Correlates of life-history variation in the livebearing fish *Poecilia vivipara* (Cyprinodontiformes: Poeciliidae) inhabiting an environmental gradient

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Understanding whether and how different habitat features affect patterns of life-history variation is an intriguing aim in evolutionary ecology. We tested how a suite of biotic and abiotic factors simultaneously affect life-history patterns in six populations of the livebearing fish *Poecilia vivipara* inhabiting coastal lagoons in southeastern Brazil, varying greatly in salinity and fish communities. We sampled potential competitors (cyprinodontiform fishes) and piscivores, and we measured nine limnological variables in the wet and dry seasons during 2 years. We found that size at maturity and offspring size were strongly and negatively influenced by the abundance of piscivores, and that reproductive allocation was strongly and positively influenced by variations in resource abundance and temperature among seasons. Effects of salinity on size at maturity, female reproductive investment and offspring size were indirect, via piscivore abundance, highlighting the potential for indirect effects that can be detected only by analysing multiple habitat features simultaneously. This suggests caution in interpreting associations between life histories and salinity in wild populations. Observed patterns generally matched theoretical predictions and prior empirical work, but some predictions were not met. Our results suggest that life histories result from a partly predictable complex combination of direct and indirect effects of habitat features, which can sometimes act antagonistically.

ADDITIONAL KEYWORDS: body size – coastal lagoons – fecundity – gonadosomatic index – livebearing fishes – offspring size – reproductive investment – Restinga de Jurubatiba – salinity gradient – seasonality.

INTRODUCTION

Given the relevance of life-history traits to population demography, the evolution of life histories has long interested evolutionary ecologists (Lack, 1947; Stearns, 1976; Roff, 2002). One of the core predictions of the demographic theory of life-history evolution is that increased mortality (e.g. by predation) should select for early maturity, increased reproductive investment (RI) and increased fecundity (Charlesworth, 1980; Stearns, 1992). Models incorporating effects of density dependence and resource availability indicate that these can also be important agents of selection on life histories (Mylius & Diekmann, 1995). Specifically, lower resource abundances should select for lower age and/or smaller size at maturity (Kozłowski & Uchmanski, 1987; Abrams & Rowe, 1996) and lower reproductive effort (Gadgil & Bossert, 1970). Importantly, these factors can interact in complex ways, for example when predation reduces the density of prey, resulting in increased prey food supply (indirect effect of predation). Depending on the relative magnitude of direct and indirect effects,

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the net effect of predation can be increased size at maturity (Abrams & Rowe, 1996).

Empirical evidence from different taxa has largely supported the prediction that higher mortality (in most cases caused by predation) induces maturation at lower age and/or smaller size, increased RI and fecundity, and smaller offspring size (Promislow & Harvey, 1990; Martin & Clobert, 1996; Hilton *et al.*, 2002; Johnson & Bagley, 2011). Other studies indicate that higher food availability is associated with higher growth rate, early maturation, higher fecundity and smaller offspring size (Reznick & Yang, 1993; Bronikowski & Arnold, 1999; Arendt & Reznick, 2005). A few studies on fishes from Trinidadian streams indicate that in high-predation sites prey population density is lower and growth rates are higher, probably as a result of increased per capita food availability (Reznick *et al.*, 2001, 2012; Walsh & Reznick, 2008, 2009). However, because productivity covaries positively with predation (Grether *et al.*, 2001), the effect of productivity and the indirect effect of predation on life histories may be confounded.

Other studies have highlighted the importance of biotic interactions other than predation, such as interspecific competition, and abiotic factors to life-history traits. For example, in the livebearing fish *Poecilia picta*, populations coexisting with its putative competitor, *Poecilia reticulata*, exhibit an increase in offspring size (Torres-Dowdall *et al.*, 2013), in line with predictions of classic *K*-selection theory (Pianka, 1970; Reznick *et al.*, 2002). Other studies on livebearing species have revealed a positive relationship between salinity and female body size and male lean weight (Gomes & Monteiro, 2007; Riesch *et al.*, 2015); female RI and fecundity (Martin *et al.*, 2009); and offspring size (Alcaraz & García-Berthou, 2007; Gomes & Monteiro, 2007). These relationships might be the result of direct effects of salinity on fish physiology that might ultimately affect life-history traits. For example, cyprinodontiforms exposed to salinities deviating from isosmotic levels expend more energy on osmoregulation and reduce routine metabolic rate and activity levels (Nordlie, 1987; Nordlie *et al.*, 1992; Haney *et al.*, 1999). However, it is still not clear whether these associations between salinity and life histories were caused by direct effects of salinity or by environmental features that happened to covary with salinity, such as environmental stability (Alcaraz & García-Berthou, 2007; Martin *et al.*, 2009) or predation (Gomes & Monteiro, 2007; Riesch *et al.*, 2015). The potential covariation between environmental features in natural habitats highlights the need to analyse them simultaneously in order to disentangle their effects.

