind no phylogenetic signal. Third, we directly quantified the amount of phylogenetic signal in the residuals of the relationships between female size and superfetation and matrotrophy by estimating lambda (Pagel, 1999), a scaling parameter that weights the influence of the phylogeny in explaining the observed data (PGLS-λ). A λ-value close to zero indicates independence from the phylogeny, whereas a λ-value close to one indicates strong similarity between related species. Fourth, we assumed that the phylogenetic relationships have not influenced the correlated evolution between female size, superfetation, and matrotrophy by fitting an ordinary least squares regression (OLS). We used AICc to compare the fit of these four evolutionary assumptions.

We conducted a phylogenetic principal components analysis [PPCA (Revell, 2009)] on both degree of superfetation and the matrotrophy index to create a combined measure of these two reproductive strategies. For this purpose, we used the R package 'phytools' (Revell, 2012). PPCA incorporates the expected covariance among phenotypic traits that would result from phylogenetic relatedness into the principal components analysis. This phylogenetic PCA resulted in a single factor with an eigenvalue >1, which means that the information contained in both variables can be summarized in a single principal component. The largest values of this first principal component (PC1) corresponded to species with the highest degrees of both matrotrophy and superfetation, whereas the lowest values corresponded to lecithotrophic and non-superfetating species.

We implemented the three PGLS models and OLS regression to examine the potential effect of female size on (1) degree of superfetation, (2) matrotrophy index, and (3) the joint presence

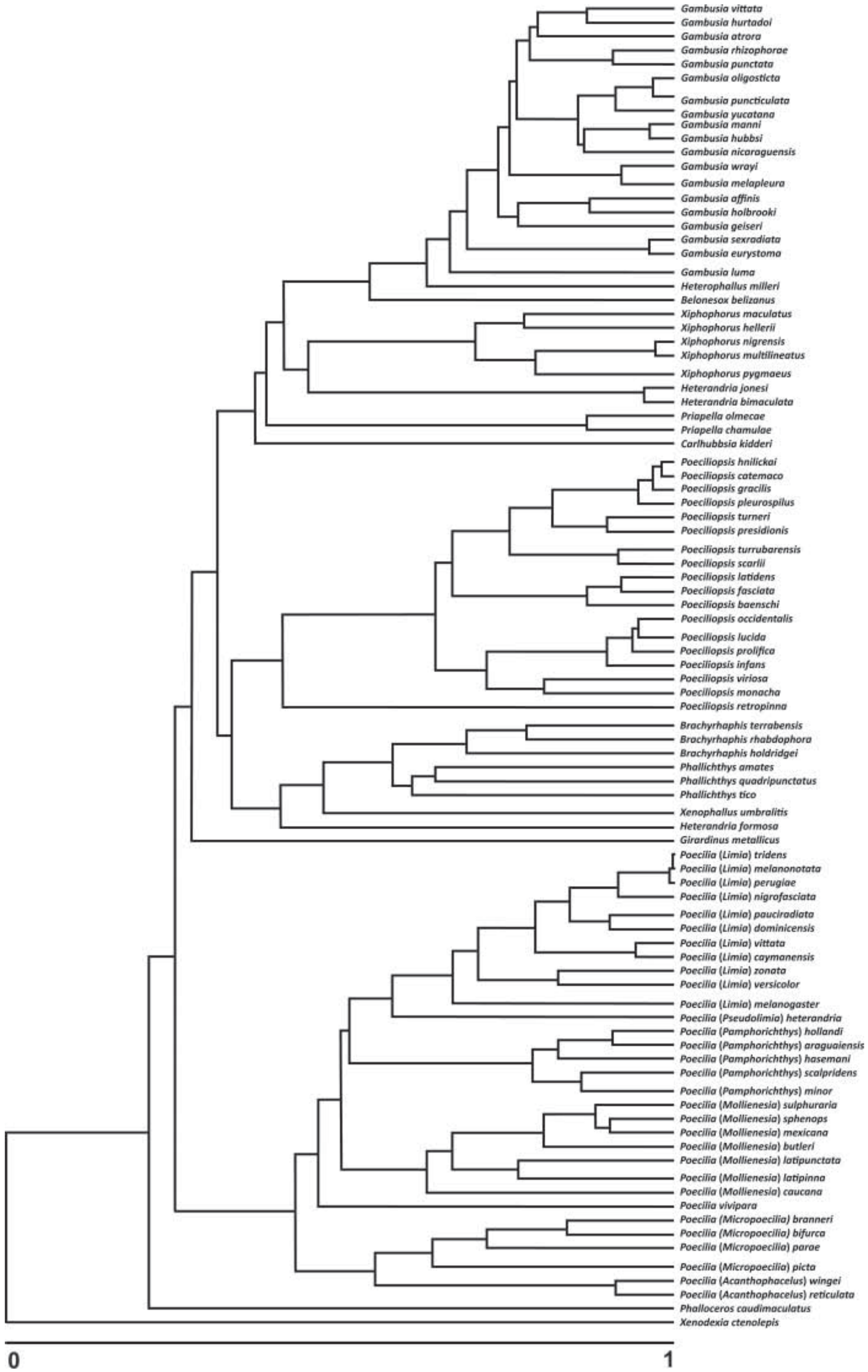


Fig. 2. Phylogenetic relationships among 91 species of fishes from the family Poeciliidae according to Pollux *et al.* (2014). Branch lengths represent relative time.

of superfetation and matrotrophy (PC1). Before running these models, we log-transformed all variables, except PC1 (which followed a normal distribution). These models test our hypothesis that a small body size promotes the evolution of a higher degree of both superfetation and matrotrophy.

Intraspecific evaluation of the effect of size

We examined the influence of female size on superfetation and matrotrophy at the intraspecific level by comparing the average degree of these two reproductive modes among three size categories: small, medium-sized, and large females. We used data from two populations of *P. gracilis* and two populations of *P. infans* (Frías-Alvarez and Zúñiga-Vega, 2016). The small size category included all females that measured less than −0.5 standard deviation (SD) of the mean standard length, the medium size category included females that measured between −0.5 and +0.5 SD, and the large size category included females that measured more than +0.5 SD. In these intraspecific analyses, we treated size as a categorical variable because the matrotrophy index cannot be calculated for each individual, but instead is calculated for a group of individuals by regressing log-transformed embryo mass on developmental stage and then dividing the predicted embryo mass at stage 11, which is the estimated size of full-term embryos according to Haynes (1995), by the predicted mass at stage 4, which is the estimated size at fertilization (Riesch *et al.*, 2013). In this regression, each data point corresponds to an individual female.

We statistically compared degree of superfetation (number of simultaneous broods per female) among size categories by means of a generalized linear model with log link function and Poisson distribution of the error term. We used AICc to compare the fit of this model against the fit of an intercept-only model. Given that the matrotrophy index is calculated for a group of individuals and not for each individual female, we conducted randomization tests (Manly, 2007) to compare the index among size categories. These randomization tests consisted of the following steps. First, we calculated an MI value for each of the three size categories. Second, we calculated the standard deviation (SD) among these three MI values. Third, we randomized all data across all size categories and drew three random samples from this pooled dataset. Fourth, we calculated MI values for each of these three random samples as well as the SD among these new MI values. We repeated the last two steps 1000 times and compared the originally observed SD with the distribution of 1000 SD values that resulted from randomizing the data. Evidence of differences among categories in the matrotrophy index would be provided by our observed SD being higher than the 950th sorted value of the resulting distribution (this would be equivalent to $P < 0.05$ in a one-tailed test). We also calculated 95% bootstrap confidence intervals for the MI values (as per Zúñiga-Vega *et al.*, 2011). These analyses were conducted separately for each population.

Fecundity comparisons

We classified our 91 species into one of the following four categories: lecithotrophy without superfetation, lecithotrophy with superfetation, matrotrophy without superfetation, and matrotrophy with superfetation, and then compared total fecundity among these four categories. For this purpose, we also implemented the PGLS and OLS regression models that we described above in which fecundity was the response variable and reproductive mode (our four categories) served as the main factor. We included average female size as a covariate in these models and predicted that, for small species, total fecundity should be greater in species that exhibit both matrotrophy and superfetation than in the other three categories (Fig. 3). This difference should

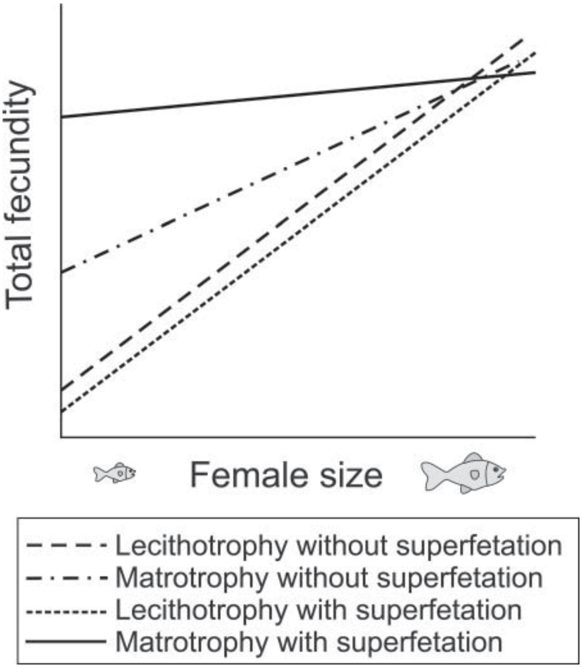


Fig. 3. Predicted relationships between average female size and total fecundity for four distinct reproductive modes. Small species that exhibit both matrotrophy and superfetation should have (1) higher fecundity than small species that exhibit the other three reproductive modes, and (2) similar fecundities to those of larger species.

be more pronounced with respect to lecithotrophic species (both with and without superfetation) (Fig. 1). We also predicted that small species with matrotrophy and superfetation should have similar fecundities to those of larger species (regardless of the particular reproductive mode of such larger taxa; Fig. 3). Evidence supporting these predictions would be provided by a regression line (between fecundity and average size) with higher intercept and less steep slope for matrotrophic and superfetating species compared with all other reproductive modes, such as we depict in Fig. 3. This means that we expected a significant interaction between reproductive mode and female size as well as a significantly different intercept for matrotrophic and superfetating species with respect to the intercepts for all other reproductive modes.

Phylogenetic signal

From PGLS-λ, we obtained estimates of phylogenetic signal in the residuals of the regressions between female size and our reproductive variables (superfetation, matrotrophy index, PC1, and total fecundity). These estimates of lambda indicate how important shared ancestry has been in the correlated evolution between female size and these reproductive traits. However, these estimates of lambda derived from PGLS do not quantify the amount of phylogenetic signal in the traits themselves (Symonds and Blomberg, 2014). Thus, we used the R package ‘phytools’ to estimate lambda for all traits and to evaluate whether lambda was significantly different than zero (i.e. significant phylogenetic signal) using likelihood ratio tests.

RESULTS

Superfetation and matrotrophy promote slender bodies

We found differences in female morphology among species and these differences were consistent with the hypothesis that pregnant females that exhibit superfetation and matrotrophy have less distended abdomens than females that lack these reproductive strategies. The intercept-only model had substantially weaker support than the model that included differences among species (ΔAICc = 638.28). *Poeciliopsis prolifica* (which exhibits substantial matrotrophy and superfetation) had the lowest scores in the first shape axis, indicating the thinnest morphologies (Fig. 4A). *Poeciliopsis infans* (incipient matrotrophy and superfetation) and *P. gracilis* (lecithotrophic with superfetation) had relatively low values in the first shape axis, also indicating slender female bodies. In contrast, *Poecilia mexicana*, *Xiphophorus hellerii* (both are lecithotrophic and lack superfetation), and *Poecilia butleri* (matrotrophic without superfetation) had higher scores in the first shape axis, indicating robust females (Fig. 4A).

In both *P. gracilis* (Fig. 4B) and *P. infans* (Fig. 4C), females from the population with higher MI values exhibited thinner bodies (lower scores in the first shape axis) than females from the population with lower MI values, which had more distended abdomens (higher scores in the first shape axis). In both species, the intercept-only model had weaker support than the model that included a difference between populations (*P. gracilis*: ΔAICc = 36.68; *P. infans*: ΔAICc = 18.79).

Superfetation and matrotrophy are not more prominent in small species

We found no significant effects of female size on either superfetation, matrotrophy index, or PC1 (Table 1). The slope that represents the effect of female size on these traits was not statistically different than zero in almost all the evolutionary models that we tested (Fig. 5). The only exception was the effect of female size on the matrotrophy index assuming that phylogeny is unimportant (OLS regression). In this case, the slope indicated a negative relationship between these two traits (Table 1). However, the relative support for this model was extremely weak (ΔAICc = 57.9 with respect to the best-fitting model).

In all cases, the models with strongest support indicated that the potential effect of female size on these reproductive strategies evolved in a way that is consistent with a Brownian motion model of evolution. PGLS-BM provided the best fit for both MI and PC1 (Table 1). In the case of the potential effect of female size on superfetation, PGLS-λ provided the best fit, with an estimate of λ equal to 1.03 (Table 1), which is also indicative of high phylogenetic signal,

Fig. 4. Morphological comparisons among six species with different reproductive modes (A) and between two populations of *Poeciliopsis gracilis* (B) and *P. infans* (C). The two populations of *P. gracilis* and *P. infans* differed in the matrotrophy index (MI). In all cases, we compared the first shape axis (first relative warp, RW1) derived from geometric morphometric analyses. Deformation grids represent deviations from the consensus shape for females with positive and negative scores in the RW1. Error bars represent 95% confidence intervals.

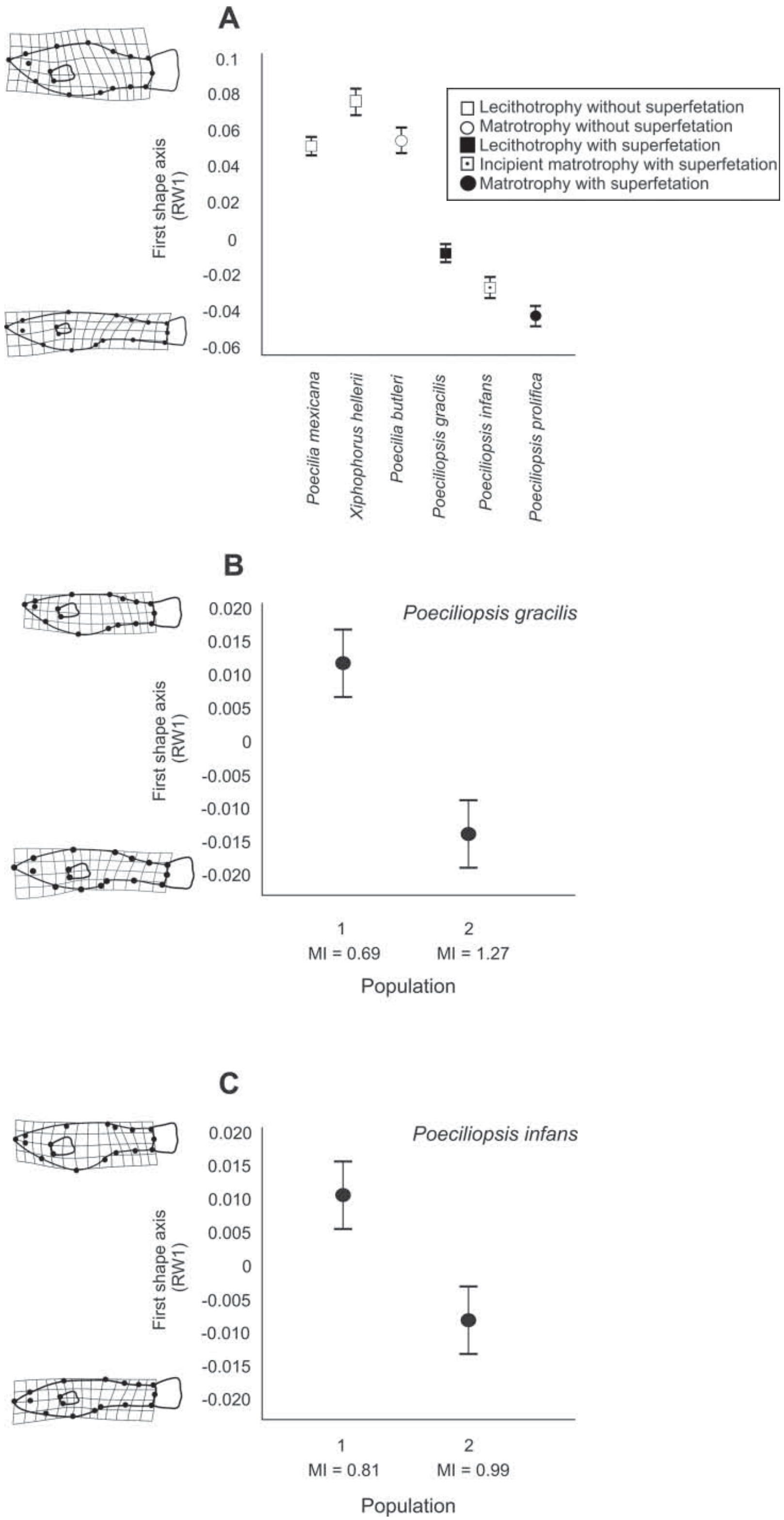

A
First shape axis (RW1)
0.1
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
☐ Lecithotrophy without superfetation
○ Matrotrophy without superfetation
■ Lecithotrophy with superfetation
⊡ Incipient matrotrophy with superfetation
● Matrotrophy with superfetation
Poecilia mexicana
Xiphophorus hellerii
Poecilia butleri
Poeciliopsis gracilis
Poeciliopsis infans
Poeciliopsis prolifica
B
Poeciliopsis gracilis
First shape axis (RW1)
0.020
0.015
0.010
0.005
0
-0.005
-0.010
-0.015
-0.020
1
MI = 0.69
2
MI = 1.27
Population
C
Poeciliopsis infans
First shape axis (RW1)
0.020
0.015
0.010
0.005
0
-0.005
-0.010
-0.015
-0.020
1
MI = 0.81
2
MI = 0.99
Population

Table 1. Results from phylogenetic generalized least squares (PGLS-BM, PGLS-OU, and PGLS-λ) and ordinary least squares (OLS) regression models fitted to test the effect of female size on superfetation and matrotrophy (MI) for 91 fish species from the family Poeciliidae

Regression model	Evolutionary assumption	AICc	ΔAICc	Regression slope	*P*	α	λ
Superfetation – female size	PGLS-λ	18.56	0	−0.00001 (0.0002)	0.97		1.03
	PGLS-BM	29.41	10.85	0.11 (0.08)	0.21		
	PGLS-OU	31.01	12.45	0.10 (0.09)	0.24	0.41	
	OLS	87.78	69.22	0.16 (0.11)	0.18		
MI – female size	PGLS-BM	244.86	0	−0.38 (0.28)	0.18		
	PGLS-λ	247.03	2.17	−0.37 (0.28)	0.19		0.99
	PGLS-OU	247.05	2.19	−0.38 (0.28)	0.18	2×10^{-7}	
	OLS	302.76	57.90	−0.97 (0.38)	0.013		
PC1 – female size	PGLS-BM	190.78	0	−0.01 (0.21)	0.97		
	PGLS-λ	192.90	2.12	−0.02 (0.20)	0.92		1.001
	PGLS-OU	192.97	2.19	−0.01 (0.21)	0.97	4×10^{-7}	
	OLS	251.49	60.71	−0.19 (0.29)	0.52		

Note: PGLS was implemented using three evolutionary models: Brownian motion (BM), Ornstein-Uhlenbeck (OU), and phylogenetic signal (λ). The fit of each model to the data was evaluated using the Akaike Information Criterion (AICc), with the smallest value indicating the best-fitting model. We also show differences in AICc values between each model and the best-fitting model (ΔAICc). Models are listed according to their AICc values. Standard errors of the regression slopes are shown within parentheses. PGLS-OU estimates an additional parameter, α, which measures the strength of stabilizing selection. λ is a scaling parameter that weights the influence of the phylogeny in explaining the observed data. PC1 represents a combined measure of superfetation and matrotrophy.

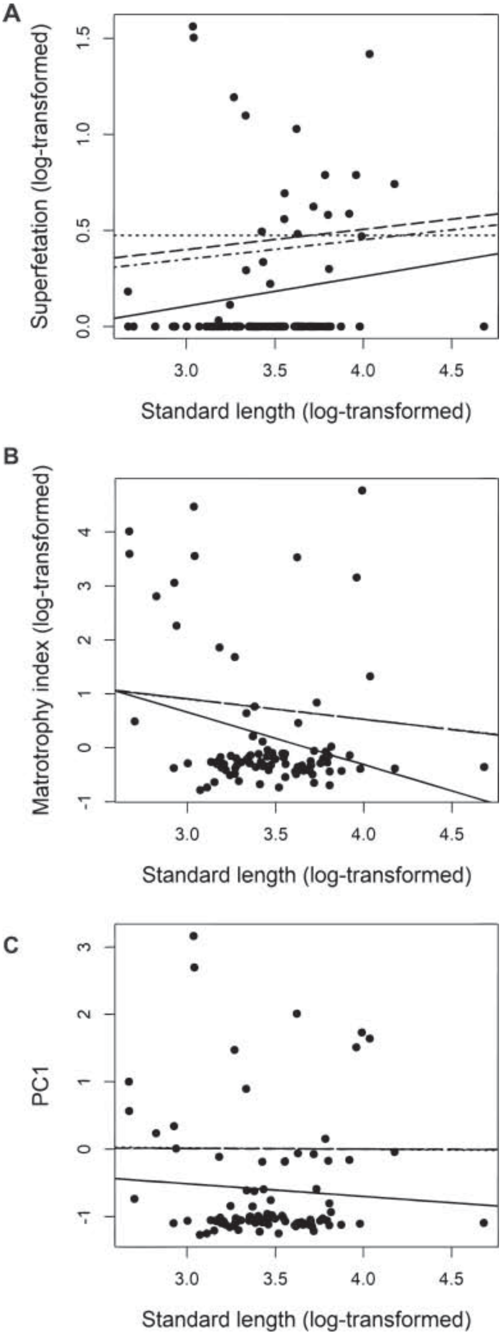


Fig. 5. Potential effect of female size (standard length) on superfetation (A), matrotrophy index (B), and a combined measure of superfetation and matrotrophy (PC1) (C) for 91 species of fishes from the family Poeciliidae. In all panels, the solid line corresponds to an ordinary (non-phylogenetic) least squares regression. The other lines represent phylogenetic generalized least squares regressions (PGLS) assuming a Brownian motion model of evolution (dashed), an Ornstein-Uhlenbeck model of evolution (dot-dashed), or estimating the amount of phylogenetic signal (λ) in the residuals (dotted).

a pattern that is consistent with the Brownian motion model. However, as we emphasized above, the estimated effects of female size derived from these models were not statistically significant (Fig. 5).

Within species, superfetation – but not matrotrophy – may increase in larger females

Contrary to our prediction that superfetation and matrotrophy would be more prominent in small females, medium-sized and large females exhibited higher degrees of superfetation than small females in the two populations of *P. gracilis* (Fig. 6A, B). In both cases, the model indicating differences among size categories had strong support (ΔAICc = 0 and 1.33) and the 95% confidence interval for small females did not overlap with those for medium-sized and large females. In one population of *P. infans*, the degree of superfetation was similar among size categories as indicated by weak support for the model that included differences among categories (ΔAICc = 4.52; Fig. 6C). In the other population of *P. infans*, the model indicating differences among size categories provided the best fit, whereas the intercept-only model had less support (ΔAICc = 2.79). In this population, large females had more pronounced superfetation than small and medium-sized females (Fig. 6D).

Regarding matrotrophy, the matrotrophy index was similar among size categories in all four populations (Fig. 6E–H). In all cases, the 95% bootstrap confidence intervals for the three size categories overlapped each other and the standard deviation of the actual MI values was similar to the SD values that we obtained after randomizing the data ($P > 0.15$ in all four populations).

Superfetation and matrotrophy do not maximize fecundity

The model that assumed independence from the phylogeny (OLS regression) provided the best fit to the fecundity data (Table 2). PGLS-OU and PGLS-λ also had considerable support (ΔAICc < 2 in both cases), with a large estimate of α (35.37) and a low estimate of λ (0.18), both of which also indicate independence from the phylogeny. PGLS-BM had little support (Table 2). For simplicity, we present and interpret results from the OLS regression (all other models yielded similar results). The main effect of female size was positive and significant ($F_{1,83} = 12.25$, $P = 0.001$). Thus, in general, females of larger species carry more embryos than females of smaller species (Fig. 7). In contrast, the main effect of reproductive mode and the interaction

Table 2. Results from phylogenetic generalized least squares (PGLS-BM, PGLS-OU, and PGLS-λ) and ordinary least squares (OLS) regression models fitted to test the effects of female size and reproductive mode on total fecundity for 91 fish species from the family Poeciliidae

Evolutionary assumption	AICc	ΔAICc	α	λ
OLS	225.25	0		
PGLS-OU	225.91	0.66	35.37	
PGLS-λ	226.60	1.35		0.18
PGLS-BM	277.45	52.20		

Note: We classified our 91 species into one of four distinct reproductive modes: lecithotrophy without superfetation, lecithotrophy with superfetation, matrotrophy without superfetation, and matrotrophy with superfetation. All abbreviations and symbols are as defined in the footnote to Table 1.

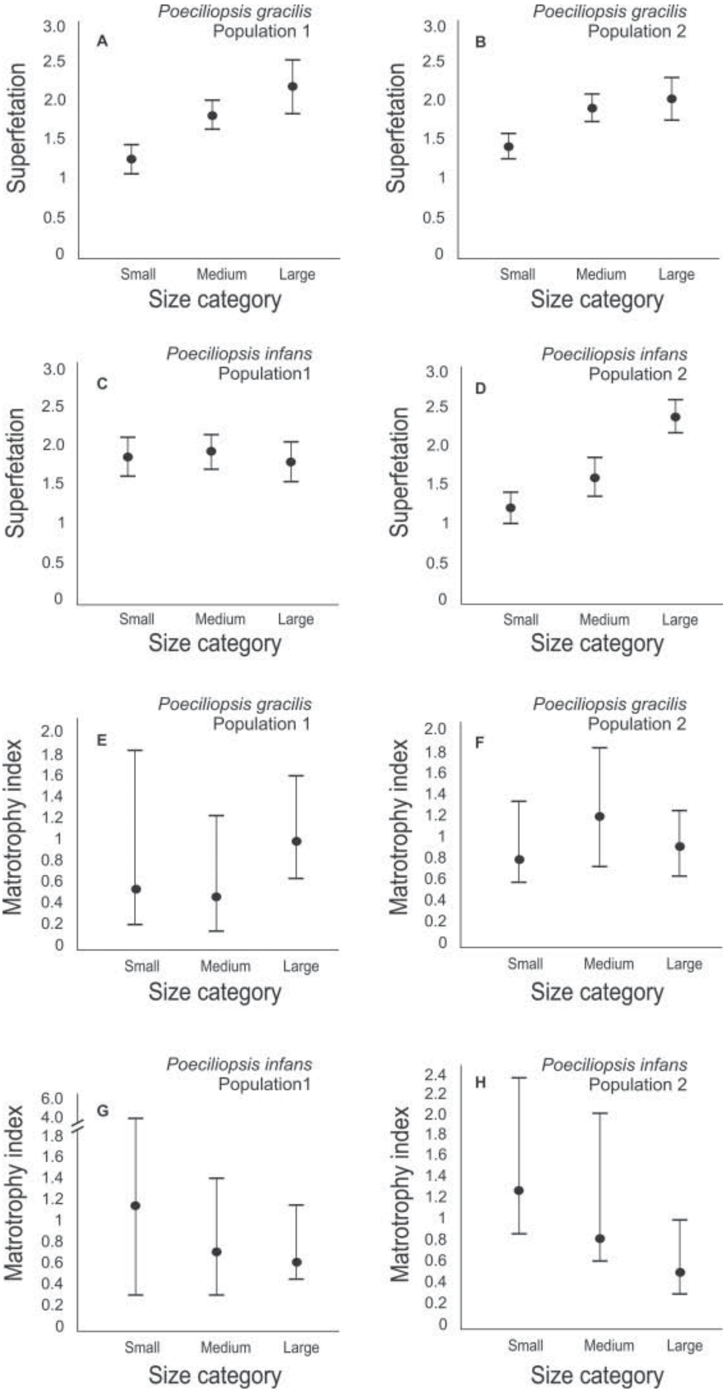


Fig. 6. Average number of simultaneous broods (i.e. degree of superfetation) (A–D) and matrotrophy index (E–H) for two populations of *Poeciliopsis gracilis* and two populations of *P. infans*. Females were classified into three different size categories. Error bars represent 95% parametric (A–D) and bootstrap (E–H) confidence intervals.

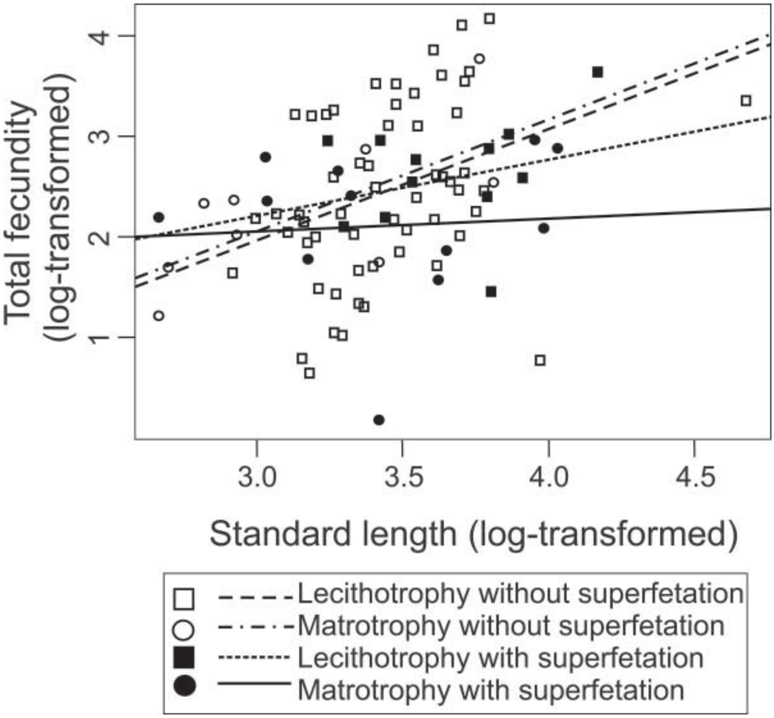


Fig. 7. Relationships between average female size and total fecundity for 91 fish species from the family Poeciliidae that we classified into four distinct reproductive modes. For species with superfetation, total fecundity was calculated as brood size multiplied by the average number of simultaneous broods. We show the fitted lines for all four reproductive modes according to an ordinary (non-phylogenetic) least squares regression.

between reproductive mode and female size were not significant ($F_{3,83} = 0.60$, $P = 0.61$ and $F_{3,83} = 0.88$, $P = 0.46$, respectively) (Fig. 7).

Table 3 shows the estimated regression coefficients from the OLS model. We used the category of matrotrophy with superfetation as the reference level. In this way, regression coefficients indicate if the intercepts and slopes of the other three categories differ with respect to those of matrotrophic and superfetating species. Neither the intercepts nor the slopes of the other three categories differed significantly from the intercept and slope estimated for matrotrophic and superfetating species (Table 3). Therefore, small species that exhibit both matrotrophy and superfetation do not produce more embryos than small species that exhibit the other three reproductive modes, and do not have similar fecundities to those of larger species (Fig. 7).

Phylogenetic signal

Our estimates of λ for individual traits revealed strong phylogenetic signal in female size ($\lambda = 0.83$), superfetation ($\lambda = 0.93$), MI ($\lambda = 0.99$), and PC1 ($\lambda = 1.00$; $P < 0.001$ in all cases). In contrast, we found no phylogenetic signal in total fecundity ($\lambda = 6.6 \times 10^{-5}$, $P = 1$).

Table 3. Estimated regression coefficients and standard errors (SE) from the ordinary least squares (OLS) regression that tested the effects of female size and reproductive mode on total fecundity for 91 fish species from the family Poeciliidae

Model terms	Regression coefficient	SE	*P*
Intercept (matrotrophy with superfetation)	1.68	1.87	0.37
Female size	0.13	0.54	0.81
Lecithotrophy with superfetation	−1.16	3.69	0.75
Matrotrophy without superfetation	−2.96	2.72	0.28
Lecithotrophy without superfetation	−3.07	2.23	0.17
Female size (lecithotrophy with superfetation)	0.43	1.02	0.67
Female size (matrotrophy without superfetation)	0.98	0.82	0.24
Female size (lecithotrophy without superfetation)	0.99	0.65	0.13

Note: We classified our 91 species into one of four distinct reproductive modes: lecithotrophy without superfetation, lecithotrophy with superfetation, matrotrophy without superfetation, and matrotrophy with superfetation. We used matrotrophy with superfetation as the reference level.

DISCUSSION

We found no patterns in support of the hypothesis that superfetation and matrotrophy are more common in smaller species or individuals. Based on the theoretical consideration that a particular number of embryos occupy a smaller volume of the reproductive tract in females of species that exhibit both reproductive modes than in females of species that lack these reproductive modes (Thibault and Schultz, 1978; Reznick and Miles, 1989; Pollux *et al.*, 2009), we proposed that matrotrophic and superfetating females would be able to fit more embryos in any given volume of the reproductive tract than lecithotrophic and non-superfetating females. This would be a major benefit for small species or individuals, given the space limitations associated with a small body size. However, we found that: (1) small species do not have more pronounced superfetation and/or matrotrophy, and (2) their joint presence does not increase the total reproductive output of small species, and hence these reproductive strategies do not function as mechanisms to compensate the physical restrictions of a small body size. Therefore, in poeciliid fishes, the evolution of small body size has not promoted the evolution of more prominent superfetation and/or matrotrophy.

The reverse direction of evolutionary causality would be that the evolution of these two reproductive modes facilitates the evolution of a small body size, because small females with more pronounced matrotrophy and superfetation would be able to produce as many embryos as large females, which relaxes the selective pressure towards attaining a large body size. However, the lack of statistical relationships between female size and these two reproductive modes does not support this reverse direction of causation either.

