Environmental drivers of demographics, habitat use, and behavior during a post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*)

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Abstract A fundamental goal of evolutionary ecology is to understand the environmental drivers of ecological divergence during the early stages of adaptive diversification. Using the model system of the post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*) inhabiting blue holes, we used a comparative field study to examine variation in density, age structure, tertiary (adult) sex ratio, habitat use, as well as adult feeding and social behaviors in relation to environmental features including predation risk, interspecific competition, productivity (e.g. chlorophyll *a*, zooplankton density), and abiotic factors (e.g. salinity, surface diameter). The primary environmental factor associated with ecological differentiation in *G. hubbsi* was the presence of piscivorous fish. *Gambusia hubbsi* populations coexisting with predatory fish were less dense, comprised of a smaller proportion of juveniles, and were more concentrated in shallow, near-shore regions of blue holes. In addition to predation risk, the presence of a competitor fish species was associated with *G. hubbsi* habitat use, and productivity covaried with both age structure and habitat

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use. Feeding and social behaviors differed considerably between sexes, and both sexes showed behavioral differences between predator regimes by exhibiting more foraging behaviors in the absence of predators and more sexual behaviors in their presence. Males additionally exhibited more aggressive behaviors toward females in the absence of predators, but were more aggressive toward other males in the presence of predators. These results largely matched a priori predictions, and several findings are similar to trends in other related systems. Variation in predation risk appears to represent the primary driver of ecological differentiation in this system, but other previously underappreciated factors (interspecific competition, resource availability) are notable contributors as well. This study highlights the utility of simultaneously evaluating multiple environmental factors and multiple population characteristics within a natural system to pinpoint environmental drivers of ecological differentiation.

Keywords Adaptive radiation · Blue holes · Competition · Ecological divergence · Habitat shift · Predation

Introduction

A fundamental question in evolutionary ecology is how environmental agents drive the early stages of species radiations (Schluter 2000; MacColl 2011). It is well known that environmental variation across space and time can promote phenotypic and ecological divergence (e.g. Reznick and Endler 1982; Schluter 2000; Rundle and Nosil 2005; Grether and Kolluru 2011), but unraveling the relative importance of particular environmental factors among the myriad potential agents (e.g. predators, competitors, parasites, resources, and abiotic factors) is a daunting task. Population characteristics potentially shaped by the environment are just as numerous, including demographics, habitat use, behaviors, morphologies, and life histories. All of these factors can significantly influence ecological and evolutionary dynamics, and may contribute to speciation (e.g. Endler 1995; Orr and Smith 1998; Coyne and Orr 2004; Magurran 2005; Hall and Colegrave 2007; Nosil 2012).

Longstanding theory suggests that divergent selection acting on multiple traits, multifarious divergent selection, may be an important contributor to speciation (Rice and Hostert 1993; Nosil et al. 2009). Put simply, with more targets of divergent selection, more opportunity exists for the evolution of reproductive isolation. Because most studies of ecological divergence focus on a single agent and a single target of selection at a time (reviewed in MacColl 2011), further study of putative cases of multifarious divergent selection is needed. Understanding the relative strengths of different selective agents, how they interact, and the breadth of traits they act upon—either directly through selection or indirectly through changes in demographics—will improve our grasp of the process of adaptive diversification. Acquiring such an understanding requires a pluralistic approach, investigating multiple environmental factors and multiple ecologically and evolutionarily important population-level characteristics (e.g. Schlichting and Pigliucci 1998; DeWitt and Langerhans 2003; Ghalambor et al. 2003).

Here we examine how four environmental factors (predation, interspecific competition, resource availability, and abiotic factors) and three population characteristics (demographics, habitat use, and behavior) may interact to shape ecological divergence in the post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*). We consider *ecological divergence* to comprise population-level differences in ecologically relevant characteristics such as density, age structure, sex ratio, and individual-level traits (e.g. habitat use, behavior) that may reflect either evolutionary divergence, phenotypic plasticity, or both.

Each environmental factor above has empirical support for promoting population-level divergence in other systems (e.g. Reznick and Endler 1982; Schluter 1994; Langerhans et al. 2004; Nosil and Crespi 2006; Riesch et al. 2010; Grether and Kolluru 2011), and is hypothesized as important in this system based on natural history (see below) and previous work (e.g. Langerhans et al. 2007; Langerhans 2009; Langerhans and Gifford 2009). Similarly, each population characteristic examined here is also known to play an important role in evolutionary diversification in other systems (Rodd and Sokolowski 1995; Coyne and Orr 2004; Kokko and Rankin 2006; Losos 2009), and is hypothesized to exhibit strong population differences in the *G. hubbsi* system.

Gambusia hubbsi has recently undergone a radiation across inland blue holes (vertical, water-filled caves) on Andros Island, The Bahamas, exhibiting adaptive phenotypic evolution between blue holes with and without predatory fish. Previous research from field and common-garden experiments has uncovered numerous traits diverging between predator regimes including life history (Downhower et al. 2000; Riesch et al. 2013), body shape (Langerhans et al. 2007), locomotor performance (Langerhans 2009; Langerhans 2010), and male genital morphology (Langerhans et al. 2005). Further, these populations are undergoing ecological speciation, as sexual isolation between populations inhabiting different predator regimes has resulted as a by-product of divergent natural selection (Langerhans et al. 2007). While this radiation has become a textbook example of adaptive diversification (e.g. Freeman and Herron 2007; Cain et al. 2008; Reece et al. 2010), no study has yet investigated population differences in demographics, habitat use, or feeding and social behaviors in this system. Moreover, the role of other environmental agents in driving ecological differentiation is currently unknown.

Here we investigate understudied features of this model system by examining a total of 17 blue holes on Andros Island (Fig. S1). Our primary hypothesis centers on predation as the dominant factor driving ecological differentiation (based on prior work), and we test a number of a priori predictions regarding differences between populations facing low and high levels of predation risk (Table 1). We also test secondary hypotheses of the effects of competitors, resource availability, and abiotic factors, though a priori predictions are generally more tenuous. Specifically, we predicted increased resource availability will lead to greater *G. hubbsi* densities, an age structure more dominated by juveniles (via increased fecundity and juvenile survivorship), greater use of shallow-water regions (where preferred prey are found), and reduced feeding behaviors (i.e. reduced search and foraging times due to higher abundance of food). Additionally, we predicted that increased interspecific competition will lead to reduced densities, a smaller proportion of juveniles, greater use of deeper and more offshore waters (in search of less preferred prey), and increased frequencies of feeding behaviors. We used comparative analyses to test these predictions and identify environmental drivers of population differences in demographics, habitat use, and behavior.

