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Variability in maternal provisioning and offspring quality in eastern mosquitofish (*Gambusia holbrooki*, Poeciliidae)

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Abstract

Due to their success as an invasive alien species, eastern mosquitofish (*Gambusia holbrooki*, Girard 1859) are now almost globally distributed, yet some aspects of their phenotype remain unexplored. Drawing on data from 50 native and invasive mosquitofish populations, we examined the extent of variation in offspring size, offspring fat content and maternal provisioning strategies. We tested two predictions: (1) Developmental changes of embryo mass (matrotrophy index, MI) and embryo fat content (the novel fat index, FatI) should be largely independent from one another. (2) Some of the observed variation in all four phenotypic measures should be associated with differences in local environmental factors, including a higher MI in populations from resource-rich habitats and greater offspring size at birth in H_2S-toxic habitats. Contrary to prediction 1, MI and FatI covaried to a large extent, but we found pronounced variation among populations in all four traits (offspring size: 0.408-5.480 mg; offspring fat: 1.2-22.1%; MI: 0.49-1.98; FatI: 0.24-1.50). In addition, while we found a negative effect of water temperature on MI, variation in maternal provisioning strategies and offspring quality were not significantly associated with H_2S-toxicity or environmental proxies of habitat productivity. Possibly, our snapshot proxies of resource availability did not adequately capture the salient aspects of resource abundance in sampled habitats and other, not quantified factors, might have been more important.

Zuzammenfassung

Aufgrund ihres Erfolgs als invasive gebietsfremde Art sind Östliche Moskitofische (*Gambusia holbrooki*, Girard 1859) heute fast weltweit verbreitet, dennoch bleiben einige Aspekte ihres Phänotyps unerforscht. Anhand von Daten von 50 einheimischen und invasiven Moskitofischpopulationen untersuchten wir das Ausmaß der Variation in der Größe der Nachkommen, dem Fettgehalt der Nachkommen und den Ernährungsstrategien der Mütter. Wir haben zwei Vorhersagen getestet: (1) Entwicklungsveränderungen der Embryomasse (Matrotrophie-Index, MI) und des Embryofettgehalts (der neuartige Fettindex, FatI) sollten weitgehend unabhängig voneinander sein. (2) Einige der beobachteten Abweichungen bei allen vier phänotypischen Maßen sollten mit Unterschieden in den lokalen Umweltfaktoren zusammenhängen, einschließlich eines höheren MI in Populationen aus ressourcenreichen Lebensräumen und einer größeren Nachkommensgröße bei der Geburt in H_2S-toxischen Lebensräumen. Entgegen Vorhersage 1 kovariierten MI und FatI stark, aber wir fanden ausgeprägte Variationen zwischen den Populationen in allen vier Merkmalen (Nachkommensgröße: 0,408-5,480 mg; Nachkommenfett: 1,2-22,1 %; MI: 0,49-1,98; FatI: 0,24-1,50). Darüber hinaus fanden wir zwar einen negativen Effekt der Wassertemperatur auf den MI, aber Unterschiede in den mütterlichen Versorgungsstrategien und der Qualität der Nachkommen waren nicht signifikant mit der H_2S-Toxizität oder Umweltproxies der Habitatproduktivität verbunden. Möglicherweise haben unsere Snapshot-Proxies der Ressourcenverfügbarkeit die hervorstechenden Aspekte der Ressourcenfülle in den beprobten Lebensräumen nicht angemessen erfasst, und andere, nicht quantifizierte Faktoren könnten wichtiger gewesen sein.

Riassunto

A causa del loro successo come specie aliena invasiva, il gambusia orientale (*Gambusia holbrooki,* Girard 1859) è ora distribuito quasi a livello globale, ma alcuni aspetti del suo fenotipo rimangono inesplorati. Basandoci sui dati di 50 popolazioni native e invasive di pesci mangiazanzara, abbiamo esaminato l'entità della variazione delle dimensioni della prole, del contenuto di grasso della prole e delle strategie di approvvigionamento materno. Abbiamo testato due previsioni: (1) i cambiamenti nello sviluppo della massa dell'embrione (indice di matrotrofia, MI) e del contenuto di grasso dell'embrione (il nuovo indice di grasso, FatI) dovrebbero essere ampiamente indipendenti l'uno dall'altro. (2) Alcune delle variazioni osservate in tutte e quattro le misure fenotipiche dovrebbero essere associate a differenze nei fattori ambientali locali, tra cui un MI maggiore nelle popolazioni provenienti da habitat ricchi di risorse e una maggiore dimensione della prole alla nascita in habitat tossici per H_2S. Contrariamente alla previsione 1, MI e FatI variavano in larga misura, ma abbiamo riscontrato variazioni pronunciate tra le popolazioni in tutti e quattro i tratti (dimensione della prole: 0,408-5,480 mg; grasso della prole: 1,2-22,1%; MI: 0,49-1,98; FatI: 0,24-1,50). Inoltre, mentre abbiamo riscontrato un effetto negativo della temperatura dell'acqua sull'MI, la variazione delle strategie di approvvigionamento materno e la qualità della prole non erano significativamente associate alla tossicità H_2S o ai proxy ambientali della produttività dell'habitat. Forse, i nostri proxy istantanei della disponibilità delle risorse non hanno catturato adeguatamente gli aspetti salienti dell'abbondanza delle risorse negli habitat campionati e altri fattori, non quantificati, avrebbero potuto essere più importanti.

Résumé

En raison de leur succès en tant qu'espèce exotique envahissante, les poissons-moustiques orientaux (*Gambusia holbrooki*, Girard 1859) sont maintenant distribués presque mondialement, mais certains aspects de leur phénotype restent inexplorés. A partir de données provenant de 50 populations de poissons-moustiques indigènes et invasives, nous avons examiné l'étendue de la variation de la taille et de la teneur en graisse de la progéniture ainsi que les stratégies d'approvisionnement maternel. Nous avons testé deux prédictions : (1) Les changements développementaux de la masse embryonnaire (indice de matrotrophie, MI) et de la teneur en graisse embryonnaire (nouvel indice de graisse, FatI) devraient être largement indépendants les uns des autres. (2) Une partie de la variation observée dans les quatre mesures phénotypiques devrait être associée à des différences dans les facteurs environnementaux locaux, y compris un MI plus élevé dans les populations provenant d'habitats riches en ressources et une plus grande taille de la progéniture à la naissance dans les habitats toxiques au H_2S. Contrairement à la prédiction 1, le MI et le FatI covarient dans une large mesure, mais nous avons trouvé une variation prononcée entre les populations pour les quatre traits (taille de la progéniture : 0,408-5,480 mg ; graisse de la progéniture : 1,2-22,1% ; MI : 0,49-1,98 ; FatI : 0,24-1,50). De plus, bien que nous ayons trouvé un effet négatif de la température de l'eau sur l'IM, la variation des stratégies d'approvisionnement maternel et la qualité de la progéniture n'étaient pas significativement associées à la toxicité du H_2S ou aux indicateurs environnementaux de la productivité de l'habitat. Il est possible que nos approximations instantanées de la disponibilité des ressources n'aient pas capturé de manière adéquate les aspects saillants de l'abondance des ressources dans les habitats échantillonnés et que d'autres facteurs, non quantifiés, aient été plus importants.