Our results also revealed that, at the intraspecific level, large females may overlap more broods than small females, a pattern that is opposite to what we predicted. Thus, at least within two species (*P. gracilis* and *P. infans*), superfetation may be more prominent in larger females than in smaller ones. In all poeciliid species, females keep growing after attaining sexual maturity, which means that the range of female sizes within populations may be quite wide (Turner and Snelson, 1984; Abney *et al.*, 2005; Riesch *et al.*, 2012). For this reason and based on our hypothesis, we predicted more pronounced superfetation and/or matrotrophy in small females. Contrary to this prediction, we found that the amount of post-fertilization provisioning is not affected by female size and, more interestingly, that larger females exhibit more prominent superfetation. These results not only provide further evidence against our hypothesis, but also imply that any interspecific comparison may be problematic if the average values of the reproductive traits cannot reflect the wide variation that occurs within species. In our case, at the interspecific level, we found no statistical relationship between average female size and superfetation, but this lack of association could have arisen erroneously if, for example, the average values of superfetation for some of the large species came from biased samples that contained only a few of the largest reproductive females, which may exhibit the greatest degree of superfetation, but which do not provide a representative value for the majority of the females from such species. Thus, the interpretation of our interspecific patterns must be treated with caution.

Two other hypotheses have been proposed to explain the potential benefits of the joint presence of both superfetation and matrotrophy. First, these two reproductive modes may enhance locomotor (swimming) performance because relatively low body mass and volume during pregnancy should allow females to: (1) exhibit less-impaired escape responses, (2) experience less drag forces during swimming, and (3) maintain a steady velocity (Thibault and Schultz, 1978; Pollux *et al.*, 2009; Zúñiga-Vega *et al.*, 2010). Therefore, these two reproductive modes should be advantageous in environments where fish must swim fast to escape from abundant predators or where there are strong water currents. This hypothesis has not been formally tested by considering the two reproductive strategies together. The only existing evidence that partially supports this hypothesis comes from a recent study by Gorini-Pacheco and colleagues (2018), who studied *Phalloceros harpagos* in three populations that differ in predation pressure and found the most pronounced matrotrophy and superfetation in the population with the highest predation risk. However, these authors did not quantify body shape or swimming performance.

Here, we examined if the presence of both superfetation and matrotrophy would result in pregnant females having less distended abdomens. Consistent with this expectation, the thinnest females were those of *P. prolifica*, a species that exhibits a high degree of both superfetation and matrotrophy [up to four simultaneous broods and MI = 5.4 (Pires *et al.*, 2007; Pollux *et al.*, 2014)]. In addition, *P. gracilis* and *P. infans*, both of which display superfetation and limited post-fertilization maternal provisioning to developing embryos, also exhibited slender bodies. However, we recognize here that this morphological comparison among few species provides only preliminary evidence in favour of superfetation and matrotrophy promoting streamlined phenotypes, because thin bodies may simply be a shared feature of the entire genus *Poeciliopsis*. Using a substantially larger number of species and accounting for phylogenetic relatedness, further comparative studies are required to examine, at the interspecific level, whether the joint presence of matrotrophy and superfetation is associated with more streamlined phenotypes and enhanced locomotor performance.

Few other studies have examined the potential benefits of either superfetation or matrotrophy (not considering them together) in terms of promoting more streamlined phenotypes. Zúñiga-Vega *et al.* (2007) found that females of *Poeciliopsis turrubarensis* with the most pronounced superfetation are thinner, more elongated, and inhabit environments with stronger water currents than females that overlap fewer broods, which are more robust and inhabit environments with less strong currents. However, studies on other congeneric species (*P. gracilis, P. infans*, and *P. baenschi*) did not observe this same pattern (Frías-Alvarez and Zúñiga-Vega, 2016; Zúñiga-Vega *et al.*, 2017). Fleuren *et al.* (2018) examined morphological differences between a matrotrophic species (*P. turneri*) and a lecithotrophic species (*P. gracilis*) during pregnancy and found that females of *P. turneri* are more slender than those of *P. gracilis* during early pregnancy. They inferred that pregnant females of *P. turneri* would experience less drag forces and better swimming performance than pregnant females of *P. gracilis*. Here, we also provided evidence, in two different species (*P. gracilis* and *P. infans*), that greater post-fertilization maternal provisioning (higher MI) may result in thinner females.

Second, if indeed a particular number of embryos weigh less and occupy a smaller volume of the reproductive tract in females that exhibit superfetation and matrotrophy, then such females may have some additional space to produce larger newborns (Olivera-Tlahuel *et al.*, 2015). Under particular ecological circumstances, larger offspring have higher fitness than smaller offspring (Bashey, 2008; Gordon *et al.*, 2009; Riesch *et al.*, 2010b). However, phylogenetically corrected evidence from 44 poeciliid species did not support this hypothesis: species that exhibit these two reproductive strategies do not produce larger newborns than lecithotrophic and non-superfetating species (Olivera-Tlahuel *et al.*, 2015). This hypothesis has yet to be tested at the intraspecific level.

Here, we failed to find support for the hypothesis that superfetation and matrotrophy are mechanisms that maximize total fecundity of small species. Small species that exhibit superfetation and matrotrophy have neither higher fecundities than similar-sized species that lack these two reproductive modes, nor similar fecundities to those of larger species. Hence, we now should ask if small poeciliids have some other way to compensate the space restrictions imposed by their small body on their reproductive output. We hypothesize that smaller poeciliid species may have faster developmental rates (i.e. shorter gestation periods) or shorter non-breeding intervals. Quantification of gestation length and yearly reproductive cycles in natural conditions remains limited (Turner, 1937; Greven, 2011).

Finally, our estimates of phylogenetic signal from both residuals and traits indicate that body size as well as the degree of both superfetation and matrotrophy have evolved by gradually accumulating changes throughout time, which resulted in substantial similarity among closely related species. This is an interesting pattern given the multiple independent times that the capacity of females to superfetate and the extensive post-fertilization maternal provisioning of nutrients to developing embryos have evolved in these livebearing fishes (Pollux *et al.*, 2009, 2014; Meredith *et al.*, 2010, 2011). Thus, once these two reproductive strategies arise, the amounts of superfetation and matrotrophy steadily accumulate small changes with little influence of the local environment. This high phylogenetic signal in both matrotrophy and superfetation has already been documented, although with fewer ($n = 44$) species (Olivera-Tlahuel *et al.*, 2015). Regarding female size, our study is the first to document that in poeciliid fishes this trait has also evolved in a way that is consistent with a Brownian motion model of evolution. In contrast, we found no phylogenetic signal in total fecundity, which indicates that this life-history trait adapts quickly to the local environment. This result is consistent with the well-documented selective effects of particular environmental factors on offspring size and number – predation, salinity, population density, food availability (Reznick *et al.*, 1990; Johnson and Belk, 2001; Bashey, 2008; Johnson and Bagley, 2011) – as well as with the low phylogenetic signal observed in the size of each individual newborn in poeciliid fishes (Olivera-Tlahuel *et al.*, 2015).

ACKNOWLEDGEMENTS

Israel Solano-Zavaleta, Pedro Mendoza-Hernández, Mariana Hernández-Apolinar, and Jhoana Díaz-Larrea provided technical assistance. Nabila Saleh-Subaie is a doctoral student from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and received fellowship #596700 from CONACYT.

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Submitted to *Evolutionary Ecology Research*

APPENDIX

Are superfetation and matrotrophy more prominent in small species?

Nabila Saleh-Subaie[1] and J. Jaime Zúñiga-Vega[2]

[1] *Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, México.*

[2] *Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México.*

*Correspondence: J.J. Zúñiga-Vega, Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, 04510, Ciudad de México, México. email: jzuniga@ciencias.unam.mx

Table S1. Female size (standard length), degree of superfetation (number of simultaneous broods), matrotrophy index, and total fecundity for 91 fish species from the family Poeciliidae.

Species	Female length (mm)	Degree of superfetation	Matrotrophy index	Total Fecundity	References
Belonesox belizanus	108.19	1	0.70	28.5	Turner and Snelson, 1984; Pollux *et al*., 2014.
Brachyrhaphis holdridgei	24.99	1	0.66	4.35	Pollux *et al*., 2014; Olivera-Tlahuel *et al*., 2015.
Brachyrhaphis rhabdophora	23	1	0.77	24.6	Reznick *et al*., 1993; Pollux *et al*., 2014.
Brachyrhaphis terrabensis	25.59	1	0.60	24.66	Stallsmith, 2018; Pollux *et al*., 2014.
Carlhubbsia kidderi	32.25	1.25	0.73	8.73	Zúñiga-Vega, unpubl. data.
Gambusia affinis	26.32	1	0.62	26.04	Milton and Arthington, 1983; Marsh-Matthews *et al*., 2005; Pollux *et al*., 2014.
Gambusia atrora	26.52	1	0.83	4.15	Torres-Mejia, 2011.
Gambusia eurystoma	23.63	1	0.76	2.19	Riesch *et al*., 2010; Riesch, unpubl. data.
Gambusia geiseri	26.90	1	0.54	9.28	Marsh-Matthews *et al*., 2001; Marsh-Matthews *et al*., 2005; Torres-Mejia, 2011.
Gambusia holbrooki	44.97	1	0.64	64.62	Pen and Potter, 1991; Pollux *et al*., 2014.
Gambusia hubbsi	28.65	1	0.86	5.32	Riesch *et al*., 2012a.
Gambusia hurtadoi	24.74	1	0.74	7.3	Torres-Mejia, 2011.
Gambusia luma	30.06	1	0.89	5.5	Torres-Mejia, 2011.

Gambusia manni	28.60	1	0.75	3.8	Torres-Mejia, 2011; Pollux *et al*., 2014.
Gambusia melapleura	33.02	1	0.81	6.3	Torres-Mejia, 2011.
Gambusia nicaraguensis	28.75	1	0.87	15.4	Torres-Mejia, 2011.
Gambusia oligosticta	22.48	1	0.48	7.7	Torres-Mejia, 2011.
Gambusia punctata	42.70	1	0.75	9.5	Ponce de León *et al*., 2011; Torres-Mejia, 2011.
Gambusia puncticulata	40.38	1	0.78	11.79	Abney and Rakocinski, 2004; Ponce de León *et al*., 2011.
Gambusia rhizophorae	29.13	1	1.24	3.7	Torres-Mejia, 2011.
Gambusia sexradiata	26.68	1	0.73	14.02	Riesch *et al*., 2010; Torres-Mejia, 2011.
Gambusia vittata	27.32	1	0.77	8.08	Torres-Mejia, 2011.
Gambusia wrayi	37.5	1	0.7	13.6	Torres-Mejia, 2011.
Gambusia yucatana	23.45	1	0.53	9.17	Olivera-Tlahuel *et al*., 2015.
Girardinus metallicus	38.41	1	0.72	13.5	Ponce de León *et al*., 2011; Pollux *et al*., 2014.
Heterandria bimaculata	43.34	1	0.82	43	Pollux *et al*., 2014; Olinger *et al*., 2016.
Heterandria formosa	20.95	4.5	34.99	10.5	Reznick *et al*., 1996; Pollux *et al*., 2014.
Heterandria jonesi	48.10	1	0.65	20.44	Olivera-Tlahuel *et al*., 2015.
Heterophallus milleri	23.76	1	0.74	8.49	Riesch, *et al*., 2011.
Phallichthys amates	41.18	1	0.52	34.67	Regus *et al*., 2013; Pollux *et al*., 2014.
Phallichthys quadripunctatus	20.13	1	0.75	8.86	Regus *et al*., 2013.
Phallichthys tico	21.60	1	0.46	9.28	Regus *et al*., 2013; Pollux *et al*., 2014.
Phalloceros caudimaculatus	29.38	1	2.14	17.55	Arias and Reznick, 2000; Pollux *et al*., 2014.

Poecilia (*Acanthophacelus*) *reticulata*	18.60	1	0.69	5.15	Pires *et al*., 2010; Pollux *et al*., 2014.
Poecilia (*Acanthophacelus*) *wingei*	24.25	1	0.83	1.9	Pires *et al*., 2010; Pollux *et al*., 2014.
Poecilia (*Limia*) *caymanensis*	37.18	1	0.61	8.7	Pollux *et al*., 2014; Cohen *et al*., 2015.
Poecilia (*Limia*) *dominicensis*	30.30	1	0.51	12	Cohen *et al*., 2015.
Poecilia (*Limia*) *melanogaster*	36.97	1	0.64	46.85	Pollux *et al*., 2014; Cohen *et al*., 2015.
Poecilia (*Limia*) *melanonotata*	38.10	1	0.67	36.6	Cohen *et al*., 2015.
Poecilia (*Limia*) *nigrofasciata*	40.53	1	0.72	7.4	Pollux *et al*., 2014; Cohen *et al*., 2015.
Poecilia (*Limia*) *pauciradiata*	31.80	1	0.66	22.2	Cohen *et al*., 2015.
Poecilia (*Limia*) *perugiae*	34.40	1	0.90	12.7	Cohen *et al*., 2015.
Poecilia (*Limia*) *tridens*	34.67	1	0.83	30.7	Pollux *et al*., 2014; Cohen *et al*., 2015.
Poecilia (*Limia*) *versicolor*	35.00	1	0.74	22.3	Cohen *et al*., 2015.
Poecilia (*Limia*) *vittata*	31.65	1	0.96	22.4	Pollux *et al*., 2014; Cohen *et al*., 2015.
Poecilia (*Limia*) *zonata*	32.50	1	0.91	33.4	Cohen *et al*., 2015.
Poecilia (*Micropoecilia*) *bifurca*	14.45	1.2	55.06	8.95	Pires *et al*., 2010.
Poecilia (*Micropoecilia*) *branneri*	20.84	4.77	86.73	16.23	Pires *et al*., 2010; Pollux *et al*., 2014.
Poecilia (*Micropoecilia*) *parae*	24.13	1.03	6.43	5.9	Pires *et al*., 2010; Pollux *et al*., 2014.
Poecilia (*Micropoecilia*) *picta*	23.99	1	0.78	6.95	Pires *et al*., 2010; Pollux *et al*., 2014.
Poecilia (*Mollienesia*) *butleri*	41.77	1	2.31	38.14	Zúñiga-Vega *et al*., 2011; Olivera-Tlahuel *et al*., 2015.
Poecilia (*Mollienesia*) *caucana*	30.40	1	0.77	33.6	Pires and Reznick, 2018.

Poecilia (*Mollienesia*) *latipinna*	45.38	1	1.02	12.6	Martin *et al*., 2009; Pollux *et al*., 2014.
Poecilia (*Mollienesia*) *latipunctata*	31.50	1	0.85	8.8	Pires and Reznick, 2018.
Poecilia (*Mollienesia*) *mexicana*	39.16	1	0.68	12.85	Riesch *et al*., 2010.
Poecilia (*Mollienesia*) *sphenops*	40.17	1	0.65	31.25	Burns, 1985; Pollux *et al*., 2014.
Poecilia (*Mollienesia*) *sulphuraria*	26.40	1	0.69	2.83	Riesch *et al*., 2010.
Poecilia (*Pamphorichthys*) *araguaiensis*	18.90	1	9.62	7.48	Pires and Reznick, 2018.
Poecilia (*Pamphorichthys*) *hasemani*	14.47	1	36.37	3.36	Pires and Reznick, 2018.
Poecilia (*Pamphorichthys*) *hollandi*	18.67	1	21.29	10.63	Pires and Reznick, 2018.
Poecilia (*Pamphorichthys*) *minor*	14.90	1	1.63	5.4	Pires and Reznick, 2018.
Poecilia (*Pamphorichthys*) *scalpridens*	16.85	1	16.58	10.3	Pires and Reznick, 2018.
Poecilia (*Pseudolimia*) *heterandria*	24.42	1	0.69	24.5	Pollux *et al*., 2014; Cohen *et al*., 2015
Poecilia vivipara	53.51	1	0.68	14.66	Rocha *et al*., 2011; Pollux *et al*., 2014.
Poeciliopsis baenschi	37.62	1.62	1.58	4.82	Pollux *et al*., 2014; Olivera-Tlahuel *et al*., 2015.
Poeciliopsis catemaco	65.10	2.1	0.68	38.22	Bassar *et al*., 2014.
Poeciliopsis fasciata	28.18	1.34	0.81	7.55	Pollux *et al*., 2014; Olivera-Tlahuel *et al*., 2015.
Poeciliopsis gracilis	30.99	1.4	0.72	19.23	Bassar *et al*., 2014; Pollux *et al*., 2014.
Poeciliopsis hnilickai	34.94	1.75	0.90	15.72	Bassar *et al*., 2014.

Poeciliopsis infans	44.72	1.79	0.86	18	Frías-Alvarez *et al*., 2014; Pollux *et al*., 2014.
Poeciliopsis latidens	25.72	1.12	0.86	19.14	Pollux *et al*., 2014; Olivera-Tlahuel *et al*., 2015.
Poeciliopsis lucida	28.08	3	1.89	11	Thibault and Schultz, 1978; Turcotte *et al*., 2008; Pollux *et al*., 2014.
Poeciliopsis monacha	34.95	2	0.58	10.84	Thibault and Schultz, 1978; Banet and Reznick, 2008; Turcotte *et al*., 2008; Pollux *et al*., 2014.
Poeciliopsis occidentalis	30.73	1.64	1.12	1.2	Pollux *et al*., 2014; Olivera-Tlahuel *et al*., 2015.
Poeciliopsis pleurospilus	45.01	1.35	0.5	4.29	Olivera-Tlahuel *et al*., 2015.
Poeciliopsis presidionis	52.44	2.2	23.48	18.94	Bassar *et al*., 2014; Pollux *et al*., 2014.
Poeciliopsis prolifica	26.29	3.3	5.40	13.33	Pires *et al*., 2007; Banet and Reznick, 2008; Pollux *et al*., 2014.
Poeciliopsis retropinna	54.06	1.6	117	9.53	Pollux *et al*., 2014; Zúñiga-Vega, unpubl. data.
Poeciliopsis scarlii	50.38	1.8	0.87	13.25	Bassar *et al*., 2014; Pollux *et al*., 2014.
Poeciliopsis turneri	37.40	2.8	34.02	5.58	Thibault and Schultz, 1978; Bassar *et al*., 2014; Pollux *et al*., 2014.
Poeciliopsis turrubarensis	41.18	1.87	0.95	13.93	Zúñiga-Vega *et al*., 2007; Bassar *et al*., 2014; Pollux *et al*., 2014.
Poeciliopsis viriosa	43.96	2.2	0.93	11.66	Pollux *et al*., 2014; Olivera-Tlahuel *et al*., 2015.
Priapella chamulae	38.76	1	0.66	6.38	Riesch, *et al*., 2012b; Pollux *et al*., 2014.
Priapella olmecae	44.38	1	0.76	11.27	Olivera-Tlahuel *et al*., 2015.

Xenodexia ctenolepis	56.58	4.13	3.74	17.53	Reznick *et al*., 2007; Pollux *et al*., 2014.
Xenophallus umbralitis	29.58	1	0.70	15	Meek, 1912; Pollux *et al*., 2014.
Xiphophorus hellerii	40.78	1	0.61	60.15	Milton and Arthington, 1983; Pollux *et al*., 2014.
Xiphophorus maculatus	32.55	1	0.85	27.27	Milton and Arthington, 1983; Pollux *et al*., 2014.
Xiphophorus multilineatus	33.73	1	0.48	7.84	Morris and Ryan, 1992; Pollux *et al*., 2014.
Xiphophorus nigrensis	30.75	1	0.71	5.66	Morris and Ryan, 1992; Pollux *et al*., 2014.
Xiphophorus pygmaeus	27.08	1	0.77	2.76	Morris and Ryan, 1992; Pollux *et al*., 2014.

Note: For species with superfetation, total fecundity was calculated as brood size multiplied by the average number of simultaneous broods.

Table S2. Results from phylogenetic generalized least squares (PGLS-BM, PGLS-OU and PGLS-λ) and ordinary least squares (OLS) regression models fitted to test the effects of superfetation and matrotrophy on average female size for 91 fish species from the family Poeciliidae.

Regression model	Evolutionary assumption	AICc	ΔAICc	Regression slope	*P*	α	λ
Female size ~ superfetation	PGLS-λ	42.00	0	0.04 (0.11)	0.75		0.84
	PGLS-OU	58.28	16.28	0.12 (0.12)	0.30	2.79	
	PGLS-BM	70.56	28.56	0.17 (0.13)	0.21		
	OLS	72.05	30.05	0.13 (0.10)	0.18		
Female size ~ matrotrophy index	PGLS-λ	43.18	0	-0.04 (0.03)	0.21		0.84
	PGLS-OU	59.37	16.19	-0.05 (0.03)	0.11	3.06	
	OLS	70.14	26.96	-0.07 (0.03)	0.013		
	PGLS-BM	72.75	29.57	-0.05 (0.04)	0.18		
Female size ~ PC1	PGLS-λ	43.72	0	-0.02 (0.04)	0.57		0.84
	PGLS-OU	61.11	17.39	-0.01 (0.05)	0.76	2.81	
	PGLS-BM	73.95	30.23	-0.002 (0.05)	0.97		
	OLS	75.26	31.54	-0.03 (0.04)	0.52		

Note: PGLS was implemented using three evolutionary models: Brownian motion (BM), Ornstein-Uhlenbeck (OU) and phylogenetic signal (λ). The fit of each model to the data was evaluated using the Akaike Information Criterion (AICc), with the smallest value indicating the best-fitting model. We also show differences in AICc values between each model and the best-fitting model (ΔAICc). Models are listed according to their AICc values. Standard errors of the regression slopes are shown within parentheses. PGLS-OU estimates an additional parameter, α, which measures the strength of stabilizing selection. λ is a scaling parameter that weights the influence of the phylogeny in explaining the observed data. PC1 represents a combined measure of superfetation and matrotrophy.

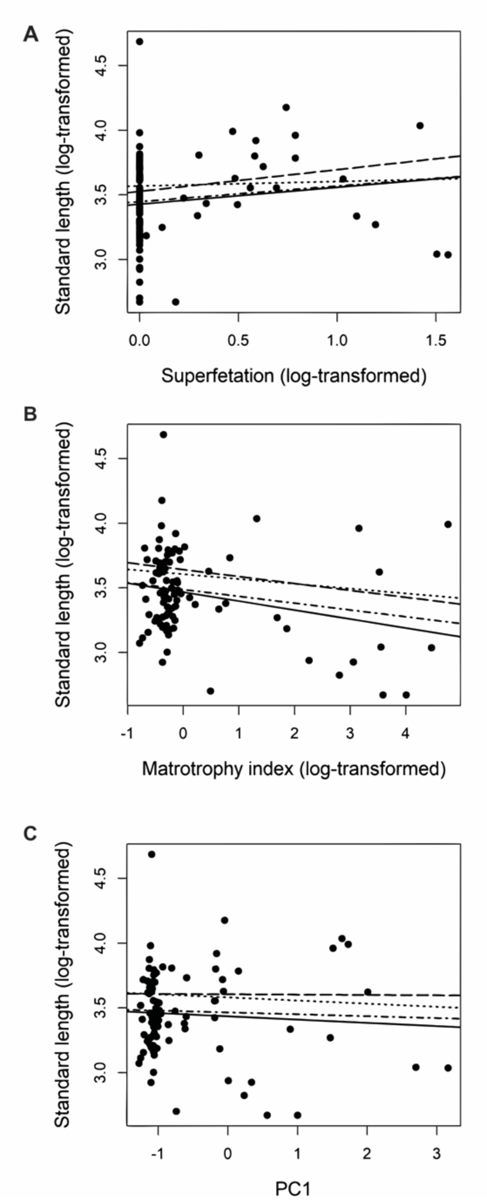

Fig. S1. Potential effects of superfetation (A), matrotrophy index (B), and a combined measure of superfetation and matrotrophy (PC1) (C) on average female size (standard length) for 91 species of fishes from the family Poeciliidae. In all panels, the continuous line corresponds to an ordinary (non-phylogenetic) least squares regression. The other lines represent phylogenetic generalized least squares regressions (PGLS) assuming a Brownian motion model of evolution (dashed), an Ornstein-Uhlenbeck model of evolution (dot-dashed), or estimating the amount of phylogenetic signal (λ) in the residuals (dotted).

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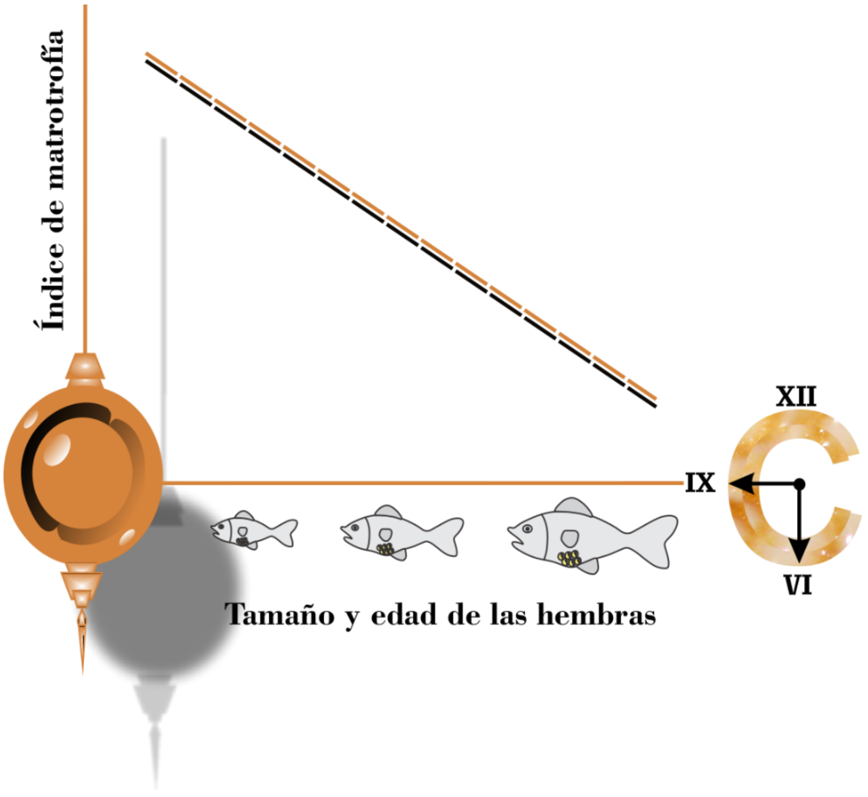
CAPÍTULO II

Small sizes, big strategies: the relationship between female size, matrotrophy and superfetation throughout the reproductive lives of poeciliid fishes

Nabila Saleh-Subaie, Jerald B. Johnson and J. Jaime Zúñiga-Vega

Artículo publicado en Journal of Zoology

2021



Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

Small sizes, big strategies: the relationship between female size, matrotrophy and superfetation throughout the reproductive lives of poeciliid fishes

N. Saleh-Subaie[1], J. B. Johnson[2] & J. J. Zúñiga-Vega[3]

[1]Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, México
[2]Department of Biology, Brigham Young University, Provo, UT, USA
[3]Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México

Keywords
internal fertilization; lecithotrophy; maternal provisioning; ontogenetic changes; Poeciliidae; reproductive modes; viviparous fishes.

Correspondence
José Jaime Zúñiga-Vega, Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, 04510, Ciudad de México, México.
Email: jzuniga@ciencias.unam.mx

Editor: Jean-Nicolas Volff

Received 19 October 2020; revised 11 June 2021; accepted 18 June 2021

doi:10.1111/jzo.12917

Abstract

In viviparous fishes, females of species that exhibit matrotrophy (post-fertilization maternal provisioning to developing embryos) and superfetation (ability of females to bear simultaneously multiple broods of embryos at distinct developmental stages) increase less in mass and volume during pregnancy than females of species that lack these traits. Such a reduction in reproductive allocation may provide greater benefits to young and small females for two reasons. First, they could devote energy and resources to both growth and reproduction. Second, they could compensate for the space restrictions that are inherent to a small body size because both superfetation and matrotrophy maximize fecundity for any given ovarian volume. In this study, we test the hypothesis that both matrotrophy and superfetation will decrease over female reproductive lifespan (i.e. as they grow larger). We examined reproductive females from 77 populations of 13 species of the family Poeciliidae. Remarkably, we found support for the matrotrophy prediction in only three populations. As expected, in these populations, small females were more matrotrophic than larger females, which in turn exhibited a predominantly lecithotrophic strategy. In one population, we found the opposite pattern—the degree of matrotrophy actually increased in larger females. With respect to superfetation, none of the populations showed a pattern consistent with our prediction. In fact, in five populations the pattern was opposite to our expectation—larger females produced more simultaneous broods than smaller females. Our findings reveal that the degree of matrotrophy and superfetation can vary throughout the adult lifespans of poeciliid fishes, but such ontogenetic shifts are not common in natural populations.

Introduction

In nature, there are two ways in which viviparous females transfer nutrients to their developing embryos: matrotrophy and lecithotrophy. In matrotrophic females, nutritional resources are actively supplied post-fertilization through specialized morphological structures (i.e. placentas; Kwan *et al*., 2015; Olivera-Tlahuel *et al*., 2019). In contrast, in lecithotrophic females, nutrients are provided to the eggs prior to fertilization in the form of yolk, with no additional provisioning by the mothers after fertilization (Marsh-Matthews, 2011). Viviparous fishes from the family Poeciliidae represent an interesting continuum in the way females transfer nutrients to their developing embryos. Some species are apparently strictly lecithotrophic whereas other species exhibit extensive matrotrophy, with several species between these two extreme modes of maternal provisioning that exhibit varying degrees of the relative amounts of pre- and post-fertilization provisioning (Reznick, Mateos & Springer, 2002; Pollux *et al*., 2009, 2014). Furthermore, the degree of post-fertilization transfer of nutrients (i.e., the degree of matrotrophy) may vary widely among populations within species (Reznick *et al*., 2007; Turcotte *et al*., 2008; Molina-Moctezuma, Hernández-Rosas & Zúñiga-Vega, 2020). This large interspecific and intraspecific variation in how females from poeciliid species provide embryos with the necessary nutrients for development has raised questions about the potential benefits of matrotrophy over lecithotrophy (Trexler & DeAngelis, 2003; Pollux *et al*., 2009, 2014; Saleh-Subaie & Zúñiga-Vega, 2019).

Superfetation, the ability of females to simultaneously carry multiple broods of embryos at different developmental stages (Scrimshaw, 1944a), is another reproductive strategy that occurs in some genera of Poeciliidae such as *Heterandria* and *Poeciliopsis* (Scrimshaw, 1944b; Thibault & Schultz, 1978), whereas it is absent in other poeciliid genera such as *Brachyrhaphis* and *Gambusia* (Johnson & Belk, 2001; Riesch, Martin

& Langerhans, 2013). The degree of superfetation (i.e. the number of simultaneous broods that females bear) also varies substantially among species as well as among populations within species (Reznick *et al*., 2007; Zúñiga-Vega, Reznick & Johnson, 2007; Frías-Alvarez *et al*., 2014; Zúñiga-Vega, Olivera-Tlahuel & Molina-Moctezuma, 2017). Special attention has been given to the potential benefits of greater degrees of superfetation (Zúñiga-Vega, Macías-Garcia & Johnson, 2010; Fleuren, Van Leeuwen & Pollux, 2019).

One hypothesis that was previously proposed by other authors to explain the advantages of both matrotrophy and superfetation suggests that reproductive allocation (defined as the proportion of female mass consisting of developing embryos) may be lower in matrotrophic and/or superfetating species than lecithotrophic and/or non-superfetating species (Thibault & Schultz, 1978; Reznick & Miles, 1989; Pollux *et al*., 2009). Such relatively low reproductive allocation must be particularly evident in terms of female body mass and volume during pregnancy. In matrotrophic females, mature ova are small, and, after fertilization, embryos gradually increase in mass because the mother actively transfers nutrients during pregnancy. In contrast, in lecithotrophic females, mature ova are large because of the yolk provided before fertilization and embryos increase even more in wet mass due to water uptake (however, the dry mass of embryos from lecithotrophic females decreases during development due to metabolic costs Reznick, Mateos & Springer, 2002). Therefore, during a significant proportion of pregnancy the wet mass and volume of reproductive females are larger in lecithotrophic species than in matrotrophic species (assuming that female size and offspring size and number are the same for both reproductive modes). Superfetation can also reduce reproductive allocation because females give birth frequently to the subset of most advanced embryos, and, hence, superfetating females usually carry fewer embryos in late stages of development (which are in general larger due to water uptake as development progresses) than non-superfetating females (Thibault & Schultz, 1978; Reznick & Miles, 1989; Pollux *et al*., 2009). Such reduction in reproductive burden may also be true for females with advanced degrees of both matrotrophy (greater amounts of post-fertilization provisioning and less yolk deposition) and superfetation (more simultaneous broods) when compared to females with lower degrees of matrotrophy and superfetation.

Based on the hypothesis that smaller increases in body mass and volume during pregnancy will occur in females with advanced degrees of matrotrophy and superfetation, compared to females that either lack these reproductive modes or exhibit low degrees of these two strategies (Pollux *et al*., 2009; Zúñiga-Vega *et al*., 2010), we propose that such reduced reproductive burden will be more beneficial for small (young) females than for larger (older) females. In organisms with indeterminate growth, such as most fishes, females keep growing after attaining sexual maturity (Hughes, 1986; Vargas & De Sostoa, 1996). Thus, small adult females need to allocate resources and energy to both reproduction and growth. Relatively low reproductive allocation derived from advanced degrees of matrotrophy and/or superfetation could allow them to devote surplus energy to growth, without compromising fecundity. In addition, a small body size implies constraints in the space that females can devote to offspring production (in numerous viviparous taxa, larger females have substantially higher fecundities Leips & Travis, 1999; Abney, Heard & Rakocinski, 2005; Karino & Ikeuchi, 2011). According to theoretical models, matrotrophy should allow females to produce more offspring per reproductive bout because initial egg size is small and less energetically costly than the large initial eggs of lecithotrophic females (Trexler & DeAngelis, 2003, 2010). Thus, advanced degrees of matrotrophy in small females would enable them to produce more embryos, thereby overcoming the space restrictions that are inherent to a small body size. As females grow, they could depend less on post-fertilization provisioning because they have more space to accommodate large yolked eggs, and, given that larger (older) females allocate less resources to body growth, they could afford a large reproductive investment since the onset of gestation (i.e. eggs full of all the necessary nutrients to support development).