Materials and methods

Study system

Blue holes are water-filled vertical caves found in some carbonate banks and islands (Mylroie et al. 1995), and Andros Island, The Bahamas harbors the greatest density of blue holes on earth. Blue holes were previously air-filled caves, filling with water during the past ~17,000 years (Fairbanks 1989) as rising sea levels lifted the freshwater lenses of the island (freshwater aquifers floating atop marine groundwater), flooding the voids. This

Table 1 Predictions of ecological divergence between predator regimes in poeciliid fishes

Character	↑ Predation risk	References
Population demographics		
Density	↓	1–7
Sex ratio (F:M)	↓	8–10
Proportion juveniles	↓	4, 6
Habitat use		
Shallow-water use	↑	1, 11–12
Offshore use	↓	1, 6, 12
Male behavior		
Foraging, feeding	↓	13–15
Sexual behaviors	↑	14–17
Male–male aggression	↑	15
Male–female aggression	↓	16–17
Female behavior		
Foraging, feeding	↓	13–15
Sexual encounters	↑	14–17
Female–female aggression	?	–
Female–male aggression	?	–

References: 1: Fraser and Gilliam (1992); 2: Gilliam et al. (1993); 3: Johnson (2002); 4: Johnson and Zuniga-Vega (2009); 5: Reznick et al. (1996); 6: Reznick et al. (2001); 7: Reznick and Endler (1982); 8: Haskins et al. (1961); 9: Liley and Seghers (1975); 10: Pettersson et al. (2004); 11: Seghers (1973); 12: R.B. Langerhans unpublished data; 13: Fraser et al. (2004); 14: Magurran and Seghers (1994); 15: Kolluru and Grether (2005); 16: Farr (1975); 17: Rodd and Sokolowski (1995)

created a unique replicate set of environments eventually colonized by aquatic organisms. Based on surveys conducted in 45 inland blue holes on Andros Island, blue holes are typically deep (35 m mean maximum depth), moderate in surface exposure (75 m mean surface diameter), and generally harbor a depauperate fish assemblage of 1–3 species (2.16 ± 0.23 species, mean ± SE). Three particular species comprise the bulk of inhabitants: the small livebearer, Bahamas mosquitofish (*G. hubbsi*, 89 % occurrence), the small pupfish, sheepshead minnow (*Cyprinodon variegatus*, 38 % occurrence; hereafter referred to as *Cyprinodon*), and the larger predatory eleotrid, bigmouth sleeper (*Gobiomorus dormitor*, 27 % occurrence; hereafter referred to as *Gobiomorus*) (R.B. Langerhans unpubl. data). Blue holes appear analogous to aquatic islands in a sea of land, as most blue holes seem to harbor their equilibrium number of species based on the theory of island biogeography (Langerhans and Gifford 2009; R.B. Langerhans unpubl. data). All existing molecular genetic evidence indicates strong isolation among fish populations inhabiting blue holes (Schug et al. 1998; Langerhans et al. 2007; Riesch et al. 2013). Moreover, blue holes represent stable, constant environments (e.g. fish communities appear to have persisted for long time periods; mosquitofish breed year-round; water temperature ranges from 25–34 °C throughout the year; no flowing water; see temporal repeatability of environmental and demographic variables below).

Environmental measurements

While our primary focus is to understand the effects of predation risk on ecological divergence in *G. hubbsi*, we are more generally interested in understanding the relative importance of the major biotic and abiotic factors that may drive ecological differences among populations of *G. hubbsi*. To this end, we selected a priori environmental agents that could play important roles in influencing *G. hubbsi* demographics, habitat use, and behavior (factors with potentially significant evolutionary implications), and selected study

sites so as to maximize variation along these environmental axes: (1) *Gobiomorus* presence (*G. hubbsi* predator), (2) *Cyprinodon* presence (*G. hubbsi* competitor), (3) resource availability (estimated with chlorophyll *a*, phycocyanin, zooplankton, phytoplankton, turbidity, and water transparency), and (4) abiotic factors (salinity, dissolved oxygen, pH, surface diameter).

Establishing the presence of *Gobiomorus* and *Cyprinodon* within each blue hole was easily accomplished with underwater visual observations due to water clarity and these fishes' active behavior. *Gobiomorus dormitor* is highly piscivorous (McKaye et al. 1979; Winemiller and Ponwith 1998; Bedarf et al. 2001; Bacheler et al. 2004) and readily hunts and consumes *G. hubbsi* in blue holes (R.B. Langerhans unpubl. data). Thus, *Gobiomorus* presence represents a high level of predation risk for *G. hubbsi*, while their absence indicates a relatively predator-free environment (e.g. no other piscivorous fish, no predatory snakes or turtles, wading birds are virtually excluded due to steep-sided shorelines and great depth, and predatory invertebrates are extremely rare).

Cyprinodon variegatus represents a potential competitor of *G. hubbsi* for both food and space. *Cyprinodon* are similarly sized to *G. hubbsi* (most adults of both species are ∼20–40 mm standard length), and while *Cyprinodon* consume more detritus and algae, their omnivorous diet overlaps considerably with the more carnivorous diet of *G. hubbsi*, perhaps inducing exploitative competition (R. B. Langerhans unpubl. data). Because male *Cyprinodon* aggressively defend territories and nests, they may additionally induce interference competition by restricting access of *G. hubbs*i to particular foraging patches and inflicting direct injuries (Itzkowitz 1977; R.B. Langerhans pers. obs.).

Because *G. hubbsi* exhibit a broad diet—primarily copepods, dipteran larvae and pupae, ostracods, cladocerans, amphipods, and adult insects (Gluckman and Hartney 2000; R.A. Martin and R.B. Langerhans unpubl. data)—it is not clear how to best estimate resource availability for these fish. Therefore, we measured a range of variables designed to capture relevant aspects of overall productivity in blue holes (Grether and Kolluru 2011). We measured four direct components of productivity in May 2011—chlorophyll *a*, phycocyanin, zooplankton, and phytoplankton—and measured two indirect correlates of productivity in blue holes over the course of multiple visits between 2002 and 2011 (see below)—turbidity and water transparency. To estimate total algal biomass and cyanobacteria biomass, we measured the photosynthetic pigments chlorophyll *a* and phycocyanin, respectively, using a fluorometer (AquaFluor model, Turner Designs, Sunnyvale, CA). Zooplankton and phytoplankton densities were estimated using a 60-m tow of a zooplankton net (20-cm diameter, 153-μm mesh) at 0.5-m depth. All plankton were counted within a 2.5-ml subsample of each plankton collection using a stereo microscope. Water turbidity was measured with an Oakton T-100 turbidimeter (Vernon Hills, IL), and water transparency was measured with a Secchi disk. While the direct estimates of productivity reflect only a single estimate, these are correlated with our indirect estimates, all of which exhibit strong repeatability across time (see below). This suggests that relevant differences across sites for the purposes of this study were likely adequately captured with this method.

For abiotic factors, surface diameter was estimated using a Bushnell Yardage Pro Legend laser rangefinder (Overland Park, KS), and all remaining environmental variables (as well as turbidity and transparency, mentioned above) were measured at the time of fish sampling (e.g. censuses and behavioral observations), as well as over the course of multiple visits between 2002 and 2011 (all blue holes but one were examined during multiple years), encompassing measurements from various times of the year (i.e. during months of March, May, July, August, November, and December). Salinity and dissolved oxygen were measured with a YSI 85 or YSI Pro2030 (Yellow Springs, OH), and pH was measured with

a Hanna HI 98128 pH meter (Woonsocket, RI). For blue holes with multiple measurements, we examined repeatability of environmental variables. As previous work indicated (Langerhans et al. 2007), pH and dissolved oxygen levels are very similar among most blue holes, with greater variance within blue holes across time than between them; thus, we did not include these variables in analysis. All other variables exhibited highly significant repeatability (intraclass correlation coefficients ranged from 0.88 to 0.98; following Lessells and Boag 1987), demonstrating that these factors remain quite consistent across seasons and years within blue holes relative to differences between sites, and thus site means were included in analyses.