INTRODUCTION

Offspring quality is an essential determinant of parental and offspring fitness (Goodenough et al. 2008; Rollinson and Hutchings 2013); yet the pathways leading to increased or decreased offspring quality are often species- and context-dependent (Brooks et al. 1997). Offspring size is generally considered a good indicator of offspring quality, and its influence on offspring performance,as well as its trade-off with offspring number, have been the focus of life-history research for decades (Smith and Fretwell 1974; Khokhlova et al. 2014). For example, larger offspring can have a survival advantage in highly competitive (e.g., Bashey 2006) or extreme environments (e.g., H_2S-toxic waters; Riesch et al. 2016). Still, other parameters, such as body colouration or the amount/concentration of body lipids (fats), may also serve as proxies of offspring quality due to their association with fitness components (Khokhlova et al. 2014; Hagmayer et al. 2018). For example, the amount of fat resources available to the offspring can be linked to enhanced performance and survival, independent of offspring size (Khokhlova et al. 2014). Multiple internal (e.g., maternal life-history traits) and external factors (e.g., resource availability and type of environment the offspring experience) influence offspring quality and consequently, offspring performance (Marshall et al. 2010; Jørgensen et al. 2011; Pollux and Reznick 2011; Hagmayer et al. 2018).

Maternal nourishment is one of the main sources of variation in offspring quality by affecting offspring development and survival (Roff 2002; Marsh-Matthews 2011; Ota and Kohda 2014). The extent and mode of maternal provisioning varies widely across taxa, from mothers fully provisioning their eggs before they are fertilized (lecithotrophy or yolk-feeding) to mothers transferring nutrients needed for the developing embryos post-fertilization (matrotrophy or mother-feeding; Marsh-Matthews 2011; Pires et al. 2011). The extent of matrotrophy itself may vary from limited amounts of nutrients being transferred (incipient matrotrophy) to mothers providing all nutrients essential to embryonic development (substantial matrotrophy; Marsh-Matthews 2011; Pires et al. 2011).

To date, maternal provisioning in fish of the family Poeciliidae has been evaluated almost exclusively using the matrotrophy index (e.g., Reznick et al. 2002; Hagmayer et al. 2018; Molina-Moctezuma et al. 2020; Furness et al. 2021). If mothers provided all resources for embryonic development prior to fertilization (lecithotrophy), their embryos are expected to lose 25-40% of the initial mass during development ($0.60 < MI \leq 0.75$; Marsh-Matthews 2011; Pires et al. 2011). By contrast, in the case of post-fertilization maternal provisioning (matrotrophy), embryos are expected to lose less or even gain mass during development (moderate matrotrophy: $0.75 < MI < 5$; extensive matrotrophy: $MI > 5$; Reznick et al. 2002; Marsh-Matthews 2011; Pires et al. 2011). Yet, other embryo characteristics, such as the amount of available body fat, might also reflect variation in maternal provisioning. Previous work has usually reported these measurements as proxies of offspring quality, but they are rarely evaluated in relation to maternal provisioning (Brooks et al. 1997; Khokhlova et al. 2014). To address these questions, we leverage a unique dataset comprising 50 populations of *Gambusia holbrooki* from their native and invasive distributional ranges. We examined maternal provisioning by calculating the well-established matrotrophy index (MI; Marsh-Matthews 2011; Pires et al. 2011), which is based on embryo dry mass. We also investigated a different aspect of offspring provisioning (and offspring physiology) based on embryo fat values by calculating the novel embryo fat index (FatI) (for more details see Methods). In short, FatI is a measure of how embryo fat storage changes during development, with values < 1 suggesting that neonates have lower lipid contents than oocytes at fertilization as a result of embryos metabolising the fat stored within the yolk. By contrast, FatI values ≈ 1 suggest that embryos have maintained the same amount of lipid reserves during their development, either by moving lipids directly from the yolk into their body fat reserves, or by converting additional resources obtained from their mother. Finally, FatI > 1 equate to neonates having higher lipid contents than unfertilized oocytes, be it through resource conversion from yolk stores and/or maternal provisioning.

We asked the following questions: First, to what extent do MI and FatI represent independent maternal provisioning strategies? Second, what is the influence of environmental parameters on offspring size at birth, fat content at birth, as well as MI and FatI? Previous studies in the genus *Gambusia* on a smaller geographic scale found offspring size to vary among populations and were able to identify several environmental variables (including H_2S-toxicity) most likely to be associated with these differences (e.g., Marsh-Matthews et al. 2005; Riesch et al. 2016, 2018; Santi et al. 2020; Hulthén et al. 2021). For example, Riesch et al. (2016) found *Gambusia* spp. to produce larger neonates in H_2S-rich environments. Moreover, the Trexler and DeAngelis (2003) model predicts that matrotrophy should be favoured in environments with high and constant levels of resource availability, which has received support from empirical studies in multiple poeciliid species (Riesch et al.

2013; Molina-Moctezuma et al. 2020). Based on these and previous considerations, we formulated two predictions: (1) MI and FatI could represent independent routes of influencing offspring quality, and so population-specific patterns of these two indices (and the associated estimates for offspring size and fat content at birth) should show no (or only weak) covariance. (2) Some of the variation in maternal provisioning strategies and offspring quality (i.e., size and fat content at birth) should be linked to differences in local environmental vari-ables. For example, MI should increase in habitats with greater resource availability (i.e., chlorophyll *a* and phycocyanin), while offspring size at birth should be greater in H_2S-toxic compared to non-toxic habitats.

MATERIAL AND METHODS

Sample collection and laboratory procedures.

We re-examined data originally collected to address different questions regarding mosquitofish life-history strategies (Riesch et al. 2016, 2018; Santi et al. 2020). Specifically, during August 2011 and May 2012, we collected pregnant *G. holbrooki* females at 16 distinct locations along the Atlantic Coast of the United States (native range; Riesch et al. 2016, 2018). During the September 2013 and July/August 2017, we collected pregnant females at 34 sites in Spain, France and Italy (invasive range; Santi et al. 2020; Fig. 1). We used dip nets, and occasionally a seine net (2 mm mesh size), euthanized the fish with an overdose of clove oil, and then preserved them in 95% ethanol. Samples collected in 2012 and a handful of populations collected in 2011, however, were fixed in 10% formaldehyde (Supplementary Information, Table S1). We measured water temperature (°C) in situ using a YSI 85 meter (Yellow Springs, Ohio, USA) in 2011 and 2012, and a Hach Rugger Feld Kit (Hach, Loveland, Colorado, USA) in 2013 and 2017. Additionally, phycocyanin and chlorophyll *a* were quantified during our 2011 (native populations) collection using an AquaFluor fluorometer (Turner Designs, Sunnyvale, CA). These measurements estimate cyanobacteria and total algal biomass, respectively, and consequently capture aspects of resource availability and overall habitat productivity (Supplementary Information, Table S1). Previously, developing offspring had been removed from preserved females, had been counted and their stage of development classified following well-established protocols (Reznick and Endler 1982; Riesch et al. 2013, 2016) before we weighed embryos, extracted all soluble fat via emersion in petroleum ether, and then reweighed them. Specifically, each embryo developmental stage was defined on a numerical scale ranging from 0 (fully yolked but unfertilised oocytes) to 50 (mature embryo; following Riesch et al. 2011). For the current study, we extracted the following variables from the datasets: offspring dry mass (mg) and offspring fat content (%). To meet statistical assumptions of normality of model residuals, embryo dry masses were $\log_{10}$-transformed.