Surprisingly few studies have tried to tease apart the relative contributions of different biotic and abiotic factors to life-history patterns. Two recent studies showed that the life histories of four species of livebearers (*Gambusia* spp.) inhabiting environmental gradients result from a complex interaction between biotic and abiotic factors (Riesch *et al.*, 2015, 2018). In the present study, we investigate how a suite of biotic and abiotic factors simultaneously affect the variation in life-history traits of the cyprinodontiform *P. vivipara* (Poeciliidae) Bloch & Schneider. We analysed populations of *P. vivipara* inhabiting lagoons varying in abiotic factors (e.g. salinity ranging from near 0 to > 20 ppt) and biotic factors (e.g. widely varying abundance of predators and competitors). Common piscivores in this system are the erythrinids *Hoplias* aff. *malabaricus* and *Hoplerythinus unitaeniatus*, the auchenipterid *Trachelyopterus striatulus* and the characid *Oligosarcus hepsetus*. These freshwater species are usually absent from lagoons at the higher end of the salinity spectrum (Caramaschi *et al.*, 2004; Di Dario *et al.*, 2013). These species preferentially inhabit the vegetated shores and are active during the day (*O. hepsetus*), at night (*T. striatulus*) or at dusk and dawn (the erythrinids; Sánchez-Botero *et al.*, 2007). Two other cyprinodontiforms, *Jenynsia darwini* (Anablepidae) and *Phalloptychus januarius* (Poeciliidae), are found at varying abundances in lagoons but are generally more common at higher salinities (Felice, 2014). These two livebearing species share the same microhabitat and have similar diets to *P. vivipara* (Aranha & Caramaschi, 1999; Iglesias *et al.*, 2008) and are therefore potential competitors.

We make the following predictions: (1) that the abundance of piscivores should have a direct positive effect on RI and fecundity and a direct negative effect on body sizes of *P. vivipara* and/or an indirect positive density-dependent effect on RI and fecundity by reducing the abundance of *P. vivipara* and other cyprinodontiforms; (2) that reproductive allocation is correlated positively with resource abundance; and (3) based on prior empirical findings, a positive effect of salinity on body size, RI, fecundity and offspring size. The life-history patterns of *P. vivipara*, therefore, should represent a balance between these multifarious effects in this system.

MATERIAL AND METHODS

STUDY AREA

To investigate temporal and spatial variation in *P. vivipara* life histories, we examined six coastal lagoons within the Parque Nacional da Restinga de Jurubatiba, northern Rio de Janeiro state, Brazil, during both wet and dry seasons. We refer readers to Araújo *et al.* (2014) for more information on the study area. Briefly, most low-salinity lagoons are

perpendicular to the coastline, being deeper and larger, having a short perimeter near the sea and freshwater inlets opposite to the sea. Owing to their longer lateral shorelines and larger catchment areas, these lagoons are colonized by dozens of aquatic macrophytes and freshwater fish species (Bove & Paz, 2009; Di Dario *et al.*, 2013). Fish assemblages in these lagoons occasionally increase in species number by the entrance of marine species under sporadic sandbar breaching caused by intense rainfall (Camara *et al.*, 2018). Most of these species are bottom or plankton feeders, i.e. species of gerreids (*Eucinostomus argenteus*) and clupeids (*Anchovia clupeoides* and *Lycengraulis grossidens*), respectively (Camara *et al.*, 2018), that occupy the deeper open-water area near the sandbar, where salinity is rapidly diluted after the closing of the sandbar by freshwater refilling. The shallower high-salinity lagoons are parallel to the coastline and more subject to the tidal regime, and freshwater inputs are limited to rainfall. Salt concentrations vary in these lagoons depending on the balance between precipitation and evaporation. Few species of aquatic macrophytes (e.g. the widgeon grass *Ruppia maritima*, *Eleocharis* spp.), macroalgae (*Chara* sp.) and fishes are able to withstand these oscillations in salinity content (Bove & Paz, 2009; Di Dario *et al.*, 2013). We investigated populations inhabiting six lagoons previously studied by our research group (Araújo *et al.*, 2014): three lagoons at the lower end of the salinity spectrum and containing two or three piscivorous species and three lagoons at the upper end of salinities with no record of piscivorous species (Table 1). The studied lagoons are completely isolated from each other, and populations are treated as independent. The climate in the area is warm (mean annual temperature, 26.6 °C), with a marked seasonality in precipitation and higher temperatures in the wet season (de Macedo-Soares *et al.*, 2010).