Underwater census

We measured density, tertiary (adult) sex ratio, age structure, and habitat use of *G. hubbsi* using underwater visual census methods (Brock 1954; English et al. 1994; Nagelkerken et al. 2000; Layman et al. 2004). Due to water clarity, ease of underwater identification of sex/age classes, and ability to approach fish without causing disturbance, visual census techniques are especially well suited for fish density estimation in inland blue holes. While snorkeling, observers recorded the number of juvenile, male, and female *G. hubbsi* present in 1-m^3 quadrats within each of four habitat types: (1) shallow near-shore (0–1 m deep, 1–2 m from shore), (2) deep near-shore (2–3 m deep, 1–2 m from shore), (3) shallow offshore (0–1 m deep, 9–10 m from shore), and (4) deep offshore (2–3 m deep, 9–10 m from shore). Counts were made immediately upon arrival within a 1-m distance of the pre-designated quadrat location to avoid disturbing the fish. For a single blue hole (Archie's), the offshore region had to be modified to a distance of 5–6 m from shore due to its comparatively small size (15 m surface diameter, while all other blue holes were >50 m diameter).

A total of 17 blue holes were censused (8 without *Gobiomorus*, 9 with *Gobiomorus*), with eight blue holes being censused multiple times (Table S1). Censuses were conducted during three sampling periods: (1) six blue holes censused 7–11 November 2009, (2) 17 blue holes censused 1–12 May 2011, and (3) six blue holes censused 15–19 July 2011. For the first two sampling periods, 10 quadrats distributed equidistant around the perimeter of each blue hole were surveyed by a single observer within each habitat type on a single day (between 8:00 and 18:00; 13:26 ± 44 min). For the final sampling period, 20 similar quadrats were surveyed by two observers (10 quadrats each) in two habitat types in both the morning (between 10:10 and 11:35) and afternoon (between 13:00 and 16:00) of a single day. The latter sampling period only examined the two near-shore habitats because this was where most *G. hubbsi* were located in previous censuses.

We found no effects of observer or time-of-day on density estimates during the latter census period. For the eight blue holes censused multiple times, we tested for repeatability among sampling points and found significant repeatability of *G. hubbsi* density (intraclass correlation coefficient across all habitats, $r = 0.64$, $P < 0.0001$). This consistency across observers, time of day (morning vs. afternoon), and season/year indicates that our "snapshot" density measurements provide reasonable estimates for comparing relative values among sites. Thus, we pooled data across observers, time-of-day, and sampling period, and calculated habitat-specific mean density estimates for each blue hole.

Density was calculated as the average number of *G. hubbsi* observed within a 1-m^3 quadrat (including all age/sex classes). Tertiary (adult) sex ratio was calculated as the density of females divided by the density of males. Age structure was calculated as the proportion of juveniles in the population (juvenile density divided by total density). Habitat

use was examined in two ways: (1) fish demographics were directly examined across habitat types, and (2) overall habitat use was estimated as the proportion of fish using shallow-water (density of fish in the two shallow-water habitats divided by the total density) and offshore regions (density of fish in the two offshore habitats divided by the total density).

Behavioral observations

Underwater behavioral observations were conducted in six blue holes (three with *Gobiomorus*, three without) during 15–19 July 2011 between 10:35 and 15:40 (13:10 ± 49 min). Using a focal animal sampling approach (Martin and Bateson 1986), we recorded the frequencies of six feeding and social behaviors of 240 *G. hubbsi*: feeding, prey inspection, male–female chase, copulation attempt, intrasexual aggression, and intersexual aggression. Behavioral observations were conducted during a single time period at each blue hole, where observers moved systematically around the blue-hole perimeter such that only one fish was observed within a given area (to avoid observing the same fish twice). Four separate observers recorded behaviors of five males and five females while snorkeling within each blue hole (i.e. total of 20 males and 20 females per blue hole) by slowly approaching a focal fish within approximately 1 m and remaining relatively still while recording the number of behavioral events exhibited during an approximately 90-s observation period (42–235 s; 92.2 ± 2.1). Although these observation times are relatively short, longer periods were not feasible without potentially disturbing the fish by following it when it left the observation area. Moreover, focal behaviors were commonly observed during observation periods (most behaviors occurred on average more than once per minute), and other studies have used similar time periods for assessing poeciliid fish behaviors (Tobler et al. 2009; Köhler et al. 2011).

The six focal behaviors were selected based on their ecological importance, potential divergence among blue holes, and ease of underwater detection. Feeding describes the act of ingesting a prey item. Prey inspection describes an obvious examination of a potential food item, comprising a change in orientation followed by an approach within half a body length of the potential prey item (often involving "mouthing" of the item) and eventual rejection (not consumption) of the item. A male–female chase is the act of a male clearly chasing a female that is actively swimming away from the male. This reflects a premating behavior in which a male attempts to position himself for copulation either through force or female receptivity. A copulation attempt occurred when a male circumducted his gonopodium and performed a rapid torque-thrust maneuver making apparent physical contact with the female (Rivera–Rivera et al. 2010). Intrasexual aggression included any agonistic behavior between members of the same sex, including body/fin nipping, nudging, rapid flank approaches/ramming, and chases (e.g. Clark et al. 1954; Magurran and Seghers 1991). Intersexual aggression is the between-sex counterpart of the agonistic behaviors just described, with the exception of male–female chases, which are considered a sexual behavior (see above). The frequency (#/min) of each behavior was calculated for each fish, and because there were significant observer effects for some behaviors, we included an "observer" term in statistical models described below.

Statistical analysis

We examined population variation in demographics, habitat use, and behavior using a two-step approach for each of two sets of data: (1) demographics and habitat use across 17 blue holes, and (2) behaviors across six blue holes. For each set, we first tested for differences between predator regimes using mixed-model nested analysis of variance (ANOVA), and

then used a model selection approach to determine whether other factors might be important and whether observed differences between predator regimes persisted after controlling for these other possible factors (see below).

We first derived independent environmental axes for analysis based on our quantitative environmental measurements (i.e. estimates of resource availability and abiotic factors) and assessed whether environmental factors strongly covaried with the presence of *Gobiomorus* or *Cyprinodon* (which would reduce our ability to distinguish among alternative explanatory variables). We log-transformed plankton densities to increase normality, while all other variables remained untransformed. We reduced dimensionality by conducting principal components analysis (PCA) using the correlation matrix of the suite of environmental variables. We retained all PC axes that explained more variation than was expected on average in the absence of correlated structure using 1,000 randomizations of the data (see Avg-Rnd rule in Peres-Neto et al. 2005). This resulted in retention of three PC axes explaining over 76 % of total variance (Table 2). These axes were subsequently used in analyses to estimate productivity and capture salient abiotic factors. Using these PC axes, we took two steps to ensure that we avoided confounding factors. We first conducted ANOVAs with each environmental PC axis to test for associations with *Gobiomorus* presence, *Cyprinodon* presence, and their interaction ($n = 17$ blue holes). Only one marginally significant term was observed: *Cyprinodon* presence with Environmental PC 2 ($F_{1,13} = 4.45$, $P = 0.055$). This indicated that *Cyprinodon* tended to be present in blue holes with higher salinity and greater zooplankton density. All other tests were non-significant (all $P > 0.29$), revealing that *Gobiomorus* presence is independent of these environmental variables. Second, we examined variance inflation factors (VIFs) in our statistical models that included multiple factors (described below), and all were small (all <1.53). Together, these results of weak to absent associations indicate no problems of multicollinearity, increasing our confidence in analyses designed to tease apart effects of these alternative factors.

For demographics and habitat use, we first used habitat-specific demographic data to conduct mixed-model nested ANOVAs testing for effects of *Gobiomorus* presence, habitat, and their interaction on log-transformed density, square-root transformed sex ratio, and arcsine square-root transformed proportional density of juveniles. Population nested within *Gobiomorus* presence was treated as a random effect in the models. For density, the habitat term included all four habitat types ($n = 68$), while for the other two variables it only included the two near-shore regions due to low sample sizes in offshore regions which would have reduced accuracy of these estimates and led to considerable missing data ($n = 28$; 6 cases were excluded as no fish were observed in the deep habitat region).