Maternal provisioning and matrotrophy index

The matrotrophy index (MI) is defined as the ratio between offspring dry mass at birth and oocyte dry mass at fertilization (Wourms et al. 1988; Reznick et al. 2002; Marsh-Matthews 2011; Pires et al. 2011). These values were estimated for each population by utilizing the slopes and intercepts from linear regressions between the means of each brood's embryo dry mass (dependent variable) and embryo developmental stage (explanatory variable). Furthermore, to investigate the extent of variation around the MI values for each population, we created artificial 95% confidence intervals (CI) for each value of MI. For this, we used the 95% CI calculated for the slopes from embryo dry mass vs. embryo developmental stage regressions described above. We then used the calculated 95% CI values as new slopes to predict offspring dry mass at birth for each population (essentially providing us with offspring mass at birth if the lower or upper bounds of the 95% CI were true). We now calculated two additional MI values for each population using the new 95%-CI-based estimates of offspring mass at birth. Whenever MI > 0.75, we followed Reznick et al. (2002) to test if this differed significantly from an MI of 0.75 by comparing the slope obtained from the embryo mass vs. development regression with that obtained if embryos lost 75% of their initial dry mass during development using one-sample t-tests.

The novel fat index

We also calculated the 'fat index' (FatI) as the ratio between offspring fat content (%) at birth and oocyte fat content (%) at fertilization, for which we used the slopes and intercepts from linear regressions between offspring fat content (dependent variable) and stage of development (explanatory variable). Using the same approach as outlined above for MI, we also calculated 95% confidence intervals for each FatI value. However, due to the presence of many outliers and abnormalities (i.e., high number of embryos from the invasive range with virtually zero percent body fat, possibly reflecting the specific conditions during collection and sample storage), we excluded individuals from the invasive range from this particular analysis.

Effects of H_2S-toxicity

Some native populations were fixed in formalin (N = 10) and some in ethanol (N = 7). Furthermore, the formalin-preserved populations were sampled from H_2S-toxic springs (N = 5) and from nearby non-toxic habitats (N = 5) in Florida (Riesch et al. 2016). To examine potential effects of H_2S on variation in MI, FatI, offspring size at birth and fat content at birth of native formalin-preserved populations, we ran several generalized linear models (GLMs), coding 'toxicity' and 'year-nested-within-toxicity' as explanatory variables to control for the fact that some habitats were sampled across two consecutive years. For the analyses with FatI and offspring dry mass at birth, a gamma error distribution and log link function accounted for a zero-bound data distribution pattern showing a skew in the positive direction. We instead ran GLMs with gaussian family for the analyses with MI and offspring fat content at birth. All statistical analyses were performed using the software R x64 3.5.1 (R Development Core Team 2019). We screened all models for overdispersion.

Effects of temperature and resource availability on maternal provisioning and offspring quality

To avoid potential confounding effects of the method of preservation on the results of the analyses investigating the effects of environmental and geographic parameters on MI, FatI, offspring dry mass and fat content at birth, these analyses were only run for the ethanol-preserved populations. For MI and offspring dry mass at birth, we compiled two separate data sets: (1) native and invasive populations, for which information on water temperature was available, and (2) native populations only, for which resource availability was quantified. For the first subset of populations (N = 41), a principal components analysis (PCA) was performed on z-transformed water temperature and information on geographic locations (latitude and longitude). We retained one principal component (PC), which had an eigenvalue >1 and explained > 70% of the total variance (Supplementary Information, Table S2). For the second subset we directly used our two proxies for resource availability. For the analyses of FatI and fat content at birth, environmental parameters were available for all ethanol-preserved native populations (N = 7; Supplementary Information, Table S1). We ran another PCA on the matrix of z-transformed environmental (water temperature, chlorophyll a and phycocyanin) and geographic data and retained the first two PCs with eigenvalues > 1.0, cumulatively explaining > 80% of the total variance (Supplementary Information, Table S3).We ran GLMs with Gaussian family for models with MI and offspring dry mass at birth as the dependent variable and either the PC or our measures of resource availability as predictor variables. For the analyses with FatI and offspring fat content at birth, we instead ran GLMs with Gaussian family with these traits as the dependent variable and the two environmental PCs as independent variables. Model validation was checked and fitted using the DHARMa R package (v0.4.5; Hartig, 2022). As multiple comparisons of data were involved, sequential false discovery rate (FDR) corrections were applied to all p-values (Benjamini and Hochberg 1995)

RESULTS

Population variation in maternal provisioning and offspring quality

An overview of the variation uncovered in our estimates of maternal provisioning and offspring quality among the 50 populations included in this study is provided in Table 1. We found pronounced variation in MI, with some populations exhibiting MI-values characteristic of lecithotrophy ($0.47 < MI \leq 0.75$), while females in other populations appeared to employ moderate amounts of post-fertilization maternal provisioning ($0.75 < MI < 1.98$); no population exhibited extensive matrotrophy though ($MI > 5$). One-sample t-tests revealed that females from 19 populations (38%) exhibited a degree of maternal provisioning that significantly exceeded the lecithotrophy threshold value of MI = 0.75 (Table 1).

Likewise, changes in embryo fat content over the course of development (FatI) varied widely among native populations, with values of FatI ranging from 0.24 to 1.50 (Table 1). However, MI and FatI exhibited a statistically significant correlation