Table 1. Biotic and abiotic habitat parameters of the six studied lagoons in the wet and dry seasons

Parameter		Lagoon					
		Cabiúnas	Bezerra	Pitanga	Catingosa	Pires	Maria Menina
N	Female	145 (63)	113 (21)	171 (125)	166 (94)	98 (16)	61 (32)
	Male	69	56	92	70	40	78
Number of surveys	Wet	2	1	2	2	1	1
	Dry	2	2	2	2	2	2
Piscivores	Wet	0.033	0	0.080	0	0	0
	Dry	0.055	0.045	0	0	0	0
Cyprinodontiforms	Wet	0.065	0.372	3.585	16.616	0.644	1.065
	Dry	0.340	1.167	7.860	5.456	2.380	8.050
Dissolved oxygen (mg/L)	Wet	5.45	6.62	7.07	8.70	7.56	5.01
	Dry	7.82	7.86	6.47	7.28	7.88	7.54
pH	Wet	6.77	6.40	7.30	8.04	8.36	7.83
	Dry	6.74	6.51	6.66	8.01	8.00	7.52
Salinity (ppt)	Wet	0.10	1.80	1.85	26.30	27.30	29.00
	Dry	0.20	2.45	11.85	19.05	28.65	34.15
Temperature (°C)	Wet	30.1	28.1	26.8	29.8	30.1	27.2
	Dry	24.8	25.0	22.5	25.3	27.7	23.8
P (µg/L)	Wet	51.09	20.23	240.82	134.33	63.56	60.22
	Dry	18.72	52.65	42.34	61.18	60.29	63.89
N (mg/L)	Wet	0.60	1.52	2.29	3.32	2.19	2.30
	Dry	0.76	2.89	2.16	3.45	2.78	2.78
Dissolved organic carbon (mg/L)	Wet	12.96	45.79	31.03	81	31.63	47.86
	Dry	8.81	38.81	14.24	53.05	37.03	39.59
Chlorophyll *a* (µg/L)	Wet	5.50	75.43	17.75	6.28	23.47	6.42
	Dry	2.17	4.92	4.21	2.01	2.68	1.67
Zooplankton (individuals/cm³)	Wet	12 117 529	597 688	36 512 068	4 798 050	8 922 167	10 197 400
	Dry	3 442 486	1 429 239	5 348 597	10 113 220	692 692	8 002 440

Sample sizes (N) of *Poecilia vivipara* and number of surveys in each season are indicated. Lagoons were surveyed from 2011 to 2013; values represent averages of two measurements for each lagoon–season combination, except when only one survey was done. Numbers in parentheses denote sample sizes of pregnant females (stage 2 or higher) examined for embryo traits. Piscivores: densities of the piscivores *Hoplias malabaricus*, *Hoplerythrinus unitaeniatus*, *Trachelyopterus striatulus* and *Oligosarcus hepsetus*. Cyprinodontiforms: densities of *P. vivipara*, *Jenynsia darwini* and *Phalloptychus januarius*.

DATA COLLECTION

Lagoons were surveyed at the peaks of the dry (July) and the wet seasons (January), in July 2011, January 2012, July 2012 and January 2013. In the last survey, we were not able to sample the lagoons Bezerra, Pires and Maria Menina, so for these three lagoons there is only one survey in the wet season (total of 21 surveys; see Table 1). To characterize the limnological features of lagoons, in every survey we measured the concentration of dissolved oxygen (DO), salinity and temperature using a YSI-85 meter (Table 1). Additionally, we collected water samples for the quantification in the laboratory of pH (using a pH meter, Digimed DM-20) and the concentrations of total phosphorous, total nitrogen and dissolved organic carbon (DOC) by spectrophotometry after standard analytical procedures (Golterman *et al.*, 1978; Hu *et al.*, 2002). To account for potential differences in resource abundance among lagoons, we quantified the abundance of chlorophyll *a* and zooplankton. Water samples were collected in plastic bottles, taken to the laboratory and filtered in fibre-glass filters (GF/C Whatman). Chlorophyll *a* was extracted with 90% ethanol, and its concentration was determined in a spectrophotometer at 665 nm. In the field, 50–100 L of water were filtered in a plankton net (mesh size 68 μm) and immediately fixed in 4% buffered formalin solution for posterior identification and quantification in the laboratory. Zooplankton species were identified using an optical microscope (Olympus BX51), and quantitative samples were counted in a Sedgewick Rafter counting cell. We acknowledge that by using these proxies we restricted our analysis to the limnetic food chain, disregarding the food chains based on periphyton and detritus, which also contribute to the diet of *P. vivipara* in the studied populations (M. S. Araújo, unpublished data).