Environmental variable	PC 1	PC 2	PC 3
Chlorophyll *a* [RFU]	**0.68**	−0.45	0.44
Phycocyanin [RFU]	**0.89**	−0.35	−0.07
Turbidity [NTU]	**0.93**	−0.05	0.09
Secchi depth [m]	**−0.65**	**−0.57**	−0.11
Log zooplankton [#/ml]	**0.54**	**0.65**	−0.16
Log phytoplankton [#/ml]	0.37	0.05	**−0.77**
Salinity [ppt]	−0.07	**0.72**	−0.04
Surface diameter [m]	−0.05	0.41	**0.71**
Variance explained	37.26	22.10	16.77

Table 2 Principal components analysis of quantitative environmental variables

Factor loadings in bold indicate variables that load strongly on each axis (loadings ≥ |0.5|)

Second, using population means for each variable ($n = 17$) we took a model selection approach to evaluate the effects of predation, interspecific competition, and environmental factors on five dependent variables: the three demographic variables (pooled across habitats), arcsine square-root transformed shallow-water use, and arcsine square-root transformed offshore use. For each dependent variable, we built general linear models that included *Gobiomorus* presence, *Cyprinodon* presence, and their interaction, and then used model selection based on Akaike's Information Criterion corrected for small sample sizes (AIC_c; Akaike 1992; Burnham and Anderson 2002) to determine whether certain environmental PC axes should be included in the models. Because we wished to discover any potentially important environmental factor (even those with weak effects, which could suggest future directions for research), we selected the best model (lowest AIC_c) that included at least one environmental PC unless that model's ΔAIC_c was greater than 2.0 or the term was clearly non-significant ($P > 0.25$), in which case no environmental PC was retained. This allowed us to potentially retain a more complex model that included any strongly suggestive environmental factors while following the convention of considering all models with ΔAIC_c less than 2.0 (Burnham and Anderson 2002).

For behaviors, we first reduced dimensionality by performing a PCA on the correlation matrix of the six behaviors ($n = 240$), retaining PC axes according to the method described above. Then we conducted mixed-model nested ANOVAs to test for effects of *Gobiomorus* presence, sex, and their interaction on the retained behavioral PC axes. In these models, observer and population nested within *Gobiomorus* presence were treated as random effects. We further employed a model selection approach analogous to that described above. We used site means of behavioral variables for each sex to construct general linear models for each behavioral PC that potentially included the following terms and all possible two-way interactions: *Gobiomorus* presence, sex, square-root transformed sex ratio, and arcsine square-root transformed proportional density of juveniles ($n = 12$). As above, models were selected using AIC_c, and focused on models with ΔAIC_c less than 2.0. The terms chosen for the initial model sets were based on a balance of hypothesized importance in explaining behavioral variation and degrees of freedom. While density could significantly affect behaviors, we could not examine this due to density's strong association with *Gobiomorus* presence (VIF > 12 when included in models). Moreover, while we chose not to include environmental PCs due to sample size constraints, we found no trends with behaviors during data exploration, and thus these factors are likely of little significance here. Finally, effects of *Cyprinodon* presence could not be adequately examined in this case as no high-predation blue holes included in this analysis contained *Cyprinodon*. However, analysis within only low-predation blue holes revealed no suggestive evidence for effects of *Cyprinodon*.

Results

Demographics and habitat use

For *G. hubbsi* density, we found significant effects of all model terms (Table S2). *Gambusia hubbsi* densities were much higher in the absence of *Gobiomorus* ($P < 0.0001$), especially in shallow near-shore habitat (interaction term, $P = 0.0104$), and most *G. hubbsi* were located in near-shore regions ($P < 0.0001$) (Fig. 1a). For *G. hubbsi* sex ratio, we found suggestive evidence for effects of *Gobiomorus* and the interaction between

predator presence and habitat type (Table S2), indicating trends where the sex ratio was slightly more female biased in the absence of *Gobiomorus*, particularly in deep near-shore habitat (both $P < 0.08$) (Fig. 1b). Age structure of *G. hubbsi* populations was only cor- related with *Gobiomorus* presence (Table S2), where a greater proportion of juveniles was observed in the absence of the predator ($P = 0.0010$) (Fig. 1c).

In our examination of the effects of predation, interspecific competition, resource availability, and abiotic variables on demographics and habitat use of *G. hubbsi* using model selection, we found that predation was the most commonly significant factor, although *Cyprinodon* presence and resource availability also had significant effects (Table 3, Table S3). *Gambusia hubbsi* densities were higher in the absence of *Gobiomorus*, and no other factors influenced density. When including resource and abiotic variation in the analysis, sex-ratio differences between predator regimes were no longer evident, but a weak trend of more female-biased sex ratios in sites with reduced salinity and zooplankton was observed (Fig. 2f). A greater proportion of juveniles was present in the absence of *Gobiomorus* and in

Fig. 1 Variation across predator regimes and habitat types in *Gambusia hubbsi* **a** density, **b** sex ratio, and **c** proportional density of juveniles (back-transformed least-squares means and standard errors depicted). *SNS* shallow near-shore, *DNS* deep near-shore, *SOS* shallow offshore, *DOS* deep offshore
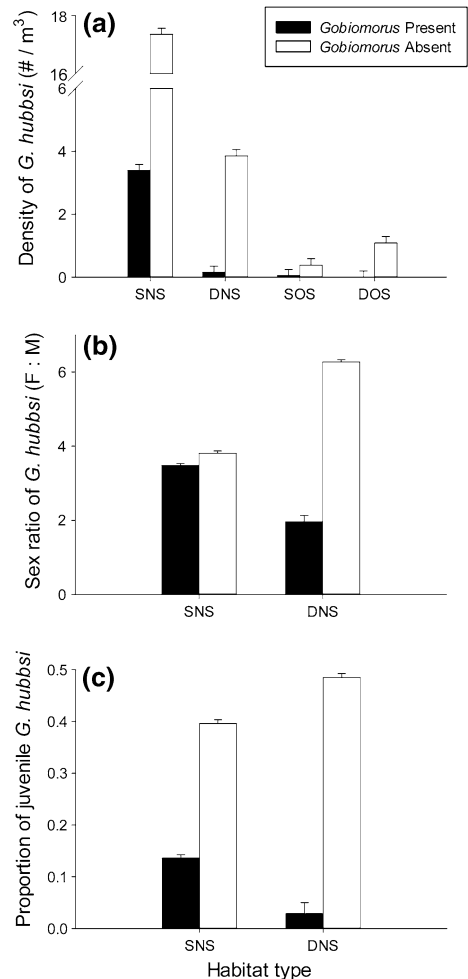
Table 3 Results of general linear models examining population demographics and habitat use as predicted by predator presence, competitor presence, and quantitative environmental factors (environmental PCs included in models based on AIC$_c$, see Table S3)

Source	Density		Sex ratio		Prop. Juveniles		Shallow-water		Offshore	
	$F_{1,13}$	P	$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,13}$	P
Gobiomorus predator (P)	48.96	<0.0001	0.94	0.3506	12.15	0.0045	55.08	<0.0001	4.59	0.0516
Cyprinodon competitor (C)	0.07	0.7899	1.23	0.2896	2.29	0.1565	6.29	0.0275	0.32	0.9022
P × C	0.06	0.8059	0.54	0.4758	0.49	0.4986	2.00	0.1831	2.66	0.1725
Environment PC1 (productivity)	–	–	–	–	4.88	0.0473	11.33	0.0056	–	–
Environment PC2 (salinity, zooplankton)	–	–	3.13	0.1022	–	–	–	–	–	–
Environment PC3 (size, phytoplankton)	–	–	–	–	–	–	–	–	–	–

sites with greater resource availability (Fig. 2a, b). Shallow-water use was greater in the presence of *Gobiomorus*, in the absence of *Cyprinodon*, and in sites with greater resource availability (Fig. 2c, d). Offshore use tended to increase in the absence of *Gobiomorus* (Fig. 2e).