Our hypothesis assumes that the greatest benefit for small females with advanced degrees of matrotrophy would come from offspring size at birth being smaller than in lecithotrophic females. In this way, higher fecundity would not imply an overall greater reproductive allotment or the need for additional space within the ovary. This assumption appears to be valid according to evidence in some poeciliid species that increased matrotrophy may result in smaller offspring (Pires *et al*., 2011; Olivera-Tlahuel *et al*., 2015; Furness *et al*., 2021). However, even assuming an equal offspring size at birth for both lecithotrophic and matrotrophic females (which is an original assumption of the model formalized by Pollux *et al*. (2009), small females with advanced degrees of matrotrophy should be able to produce a relatively large number of embryos within a relatively small ovarian volume and by means of a low energetic investment during a large proportion of pregnancy, because embryos in early and intermediate stages of development are quite small. At the end of pregnancy, when embryos reach their maximum size, these matrotrophic females may experience a high reproductive allocation and disproportionally large abdomen distention, but only during a very short period of time. Therefore, even if offspring size at birth were equally large in both matrotrophic and lecithotrophic females, the benefits that advanced matrotrophy provides to small females during most of pregnancy (low reproductive allocation and numerous embryos occupying a small ovarian volume) must outweigh the costs of the final phase of gestation, when reproductive investment and burden may be greater for such small matrotrophic females than similar-sized lecithotrophic females.

Superfetation in turn may provide a way to maintain a high reproductive output despite restrictions in the amount of space that females can devote to developing embryos, because this reproductive mode splits a large reproductive bout into smaller broods at distinct developmental stages. In this way, females with advanced degrees of superfetation carry fewer large full-term embryos at any given time than females with lower degrees of superfetation (Thibault & Schultz, 1978; Zúñiga-Vega *et al*., 2010). Given that large late-stage embryos occupy more space within the reproductive tract, producing fewer large embryos through increased superfetation would allow females to fit more embryos of earlier stages. Therefore, similar to what we expect for matrotrophy, small females would benefit from increased superfetation because they would be able to fit a relatively large number of embryos (across all broods) into their reduced ovarian cavity.

In this study, we propose the hypothesis that the amount of post-fertilization maternal provisioning, as well as the degree of superfetation, will change predictably throughout the reproductive lives of viviparous fishes. Specifically, we expect greater degrees of both matrotrophy and superfetation in small females (young adults) and a switch towards increased lecithotrophy and fewer simultaneous broods in larger females (older adults). Increased matrotrophy and/or superfetation could allow small females to somewhat overcome the space restrictions of a small reproductive tract. Therefore, our main objective was to test this hypothesis in different poeciliid species by examining if matrotrophy and superfetation vary among females within populations depending on their size (and age), a pattern that in turn would represent evidence of changes in reproductive mode within individual females throughout their reproductive lives.

Materials and methods

Study species and life-history data

We focused on the fish family Poeciliidae because of the wide variation among species in both matrotrophy and superfetation (Pollux *et al*., 2009, 2014; Marsh-Matthews, 2011). We used data from a total of 77 populations that belong to 13 species (Table 1). Geographic coordinates of all these populations are given in Table S1. In this dataset, we included species with and without superfetation, strictly lecithotrophic species, and species with varying degrees of matrotrophy (Table 1). To test our hypothesis, we gathered data on four variables: female size (mm standard length [SL]), average dry mass of individual embryos (g), developmental stage (using the classification proposed by Haynes (1995)), and number of simultaneous broods (only for species with superfetation). We collected these data from previously published studies as well as from our own dissections of pregnant females, which we obtained from the National Collection of Fishes – Universidad Nacional Autónoma de México. In Appendix S1, we provide additional details about our datasets and describe the protocol that we implemented to quantify these four variables from preserved females.

Statistical analyses

To test the hypothesis that matrotrophy should be more prominent in small (young) females, we implemented linear models using the R statistical software v. 3.4.2 (R Core Team, 2018). We used embryo dry mass (log-transformed) as response variable and developmental stage as an explanatory variable because embryo size can change as development progresses (embryo dry mass decreases in lecithotrophic females and increases in matrotrophic females Reznick *et al*., 2002). In addition, we included female SL (log-transformed) as another predictor of embryo dry mass to examine if the magnitude and direction of change in embryo mass during development depend on the size (and age) of the females. The model that represents our prediction includes the interaction between developmental stage and female size affecting embryo mass. According to our hypothesis, we expected a greater slope between embryo mass and developmental stage in smaller females, indicating greater degrees of post-fertilization provisioning (greater matrotrophy). In contrast, we expected a less steep slope or even a negative slope in larger females, indicating less post-fertilization provisioning (a less matrotrophic strategy).

We also fitted four additional models. First, embryo dry mass as a function of developmental stage. This model represents the traditional way to calculate the degree of matrotrophy (Marsh-Matthews, 2011). Second, embryo mass as a function of female size. This model takes into account that larger females may produce larger embryos. Third, an additive effect of developmental stage and female size on embryo mass. Fourth, an intercept-only model in which neither female size nor developmental stage affect embryo dry mass. We fitted our five competing models separately for each population. We used the Akaike information criterion adjusted for small sample sizes (AICc Akaike, 1973) to select the best model for each population (i.e. the model with smallest AICc). We also calculated Akaike weights (w) as measures of the relative support for each model in the data (Burnham & Anderson, 2002). We compared the support of two models relative to each other by means of evidence ratios, calculated as the quotient between their Akaike weights. Given that models that differ by less than seven units of the AICc (ΔAICc < 7) with respect to the best model also have some support in the data (Burnham, Anderson, & Huyvaert, 2011), we considered that unequivocal evidence in favor of our hypothesis would come from three complementary lines of evidence. First, the model including the interaction between stage and female SL must be the best-fitting model. Second, the 95% confidence interval for the regression coefficient of this interaction must not include zero. Third, this model must have at least three times stronger support than any other model (i.e., an evidence ratio ≥ 3).

With respect to superfetation, we predicted that smaller females should exhibit greater degrees of superfetation than larger females. To test this prediction, we implemented generalized linear models with log link function and Poisson distribution of the error term in which the response variable was the degree of superfetation (i.e. number of simultaneous broods within each female) and the explanatory variable was female SL. We also used AICc, evidence ratios, and the 95% confidence interval for the regression coefficient of female SL to compare the fit of this model against the fit of an intercept-only model. We used R to implement these models separately for each population of the six species that exhibit superfetation: *Poeciliopsis baenschi, P. gracilis, P. infans, P. latidens, P. prolifica,* and *P. turrubarensis* (Table 1).

Finally, we examined how our sample sizes and the amount of variability in female size that we were able to capture in each sample influenced the probability of detecting size-dependent changes in the amount of post-fertilization maternal

Table 1 Type of maternal provisioning to developing embryos (matrotrophy, incipient matrotrophy, or lecithotrophy), matrotrophy index (MI), presence or absence of superfetation, number of populations per species, and sample size (*n* = number of reproductive females) per population for the 13 fish species from the family Poeciliidae that we examined

Species	Type of maternal provisioning	MI	Superfetation	Number of populations	*n* per population	References
Gambusia sexradiata	Lecithotrophy	0.73	Absent	3	21, 22, 15	Riesch *et al.* (2010); Torres-Mejia (2011); this study
Gambusia vittata	Incipient matrotrophy	0.77	Absent	3	56, 63, 26	Torres-Mejia (2011); Weldele *et al.* (2014); this study
Pseudoxiphophorus jonesii	Lecithotrophy	0.65	Absent	3	22, 17, 15	Olivera-Tlahuel *et al.* (2015); this study
Poecilia butleri	Matrotrophy	2.31	Absent	9	28, 31, 31, 18, 16, 20, 11, 17, 42	Zúñiga-Vega *et al.* (2011); Olivera-Tlahuel *et al.* (2015); this study
Poecilia mexicana	Lecithotrophy	0.68	Absent	4	32, 28, 30, 27	Riesch *et al.* (2010); this study
Poeciliopsis baenschi	Matrotrophy	1.58	Present	6	48, 38, 32, 29, 45, 34	Pollux *et al.* (2014); Olivera-Tlahuel *et al.* (2015); Zúñiga-Vega *et al.* (2017); this study
Poeciliopsis gracilis	Lecithotrophy	0.72	Present	11	15, 58, 78, 116, 54, 64, 74, 23, 90, 85, 91	Bassar, Auer & Reznick (2014); Pollux *et al.* (2014); Frías-Alvarez & Zúñiga-Vega (2016); this study
Poeciliopsis infans	Incipient matrotrophy	0.86	Present	11	50, 74, 131, 56, 60, 57, 38, 52, 50, 65, 63	Frías-Alvarez *et al.* (2014); Pollux *et al.* (2014); Frías-Alvarez and Zúñiga-Vega (2016); this study
Poeciliopsis latidens	Incipient matrotrophy	0.86	Present	4	52, 52, 25, 34	Pollux *et al.* (2014); Olivera-Tlahuel *et al.* (2015); this study
Poeciliopsis prolifica	Matrotrophy	5.40	Present	8	19, 32, 145, 18, 93, 49, 78, 44	Pires, McBride & Reznick (2007); Banet & Reznick (2008); Pollux *et al.* (2014); this study
Poeciliopsis turrubarensis	Incipient matrotrophy	0.95	Present	9	37, 71, 49, 40, 38, 77, 21, 40, 14	Zúñiga-Vega *et al.* (2007); Bassar *et al.* (2014); Pollux *et al.*, (2014)
Priapella intermedia	Matrotrophy	1.03	Absent	3	45, 25, 28	This study
Xiphophorus hellerii	Lecithotrophy	0.61	Absent	3	33, 29, 29	Milton & Arthington (1983); Pollux *et al.* (2014); this study

provisioning and/or in the degree of superfetation. Small samples or samples in which most females have a similar body length (low variability in the size of females) may have a lower probability of detecting statistical effects of female size. Thus, we visually examined if the populations in which we detected a change in matrotrophy or superfetation with female size corresponded to populations with either a large sample size or a wide variability in the size of the females included in such samples (calculated for each population as the coefficient of variation of female SL). In addition, we conducted more formal tests by implementing logistic regressions in which the response variable was binomial and included whether an effect of female size on matrotrophy or superfetation was detected or not. These logistic regressions were conducted separately for matrotrophy and superfetation. The explanatory variables were sample size and coefficient of variation of female size. We compared the fit of models including either of these predictors against an intercept-only model by means of AICc and evidence ratios.

Results

The degree of matrotrophy may change as females grow

Only in the following four populations (of the 77 that we examined), the model representing our prediction (an interaction between female size and developmental stage affecting embryo mass) had unequivocal support: populations 2 and 8 of *Poeciliopsis infans*, population 3 of *P. prolifica*, and population 6 of *P. turrubarensis* (Table 2). In these four populations the interactive model ranked first and had an evidence ratio ≥ 3 relative to the model that ranked second (Table 2). In population 8 of *P. infans*, small females were more matrotrophic than larger females as indicated by a positive relationship between developmental stage and embryo mass. In contrast, larger females from this population exhibited the opposite pattern, namely a negative relationship between stage and embryo mass, which indicates a predominantly lecithotrophic strategy (Fig. 1a). The interaction term between female SL and stage was negative and statistically different than zero (hereafter we report regression coefficients, followed by 95% confidence intervals: $\beta = -1.14$, -1.91–-0.36).

In population 2 of *P. infans* and population 6 of *P. turrubarensis*, we observed a similar pattern: in small females, embryo mass remained relatively constant as development progresses, indicating incipient matrotrophy. In contrast, in larger females, embryo mass decreased throughout gestation indicating a lecithotrophic strategy (Fig. 1b,c). In addition, in these two populations, the interaction term was negative and statistically different than zero (population 2 of *P. infans*: $\beta = -0.22$, -0.41–-0.031; population 6 of *P. turrubarensis*: $\beta = -0.10$, -0.19–-0.01).

In population 3 of *P. prolifica* we observed the opposite pattern to what we expected. Small females exhibited a low degree of matrotrophy as indicated by a slight increase in embryo mass throughout development, whereas larger females exhibited a much greater degree of matrotrophy as indicated by a steeper positive slope between embryo mass and developmental stage (Fig. 1d). In this case, the interaction term between stage and female SL was positive and statistically different than zero ($\beta = 0.54$, 0.17–0.91).

Non-interactive effects of female size and developmental stage

In 10 other populations we detected an additive effect of female SL and developmental stage on embryo mass (Table 2). In nine of these 10 populations, female SL had a positive effect on embryo mass, indicating that larger females produced larger embryos, regardless of the particular developmental stage (Fig. 2). The only exception was population 5 of *P. prolifica* in which larger females produced slightly smaller embryos than smaller females (Fig. 2f). In addition to the effect of female size, stage had a negative effect on embryo mass in eight of these 10 populations (Fig. 2), indicating that embryo mass decreased during development. This pattern is characteristic of lecithotrophic species. In the remaining two populations (populations 5 and 7 of *P. prolifica*), stage had a positive influence on embryo mass, indicating a matrotrophic mode of maternal provisioning (Fig. 2f,g).

In all other 63 populations we observed less complex patterns. In 28 of these 63 populations, the intercept-only model ranked first or had similar support compared to the top model, indicating little evidence of effects of stage or female size (Table 2). In 25 populations we detected an evident effect of female SL on embryo mass (with no effect of stage; Table 2). In all these 25 populations, the effect of female size on embryo mass was positive (Table S2). In the remaining 10 populations, only developmental stage affected embryo mass (with no effect of female SL; Table 2). In four of these 10 populations, the effect of stage on embryo mass was negative, indicating a lecithotrophic strategy, whereas in the other six populations, embryo mass increased as a function of stage, indicating a matrotrophic strategy (Table S3).

In a few populations, large females exhibited advanced degrees of superfetation

Contrary to our prediction, in the following five populations of superfetating species (of the 49 that we examined), female SL had a positive influence on the degree of superfetation (the model including an effect of female SL ranked first and had an evidence ratio ≥ 3 relative to the intercept-only model): populations 4 ($\beta = 2.04$, 0.24–3.79), 5 ($\beta = 2.67$, 1.22–4.12), and 11 ($\beta = 1.36$, 0.11–2.64) of *P. infans*, population 6 of *P. prolifica* ($\beta = 2.66$, 0.57–4.79), and population 7 of *P. turrubarensis* ($\beta = 1.86$, 0.18–3.80; Table 3). In these five populations, larger females tended to carry more simultaneous broods than smaller females (Fig. z3). In all other 44 populations, we found no effect of female size on degree of superfetation (Table 3).

Table 2 Differences in the Akaike information criterion adjusted for small sample sizes (ΔAICc) for five different linear models that examined the potential effects of female size and developmental stage on individual embryo mass for 77 populations of 13 species from the family Poeciliidae

Species/population	Intercept only	Developmental stage	Female size	Developmental stage + female size	Developmental stage × female size
Gambusia sexradiata					
1	0 (0.58)	2.19 (0.19)	2.59 (0.16)	5.13 (0.04)	6.25 (0.03)
2	0 (0.61)	2.63 (0.16)	2.53 (0.17)	5.38 (0.04)	8.05 (0.01)
3	0 (0.65)	2.74 (0.17)	2.84 (0.16)	6.37 (0.03)	10.20 (0)
Gambusia vittata					
1	15.66 (0)	17.90 (0)	0 (0.67)	2.04 (0.24)	4.20 (0.08)
2	15.89 (0)	0 (0.56)	16.83 (0)	1.46 (0.27)	2.31 (0.18)
3	16.05 (0)	18.51 (0)	3.30 (0.12)	1.59 (0.27)	0 (0.61)
Pseudoxiphophorus jonesii					
1	0 (0.62)	2.63 (0.17)	2.66 (0.17)	5.59 (0.04)	8.98 (0.01)
2	9.64 (0.01)	12.35 (0)	0 (0.76)	3.47 (0.13)	4.04 (0.10)
3	3.14 (0.14)	5.10 (0.05)	0 (0.69)	3.82 (0.10)	7.79 (0.01)
Poecilia butleri					
1	0 (0.52)	2.17 (0.18)	1.72 (0.22)	4.25 (0.06)	6.03 (0.03)
2	1.76 (0.19)	3.93 (0.07)	1.55 (0.21)	3.73 (0.07)	0 (0.46)
3	0.66 (0.31)	2.98 (0.10)	0 (0.44)	2.58 (0.12)	5.37 (0.03)
4	0 (0.36)	1.05 (0.21)	0.46 (0.29)	2.17 (0.12)	6.05 (0.02)
5	12.70 (0)	14.77 (0)	1.38 (0.28)	0 (0.56)	2.48 (0.16)
6	2.98 (0.12)	0 (0.54)	3.56 (0.09)	2.02 (0.20)	4.49 (0.06)
7	0.18 (0.36)	0 (0.39)	3.70 (0.06)	1.43 (0.19)	8.76 (0)
8	2.39 (0.17)	4.85 (0.05)	0 (0.55)	2.05 (0.20)	5.07 (0.04)
9	0 (0.50)	2.27 (0.16)	1.44 (0.24)	3.60 (0.08)	6.19 (0.02)
Poecilia mexicana					
1	12.17 (0)	14.25 (0)	0 (0.67)	2.46 (0.20)	3.32 (0.13)
2	4.68 (0.05)	1.03 (0.30)	6.28 (0.02)	0 (0.50)	2.72 (0.13)
3	0.35 (0.33)	0 (0.40)	2.72 (0.10)	0.25 (0.13)	4.63 (0.04)
4	1.75 (0.22)	0 (0.53)	4.00 (0.07)	2.49 (0.15)	5.53 (0.03)
Poeciliopsis baenschi					
1	2.48 (0.14)	4.54 (0.05)	0 (0.47)	1.20 (0.26)	3.22 (0.09)
2	7.80 (0.01)	10.14 (0)	0 (0.6)	1.82 (0.24)	2.98 (0.14)
3	2.14 (0.18)	0 (0.51)	2.91 (0.12)	2.51 (0.15)	4.62 (0.05)
4	1.01 (0.29)	0 (0.47)	3.51 (0.08)	2.68 (0.12)	5.26 (0.03)
5	1.04 (0.25)	3.26 (0.08)	0 (0.42)	1.63 (0.19)	3.98 (0.06)
6	0 (0.56)	2.25 (0.18)	2.34 (0.18)	4.61 (0.06)	6.42 (0.02)
Poeciliopsis gracilis					
1	1.70 (0.23)	3.35 (0.10)	0 (0.55)	3.40 (0.10)	7.19 (0.02)
2	1.86 (0.20)	3.74 (0.08)	0 (0.51)	2.31 (0.16)	4.61 (0.05)
3	6.95 (0.02)	7.57 (0.02)	0 (0.67)	2.22 (0.22)	4.29 (0.08)
4	16.54 (0)	17.48 (0)	2.99 (0.13)	0 (0.58)	1.39 (0.29)
5	7.00 (0.01)	8.83 (0.01)	0 (0.41)	0.88 (0.26)	0.54 (0.31)
6	5.84 (0.02)	1.19 (0.23)	7.52 (0.01)	0 (0.42)	0.56 (0.32)

Table 2 Continued.

Species/population	Intercept only	Developmental stage	Female size	Developmental stage + female size	Developmental stage × female size
7	35.02 (0)	37.17 (0)	0 (0.48)	0.59 (0.36)	2.24 (0.16)
8	5.68 (0.04)	0 (0.69)	8.32 (0.01)	2.40 (0.21)	5.38 (0.05)
9	9.55 (0.01)	11.69 (0)	0 (0.59)	1.64 (0.26)	2.77 (0.15)
10	0 (0.27)	0.72 (0.19)	0.80 (0.18)	0.08 (0.26)	1.80 (0.11)
11	10.49 (0)	9.34 (0.01)	3.36 (0.12)	0 (0.65)	2.14 (0.22)
Poeciliopsis infans					
1	5.24 (0.04)	6.46 (0.02)	0 (0.56)	1.76 (0.23)	2.63 (0.15)
2*	4.82 (0.06)	4.73 (0.06)	3.62 (0.11)	3.21 (0.13)	0 (0.65)
3	0 (0.37)	0.85 (0.24)	1.28 (0.19)	2.19 (0.12)	3.17 (0.08)
4	3.78 (0.06)	6.00 (0.02)	0 (0.40)	0.60 (0.30)	1.21 (0.22)
5	0 (0.44)	1.81 (0.18)	2.16 (0.15)	3.14 (0.09)	2.26 (0.14)
6	20.91 (0)	23.14 (0)	0.34 (0.39)	0 (0.46)	2.15 (0.16)
7	0 (0.37)	1.64 (0.17)	1.09 (0.22)	1.96 (0.14)	2.53 (0.11)
8*	8.72 (0.01)	3.96 (0.12)	9.45 (0.01)	6.25 (0.04)	0 (0.83)
9	10.42 (0)	0 (0.50)	8.21 (0.01)	0.67 (0.36)	2.78 (0.13)
10	16.29 (0)	15.74 (0)	0 (0.68)	2.24 (0.22)	3.88 (0.10)
11	7.00 (0.01)	7.65 (0.01)	0 (0.47)	2.27 (0.15)	0.51 (0.36)
Poeciliopsis latidens					
1	4.84 (0.06)	0 (0.66)	7.00 (0.02)	2.31 (0.21)	4.72 (0.06)
2	12.93 (0)	9.90 (0.01)	8.42 (0.01)	0 (0.76)	2.45 (0.22)
3	1.76 (0.21)	3.19 (0.10)	0 (0.50)	2.85 (0.12)	3.67 (0.08)
4	16.73 (0)	19.13 (0)	3.86 (0.09)	1.21 (0.32)	0 (0.59)
Poeciliopsis prolifica					
1	0.07 (0.38)	0 (0.37)	1.83 (0.15)	3.23 (0.08)	5.79 (0.02)
2	16.04 (0)	0 (0.72)	17.89 (0)	2.51 (0.21)	4.47 (0.08)
3*	59.72 (0)	5.27 (0.06)	59.58 (0)	6.20 (0.04)	0 (0.90)
4	7.60 (0.01)	2.94 (0.13)	0 (0.54)	1.37 (0.27)	4.97 (0.05)
5	38.39 (0)	2.31 (0.16)	40.42 (0)	0 (0.49)	0.69 (0.35)
6	18.95 (0)	0 (0.53)	21.13 (0)	1.06 (0.31)	2.47 (0.16)
7	26.13 (0)	22.36 (0)	4.62 (0.01)	0.21 (0.49)	0 (0.50)
8	0 (0.49)	2.04 (0.18)	1.54 (0.23)	3.77 (0.08)	5.64 (0.03)
Poeciliopsis turrubarensis					
1	4.28 (0.05)	4.00 (0.06)	2.47 (0.12)	0 (0.41)	0.18 (0.37)
2	18.87 (0)	20.76 (0)	0 (0.69)	2.23 (0.23)	4.33 (0.08)
3	63.19 (0)	61.2 (0)	13.95 (0)	0 (0.54)	0.32 (0.46)
4	19.09 (0)	11.39 (0)	4.52 (0.05)	0.25 (0.44)	0 (0.50)
5	28.08 (0)	30.30 (0)	0 (0.37)	0.55 (0.28)	0.04 (0.36)
6*	52.64 (0)	53.77 (0)	4.37 (0.08)	2.23 (0.23)	0 (0.70)
7	30.31 (0)	24.41 (0)	0.26 (0.43)	3.26 (0.10)	0 (0.48)
8	10.92 (0)	12.76 (0)	0 (0.58)	1.74 (0.24)	2.48 (0.17)
9	13.66 (0)	15.30 (0)	0 (0.85)	3.67 (0.14)	7.59 (0.02)

Table 2 Continued.

Species/population	Intercept only	Developmental stage	Female size	Developmental stage + female size	Developmental stage × female size
Priapella intermedia					
1	9.12 (0.01)	11.31 (0)	0 (0.71)	2.35 (0.22)	4.85 (0.06)
2	8.42 (0.01)	10.72 (0)	0 (0.68)	2.09 (0.24)	4.49 (0.07)
3	0 (0.39)	2.50 (0.11)	0.14 (0.37)	2.75 (0.10)	5.49 (0.03)
Xiphophorus hellerii					
1	0 (0.57)	2.25 (0.18)	2.30 (0.18)	4.80 (0.05)	7.03 (0.02)
2	1.77 (0.21)	3.13 (0.10)	0 (0.50)	2.66 (0.13)	4.14 (0.06)
3	0.08 (0.28)	0 (0.30)	0.56 (0.22)	1.21 (0.16)	4.15 (0.04)

The best-fitting model is indicated by ΔAICc = 0. Asterisks indicate populations in which the model representing our hypothesis (i.e., the interaction between developmental stage and female size affecting individual embryo mass) had unequivocal support. For all models, we also show Akaike weights within parentheses.

Potential effects of sample characteristics

Sample size influenced the probability of detecting an interaction between developmental stage and female SL affecting embryo mass (Fig. S3). Three of the four populations in which we detected such interactive effect had relatively large sample sizes (n = 74, 77, and 145; as reference, the smallest sample size was 11 females). In fact, one of these three populations had the largest sample size of all populations (n = 145). The logistic regression that included sample size as a predictor had stronger support than the intercept-only model (evidence ratio = 12.9; Table S4) and indicated that the probability of detecting this interactive effect is substantially low (<0.1) if $n < 70$ and increases markedly in larger samples (Fig. S3). The coefficient of variation of female size (i.e. amount of variability in female size included in our samples) did not influence the probability of detecting this interactive effect (Fig. S3, Table S4). Neither sample size nor coefficient of variation of female size influenced the probability of detecting an effect of female SL on superfetation (Fig. S3, Table S4).

Discussion

In this study, we tested the hypothesis that matrotrophy and superfetation should be more prominent in small females to compensate the energetic demands and the space restrictions inherent to a small body size. We found modest evidence in support of this hypothesis: in three populations from two different species (*Poeciliopsis infans* and *P. turrubarensis*), smaller females provided more nutrients after fertilization than larger females, which relied more on pre-fertilization provisioning. These findings indicate that the degree of matrotrophy can change throughout the adult life of females, being relatively matrotrophic when they are young and switching towards a lecithotrophic strategy as they get older. Ours is the first study to demonstrate this ontogenetic change in the mode of maternal provisioning to developing embryos in poeciliid fishes. Being matrotrophic at early stages of adulthood could allow females to devote energy and resources to both growth and reproduction and, assuming that matrotrophy allows the production of more offspring compared to lecithotrophy (Trexler & DeAngelis, 2003), allocating energy to body growth should not compromise overall fecundity of these young and small females. Although we found evidence that the degree of matrotrophy can change ontogenetically in some populations, this change did not occur in most populations.

Intriguingly, we observed that in one population of *P. prolifica* the amount of post-fertilization transfer of nutrients to embryos also varied as a function of female size, but in the opposite direction to what we predicted. At this site, large females were more matrotrophic than small females. A possible explanation for this unexpected pattern is a difference between small and large females in food intake. According to the Trexler-DeAngelis model of maternal provisioning, advanced degrees of matrotrophy are only possible if food is relatively abundant and constantly available (Trexler & DeAngelis, 2003, 2010). Only under such favorable conditions, females would be able to provide a constant supply of nutrients to developing

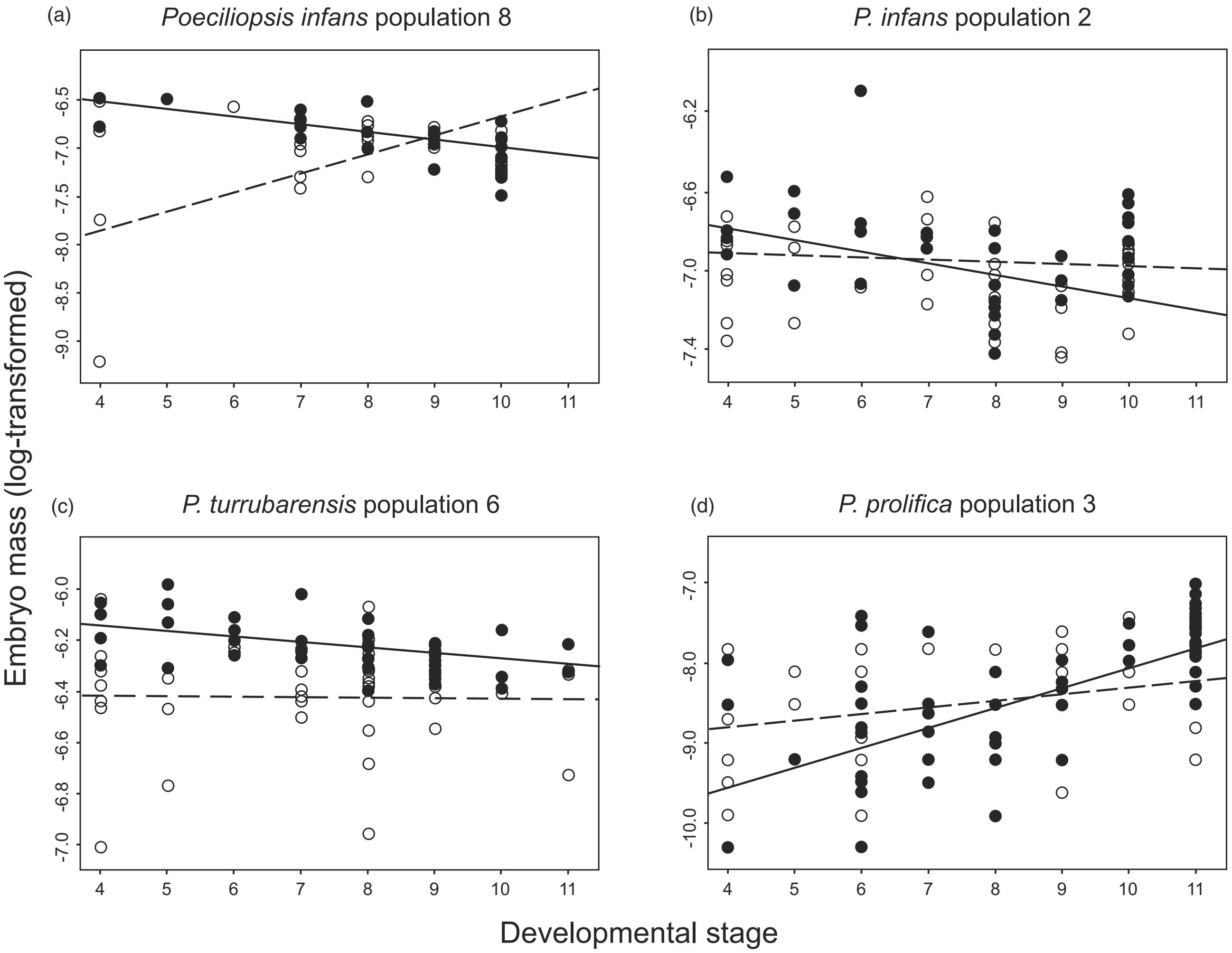


Figure 1 Changes in the mass of individual embryos as development progresses for small (white circles and dashed lines) and large (black circles and continuous lines) females. These two size categories correspond to females with a standard length below (small) or above (large) the population median. In these four populations we detected an interactive effect of female length and developmental stage on individual embryo mass. (a) Population 8 of *Poeciliopsis infans*: small females are matrotrophic whereas large females are lecithotrophic. (b) Population 2 of *P. infans* and (c) population 6 of *P. turrubarensis*. In these two populations, small females exhibit incipient matrotrophy whereas large females are predominantly lecithotrophic. (d) Population 3 of *P. prolifica*: small females exhibit a low degree of matrotrophy whereas large females are highly matrotrophic. In Fig. S1 we use three-dimensional scatterplots to represent these same associations but using female length as a continuous variable.

embryos while at the same time meeting their own energetic demands. If intraspecific competition is intense in this population (for example as a result of high population density) smaller females may be outcompeted by larger females, which in turn would consume more food. Greater food intake may allow larger females to be more matrotrophic, whereas small females must allocate most of their restricted food intake to self-maintenance and, only whenever they have some surplus energy, they could store nutrients in the form of yolk. Previous studies have demonstrated that intraspecific competition is an important driver of variation in reproductive traits of poeciliid fishes (Leips & Travis, 1999; Bashey, 2006, 2008; Schrader & Travis, 2012).