Specimens of *P. vivipara* were sampled with a seine net (1.5 m high, 5 m wide, mesh size 0.5 mm), which was slowly pulled by two of us along the lagoon shore (< 1.5 m deep; Petry *et al.*, 2003). Sampling effort varied among lagoons depending on the abundance of *P. vivipara*. During sampling with the seine net, we sampled the livebearers *J. darwini* and *P. januarius*, in addition to piscivores. These livebearers were counted and released back into the lagoons while still alive, whereas piscivores were taken to the laboratory for gut-content analysis. In the field, *P. vivipara* and piscivore specimens were killed in eugenol before preservation in 10% formalin, and later (7 days) transferred to 70% ethanol.

In the laboratory, specimens of *P. vivipara* were measured for standard length (SL) with a digital calliper (0.01 mm), dissected for the removal of gonads and digestive tracts, oven dried (60 °C, > 48 h) and weighed (0.01 mg). The determination of age (juveniles vs. adults) and sex was based on secondary sexual characters and gonads, and only adults were analysed. Male gonads were oven dried and weighed. Female gonads were examined under a stereomicroscope, and embryo development was categorized in five stages adapted from Haynes (1995): (1) immature, small ova, opaque white in colour (Haynes' stages 1–2); (2) mature ova, opaque or translucent orange in colour; blastodisc embryo or embryonic shield might be present (Haynes' stages 3–4); (3) embryo with eye cups or pigmented eyes; pectoral and caudal fin buds might be present, but not dorsal fin bud (Haynes' stages 5–7); (4) pectoral and caudal fins with rays present; caudal fin may sit around the tip of the snout; dorsal fin bud might be present, but no rays present (Haynes' stages 8–9); and (5) dorsal fin with rays; yolk sac mostly or completely absorbed; embryo much more elongate (Haynes' stages 10–11). All offspring at stages 2 or higher (Supporting Information, Fig. S1) were counted, oven dried and weighed. Immature ova and reproductive tissues were dried and weighed separately.

We measured the following life-history traits in *P. vivipara*: size at maturity (SL) in males and females; gonadosomatic index (GSI) in males; and reproductive investment, fecundity and offspring size in females. By counting both fertilized and unfertilized eggs (pooled as stage 2 herein), our measures of fecundity might be slightly overestimated (compared with more conventional measures of fecundity), but this should not affect our comparisons among lagoons. We used the SL of the smallest reproductive female in each survey in each population (Zandonà *et al.*, 2011) as measures of female size at maturity. Using the average female body size instead of the minimum size of pregnant females in the analyses gave similar results. We used the mean SL of all males in each survey in each population as measures of male size. We refrained from using the term 'male size at maturity' because males in this species can still grow after maturation (albeit at greatly reduced growth rates), and our measure has probably overestimated size at maturity. The GSI was calculated by dividing male gonad mass by total body mass. Reproductive investment was calculated as the summed mass of offspring and reproductive tissues divided by total body mass. Fecundity was determined by counting the number of offspring (stage 2 or higher), and offspring size was estimated by dividing offspring mass (stage 2 or higher, not including reproductive tissues) by fecundity.

DATA ANALYSIS

In order to investigate the effects of environmental variation among lagoons and seasons in influencing fish densities and *P. vivipara* life histories, we first needed to summarize limnological variation among our

surveys. To accomplish this, we performed a principal components analysis (using the correlation matrix) on the nine environmental variables measured in each lagoon during each survey (21 surveys; Table 1). We retained the first four principal component (PC) axes (eigenvalues > 1.0), accounting for 77.1% of the variance (Table 2). These axes describe environmental variation observed across all 21 surveys. We interpreted PC axes by inspecting loadings (correlations with original variables) and by testing for differences among lagoons and seasons using ANOVA (lagoon, season and their interaction served as independent variables). Principal component 1 was associated positively with nitrogen, DOC, pH, salinity, phosphorus concentration and DO. This axis largely captured inter-lagoon variation, irrespective of season, weighting variables with relatively low inter-season variation more strongly (lagoon, $P = 0.0213$; other terms, $P > 0.80$; $R^2 = 0.74$). Principal component 2 was associated positively with higher temperature, chlorophyll *a* concentration and zooplankton abundance. This axis clearly captured variation between wet and dry seasons that was consistent across all lagoons (season, $P < 0.0001$; other terms, $P > 0.36$; $R^2 = 0.87$). Principal component 3 was associated positively with DO and negatively with salinity. This axis captured environmental variation that did not clearly differentiate lagoons or seasons (all $P > 0.20$; $R^2 = 0.55$). Principal component 4 was associated positively with zooplankton and phosphorus and negatively with temperature. This axis described seasonal variation that differed among lagoons (interaction between lagoon and season, $P = 0.0414$; lagoon, $P = 0.0024$; season, $P = 0.4879$; $R^2 = 0.88$).