Behavior

We retained three PC axes describing variation in social and foraging behaviors, explaining approximately 64 % of behavioral variance (Table 4). We interpret the first PC axis as a trade-off between foraging and sexual behaviors, the second axis as a trade-off between within-sex and between-sex aggression, and the third axis as a trade-off between aggressive behaviors and sexual/foraging behaviors (Table 4). We first examined effects of predation, sex, and their interaction on *G. hubbsi* behaviors, finding many significant influences on behavioral variation (Table S4). For PC 1, we found that (1) males exhibited more sexual behaviors and less foraging behaviors than females ($P < 0.0001$), (2) the presence of *Gobiomorus* was associated with an increase in sexual behaviors and a decrease in foraging behaviors ($P = 0.0157$), and (3) the latter effect was more pronounced for females than for males ($P = 0.0303$) (Fig. 3a). For PC 2, we found that males exhibited much more intrasexual aggression and less intersexual aggression in the presence of *Gobiomorus*, while females exhibited only a slight trend in this direction—this resulted in strong sexual differences in the presence, but not absence, of *Gobiomorus* (interaction term, $P = 0.0001$) (Fig. 3b). For PC 3, we found that both sexes exhibited similar values in the absence of *Gobiomorus*, but strongly diverged in the predator's presence, where females exhibited more sexual/foraging behaviors and less aggressive behaviors than males (interaction term, $P = 0.0002$) (Fig. 3c).

Using model selection, we found that demographic variables were associated with two of the three behavioral PC axes (Table 5, Table S5). For PC 1, this analysis revealed that
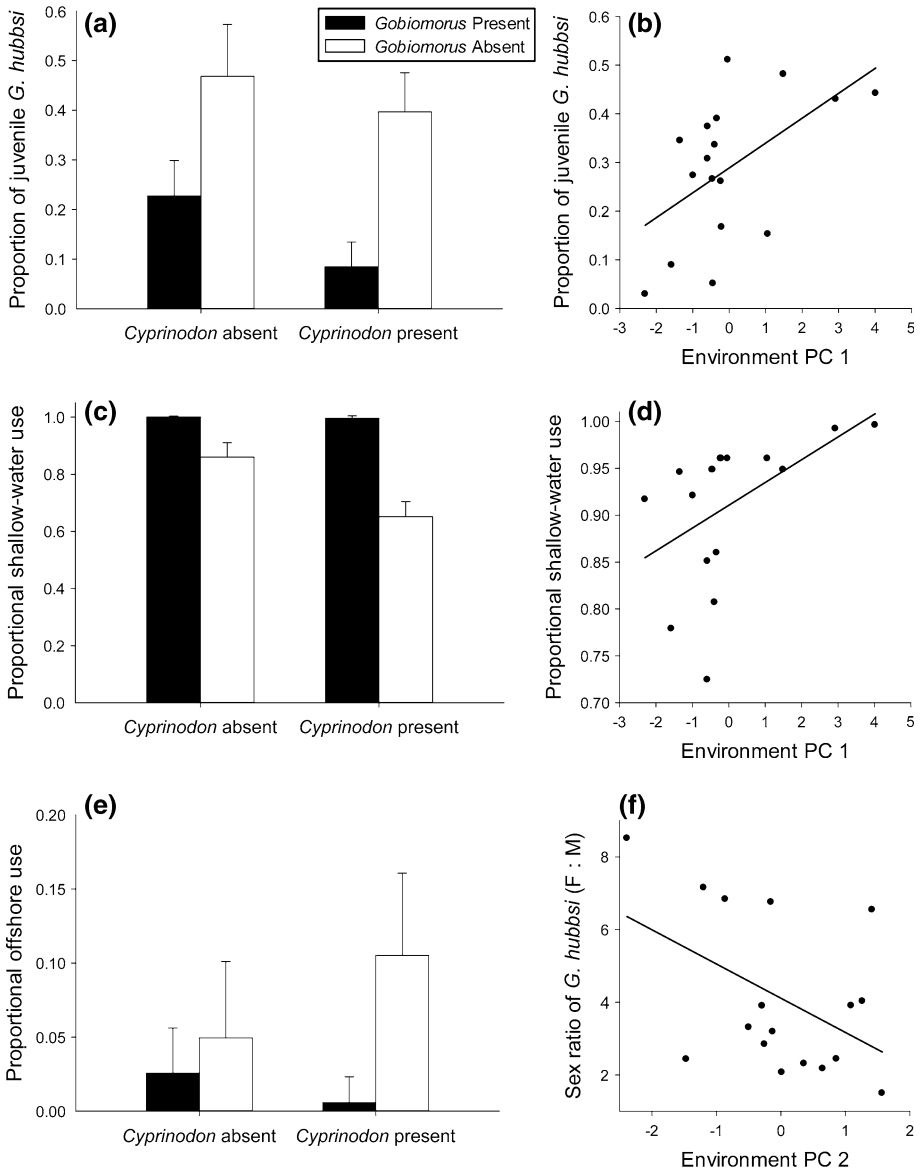
Fig. 2 Variation across *Gobiomorus* presence, *Cyprinodon* presence, and environmental PCs for *Gambusia hubbsi* **a**, **b** proportional density of juveniles, **c**, **d** shallow-water use, **e** offshore use, and **f** sex ratio. Back-transformed least-squares means and standard errors depicted in *bar graphs*; back-transformed residuals depicted in *scatter plots*

a sex-dependent effect of sex-ratio, where females exhibited less sexual behavior and more foraging behavior in sites with a more female-biased sex ratio (Fig. 3d), apparently explained the sex-dependent response to predation observed above. For PC 2, results were consistent with those described above, with no additional effects of demographic

Table 4 Principal components analysis of foraging and social behaviors

Behavior	PC 1	PC 2	PC 3
Feeding	**−0.53**	0.23	0.28
Prey inspection	**−0.54**	−0.27	**0.49**
Copulation attempt	**0.71**	0.12	**0.47**
Male–female chase	**0.68**	−0.09	0.38
Intrasexual aggression	0.25	**−0.68**	**−0.48**
Intersexual aggression	0.15	**0.74**	−0.38
Variance explained	27.25	19.35	17.65

