



Predicting ecological and phenotypic differentiation in the wild: a case of piscivorous fish in a fishless environment

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Environmental variation drives ecological and phenotypic change. How predictable is differentiation in response to environmental change? Answering this question requires the development and testing of multifarious a priori predictions in natural systems. We employ this approach using *Gobiomorus dormitor* populations that have colonized inland blue holes differing in the availability of fish prey. We evaluated predictions of differences in demographics, habitat use, diet, locomotor and trophic morphology, and feeding kinematics and performance between *G. dormitor* populations inhabiting blue holes with and without fish prey. Populations of *G. dormitor* independently diverged between prey regimes, with broad agreement between observed differences and a priori predictions. For example, in populations lacking fish prey, we observed male-biased sex ratios, a greater use of shallow-water habitat, and larger population diet breadths as a result of greater individual diet specialization. Furthermore, we found predictable differences in body shape, mouth morphology, suction generation capacity, strike kinematics, and feeding performance on different prey types, consistent with the adaptation of *G. dormitor* to piscivory when coexisting with fish prey and to feeding on small invertebrates in their absence. The results of the present study suggest great potential in our ability to predict population responses to changing environments, which is an increasingly important capability in a human-dominated, ever-changing world. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, 114, 588–607.

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INTRODUCTION

A major topic in evolutionary ecology centres on understanding the role of environmental variation, both biotic and abiotic, in driving predictable differentiation between populations. Despite renewed interest in the importance of environmental factors that might drive predictable phenotypic differentiation, evolutionary divergence, and ultimately speciation (Langerhans, Gifford & Joseph, 2007; Langerhans, 2008; Schluter, 2009; Nosal, 2012;

Langerhans & Riesch, 2013), we still have much to learn about the predictability of population differentiation during times of environmental change (Langerhans, 2010; Heinen *et al.*, 2013; de Visser & Krug, 2014; Wellborn & Langerhans, in press). In particular, the joint investigation of the predictability of changes in both ecological parameters (e.g. population density, sex ratio) and phenotypes (e.g. morphology, behaviour) is rare but important, especially in light of increasing evidence for potentially widespread eco-evolutionary feedbacks (Palkovacs & Hendry, 2010; Farkas *et al.*, 2013). Addressing these topics is particularly crucial today, as humans dramatically impact

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natural systems, altering natural regimes of selection and gene flow.

To further the goals described above, in this study we ask to what extent can we predict organisms' ecological and phenotypic responses to environmental variation a priori based on prior theoretical knowledge and an understanding of the natural history of relevant systems? To gain insights into this question, it is ideal to begin with a relatively simple system. Here, we use the model system of inland blue holes in The Bahamas. Such a simple system allows us to focus on specific environmental factors, develop clear predictions based on strong theoretical and empirical foundations, and more directly test the predictability of change in both ecological parameters and phenotypic traits during population differentiation.

STUDY SYSTEM

The bigmouth sleeper [*Gobiomorus dormitor* (Lacepède, 1800)] is an eleotrid fish inhabiting tropical and subtropical coastal freshwater and brackish environments in the Caribbean, south-eastern Florida, southern Texas, and the Atlantic slope of Central and northern South America (Lindquist, 1980; Gilmore, 1992). *Gobiomorus dormitor* is commonly catadromous or amphidromous (Darnell, 1962; Winemiller & Ponwith, 1998; Adelsberger, 2009; Nordlie, 2012), using saltwater regions during egg laying or larval development, but is also known to reproduce in some landlocked lakes and reservoirs (Darnell, 1962; McKaye, Weiland & Lim, 1979; Holmquist, Schmidt-Gengenbach & Yoshioka, 1998; Bedarf *et al.*, 2001; Bacheler, Neal & Noble, 2004a). In coastal streams and lakes (its ancestral environment), *G. dormitor* is a benthic, ambush predator, feeding primarily on fish and large invertebrates (McKaye, Weiland & Lim, 1979; Winemiller & Ponwith, 1998; Bedarf *et al.*, 2001; Bacheler, Neal & Noble, 2004b).

Although widespread across the Greater Antillean islands, within the Bahama Archipelago the species is only known from the northern half of Andros Island. In this region, bigmouth sleepers have colonized numerous land-locked blue holes (water-filled, vertical caves; Mylroie, Carew & Moore, 1995) during the past approximately 15 000 years when rising sea levels pushed the freshwater aquifers of the island upward, flooding the ancient cave systems (Fairbanks, 1989). These blue holes average approximately 127 m in surface diameter (range 52–304 m), with very steep shorelines dropping to a mean depth of approximately 40 m (range 7–105 m). In blue holes, bigmouth sleepers are readily observed in shallow regions, swimming throughout the water column and

vertically positioned along the steep-sided cave walls, unlike their more typical stream and lake environments where they generally remain cryptic, resting on the bottom. Previous research has revealed that other fish species are strongly isolated among different blue holes (Schug *et al.*, 1998; Langerhans *et al.*, 2007; Heinen-Kay & Langerhans, 2013; Riesch, Martin & Langerhans, 2013), and recent studies have demonstrated the utility of blue holes as 'natural laboratories' of evolution, serving as a model system for the study of divergent adaptation and ecological speciation (Langerhans, Layman & DeWitt, 2005; Langerhans *et al.*, 2007; Langerhans, 2010; Heinen *et al.*, 2013; Heinen-Kay & Langerhans, 2013; Langerhans & Makowicz, 2013; Riesch *et al.*, 2013; Martin *et al.*, 2014). These isolated blue holes usually harbour depauperate fish communities, with *G. dormitor* often co-occurring with only one other fish species, the small, livebearing Bahamas mosquitofish [*Gambusia hubbsi* (Breder, 1934); Family Poeciliidae]. However, it was recently discovered that *G. dormitor* inhabit two isolated blue holes in which no other fish species is present. Has this environmental shift driven predictable changes in population and phenotypic characters?

Before describing our predictions for population differentiation, we should first highlight an important prerequisite for testing associations between environmental features (the presence of fish prey) and population/phenotypic attributes: that populations represent independent replicates, where similarities in *G. dormitor* characters among populations in similar environments do not simply reflect shared ancestry or recent/ongoing gene flow. To assess this alternative explanation to replicated ecological and phenotypic change, we evaluated the population genetic structure of *G. dormitor* among blue holes and tested whether populations within the two prey regimes (populations with and without potential fish prey) were more closely related to one another than to populations in the alternative prey regime. Informed by the general geographical isolation of blue holes, and from previous work on *G. hubbsi* populations in inland blue holes (Schug *et al.*, 1998; Langerhans *et al.*, 2007; Heinen-Kay & Langerhans, 2013; Riesch *et al.*, 2013), we expected *G. dormitor* populations to exhibit substantial genetic structure among blue holes, and to reflect a history of haphazard colonization and isolation, unassociated with the presence/absence of potential fish prey.