In order to estimate variation in fish communities, we calculated the density of cyprinodontiforms (*P. vivipara*, *J. darwini* and *P. januarius*) and piscivores

for each lagoon during each of the 21 surveys. Given that sampling effort varied among lagoons, we standardized fish densities by dividing the number of specimens sampled by the distance (in metres) that the seine net was pulled in each sampling (Table 1). Given that we used the same seine net in all samplings, this distance is a simple and appropriate measure of sampling effort. Owing to the similarity in body size, microhabitat use and diet between cyprinodontiform species, we believe that combining the abundances of cyprinodontiforms captures the primary sources of competition experienced by *P. vivipara* more accurately than its abundance alone. We acknowledge that our quantitative estimate of piscivore abundance might underrepresent the real densities of these larger-bodied predators in these lagoons. However, this should not affect our results because: (1) we wished to provide relative estimates for standardized comparisons among sites and seasons, not provide estimates of the absolute densities; and (2) if we instead treat piscivore abundance as a categorical variable (presence vs. absence), all results presented are qualitatively unchanged.

For all types of data (environmental PCs, cyprinodontiform density, piscivore density and life histories), we obtained site–season average values for analysis, because our research questions centre on variation across seasons and lagoons and thus require the units of replication to comprise these site–season values ($N = 12$). For environmental PCs, cyprinodontiform density and piscivore density, we simply calculated means for each season for each lagoon. For female size at maturity (SL), we used the minimum SL of reproductive females for each season for each lagoon (Zandonà *et al.*, 2011). For other life-history traits, we calculated means adjusted to control statistically for effects of body size and embryo stage. We accomplished this by conducting general linear mixed models (GLMMs) with each variable to obtain least-squares means of each trait for each population in each season, controlling for effects of body size and embryo stage. In all models, life-history traits were dependent variables, and population, season and their interaction were independent variables, with survey nested within the interaction term included as a random effect. We included embryo stage and female somatic body mass as covariates for reproductive investment, fecundity and offspring size; and male somatic body mass as a covariate for GSI. No covariate was included for male size. In a small number of the females containing embryos in stage 2 or higher, we found two embryo developmental stages within a single female (17% of females). In most cases, these stages were adjacent (e.g. 2–3 or 3–4); therefore, this is probably caused by a developmental asynchrony of a few hours to a few days, rather than superfetation.

Table 2. Results of principal components analysis of the nine limnological variables measured at the six studied lagoons

Factor	PC1	PC2	PC3	PC4
Dissolved oxygen	0.50	−0.20	0.59	−0.19
pH	0.76	0.29	−0.35	−0.06
Salinity	0.76	−0.02	−0.52	−0.16
Temperature	−0.03	0.77	0.06	−0.48
Total phosphorous	0.52	0.16	0.52	0.47
Total nitrogen	0.87	−0.13	−0.05	0.25
Dissolved organic carbon	0.86	0.25	0.16	−0.04
Chlorophyll *a*	−0.26	0.58	0.29	−0.03
Zooplankton	−0.27	0.49	−0.27	0.67
Percentage variance	36.81	15.59	13.36	11.37

For each variable, we had a total of 21 measurements corresponding to three or four surveys for each lagoon from 2011 to 2013.
Abbreviation: PC, principal component.

In these cases, we used the number of embryos in each stage and the nominal value of each stage (2–5) to calculate an average embryo developmental stage for each female, which was used as a covariate. In all models, size at maturity (SL) and somatic body mass were ln transformed; GSI and reproductive investment were arcsine–square root transformed; fecundity was square root transformed; and offspring size was ln transformed.

We used path analysis (e.g. Kline, 2005) to investigate direct and indirect effects of environmental variables (four PCs) and estimated densities of piscivores and cyprinodontiforms on *P. vivipara* life histories. Using the site–season values described above, we constructed a full path diagram based on hypotheses regarding how environmental variation might lead to life-history variation in *P. vivipara*. Our full path diagram included potential pathways leading from: (1) all four environmental PCs to piscivore density; (2) all four environmental PCs and piscivore density to Cyprinodontiform density; and (3) all four environmental PCs, piscivore density and Cyprinodontiform density to each life-history variable. We examined sexes separately. We carried out model selection using Akaike's information criterion corrected for small sample sizes (AIC$_c$; Akaike, 1992; Burnham & Anderson, 2002) to select the best subset of paths leading to each endogenous variable. Multicollinearity was low in all considered models (all variance inflation factors (VIFs) < 5), with the highest VIF in the final analyses being 1.41.