Population	Embryo stages	MI (95% CI)	Estimated dry mass at birth (mg)	FatI (95% CI)
United States				
Charleston, SC	2-40	0.64 (0.44, 0.93)	1.033	0.51 (0.05, 0.97)
Ditch off Hwy 98, FL	2-50	0.72 (0.57, 0.90)	1.958	1.02 (0.32, 1.03)
Ditch St Marks, FL	2-50	**1.03 (0.88, 1.22)**	2.509	0.81 (0.67, 0.95)
Green Springs, FL	2-50	**1.27 (1.11, 1.47)**	5.480	1.50 (1.27, 1.73)
Hwy 45, NC	2-50	0.50 (0.42, 0.61)	0.721	0.61 (0.31, 0.91)
Lake Lilly, NJ	2-50	0.58 (0.42, 0.61)	0.645	0.39 (0.11, 0.66)
Lake Monroe, FL (2011)	2-50	**1.03 (0.85, 1.24)**	1.318	0.71 (0.32, 1.10)
Lake Monroe, FL (2012)	2-50	**0.88 (0.77, 1.01)**	1.871	0.55 (0.39, 0.72)
Melbourne, FL (formalin)	2-50	**0.96 (0.83, 1.11)**	2.041	1.20 (0.99, 1.41)
Melbourne, FL (ethanol)	2-50	0.72 (0.56, 0.93)	1.725	0.88 (0.55, 1.38)
Newport Springs, FL (2011)	2-50	0.72 (0.58, 0.90)	1.387	0.34 (-1.00, 1.68)
Newport Springs, FL (2012)	2-50	0.62 (0.53, 0.73)	2.478	0.92 (0.74,1.10)
Panacea Mineral Springs, FL (2011)	2-50	0.66 (0.54, 0.79)	1.196	0.24 (-0.31, 0.79)
Panacea Mineral Springs, FL (2012)	2-50	0.91 (0.72, 1.14)	2.575	0.87 (0.71, 1.03)
Rehoboth Beach, DE	5-45	0.70 (0.57, 0.86)	0.632	0.61 (0.33, 0.90)
St Simon, GA	10-45	0.49 (0.31, 0.78)	1.013	0.62 (0.20, 1.03)
Suffolk, VA	2-50	0.71 (0.54, 0.94)	1.030	0.75 (0.29, 1.21)
Italy				
Comacchio	2-50	**1.00 (0.87, 1.16)**	0.845	-
Ditch Marina di Grosseto	30-45	**1.53 (0.75, 3.11)**	0.633	-
Lago di Bolsena	2-25	**1.10 (0.82, 1.48)**	1.025	-
Lago di Fimon	2-50	**1.13 (0.85, 1.51)**	0.916	-
Lago di Garda	2-25	**1.98 (1.14, 3.44)**	1.242	-
Marina di Grosseto	2-30	0.82 (0.45, 1.49)	0.691	-
Porto Cesareo	2-50	**1.15 (1.02, 1.29)**	1.196	-
Torre Castiglione (2013)	2-50	**1.00 (0.85, 1.18)**	1.218	-
Torre Castiglione (2017)	2-45	0.87 (0.67, 1.13)	1.028	-
France				
Arles	5-50	0.73 (0.52, 1.02)	0.852	-
Avignon	2-50	0.58 (0.51, 0.66)	0.408	-
Briere	2-50	0.64 (0.47, 0.86)	0.671	-
Garonne	2-50	**0.87 (0.75, 1.00)**	0.593	-
La Charente	2-50	**1.02 (0.82, 1.28)**	0.662	-
La Ligneron	2-50	0.73 (0.65, 0.83)	0.667	-
Montpellier	2-50	0.76 (0.64, 0.91)	0.593	-
Spain				
Almanzora	2-50	**0.93 (0.73, 1.20)**	0.915	-
Barcelona	2-50	**1.25 (1.05, 1.50)**	1.084	-
Doñana North	2-50	0.78 (0.53, 1.15)	0.936	-
Ebro Delta	2-50	0.59 (0.52, 0.67)	0.523	-
El Palmar	2-50	**1.09 (0.77, 1.53)**	0.719	-
Guadalquivir (2013)	2-50	0.89 (0.67, 1.18)	0.953	-
Guadalquivir (2017)	2-50	0.72 (0.46, 1.12)	0.936	-
Guadiaro	2-50	0.47 (0.38, 0.58)	0.504	-
Gualta	2-45	0.82 (0.63, 1.06)	0.703	-
Lebrija	2-45	**0.98 (0.74, 1.30)**	1.155	-
Rio Ebro - Tortosa	10-50	0.59 (0.47, 0.74)	0.626	-
Rio Segura	5-50	0.53 (0.36, 0.78)	0.607	-
Riu Ter	20-50	0.67 (0.46, 0.98)	0.787	-
Rio Vaca	2-45	0.96 (0.59, 1.54)	0.732	-
Rio Xuquer (2013)	2-50	**0.90 (0.78, 1.04)**	0.952	-
Rio Xuquer (2017)	2-45	0.71 (0.48, 1.03)	0.633	-
Sagunt	2-50	0.51 (0.36, 0.72)	0.759	-
Zadorra	2-50	0.76 (0.65, 0.89)	0.485	-

Estimated fat at birth (%)	t-value	Sample size	Sig.
6.70	-	-	-
16.20	-	-	-
14.40	3.90	48	**<0.0005**
22.10	7.68	35	**<0.0005**
10.50	-	-	-
15.20	-	-	-
5.10	3.36	54	**<0.001**
10.60	2.47	35	**<0.01**
17.30	3.49	78	**<0.0005**
12.20	-	-	-
1.20	-	-	-
16.50	-	-	-
2.30	-	-	-
15.10	1.69	32	NS
7.80	-	-	-
8.50	-	-	-
12.10	-	-	-
-	4.02	53	**<0.0005**
-	2.24	12	**<0.025**
-	2.64	47	**<0.01**
-	3.05	20	**<0.005**
-	3.56	42	**<0.0005**
-	0.31	20	NS
-	7.45	66	**<0.0005**
-	3.54	49	**<0.001**
-	1.19	22	NS
-	-	-	-
-	-	-	-
-	-	-	-
-	1.97	46	**<0.05**
-	2.83	64	**<0.005**
-	-	-	-
-	0.18	48	NS
-	1.87	21	**<0.05**
-	5.77	34	**<0.0005**
-	0.24	18	NS
-			
-	2.28	20	**<0.025**
-	1.21	49	NS
-	-	-	-
-	-	-	-
-	0.69	19	NS
-	2.04	15	**<0.05**
-	-	-	-
-	-	-	-
-	-	-	-
-	1.16	11	NS
-	2.59	60	**<0.01**
-	-	-	-
-	-	-	-
-	0.24	91	NS

Table 1. Range of embryonic stages (following Riesch et al., 2011), MI (matrotrophy index), FatI (fat index), estimated dry mass (mg) and fat content (%) at birth for populations of *Gambusia holbrooki* sampled across their invasive and native range. FatI values were calculated only for populations sampled in the native range. CI are 95% confidence intervals calculated using the 95% confidence intervals obtained for the slopes from regressions of embryo dry mass and embryo fat content against embryonic stage of development. Populations of *G. holbrooki* with an MI > 0.75 were tested for significant deviation from lecithotrophy (i.e., MI = 0.75; slope = -0.00227161) via one-sample t-tests; significant p-values and associated MI in bold.

(Spearman's $r_s = 0.49$, $p = 0.045$; Supplementary Information, Fig. S1). Specifically, in four populations either embryo dry mass or fat content increased during development while the other variable remained comparatively unaffected (i.e., values ≈ 1), and in one population, embryos gained both mass and fat during their development (i.e., both values > 1). In most populations (N = 12), however, embryos lost both mass and body fat over the course of gestation (i.e., both values < 1; Table 1; Fig. 2).