PREDICTIONS

We derived a set of ecological and phenotypic predictions for *G. dormitor* populations inhabiting divergent prey regimes based on our understanding of the

Table 1. Predictions of population differentiation among *Gobiomorus dormitor* populations inhabiting Bahamas blue holes tested in the present study

Prediction type	Trait type	A priori predictions	
		Fish prey absent	Fish prey present
Ecological	Demographics	↓ density, ↓ proportion small size-class, male-biased sex ratio	↑ density, ↑ proportion small size-class, even sex ratio
	Habitat use	↑ habitat breadth, ↑ shallow and offshore use	↓ habitat breadth, ↑ deep and near-shore use
	Diet	↑ small invertebrates, ↑ diet breadth, ↑ specialization	↑ fish, ↓ diet breadth, ↓ specialization
Phenotypic	Locomotor Morphology	↑ anterior region	↑ posterior region, ↓ frontal area
	Trophic morphology	↓ mouth size, ↑ post. head depth, more terminal mouth	↑ mouth size, ↓ post. head depth, upturned mouth
	Feeding kinematics	↓ attack speed, ↓ strike distance, ↓ gape, ↑ gape speed, ↓ hyoid depression, ↓ head angle	↑ attack speed, ↑ strike distance, ↑ gape, ↓ gape speed, ↑ hyoid depression, ↑ head angle
	Feeding performance	↑ suction index, ↑ force exerted on non-evasive prey, ↑ fish capture time, ↓ strike efficiency on fish prey	↓ suction index, ↑ ability to capture evasive prey, ↓ fish capture time, ↑ strike efficiency on fish prey

natural history of these blue-hole environments, prior theoretical and empirical work on ecological responses to different resource environments, and hypothesized divergent selection on morphology and feeding performance based on ecological and biomechanical relationships (Table 1). To generate ecological predictions, we centred on optimal foraging theory, dietary energetics, and life-history theory. For phenotypic predictions, we used the approach of generalized models of divergent selection (*sensu* Langerhans, 2010) based on previous ecological and biomechanical research to generate testable predictions of changes in phenotypes between prey regimes.

For population demographics, we predict that, in the presence of fish prey, the greater energy-content resource they provide could lead to greater overall *G. dormitor* densities and greater proportion of juveniles, compared to their absence (e.g. higher energy acquisition can lead to greater fecundity, higher larval survival, reduced cannibalism; Reznick, 1985; Manica, 2002). At the same time, reduced energy acquisition in the absence of fish prey could lead to a sex ratio more biased toward males because female development and survival during reproductive periods can be reduced (Bell, 1980; Reznick, 1985) and may require more energy than commonly available in the absence of fish prey. These predictions assume that differences in energy content of consumed prey exist, and that energetic effects on demographics transcend effects of other potentially influential variables; although reasonable for this

system, the validity of these assumptions is unknown, and thus these predictions reflect the weakest predictions tested in the present study.

Regarding habitat use, we predict that, in the absence of fish prey, bigmouth sleepers will expand or otherwise alter their foraging niche in search of typically less-favourable prey. To accomplish this, *G. dormitor* might exhibit a greater overall habitat breadth in the absence of fish prey, or utilize more 'atypical' environments for this species, such as increased use of offshore or shallow-water habitats to feed on prey that typically would have been consumed by *G. hubbsi*.

Gobiomorus dormitor may experience increased levels of competition for invertebrate prey in the absence of fish prey, leading to changes in diet breadth and individual resource utilization. Specifically, a potential response to increased resource competition involves the addition of alternative diet items, where individuals differ in their choice of alternative prey. This can result in both an enlarged population niche width and increased individual diet specialization (increased between-individual variance in diet; Bolnick *et al.*, 2003; Svanbäck & Bolnick, 2007; Araújo, Bolnick & Layman, 2011). Therefore, we predict that, in the absence of fish prey, *G. dormitor* will exhibit an enlarged total niche width, comprising a variety of small invertebrates, and elevated levels of individual diet specialization. In the presence of fish prey, we expect *G. dormitor* to feed primarily on fish and large invertebrates, exhibiting a smaller total niche width and reduced

individual diet specialization because interactions with fish prey should dominate and lessen competition for invertebrates.

To facilitate effective foraging behaviours in the face of divergent prey regimes, we expect natural selection to favour alternative locomotor morphologies in the presence versus absence of fish prey. In the presence of fish prey, we hypothesize that selection will favour morphological features that increase S-start performance during a predatory strike (e.g. ram speed), or facilitate a closer approach to fish prey during a hunting sequence prior to startling the prey, compared to localities without fish prey. Specifically, we predict these predators to exhibit a greater allocation to the posterior (mid-body/caudal) region, especially in the lateral profile (to increase thrust generation), and this should additionally manifest as a more posterior placement of maximum body thickness (Blake, 1983; Webb & Weihs, 1983, 1986; Webb, 1984; Jayne & Lauder, 1994; Walker, 1997; Blake, 2004). Additionally, bigmouth sleepers may exhibit a smaller frontal profile (frontal area of predator, as seen by prey) that would reduce the reaction distance of fish prey and enhance prey-strike success (Dill, 1974; Webb, 1982; Domenici, 2002), although this could be counteracted by increased posterior body depth that would facilitate S-start performance. In the absence of fish prey, selection should not favour these features, permitting counteracting selection and drift to result in divergent morphologies.

Divergent selection on *G. dormitor* morphology should also derive from differential selection on feeding performance (not only *foraging* performance). We expect natural selection to favour morphological features that increase suction force for effective feeding on small invertebrates in the absence of fish prey but, instead, favour features that increase the reach of the suction flow in localities with fish prey. To accomplish these competing feeding demands, we expect bigmouth sleepers to exhibit a smaller mouth, a deeper posterior head depth (to accommodate enlarged epaxial muscles), and a more terminally oriented mouth in localities without fish prey relative to sites with mosquitofish (Carroll *et al.*, 2004; Collar & Wainwright, 2006; Wainwright *et al.*, 2007).

Similarly, to enhance feeding performance on divergent prey items, we expect *G. dormitor* to exhibit different feeding kinematics between populations with and without fish prey. In the presence of fish prey, we predict that *G. dormitor* will close the distance to its prey more rapidly, strike from a distance farther away from the prey, produce a larger gape, reach peak gape more slowly, and produce greater, more rapid hyoid depression and head elevation (Norton & Brainerd, 1993; Wainwright & Shaw,

1999; Higham, Day & Wainwright, 2006a, 2006b; Wainwright *et al.*, 2007; Holzman *et al.*, 2012; Oufiero *et al.*, 2012).

Finally, we predict that bigmouth sleepers evolving in the absence of fish prey will exhibit increased feeding performance on small invertebrates but reduced feeding performance on fish prey. Specifically, bigmouth sleeper populations without fish prey should generate greater suction forces during feeding on non-evasive prey but experience reduced abilities to capture highly evasive prey, resulting in a lower strike efficiency and longer hunting times prior to capture when feeding on fish prey (Higham *et al.*, 2006a; Wainwright *et al.*, 2007; Holzman *et al.*, 2012).