All path coefficients were calculated as standardized (partial) regression coefficients estimated using 1000 bootstraps of the data set. We assessed the significance of direct effects (path coefficients) and total effects (sum of direct and indirect effects) using a bootstrap approximation obtained by constructing two-sided bootstrapped confidence intervals. These bootstrapping approaches provide more accurate estimates of path coefficients and their errors for data sets with relatively small sample sizes (Bollen & Stine, 1990; MacKinnon *et al.*, 2004). The GLMMs were conducted with JMP Pro v.12.1.0 (SAS Institute Inc., Cary, NC, USA) and path analysis with Amos v.24 (Arbuckle, 2003).

RESULTS

The best subset of paths for our path analysis based on AIC$_c$ resulted in a total of seven direct paths for males (out of 21 possible) and ten direct paths for females (out of 33 possible). In most cases, the best model was selected unambiguously, but in some cases multiple models exhibited similar AIC$_c$ values (within two AIC$_c$ units; see Supporting Information, Model

selection results). The only ambiguous cases involved paths to piscivore density, male GSI and female RI, and alternative models were either subsets of the selected model (omitting highly significant paths) or added paths that resulted in non-significant paths. Thus, in all cases, we selected the top model. The analyses identified several environmental drivers of fish densities and *P. vivipara* life histories (Fig. 1). Regarding environmental determinants of fish densities, our analysis revealed two key findings. First, we found that cyprinodontiform density was greater in sites with higher nitrogen content, DOC, pH, salinity, phosphorus and DO (i.e. positive association with PC1). Second, piscivore density increased with increasing DO and reduced salinity (i.e. positive association with PC3) and tended to increase with increasing zooplankton abundance and phosphorus concentration (i.e. positive association with PC4).

We found that several environmental factors and piscivore density influenced *P. vivipara* life histories, but density of cyprinodontiforms was not associated with *P. vivipara* life-history variation. The strongest effects observed described the influence of piscivore density on body and offspring size; higher densities of predatory fish were associated with smaller male size and female size at maturity and with smaller offspring size (Fig. 1; Table 3). Allocation toward reproduction was strongly associated with seasonal environmental variation; during wet seasons, characterized by higher temperature and resource productivity (PC2), *P. vivipara* exhibited relatively larger testes (GSI), greater female reproductive investment and higher fecundity (Fig. 1; Table 3). Female reproductive investment was also associated positively with piscivore density. Higher DO and lower salinity (PC3) were associated with smaller size at maturity, smaller male GSI, smaller offspring size and greater female RI (Table 3), but these patterns sometimes resulted from direct effects (male GSI) and sometimes from indirect effects via piscivore density (size at maturity, female RI and offspring size; Fig. 1). Although PC4 had strong direct effects on size at maturity and offspring size, these effects were opposed by indirect effects via piscivore density (Fig. 1), resulting in weakened to non-existent total effects of PC4 on these traits (Table 3). Principal component 4 also had a weak, suggestive positive effect on female RI (Table 3) through an indirect effect via piscivore density (Fig. 1).

DISCUSSION

By simultaneously considering a number of environmental agents, we uncovered a range of environmental drivers of *P. vivipara* life histories in this study. We found strong, clear effects of predation regime
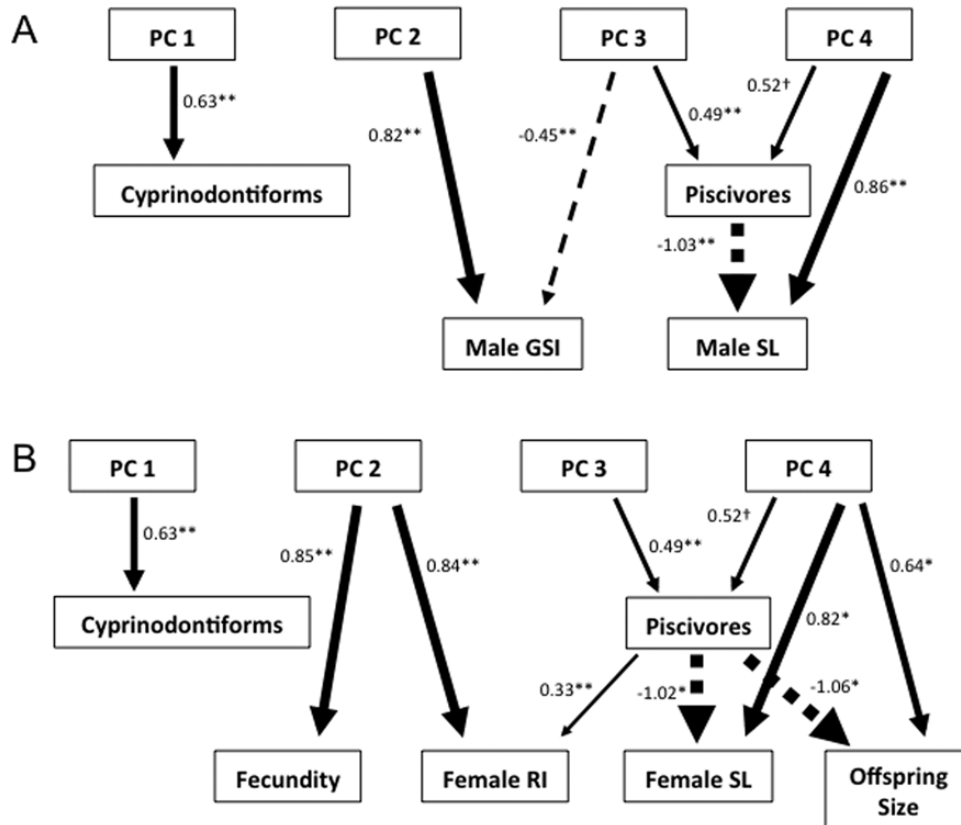
Figure 1. Path analysis results for males (A) and females (B). Numerical values indicate standardized path coefficients. Line thickness reflects the strength of the path; continuous lines represent positive effects, and dashed lines represent negative effects. *$P \leq 0.05$. **$P \leq 0.01$. †$P \leq 0.10$. Abbreviations: GSI, gonadosomatic index; PC, principal component; RI, reproductive investment; SL, standard length.