Offspring dry mass and fat content at birth also varied considerably between populations, with the former ranging from 0.408 to 5.480 mg and the latter from 1.2% to 22.1% (Table 1). When testing for a potential association between estimated dry mass and fat content at birth, we again found a moderate positive correlation (N = 17, Spearman's $r_s = 0.57$, $p = 0.017$), indicating that larger offspring tended to have greater fat reserves.

Effects of H_2S-toxicity

While we did not discover significant effects of H_2S-toxicity and 'year-nested-within-toxicity' on MI (toxic: 0.84 ± 0.27; non-toxic: 0.92 ± 0.13; Figure 3a), we found significant effects of these factors on FatI (Table 2, Figure 3b). Specifically, populations from toxic habitats exhibited lower FatI values than populations from non-toxic habitats (toxic: 0.77 ± 0.51; non-toxic: 0.86± 0.26). Moreover, post-hoc analysis revealed that populations sampled in 2012 from toxic-habitats had higher FatI values compared to the populations sampled in 2011 in toxic habitats. By contrast this pattern was reversed with respect to populations from non-toxic habitats. H_2S-toxicity had no statistically significant effect on offspring dry mass, even though the observed pattern qualitatively showed the expected direction (toxic: 2.62 ± 1.71; non-toxic: 1.94 ± 0.43). However, we found a significant effect of 'year-nested-within-toxicity' indicating significant variation between years for toxic and non-toxic populations (Table 2, Figure 3c). Specifically, neonates of non-toxic and toxic-populations sampled in 2012 were larger than neonates of non-toxic and toxic-populations sampled in 2011. Finally, we identified a significant difference between populations from toxic and non-toxic habitats in offspring fat content at birth, with neonates from non-toxic habitats exhibiting greater fat content (toxic: 11.44 ± 9.23%; non-toxic: 12.72 ± 4.96%). Again, we found a significant effect of the term 'year-nested-within-toxicity', with neonates of toxic and non-toxic populations sampled in 2012 having more fat reserves compared to neonates of populations sampled from toxic and non-toxic habitats in 2011 (Table 2; Figure 3d).

Effects of environmental/geographic parameters on MI but not on FatI and offspring quality

The PC condensing water temperature and geographic parameters had a significant effect on MI after FDR-correction (Table 3; Supplementary Information, Table S2). *Post-hoc* evaluations of the associations between MI and the actual variables revealed that this effect was mostly due to a positive correlation with longitude ($r_S = 0.53$), a weaker positive correlation with latitude ($r_S = 0.38$), and a weak, negative correlation with temperature ($r_S = -0.29$; Fig. 4, Supplementary Information, Table S2). By contrast, we did not find any significant effects of environmental and geographic variables on FatI (Table 3, Supplementary information, Table S3). Similarly, we detected no significant effects of environmental and geographic variables on offspring dry mass and fat content at birth ($p \geq 0.13$ for all models; Table 4).

DISCUSSION

Examining offspring quality and maternal provisioning strategies for 50 populations of eastern mosquitofish sampled across their native and invasive range, we found considerable variation that mostly did not match our *a priori* predictions. Moreover, at least some variation in MI appears to be influenced by water temperature and geography.

Relationship between MI and the novel fat index (FatI)

In partial disagreement with our prediction 1, population-wise differences in MI and FatI showed moderate covariance, and offspring size at birth was also moderately correlated with fat content at birth. This suggests that the two different routes of influencing higher offspring quality at birth-increasing body mass and/or fat reserves-are often employed in combination. A higher proportion of body fat has been suggested to improve the survival of fish neonates and to result in competitive superiority, as this energy resource can be mobilized quickly and effectively (Berg et al. 2001). Both variables might, therefore, partially reflect complementary aspects of offspring quality. However, a previous study by Riesch et al. (2011) on two

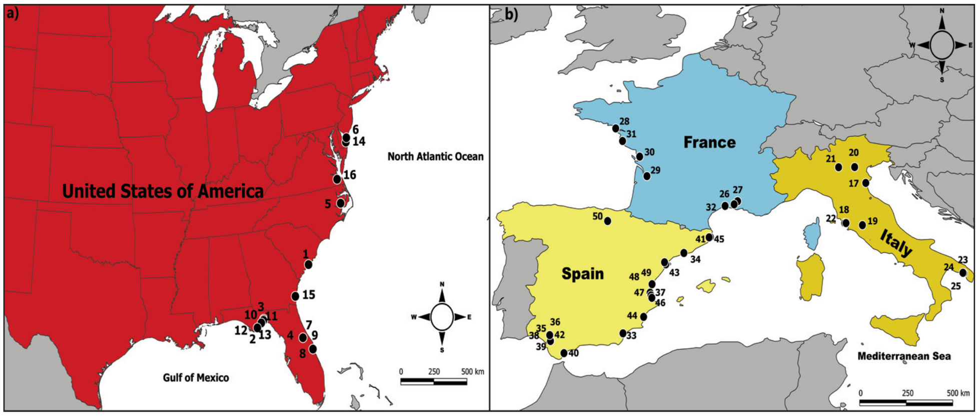

Fig. 1 Map of the sampling sites of *Gambusia holbrooki* in the United States (a) and Europe (b). The maps were generated using QGIS 3.2.