MATERIAL AND METHODS

We examined a total of nine *G. dormitor* populations in inland blue holes on Andros Island, The Bahamas (see Supporting information, Fig. S1). We focus on two categories of blue holes based on prey regime: presence versus absence of fish prey. Prey regime comprises the key environmental axis of variation among blue holes for *G. dormitor*, and no known environmental factor covaries with the presence of potential fish prey (see Supporting information, Doc. S1, Supplemental Methods A). Bigmouth sleepers comprise the sole fish species within two of these populations (Captain Bill's, Paul's), whereas they coexist with only *G. hubbsi* in five localities (Cousteau's, Hard Mile, Runway, Stalactite, West Twin), and coexist with *G. hubbsi* and at least one other potential prey fish species in the remaining two blue holes (Gibson, Rivean's). We gathered data from all, or a subset, of these nine populations to test each of our predictions (Table 2).

To evaluate population genetic structure and test whether populations within prey regimes are more closely related to one another than to populations in the alternative prey regime, we examined molecular genetic data: a 983-bp fragment of the mitochondrial (mt)DNA NADH subunit 2 (ND2 gene) for 45 *G. dormitor* individuals (Table 2; see also see Supporting information, Doc. S1, Supplemental Methods B). Although modest, our sample sizes within populations should prove adequate if substantial genetic structure exists, as expected (Heinen-Kay & Langerhans, 2013). Moreover, the number of populations examined provides sufficient statistical power to detect whether the hierarchical grouping by prey regime indeed represents the maximal genetic structure (F_{CT}) among possible permutations (Fitzpatrick, 2009).

We measured density, size structure, and habitat use of *G. dormitor* using underwater visual census methods (Brock, 1954; English, Wilkinson & Baker, 1994; Nagelkerken *et al.*, 2000; Layman *et al.*, 2004).

Table 2. Sampling scheme for each component of the present study

Blue Hole	Fish prey presence	Mitochondrial DNA	Density, habitat use, size structure	Sex ratio	Diet	Morphology	Feeding kinematics	Fish-feeding performance
Captain Bill's	Absent	8	3	4	70	41	4	4
Paul's	Absent	7	3	3	55	34	–	–
Cousteau's	Present	8	3	3	49	31	5	6
Gibson	Present	3	1	–	–	–	–	–
Hard Mile	Present	5	1	2	–	6	–	–
Rivean's	Present	–	1	2	–	7	–	–
Runway	Present	2	1	–	–	–	–	–
Stalactite	Present	7	3	3	43	38	–	–
West Twin	Present	5	2	2	20	18	–	–

For demographics and habitat use, numbers indicate separate surveys or collections conducted within each site; for all other components, numbers reflect sample size of specimens examined per site.

This technique proves effective in blue holes as a result of water clarity, the ease of underwater identification of size classes, and the ability to approach fish without causing disturbance (Heinen *et al.*, 2013). One investigator (RBL) recorded the number of *G. dormitor* within two size classes [small: total length (TL) < 10 cm, large: TL ≥ 10 cm] present in 1-m³ 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. During each census, we surveyed 10 quadrats within each habitat type distributed equidistant around the perimeter of the blue hole. All nine blue holes were censused, with five blue holes being censused multiple times (Table 2). We conducted censuses in November 2009, May 2011, July 2012, and April 2013. We calculated density as the mean number of *G. dormitor* observed within a 1-m³ quadrat (including both size classes and all four habitats); size structure as the proportional density of small size-class sleepers; habitat breadth as Shannon's diversity index for density estimates across the four habitat types; shallow-water use as the proportion of *G. dormitor* using shallow water (density of *G. dormitor* in the two shallow-water habitats divided by total density); and offshore use as the proportion of *G. dormitor* using offshore regions (density of *G. dormitor* in the two offshore habitats divided by total density).

We calculated the adult sex ratio as the proportion of males within each of seven blue holes (Table 2) using *G. dormitor* captured by hook and line, and minnow traps for diet or morphological analysis;

underwater identification of sexes during censuses was too difficult for accurate estimation. The mean sex ratio across collections within each blue hole was used in analysis.

To investigate diet, we conducted stomach-content analysis for two size classes of *G. dormitor* (small: TL < 10 cm, large: TL ≥ 10 cm) within five blue holes (Table 2). We employed these two size categories because a previous study suggested piscivory primarily occurs in *G. dormitor* with a TL larger than approximately 10 cm (Bacheler *et al.*, 2004a). We focused our sampling and analyses on the larger size-class individuals, although we also achieved moderate sample sizes of the small size-class individuals for several blue holes. In total, we examined the stomach contents of 184 live *G. dormitor* individuals using gastric lavage (see Supporting information, Doc. S1, Supplemental Methods C) and 53 preserved individuals using stomach dissection (total fish = 237; TL 12.9 ± 4.1 cm, mean ± SD). We recorded prey items to the lowest taxonomic category possible (usually Order), comprising 20 prey taxonomic categories. For the examination of diet composition, we summarized our findings into seven major prey categories based on results using both percentage occurrence (number of stomachs with prey *i*/number non-empty stomachs) and percentage by number (number of prey *i* in stomach/number prey in non-empty stomach) where *i* indicates a given prey category. To investigate variation in size of prey among blue holes, we measured the volume of several representative intact prey items as an approximation of mean prey size for each category, lumping prey taxa into small (< 0.1 mL) and large (> 0.1 mL) prey categories. These two categories neatly summarized the observed size variation among prey categories because prey volume typically ranged from 0.01–

0.25 mL, with a natural break at 0.1 mL. We then calculated the proportion of large prey items consumed for each population. We examined the diet overlap between blue holes using the Pianka index, O (Pianka, 1973), based on the seven major prey categories. Values of O can range from 0 (no diet overlap) to 1 (complete diet overlap). For diet breadth within each blue hole, we measured total niche width (TNW) using Shannon's diversity index for prey items found in stomach contents (using all 20 prey taxonomic categories; Roughgarden, 1972). We measured individual diet specialization for each blue hole using V , which is $1 - IS$, where IS equals the average overlap between each individual's diet niche and the population niche (Bolnick *et al.*, 2002, 2007). We calculated V based on all 20 prey taxonomic categories; V equals zero in the absence of individual specialization and assumes higher decimal values as individuals become more specialized.

We examined three categories of morphometric data: (1) landmark-based geometric morphometrics for investigation of overall lateral body shape; (2) functional measurements of foraging/locomotor morphology; and (3) functional measurements of trophic morphology. We examined a total of 175 live adult *G. dormitor* individuals for morphology (Table 2). The fish spanned a broad range of body size (TL 8.8–24.6 cm) and, because we observed no differences between prey regimes in body length, weight or condition (length-specific weight), we thus have a highly appropriate dataset for examining body shape differences among populations (see Supporting information, Doc. S1, Supplemental Methods D). We collected all morphometric data from lateral- and dorsal-perspective photographs taken of live fish in the field.

For geometric morphometrics, we digitized 17 homologous landmarks on lateral photographs (see Supporting information, Fig. S2) using TPSDIG2 (Rohlf, 2010a). We used TPSRELW (Rohlf, 2010b) to perform generalized Procrustes analysis (i.e. aligned landmark coordinates by rotating, translating, and scaling coordinates to remove positioning effects and isometric size effects; Bookstein, 1991; Marcus *et al.*, 1996) and obtain geometric shape variables for analysis [relative warps (RWs)]. We retained the first 23 RWs to ensure inclusion of most shape data (98.7%) and maintain sufficient degrees of freedom for mixed-model multivariate analysis. We also extracted centroid size (square root of sum of squared distances of all landmarks from their centroid) for each fish as an estimate of body size for use as a covariate in analysis, controlling for multivariate allometry.