Table 3. Summary of total effects (combined direct and indirect effects) revealed by path analysis

Effect on	PC1		PC2		PC3		PC4		Piscivore density	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Piscivore density					0.49	< 0.001*	0.52	0.066		
Cyprinodontiform density	0.63	0.006*								
Male standard length					−0.51	0.003*	0.33	0.043*	−1.03	0.004*
Male gonadosomatic index			0.82	0.003*	−0.45	0.002*				
Female standard length					−0.50	< 0.001*	0.29	0.018*	−1.02	0.031*
Female reproductive investment			0.84	0.002*	0.16	< 0.001*	0.17	0.073	0.33	0.002*
Fecundity			0.85	0.002*						
Offspring size					−0.52	0.001*	0.09	0.455	−1.06	0.043*

Abbreviation: PC, principal component.
*Significant *P*-values.

and seasonality on life histories, largely matching our a priori predictions. We also found phenotype–environment associations that can be obscured by complex interactions among environmental variables: (1) strong, direct effects of zooplankton abundance and phosphorus concentration (PC4) on several traits

were opposed by indirect effects via piscivore density, resulting in small to no total effect of PC4 on these traits; and (2) associations between DO/salinity (PC3) and size at maturity, female RI and offspring size result from indirect effects via piscivore abundance. Together, these findings contribute to our understanding of the predictability of phenotypic change in response to environmental variation and highlight the potential for phenotypic effects of complex interactions between habitat features that can be detected only by the simultaneous analysis of multiple environmental factors and multiple traits.

Consistent with theoretical expectations and prior empirical work, piscivore abundance had a direct and positive influence on male and female body size and female reproductive investment and a negative influence on offspring size. Thus, predator-driven variation in mortality has probably selected for different life histories across the lagoons of our study, reinforcing the generality of predator-driven life-history shifts in prey taxa (Reznick & Endler, 1982; Reznick *et al.*, 1996; Hilton *et al.*, 2002; Johnson & Zúñiga-Vega, 2009; Riesch *et al.*, 2013). That said, predation did not influence all traits that we predicted.

Contrary to expectations, piscivore abundance had no effects (direct or indirect) on male GSI or female fecundity. One explanation for these results is that higher piscivore abundance did not, in fact, impart higher predation pressure in this system. We find this explanation unlikely for several reasons. First, there are several studies showing that the piscivore species found in this system are effective predators of cyprinodontiforms (Deus & Petrere-Junior, 2003; Magurran, 2005; Mazzoni & da Costa, 2007; Mazzeo *et al.*, 2010; Petry *et al.*, 2010). This is corroborated by both the finding of specimens of *P. vivipara* in piscivore gut contents in a preliminary analysis and the successful use of cyprinodontiforms as bait in a previous study at the same sites (Araújo *et al.*, 2014). Second, piscivore abundance did influence *P. vivipara* body size, female RI and offspring size in the direction predicted by theory and prior empirical work. Finally, previous work in this system indicates that predation acts as a selective agent on *P. vivipara* functional body morphology, because populations in lagoons lacking piscivores have more streamlined bodies, with more anterior allocation (larger heads tapering into smaller caudal peduncles; Monteiro & Gomes, 2005; Gomes & Monteiro, 2008; Araújo *et al.*, 2014), in line with theory on the effects of predation on body shape and swimming performance of prey species (Langerhans, 2010). A more likely explanation is that selection caused by piscivores on reproductive allocation might be relatively weak compared with the effects of resource abundance associated with seasonality. Moreover, prior work in poeciliid fishes has suggested that direct selection on

offspring size via environmental stressors might be more common than direct selection on fecundity, with population divergence in fecundity often reflecting a correlated response owing to its genetic association with offspring size (Riesch *et al.*, 2014). Regardless, major patterns in these traits were explained by factors other than predation in this system.