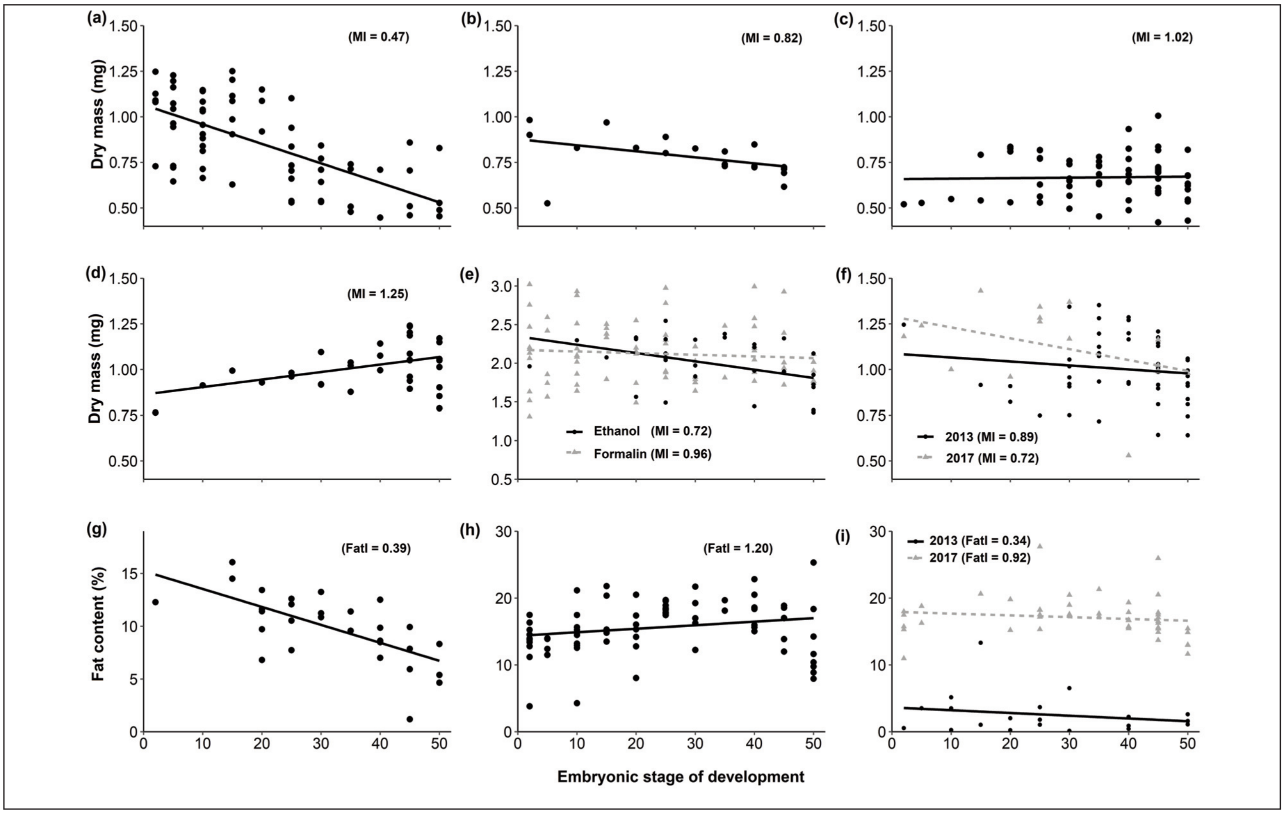

Fig. 2 Scatter plots depicting embryo dry mass versus embryonic stage of development for (a) the population of Guadiaro (lecithotrophy), (b) the population of Gualta (moderate matrotrophy), (c) the population of La Charente (moderate matrotrophy), (d) the population of Barcelona (moderate matrotrophy), (e) the population of Melbourne (ethanol-preserved: black line with circles; formalin-preserved: grey line with triangles, moderate matrotrophy) and for (f) the populations of Guadalquivir (sampled in 2013: black line with circles, moderate matrotrophy; sampled in 2017: grey line with triangles, lecithotrophy); Scatter plots depicting embryo fat content versus embryonic stage of development for (g) the population in Lake Lilly (low FI), (h) the population at Melbourne (high FI) and (i) the population in Newport Springs (sampled in 2011: black line with circles; sampled in 2012: grey line with triangles). The populations above were chosen to showcase the variability of maternal provisioning and embryonic fat content during development.

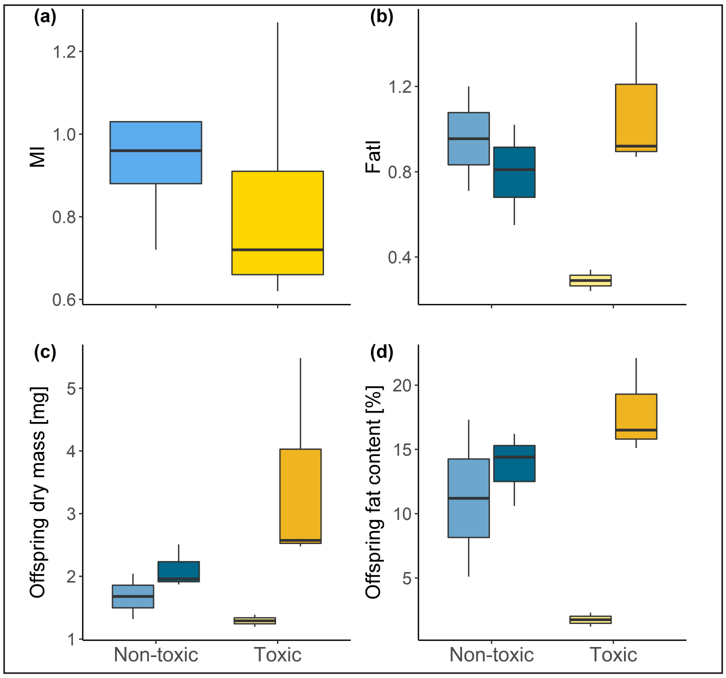

Fig. 3 Box plots showing differences in (a) MI, (b) FatI, (c) offspring dry mass and (d) offspring fat content at birth between native Florida populations sampled from toxic (yellow) and non-toxic (blue) habitats. Light and dark shading indicate life-history estimates for populations sampled in 2011 and 2012, respectively.

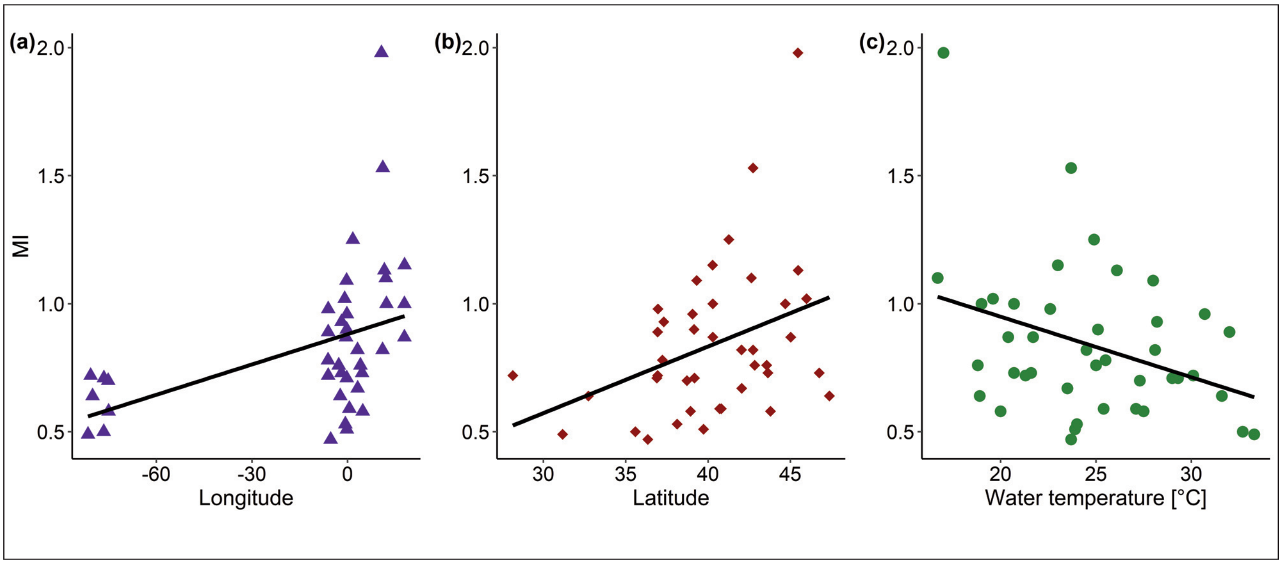

Fig. 4 Scatterplots depicting effects of (a) longitude, (b) latitude and (c) water temperature [°C] on MI.

species of *Poecilia* indicated the two could be largely independent, and some populations in the present study also showed clear signs that both processes can be uncoupled (i.e., one variable increased and the other decreased).