For foraging/locomotor morphology, we measured the position of maximum body thickness and frontal profile area using both lateral- and dorsal-perspective photographs. The position of maximum body thick-

ness was measured as the mean of k_{depth} and k_{width} , which represent the distance between the snout tip and position of maximum depth (or width) divided by standard length (SL) (i.e. proportional distance along the body from the snout). We measured the frontal profile area as the area of an ellipse based on maximum depth and width: $\pi \times \text{depth}_{\text{max}}/2 \times \text{width}_{\text{max}}/2$. Because dorsal photographs were not taken for 20 fish, we had a reduced sample size of 155 fish for frontal profile area. However, we retained all 175 fish for maximum body thickness because we used k_{depth} instead of the mean of k_{depth} and k_{width} for our estimate of maximum body thickness for the 20 fish with missing dorsal photographs; the two thickness variables were highly correlated ($r = 0.67$, $P < 0.0001$) and the results for maximum body thickness were almost identical when including or excluding these 20 fish.

For examination of feeding morphology, we measured mouth area (lower jaw length \times mouth width), head depth (distance between landmarks 2–14), and jaw angle (angle formed by lines connecting landmarks 1–16–17) (see Supporting information, Fig. S2). Although dorsal photographs were not taken for 20 fish, mouth width was measured manually for 12 of these fish, and thus a total of 167 fish were examined for mouth size. One hundred and seventy-five fish were examined for other measurements.

To estimate suction feeding performance based on morphology, we calculated a modification of the suction index (SI) (Carroll *et al.*, 2004; Wainwright *et al.*, 2007; McGee & Wainwright, 2013; see also Supporting information, Doc. S1, Supplemental Methods E). SI has been shown to strongly predict the capacity to generate suction pressure in the buccal cavity for several fish species morphologically similar to *G. dormitor* (Carroll *et al.*, 2004; Wainwright *et al.*, 2007).

To measure feeding kinematics and estimate feeding abilities on both evasive and non-evasive prey, we conducted high-speed video analysis of nine *G. dormitor* feeding on *G. hubbsi* (Table 2) (TL 16.5 ± 2.3 cm). To measure these detailed performance attributes, we selected fish from only one representative population from each prey environment. Although the results indicate that these populations provide a representative subset for the overall patterns of differentiation (see below), they should be interpreted with caution because of the lack of replication of populations within the prey regime in this case. We conducted trials at Forfar Field Station on Andros Island, filming multiple feeding events per fish, and using average values per individual for analysis (see Supporting information, Doc. S1, Supplemental Methods F). We recorded nine kinematic variables from high-speed videos using the DLTDV3 package (Hedrick, 2008) implemented in MATLAB,

version 7.9 (The MathWorks, Inc.): attack speed, strike distance, maximum gape, time to peak gape, gape speed, maximum hyoid depression, time to maximum hyoid depression, maximum head angle, and time to peak head angle (all measured *sensu* Oufiero *et al.*, 2012). These variables were selected based on their hypothesized importance in prey capture abilities (Holzman *et al.*, 2012; Oufiero *et al.*, 2012). We then used the kinematic data, combined with morphological measurements, to model the suction-feeding performance of these *G. dormitor* individuals using the suction-induced force-field (SIFF) model recently described in Holzman *et al.*, (2012) (see Supporting information, Doc. S1, Supplemental Methods G).

We conducted two separate SIFF model iterations for each video. First, we modelled suction-feeding performance on an evasive fish prey by simulating an average *G. hubbsi* prey (SL 26 mm, body depth 7 mm, C-start escape data from Langerhans 2009a). All strikes were positioned so that each predator was at 20% of peak gape 13 mm away (i.e. half the prey's body length) from the prey, which triggered an escape response from the simulated *G. hubbsi*. For each video, we determined the maximal escape force exerted by the evasive prey that still resulted in capture, and used average values per individual in analysis. Second, we modelled suction-feeding performance on non-evasive prey by simulating a circular prey 10 mm in diameter that did not attempt to evade capture, and calculated the maximum force exerted on the prey when the strike was initiated one gape length away from the prey. Average values per individual were used in analyses.

We measured fish-feeding performance of *G. dormitor* using feeding trials with 10 fish (Table 2) (TL 16.1 ± 2.7 cm) feeding on *G. hubbsi*. *Gobiomorus dormitor* were collected from the wild and starved at least 48 h prior to experimentation at Forfar Field Station. We conducted three separate feeding trials with each *G. dormitor* (30 total trials), waiting 48 h (without feeding) between each trial (see Supporting information, Doc. S1, Supplemental Methods H). We included multiple trials per individual over a 5-day period to evaluate any 'learning' effect because *G. dormitor* from a blue hole without fish prey might improve their feeding performance with practice if plastic behaviours largely explained any initial differences in feeding performance between prey regimes. From the videos, we measured capture time (i.e. time from initial orientation of *G. dormitor* toward prey until capture) and strike efficiency (i.e. number of strikes prior to capture). As with the kinematic trials, we only examined one representative population from each prey environment, and thus the results should again be interpreted with caution.

STATISTICAL ANALYSIS

Using haplotype sequence variation of the ND2 gene, we conducted analysis of molecular variance (AMOVA) using ARLEQUIN, version 3.5.1.2 (Excoffier, Laval & Schneider, 2005) to summarize the proportion of total genetic variation attributable to variation among prey regimes, variation among populations within prey regimes, and variation within populations of *G. dormitor*. This analysis specifically tests whether populations within prey regimes are more closely related to one another than to populations of the alternative prey regime.

We had a total of 21 estimates of density, size structure, and habitat use for the analyses. Although all surveys were included in these analyses (including multiple surveys for five blue holes), the population was effectively treated as the unit of replication. We conducted mixed model nested analysis of variance (ANOVA) for each variable with fish prey presence as the independent variable and population nested within fish prey presence as a random term. We conducted ANOVA to test for differences in mean sex ratio (proportion of males) between prey regimes using site means ($N = 7$). Our data met assumptions for homogeneity of variance, and none of these data needed transformation to meet assumptions of normality, and thus the nontransformed results are reported here.

We conducted one-way ANOVAs using population means to test for differences between prey regimes in piscivory (both percentage occurrence and percentage by number), proportion of large prey consumption, TNW, and *V*. For both percentage occurrence and percentage by number, we tested significance of diet overlap *O* between each population pair using 10 000 randomizations with ECOSIM, version 7.0 (Gotelli & Entsminger, 2006) and conducted Mantel tests to test whether the degree of diet overlap between populations was greater within the same prey regime than between prey regimes.