Factor loadings in bold indicate variables that load strongly on each axis (loadings ≥ |0.4|)
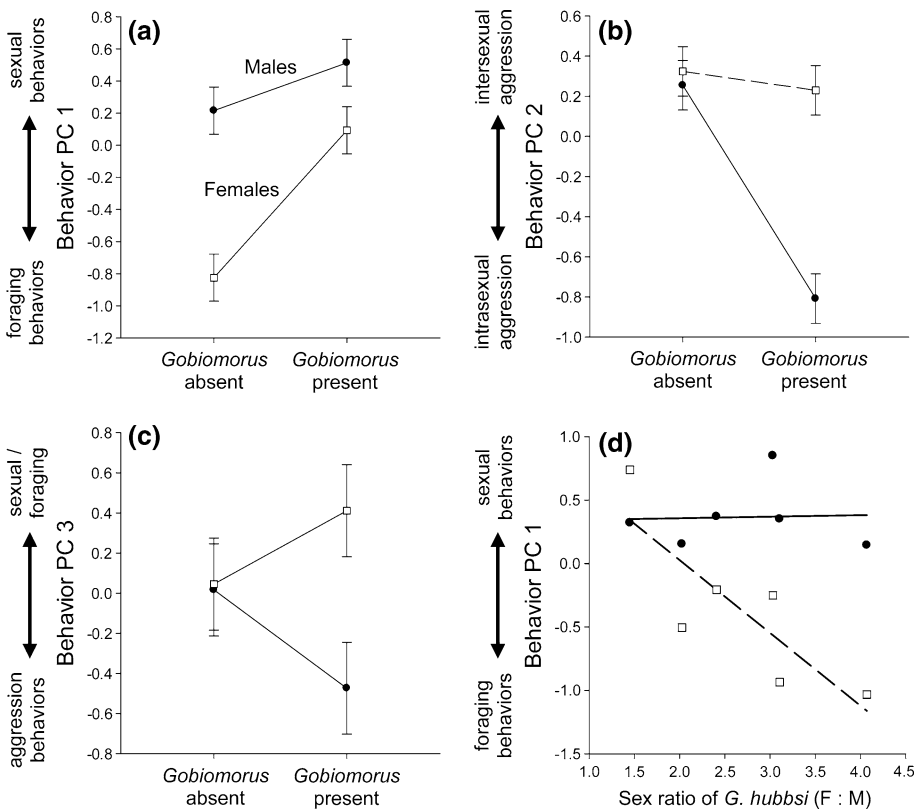

Fig. 3 Variation in foraging and social behaviors of *Gambusia hubbsi* in relation to sex, *Gobiomorus* presence, and demographics. Effects of sex and predation on **a** behavioral PC 1, **b** behavioral PC 2, and **c** behavioral PC 3; and **d** effect of sex ratio on behavioral PC 1. Least-squares means and standard errors depicted in **a**–**c**; site means depicted in **d**

variables. For PC 3, additional effects of sex ratio (more sexual/foraging behavior and less aggression with a more female-biased sex ratio) and age structure (more sexual/foraging behavior and less aggression with a greater proportion of juveniles) were observed.

Table 5 Results of general linear models examining foraging and social behaviors as predicted by predator presence, sex, and demographics (models selected based on AICc)

Trait	Source	F	df	P
Behavior PC1	*Gobiomorus* predator	10.24	1,7	0.0151
	Sex (S)	28.20	1,7	0.0011
	Sex ratio (SR)	2.54	1,7	0.1552
	S × SR	12.85	1,7	0.0089
Behavior PC2	*Gobiomorus* predator (P)	27.87	1,8	0.007
	Sex (S)	25.50	1,8	0.0010
	P × S	19.53	1,8	0.0022
Behavior PC3	*Gobiomorus* predator (P)	31.28	1,6	0.0014
	Sex (S)	55.50	1,6	0.0003
	P × S	48.62	1,6	0.0004
	Sex ratio	65.36	1,6	0.0002
	Prop. juvenile	36.24	1,6	0.0009

Discussion

In nature, most organisms inhabit complex environments where they experience multiple selective agents to which multiple individual traits and population characteristics may respond. Tackling such complexity is difficult, and most studies to date have focused on atomized components of the environment and phenotype. We investigated how multiple environmental factors are correlated with demographics, habitat use, and behavior across *G. hubbsi* populations inhabiting inland blue holes on Andros Island, The Bahamas. Based on prior knowledge of the system, we predicted that presence or absence of the predatory fish *Gobiomorus dormitor* would be the most influential ecological factor associated with population-level differentiation, but that the presence of a competitor species (*Cyprinodon variegatus*), resource availability, and abiotic factors may also contribute.

Our results suggest that variation in predation risk indeed represents the environmental agent most commonly associated with ecological differentiation across *G. hubbsi* populations. *Gobiomorus* presence was associated with differences in population density, age structure, habitat use, and a variety of behaviors—a weak association with *G. hubbsi* sex ratio was also observed. We found support for every a priori prediction regarding predation described in Table 1, and we found that other environmental agents were correlated with population characteristics as well. Together, these results provide a more complete and integrative understanding of the complex interactions that can contribute to ecological differentiation, and sheds light on which environmental factors may prove most potent during adaptive diversification. Further, this study is the first to investigate variation in demographics, habitat use, and behavior in the model system of Bahamas blue holes.

Demographics

Gambusia hubbsi population density was only correlated with predation risk. Consistent with our prediction, density was greatly reduced in the presence of *Gobiomorus*, presumably from higher mortality rates. These results are consistent with trends in other poeciliid fishes (*Poecilia reticulata*: Gilliam et al. 1993; Reznick et al. 2001; Palkovacs

et al. 2011; *Brachyrhapis*: Johnson 2002; *Gambusia*: Araujo et al. submitted), suggesting fish predators commonly reduce poeciliid fish densities. In contrast with our predictions, *G. hubbsi* did not exhibit higher densities in sites with greater resource availability. It is unlikely that this resulted from imprecision in our estimates as we did find that resource availability was associated with other factors (see below). It is also unlikely that overall densities are influenced by cumulative resource availability over longer time frames (e.g. previous year) due to the general stability of blue holes and the high temporal repeatability of most environmental variables. Rather, the dramatic impacts of predation appear to negate virtually any effect of resource availability on *G. hubbsi* densities in blue holes. Moreover, the presence of an interspecific competitor was not associated with reduced densities of *G. hubbsi*. While this may suggest that *Cyprinodon* does not strongly compete with *G. hubbsi*, we did find that *Cyprinodon* presence seemed to influence *G. hubbsi* habitat use (see below). This habitat shift may alleviate some of the negative impacts of competition with *Cyprinodon*.

We predicted that *G. hubbsi* populations with predators would have a more even sex ratio, while low-predation populations would be more biased toward females. Our results suggest a weak relationship of this type, especially in deep-water habitats. Our prediction was largely based on (1) potentially increased predation rates on larger, more energetically valuable females by *Gobiomorus*, and (2) males potentially being worse competitors for food than females and more susceptible to starvation (Schultz 1977). However, our results actually seem to reflect a habitat shift where females increase use of more marginal, deep-water regions to reduce sexual harassment from males in high density, low-predation populations (Croft et al. 2006; Darden and Croft 2008). This is supported by the significant interaction term between predator regime and habitat, and by the elimination of sex-ratio differences when pooled across habitats—both of which suggest sex ratio only tended to differ between predator regimes within deep habitats. Additionally, the more even sex ratio in the presence of *Gobiomorus* could partially reflect greater predation rates on females in deep-water regions, where *Gobiomorus* are primarily found. While a previous study did not find differential predation rates of *Gobiomorus* among the sexes of *G. hubbsi* (Langerhans 2009), that experimental study only examined predation in shallow water. Future work is required to more accurately quantify sex-ratio differences across habitat types and blue holes, and determine which mechanism(s) might be responsible.

Juveniles comprised a larger proportion of *G. hubbsi* populations in blue holes without *Gobiomorus*, matching our predictions based on elevated survival probabilities in the absence of predation. Also matching predictions, the proportional density of juveniles was positively associated with resource availability, possibly due to higher survivorship in sites with reduced intraspecific resource competition (Clutton-Brock et al. 2001; Daunt et al. 2007), and also potentially from increased fecundity in sites with higher resource levels (Grether et al. 2001; Johnson 2002; Riesch et al. 2013). We did not find significant evidence that *Cyprinodon* presence reduced juvenile recruitment (although a weak trend in this direction was observed), as we predicted might occur due to potentially increased competition for space and food.