FatI and pathways to increase offspring quality

The fat content we extracted from early-stage embryos likely represents fat reserves stored within the yolk. However, at some point during embryo development, embryos will actively start converting available resources into body fat (Fraher et al. 2016). While it remains unknown exactly when poeciliid embryos start this conversion, important phenotypic changes-including emergent body pigmentation-occur around stage 25 (Riesch et al. 2011), and we speculate that changes in embryo physiology (lipogenesis and fat deposition) might also be activated at this stage. If this scenario was true, then the fat extracted from mid- to late-stage embryos might be the result of a combination of maternal provisioning and processes controlled by the embryos themselves, and this could be seen as an indication of parent-offspring conflict (Trivers 1974). Indeed, since *G. holbrooki* are primarily lecithotrophic, it seems unlikely that FatI-values substantially greater than 1.0 merely reflect significant maternal nourishment, suggesting that embryos convert yolk resources into body fat reserves at a relatively high rate. This raises the question of how this pattern unfolds in species with extensive matrotrophy: do mothers of those species have more control over embryonic body fat content as well? In *Poeciliopsis retropinna*, for example, fat content also only increased towards the end of embryo development (Hagmayer et al. 2018). While future research is needed to unravel patterns of FatI in more species with different maternal provisioning strategies, evaluating the novel FatI in combination with the MI likely allows for a more nuanced understanding of how offspring quality is achieved in livebearing fishes.

Population differences in maternal provisioning

The genus *Gambusia* has been classified as primarily lecithotrophic (based on analyses of the MI; Pollux et al. 2014; Olivera-Tlahuel et al. 2015), but some studies suggest they are instead capable of at least small amounts of post-fertilization maternal nourishment of developing embryos, i.e., moderate matrotrophy (Thibaut et al. 2002; Marsh-Matthews et al. 2010; Riesch et al. 2013). Congruent with this, our analyses using the MI suggest varying degrees of low-level maternal provisioning in eastern mosquitofish. Specifically, only 45% of populations were found to be strictly lecithotrophic, while 55% showed a low degree of matrotrophy. Moreover, 38% of the populations showed MI-values that differed significantly from the threshold-value characteristic of lecithotrophy (MI 0.75; Reznick et al. 2002), with 68% of these showing MI ≥ 1.0. This suggests that previous classifications of species (or even entire genera) as either lecithotrophic or matrotrophic, based on relatively few population replicates, might have been premature. Our current findings, therefore, add to the growing body of literature on population-level variation in offspring provisioning strategies, as well as temporal variation, within the family Poeciliidae (e.g., *Poecilia latipinna*: Trexler 1985, 1997; *Heterandria formosa*: Schrader and Travis 2005; *Gambusia hubbsi*: Riesch et al. 2013; *Poeciliopsis gracilis* and *P. infans*: Molina-Moctezuma et al. 2020).

Environmental effects on maternal provisioning

We uncovered a significant effect of H_2S-toxicity on differences in FatI between native *G. holbrooki* populations, with populations from toxic habitats showing lower FatI values than those from non-toxic habits. However, we did not find evidence of toxicity driving population differences in MI, suggesting that toxicity has a different effect on both aspects of maternal provisioning and embryo physiology. Specifically, our results indicate that in sulfidic habitats mosquitofish display similar degrees of maternal provisioning (as estimated from embryonic mass loss) to those observed in non-sulfidic habitats, whereas the rate by which embryos convert resources into fat content (as estimated via FatI) is strongly influenced by H_2S concentrations. These findings are in contrast with previous work of Riesch et al. (2011) who compared patterns of embryonic development between two species of *Poecilia* from toxic and non-toxic habitats. However, we cannot rule out that these patterns were an artefact of the low sample size (we tested toxicity effects on only 10 native *G. holbrooki* populations). Furthermore, the finding of a significant variation in FatI between years of sampling suggests that other factors that we did not quantify could have masked or even overriden the response to toxicity. According to the Trexler and DeAngelis (2003) model, matrotrophy should evolve in environments with continuous and abundant resource availability. However, our data did neither support the Trexler and DeAngelis (2003) model nor our prediction 2, as we found no association between variation in MI or FatI and our proxies of habitat productivity (i.e., chlorophyll *a* and phycocyanin). Our results also contrast with several studies on other poeciliids (e.g., Marsh-Matthews and Deaton 2006; Riesch et al, 2013; Tobler and Culumber 2019; Molina-Moctezuma et al. 2020) but are in accordance with a recent study of Furness et al. (2021), in which no association between MI and proxies of resource availability was found. While it is tempting to argue that variation in maternal provisioning in *G. holbrooki* might be largely independent of variation in resource availability, we think this is again more likely the result of small sample size ($N = 7$). Also, we considered single-point measures of phycocyanin and chlorophyll *a* as proxies for resource availability, while other studies investigating the relationship between resource availability and maternal provisioning had estimated additional parameters, such as zooplankton and phytoplankton densities (Riesch et al. 2013). It is likely that our measures did not fully capture (long-term) resource abundance in the sampled habitats. Due to the broad diet of eastern mosquitofish (e.g., Langerhans et al. 2021; Pirroni et al. 2021), assessing a wider range of parameters related to resource availability likely provides more reliable estimates. Our analyses uncovered significant associations between MI and longitude (positive), latitude (positive), and water temperature (negative). Water temperature has been linked to latitudinal variation in other studies on life-history variation of our study species (Riesch et al. 2018). Elevated environmental temperatures in southern latitudes bring about higher metabolic rates (e.g., Clarke and Fraser 2004), and higher energy expenditure of females in those populations could partially explain the weak associations between MI and both water temperature and latitude. The observed association between MI and longitude, however, was stronger. Given the west (USA, native range)/east (Europe, invasive range) dichotomy in our data set, and that the range of invasive European populations is shifted into higher latitudes, the patterns for latitude and longitude mainly reflect increased rates of maternal provisioning in invasive (European) populations. Potentially, this finding could be related to an overall colder climate and consequently, lower metabolic rates in the invasive range. Another, not mutually exclusive explanation, would be that the observed patterns are driven by different predatory and competitive regimes between native and invasive populations, as species richness in freshwater habitats in Europe differ from those of eastern North America, and climatic effects on species richness are also stronger (Griffiths et al. 2014). Unfortunately, we do not have reliable data on population densities and co-habiting predators for populations from the invasive range to directly evaluate this.