For our geometric morphometric analysis, we performed a mixed-model multivariate analysis of covariance (MANCOVA) to test for body shape divergence between prey regimes in *G. dormitor*. The 23 RWs served as dependent variables; fish prey presence, sex, and their interaction served as independent variables; centroid size served as a covariate (controlling for multivariate allometry); and population nested within fish prey presence served as a random effect. Statistical significance was determined using an *F*-test based on Wilks's Λ for all terms except fish presence, which used an *F*-test employing restricted maximum likelihood and the Kenward–Roger degrees of freedom adjustment (Kenward & Roger, 1997, 2009), which allowed us to use population as the unit

of replication, effectively treating population as a random effect (Wesner *et al.*, 2011; Hassell *et al.*, 2012; Heinen-Kay & Langerhans, 2013; Riesch *et al.*, 2013; Martin *et al.*, 2014). The latter significance test was conducted using the MIXED procedure in SAS (SAS Institute Inc.), whereas all other tests were conducted in JMP (SAS Institute Inc.). We used the full shape space (all 30 RWS) to calculate the relative importance of model terms using the effect size measurement of Wilks's partial η^2 , and to calculate the divergence vector, \mathbf{d} , derived from the MANCOVA fish prey presence term *sensu* Langerhans (2009b). \mathbf{d} represents a principal components analysis on the sums of squares cross-products matrix of the fish presence term from MANCOVA, resulting in a multivariate axis describing the linear combination of dependent variables exhibiting the greatest difference between prey regimes, controlling for other terms in the model. We visualized shape variation along \mathbf{d} using thin-plate spline transformation grids with TPSREGR (Rohlf, 2010c). There were no significant heterogeneity of slopes, and data met assumptions of MANCOVA.

For all functional morphological measurements, we conducted separate mixed-model nested ANCOVAs. All models included fish prey presence as the main effect and population nested within fish prey presence as a random effect. Additionally, we included log-transformed TL as a covariate to control for allometry in all models except that for maximum body thickness (excluded as a result of nonsignificance, $P = 0.87$). All length and area measurements were log-transformed for analysis. Angles and ratios were not transformed as assumptions of normality were met without any transformation. No heterogeneity of slopes was detected.

For analysis of feeding kinematics from our high-speed video feeding trials, we first log₁₀-transformed all variables, and calculated residuals from regression on log₁₀ TL for all variables significantly associated with body size to obtain size-corrected variables. Only maximum gape, time to peak gape, and maximum hyoid depression were correlated with body size. We conducted principal components analysis (PCA) using the correlation matrix of these nine kinematic traits to reduce dimensionality, and retained those axes explaining more variance than that expected based on the broken stick criterion. This resulted in retention of two PC axes (see Supporting information, Table S1). We tested for differences in feeding kinematics of *G. dormitor* between the two blue holes by conducting separate one-way ANOVAs for each of the two PC axes.

To test for differences between the two populations in modelled suction-feeding performance on evasive prey (maximum force produced by evading *G. hubbsi*

that would have been successfully captured) and non-evasive prey (maximum force exerted on non-evasive prey when approached from one gape distance away), we first log₁₀-transformed both variables and examined whether size correction was necessary (neither variable exhibited a significant association with log₁₀ TL), and then performed separate one-way ANOVAs for each variable.

To examine the feeding-performance trials of *G. dormitor* feeding on *G. hubbsi*, we performed a general linear model for log₁₀-transformed capture time (time from orientation to capture), and performed a generalized linear model with a Poisson distribution and log-link function (Laplace approximation) for strike efficiency (number of strikes until capture). For each model, we initially included both *G. dormitor* and *G. hubbsi* body size as covariates, but because *G. dormitor* TL was always nonsignificant, we excluded it from our final analyses. Final models included sleeper ID and trial number as random effects, and prey size (log₁₀ SL of *G. hubbsi*) and prey regime as fixed effects.

Throughout, we employ one-tailed tests to test clear, a priori predictions of directional differences between blue holes with and without fish prey (Underwood, 1997; Freedman, Pisani & Purves, 1998). To facilitate interpretation of the magnitude of differences in all variables between prey regimes, we report standardized effect sizes (Cohen's d , describing the difference between means in standard deviation units; Cohen, 1988), with positive values indicating differences that matched our a priori predictions and negative values indicating differences that were opposite to our predictions.

Because we conducted multiple significance tests based on the same populations of fish, and some of the same individuals in some cases, we can experience inflated Type I error rates. To correct for this without suffering the substantial increase in Type II error rates (i.e. a reduction of statistical power) associated with Bonferroni correction procedures (García, 2004; Nakagawa, 2004; Verhoeven, Simonsen & McIntyre, 2005), we controlled the false discovery rate (FDR) for all of our tests of differences between fish-prey regimes (Benjamini & Hochberg, 1995; Storey, 2003; Storey & Tibshirani, 2003). FDR describes the proportion of significant tests that are actually null. We used QVALUE (Storey, 2003) to control the FDR at 5%, using the bootstrap procedure to estimate π_0 (probability of a true null hypothesis). We determined tests were significant when both the P -value and q -value (FDR equivalent of P -value; determined by QVALUE) were less than or equal to 0.05. In the present study, all observed P -values ≤ 0.05 remained significant at the FDR of 5%. Thus, we straightforwardly interpret P -values ≤ 0.05 as being significant.

RESULTS

We observed seven total mtDNA haplotypes (see Supporting information, Fig. S3), with a mean percentage nucleotide divergence of 0.43% (uncorrected p distance). AMOVA revealed that genetic variation was not associated with the presence of potential fish prey, although populations exhibited strong genetic structuring ($F_{ST} = 0.73$) indicative of strong genetic isolation among blue holes (Table 3).

For all subsequent tests, we provide a summary of results in Figure 1 illustrating the magnitudes of differences between prey regimes, as well as their correspondence to our a priori predictions. Below, we provide detailed results for all of our analyses.

We found no differences between blue holes with and without fish prey in *G. dormitor* density ($F_{1,5,2} = 0.07$, one-tailed $P = 0.4011$), size structure ($F_{1,5,4} = 0$, one-tailed $P = 0.5078$), habitat breadth ($F_{1,5,3} = 0.00$, one-tailed $P = 0.5063$) or offshore habitat use ($F_{1,6,2} = 0.14$, one-tailed $P = 0.6408$). However, *G. dormitor* populations in the absence of fish prey tended to exhibit a more male-biased sex ratio ($F_{1,5} = 25.66$, one-tailed $P = 0.0020$) (see Supporting information, Fig. S4) and utilized more shallow-water habitat ($F_{1,4,5} = 5.85$, one-tailed $P = 0.0327$) (see Supporting information, Fig. S4), matching predictions for those variables.

Of the 237 *G. dormitor* examined for diet, 125 individuals had prey items in their stomach (53%), with a mean of 2.5 prey items present; 75 individuals had multiple prey items present in the stomach (60% of *G. dormitor* with prey items present). Insects comprised the majority of prey items in blue holes lacking potential fish prey, whereas fish comprised a substantial part of the diet in blue holes with potential fish prey present (Table 4; see also Supporting information, Fig. S5). Insect prey largely reflected dipterans (30% occurrence across all fish) and coleopterans (16% occurrence). The consumption of fish was not restricted to any particular size-class because the TL

in 42 *G. dormitor* with fish prey in their stomachs (34% occurrence) ranged from 3.1 to 18.3 cm. Crustacean consumption was more common in the absence of fish prey [mostly amphipods (8% occurrence)]. The consumption of arachnids (water mites) was rare. Consumption of gastropods was fairly common (18% occurrence). We observed plant matter (including algae and cyanobacteria) in stomachs more frequently than expected, although this likely reflected incidental ingestion during animal prey capture.