Our findings also revealed that a switch in the relative amount of post-fertilization provisioning as females grow was uncommon. We detected different strategies between small (younger) and large (older) females only in four of the 77 populations that we examined (5% of our samples). Hagmayer *et al*., (2018) examined four populations of *Poeciliopsis retropinna* and found no effect of female size on the degree of matrotrophy. Thus, changes from incipient matrotrophy to a predominantly lecithotrophic mode of maternal provisioning or, alternatively, to more advanced degrees of matrotrophy throughout the reproductive lifespans of individual females are certainly possible, but these changes do not occur frequently in natural conditions. One plausible explanation for this lack of

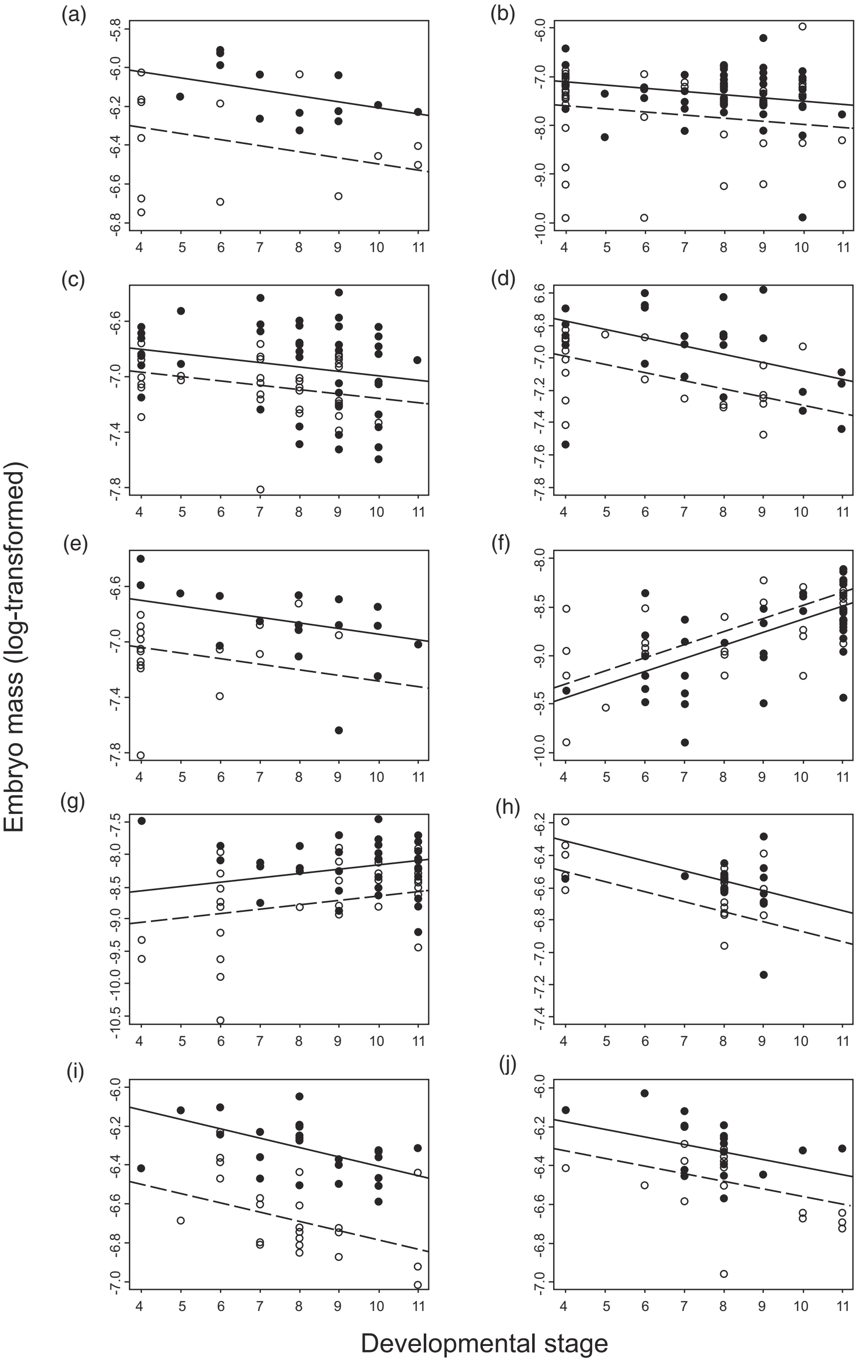


Figure 2 Changes in the mass of individual embryos as development progresses for small (white circles and dashed lines) and large (black circles and continuous lines) females. These two size categories correspond to females with a standard length below (small) or above (large) the population median. In these 10 populations we detected an additive effect of female length and developmental stage on individual embryo mass. (a) Population 3 of *Gambusia vittata*, populations (b) 4 and (c) 11 of *Poeciliopsis gracilis*, populations (d) 2 and (e) 4 of *P. latidens*, populations (f) 5 and (g) 7 of *P. prolifica*, populations (h) 1, (i) 3, and (j) 4 of *P. turrubarensis*. In Fig. S2 we use three-dimensional scatterplots to represent these same associations but using female length as a continuous variable.

ontogenetic variation in most populations is that selection towards one or the other strategy (matrotrophy over lecithotrophy or vice versa) is too strong on all size and age classes to allow for the expression of the alternative strategy. Another explanation is the existence of physiological restrictions that may be difficult to overcome for a change to occur in the main

Table 3 Differences in the Akaike information criterion adjusted for small sample sizes (ΔAICc) for two different generalized linear models that examined the potential effect of female size on the number of simultaneous broods (i.e. degree of superfetation) for 49 populations of six species from the family Poeciliidae

Species/population	Intercept only	Female size
Poeciliopsis baenschi		
1	0 (0.65)	1.24 (0.35)
2	0 (0.75)	2.18 (0.25)
3	0 (0.69)	1.62 (0.31)
4	0 (0.76)	2.32 (0.24)
5	0 (0.70)	1.71 (0.29)
6	0 (0.73)	1.96 (0.27)
Poeciliopsis gracilis		
1	0 (0.76)	2.32 (0.24)
2	0 (0.70)	1.71 (0.30)
3	0.93 (0.39)	0 (0.61)
4	1.94 (0.28)	0 (0.73)
5	0 (0.72)	1.90 (0.28)
6	0 (0.66)	1.30 (0.34)
7	0.30 (0.46)	0 (0.54)
8	0.57 (0.43)	0 (0.57)
9	0 (0.74)	2.06 (0.26)
10	0 (0.54)	0.31 (0.46)
11	0 (0.74)	2.08 (0.26)
Poeciliopsis infans		
1	0 (0.75)	2.17 (0.25)
2	0 (0.58)	0.65 (0.42)
3	0.25 (0.47)	0 (0.53)
4*	2.75 (0.20)	0 (0.80)
5*	10.68 (0.01)	0 (0.99)
6	0 (0.60)	0.84 (0.40)
7	0 (0.63)	1.02 (0.38)
8	0 (0.59)	0.77 (0.41)
9	0 (0.63)	1.02 (0.38)
10	0 (0.62)	0.93 (0.39)
11*	2.75 (0.20)	0 (0.80)
Poeciliopsis latidens		
1	0.31 (0.46)	0 (0.54)
2	0 (0.74)	2.12 (0.26)
3	0 (0.69)	1.62 (0.31)
4	0 (0.53)	0.25 (0.47)
Poeciliopsis prolifica		
1	0 (0.74)	2.08 (0.26)
2	0 (0.75)	2.15 (0.25)
3	0 (0.57)	0.57 (0.43)
4	0 (0.73)	2.00 (0.27)
5	0 (0.68)	1.52 (0.32)
6*	4.07 (0.12)	0 (0.89)
7	0.24 (0.47)	0 (0.53)
8	0 (0.75)	2.17 (0.25)
Poeciliopsis turrubarensis		
1	0 (0.74)	2.13 (0.26)
2	0 (0.68)	1.46 (0.33)
3	0 (0.60)	0.80 (0.40)
4	0.09 (0.49)	0 (0.51)
5	0 (0.52)	0.13 (0.48)
6	0 (0.65)	1.27 (0.35)
7*	2.35 (0.24)	0 (0.76)
8	0 (0.75)	2.19 (0.25)
9	0 (0.76)	2.29 (0.24)

The best-fitting model is indicated by ΔAICc = 0. Asterisks indicate populations in which the model including an effect of female size on superfetation had unequivocal support. For all models, we also show Akaike weights within parentheses.

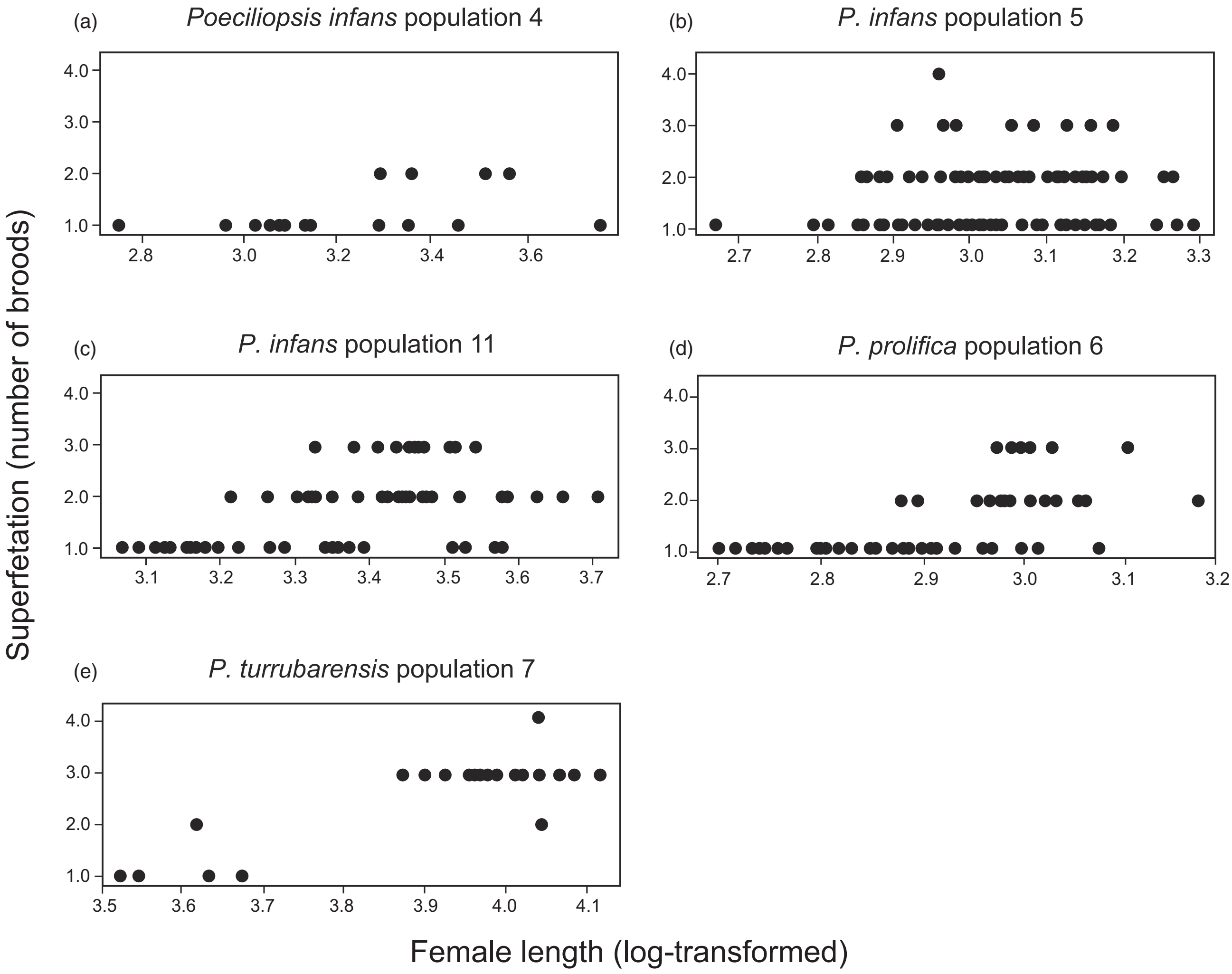


Figure 3 Effects of female length on the number of simultaneous broods (i.e. degree of superfetation). Populations (a) 4, (b) 5, and (c) 11 of *Poeciliopsis infans*, (d) population 6 of *P. prolifica*, and (e) population 7 of *P. turrubarensis*.

source of embryo nourishment. For instance, modifications in the concentrations of particular hormones, such as prolactin, estradiol, or progestins may be essential to promote a change from placental nutrition to substantial yolk deposition or vice versa (Venkatesh, Tan & Lam, 1990; Guernsey *et al*., 2020). Ontogenetic changes in hormonal profiles may be difficult to occur. Nonetheless, we did detect an effect of female size on the degree of matrotrophy in populations with large sample sizes. Our data show that the probability of detecting this effect was low in samples with fewer than 70 females. Therefore, changes in the relative amounts of pre- and post-fertilization provisioning as females grow may be more common than we observed here, but larger samples are needed to detect such changes. In fact, of the 14 populations where we sampled at least 70 females, three of them revealed an effect of female size (Fig. S3), a much higher percentage (21%) than we detected in our study overall (5%).

In addition, contrary to our expectations, in only a few populations (five of 49) we found that superfetation increased in large females. This finding is opposite to the predicted negative association between superfetation and female size and indicates that young females do not compensate for a small ovarian cavity by overlapping more broods. Instead, we observed a positive effect of female size (and presumably age) on superfetation that was also uncommon. In this case, the probability of detecting this positive association was unrelated to the characteristics of our samples (sample size or amount of variability in the size of females). Thus, we can confidently conclude that increases in the number of simultaneous broods as females grow are relatively infrequent in natural populations of poeciliid fishes. Frías-Alvarez & Zúñiga-Vega (2016) similarly found that larger females exhibited more simultaneous broods only in three of 11 populations of *Poeciliopsis gracilis* and only in one of 11 populations of *P. infans*. Leips & Travis (1999) observed that, only in two of four populations, larger females of *Heterandria formosa* overlapped more broods compared to smaller females. Therefore, evidence from other studies also reveals a pattern that contradicts our hypothesis: small (young) females do not exhibit increased superfetation, but instead there is a relatively infrequent positive effect of female size on superfetation. Fast water velocity or high predation pressure may promote advanced degrees of superfetation in large (older) females as a byproduct of their strong selection towards elongated phenotypes (Zúñiga-Vega *et al*., 2007; Gorini-Pacheco, Zandonà & Mazzoni, 2018). Given that fecundity is higher in larger females and that high fecundity entails a substantial increase in both body volume and drag forces during swimming (Ghalambor, Reznick & Walker, 2004), more simultaneous broods could reduce the reproductive burden and improve streamlining of large females because they would carry fewer large full-term embryos without compromising total fecundity (Fleuren *et al*., 2019). In contrast, small females do not carry as many total embryos as large females, and their increase in body volume during pregnancy is not as steep as to compromise their swimming performance, making unnecessary the production of more simultaneous broods even in sites with fast currents or abundant predators.

An important implication of our findings is that approximately 5% of the matrotrophy indices that have been previously reported are likely biased, because we detected a confounding effect of female size in 4 of 77 populations (5% of our samples). Overlooking a potential effect of female size on the amount of post-fertilization provisioning may bias the MI in two ways. First, a small sample of reproductive females may not capture most of the variation in female size, which in turn may result in an MI biased towards the strategy of relatively small females or, alternatively, towards the strategy of larger females, depending on which range of sizes is best represented in the sample. Second, a large sample of females may result in an MI that does not reflect the difference in the degree of matrotrophy between small and large females. In this case, the MI would indicate an intermediate mode of maternal provisioning that in fact is not representative of neither small nor large females. Therefore, further refinement of the MI is needed to account for the potentially confounding effect of female size.

Substantial variation exists among and within populations of poeciliid species in both matrotrophy and superfetation (e.g., Frías-Alvarez *et al*., 2014; Gorini-Pacheco *et al*., 2018). Experimental studies have demonstrated that changes in food availability can promote changes in both the number of simultaneous broods and the relative amounts of pre- and post-fertilization provisioning to developing embryos (Travis *et al*., 1987; Molina-Moctezuma *et al*., 2020). Hagmayer *et al*. (2018) recently found that maternal body fat positively influenced the degree of matrotrophic nourishment in *Poeciliopsis retropinna*. Here, we provide evidence that the degrees of matrotrophy and superfetation can also change within individual females throughout their reproductive lifespans. We recognize that we used data from different females that varied in size, assuming that female length is an accurate proxy for female age (Hughes, 1986; Vargas & De Sostoa, 1996). To more accurately compare the amount of nutrients that are actively transferred by the mothers to embryos between early and late stages of their reproductive lives, future studies could administer exogenous markers (e.g. radiolabeled or immunostained proteins) to individual females at different points of their lives, to trace these compounds in their offspring (e.g. Nakamura *et al*., 2004).

Acknowledgments

Israel Solano-Zavaleta provided technical assistance. Nabila Saleh-Subaie is a doctoral student from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and received fellowship 596700 from CONACYT.

Funding information

This study was funded by Consejo Nacional de Ciencia y Tecnología through a fellowship awarded to NSS (number 596700) as well as by the Programa de Apoyo a Estudiantes de Posgrado (PAEP) from Universidad Nacional Autónoma de México.

Author contributions

JJZV conceived the study and designed the analyses. JBJ refined the hypothesis and provided museum data. NSS dissected museum specimens, compiled all data, conducted analyses, and wrote the first draft. All authors edited the manuscript and approved its final version.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Additional details about our datasets and protocol to quantify reproductive traits from preserved specimens.

Table S1. Geographic coordinates, in decimal degrees, for the 77 populations that we examined of 13 fish species from the family Poeciliidae.

Table S2. Regression slopes and 95% confidence intervals estimated for 25 populations in which we detected an evident effect of female length on individual embryo mass.

Table S3. Regression slopes and 95% confidence intervals estimated for 10 populations in which we detected an evident effect of developmental stage on individual embryo mass.

Table S4. Values of the Akaike information criterion adjusted for small sample sizes (AICc) and Akaike weights (*w*) for generalized linear models (logistic regressions) that examined the effects of both sample size and coefficient of variation of female size on the probability of detecting size-dependent changes in the degrees of matrotrophy and superfetation.

Figure S1. Three-dimensional scatterplots depicting interactive effects of female length and developmental stage on individual embryo mass.

Figure S2. Three-dimensional scatterplots depicting additive effects of female length and developmental stage on individual embryo mass.

Figure S3. Examination of the potential effects of sample size and coefficient of variation of female size on the probability of detecting size-dependent changes in the degrees of matrotrophy and superfetation.

Appendix S1

Additional details about our study species and datasets

We obtained data on female size (mm standard length), average dry mass of individual embryos (g), developmental stage, and number of simultaneous broods (only for species with superfetation) from previously published studies as well as from our own dissections of pregnant females, which we obtained from the National Collection of Fishes (Instituto de Biología, Universidad Nacional Autónoma de México). For two species, *Gambusia vittata* and *Poeciliopsis turrubarensis*, we used the databases from previously published studies (Zúñiga-Vega, Reznick & Johnson, 2007; Weldele, Zúñiga-Vega & Johnson, 2014). For four other species, *Poecilia butleri*, *Poeciliopsis baenschi*, *P. gracilis*, and *P. infans*, we combined data from published studies (Zúñiga-Vega *et al.*, 2011; Frías-Alvarez *et al.*, 2014; Frías-Alvarez & Zúñiga-Vega, 2016; Zúñiga-Vega, Olivera-Tlahuel & Molina-Moctezuma, 2017) and from our dissections of pregnant females. For the remaining seven species (*Gambusia sexradiata, Pseudoxiphophorus jonesii*, *Poecilia mexicana*, *Poeciliopsis latidens, P. prolifica, Priapella intermedia,* and *Xiphophorus hellerii*), all the data were obtained by dissecting preserved females, also from the National Collection of Fishes (see Table 1).

Quantification of reproductive traits from preserved specimens

Before dissecting preserved females, we measured their standard length (SL) from the tip of the mouth to base of the caudal fin at the level of the lateral line with a digital caliper (± 0.1 mm). We then dissected females to quantify brood size (number of embryos in the same developmental stage) and, in the case of species with superfetation, the number of simultaneous broods. We used the classification of embryonic stages proposed by Haynes (1995) to identify the developmental stage of each brood. Individual embryo mass was quantified by drying an entire brood for 48 h at 55 °C, weighing it with a precision scale (± 0.05 mg), and dividing brood dry mass by the number of embryos in the brood. In the cases of superfetating species, we randomly chose one brood from each superfetating female to quantify individual embryo mass. In this way, we ensured that each female was represented only once in our analyses to avoid the problem of non-independence in the data. This single-brood approach has been implemented in previous studies that analyzed life-history traits of superfetating species (Zúñiga-Vega *et al.*, 2007; Frías-Alvarez *et al.*, 2014; Frías-Alvarez & Zúñiga-Vega, 2016; Gorini-Pacheco, Zandonà & Mazzoni, 2018). Thus, our estimates of post-fertilization provisioning are comparable to those reported in studies that similarly used only one brood from females that had two or more simultaneous broods. We extracted data on female SL, number of simultaneous broods, individual embryo mass, and developmental stage from the datasets of the published studies that we mentioned above. The authors of these studies also quantified these traits from reproductive females following the same protocol that we implemented here.

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Table S1 Geographic coordinates, in decimal degrees, for the 77 populations that we examined of 13 fish species from the family Poeciliidae.

Species/Populations	Latitude	Longitude
Gambusia sexradiata		
1	20.16	-103.04
2	23.06	-105.84
3	17.73	-95.87
Gambusia vittata		
1	21.28	-97.96
2	21.93	-98.87
3	21.33	-97.75
Pseudoxhiphophorus jonesii		
1	17.73	-95.87
2	20.04	-100.68
3	20.70	-103.96
Poecilia butleri		
1	19.60	-105.10
2	19.25	-104.17
3	19.39	-104.53
4	19.21	-104.21
5	19.32	-103.82
6	21.05	-103.43
7	23.52	-106.50
8	25.09	-107.69
9	16.78	-99.51
Poecilia mexicana		
1	17.73	-95.87
2	17.15	-95.17
3	18.61	-95.65
4	17.15	-95.12

Poeciliopsis baenschi		
1	18.54	-103.39
2	19.20	-103.83
3	19.17	-103.83
4	20.19	-104.55
5	19.39	-104.53
6	19.49	-104.31
Poeciliopsis gracilis		
1	21.97	-99.26
2	21.99	-99.26
3	21.98	-98.96
4	17.43	-95.02
5	17.20	-95.05
6	17.15	-95.12
7	17.14	-95.13
8	17.15	-95.17
9	17.45	-95.44
10	17.56	-95.55
11	17.84	-95.82
Poeciliopsis infans		
1	20.58	-103.86
2	21.65	-102.97
3	21.21	-103.37
4	21.24	-102.33
5	21.16	-102.47
6	21.05	-103.43
7	20.56	-103.96
8	20.55	-103.95
9	20.54	-104.05
10	20.58	-104.15
11	20.16	-103.04
Poeciliopsis latidens		
1	23.06	-105.84
2	22.46	-105.37
3	23.89	-106.62
4	23.28	-106.24

Poeciliopsis prolifica		
1	21.65	-102.97
2	21.51	-103.09
3	21.48	-103.09
4	21.04	-104.38
5	23.89	-106.62
6	22.46	-105.37
7	23.28	-106.24
8	23.06	-105.84
Poeciliopsis turrubarensis		
1	8.68	-83.49
2	8.61	-83.42
3	9.58	-84.33
4	10.03	-85.18
5	10.13	-85.27
6	10.31	-85.17
7	10.56	-85.39
8	10.31	-85.21
9	10.35	-85.16
Priapella intermedia		
1	17.15	-95.17
2	17.14	-95.13
3	17.15	-95.14
Xiphophorus hellerii		
1	17.15	-95.12
2	20.58	-104.07
3	20.58	-103.86

Table S2 Regression slopes and 95% confidence intervals estimated for 25 populations in which we detected an evident effect of female length on individual embryo mass.

Species/population	Regression slope	Confidence interval
Gambusia vittata		
1	1.02	0.57 – 1.47
Pseudoxiphophorus jonesii		
2	1.28	0.61 – 1.95
3	2.44	0.42 – 4.46
Poecilia butleri		
5	1.60	0.84 – 2.36
8	0.98	0.10 – 1.86
Poecilia mexicana		
1	1.08	0.54 – 1.61
Poeciliopsis baenschi		
1	0.75	0.06 – 1.45
2	1.21	0.47 – 1.94
Poeciliopsis gracilis		
3	0.67	0.24 – 1.11
5	1.12	0.40 – 1.84
7	1.23	0.87 – 1.58
9	0.76	0.33 – 1.19
Poeciliopsis infans		
1	1.54	0.43 – 2.64
4	0.88	0.17 – 1.59
6	1.44	0.88 – 1.99
10	2.09	1.17 – 3.00
11	0.82	0.35 – 1.29
Poeciliopsis prolifica		
4	1.85	0.75 – 2.95

Poeciliopsis turrubarensis		
2	0.73	0.43 – 1.03
5	1.07	0.74 – 1.40
7	1.96	1.47 – 2.44
8	0.90	0.43 – 1.38
9	1.47	0.87 – 2.07
Priapella intermedia		
1	1.71	0.73 – 2.69
2	2.11	0.89 – 3.33

Table S3 Regression slopes and 95% confidence intervals estimated for 10 populations in which we detected an evident effect of developmental stage on individual embryo mass.

Species/population	Regression slope	Confidence interval
Gambusia vittata		
2	-0.07	-0.09 – -0.04
Poecilia butleri		
6	0.07	0.01 – 0.14
Poecilia mexicana		
2	0.08	0.01 – 0.14
Poeciliopsis baenschi		
3	0.08	0.004 – 0.16
Poeciliopsis gracilis		
6	-0.05	-0.08 – -0.01
8	-0.10	-0.18 – -0.03
Poeciliopsis infans		
9	0.11	0.05 – 0.16
Poeiliopsis latidens		
1	-0.04	-0.07 – -0.01
Poeciliopsis prolifica		
2	0.23	0.13 – 0.32
6	0.18	0.11 – 0.26

Table S4 Values of the Akaike information criterion adjusted for small sample sizes (AICc) and Akaike weights (*w*) for generalized linear models (logistic regressions) that examined the effects of both sample size and coefficient of variation of female size on: (a) the probability of detecting an interaction between developmental stage and female size affecting embryo mass, and (b) the probability of detecting an effect of female size on superfetation. ΔAICc represents the difference in AICc values between each model and the best-fitting model (which is indicated by ΔAICc = 0). Models are listed according to their fit to the data, from best to worst.

Response variable	Competing model (explanatory variable)	AICc	ΔAICc	*w*
(a) Probability of detecting an interaction between developmental stage and female size affecting embryo mass	Sample size	28.50	0	0.90
	Intercept only	33.50	5.00	0.07
	Coefficient of variation of female size	35.36	6.86	0.03
(b) Probability of detecting an effect of female size on superfetation	Intercept only	39.06	0	0.53
	Coefficient of variation of female size	40.44	1.37	0.27
	Sample size	41.04	1.98	0.20

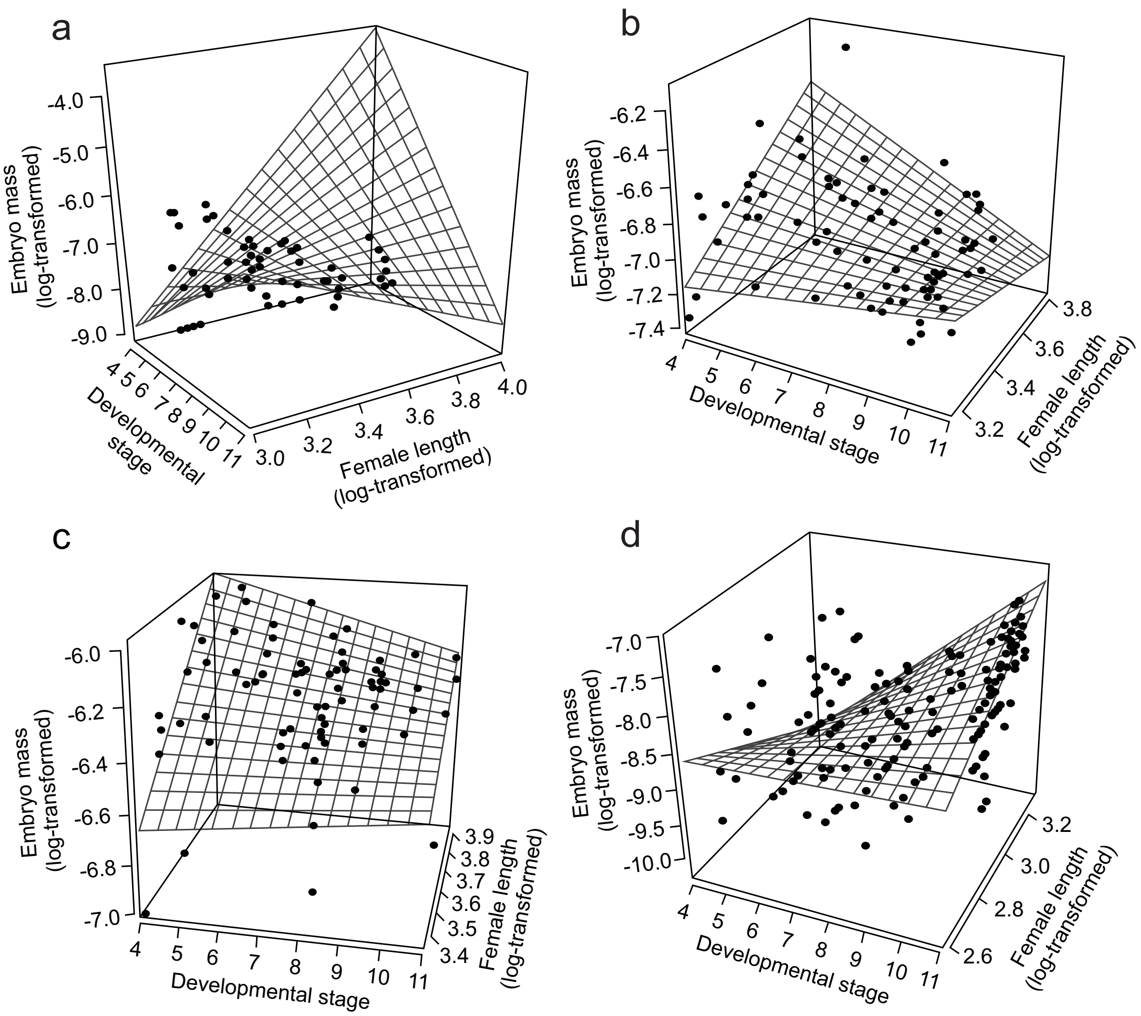

Figure S1 Interactive effects of female length and developmental stage on individual embryo mass. (a) Population 8 of *Poeciliopsis infans*: small females are matrotrophic whereas large females are lecithotrophic. (b) Population 2 of *P. infans* and (c) population 6 of *P. turrubarensis*. In these two populations, small females exhibit incipient matrotrophy whereas large females are predominantly lecithotrophic. (d) Population 3 of *P. prolifica*: small females exhibit a low degree of matrotrophy whereas large females are highly matrotrophic.

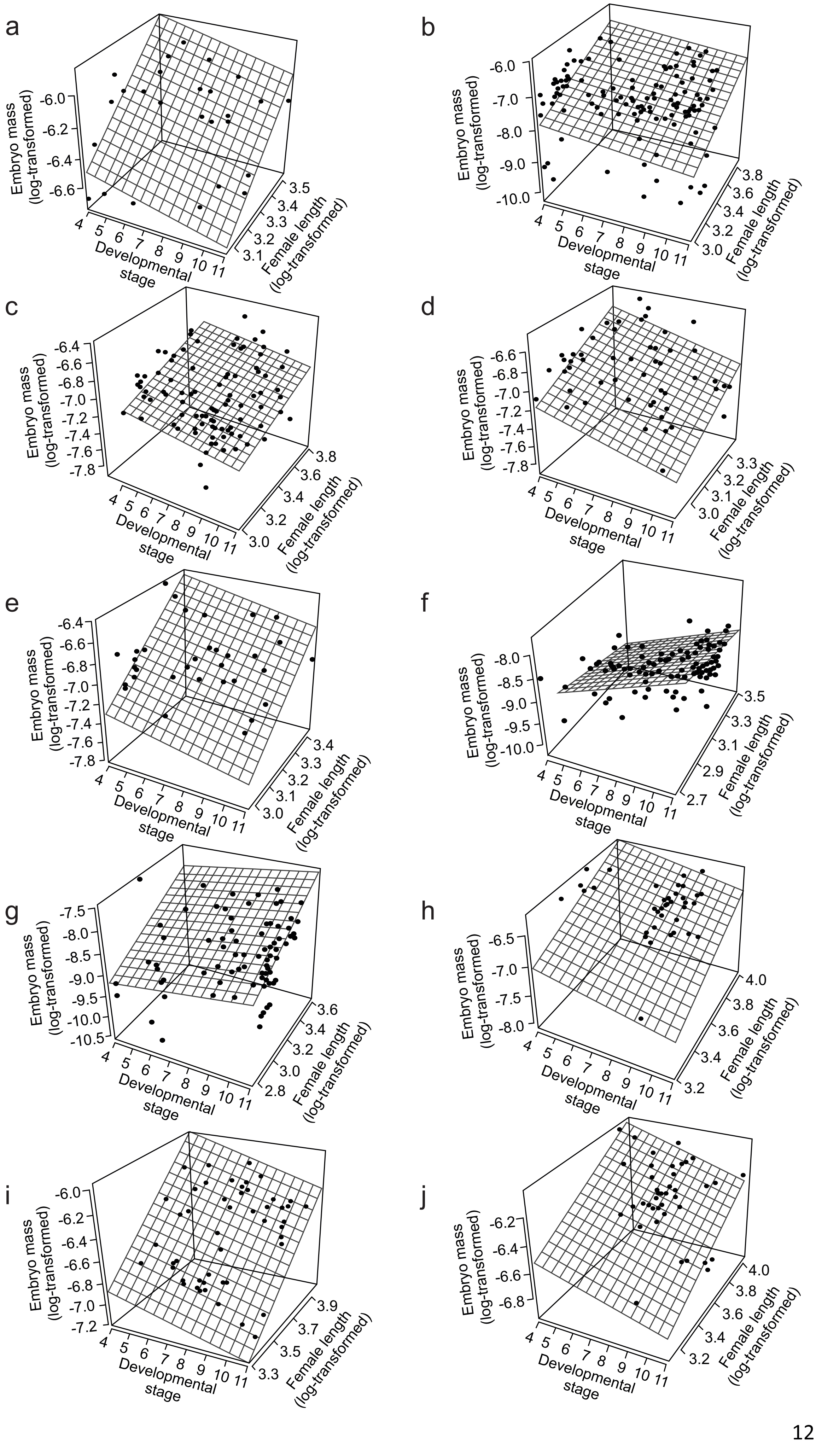
a
Embryo mass (log-transformed)
-6.0
-6.2
-6.4
-6.6
4
5
6
7
8
9
10
11
Developmental stage
3.1
3.2
3.3
3.4
3.5
Female length (log-transformed)
b
Embryo mass (log-transformed)
-6.0
-7.0
-8.0
-9.0
-10.0
4
5
6
7
8
9
10
11
Developmental stage
3.0
3.2
3.4
3.6
3.8
Female length (log-transformed)
c
Embryo mass (log-transformed)
-6.4
-6.6
-6.8
-7.0
-7.2
-7.4
-7.6
-7.8
4
5
6
7
8
9
10
11
Developmental stage
3.0
3.2
3.4
3.6
3.8
Female length (log-transformed)
d
Embryo mass (log-transformed)
-6.6
-6.8
-7.0
-7.2
-7.4
-7.6
-7.8
4
5
6
7
8
9
10
11
Developmental stage
3.0
3.1
3.2
3.3
Female length (log-transformed)
e
Embryo mass (log-transformed)
-6.4
-6.6
-6.8
-7.0
-7.2
-7.4
-7.6
-7.8
4
5
6
7
8
9
10
11
Developmental stage
3.0
3.1
3.2
3.3
3.4
Female length (log-transformed)
f
Embryo mass (log-transformed)
-8.0
-8.5
-9.0
-9.5
-10.0
4
5
6
7
8
9
10
11
Developmental stage
2.7
2.9
3.1
3.3
3.5
Female length (log-transformed)
g
Embryo mass (log-transformed)
-7.5
-8.0
-8.5
-9.0
-9.5
-10.0
-10.5
4
5
6
7
8
9
10
11
Developmental stage
2.8
3.0
3.2
3.4
3.6
Female length (log-transformed)
h
Embryo mass (log-transformed)
-6.5
-7.0
-7.5
-8.0
4
5
6
7
8
9
10
11
Developmental stage
3.2
3.4
3.6
3.8
4.0
Female length (log-transformed)
i
Embryo mass (log-transformed)
-6.0
-6.2
-6.4
-6.6
-6.8
-7.0
-7.2
4
5
6
7
8
9
10
11
Developmental stage
3.3
3.5
3.7
3.9
Female length (log-transformed)
j
Embryo mass (log-transformed)
-6.2
-6.4
-6.6
-6.8
4
5
6
7
8
9
10
11
Developmental stage
3.2
3.4
3.6
3.8
4.0
Female length (log-transformed)

Figure S2 Additive effects of female length and developmental stage on individual embryo mass. (a) Population 3 of *Gambusia vittata*, populations (b) 4 and (c) 11 of *Poeciliopsis gracilis*, populations (d) 2 and (e) 4 of *P. latidens*, populations (f) 5 and (g) 7 of *P. prolifica*, populations (h) 1, (i) 3, and (j) 4 of *P. turrubarensis*.