Between-population differences in density, sex ratio, and age structure can have important evolutionary consequences, as these parameters can influence social interactions, intraspecific resource competition, the relative intensity of intra- and intersexual selection, life-history traits, and rate of evolutionary responses to selection (e.g. Clutton-Brock and Parker 1992; Charlesworth 1994; Roff 2002; Kokko and Rankin 2006; Smith and Sargent 2006; Knell 2009). Future research should investigate the consequences of the patterns of demographic variation observed here.

Habitat use

Gambusia hubbsi generally occupy the shallow, near-shore areas of blue holes where food (e.g. allochthonous input and small organisms living in the substrate) and shelter, provided by complex cave walls and aquatic vegetation, are most abundant. This tendency to use shallow, near-shore habitat was especially evident in the presence of *Gobiomorus*, matching our predictions based on avoidance of deep and offshore regions where *Gobiomorus* are more abundant and no structural refugia exist. This pattern is also consistent with some other poeciliids, which use shallow, near-shore regions more often under higher risk of predation (Fraser and Gilliam 1992; Reznick et al. 2001). While most *G. hubbsi* within a given blue hole were observed in shallow, near-shore regions, they commonly used deep water in the absence of *Gobiomorus*—especially in near-shore regions, where densities were similar to total densities combined across all habitats in the presence of *Gobiomorus* (see Fig. 1a). This deep-water use is rarely observed in other poeciliid fishes.

Predation was not the only factor associated with habitat use of *G. hubbsi*. First, *G. hubbsi* increased deep-water use in the presence of the interspecific competitor *Cyprinodon*, as predicted. Male *Cyprinodon* are highly territorial, often defending sections of the cave walls, and can be aggressive toward *G. hubbsi* (R. B. Langerhans, pers. obs.). Such interference competition could result in increased use of marginal habitat to avoid antagonistic encounters with *Cyprinodon*. Second, shallow-water use increased in sites with greater resource availability. This could reflect the fact that most productivity in the blue holes relevant to *G. hubbsi* is confined to relatively shallow areas, and thus this region experiences the greatest increase in density as resource levels increase.

Regardless of the source of the habitat shift—be it predation, competition, or resource availability—this may result in concurrent shifts in diet and changes in selection pressures. The different habitat types examined in blue holes likely possess different distributions of prey items, requiring different detection, locomotor, foraging, and feeding strategies. Previous work has shown both heritable and induced morphological responses to varying food regimes in *Gambusia* and other poeciliid fishes (Robinson and Wilson 1995; Ruehl and DeWitt 2005). Moreover, these different habitats likely differ in ambient background color and light environment, potentially influencing the evolution of color signals (e.g. *G. hubbsi* males possess bright orange dorsal fins) (Endler 1992; Boughman 2001; Leal and Fleishman 2002). Future work can examine whether observed differences in habitat use might reflect a plastic response to environmental cues or genetic divergence—but either source can result in evolutionary change.

Behavior

The presence of predators is a major source of behavioral differences in *G. hubbsi*, with substantial associations with foraging, sexual, and aggressive behaviors. First, female *G. hubbsi* exhibited less sexual and more foraging behaviors than males, regardless of predator regime. This pattern is widespread across many taxa as a consequence of anisogamy, and is consistent with behavior in other poeciliid fishes (Houde 1997; Magurran 2005). In line with our prediction, both sexes reduced foraging and increased sexual behaviors in the presence of *Gobiomorus*. For females, this increase in sexual behavior involves passive sexual behaviors (e.g. experiencing a copulation attempt, being chased by a male). We expected this pattern for two primary reasons. First, high-predation populations are less dense than low-predation populations, but do not differ in resource

availability. This presumably results in reduced resource competition, allowing these fish to spend less time searching for food and more time searching for mates. Second, because life expectancy is likely shorter for *G. hubbsi* in high-predation sites, and because mating occurs under the constant risk of mortality, selection may favor individuals that mate early, often, and rapidly to maximize fitness (Magnhagen 1991; Godin 1995). The magnitude of behavioral differences between predator regimes was greater in females than males apparently because of a sex-dependent effect of sex ratio on foraging and sexual behaviors. That is, females exhibited more foraging behaviors and fewer sexual behaviors as the sex ratio became more female biased. In environments with a higher relative abundance of females, sexual encounters may be less frequent simply due to reduced encounter probabilities with males. After controlling for sex-ratio effects in our model-selection analysis, there was no longer any indication of a difference between the sexes in the strength of foraging and sexual behavioral differences between predator regimes. This highlights the importance of simultaneously considering multiple population characteristics when investigating patterns of ecological differentiation.

Sexes strongly differed in their relative frequencies of inter- and intra-sexual aggressive behaviors in the presence of *Gobiomorus*, but not in its absence. While females maintained a high level of aggression toward males in all sites, males exhibited much more male–male aggression and much less male–female aggression in the presence of *Gobiomorus*, matching our predictions. Elevated male–male aggression in high-predation populations may reflect more intense competition among males for access to females in a social environment relying less on female receptivity and more on forced mating (Endler 1995), and having fewer total females in these less dense populations (Jirotkul 1999). Moreover, if high-predation males are in better condition as a result of greater access to food—which recent work suggests may be the case (Riesch et al. 2013)—they may have the energetic resources needed to engage in costly male–male contests more frequently (Kolluru and Grether 2005). Increased levels of aggression between the sexes in low-predation populations may largely represent stronger intraspecific resource competition in these high-density environments, but may additionally reflect a shift toward more aggressive tactics to assess female receptivity and secure matings (Jirotkul 1999).

Population differences in any of these behaviors can have evolutionary consequences, affecting reproductive success, resource acquisition, and survival (e.g. Clark et al. 1954; Farr 1976; Horth 2003; Köhler et al. 2011). Moreover, such behavioral differences can lead to premating sexual isolation and assortative mating, driving the speciation process (Mayr 1963; Coyne and Orr 2004; Gavrilets 2004; Price 2008). Future work is needed to uncover the environmental (i.e. phenotypic plasticity) and genetic bases of observed behavior patterns, as well as their importance in facilitating ecological and evolutionary divergence.

Our results provide a more integrative understanding of how multiple environmental agents interact to drive ecological differentiation during the early stages of species radiations. Our study confirms predation's important role in promoting ecological divergence and demonstrates that interspecific competition and resource availability are also notable contributors. Future experimental work could build on this comparative study to confirm the direction of causation for the trends we revealed between ecological factors and population attributes.

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"Environmental drivers of demographics, habitat use, and behavior during a post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*)"