Although we found strong positive effects of resource abundance (PC2: chlorophyll *a* and zooplankton) on reproductive allocation (fecundity, female RI and male GSI), consistent with predictions and prior empirical work (Gadgil & Bossert, 1970; Reznick & Yang, 1993; Bronikowski & Arnold, 1999), cyprinodontiform abundance had no effect on any measured life-history trait. We estimated competition using cyprinodontiform abundance, but our results are similar if we instead separate this estimate into interspecific competitor abundance (*J. darwini* and *P. januarius*) and conspecific population abundance (*P. vivipara*); no effects of either variable on any life-history trait are evident. This suggests that there are no density-dependent effects on life histories in *P. vivipara*. Thus, competition might be relatively weak compared with the large seasonal variation in productivity in our study sites.

In line with some previous findings in several poeciliid fishes (Alcaraz & García-Berthou, 2007; Martin *et al.*, 2009), salinity (PC3) had a positive direct effect on male GSI. The mechanism underlying this pattern is not known. *Poecilia vivipara* is able to maintain stable plasma ionic concentrations (Na^+ and Cl^-) in moderate to high salinity levels (10–30 ppt), but not in salinities outside this range (S. N. Bolasina, unpublished data). As a consequence, *P. vivipara* should expend less energy on osmoregulation in the lagoons at the upper end of salinities examined here and should therefore be able to invest more in reproduction. This hypothesized mechanism is consistent with the positive direct association between salinity (PC1) and the abundance of cyprinodontiforms, but it is hard to explain why salinity was not correlated with either fecundity or female RI. However, a recent study of three poeciliid species inhabiting Bahamian tidal creeks spanning a wide range of salinities also failed to uncover any direct effects of salinity on these traits (Riesch *et al.*, 2015). Importantly, Riesch *et al.* (2015) measured several environmental factors in addition to salinity and found that an apparently strong positive association between salinity and male GSI was, in fact, explained by an indirect effect via conspecific density. We did not find that specific pattern here, but this emphasizes the utility of measuring multiple environmental factors and the complexity of some seemingly simple phenotype–environment correlations.

More generally, previous findings of strong effects of salinity on a variety of life-history traits, e.g. body size, female RI, fecundity and offspring size (Alcaraz

& García-Berthou, 2007; Martin *et al.*, 2009), might often result from other ecological factors that happen to covary with salinity. We found no direct effects of salinity on any life-history traits other than male GSI, although it did have indirect effects on all other measured life-history traits except fecundity. Indirect effects in this study arose from the influence of salinity on piscivore abundance, as previously hypothesized (Gomes & Monteiro, 2007). The effects of salinity on life histories might vary among systems, but our results, in combination with the findings in three other livebearing fishes (Riesch *et al.*, 2015), might help to explain the apparent inconsistent effects of salinity on life-history traits in other systems (Moore *et al.*, 2016).

In conclusion, our results support the notion that abiotic factors (e.g. salinity) and seasonal variation in resources can have major effects on the life histories of prey species comparable to those caused by predation. Although we found many strong environmental drivers of life-history variation that matched our a priori predictions, we also failed to find some predicted patterns (e.g. no effect of predation on male GSI or fecundity) and uncovered some unexpected ones (e.g. complex effects of PC4). We also cannot determine the extent to which observed phenotypic variation reflects phenotypic plasticity or genetic differentiation. Both sources of variation have been documented in the life histories of poeciliid fishes (Stearns, 1983; Trexler *et al.*, 1990; Reznick & Bryga, 1996; Johnson, 2001; Riesch *et al.*, 2009), and both are likely to play roles in this system. Altogether, our results suggest that life histories result from a partly predictable complex combination of direct and indirect effects of habitat features, which can sometimes act antagonistically. Future studies should use approaches that are able to disentangle these multifarious effects on life-history patterns across environmental gradients.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. *Poecilia vivipara* embryos at the four developmental stages used to measure fecundity and offspring size: A, stage 2; B, stage 3; C, stage 4; and D, stage 5. Scale bar: 1 mm.
Model selection results. We present the top ten models in each case.