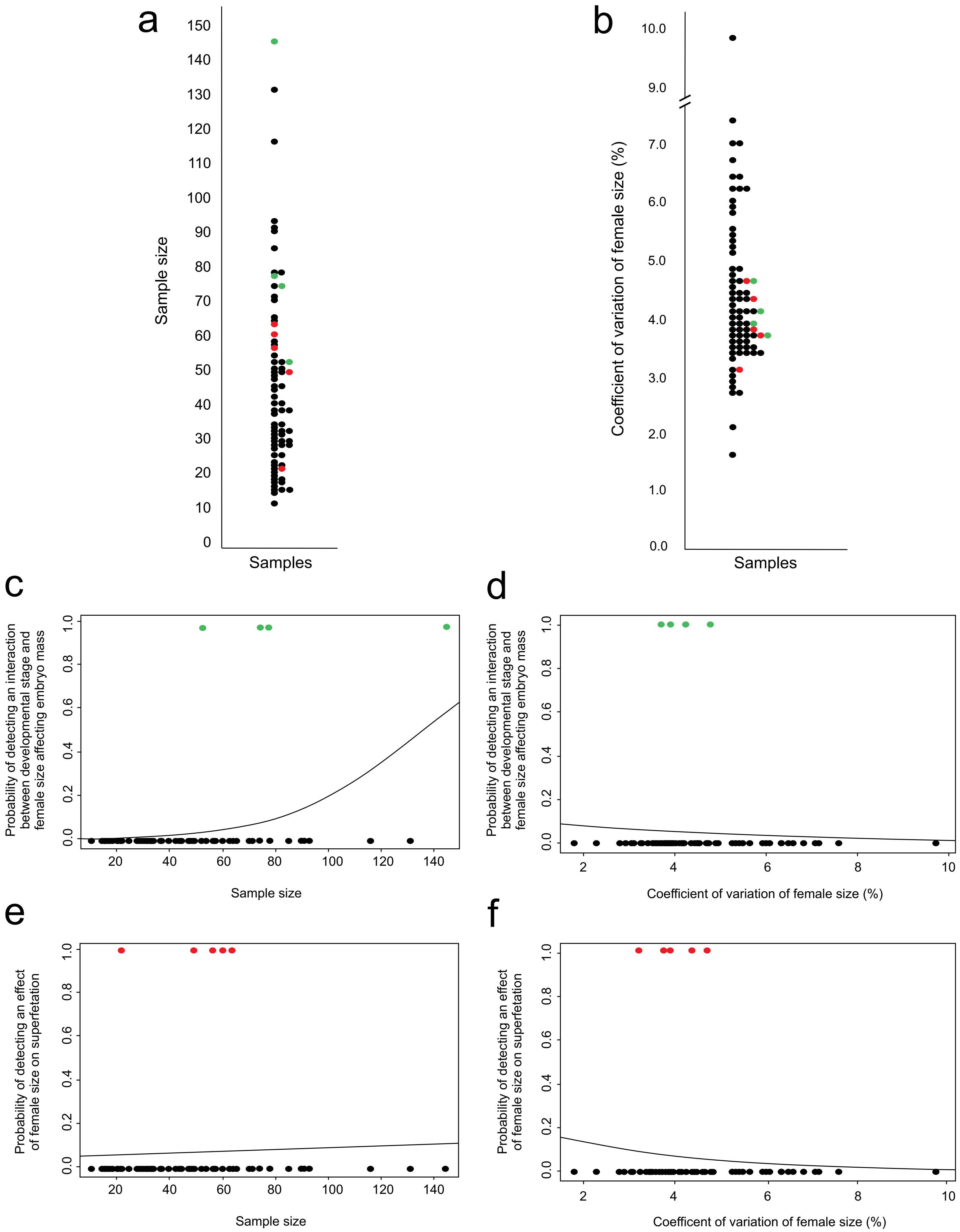
a
Sample size
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Samples
b
Coefficient of variation of female size (%)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
9.0
10.0
Samples
c
Probability of detecting an interaction between developmental stage and female size affecting embryo mass
0.0
0.2
0.4
0.6
0.8
1.0
20
40
60
80
100
120
140
Sample size
d
Probability of detecting an interaction between developmental stage and female size affecting embryo mass
0.0
0.2
0.4
0.6
0.8
1.0
2
4
6
8
10
Coefficient of variation of female size (%)
e
Probability of detecting an effect of female size on superfetation
0.0
0.2
0.4
0.6
0.8
1.0
20
40
60
80
100
120
140
Sample size
f
Probability of detecting an effect of female size on superfetation
0.0
0.2
0.4
0.6
0.8
1.0
2
4
6
8
10
Coefficent of variation of female size (%)

Figure S3 Examination of the potential effects of sample size and coefficient of variation of female size on the probability of detecting size-dependent changes in the degrees of matrotrophy and superfetation. Populations in which we found an interactive effect of developmental stage and female length on individual embryo mass are denoted by green circles, whereas populations in which we found an effect of female length on the number of simultaneous broods are denoted by red circles. (a) Sample size and (b) coefficient of variation of female size for our 77 study populations. Probability of detecting an interaction between developmental stage and female size affecting embryo mass as a function of (c) sample size and (d) coefficient of variation of female size. Probability of detecting an effect of female size on superfetation as a function of (e) sample size and (f) coefficient of variation of female size.

CAPÍTULO III

Examination of the Trexler and DeAngelis model of maternal provisioning reveals that matrotrophy is costly

Nabila Saleh-Subaie, G. Ángel Ramírez-Cruz and J. Jaime Zúñiga-Vega

Artículo enviado a Frontiers in Ecology and Evolution

2021

Examination of the Trexler-DeAngelis model of maternal provisioning reveals that matrotrophy is costly

Nabila Saleh-Subaie[1], Gonzalo A. Ramírez-Cruz[1], J. Jaime Zúñiga-Vega[1*]

[1]National Autonomous University of Mexico, Mexico

Submitted to Journal:
Frontiers in Ecology and Evolution

Specialty Section:
Behavioral and Evolutionary Ecology

Article type:
Original Research Article

Manuscript ID:
690430

Received on:
02 Apr 2021

Revised on:
29 Jul 2021

Journal website link:
www.frontiersin.org

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

The authors have contributed to the preparation, research, and writing of the manuscript.

JJZV conceived the idea for the study and designed the statistical analyses. NSS dissected museum specimens, compiled all the data, conducted analyses, prepared figures, and wrote the first draft of the manuscript. GARC provided ideas in all stages. All authors reviewed and edited the manuscript and approved its final version.

Keywords

Lecithotrophy, Matrotrophy, Maternal provisioning, Poeciliidae, Poeciliopsis, Reproductive modes, Viviparous fishes

Abstract

Word count: 282

The evolution of matrotrophy (post-fertilization maternal provisioning to developing embryos) has been explained through several hypotheses. Trexler and DeAngelis proposed in 2003 a theoretical model that defines the ecological conditions under which matrotrophy would be favored over lecithotrophy (pre-fertilization maternal provisioning). According to this model, matrotrophy offers a selective advantage in environments with abundant and constantly available food, whereas environments with limited and fluctuating food resources should instead promote a lecithotrophic mode of maternal provisioning. This model also proposes that matrotrophy entails the consequence of leaner reproductive females and in turn shorter lifespans. In this study, we examined the Trexler-DeAngelis model using data from 45 populations of five viviparous species from the fish genus Poeciliopsis (family Poeciliidae). We used the matrotrophy index as a measure of post-fertilization maternal provisioning, and the index of stomach fullness and individual body condition as proxies for food availability. We also estimated the magnitude of fluctuations in food availability by calculating the temporal variances of these two proxies. Neither abundant nor constantly available food were associated with greater degrees of matrotrophy, which fails to support the predictions of the Trexler-DeAngelis model with respect to the ecological drivers of increased post-fertilization provisioning to embryos. Nonetheless, in all five species we observed that females with greater degrees of matrotrophy had poorer body condition compared to females that provided less nutrients to embryos after fertilization. This finding is consistent with one of the expected consequences of advanced matrotrophy according to the Trexler-DeAngelis model, namely, a detriment to the nutritional status of females. Our study provides compelling evidence that gestating females experience a trade-off between post-fertilization provisioning to embryos and self-maintenance, revealing in turn that matrotrophy is a costly reproductive strategy.

Contribution to the field

The evolution of matrotrophy (post-fertilization allocation of resources to developing embryos) from lecithotrophy (pre-fertilization allocation of resources to mature eggs in the form of yolk) has received much attention in recent years. A theoretical model proposed by Trexler and DeAngelis defines the ecological conditions that would favor a matrotrophic strategy over a lecithotrophic one. Abundant and constantly available food should promote high degrees of matrotrophy, whereas limited and fluctuating food availability should promote less post-fertilization provisioning. This model also predicts two consequences of advanced matrotrophy: leaner gestating females and shorter life spans. Our study focuses on five fish species from the genus Poeciliopsis to test the predictions from the Trexler-DeAngelis model. We evaluated if food abundance and fluctuations in food availability could explain the observed variation among species, populations, and individuals in the degree of matrotrophy. Most previous evaluations of this model have focused on the predicted causes of increased matrotrophy and have not examined if the expected consequences of this reproductive strategy actually occur in natural populations. Our findings provide compelling evidence of one of the expected consequences of matrotrophy: in all five species, body condition of gestating females was negatively affected by advanced degrees of post-fertilization provisioning.

Funding statement

This study was funded by Consejo Nacional de Ciencia y Tecnología through a fellowship awarded to NSS (number 596700).

Ethics statements

Studies involving animal subjects

Generated Statement: Ethical review and approval was not required for the animal study because We used only museum specimens.

Studies involving human subjects

Generated Statement: No human studies are presented in this manuscript.

Inclusion of identifiable human data

Generated Statement: No potentially identifiable human images or data is presented in this study.

Data availability statement

Generated Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Submitted to Frontiers in Ecology and Evolution

Revised version submitted: July 29, 2021

Examination of the Trexler-DeAngelis model of maternal provisioning reveals that matrotrophy is costly

Nabila Saleh-Subaie[1], Gonzalo A. Ramírez-Cruz[2] and J. Jaime Zúñiga-Vega[2,*]

[1]Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, México.

[2]Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México.

*Correspondence: J. J. Zúñiga-Vega

Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, 04510, Ciudad de México, México.

email: jzuniga@ciencias.unam.mx

Number of words: 7438

Number of figures: 5

Number of tables: 5

Keywords: lecithotrophy, matrotrophy, Trexler-DeAngelis model, maternal provisioning, Poeciliidae, *Poeciliopsis*, reproductive modes, viviparous fishes.

ABSTRACT

The evolution of matrotrophy (post-fertilization maternal provisioning to developing embryos) has been explained through several hypotheses. Trexler and DeAngelis proposed in 2003 a theoretical model that defines the ecological conditions under which matrotrophy would be favored over lecithotrophy (pre-fertilization maternal provisioning). According to this model, matrotrophy offers a selective advantage in environments with abundant and constantly available food, whereas environments with limited and fluctuating food resources should instead promote a lecithotrophic mode of maternal provisioning. This model also proposes that matrotrophy entails the consequence of leaner reproductive females and in turn shorter lifespans. In this study, we examined the Trexler-DeAngelis model using data from 45 populations of five viviparous species from the fish genus *Poeciliopsis* (family Poeciliidae). We used the matrotrophy index as a measure of post-fertilization maternal provisioning, and the index of stomach fullness and individual body condition as proxies for food availability. We also estimated the magnitude of fluctuations in food availability by calculating the temporal variances of these two proxies. Neither abundant nor constantly available food were associated with greater degrees of matrotrophy, which fails to support the predictions of the Trexler-DeAngelis model with respect to the ecological drivers of increased post-fertilization provisioning to embryos. Nonetheless, in all five species we observed that females with greater degrees of matrotrophy had poorer body condition compared to females that provided less nutrients to embryos after fertilization. This finding is consistent with one of the expected consequences of advanced matrotrophy according to the Trexler-DeAngelis model, namely, a detriment to the nutritional status of females. Our study provides compelling evidence that gestating females experience a trade-off between post-fertilization provisioning to embryos and self-maintenance, revealing in turn that matrotrophy is a costly reproductive strategy.

INTRODUCTION

Viviparity is a reproductive strategy in which the embryo develops inside a specialized structure or cavity within the female after fertilization (Blackburn, 1999). This reproductive strategy can be found in mammals, sharks, anurans, salamanders, snakes, lizards, and fishes (Shine and Bull, 1979; Guillette and Jones, 1985; Greven and Guex, 1994; Castro, 2009; Renfree et al., 2013; Wake, 2015). In addition, viviparity also occurs in numerous invertebrate taxa, including several species from the following classes: Arachnida, Chromadorea, Digenea, Demospongiae, Insecta, and Stenolaemata, among others (Hagan, 1931; Meier et al., 1999; Ostrovsky et al., 2009, 2016). Females of viviparous species provide nutrients to their developing embryos by means of two different mechanisms. The first mechanism is lecithotrophy, in which the mother transfers all nutrients to her embryos before fertilization in the form of yolk and does not transfer additional resources during their development (Blackburn, 1992). The second mechanism is matrotrophy, which unlike lecithotrophy, involves the continuous provisioning of nutrients from the mother to the developing embryos after fertilization, usually through specialized structures (e.g., placentas; Wourms, 1981; Lombardi and Wourms, 1985; Blackburn, 1992; Marsh-Matthews, 2011; Kwan et al., 2015; Olivera-Tlahuel et al., 2019).

Theoretical models about the evolution of matrotrophy from a lecithotrophic strategy suggest that food availability is an ecological factor that could promote changes in the way that mothers provide nutrients to their developing offspring. Trexler and DeAngelis (2003, 2010) proposed a model that suggests that the amount of food available in the environment may favor the evolution of maternal adaptations such as matrotrophy. They suggested that one of the benefits of matrotrophy is that females may be able to produce a greater number of embryos than lecithotrophic females. The reason for this expected difference in fecundity is that lecithotrophic eggs are larger and more energetically costly than matrotrophic eggs because they contain all the necessary nutrients to complete embryo development. In contrast, matrotrophic eggs are notably smaller and imply a lower energy demand at the onset of reproduction, which should allow females to initiate gestation with a larger brood. However, this benefit of higher fecundity for matrotrophic females would only be possible if pregnant females have constant access to enough food to simultaneously meet their own energetic demands (i.e., self-maintenance) and to provide nutrients to their embryos all throughout development. Therefore, matrotrophy should be favored in environments with constant and high food availability. Lecithotrophy, on the other hand, should be favored in environments where resource availability is low or highly variable, because lecithotrophic females do not need a constant surplus of nutrients to support embryo development, but instead can store nutrients in the form of yolk during periods of relatively high food availability (Trexler and DeAngelis, 2003, 2010).

Viviparous fishes of the family Poeciliidae exhibit wide variation in the amount of nutrients that females can transfer to their developing embryos before and after fertilization. Some species are strictly lecithotrophic (e.g., *Gambusia hubbsi* and *G. alvarezi*), whereas other species exhibit extensive matrotrophy (e.g., *Poecilia parae* and *Poeciliopsis prolifica*), with several species showing varying degrees of the relative amounts of pre- and post-fertilization provisioning (Reznick et al., 2002; Pires et al., 2007, 2010; Pollux et al., 2009; Pollux and Reznick, 2011; Torres-Mejia, 2011; Olivera-Tlahuel et al., 2015). In addition, some studies have demonstrated that the degree of matrotrophy (i.e., the amount of post-fertilization maternal provisioning) may also vary among different populations of the same species (Reznick et al., 2007; Turcotte et al., 2008; Pires et al., 2010; Molina-Moctezuma et al., 2020). Due to the large variation in the way poeciliid females provide resources to their embryos, some studies have attempted to understand the potential benefits of matrotrophy over lecithotrophy (Trexler and DeAngelis, 2003, 2010; Pollux et al., 2009, 2014; Saleh-Subaie and Zúñiga-Vega, 2019).

Several studies have previously tested the Trexler-DeAngelis model under laboratory (Marsh-Matthews and Deaton, 2006; Pires et al., 2007; Banet and Reznick, 2008; Banet et al., 2010; Pollux and Reznick, 2011; Itonaga et al., 2012; Van Dyke et al., 2014; Molina-Moctezuma et al., 2020) and natural conditions (Schrader and Travis, 2008, 2012; Riesch et al., 2013; Molina-Moctezuma et al., 2020) obtaining mixed results. Interestingly, most of these studies have been conducted on poeciliid fishes and only three studies have evaluated this model with non-fish taxa, specifically using lizards as model systems (Cadby et al., 2011; Itonaga et al., 2012; Van Dyke et al., 2014). Some authors suggest that the Trexler-DeAngelis model is at least partially supported according to the relatively high levels of matrotrophy and the tendency to decrease the size of oocytes observed in sites with high food availability in both natural and experimental conditions (Pires et al., 2007; Schrader and Travis, 2008, 2012; Pollux and Reznick, 2011; Itonaga et al., 2012; Riesch et al., 2013; Van Dyke et al., 2014; Molina-Moctezuma et al., 2020). Nonetheless, other authors suggest that the model is not supported because they did not observe a significant decrease in oocyte size in matrotrophic females, nor did they find differences in the level of matrotrophy in experiments where the amount of food was constant or variable (Marsh-Matthews and Deaton, 2006; Banet et al., 2010; Pollux and Reznick, 2011; Riesch et al., 2013).

Since no conclusive results have been found, neither under experimental or natural conditions, it is important to study the generality of this phenomenon to determine if a higher degree of matrotrophy (i.e., greater amount of post-fertilization provisioning) indeed occurs with greater quantity and stability of food. Therefore, the main objective of this study is to evaluate the Trexler-DeAngelis model (Trexler and DeAngelis, 2003, 2010). In particular, we address the specific conditions that would lead to increased matrotrophy by comparing natural populations of five species of the genus *Poeciliopsis*. We predict that females from populations where the amount of food is relatively high and constant, will show higher levels of matrotrophy. In contrast, we expect less post-fertilization maternal provisioning in populations that inhabit sites where there is evidence that the amount of food is lower or fluctuating. We used two proxies for food availability: an index of stomach fullness (IF) and an estimate of body condition (BC). High IF and/or BC values indicate that females inhabit an environment with high food availability, whereas low IF and/or BC values indicate that females inhabit an environment with low food availability. In addition, we estimated the variance among months of both IF and BC to quantify the degree of temporal variability in food availability. In environments where food resources are constantly available (i.e., populations with small temporal variances in IF and/or BC), we expect to find higher degrees of matrotrophy, whereas in environments where food availability fluctuates (i.e., populations with large temporal variances in IF and/or BC), we expect to find females exhibiting less post-fertilization provisioning. Our study represents an empirical test of this important theoretical model that attempts to explain how different strategies for embryo nourishment have evolved in viviparous organisms.

MATERIALS AND METHODS

Study Species and Datasets

We focused on five species of the genus *Poeciliopsis* (family Poeciliidae) with relatively wide geographic distributions, which allowed us to examine interpopulation differences in food availability and how such differences affect the patterns of embryo nourishment. All five species exhibit superfetation, which is the ability of females to simultaneously bear two or more groups of embryos at different developmental stages (Scrimshaw, 1944), but differ in the degree of matrotrophy (i.e., lecithotrophy, matrotrophy, and incipient matrotrophy) (**Table 1**). The number of populations differed among species, from 6 to 11, for a total of

45 populations. The number of females per population varied from 14 to 141 (**Table1**). Geographic coordinates of all populations can be found in **Supplementary Table 1**. For *Poeciliopsis gracilis*, *P. infans*, and *P. turrubarensis* we combined data from published studies (Zúñiga-Vega et al., 2007; Frías-Alvarez et al., 2014; Frías-Alvarez and Zúñiga-Vega, 2016), and from our own dissections of pregnant females, which we obtained from the National Collection of Fishes (Instituto de Biología, Universidad Nacional Autónoma de México). For the remaining two species (*P. baenschi* and *P. prolifica*), all data was obtained by dissecting preserved females, also from the National Collection of Fishes (**Table 1**). All specimens were preserved in ethanol (70%) until they were dissected.

Laboratory Measurements

To test our hypothesis, we calculated the matrotrophy index (MI), and two proxies for food availability: the index of fullness (IF) and individual body condition (BC). To quantify these variables we measured the standard length (SL) of females before dissection with a digital caliper (± 0.1 mm), from the tip of the mouth to the base of the caudal fin at the level of the lateral line. We then dissected females to quantify brood size (number of embryos in the same developmental stage) and superfetation (number of simultaneous broods). We used the classification of embryonic stages proposed by Haynes (1995) to identify the developmental stage of each brood. Individual embryo mass was quantified by drying an entire brood for 48 h at 55 °C, weighing it with a precision scale (± 0.05 mg), and dividing brood dry mass by the number of embryos in the brood. Upon dissection, the entire digestive tract (stomach and intestines) was removed from each female. We quantified the somatic dry mass of each female (excluding digestive and reproductive tracts) as well as the dry mass of the digestive tract (including all its content) following the same drying and weighing protocol that we implemented for embryos.

Matrotrophy Index

The matrotrophy index (MI) is a standard measure of post-fertilization maternal provisioning, and is calculated as the dry mass of the offspring at birth divided by the dry mass of the egg at fertilization (Reznick et al., 2002; Marsh-Matthews, 2011). MI values < 0.75 indicate that females provide all nutrients before fertilization in the form of yolk. MI values between 0.75 and 1 indicate that females provide most nutrients before fertilization and small amounts of nutrients during embryo development (i.e., incipient matrotrophy). MI values > 1 indicate that a substantial amount of nutrients are provided after fertilization. MI values > 5 indicate extensive matrotrophy (Marsh-Matthews, 2011).

We calculated the MI in two different ways. First, we calculated an MI separately for each population by using data from all females to estimate the dry mass at birth and the dry mass of the egg at fertilization (Reznick et al., 2002; Marsh-Matthews, 2011) (**Supplementary Table 2**). For this purpose, we fitted a linear regression to our data on individual embryo mass (response variable, log-transformed) and developmental stage (predictor variable), separately for each population. From this regression, we used the predicted dry mass at stage 4 (recently fertilized eggs according to Haynes [1995]) as well as the predicted dry mass at stage 11 (last stage of development and, thus, an estimate of mass at birth) to calculate the MI. In this regression, each female was represented by a single data point (i.e., the average dry mass of individual embryos in a particular developmental stage). Because all species in this study exhibit superfetation, we randomly chose one brood from each female to quantify individual embryo mass (as per Frías-Alvarez et al., 2014; Gorini-Pacheco et al., 2018). In this way, we ensured that each female was represented only once in our linear regressions to avoid the problem of non-independence in the data.

Second, we calculated an MI per individual female (MI_{ind}) by selecting females that had simultaneous broods in developmental stages 4 (recently fertilized) and 11 (close to be born). We calculated an MI_{ind} for each of these females by dividing the average dry mass of her embryos at stage 11 by the average dry mass of her embryos at stage 4. In total, 51 females across all species had broods in stages 4 and 11 simultaneously. Our MI_{ind} values represent how embryo mass changes throughout development within individual females and, hence, are individual estimates of the amount of post-fertilization provisioning. In contrast, our MI values per population are an average representation of the degree of matrotrophy across all the females that inhabit a population.

Index of Fullness and Body Condition as Proxies for Food Availability

To estimate food availability, we used the index of stomach fullness (IF), which was calculated for each female as the dry mass of her digestive tract (including all its content) divided by her somatic dry mass, and multiplying the result by 100 (Hyslop, 1980). The IF is an indirect measure of the amount of available food and, more specifically, it is a measure of how much females ate at a certain time. It gives us a proxy for food availability at the time when we collected the females, and maybe for a few days before sampling. High IF values indicate that females were in an environment with high food availability, whereas low IF values indicate that females were in an environment with low food availability. Once we obtained the IF for each female, we calculated an average IF (IF_A) for each population (**Supplementary Table 2**). Given our hypothesis, we expected that in environments where food is abundant (i.e., populations with high IF_A), females will have higher degrees of matrotrophy, whereas in environments where food is less abundant (i.e., populations with low IF_A), we expected to find less matrotrophic females.

In addition, to quantify the magnitude of fluctuations in food availability, we calculated for each population the variance of the IF among months ($VarIF_{months}$) (**Supplementary Table 2**). Given that the amount of available food can vary over time, in those cases in which we had several collection dates (i.e., conducted in different months) from the same population (**Supplementary Table 3**), we calculated an average IF (IF_A) per month and then the variance among these monthly IF_A values. Populations with large values of $VarIF_{months}$ are those with pronounced temporal fluctuations (among months or between seasons) in food availability. According to the Trexler-DeAngelis model (Trexler and DeAngelis, 2003, 2010), we expected a negative relationship between MI and $\mathrm{VarIF}_{\mathrm{months}}$. That is, matrotrophy should be favored in populations where food availability is relatively constant, whereas lecithotrophy should be favored in environments with substantial variability in the access to food resources. Our data from populations with several collection dates revealed that, in some of these populations, both the matrotrophy index and the index of stomach fullness varied substantially among months (**Supplementary Figures 1, 2**).

In most of the populations that were sampled in different occasions, the collection dates occurred within a period of two years (**Supplementary Table 3**). Given that the lifespan of poeciliid fishes varies between two and four years (Coad 2017; Young et al., 2017), our multiple samples provide a reliable representation of the temporal fluctuations in food availability that a single generation of females experienced. Therefore, this is the appropriate temporal scheme to test the prediction that females facing changing conditions, in terms of food supply, over the course of their lifespan would increase their pre-fertilization investment (a less matrotrophic strategy).

We also calculated body condition (BC) as another proxy for food availability. We used the scaled mass index as an estimate of individual body condition (Peig and Green, 2009). This index is a more reliable indicator of the relative size of energy reserves compared to other indices of body condition (Peig and Green, 2010). We calculated the scaled mass index, which we denote here as BC because it is our measure of body condition, for each female as follows:

$$\mathrm{BC}_i = \mathrm{M}_i \left[\frac{\mathrm{SL}_0}{\mathrm{SL}_i}\right]^{b/r}$$

where M_i and SL_i are the somatic dry mass and standard length of female *i*, respectively, SL_0 is the average SL of the corresponding species, *b* is the slope from a linear regression of female dry mass on SL (both variables log-transformed), and *r* is the correlation coefficient between log-transformed female dry mass and SL. Given that female mass and SL differed substantially among species, we calculated the scaled mass index separately for each species, but pooling data from different populations. This index (BC_i) is an estimate of the dry mass that each female *i* would have at a fixed body length, which in our case is the average SL of each species (SL_0). Thus, smaller values of BC indicate poorer nutritional status and less energetic reserves, whereas larger values of BC indicate better nutritional status and greater energetic reserves (Peig and Green, 2009, 2010).

We then calculated an average of this scaled mass index separately for each population (BC_A) (**Supplementary Table 2**). BC is an estimate of food availability on a long time scale (weeks before capturing the females) because it reflects if females were able to gain somatic mass as a result of abundant food intake during the past weeks or, alternatively, if they lost mass as a consequence of food scarcity. According to our hypothesis, females will have good BC in environments with plenty of available food and, therefore, high degrees of matrotrophy. In contrast, poor BC indicates that females have experienced reduced food availability and, thus, they should exhibit lower degrees of matrotrophy.

Estimates of body condition may be affected by morphological differences among females. For instance, heavier females may also have deep bodies and extended abdomens whereas leaner females may have thinner and elongated bodies. Given that body shape may be affected by ecological factors other than food availability, such as predation risk and water velocity (Zúñiga-Vega et al., 2007; Langerhans, 2009; Langerhans and Makowicz, 2009), an association between morphology and body condition could imply that the observed differences among populations in average BC may have been driven by these other factors. This in turn would imply that our estimates of body condition do not reflect food availability but indicate instead interpopulation differences in predation intensity or water velocity. We discarded a confounding effect of body shape on our estimates of body condition by means of a morphological analysis based on geometric morphometric techniques (**Supplementary Material: Methods**). This analysis was conducted on four of our five study species because no digital photographs were available for one species (*P. turrubarensis*). The correlations between the scaled mass index and the first two axes of shape variation were rather weak in all four species (all correlation coefficients ranged between -0.20 and 0.31; **Supplementary Table 4**). Thus, in these *Poeciliopsis* species, the observed intraspecific variation in body condition cannot be explained by morphological differences among females.

Similar to what we did for the IF, for each population we quantified temporal fluctuations in food availability by means of the variance of BC among months ($VarBC_{months}$) (**Supplementary Table 2**). This temporal variance was calculated only for those populations that were sampled in different months (**Supplementary Table 3**). First, we calculated an average BC (BC_A) per month and then the variance among these monthly BC_A values. We expected a negative relationship between MI and $VarBC_{months}$. Consistent with the observed temporal variation in both the matrotrophy index and the index of fullness, body condition also varied substantially among months within some of the populations that were sampled in different occasions (**Supplementary Figure 3**).

Quantifications of stomach contents and body condition indices have been used previously as indicators of food availability in the surrounding environment. In particular, studies on other fish species have demonstrated that both the index of stomach fullness and individual body condition correlate positively with the abundance of important food sources, such as nutrient-rich prey (Simpkins and Hubert, 2000; Pothoven et al., 2001; Rikardsen et al., 2006). These previous observations give support to the use of IF and BC as reliable proxies for food availability.

Statistical Analyses

We implemented different sets of linear models using the R statistical software v. 3.4.2 (R Core Team, 2019). We used the Akaike information criterion adjusted for small sample sizes (AICc) (Akaike, 1973) to select the best model (i.e., the model with smallest AICc). However, models that differ by less than seven units of the AICc with respect to the best model (ΔAICc < 7) also have some support in the data (Burnham and Anderson, 2002; Burnham et al., 2011). To deal with model uncertainty, we calculated Akaike weights (w_i), which are measures of the relative support for each model *i* in the data (Burnham and Anderson, 2002). Then, we compared the support of two models relative to each other using evidence ratios, which are calculated as the quotient of their respective Akaike weights (w_1/w_2). These evidence ratios indicate how much greater the evidence is in favor of model 1 compared to that of model 2. We considered that strong evidence in support of the Trexler-DeAngelis model would come from two complementary lines of evidence. First, the best-fitting model must include one of our predictors of interest (IF, IF_A, $\text{VarIF}_\text{months}$, BC, BC_A, or $\text{VarBC}_\text{months}$). Second, this best-fitting model must have at least four times stronger support than models that do not include this predictor (i.e., an evidence ratio ≥ 4). In those cases where two or more supported models (models within ΔAICc < 7) had smaller evidence ratios (because such supported models had relatively large and similar Akaike weights), we chose the model that contained fewer parameters (the simplest model), since adding the additional parameters did not substantially improve model fit.

In addition, based on the Akaike weights, we calculated model-averaged regression coefficients, as instructed by Burnham and Anderson (2002), for those predictor variables with evident statistical effects. These model-averaged slopes account for model uncertainty and are more robust than those derived from any single model (Johnson and Omland, 2004). We used the R package "MuMIn" to implement all these procedures for model selection and multi-model inference (Bartoń, 2019).

For the first model set we used the MI per population (log-transformed) as response variable and IF_A as the main explanatory variable. We also considered the factor "species" to take into account differences among species in the degree of matrotrophy. We included additive and interactive effects of IF_A and species, as well as an intercept-only model, for a total of five competing models (**Table 2**). In this first model set, we had 45 data points, one for each of our study populations.

In our second model set we also used MI per population (log-transformed) as response variable, but in this case, we analyzed the effects of temporal fluctuations in the IF by using $\text{VarIF}_\text{months}$ as explanatory variable. We also considered differences among species, additive and interactive effects of $\text{VarIF}_\text{months}$ and species, and an intercept-only model, for a total of five competing models (**Table 2**). Given that not all our study populations were visited in different months (**Supplementary Table 3**), we had a reduced number of data points (28) for this model set. In fact, all populations of *P. baenschi* and *P. turrubarensis* lacked data from different months and, thus, these two species were excluded from this model set.

The third model set was implemented using the matrotrophy index per individual female (MI_{ind}, log-transformed) as response variable. The main explanatory variable was the IF calculated for each female. In addition, we considered differences among species, additive and interactive effects of IF and species, and an intercept-only model, for a total of five competing models (**Table 3**). In this case, we expected a positive relationship between MI_{ind} and the IF. Unlike the previous two model sets, which were conducted at the population level, this third model set was conducted at the individual level with 51 data points, one for each female with an estimate of MI_{ind}.

Additionally, we implemented all three model sets, but using body condition (BC) as proxy for food availability, instead of the IF. This means that the response variable was the same (MI per population in the first and second model sets, and MI_{ind} in the third model set), but we used BC_A as the main predictor of interest in the first model set (**Table 4**), $VarBC_{months}$ in the second (**Table 4**), and BC per individual female in the third (**Table 5**). We also implemented all other models that we described above (i.e., differences among species, additive and interactive effects, and an intercept-only model).

RESULTS

Matrotrophy and the Index of Fullness

In the first model set, using IF as proxy for food availability, we found no evident effect of IF_A on the MI per population (**Figure 1A**). The top model indicated that MI per population only differed among species (**Table 2**). Two other models were within $\Delta AICc < 7$ and both included the effect of IF_A in addition to differences among species (additive effect in the second model and interactive effect in the third model). However, these additional effects of IF_A did not improve model fit compared to the simpler model that only included differences among species (**Table 2**). Similarly, in the second model set, we found no evidence of an effect of $VarIF_{months}$ on the MI per population (**Figure 1B**). Also in this case, the top model indicated differences among species and adding the effect of $VarIF_{months}$ did not substantially improve model fit (**Table 2**). The highest degrees of matrotrophy occurred in four populations of *P. prolifica*, and the lowest (indicating a lecithotrophic mode of maternal provisioning) in one population of *P. gracilis* (**Figure 1**).