For statistical analyses of diet, we exclusively examined large size-class individuals as a result of the low sample sizes of small size-class *G. dormitor* within two blue holes. Piscivory was more frequent in the presence of potential fish prey (percentage occurrence: $F_{1,3} = 28.26$, one-tailed $P = 0.0065$; percentage by number: $F_{1,3} = 10.97$, one-tailed $P = 0.0227$) (see Supporting information, Fig. S4). *Gobiomorus dormitor* tended to consume large prey (>0.1 mL) more commonly in blue holes with fish prey present ($F_{1,3} = 34.72$, one-tailed $P = 0.0049$). Population diet breadth (TNW) tended to be greater in the absence of fish prey ($F_{1,3} = 3.47$, one-tailed $P = 0.0798$) (see Supporting information, Fig. S4), although the difference was not significant, and individual diet specialization increased in the absence of fish prey ($F_{1,3} = 26.85$, one-tailed $P = 0.0070$) (see Supporting information, Fig. S4). Comparing overall diets among populations, diet overlap ranged from low to high (see Supporting information, Table S2), with greater overlap, although nonsignificant for percentage occurrence, tending to occur between blue holes of the same prey regime (Mantel tests: percentage occurrence: one-tailed $P = 0.0503$; percentage by number: one-tailed $P = 0.0467$). Five of the six population pairs exhibiting significant diet overlap came from the same prey regime (see Supporting information, Table S2).

In our analysis of geometric morphometric data, we found significant effects for all terms except the interaction between the presence of fish prey and the sex of *G. dormitor*, indicating the presence of strong

Table 3. Results of analysis of molecular variance examining mitochondrial DNA sequence variation among eight *Gobiomorus dormitor* populations in blue holes

Source of variation	d.f.	% of variation	P	F -statistic
Among prey regimes (with and without <i>Gambusia</i>)	1	14.60	0.2268	$F_{CT} = 0.15$
Among populations within prey regimes	6	58.27	< 0.0001	$F_{SC} = 0.68$
Within populations	37	27.13	< 0.0001	$F_{ST} = 0.73$
Total	44			

F_{CT} , correlation for random pairs of haplotypes within a prey regime (fish prey present versus absent), relative to random pairs of haplotypes drawn from the whole system. F_{SC} , correlation for random pairs of haplotypes within populations, relative to random pairs of haplotypes drawn from the same prey regime. F_{ST} , correlation for random pairs of haplotypes within populations, relative to random pairs of haplotypes drawn from the whole system.

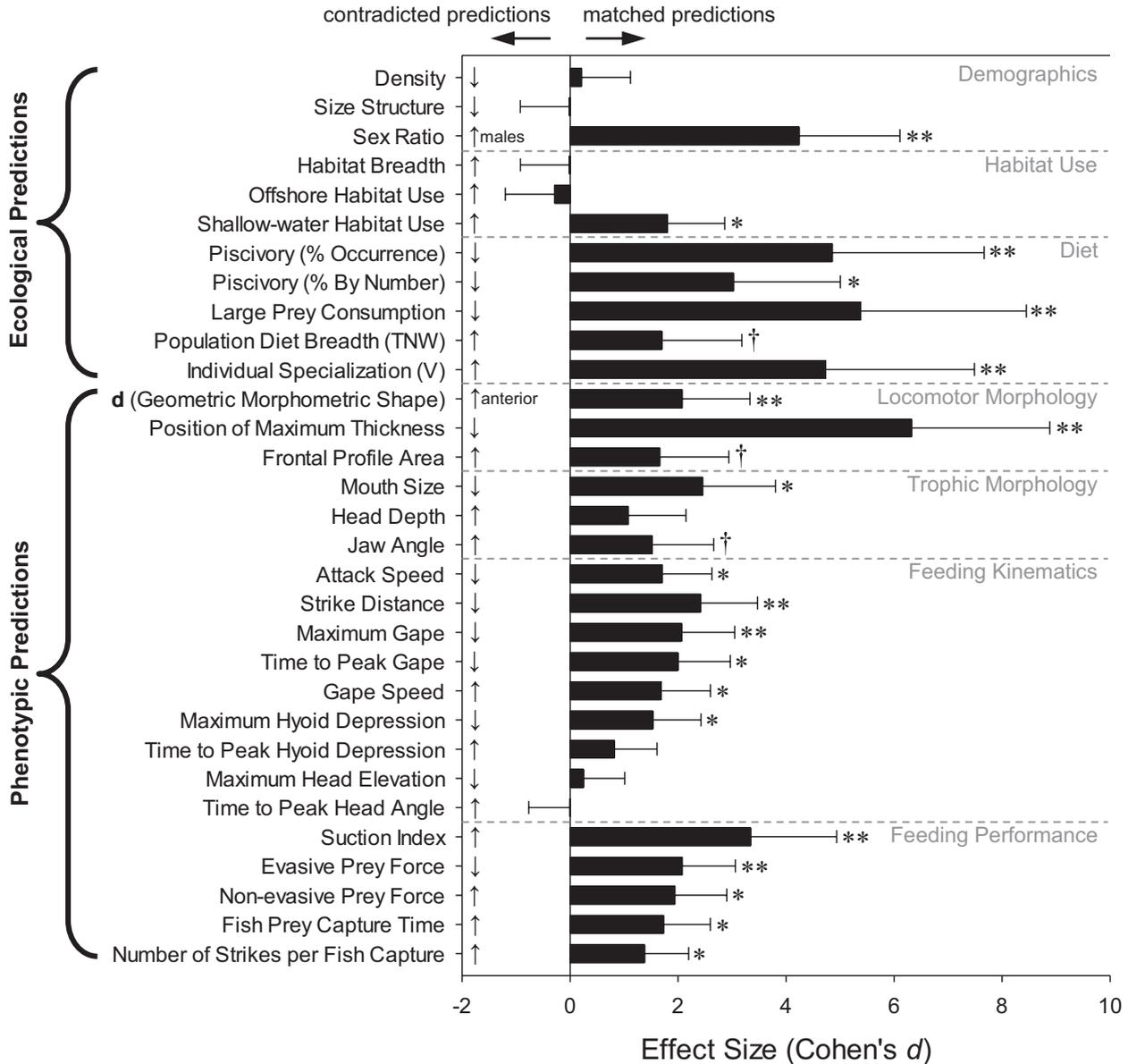


Figure 1. Summary of standardized effect sizes and associated standard errors for differences between prey regimes in the many variables examined in the present study. Positive values indicate that observed differences matched our a priori predictions, whereas negative values denote differences that were contrary to our predictions. Arrows depict the predicted direction of change in trait values for bigmouth sleepers in the absence of fish prey. We report the results for each feeding kinematic variable, even though the statistical tests focused on principal component axes. Symbols represent one-tailed *P*-values: †*P* < 0.1, **P* < 0.05, ***P* < 0.01.

multivariate allometry, that body shape differs between prey regimes, that sexes differ in body shape, and that differences in body shape between prey regimes are relatively similar for both male and female *G. dormitor* (Table 5). The strong effect size of allometry was expected because our dataset spanned a broad range of body size. However, variation as a result of the presence of fish prey comprised the next most important term, with *G. dormitor* inhabiting

blue holes with fish prey tending to exhibit a longer and more upturned jaw, shallower and longer head, a relatively deeper mid-section, and an overall more elongate body (Fig. 2).