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Figure S1 Map of 17 blue holes where *Gambusia hubbsi* were collected (blue holes without piscivorous fish: blue circles, blue holes with piscivorous fish: red circles). Site numbers follow Table S1.
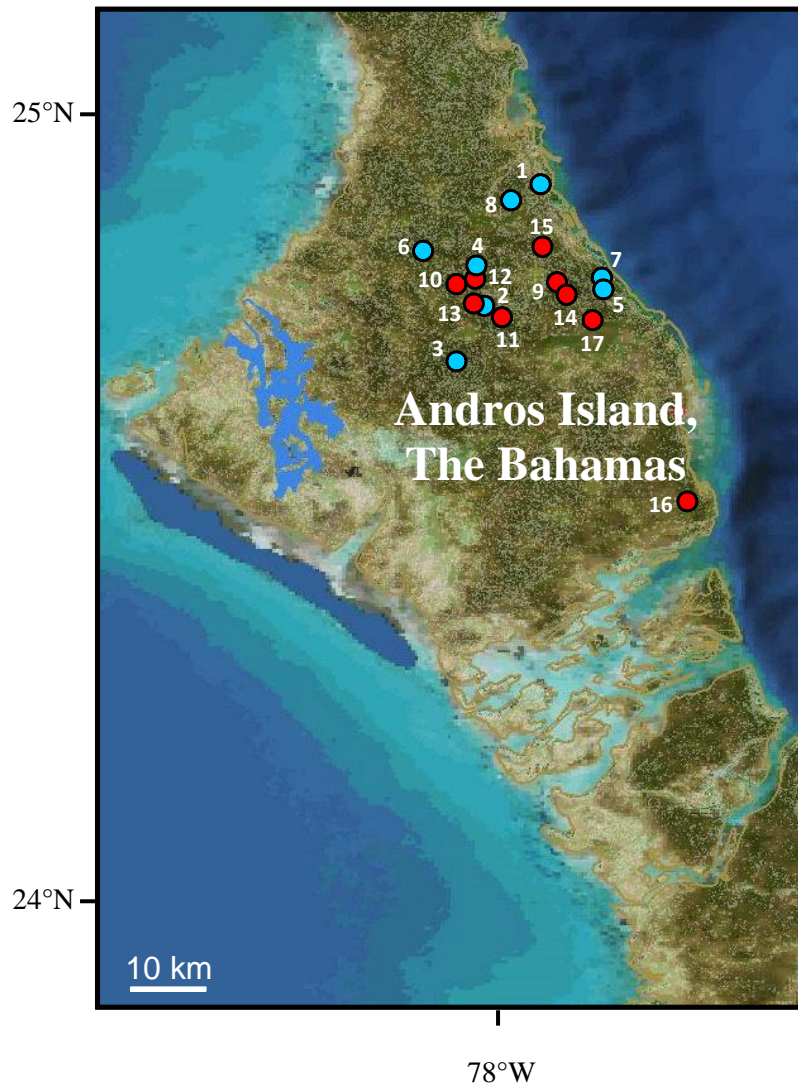
Table S1 Summary of study sites (those censused in Summer 2011 were additionally examined for adult feeding and social behaviors).

Blue Hole	Census Sampling Period(s)	*Gobiomorus* Presence	*Cyprinodon* Presence	Fig. S1 Label
Archie's	Spring 2011	Absent	Absent	1
East Twin	Fall 2009, Spring 2011, Summer 2011	Absent	Absent	2
Pigskin	Spring 2011	Absent	Absent	3
Gollum	Spring 2011	Absent	Present	4
Hubcap	Spring 2011, Summer 2011	Absent	Present	5
Ken's	Spring 2011	Absent	Present	6
Rainbow	Fall 2009, Spring 2011, Summer 2011	Absent	Present	7
Voy's	Spring 2011	Absent	Present	8
Cousteau's	Fall 2009, Spring 2011, Summer 2011	Present	Absent	9
Hard Mile	Spring 2011	Present	Absent	10
Runway	Spring 2011	Present	Absent	11
Stalactite	Fall 2009, Spring 2011, Summer 2011	Present	Absent	12
West Twin	Spring 2011, Summer 2011	Present	Absent	13
Gibson	Spring 2011	Present	Present	14
Goby Lake	Fall 2009, Spring 2011	Present	Present	15
Rivean's	Spring 2011	Present	Present	16
Shawn's	Fall 2009, Spring 2011	Present	Present	17

Table S2 Mixed-model nested ANOVA results examining variation in population demographics across predator regimes and habitat types.

Source	Density			Sex Ratio			Prop. Juveniles		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
Gobiomorus Predator (P)	101.99	1,15	< 0.0001	3.55	1,17.4	0.0765	15.68	1,16.9	0.0010
Habitat (H)	42.89	3,45	< 0.0001	0.03	1,14.8	0.8750	0.39	1,13.8	0.5401
P x H	4.21	3,45	0.0104	3.57	1,14.8	0.0787	2.52	1,13.8	0.1349

Table S3 Model selection results examining variation in population demographics and habitat use. Model term abbreviations: P = *Gobiomorus* predator presence, C = *Cyprinodon* competitor presence, E-PC# = environmental principal components. Selected models in bold type. For sex ratio, we selected a more complex model within 2 AIC_c units of the best model, as we were particularly interested in identifying any influential environmental factors.

Model	Density AIC_c	ΔAIC_c	Sex Ratio AIC_c	ΔAIC_c	Prop. Juveniles AIC_c	ΔAIC_c	Shallow-water AIC_c	ΔAIC_c	Offshore AIC_c	ΔAIC_c
P + C + P x C	**12.46**	**0.00**	40.37	0.00	5.42	0.86	-1.79	6.36	**6.05**	**0.00**
P + C + P x C + E-PC1	16.30	3.84	44.50	4.13	**4.57**	**0.00**	**-8.15**	**0.00**	10.80	4.75
P + C + P x C + E-PC2	17.21	4.75	**41.38**	**1.00**	10.20	5.63	0.70	8.85	8.56	2.51
P + C + P x C + E-PC3	16.83	4.38	45.16	4.79	8.81	4.24	1.05	9.19	10.42	4.37
P + C + P x C + E-PC1 + E-PC2	21.93	9.47	46.98	6.61	9.71	5.14	-4.39	3.76	14.04	7.99
P + C + P x C + E-PC1 + E-PC3	21.57	9.11	50.44	10.07	7.51	2.95	-4.97	3.18	16.20	10.16
P + C + P x C + E-PC2 + E-PC3	22.70	10.24	47.29	6.92	14.71	10.14	4.49	12.64	14.05	8.00
P + C + P x C + E-PC1 + E-PC2 + E-PC3	28.72	16.26	54.43	14.06	14.08	9.51	0.01	8.15	20.91	14.86

Table S4 Mixed-model nested ANOVA results examining variation in foraging and social behaviors across predator regimes and sexes.

Source	Behavior PC1 F	df	P	Behavior PC2 F	df	P	Behavior PC3 F	df	P
Gobiomorus Predator (P)	16.26	1,4	0.0157	22.71	1,4	0.0089	0.04	1,4	0.8470
Sex (S)	26.32	1,229	< 0.0001	19.69	1,229	< 0.0001	16.62	1,229	< 0.0001
P x S	4.75	1,229	0.0303	15.08	1,229	0.0001	14.56	1,229	0.0002

Table S5 Model selection results examining variation in foraging and social behaviors. Models with $\Delta\,AIC_c < 8.0$ presented. Model term abbreviations: P = *Gobiomorus* predator presence, S = sex, SR = sex ratio, J = proportional density of juveniles. Selected models in bold type. For behavioral PC1, we selected the more complex model with $\Delta\,AIC_c$ of 1.14, as we were particularly interested in identifying any influential demographic factors (i.e. including any demographic term with a suggestive *P* value < 0.25).

Dependent Variable	Model	AIC_c	ΔAIC_c
Behavior PC1	P + S	20.82	0.00
	P + S + SR + S x SR	**21.95**	**1.14**
	S	23.63	2.81
	S + SR+ S x SR	23.97	3.15
	P + S + P x S	24.01	3.19
	S + J	24.25	3.43
	S + SR	24.37	3.55
	P + S + SR	25.66	4.84
	P	25.89	5.07
	S + SR + J + S x SR	25.98	5.16
	P + J + S	26.48	5.66
	S + SR + J	27.24	6.42
	J	27.53	6.71
	SR	27.59	6.77
	S + J + S x J	27.81	6.99
Behavior PC2	**P + S + P x S**	**9.31**	**0.00**
	P + S + SR + P x S	12.08	2.76
Behavior PC3	**P + S + SR + J + P x SR**	**13.93**	**0.00**
	S + SR	19.17	5.24
	S	19.83	5.90
	SR	19.84	5.91
	J	21.88	7.95