We obtained a similar result when we analyzed the potential effect of the individual IF on MI_{ind} in our third model set. The model with the best fit only included differences among species in the MI_{ind} (**Table 3**). The second model was within 7 AICc units from the best-fitting model and included an additive effect of IF and species. Hence, evidence of an effect of IF on MI_{ind} was weak because adding IF did not substantially improve model fit with respect to the simpler model that only included differences among species (**Table 3**). Most females of *P. prolifica* had higher degrees of matrotrophy compared to females from all other species, whereas three females of *P. gracilis* had the lowest values of the MI_{ind} (**Figure 2**).

Matrotrophy and Body Condition

In our first model set, using BC as proxy for food availability, BC_A did not affect the MI per population (**Figure 3**). Again in this case, the best-fitting model indicated differences among species in the MI (**Table 4**). The model that ranked second was within ΔAICc < 7 and included the additive effect of species and BC_A. However, adding BC_A did not improve model fit compared to the model that only included differences among species. Similarly, in our second model set, the top model indicated that MI only differs among species (**Table 4**). Two other models had support (i.e., ΔAICc < 7) and both included the effect of $VarBC_{months}$ (interacting with species in the second model and as an additive effect in the third model). Hence, adding $VarBC_{months}$ did not substantially improve model fit, indicating a weak effect of this predictor on the MI per population (**Figure 4**). In *P. prolifica*, MI apparently decreased as $VarBC_{months}$ increased (**Figure 4C**). However, this negative association was caused by a single population with large temporal variance and quite small MI. For this reason, the model including the interaction between $VarBC_{months}$ and species did not outperform the simpler model that only included differences among species (**Table 4**).

Finally, in the third model set in which we used BC as proxy for food availability, the model that included an interactive effect of individual BC and species on MI_{ind} ranked first (**Table 5**). The model that included an additive effect of these two predictors ranked second. The top model had 2.6 times stronger support than the second model (evidence ratio: 0.63/0.24 = 2.63). The third model only included differences among species with no effect of BC. However, the top model had almost five times stronger support than this third model (evidence ratio: 0.63/0.13 = 4.85) (**Table 5**), which in this case represents compelling evidence of a combined effect of both BC and species on MI_{ind}. Contrary to our prediction, model-averaged regression coefficients revealed a negative relationship between MI_{ind} and individual BC for females of all five species (**Figure 5**). This negative relationship was weaker (i.e., a less steep negative slope) for females of *P. prolifica* (**Figure 5D**).

DISCUSSION

Matrotrophy Negatively Affects Body Condition and Is Apparently Not Influenced by Food Availability

In this study, we examined the Trexler-DeAngelis model of maternal provisioning to developing embryos (Trexler and DeAngelis, 2003, 2010). According to this model, higher levels of matrotrophy are only possible if food is relatively abundant and constantly available. To test this prediction, we used the index of stomach fullness and body condition as proxies for the amount of available food in the environment. Based on these two variables, we also quantified the degree of temporal variability in food availability. We found that females with higher levels of matrotrophy had poorer body condition compared to females that provided less nutrients to embryos after fertilization. This negative association was evident in females of all five species and is opposite to our prediction of greater matrotrophy in females that exhibit better body condition, presumably as a result of constant access to abundant food resources. In contrast, our main finding implies that providing relatively large amounts of nutrients to embryos after fertilization has a negative consequence on the nutritional status of the females. Possibly, females experience a trade-off between active provisioning of embryos during gestation and allocation of energy and nutrients to somatic tissues, suggesting that matrotrophy is a costly physiological process. Severe demands for energy and nutrients are well-known consequences of large investments in reproduction (Koskela et al., 1998; Jonsson et al., 2002; Velando and Alonso-Alvarez, 2003).

According to the Trexler-DeAngelis model, one of the main benefits of matrotrophy is a potentially higher fecundity, because lecithotrophy implies that eggs must be full of yolk before fertilization and thus represent a large energetic investment since the onset of gestation. In contrast, matrotrophic eggs are considerably smaller and less energetically costly, which could allow females to initiate gestation with a higher number of these smaller eggs. If resources are abundant enough to provide embryos with the necessary nutrients all throughout gestation, then matrotrophy would be favored over lecithotrophy (Trexler and DeAngelis, 2003, 2010). Here, we have shown that neither the amount of food that is present in the digestive tract of females nor their nutritional status reflected in their body condition are positively associated with the degree of matrotrophy, which in turn suggests that high food availability is not the main driver of advanced degrees of matrotrophy. In contrast, we detected in all five species that females that provided more nutrients to embryos after fertilization were in poorer body condition compared to females that relied more on pre-fertilization provisioning. This observed pattern represents compelling evidence that, if advanced matrotrophy allows females to gestate more embryos, such increased fecundity comes at the cost of a reduced investment in self-maintenance.

Our work with 45 populations of five species of poeciliid fishes did not support the expectation of increased matrotrophy in sites with abundant food. Furthermore, sites where food availability fluctuates over time (i.e., sites with large temporal variances in the index of stomach fullness and in body condition) did not promote reduced levels of matrotrophy. According to the Trexler-DeAngelis model, if food-limiting conditions are likely to arise, then females must rely more on pre-fertilization provisioning because they can store as much surplus energy as possible in the form of yolk whenever food becomes available (during periods when food intake exceeds metabolic demands) (Trexler and DeAngelis, 2003, 2010). Our data did not support this prediction either. Interestingly, however, the original model specifies that the total cost per brood may be greater for matrotrophic females compared to lecithotrophic females, because the former initially invest less per egg (because eggs are smaller) which allows them to initiate gestation with larger broods. If the size of offspring at birth is the same between matrotrophic and lecithotrophic females, then producing a larger number of similar-sized young will result in an overall higher reproductive cost for females with advanced matrotrophy. As a consequence, Trexler and DeAngelis (2003, 2010) predicted leaner bodies and shorter lifespans in females that allocate more resources to developing embryos after fertilization. Our findings revealed poor body condition in females with advanced matrotrophy and are thus consistent with this particular prediction of the model that is related to the consequences of matrotrophy. In conclusion, we failed to provide evidence in support of the expected ecological causes of this reproductive mode (high and constant food availability), but we have demonstrated a critical consequence of advanced matrotrophy that appears to be pervasive because it occurred in five different species.

The Costs of Matrotrophy Decrease as Yolk Dependency Decreases

Our findings, along with previous evidence, suggest that the severity of the physiological costs imposed by greater post-fertilization provisioning depends on the degree to which females rely on yolk reserves for embryo nutrition. A previous study in the congeneric *P. retropinna* found that the amount of post-fertilization provisioning was positively associated with maternal body fat and unrelated to maternal lean mass (Hagmayer et al., 2018). This finding is somehow contrary to what we observed in this study, because our estimates of body condition included both lipids and lean tissue (i.e., we did not extract lipids from the females). Thus, our observed reduction in body condition associated with higher degrees of matrotrophy likely entailed reductions in both body fat and lean mass. The reasons why in *P. retropinna* a higher investment in embryo development after fertilization does not entail a reduction in maternal body fat or in lean mass, whereas in the five congeneric species that we studied advanced matrotrophy clearly resulted in poorer body condition, remain unknown. A tentative explanation may involve the remarkably high degree of matrotrophy of *P. retropinna*, in which embryos increase in mass more than 100-fold during development (MI = 117; Reznick et al., 2002). Such an extensive degree of matrotrophy could substantially reduce the costs derived from reproduction because mature ova are notably small with no nutrients before fertilization and, hence, overall investment in reproduction is low during a significant proportion of pregnancy. All our study species exhibit considerably lower degrees of matrotrophy, from an MI = 0.72 in *P. gracilis* (which in fact indicates strict lecithotrophy) to an MI = 5.40 in *P. prolifica* (**Table 1**). Therefore, energetic demands derived from a higher investment in embryos since the onset of gestation (in our study species mature ova already contain some nutrient-rich yolk before fertilization) are likely higher in our five study species than in *P. retropinna*.

The weaker negative relationship between degree of matrotrophy and body condition that we observed in *P. prolifica* confirms a lower physiological cost of actively providing embryos with nutrients during gestation for species that depend less on yolk reserves. Unlike *P. prolifica*, which exhibits a substantial amount of post-fertilization provisioning (MI = 5.40) and, hence, smaller amounts of yolk in mature ova, the other four species that we studied have a greater dependency on the yolk that is deposited into the eggs before fertilization for embryo nourishment (their MI values range between 0.72 and 1.58; **Table 1**) (Reznick et al., 2002; Olivera-Tlahuel et al., 2015). Females of these four species lack specialized placentas that facilitate the transfer of nutrients from the mother to developing embryos and, instead, they have relatively simple maternal follicles that have the primary functions of gas exchange and osmoregulation (Kwan et al., 2015; Olivera-Tlahuel et al., 2019). Certainly, the observed variation in the degree of matrotrophy among individual females (**Figure 2**) as well as among populations (**Figure 1**) of these four species indicates that females are able to increase the amount of nutrients that are actively transferred to developing embryos after fertilization. Apparently, doing so without the specialized anatomical structures that are present in placental species such as *P. prolifica* and *P. turneri* (i.e., abundant microvilli and capillaries, thicker maternal follicles, numerous enlarged vesicles; Kwan et al., 2015; Olivera-Tlahuel et al., 2019) entails a greater energetic expenditure for gestating females of non-placental and yolk-dependent species that causes a detrimental effect on their somatic tissues and lipid reserves.

Caveats and Limitations

Even though previous studies have demonstrated that the index of stomach fullness and estimates of body condition are accurate indicators of the amount and quality of food that is available in the surrounding environment (Simpkins and Hubert, 2000; Pothoven et al., 2001; Rikardsen et al., 2006), we recognize the potential limitations of using these proxies for food availability. These limitations may explain, at least partially, the lack of association between food availability and degree of matrotrophy that we observed in all five species. First, stomachs of females could be quite full, but most of their content may be of low nutritional value. For instance, females may have consumed abundant sediment or shells of mollusks that provide little to no energy (Langerhans et al., 2021). If this were the case, stomach fullness may not be correlated with the actual availability of nutritious food items.

Second, food availability, as well as stomach fullness and body condition, may vary substantially over relatively short timescales (e.g., between weeks) and, hence, our collections of females may have occurred during an uncommon episode of low or high food abundance that does not reflect the general pattern of food availability in our study sites. Therefore, the lack of association between food availability and matrotrophy that we observed in all our focal species may have been caused by a mismatch between food availability at the time when our collections took place (e.g., low food availability during the past couple of days) and the observed degree of matrotrophy that was likely driven by the general long-term pattern of food availability of each population (e.g., high food availability during most of the past months).

Third, both stomach fullness and body condition may be affected by factors other than food availability, such as temperature (which has a direct influence on digestive efficiency in ectotherms; Harlow et al., 1976), prevalence of pathogens (sick females may have poor body condition even if food is abundant in the surrounding environment; Ageze and Menzir, 2018), and rates of activity (e.g., fish may maintain low foraging rates in the presence of abundant predators; Botham et al., 2006). Hence, females from populations where food is abundant may have less full stomachs or poor body condition if these ecological conditions are suboptimal (low temperatures or abundant pathogens and predators). In these cases, neither stomach fullness nor body condition would be reliable indicators of food availability. Therefore, the lack of associations between degree of matrotrophy and our proxies for food availability must be interpreted with caution, since future examination of additional measures of food availability, such as in situ abundances of algae and aquatic invertebrates (Quintans et al., 2009; Carbajal-Becerra et al., 2020), may provide supporting evidence for this prediction of the Trexler-DeAngelis model.

Finally, we must notice that our study is based on two important assumptions. (1) Populations differ in the average amount of food availability, which means that over the course of a relatively long period, a year for example, food availability is overall higher in a particular population whereas it is constantly lower in another. This assumption implies that our two proxies for food availability should reflect this same situation, namely, in the former population females must have fuller stomachs and better body condition, whereas in the latter, females must have less full stomachs and poorer body condition, regardless of the particular time of collection. If this assumption is true, then females experience the same regime of food availability during most of their reproductive lives (i.e., during most of their pregnancies), which in turn must promote the evolution of either reduced or advanced matrotrophy. (2) Fluctuations in food availability occur on relatively long timescales, such as among months or seasons. This means that a female inhabiting a population where food supply fluctuates over time, experiences some months of food restriction and other months of abundant food. Given that gestation lasts approximately 30 days in poeciliid fishes (Veggetti et al., 1993; Bizzaza and Marin, 1995), some broods are produced under limiting conditions and others under abundant food, which in the long term must promote the evolution of a predominantly lecithotrophic strategy. If these two assumptions are incorrect and food availability fluctuates drastically on shorter timescales (among weeks or days), then our statistical approach to estimate the degree of post-fertilization provisioning (a linear regression that predicts how embryo mass changes from fertilization to birth) may be invalid because the mass of embryos at different developmental stages, which depends to a large extent on the amount of food that females consume, would also change drastically within months or even weeks (during a single pregnancy). This, in turn, would promote short-term temporal changes in the overall degree of matrotrophy (i.e., in the matrotrophy index). Thus, in summary, the temporal scale at which food availability fluctuates may be a critical aspect that could determine whether evidence in support of the Trexler-DeAngelis model is found or not.

Future Directions

The studies that have tested the Trexler-DeAngelis model of maternal provisioning have yielded mixed results, which may be attributed to different methodologies. Some studies were based on laboratory experiments (Marsh-Matthews and Deaton, 2006; Pires et al., 2007; Banet and Reznick, 2008; Banet et al., 2010; Pollux and Reznick, 2011; Itonaga et al., 2012; Van Dyke et al., 2014; Molina-Moctezuma et al., 2020), whereas others were conducted under natural conditions (Schrader and Travis, 2008, 2012; Riesch et al., 2013; Molina-Moctezuma et al., 2020). In controlled experiments, the amount of food and fluctuations in its availability are the only variables at play. Such experimental studies have provided evidence that abundant and constantly available food are positively associated with the amount of post-fertilization maternal provisioning, which supports the Trexler-DeAngelis model (Van Dyke et al., 2014; Molina-Moctezuma et al., 2020). In contrast, when data are obtained under natural conditions, several ecological factors interact and may mask the potential effects of food availability. Some of these studies have not found evidence in support of the Trexler-DeAngelis model (Schrader and Travis, 2008, Riesch et al., 2013). In addition to high and constant food availability, other ecological conditions may select for increased matrotrophy, such as a high predation risk. Given that matrotrophic embryos are substantially smaller during a large proportion of pregnancy, the ovarian mass and volume remain relatively small in gestating females, which provides them with an advantage in terms of swimming performance and escape velocity. Consistent with this hypothesis, in two poeciliid species, *P. retropinna* and *Phalloceros harpagos*, females from high-predation environments exhibit greater degrees of matrotrophy compared to females from low-predation environments (Gorini-Pacheco et al., 2018; Hagmayer et al., 2020). The combined effects of predation intensity and food availability on the relative amounts of pre- and post-fertilization provisioning to developing embryos have not been investigated yet and deserve further examination.

The Trexler-DeAngelis model was originally proposed using poeciliid fishes as model systems (Trexler and DeAngelis, 2003, 2010). To date, most empirical tests of this model have been conducted also on species of this group of viviparous fishes ((Pires et al., 2007; Schrader and Travis, 2008, 2012; Pollux and Reznick, 2011). The few notable exceptions are three studies conducted on lizards (Cadby et al., 2011; Itonaga et al., 2012; Van Dyke et al., 2014). This means that we are still far from understanding if the causes and consequences of advanced degrees of matrotrophy are the same across diverse phylogenetic groups. The search for generalizations about the influence of food availability on post-fertilization maternal provisioning requires additional studies in other viviparous taxa such as some cartilaginous fishes and numerous invertebrates, which also exhibit wide variation in the relative amounts of pre- and post-fertilization maternal investment (Hamlett et al., 2005; Ostrovsky et al., 2016; Carter and Soma, 2020). These other taxa would be excellent model systems to test the predictions of the Trexler-DeAngelis model.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

The authors have contributed to the preparation, research, and writing of the manuscript.

JJZV conceived the idea for the study and designed the statistical analyses. NSS dissected museum specimens, compiled the data, conducted analyses, prepared figures, and wrote the first draft of the manuscript. GARC compiled part of the data and provided ideas in all stages. All authors reviewed and edited the manuscript and approved its final version.

FUNDING

This study was funded by Consejo Nacional de Ciencia y Tecnología through a fellowship awarded to NSS (number 596700).

ACKNOWLEDGMENTS

Israel Solano-Zavaleta, Pedro Mendoza-Hernández and Mariana Hernández-Apolinar provided technical assistance. Nabila Saleh-Subaie is a doctoral student from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and received fellowship 596700 from CONACYT. NSS thanks her brothers for all their moral support, so she does it by saying: thank you "Oso-Republic".

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TABLE 1 | List of the five study species from the fish genus *Poeciliopsis* with information on the type of maternal provisioning to developing embryos (matrotrophy, incipient matrotrophy, or lecithotrophy), matrotrophy index (MI), number of populations per species, and sample size (*n* = number of reproductive females) per population.

Species	**Type of maternal provisioning**	**MI**	**Number of populations**	***n* per population**	**References**
Poeciliopsis baenschi	Matrotrophy	1.58	6	48, 38, 32, 29, 45, 34	Pollux et al., 2014; Olivera-Tlahuel et al., 2015; Zúñiga-Vega et al., 2017; this study
Poeciliopsis gracilis	Lecithotrophy	0.72	11	15, 58, 78, 116, 52, 64, 74, 23, 90, 85, 91	Bassar et al., 2014; Pollux et al., 2014; Frías-Alvarez and Zúñiga-Vega, 2016; this study
Poeciliopsis infans	Incipient matrotrophy	0.86	11	50, 74, 131, 55, 59, 56, 38, 52, 50, 65, 63	Frías-Alvarez et al., 2014; Pollux et al. 2014; Frías-Álvarez and Zúñiga-Vega, 2016; this study
Poeciliopsis prolifica	Matrotrophy	5.40	8	19, 32, 145, 18, 93, 49, 78, 44	Pires et al., 2007; Banet and Reznick, 2008; Pollux et al., 2014; this study
Poeciliopsis turrubarensis	Incipient matrotrophy	0.95	9	37, 71, 49, 40, 38, 77, 21, 40, 14	Zúñiga-Vega et al., 2007; Bassar et al., 2014; Pollux et al., 2014.

TABLE 2 | Values of the Akaike information criterion adjusted for small sample sizes (AICc), differences in AICc with respect to the best-fitting model (ΔAICc), and Akaike weights (*w*) for competing models that examined variation in the matrotrophy index (MI) per population. In the first model set, we examined the effects of the average index of fullness (IF_A) while accounting for differences among species. In the second model set, we examined the effect of the variance of the index of fullness among months ($VarIF_{months}$) also accounting for differences among species. The best-fitting models are indicated by ΔAICc = 0. Models are listed according to their fit to the data, from best to worst.

	Models	**AICc**	**ΔAICc**	***w***
First model set	Species	47.52	0	0.70
	Species + IF_A	50.27	2.75	0.18
	Species × IF_A	51.00	3.48	0.12
	IF_A	65.48	17.96	0
	Intercept only	67.47	19.95	0
Second model set	Species	22.39	0	0.75
	Species + $VarIF_{months}$	24.70	2.31	0.24
	Species × $VarIF_{months}$	30.33	7.94	0.01
	Intercept only	52.31	29.92	0
	$VarIF_{months}$	54.38	31.99	0

TABLE 3 | Values of the Akaike information criterion adjusted for small sample sizes (AICc), differences in AICc with respect to the best-fitting model (ΔAICc), and Akaike weights (*w*) for competing models that examined variation in the matrotrophy index per individual female ($\mathrm{MI}_{\mathrm{ind}}$). In this third model set, we examined the effect of the index of fullness per individual (IF) while accounting for differences among species. The best-fitting model is indicated by ΔAICc = 0. Models are listed according to their fit to the data, from best to worst.

Models	**AICc**	**ΔAICc**	***w***
Species	50.02	0	0.65
IF + Species	51.28	1.26	0.35
IF × Species	60.63	10.61	0.003
Intercept only	85.18	35.16	0
IF	85.18	35.16	0

TABLE 4 | Values of the Akaike information criterion adjusted for small sample sizes (AICc), differences in AICc with respect to the best-fitting model (ΔAICc), and Akaike weights (*w*) for competing models that examined variation in the matrotrophy index (MI) per population. In the first model set, we examined the effects of the average body condition (BC_A) while accounting for differences among species. In the second model set, we examined the effect of the variance of body condition among months ($VarBC_{months}$) also accounting for differences among species. The best-fitting models are indicated by ΔAICc = 0. Models are listed according to their fit to the data, from best to worst.

	Models	**AICc**	**ΔAICc**	***w***
First model set	Species	47.52	0	0.80
	Species + BC_A	50.34	2.82	0.20
	Species × BC_A	57.62	10.10	0.01
	BC_A	65.59	18.07	0
	Intercept only	67.47	19.95	0
Second model set	Species	22.39	0	0.46
	Species × $VarBC_{months}$	22.82	0.43	0.38
	Species + $VarBC_{months}$	24.50	2.11	0.16
	$VarBC_{months}$	46.53	24.14	0
	Intercept only	52.31	29.92	0

TABLE 5 | Values of the Akaike information criterion adjusted for small sample sizes (AICc), differences in AICc with respect to the best-fitting model (ΔAICc), and Akaike weights (*w*) for competing models that examined variation in the matrotrophy index per individual female (MI_{ind}). In this third model set, we examined the effect of individual body condition (BC) while accounting for differences among species. The best-fitting model is indicated by ΔAICc = 0. Models are listed according to their fit to the data, from best to worst.

Models	**AICc**	**ΔAICc**	***w***
BC × Species	46.90	0	0.63
BC + Species	48.78	1.88	0.24
Species	50.02	3.12	0.13
Intercept only	85.18	38.28	0
BC	86.57	39.67	0

FIGURE 1 | (A) Lack of association between the matrotrophy index per population (MI) and average index of fullness (IF_A) for 45 populations of five fish species from the genus *Poeciliopsis*. **(B)** Lack of association between the matrotrophy index per population (MI) and the variance of the index of fullness among months ($VarIF_{months}$) for 28 populations of three fish species from the genus *Poeciliopsis*.

FIGURE 2 | Lack of association between the matrotrophy index per individual (MI_{ind}) and the individual index of fullness (IF) for 51 females of five fish species from the genus *Poeciliopsis*.

FIGURE 3 | Lack of association between the matrotrophy index per population (MI) and average body condition (BC_A) for 45 populations of five fish species from the genus *Poeciliopsis*. Each species is shown in a different panel because of substantial differences among species in the values of body condition.

FIGURE 4 | Lack of association between the matrotrophy index per population (MI) and the variance of body condition among months ($VarBC_{months}$) for 28 populations of three fish species from the genus *Poeciliopsis*. Each species is shown in a different panel because of substantial differences among species in the values of body condition.

FIGURE 5 | Negative association between the matrotrophy index per individual (MI_{ind}) and individual body condition (BC) for 51 females of five fish species from the genus *Poeciliopsis*. Each species is shown in a different panel because of substantial differences among species in the values of body condition. Fitted lines represent model-averaged regression coefficients.