We revealed differences in functional measurements of locomotor/foraging morphology between prey regimes in *G. dormitor*. First, we found that maximum body thickness was more posteriorly positioned in blue holes with fish prey ($F_{1,4.5} = 62.39$,

Table 4. Prey composition of bigmouth sleepers in Bahamas blue holes based on stomach-content analysis

Fish presence	Blue hole	Size class	N	Insect	Arachnid	Gastropod	Crustacea	Plant/algae	Other	Fish	TNW	V
Absent	Captain Bill's	Small	15 (8)	75.0/67.7	25.0/10.4	0/0	12.5/8.3	25.0/10.4	0/0	12.5/3.1		
		Large	55 (26)	69.2/62.2	0/0	11.5/6.1	7.7/5.8	26.9/20.2	7.7/4.8	3.8/1.9	1.94	0.76
Absent	Paul's	Small	26 (17)	76.5/65.0	0/0	23.5/11.3	11.8/8.8	11.8/7.1	0/0	11.8/7.8		
		Large	29 (7)	28.6/21.4	0/0	14.3/14.3	14.3/14.3	28.6/28.6	14.3/7.1	14.3/14.3	1.91	0.82
Present	Cousteau's	Small	12 (9)	100/41.5	0/0	44.4/15.5	33.3/21.1	33.3/9.0	0/0	44.4/10.2		
		Large	37 (24)	33.3/23.3	0/0	16.7/11.5	0/0	20.8/13.9	4.2/2.2	62.5/49.3	1.58	0.63
Present	Stalactite	Small	2 (1)	0/0	0/0	0/0	0/0	0/0	0/0	100/100		
		Large	41 (20)	35.0/17.8	0/0	35.0/16.6	0/0	35.0/17.7	15.0/5.4	55.0/42.5	1.81	0.67
Present	West Twin	Small	2 (0)	—	—	—	—	—	—	—		
		Large	18 (14)	64.3/38.1	0/0	7.1/3.6	0/0	42.9/23.8	14.3/7.1	42.9/27.4	1.82	0.66

For N, numbers in parentheses indicate the number of individuals with prey items present in the stomach. For prey categories, numbers represent percentage occurrence and percentage by number, respectively. TNW, total niche width as measured by Shannon's Diversity Index. V, estimate of individual diet specialization (1 - IS).

Table 5. Mixed-model multivariate analysis of covariance results for morphological differentiation of *Gobiomorus dormitor* among seven blue holes using geometric morphometric data (treating population as a random effect)

Source	F	d.f.	P	Partial η ²
Centroid size	12.85	23,143	< 0.0001	71.11
Fish presence	2.86	22,1438	< 0.0001	58.88
Sex	3.59	23,143	< 0.0001	44.45
Fish presence × Sex	1.48	23,143	0.0859	23.12
Population (fish presence)	5.38	115,706.6	< 0.0001	51.72

The F-statistics and partial variance (partial η²) were calculated as described in the text.

one-tailed $P = 0.0004$), that this effect was greater for females than for males ($F_{1,167.8} = 8.48, P = 0.0041$), and that the sexes did not differ in their position of maximum body thickness ($F_{1,167.8} = 0.83, P = 0.37$) (see Supporting information, Fig. S6). Second, we found a nonsignificant trend for a difference between prey regimes in frontal profile area ($F_{1,3.2} = 3.80$, one-tailed $P = 0.0709$), that males tended to have a larger frontal profile area than females ($F_{1,147.4} = 9.81, P = 0.0021$), and no interaction between sex and prey regime ($F_{1,146.2} = 0.31, P = 0.5779$) (see Supporting information, Fig. S6). Note that the test of fish prey presence had reduced statistical power in this case (only six populations in this analysis); upon further inspection, all four populations coexisting with fish prey exhibited a reduced frontal profile area on average, and thus we interpret this as providing cautious support for the prediction of reduced frontal profile area in the presence of fish prey.

Our functional morphological measurements of trophic morphology also revealed shifts between prey regimes. First, *G. dormitor* exhibited larger mouths in the presence of fish prey ($F_{1,4.0} = 9.59$, one-tailed $P = 0.0183$), no difference between the sexes ($F_{1,160.6} = 0.05, P = 0.8253$), and greater divergence in females than males ($F_{1,159.5} = 7.51, P = 0.0068$) (see Supporting information, Fig. S6). Upon inspection of lower jaw length and mouth width separately, these results were apparently caused by both sexes exhibiting longer jaws in the presence of fish prey (with this effect being stronger in females), although only females exhibiting wider mouths in the presence of fish prey. Second, we found that *G. dormitor* did not exhibit significant differences in head depth between prey regimes, although trends were in the predicted direction ($F_{1,3.06} = 1.73$, one-tailed $P = 0.1390$), that