Environmental effects on offspring quality

There are several examples of how environmental conditions influence offspring quality in fish (e.g., Hagmayer et al. 2018; Santi et al. 2020). For example, *G. holbrooki* and other poeciliid fishes from H_2S-toxic habitats were reported to produce larger offspring, often with greater fat reserves (Riesch et al. 2014, 2016). Here, contrary to our expectations, we found no significant effects of environmental (including toxicity) and geographic variables on offspring size at birth but a significant effect of toxicity on offspring fat content at birth. However, in contrast with above studies, we found offspring fat content at birth to be lower in H_2S-toxic habitats compared to non-toxic habitats, and toxicity effects to be significantly influenced by the year of sampling. These patterns could be partially due to Newport Springs and Panacea Mineral Springs showing only a weak signal of phenotypic adaptation to toxicity and few signs of genetic differentiation from surrounding non-toxic habitats (Riesch et al. 2016). Moreover, we tentatively argue that our data set included more levels of environmental variation than we were able to capture here, and this may have induced unexpected signals, e.g., increased FatI-values in certain populations, thereby obscuring the expected parameter-related signals (such as those resulting from H_2S). As with FatI, we also found significant temporal variation in offspring size and offspring fat content at birth for toxic and non-toxic sites. This could be in indication of seasonal variation (we sampled late in the reproductive season in 2011, and relatively early in the reproductive season in 2012) in reproductive strategies (Edwards et al. 2010; Rius et al. 2019), but could also be an indication of temporal changes in toxicity within toxic habitats, potentially linked to changes in groundwater levels. Due to low sample size and *p*-value correction, we generally interpret our results on how environmental fac-

Table 2. Wald Chi-Square statistics for generalised linear models (GLMs) investigating the influence of H_2S water toxicity and year of sampling on (a) MI, (b) FatI, (c) offspring dry mass at birth and (d) fat content at birth across the native range. (b & c) GLMs with a gamma error distribution and log link function; (a & d) GLMs with gaussian family. Significant p-val-ues are highlighted in bold.

Variable	df	$\chi 2$	*p*
a) MI model			
Toxicity	1	44.936	0.146
Year (Toxicity)	2	1.994	0.369
b) FatI model			
Toxicity	1	14.922	**<0.001**
Year (Toxicity)	2	22.737	**<0.001**
c) Offspring size model			
Toxicity	1	0.658	0.417
Year (Toxicity)	2	12.044	**0.002**
d) Offspring fat content model			
Toxicity	1	4.510	**0.034**
Year (Toxicity)	2	16.195	**<0.001**

tors affect maternal provisioning and offspring quality with caution. Although FDR correction reduces the likelihood of false positives (i.e., type I errors), it does so at the expense of type II errors (i.e., false negatives). For instance, even though offspring dry mass at birth was greater in toxic compared to non-toxic habitats, the effect was not significant. As such, our findings contradict previous work, e.g., on members of the genera *Poecilia* and *Gambusia* from toxic and non-toxic habitats, reporting increased offspring size in toxic habitats (Riesch et al. 2011, 2014, 2016). Indeed, detoxification of H_2S is energetically costly (Bagarinao 1992; Cooper and Brown 2008), and neonates should have a survival advantage when born at a large body size and with ample fat reserves that serve as an ATP reservoir for cellular detoxification of H_2S. Furthermore, although investigating the effects of preservatives on the observed patterns was beyond the scope of our study, closer inspection of the one population for which both ethanol- and formalin-preserved specimens were collected at the same time (i.e., Melbourne) uncovered MI, FatI, offspring mass at birth, and offspring fat content at birth all being greater in formalin-preserved samples. We therefore believe that future research, potentially comparing ethanol- and formalin-preserved samples collected from the same populations, is needed to shed more light on the potential pitfalls arising from preservation effects in life-history analyses.

Conclusions and outlook

We demonstrate that eastern mosquitofish are capable of varying degrees of maternal provisioning, resulting in highly variable patterns of offspring quality. Our study, alongside other studies demonstrating population variation in life-history strategies, calls for careful consideration of this variation when making an attempt to characterize an entire species as either lecitho- or matrotrophic. Future studies should try to capture the full extent of the range of life-history strategies employed by including populations from a wide geographic range and ecological spectrum (to the extent that species distributions allow). Combined with standardized laboratory rearing (i.e., common-garden) or translocation experiments, future studies will also need to address the question as to what portion of the observed variation in maternal provisioning strategies is caused by either phenotypic plasticity or evolutionary divergence (i.e., local adaptation); patterns of temporal variation, as uncovered here, strongly support the former. Finally, we introduce a novel index, the fat index. Only about half of the variation in FatI was explained by its moderate, positive association with MI. Hence, FatI should provide additional, independent information on how poeciliid fishes alter the quality of their offspring, and more generally, how the parent-offspring conflict might play out in this taxonomic group. We therefore advocate evaluation of FatI in poeciliid fishes in future research.

Table 3. Parameter estimates of the general linear models (GLMs) investigating (a) the influence of water temperature and geographic parameters (via principal component 1; PC1) on matrotrophy index (MI) of populations of G. holbrooki; (b) the influence of chlorophyll *a* and phycocyanin on MI of only native *G. holbrooki* populations; and (c) the influence of environmental and geographic parameters on variation in fat index (FatI) of native *G. holbrooki* populations. Significant FDR-adjusted *p*-values are highlighted in bold.

	Estimate	**SE**	**t-value**	***p* (FDR)**
a) MI-model: Water temperature, latitude and longitude				
Intercept	0.839	0.042	19.995	**<0.001**
PC1	0.127	0.042	0.042	**0.008**
b) MI-model: Chlorophyll* a *and phycocyanin Intercept				
Chlorophyll *a*	0.648	0.083	7.818	**0.002**
Phycocyanin	0.008	0.006	1.263	0.275
c) FatI-model: All predictors	-0.027	0.021	-1.293	0.275
Intercept				
	0.624	0.066	9.465	**<0.001**
PC1	-0.055	0.071	-0.778	0.609
PC2	0.039	0.071	-0.778	0.609

Table 4. Parameter estimates of general linear models (GLMs) investigating (a) the influence of water temperature, latitude and longitude (via principal component 1; PC1) on estimated offspring dry mass at birth (mg) of populations of *G. holbrooki*; (b) the influence of chlorophyll *a* and phycocyanin on estimated offspring size at birth (mg) of only native *G. holbrooki* populations; (c) the influence of environmental and geographic parameters (PC1 and PC2) on estimated fat content at birth (%). Significant FDR-adjusted *p*-values are highlighted in bold.

	Estimate	**SE**	**t-value**	***p*(FDR)**
a) Offspring dry mass: Water temperature, latitude and longitude				
Intercept	0.830	0.039	21.074	<0.001
PC1	-0.072	0.040	-1.819	0.128
b) Offspring dry mass: Chlorophyll* a *and phycocyanin				
Intercept	0.867	0.284	3.047	0.095
Chlorophyll *a*	0.042	0.022	1.923	0.159
Phycocyanin	-0.081	0.071	-1.133	0.320
c) Offspring fat content: Water temperature, phycocyanin, chlorophyll* a*, latitude and longitude				
Intercept	10.429	1.261	8.267	0.003
PC1	1.197	1.363	0.879	0.643
PC2	0.113	1.363	0.083	0.938

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