Figure 1.JPEG

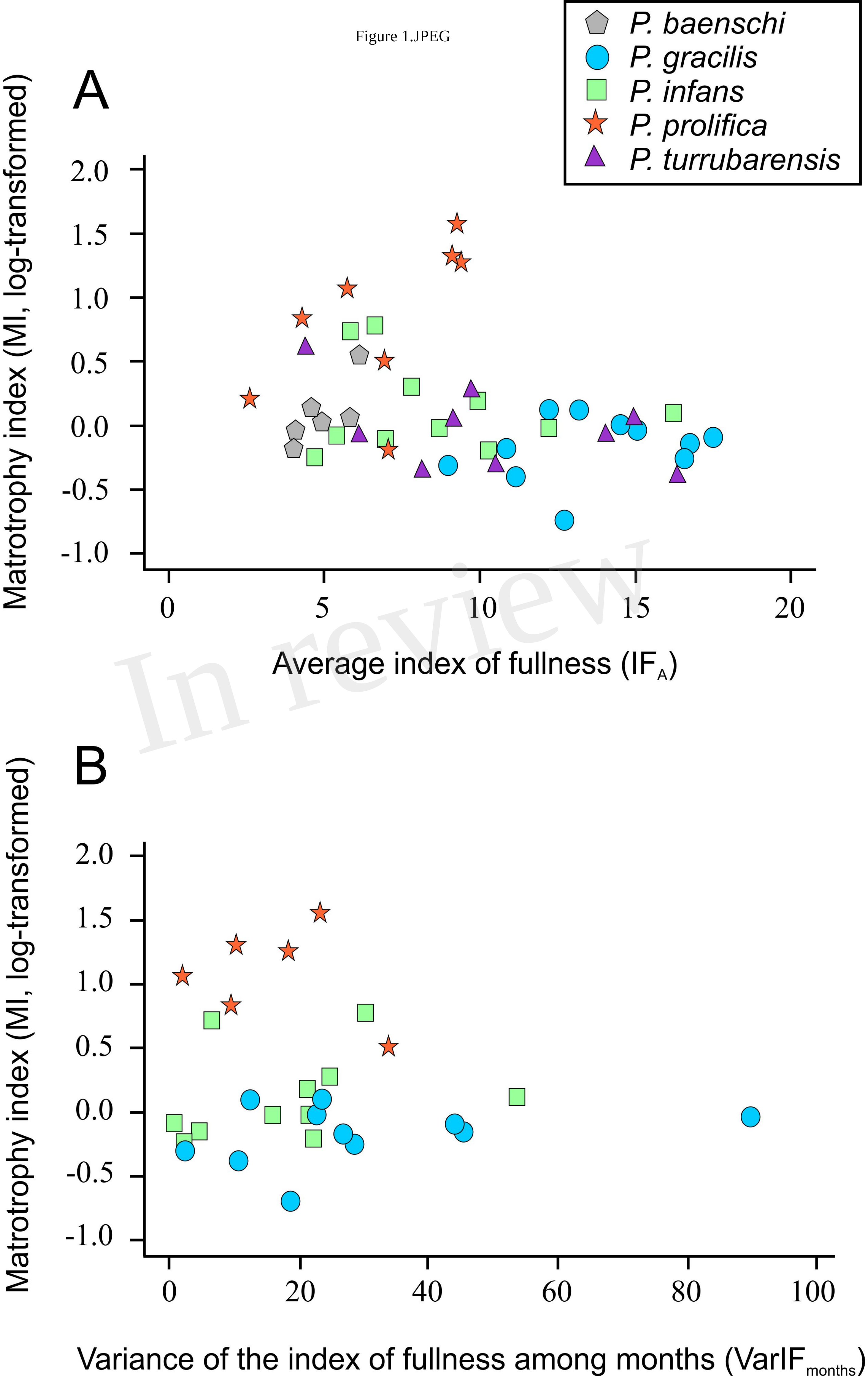


Figure 2.JPEG

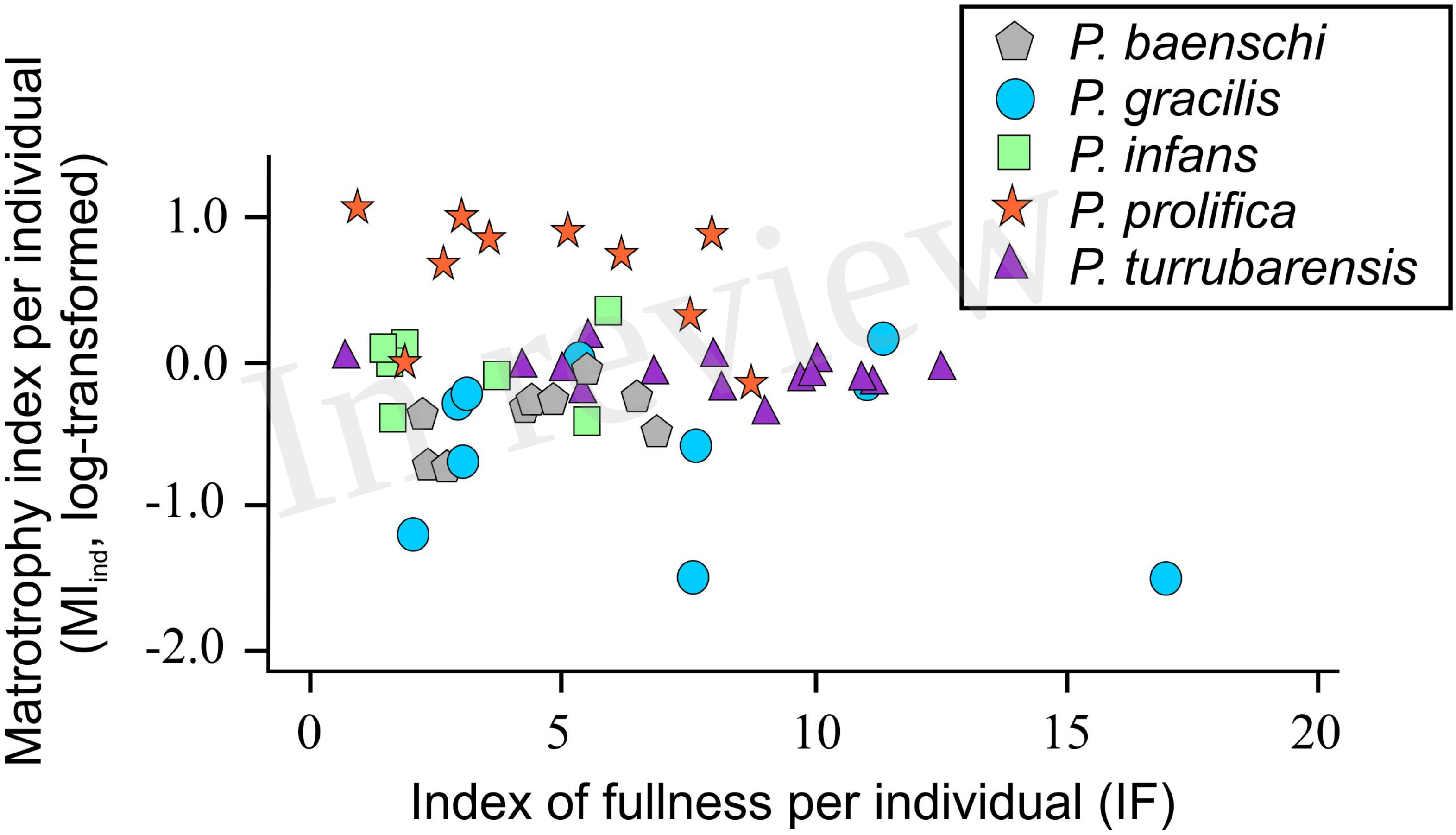


Figure 3.JPEG

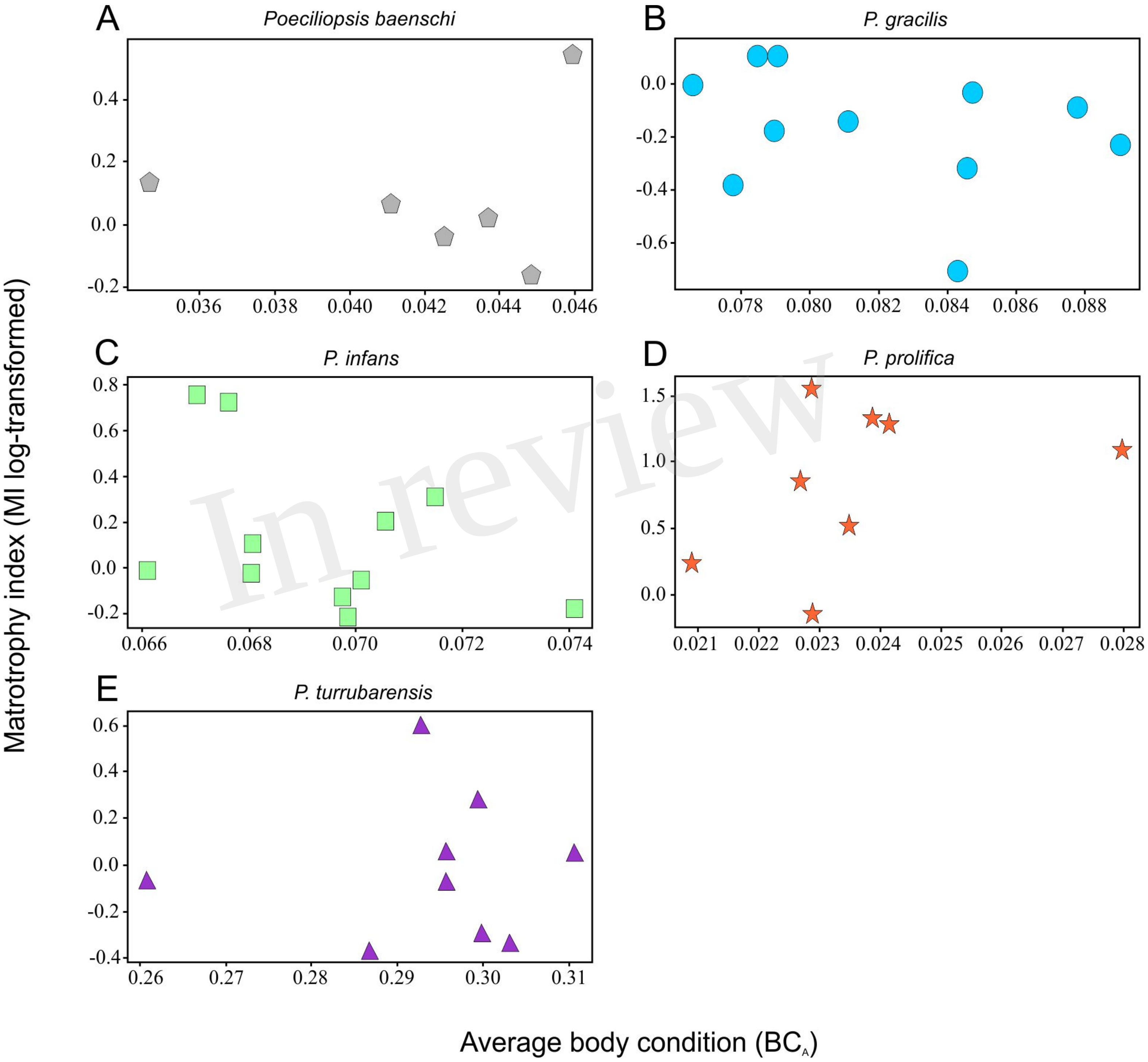


Figure 4.JPEG

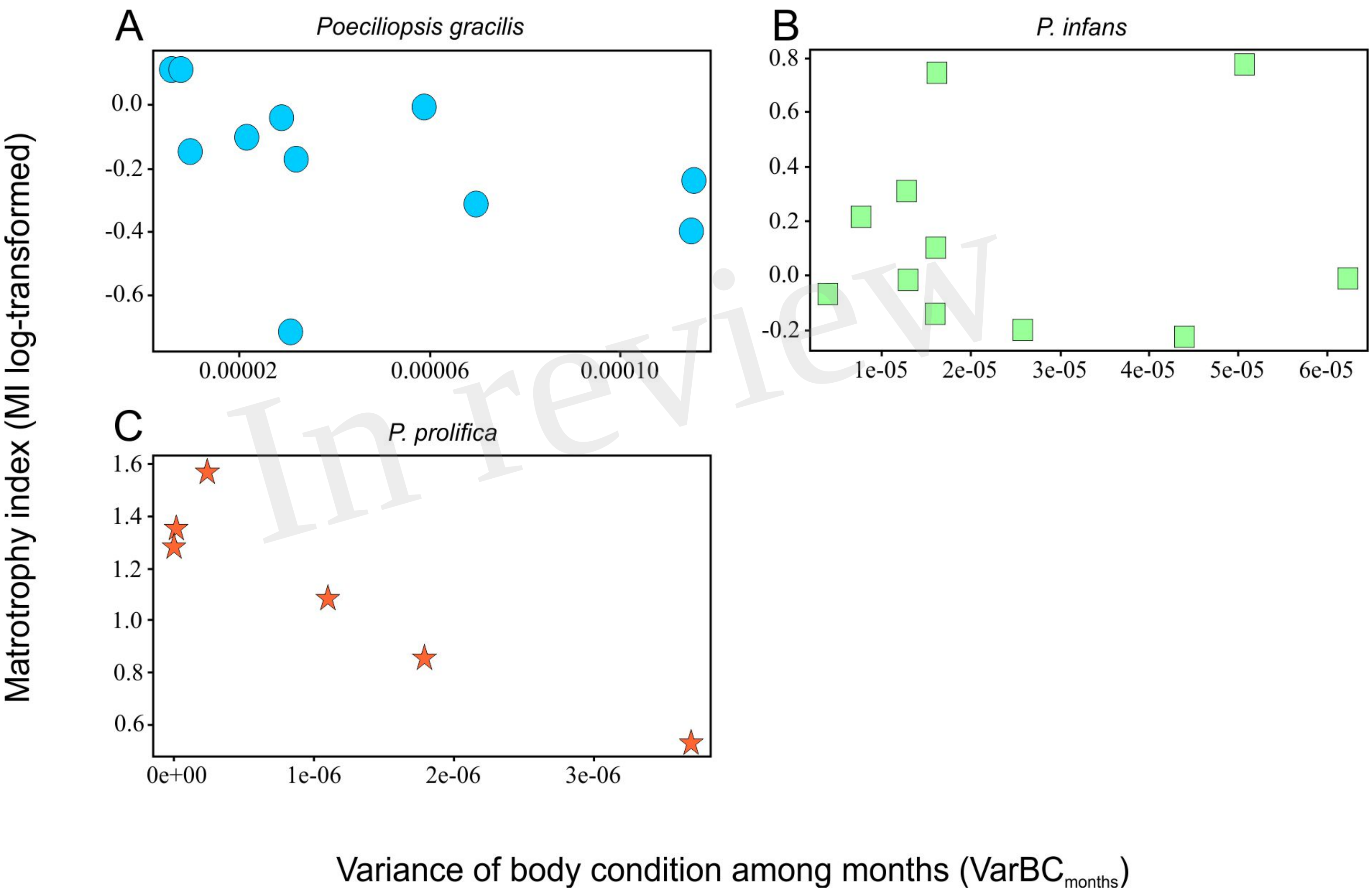
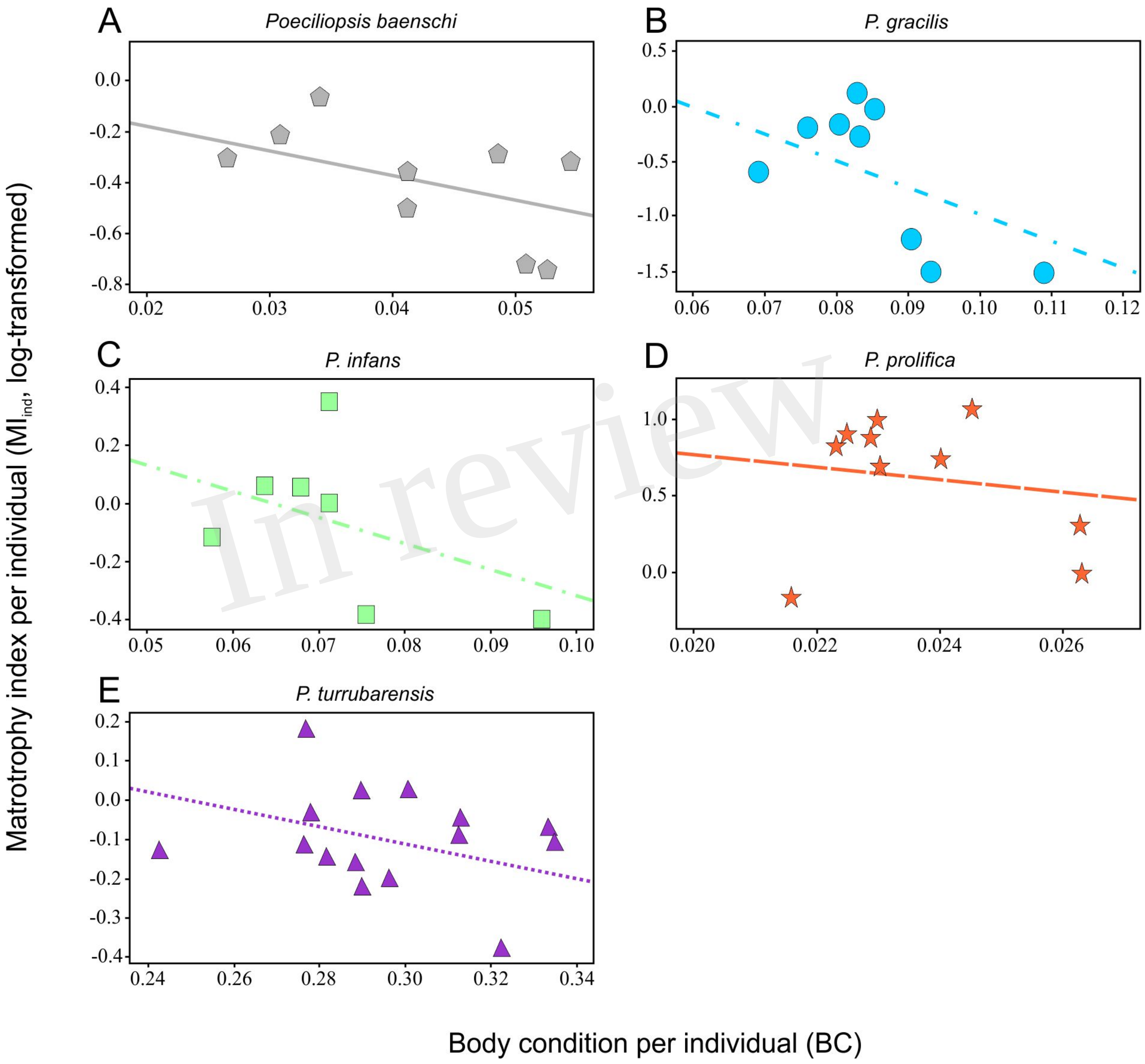


Figure 5.JPEG



SUPPLEMENTARY MATERIAL

METHODS

Examination of Body Shape and Its Potential Effect on Body Condition

Based on digital photographs of the lateral profile of females of our study species, we used geometric morphometric techniques to examine if the observed intraspecific variation in body condition could be explained by morphological differences among females. This morphological analysis was conducted separately for each species (pooling data from different populations), excluding *Poeciliopsis turrubarensis* because no photographs were available for this species. Using the program tpsDIG2 (Rohlf, 2010), we scored 17 anatomical landmarks on the lateral profile of each female. We used the landmarks defined by Zúñiga-Vega et al. (2011) in their morphological analysis of another poeciliid species (*Poecilia butleri*). Based on these landmarks, we computed two different measures of shape variation for each female using the thin-plate spline approach (Zelditch et al., 2004) implemented in the program tpsRELW (Rohlf, 2017). First, we calculated a set of uniform shape components, which are geometrically uniform changes in shape across the entire body of the fish (general increases in width or length with respect to an average or consensus shape). Second, we calculated a set of non-uniform shape components, also called "partial warps", which describe non-uniform changes in the position of a subset of landmarks with respect to other landmarks (Zelditch et al., 2004).

To reduce dimensionality, we applied a principal components analysis (PCA), also implemented in the program tpsRELW, to both uniform and non-uniform shape variables. From this PCA we obtained "relative warps" (RW), which are orthogonal axes of shape variation. Relative warps represent a summary of how the shape of each individual deviates from the consensus shape (among all individuals and across all populations). We used the first two relative warps (RW1 and RW2), which explained the largest amount of morphological variation, in our examination of the potential association between body condition and morphology. For each study species, we calculated Pearson correlation coefficients between the scaled mass index (our estimate of body condition, denoted as BC) and both RW1 and RW2 (**Supplementary Table 4**).

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Supplementary Table 1 | Geographic coordinates, in decimal degrees, for the 45 populations that we examined of five fish species from the genus *Poeciliopsis*.

Species/Population	Latitude	Longitude
Poeciliopsis baenschi		
1	18.54	-103.39
2	19.2	-103.83
3	19.17	-103.83
4	20.19	-104.55
5	19.39	-104.53
6	19.49	-104.31
Poeciliopsis gracilis		
1	21.97	-99.26
2	21.99	-99.26
3	21.98	-98.96
4	17.43	-95.02
5	17.2	-95.05
6	17.15	-95.12
7	17.14	-95.13
8	17.15	-95.17
9	17.45	-95.44
10	17.56	-95.55
11	17.84	-95.82
Poeciliopsis infans		
1	20.58	-103.86
2	21.65	-102.97
3	21.21	-103.37
4	21.24	-102.33
5	21.16	-102.47
6	21.05	-103.43
7	20.56	-103.96
8	20.55	-103.95
9	20.54	-104.05
10	20.58	-104.15

11	20.16	-103.04
Poeciliopsis prolifica		
1	21.65	-102.97
2	21.51	-103.09
3	21.48	-103.09
4	21.04	-104.38
5	23.89	-106.62
6	22.46	-105.37
7	23.28	-106.24
8	23.06	-105.84
Poeciliopsis turrubarensis		
1	8.68	-83.49
2	8.61	-83.42
3	9.58	-84.33
4	10.03	-85.18
5	10.13	-85.27
6	10.31	-85.17
7	10.56	-85.39
8	10.31	-85.21
9	10.35	-85.16

Supplementary Table 2 | Matrotrophy index (MI), average index of fullness (IF_A), variance of the index of fullness among months ($VarIF_{months}$), average body condition (BC_A), and variance of body condition among months ($VarBC_{months}$) for 45 populations of five fish species from the genus *Poeciliopsis*.

Species/ Population	**MI**	**IF_A**	**$VarIF_{months}$**	**BC_A**	**$VarBC_{months}$**
Poeciliopsis baenschi					
1	1.06	5.84	-	0.04	-
2	1.02	4.91	-	0.04	-
3	1.75	6.14	-	0.05	-
4	0.84	4.00	-	0.04	-
5	0.96	4.06	-	0.04	-
6	1.14	4.60	-	0.03	-
Poeciliopsis gracilis					
1	0.68	11.15	10.48	0.08	0.00012
2	1.14	12.25	23.65	0.08	0.000007
3	1.14	13.17	12.18	0.08	0.000006
4	0.79	16.60	28.37	0.09	0.00012
5	0.91	17.45	44.60	0.09	0.00002
6	0.73	8.94	1.63	0.08	0.00007
7	0.97	15.03	90.24	0.08	0.00003
8	0.48	12.74	18.98	0.08	0.00003
9	1.00	14.49	22.76	0.08	0.00006
10	0.87	16.70	45.68	0.08	0.00001
11	0.84	10.87	27.15	0.08	0.00003
Poeciliopsis infans					
1	0.79	4.68	1.65	0.07	0.000044
2	0.87	6.98	4.14	0.07	0.000016
3	1.11	16.21	53.99	0.07	0.000016
4	0.99	12.21	22.22	0.07	0.000013
5	0.93	5.38	0.25	0.07	0.000004
6	0.99	8.68	16.07	0.07	0.000062
7	0.82	10.27	22.38	0.07	0.000026
8	2.19	6.66	30.63	0.07	0.000051

9	2.09	5.84	6.29	0.07	0.00002
10	1.37	7.79	25.28	0.07	0.00001
11	1.24	9.91	21.40	0.07	0.000008
Poeciliopsis prolifica					
1	0.85	7.06	-	0.02	-
2	4.86	9.27	23.19	0.02	0.0000002
3	3.81	9.10	10.03	0.02	0.00000002
4	2.95	5.73	1.73	0.03	0.000001
5	2.35	4.31	9.20	0.02	0.000002
6	3.59	9.43	18.06	0.02	0.0000000007
7	1.68	6.94	33.70	0.02	0.000004
8	1.26	2.60	-	0.02	-
Poeciliopsis turrubarensis					
1	0.71	8.16	-	0.30	-
2	1.05	14.97	-	0.31	-
3	0.74	10.54	-	0.30	-
4	0.68	16.36	-	0.29	-
5	1.05	9.14	-	0.30	-
6	0.93	6.14	-	0.30	-
7	1.85	4.45	-	0.29	-
8	0.93	14.06	-	0.26	-
9	1.32	9.72	-	0.30	-

Supplementary Table 3 | Collection dates for the 45 populations that we examined of five fish species from the genus *Poeciliopsis*.

Species/ Population	**Dates**
Poeciliopsis baenschi	
1	January 2010
2	February 2010
3	January 2010
4	November 2009
5	November 2009
6	March 2012
Poeciliopsis gracilis	
1	April 2012 September 2012 November 2012
2	June 2012 November 2012 January 2013 March 2013
3	April 2012 June 2012 September 2012 November 2012 March 2013
4	March 2012 April 2012 June 2012 November 2012 January 2013

	March 2013
5	April 2012
	June 2012
	September 2012
	March 2013
6	September 2012
	November 2012
	January 2013
	March 2013
7	March 2012
	April 2012
	November 2012
	January 2013
	March 2013
	October 2013
8	March 2012
	April 2012
	November 2012
	March 2013
9	April 2012
	June 2012
	September 2012
	November 2012
	January 2013
	March 2013
10	March 2012
	June 2012
	September 2012
	November 2012
	January 2013
	March 2013
11	March 2012
	April 2012
	June 2012
	November 2012
	January 2013
	March 2013

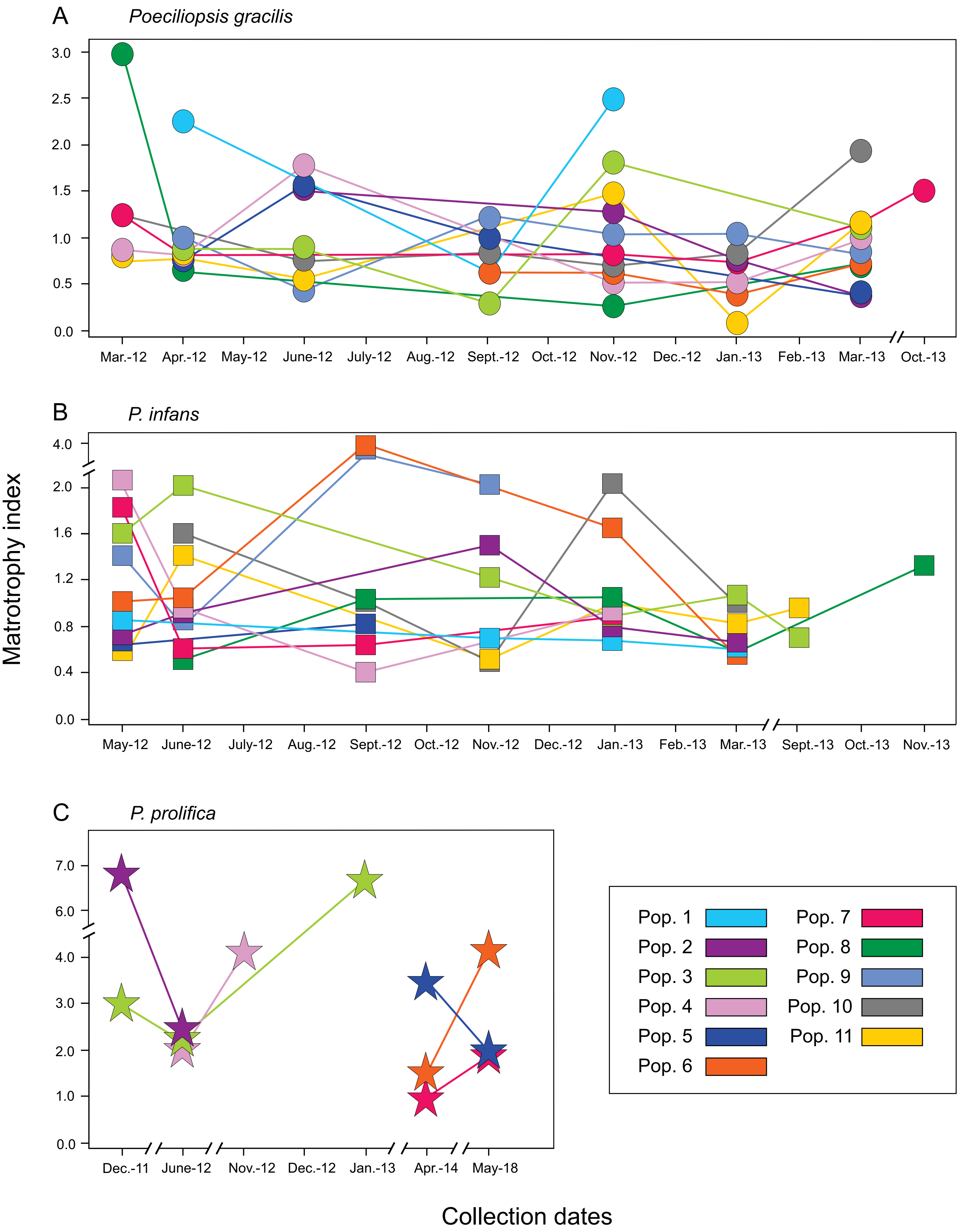
Poeciliopsis infans

1	May 2012 November 202 January 2013 March 2013
2	May 2012 June 2012 November 2012 January 2013 March 2013
3	May 2012 June 2012 November 2012 January 2013 March 2013 September 2013
4	May 2012 June 2012 September 2012 January 2013
5	May 2012 September 2012
6	May 2012 June 2012 September 2012 January 2013 March 2013
7	May 2012 June 2012 September 2012 January 2013
8	June 2012 September 2012 November 2012 January 2013 March 2013 November 2013
9	May 2012 June 2012 September 2012

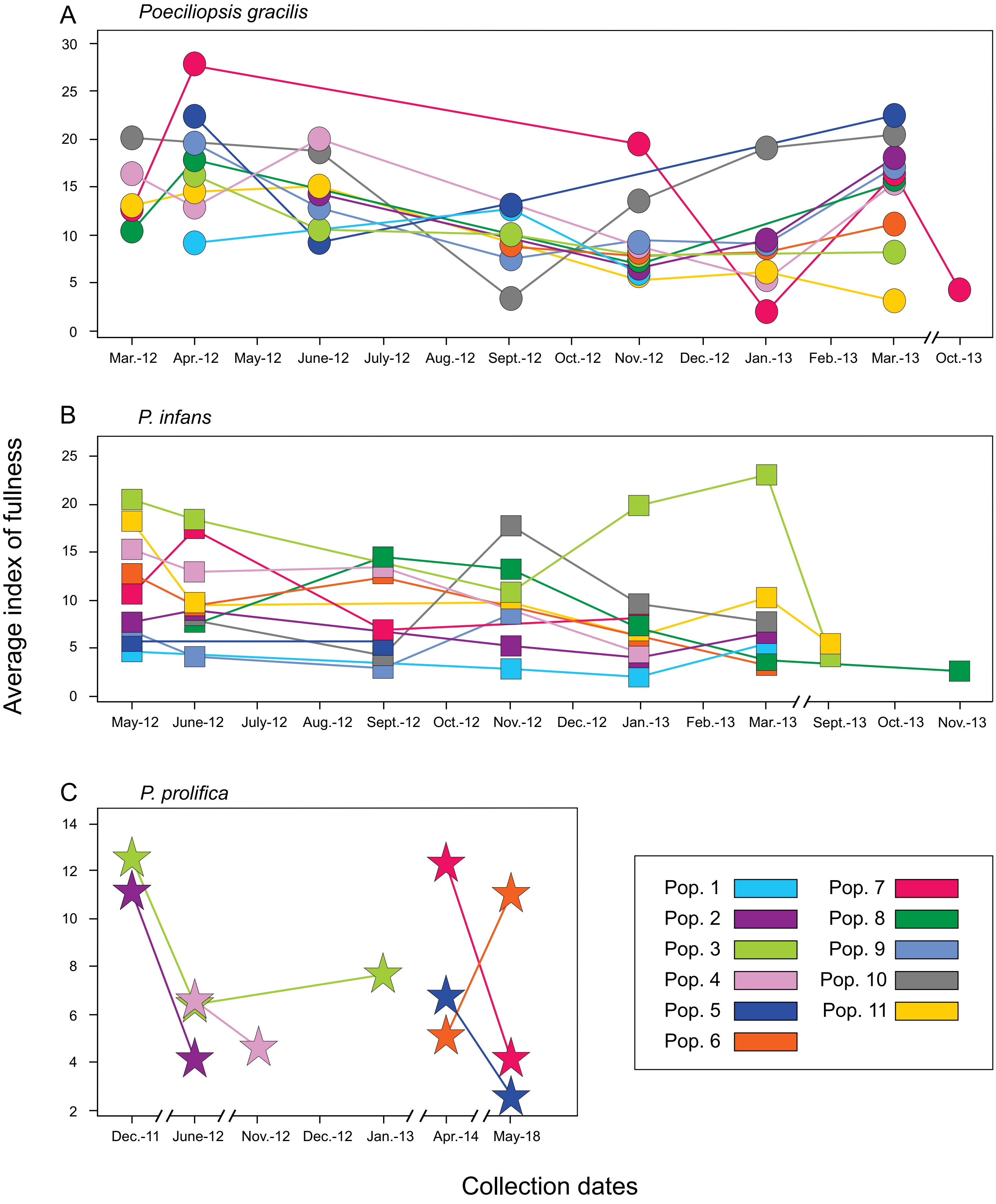
	November 2012
10	June 2012 September 2012 November 2012 January 2013 March 2013
11	May 2012 June 2012 November 2012 January 2013 March 2013 September 2013
Poeciliopsis prolifica	
1	March 2011
2	December 2011 June 2012
3	December 2011 June 2012 January 2013
4	June 2012 November 2012
5	April 2014 May 2018
6	April 2014 May 2018
7	April 2014 May 2018
8	May 2018
Poeciliopsis turrubarensis	
1-9	February 2006

Supplementary Table 4 | Pearson correlation coefficients that examine the association between body condition (BC) and the first two axes of shape variation (first and second relative warps, RW1 and RW2) for four fish species from the genus *Poeciliopsis*. We also show sample sizes (*n* = number of females).

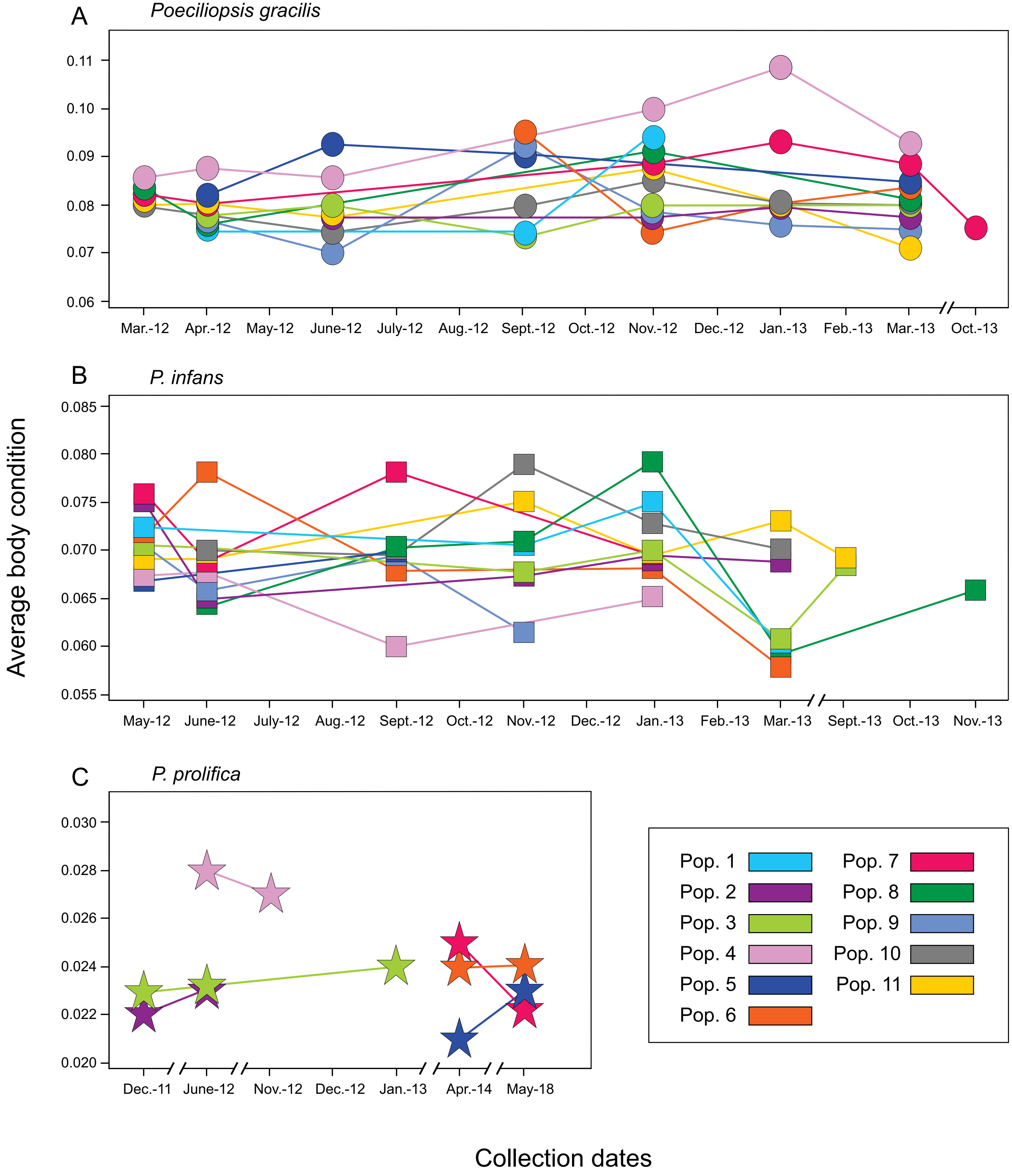
Species	**RW1**	**RW2**	***n***
Poeciliopsis baenschi	0.09	0.23	226
P. gracilis	-0.10	0.31	543
P. infans	-0.07	-0.13	614
P. prolifica	-0.20	0.03	473



Supplementary Figure 1 | Temporal variation of the matrotrophy index for different populations of three fish species from the genus *Poeciliopsis*.



Supplementary Figure 2 | Temporal variation of the index of stomach fullness for different populations of three fish species from the genus *Poeciliopsis*.



Supplementary Figure 3 | Temporal variation of body condition (estimated by means of the scaled mass index) for different populations of three fish species from the genus *Poeciliopsis*.

V DISCUSIÓN GENERAL

La matrotrofía es un modo de aprovisionamiento materno que sucede después de que el ovocito ha sido fecundado. La madre transfiere nutrientes después de la fecundación y durante todo el desarrollo del embrión hasta que llega al nacimiento (Blackburn, 1992; Reznick et al., 2002; Marsh-Matthews, 2011). La superfetación es la capacidad de las hembras de tener simultáneamente múltiples camadas en diferentes etapas de desarrollo (Scrimshaw, 1944; Zúñiga-Vega et al., 2010, 2017; Olivera-Tlahuel et al., 2015). Poco se ha estudiado sobre las funciones que tienen la matrotrofía y la superfetación de manera conjunta. Las hembras que presentan superfetación y matrotrofía presentan un menor aumento de masa y volumen corporal cuando producen un número particular de embriones en comparación con las hembras de especies que carecen de estos modos reproductivos (Pollux et al., 2014).

Dado lo anterior propuse dos hipótesis que están asociadas con el tamaño de las hembras y su efecto en la matrotrofía y superfetación. Dado lo anterior sugerí dos hipótesis: 1) la primera propone que la presencia conjunta de matrotrofía y superfetación debe ser ventajoso para especies de tamaño pequeño, ya que si tienen altos grados de ambas estrategias reproductivas sería posible tener fecundidades relativamente altas aunque tengan restricciones de espacio debido a que son de tamaño pequeño y 2) la segunda propone que la matrotrofía y la superfetación cambian a lo largo de la vida reproductiva de las hembras, de esta forma funcionarían como mecanismos que compensan las restricciones de espacio cuando las hembras son de tamaño pequeño (jóvenes). Este es el primer estudio que considera que pueden existir cambios en el nivel de aprovisionamiento materno y superfetación a lo largo de la vida reproductiva de las hembras. Aunado a estas dos hipótesis, en esta tesis busqué probar el modelo teórico de aprovisionamiento materno de Trexler y DeAngelis, que sugiere que un posible valor adaptativo de la matrotrofía radica en la cantidad y variabilidad de alimento disponible en el amiente. ya que en la literatura hay resultados a favor del modelo y otros más que indican que el modelo no se cumple. A continuación, describí brevemente que fue lo que encontré en cada uno de mis capítulos, así como su discusión.

V.1 ¿La matrotrofía y la superfetación son más prominentes en especies de tamaño pequeño?

Para mi primer capítulo mi predicción fue que la superfetación y la matrotrofía deberían ser más comunes en especies de tamaño pequeño. Me basé en la teoría que menciona que las hembras que presentan superfetación y matrotrofía deberían tener cierto número particular de embriones pequeños al menos en las primeras fases de desarrollo comparado con hembras que carecen de estas estrategias, en donde el tamaño de los embriones es mayor desde el inicio del desarrollo, debido al almacenamiento de vitelo (Thibault and Schultz, 1978; Reznick and Miles, 1989; Pollux et al., 2009). Respecto a la superfetación, se ha propuesto que las hembras que son superfetadoras dividen su esfuerzo reproductivo en dos o más camadas con un número pequeño de embriones (Zúñiga-Vega et al., 2010). Debido a que tienen camadas en diferentes estadios de desarrollo el incremento durante la gestación en masa y en volumen de las hembras superfetadoras es menor, ya que los embriones en etapas de desarrollo temprano son de tamaños más pequeños que los embriones en etapas tardías. Considerando lo anterior, el volumen de las hembras superfetadoras suele ser menor que el de las hembras que no son superfetadoras debido a que estas últimas producen todos sus embriones en un solo evento reproductivo y todos los embriones son del mismo estadio de desarrollo, por lo que el tamaño que ocupan en masa y volumen dentro de la hembra en los últimos estadios de desarrollo es mayor (Zúñiga-Vega et al., 2010).

Debido a que las hembras que presentan mayores grados de matrotrofía y superfetación aumentan menos en volumen y masa durante el desarrollo de sus embriones comparado con hembras lecitotróficas y no superfetadoras propuse que ambas estrategias deberían ser más prominentes para las especies pequeñas, ya que las especies matrotróficas y con presencia de superfetación podrían compensar las restricciones de espacio derivadas de tener cuerpos pequeños al tener un mayor número de embriones que aquellas especies lecitotróficas y sin superfetación. Esta predicción supondría un beneficio importante considerando las limitaciones de espacio que puede tener una hembra de una especie de tamaño pequeño, ya que la presencia de ambas estrategias podría permitirles a las especies de tamaño pequeño presentar una fecundidad que se asemeje a la de especies de tamaño mayor, pero sin la consecuencia de un exacerbado aumento de la masa y volumen del abdomen durante la gestación.

No encontré evidencia que sustente la hipótesis planteada en donde la matrotrofía y la superfetación deberían ser más prominentes en especies de tamaño pequeño. Los resultados indican que las especies de tamaño menor no tienen altos grados de matrotrofía y superfetación. Además, la presencia de ambas estrategias (matrotrofía y superfetación) no incrementa la fecundidad total de las especies. Por lo tanto, estas estrategias no son posibles mecanismos para compensar las restricciones debidas a ser hembras de especies con tamaños pequeños. Los resultados que obtuve revelaron que, las hembras grandes tienen un mayor número de camadas simultáneas (superfetación) que las hembras pequeñas. Contrario a mis predicciones, encontré que la cantidad de aprovisionamiento materno después de la fecundación, es decir la matrotrofía no se ve afectada por el tamaño de las hembras.

Se han realizado pocas investigaciones sobre los beneficios de la matrotrofía y la superfetación, (aunque no de forma conjunta) en términos de promover fenotipos hidrodinámicos o delgados. En un estudio realizado por Zúñiga-Vega y colaboradores (2007), encontraron que las hembras de *Poeciliopsis turrubarens*is con grados más avanzados de superfetación tienen cuerpos más delgados, son más alargadas y habitan sitios con corrientes de agua más rápidas en comparación con las hembras que tienen menos superfetación, las cuales son más robustas y habitan sitios con corriente de agua lentas. Considerando mis resultados y los de los investigadores, es posible que la superfetación esté afectada por el tamaño y las condiciones ecológicas en donde habiten. Es posible que la superfetación sea más prominente en hembras grandes y que la misma permita cuerpos más hidrodinámicos (Furness et al., 2021). Sin embargo, hay que resaltar que mi estudio fue realizado a nivel de especies y el de estos investigadores a nivel poblacional, es posible que estos resultados no sean comparables debido a la escala en la que fueron obtenidos, ya que, el estudio realizado con las hembras de *Poeciliopsis turrubarens*is fue a nivel poblacional y el estudio que yo realicé es a nivel de especies.

Se conocen al menos 273 especies de la familia Poeciliidae (recuperado en 2021 de fishbase.org). En la literatura encontré información de menos de la mitad de las especies. Aún es necesario continuar con las investigaciones sobre las historias de vida de las especies que comprenden esta familia con la finalidad de saber más sobre los mecanismos fisiológicos de cada especie, así como sus estrategias reproductivas. Es necesario realizar mis análisis con un mayor número de especies para poder llegar a una conclusión sobre cómo y por qué varían los niveles de superfetación y matrotrofía entre especies y si el tamaño de las hembras es una variable que definitivamente debe ser descartada como fuente de variación de estos interesantes modos de reproducción.

Las estimaciones que realicé de la señal filogenética (tendencia de las especies a parecerse más entre sí que con un conjunto aleatorio de especies, Pagel, 1999) con 91 especies de la familia Poeciliidae indicaron que el tamaño de las hembras, así como la superfetación y la matrotrofía han evolucionado mediante la acumulación gradual de cambios a través del tiempo, lo cual originó una similitud entre especies estrechamente relacionadas. Este patrón es interesante debido a los múltiples orígenes independientes tanto de la matrotrofía como de la superfetación (Pollux et al., 2009, 2014; Meredith et al., 2010, 2011; Furness et al., 2019, 2021). Una vez que surgen estas estrategias, acumulan de forma constante pequeños cambios con poca influencia del ambiente. Esta alta señal filogenética ha sido reportada previamente con 44 especies de la familia Poeciliidae (Olivera-Tlahuel et al., 2015) y es consistente con mis resultados obtenidos al analizarla con 91 especies.

En cuanto al tamaño de las hembras, mi estudio es el primero en documentar que este rasgo también ha evolucionado de manera consistente con un modelo browniano (los caracteres acumulan cambios de manera constante a lo largo del tiempo; Felsenstein, 1985). En contraste, no encontré señal filogenética en la fecundidad total por unidad de tiempo, lo que indica que este rasgo se adapta rápidamente al entorno local. Este resultado confirma que diversos factores ambientales ejercen fuertes efectos selectivos sobre el tamaño y el número de las crías que producen estas especies de peces vivíparos (i.e., depredación, disponibilidad de alimento, densidad de población, salinidad (Dahlgren, 1979; Johnson and Belk, 2001; Gomes and Monteiro, 2007; Bashey, 2008; Trexler and DeAngelis 2003;2010; Bosker et al., 2017). En un estudio realizado utilizando como sistema de estudio a *Brachyrhaphis rhabdophora* se encontró que las poblaciones que coexisten con depredadores alcanzan una madurez sexual de forma temprana y producen camadas más grandes con embriones más pequeños comparado con poblaciones que tienen pocos depredadores (Johnson and Belk, 2001). Los resultados de los autores sugieren que existen diferencias en la tasa de mortalidad de los peces adultos frente a los jóvenes. Entonces se maximiza la reproducción en hembras de tamaños pequeños (Johnson and Belk, 2001). Estos resultados fueron similares en un estudio realizado con *Poecilia Reticulata*. Los guppies (*Poecilia reticulata*) de poblaciones de baja depredación han evolucionado para producir menos crías y más grandes que sus contrapartes de poblaciones de alta depredación (Bashey, 2008).

En relación con la disponibilidad de alimento, se ha sugerido que en ambientes donde la disponibilidad de alimento es alta, se podría favorecer la matrotrofía debido a que las hembras necesitan transferir nutrientes a sus embriones de manera constante. Los autores sugieren que una de las ventajas de la matrotrofía sería aumentar el número de embriones por camada si la hembra tiene los recursos necesarios para llevar a término la camada (Trexler and DeAngelis 2003;2010). Respecto a la densidad poblacional, se han realizado experimentos con *Poecilia reticulata* en donde evaluaron los efectos de la densidad poblacional sobre la fecundidad. Los autores demostraron que la fecundidad fue significativamente más alta en condiciones de baja densidad poblacional comparado con la fecundidad de las hembras que tuvieron una densidad poblacional mayor (Dahlgren, 1979). Los autores sugieren que sus resultados se deben a que una alta densidad poblacional puede inducir estrés y por ende una disminución en la producción de vitelo lo que resultaría en una fecundidad menor (Dahlgren, 1979). El último de los factores ambientales que ejerce fuertes efectos selectivos sobre el tamaño y el número de las crías que producen estas especies de peces vivíparos es la salinidad. Un estudio demostró que el tamaño de los embriones de *Poecilia vivipara* está asociada de forma positiva con la salinidad (Gomes and Monteiro, 2007; Bosker et al., 2017). La salinidad del agua puede tener una influencia directa sobre el crecimiento de los peces, a través de la reducción de las tasas metabólicas basales, el aumento de la alimentación y la optimización de la osmorregulación (Gomes and Monteiro, 2007; Bosker et al., 2017).

Mis resultados del primer capítulo indican que la matrotrofía y la superfetación no son mecanismos que funcionen como estrategias para compensar las restricciones debidas al espacio reducido de las hembras de especies pequeñas. Sería interesante evaluar los efectos de la matrotrofía y la superfetación de forma conjunta en otros aspectos de las historias de vida. Por ejemplo, la posibilidad de que estas estrategias permitan iniciar de manera temprana el ciclo reproductivo permitiendo tener un mayor número de camadas a lo largo de la vida de las hembras. Otro factor podría ser que estas estrategias permitan reducir los tiempos de gestación y con ello favorecer un aumento en la fecundidad a lo largo de la vida ya que podrían tener un mayor número de camadas en menor tiempo y aumentar las probabilidades de supervivencia al permitir que las hembras permanezcan preñadas el menor tiempo posible, reduciendo costos de movilidad y con ello la probabilidad de ser depredadas (Turner, 1937; Zúñiga-Vega et al., 2007; Pollux et al., 2009; Greven, 2011; Pires et al., 2011).

V.2 ¿Los niveles de matrotrofía y superfetación pueden variar dependiendo del tamaño (edad) de las hembras?

En el segundo capítulo de mi tesis analicé el efecto del tamaño de la hembra sobre los grados de matrotrofía y superfetación a nivel poblacional (intraespecífico, comparando hembras de la misma especie) utilizando 77 poblaciones correspondientes a 13 especies de la familia Poeciliidae. En esta parte de mi investigación tomé en cuenta que las hembras tienen crecimiento indeterminado, es decir las hembras continúan creciendo después de alcanzar la madurez sexual (Hughes, 1986; Vargas and De Sostoa, 1996). Considerando esto puse a prueba la hipótesis de que, tanto la matrotrofía como la superfetación deberían estar más presentes (i.e., con grados más avanzados de ambas estrategias reproductivas) en hembras de tamaño pequeño para compensar las restricciones impuestas por tener poco espacio corporal y las demandas energéticas que esto conlleva ya que, las hembras pequeñas (jóvenes) tienen que invertir recursos en continuar creciendo y en reproducirse. La matrotrofía les permitiría a las hembras depositar pocos recursos de manera inicial (vitelo) e ir alimentando a sus embriones conforme ellas crecen. De esta forma, podrían dividir los recursos que obtienen para destinarlos tanto a la reproducción como a su crecimiento corporal.

Por otro lado, la superfetación les permitiría reducir el esfuerzo reproductivo al tener camadas simultáneas con un menor número de embriones lo que les conferiría (al igual que la matrotrofía) dividir los recursos a los cuales tienen acceso entre su crecimiento y su reproducción. La otra parte de la hipótesis consiste en que conforme las hembras crecen (y envejecen) modificarán sus estrategias, siendo más lecitotróficas y menos superfetadoras conforme crecen, debido a que 1) ya pueden destinar más recursos a la reproducción que al crecimiento y 2) ya han alcanzado un tamaño más grande. Ser de mayor tamaño les otorga más espacio en sus cavidades reproductoras, con lo que pueden almacenar embriones que sean de un tamaño mayor (i.e., embriones lecitotróficos grandes porque están llenos de vitelo desde antes de ser fecundados).

Encontré evidencia que sustenta esta hipótesis (sin considerar a la superfetación) solamente en tres poblaciones de dos especies (*Poeciliopsis infans* y *P. turrubarensis*). En estas tres poblaciones, las hembras comienzan su vida reproductiva siendo un poco más matrotróficas y conforme envejecen se vuelven un poco más lecitotróficas. Estos resultados indican que el grado de matrotrofía cambia a lo largo de la vida de las hembras, siendo matrotróficas cuando son jóvenes y cambiando a lecitotróficas cuando envejecen. Este es el primer estudio que demuestra un cambio ontogenético en el modo de aprovisionamiento materno para embriones en desarrollo en peces vivíparos. Ser una hembra matrotrófica en las primeras etapas de la edad adulta podría permitirles a las hembras dedicar energía y recursos tanto al crecimiento como a la reproducción (aunque mi estudio no prueba cual es el mecanismo a través del cual las hembras conceden recursos a su crecimiento o a su reproducción). A pesar de que encontramos evidencia de que el grado de matrotrofía puede cambiar ontogenéticamente en algunas poblaciones, claramente no es común en la mayoría de las poblaciones, debido a que este patrón solo se observó en 3 de las 77 poblaciones que examiné.

Una explicación probable por la cual no observé este patrón en un mayor número de poblaciones es que existan modificaciones fisiológicas como las fluctuaciones hormonales que permitan un cambio de estrategia de ser hembras matrotróficas a ser hembras lecitotróficas. Se han estudiado los cambios fisiológicos durante el crecimiento y desarrollo temprano en algunos peces como *Salmo gairdneri, Fundulus heteroclitus, Oncorhynchus keta* y *Oncorhynchus rhodurus* encontrando que los patrones hormonales cambian durante los procesos de crecimiento y maduración sexual. Aunado a esto, es necesario un equilibrio apropiado de todos los aspectos fisiológicos para que la fecundación pueda ocurrir, sin este equilibrio no es factible la reproducción (Young et al., 1983; Wagner et al., 1985; Venkatesh et al., 1989; Van Der Kraak et al., 1990; Degani et al., 1998). Un estudio realizado con *Poecilia reticulata* reveló que los niveles de estradiol y testosterona incrementan cuando las hembras se encuentran en la fase de desarrollo de vitelo y después de la fecundación disminuyen (Venkatesh et al., 1989). El balance para obtener estos cambios en los niveles de estradiol y testosterona se logra en las primeras fases de la madurez sexual (Young et al., 1983; Wagner et al., 1985; Venkatesh et al., 1989; Van Der Kraak et al., 1990; Degani et al., 1998). Es posible que todos los aspectos fisiológicos asociados a la reproducción o a la producción de vitelo (como el estradiol y la testosterona, Venkatesh et al., 1989) se encuentren perfectamente desbalanceados al inicio de la vida reproductiva de las hembras. Si esto fuera cierto, es posible que en las etapas tempranas de la reproducción la matrotrofía funja como un mecanismo que les permita a las hembras iniciar la reproducción de forma temprana, aunque todavía no tengan completamente balanceados todos los aspectos fisiológicos necesarios para la producción de vitelo. Es decir, es posible para producir vitelo necesitan una mayor madurez fisiológica y que la transferencia activa de nutrientes sólo requiere de los tejidos placentarios. De esta forma, la hembra podría transferir recursos después de la fecundación a sus embriones en desarrollo sin afectar su peso al nacer y de manera simultánea destinar recursos a su propio crecimiento. Comenzar a tener embriones desde una edad muy temprana podría permitirles a las hembras maximizar su fecundidad total, puesto que las hembras producirían mayor número de camadas a lo largo de sus vidas reproductivas. Después, cuando todos los aspectos fisiológicos asociados a la reproducción o a la producción de vitelo (como el estradiol y la testosterona, Venkatesh et al., 1989.) se encuentren perfectamente balanceados podría darse el cambio de estrategia de ser muy matrotrófica a ser lecitotrófica. Esta idea asume que después del cambio de estrategia se conserven grados intermedios o avanzados de matrotrofía. Estos grados de matrotrofía serían menores comparados con el momento en que las hembras producen sus primeras camadas. La conservación de cierto grado de matrotrofía después de que las hembras alcanzaron completamente la madurez sexual dependería de las estructuras especializadas de las hembras como las placentas foliculares, las cuáles podrían permitir que las hembras continúen transfiriendo nutrientes a sus embriones a lo largo de sus vidas reproductivas (Kwan et al., 2015; Olivera-Tlahuel et al., 2019).

Una explicación tentativa de porqué encontré en pocas poblaciones el cambio de estrategia de las hembras de ser matrotróficas de jóvenes a mas lecitotróficas conforme envejecen es que este cambio de estrategia ocurra de una forma relativamente rápida cuando las hembras se encuentran produciendo sus primeras camadas, de forma tal que, aunque las hembras que se observen sean de un tamaño relativamente pequeño estas ya hayan realizado el cambio de estrategia hacia lecitotróficas. Esto es posible debido a que el tamaño en el cual alcanzan la madurez sexual no es el mismo para todas las hembras (Ponce de León et al., 2013). Es decir, no todas las hembras realizarán el cambio de estrategia cuando son de un mismo tamaño pequeño, sino que debería existir variación del tamaño de las hembras en el momento en el que realizan el cambio de matrotrofía a lecitotrofía. Es posible que algunas de las hembras que tuve en mis muestras a pesar de ser tamaños relativamente pequeños (dependiendo también de la especie), ya hubiesen realizado un cambio de estrategia de matrotrofía predominante a una combinación de ambas estrategias (o solo lecitotrofía) y que esta sea la razón por la cual el patrón no sea común o fácil de detectar.

Por otro lado, encontré en una población de *P. prolifica* que la cantidad de transferencia de nutrientes a los embriones después de la fertilización también varió en función del tamaño de la hembra, pero en la dirección opuesta a lo que se esperaba dado mis predicciones. En este sitio, las hembras se vuelven más matrotróficas a medida que crecen y envejecen. Una posible explicación por la cual observé este patrón es que exista una diferencia en la ingesta de comida y competencia por este recurso entre hembras de tamaño pequeño y hembras grandes. De acuerdo con el modelo de provisión materna de Trexler-DeAngelis, los grados avanzados de matrotrofía sólo son posibles si las fuentes de alimento son relativamente abundantes y están constantemente disponibles (Trexler y DeAngelis, 2003, 2010). Por otra parte, algunos estudios han demostrado que la competencia intraespecífica es un importante impulsor de la variación en los rasgos reproductivos de los peces poecílidos (Leips y Travis, 1999; Bashey, 2006, 2008; Schrader y Travis, 2012). Tomando en cuenta que para ser una hembra matrotrófica las hembras necesitan alimento constante y que puede existir competencia por la obtención de los recursos, es posible que al menos para esta población de *P. prolífica* exista una competencia por el alimento en donde las hembras grandes, al ser de mayor edad sean mejores competidoras por tener más experiencia en la obtención de recursos, impidiendo que las hembras de menor tamaño logren obtener suficientes recursos. De este modo las hembras grandes podrían ser matrotróficas mientras que las hembras chicas solo pueden optar por almacenar energía en forma de vitelo (lecitotrofía) cuando logran obtener alimento suficiente.

Los resultados que encontramos sobre la superfetación son contrarios a lo que esperaba dada mi hipótesis. Esperaba que las hembras de menor tamaño presentaran mayores grados de superfetación. Sin embargo, encontré en 5 poblaciones que la superfetación es mayor en hembras de tamaño mayor. Estos resultados indican que las hembras de tamaño pequeño no compensan las restricciones de espacio teniendo mayores grados de superfetación. En un estudio realizado por Zúñiga-Vega y colaboradores (2017) utilizando como modelo a *Poeciliopsis baenschi* investigaron una asociación entre la velocidad del flujo de agua, la mortalidad y la densidad poblacional afectando a la superfetación (Zúñiga-Vega et al., 2017). No encontraron una asociación entre las variables ecológicas antes mencionadas y la superfetación. Un factor importante es que en este trabajo se encontró que existe una asociación positiva entre la superfetación y el número total de embriones (Zúñiga-Vega et al., 2017). Es decir, las hembras que tuvieron un mayor número de camadas simultáneas tuvieron una fecundidad total mayor. La superfetación podría permitir que las hembras incrementen su fecundidad total, aunque según lo que encontré en este capítulo de la tesis sólo sería posible para las hembras de tamaño grande, debido a que estas hembras grandes presentan mayores grados superfetación.

Por otra parte, se han realizado experimentos sobre los posibles costos de la reproducción (Curtis Creighton et al., 2009; Belk and Tuckfield, 2010). La hipótesis de la inversión terminal postula que la reproducción es costosa en términos de supervivencia y reproducción futuras. Una de las predicciones de esta hipótesis sugiere que, si el valor reproductivo disminuye a medida que las hembras envejecen, entonces las hembras jóvenes deberían destinar menos energía disponible a la fecundidad actual y más a la reproducción futura, mientras que las hembras con mayor edad deberían destinar más energía a la fecundidad actual y menos a la reproducción en el futuro (Curtis Creighton et al., 2009). En resumen, la hipótesis sugiere que conforme las hembras se van acercando al final de su vida deberían incrementar de forma exacerbada su esfuerzo reproductivo, ya que son los últimos eventos reproductivos que la hembra tendrá en su vida (Curtis Creighton et al., 2009). Un experimento realizado con *Gambusia affinis* demostró que las hembras de mayor edad destinan más recursos a la reproducción, aunque esto genera un posible impacto en la supervivencia. La manera en que los investigadores midieron el impacto en la supervivencia fue a través de medir la velocidad de escape que tuvieron las hembras jóvenes y viejas mientras sus embriones se desarrollaban (Belk and Tuckfield, 2010). Esto debido a que existe una asociación entre la velocidad de escape y la probabilidad de escapar de los depredadores. Si una hembra reacciona de forma veloz puede huir de sus depredadores, contrario a lo que pasaría si su velocidad de reacción fuera más lenta (Belk and Tuckfield, 2010). Se encontró que, las hembras jóvenes no tienen una diferencia en la velocidad de escape a medida que sus embriones se van desarrollando. Por el contrario, con las hembras viejas encontraron una reducción en la velocidad de escape a medida que sus embriones pasaban a etapas tardías del desarrollo (Belk and Tuckfield, 2010). Considerando la hipótesis de inversión terminal, los resultados obtenidos por Zúñiga-Vega y colaboradores (2017) (en *P. baenschi*, la superfetación aumenta la fecundidad total) y los resultados que obtuve en mi tesis (observé mayores grados de superfetación en hembras de tamaño grande, es decir hembras con mayor edad) es posible que grados avanzados de superfetación le permitan a las hembras aumentar (aún más) su inversión reproductiva conforme se acerca el final de su ciclo reproductivo o el final de sus vidas.

En consonancia con trabajos publicados anteriormente, mis resultados sugieren que los grados de matrotrofía y superfetación podrían ser mecanismos con cierta plasticidad. Existe una variación sustancial en ambos modos reproductivos entre poblaciones dentro de las especies (Frías-Alvarez et al., 2014; Zúñiga-Vega et al., 2017; Gorini-Pacheco et al., 2018). Además, los estudios experimentales han demostrado que los cambios en la disponibilidad de alimento pueden promover cambios tanto en el número de crías simultáneas como en las cantidades relativas de suministro previo y posterior a la fertilización para los embriones en desarrollo (Travis et al., 1987; Molina-Moctezuma et al., 2020).

Mi capítulo 2 proporciona evidencia adicional de la plasticidad que existe sobre el grado de matrotrofía y superfetación que presentan las hembras a lo largo de su vida. Sin embargo, se requiere de mayor investigación para poder conocer a profundidad los mecanismos fisiológicos de las especies de la familia Poeciliidae, con el fin de comprender mejor el funcionamiento de aspectos reproductivos como la matrotrofía y la superfetación. En este trabajó evalué el efecto del tamaño de las hembras sobre la matrotrofía y la superfetación. Mis resultados sugieren que existen cambios ontogenéticos a lo largo de la vida de las hembras. No obstante, este resultado es tentativo ya que solo analicé hembras de diferentes tamaños pertenecientes a una misma población. Sería necesario en un futuro evaluar los cambios que pudiesen tener las hembras en la transferencia de nutrientes a lo largo de sus vidas reproductivas. Concretamente evaluar a cada una de las hembras y no solo tener datos reproductivos en un punto específico del tiempo. Una manera de realizarlo podría ser a través del marcaje de la comida con la que se alimente a la hembra utilizando radiotrazadores y el uso de proteínas marcadas. De esta manera se podría identificar de manera clara cuál es el nivel de transferencia de nutrientes que tienen las hembras a lo largo de sus vidas, ya que estos marcadores se pueden encontrar en los embriones gestantes (Swain and Jones, 1997). Sería necesario realizar ultrasonidos periódicos a las hembras gestantes con el fin de medir el volumen de los embriones en desarrollo, otra medida que indicaría que tantos nutrientes transfieren las hembras (dependiendo de su edad) a sus embriones en desarrollo (Pareja, et al., 2003).

V.3 ¿El alimento es un factor determinante en el grado de matrotrofía que presentan las hembras? Posible valor adaptativo de la matrotrofía. Modelo de Trexler y DeAngelis de aprovisionamiento materno.

Por último, en el tercer capítulo de esta tesis evalué una de las teorías que explica el posible valor adaptativo de la matrotrofía. La disponibilidad de alimento es una condición ecológica que podría favorecer un cambio en el tipo de transferencia de nutrientes de las madres a su descendencia. Es decir, el modelo teórico de Trexler y DeAngelis sugiere que la cantidad de alimento disponible en el ambiente puede favorecer la evolución de adaptaciones como la matrotrofía (Trexler and DeAngelis, 2003, 2010). Los autores sugieren que, en ambientes donde la cantidad de alimento es alta y se encuentra de manera constante, la estrategia que se favorecería sería la matrotrofía. Por otra parte, en ambientes en donde los recursos son escasos y fluctuantes a través del tiempo, la estrategia reproductiva que se favorecería sería la lecitotrofía (Trexler and DeAngelis, 2003, 2010). Para poner a prueba el modelo de Trexler y DeAngelis utilicé datos de 45 poblaciones que corresponden a 5 especies del género *Poeciliopsis*. Para estimar la cantidad de recursos alimenticios disponibles en el ambiente, utilizamos el índice de llenado estomacal (Hyslop, 1980) y la condición corporal (Labocha et al., 2014). Ambas son medidas indirectas de la cantidad de alimento disponible. Evalué la cantidad de recursos en un punto fijo en el tiempo y también su variabilidad.

No encontré evidencia a favor del modelo de Trexler y DeAngelis con ninguna de mis aproximaciones de disponibilidad de alimento. No existe asociación entre la cantidad de alimento disponible, su variación ambiental y el tipo de transferencia de nutrientes que presentan las hembras. No obstante, en las 5 especies observé que las hembras con mayores grados de matrotrofía tenían una condición corporal más pobre en comparación con las hembras que proporcionaban menos nutrientes a los embriones después de la fertilización.

Una de las bases teóricas del modelo de Trexler y DeAngelis es que las hembras que presentan matrotrofía podrían sufrir un deterioro físico al destinar una mayor cantidad de recursos a sus embriones en desarrollo y una cantidad menor a su mantenimiento corporal (Trexler and DeAngelis, 2003, 2010). Los resultados que encontré sugieren que, en efecto, las hembras gestantes experimentan una disyuntiva ("trade-off") entre la cantidad de recursos que destinan a sus embriones y la cantidad de nutrientes que otorgan para su continuo crecimiento. Lo que genera esta disyuntiva es que la condición corporal de las hembras se ve afectada debido a los recursos que las hembras asignan a sus embriones en desarrollo. Mis resultados sugieren que la matrotrofía es una estrategia reproductiva costosa para las hembras.

La matrotrofía ha evolucionado de forma repetitiva en animales vivíparos, aunque también hay viviparidad lecitotrófica como en el caso de algunos lagartos (Blackburn, 1992; Ostrovsky et al. 2016). Se ha discutido ampliamente sobre los costos y beneficios que pudiese tener la matrotrofía. Dentro de los beneficios que se han sugerido de la matrotrofía se encuentran: 1) aumentar la probabilidad de supervivencia de la descendencia al menos en las primeras etapas del desarrollo (hipótesis que está fuertemente ligada con la evolución de la viviparidad) (Schrader and Travis, 2009; Pollux et al., 2014) y 2) permitirle a la hembra invertir sus recursos de forma más eficiente de manera tal que puedan modificar su inversión de acuerdo con el genotipo de sus embriones en desarrollo (selección post copulatoria) (Schrader and Travis, 2009; Pollux et al., 2014). Algunos de los costos de la matrotrofía que se han propuesto son 1) que la matrotrofía sea en realidad una “mal-adaptación” en ambientes que se caracterizan por presentar fluctuaciones en la disponibilidad de recursos debido a que las hembras no pueden conseguir recursos suficientes para alimentar a sus embriones durante todo su desarrollo (Trexler and DeAngelis, 2003, 2010) y 2) que la matrotrofía favorezca un aumento en la competencia entre miembros de la misma camada por obtener recursos de la madre (Schrader and Travis, 2009; Pollux et al., 2009; Banet and Reznick, 2010). Aún no es claro si la matrotrofía representa un beneficio o una “mal-adaptación” o bajo qué circunstancias podría ser verdaderamente beneficioso para las hembras ser matrotróficas (Schrader and Travis, 2005; Pollux et al., 2009; Furness et al., 2021). Se ha planteado una hipótesis que sugiere que existe un conflicto madre-embrión en donde el embrión influye en la forma en la que las madres destinan sus recursos, de tal forma que los embriones influyan de manera activa manipulando la interacción con la madre para obtener un mayor número de recursos (Crespi and Semeniuk, 2004). En mi trabajo encontré un costo claro de la matrotrofía al mostrar que las hembras tienen una menor condición corporal cuando destinan recursos de manera constante durante todo el desarrollo embrionario. Es posible que esto se deba a la hipótesis que mencione previamente, en donde los embriones pueden manipular de manera activa la interacción con la madre, de manera que puedan obtener un mayor número de recursos antes de su nacimiento, lo que les permitiría ser crías de mayor calidad y tendiendo como consecuencia un deterioro corporal de la madre.

Como mencioné anteriormente, varios estudios han puesto a prueba el modelo de Trexler y DeAngelis, tanto en condiciones de laboratorio (Marsh-Matthews and Deaton, 2006; Pires et al., 2007; Banet and Reznick, 2008; Banet et al., 2010; Pollux and Reznick, 2011; Itonaga et al., 2012; Van Dyke et al., 2014; Molina-Moctezuma et al., 2020) así como en condiciones naturales (Schrader and Travis, 2008, 2012; Riesch et al., 2013; Molina-Moctezuma et al., 2020), obteniendo resultados contradictorios. Nuestros resultados con hembras obtenidas en condiciones naturales muestran que no existe asociación entre la cantidad de alimento disponible y el tipo de transferencia de nutrientes. Es posible que en condiciones experimentales el factor alimento sea de suma importancia, mientras que en condiciones naturales existen otros factores que podrían estar involucrados. Se ha documentado en dos especies: *Poeciliopsis retropinna* y *Phalloceros harpagos* que las hembras que se encuentran en ambientes con altos niveles de depredación tienen altos niveles de matrotrofía comparado con hembras que se encuentran en ambientes con baja depredación (Gorini-Pacheco et al., 2018; Hagmayer et al., 2018). Los autores sugieren que los altos niveles de matrotrofía en ambientes con alta depredación permiten que las hembras conserven un rendimiento de nado óptimo para poder escapar o evadir a sus depredadores sin sacrificar su éxito reproductivo. (Gorini-Pacheco et al., 2018; Hagmayer et al., 2018). Esto sería posible ya que los embriones de las hembras matrotróficas son pequeños durante gran parte de su desarrollo, permitiendo que la hembra conserve una forma hidrodinámica mientras gesta a sus embriones (Thibault and Schultz, 1978; Pollux et al., 2009). Un estudio realizado en 2019 sugiere que altas concentraciones de oxígeno disuelto en el agua pueden afectar los niveles de matrotrofía en *P. januarius,* sin embargo, no concluyen una posible explicación para este hecho y sugieren que sus resultados pueden deberse a factores como la depredación (Santi et al., 2019).

Debido a los resultados que obtuve en el presente trabajo y las investigaciones preliminares, es evidente que no es un solo factor el que determina la cantidad de nutrientes que una hembra transfiere a sus embriones en desarrollo. Existen factores intrínsecos de la hembra, como la presencia de estructuras especializadas como las placentas foliculares (Kwan et al., 2015; Olivera- Tlahuel et al., 2019), así como factores ecológicos externos (depredación y composición del agua) que podrían facilitar o disminuir la transferencia de nutrientes de la madre a los embriones en desarrollo. Aún no se han estudiado a profundidad los aspectos ecológicos que contribuyen o afectan la presencia de matrotrofía, sería interesante evaluar de forma conjunta los efectos de la disponibilidad de alimento, la depredación y las condiciones químicas del agua sobre la matrotrofía.

V.4 Nuevas hipótesis y posibles direcciones futuras

En esta sección haré un resumen de lo que discutí con anterioridad. Me enfocaré en las nuevas hipótesis y posibles direcciones que podría tomar la investigación acerca de la matrotrofía y la superfetación.

Las hipótesis que surgen de mi primera pregunta de investigación (¿La matrotrofía y la superfetación son más prominentes en especies de tamaño pequeño?) son: 1) que la matrotrofía y la superfetación permitan iniciar de manera temprana el ciclo reproductivo permitiendo tener un mayor número de camadas a lo largo de la vida de las hembras y 2) que estas estrategias permitan reducir los tiempos de gestación y con ello favorecer un aumento en la fecundidad a lo largo de la vida (Turner, 1937; Zúñiga-Vega et al., 2007; Pollux et al., 2009; Greven, 2011; Pires et al., 2011). Sugiero que es posible evaluar ambas hipótesis de manera observacional en laboratorio, teniendo especies con diferentes grados de matrotrofía y superfetación para poder hacer una comparación entre los tiempos de gestación, número de embriones y número de camadas a lo largo de la vida entre las diferentes especies. Así se podría concluir si existen diferencias entre las hembras dependiendo de los grados de matrotrofía y superfetación.

Respecto a mi segunda pregunta de investigación (¿Los niveles de matrotrofía y superfetación pueden variar dependiendo del tamaño (edad) de las hembras?) es posible que las nuevas hipótesis consideren que ahora hay evidencia que sugiere que existe plasticidad sobre el grado de matrotrofía y superfetación que presentan las hembras a lo largo de su vida. Es indispensable continuar con la investigación para poder conocer a profundidad los mecanismos fisiológicos de las especies de la familia Poeciliidae, con el fin de comprender mejor el funcionamiento de aspectos reproductivos como la matrotrofía y la superfetación. Sería necesario en un futuro evaluar los cambios que pudiesen tener las hembras en la transferencia de nutrientes a lo largo de sus vidas reproductivas. Sería necesario realizar ultrasonidos periódicos a las hembras gestantes con el fin de medir el volumen de los embriones en desarrollo, otra medida que indicaría que tantos nutrientes transfieren las hembras (dependiendo de su edad) a sus embriones en desarrollo. Una idea que surgió de mi tesis, considerando que los niveles de matrotrofía pueden cambiar a lo largo de la vida de las hembras, es que no solo cambien los grados en lo que se presenta esta estrategia, si no que las funciones que la matrotrofía tiene podrían cambiar a lo largo de la vida de las hembras.

Por último, mi tercera pregunta de investigación (¿El alimento es un factor determinante en el grado de matrotrofía que presentan las hembras?) fue básicamente una prueba del modelo de aprovisionamiento materno. A pesar de no encontrar evidencia a favor del modelo, encontré que la matrotrofía es una estrategia que demanda demasiados recursos a las hembras. Se podría considerar a esta estrategia como una “mal adaptación” ya que es muy costosa. Otra hipótesis podría ser que el costo de esta estrategia es diferencial dependiendo del grado de especialización de las estructuras reproductivas de las hembras. Una de las direcciones futuras podría ser evaluar de manera conjunta aspectos ecológicos que podrían influir en los grados de matrotrofía que presentan las hembras, como disponibilidad de alimento, la depredación y las condiciones químicas del agua.

VI CONCLUSIONES

- A nivel de especies, la matrotrofía y la superfetación no funcionan como mecanismos para compensar las restricciones de espacio disponible dentro de las estructuras maternas particularmente en especies de tamaño menor.
- A nivel de especies, las hembras de tamaño grande son las que producen mayor cantidad de camadas (superfetación).
- A nivel de especies, la fecundidad total de las hembras pequeñas es menor que el de las hembras de mayor tamaño, por lo que la matrotrofía y la superfetación no funcionan como mecanismos para alcanzar una fecundidad similar a la de las hembras grandes.
- Las estimaciones de la señal filogenética con 91 especies de la familia Poeciliidae indican que el tamaño de las hembras, la superfetación y la matrotrofía han evolucionado mediante la acumulación gradual de cambios a través del tiempo, mientras que la fecundidad parece adaptarse rápidamente a las condiciones locales.

- A nivel poblacional las hembras de tamaño menor presentan mayores grados de matrotrofía que las hembras de tamaño mayor, lo que sugiere que la matrotrofía podría estar funcionando como mecanismo para compensar las restricciones de tamaño. Sin embargo, este patrón no es común, solo lo observé en 3 de 77 poblaciones. Aunque es un patrón poco común, encontré evidencia de que el nivel de matrotrofía puede variar a lo largo de la vida reproductiva de las hembras.
- A nivel poblacional, las hembras de tamaño más grande tienen mayor número de superposición de camadas. En este caso la superfetación no es predominante en hembras pequeñas.
- No encontré evidencia a favor del modelo de Trexler y DeAngelis con ninguna de mis aproximaciones de disponibilidad de alimento. No existe asociación entre la cantidad de alimento disponible, su variación ambiental y el tipo de transferencia de nutrientes que presentan las hembras.
- La matrotrofía es costosa. Las hembras de cinco especies del género *Poeciliopsis* con mayores grados de matrotrofía tienen una condición corporal pobre. Es posible que esto se derive de una disyuntiva fisiológica entre la cantidad de nutrientes que se transfieren a los embriones y la energía que las hembras destinan a su mantenimiento corporal.

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