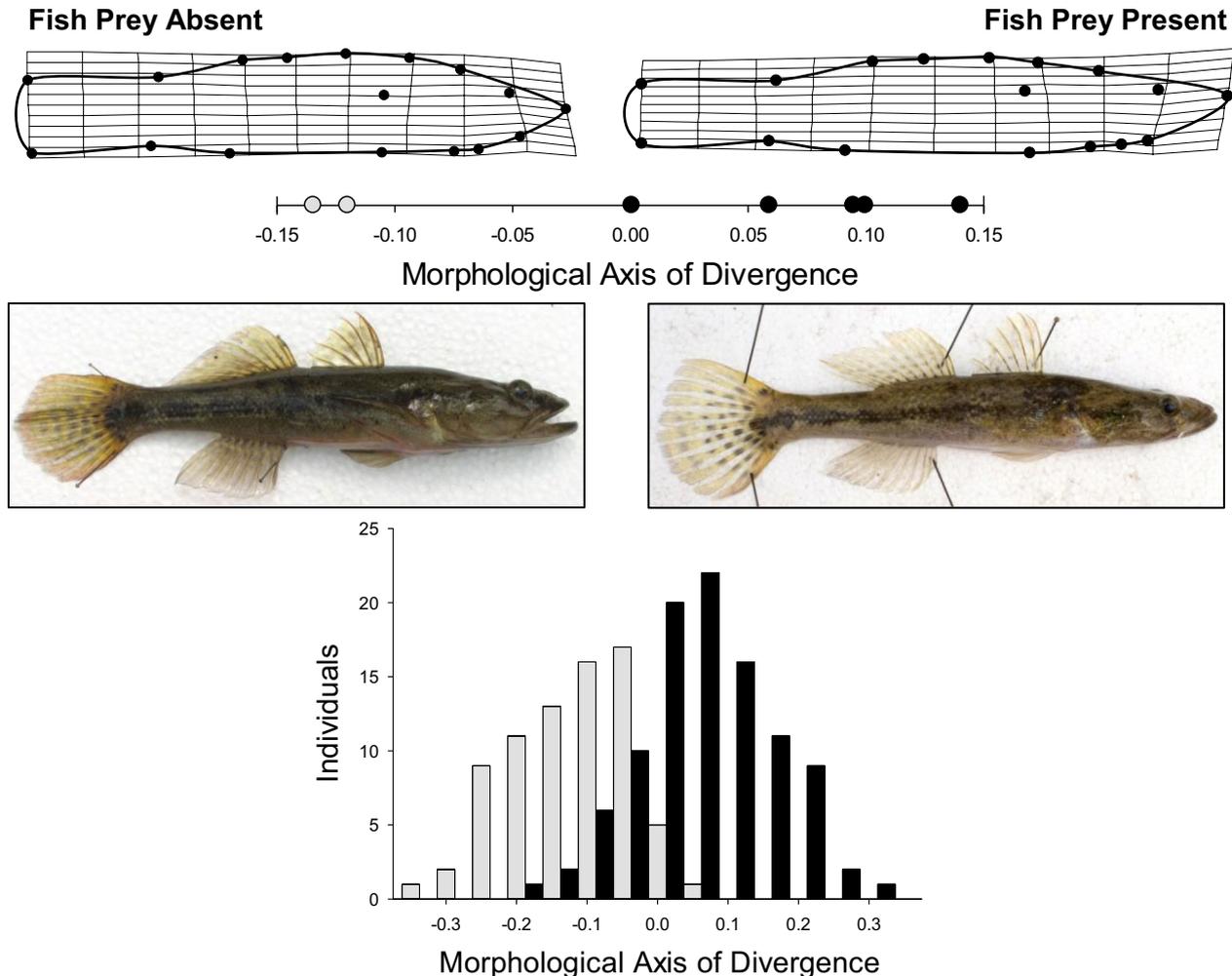


Figure 2. Thin-plate spline transformation grids illustrating differences in body shape of *Gobiomorus dormitor* between blue holes with (black symbols) and without (grey symbols) fish prey, with population means and frequency histogram along **d** depicted (grids magnified $\times 2$ to aid interpretation). Lines are drawn on grids to aid visualization. Photographs of representative individuals from blue holes without (left) and with (right) fish prey are presented (individuals selected near the lower and upper 10% of **d** distribution).

males had deeper heads than females ($F_{1,164.4} = 5.38$, $P = 0.0216$), and no interaction between sex and prey regime ($F_{1,164} = 0.68$, $P = 0.4102$) (see Supporting information, Fig. S6). Finally, we found suggestive but nonsignificant support for *G. dormitor* having smaller jaw angles in blue holes with fish prey ($F_{1,4.03} = 3.39$, one-tailed $P = 0.0696$; i.e. more upturned mouths), with no effects of sex ($F_{1,165} = 0.10$, $P = 0.7535$) or the interaction between sex and prey regime ($F_{1,164.8} = 0.02$, $P = 0.8771$) (see Supporting information, Fig. S6). For all functional measurements where the covariate \log_{10} TL was included in the statistical model (all but maximum body thickness), it was highly significant ($P < 0.0001$).

We found that *G. dormitor* populations in the absence of fish prey exhibited higher suction index

values ($F_{1,4.6} = 17.16$, one-tailed $P = 0.0053$), whereas sexes did not differ ($F_{1,159.8} = 0.73$, $P = 0.7873$), no interaction between sex and fish prey presence was found ($F_{1,159.2} = 1.13$, $P = 0.2900$), and \log_{10} TL did not influence suction index ($F_{1,132} = 1.27$, $P = 0.2619$) (see Supporting information, Fig. S7).

In our analysis of feeding kinematics, the two populations examined differed strongly in PC1 scores ($F_{1,7} = 10.13$, $P = 0.0154$) (see Supporting information, Fig. S7), although no differences were evident for PC2 ($F_{1,7} = 0.59$, $P = 0.4690$). Based on PC loadings (and post-hoc univariate tests), this indicated that *G. dormitor* from Cousteau's blue hole (fish prey present) tended to exhibit a greater, more delayed peak gape, a slower gape speed, a greater maximum hyoid depression, and also tended to strike from

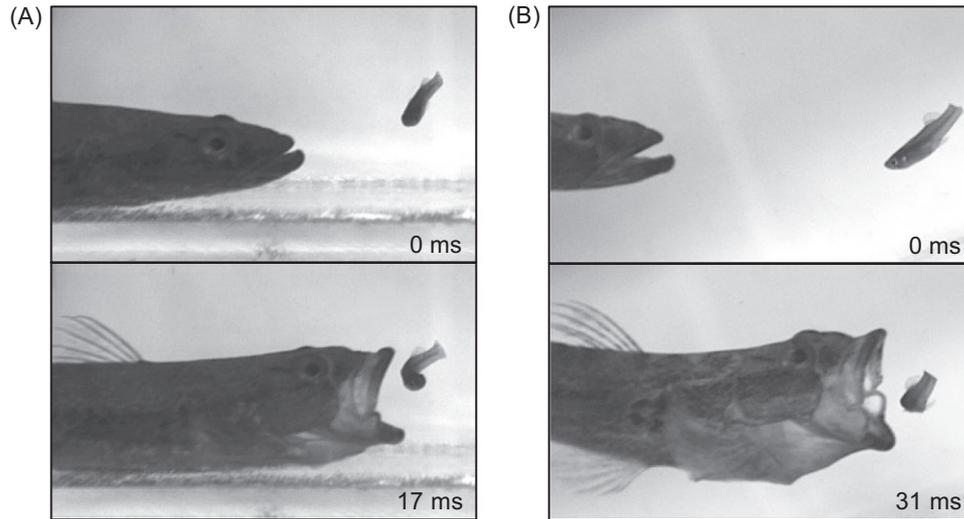


Figure 3. Representative kinematic feeding sequence of bigmouth sleepers from (A) Captain Bill's blue hole (*Gambusia* absent) and (B) Cousteau's blue hole (*Gambusia* present), showing that sleepers from Captain Bill's blue hole have a shorter strike distance, faster gape speed, smaller gape, and reduced hyoid depression, resulting in stronger suction generation. Top: showing the point at which the fish reached 20% of peak gape (designated time 0 ms). Bottom: fish just prior to prey capture (elapsed time given in lower-right corner).

farther away with a greater attack speed (Fig. 3) than their counterparts from Captain Bill's blue hole (fish prey absent); all differences matched our a priori predictions (Fig. 1; see also Supporting information, Table S1). Predicted values for feeding performance on evasive *G. hubbsi* prey were significantly higher for sleepers from Cousteau's blue hole than Captain Bill's blue hole ($F_{1,7} = 9.56$, one-tailed $P = 0.0088$) (see Supporting information, Fig. S7); at the same time, the predicted force exerted on nonevasive prey was significantly higher for *G. dormitor* from Captain Bill's blue hole compared to Cousteau's ($F_{1,7} = 8.37$, one-tailed $P = 0.0116$) (see Supporting information, Fig. S7); both results matched a priori predictions.

In our fish-feeding experiment, the *G. hubbsi* prey was captured and consumed in all 18 trials for the *G. dormitor* from Cousteau's blue hole (fish prey present), and nine of the 12 trials for Captain Bill's (fish prey absent). *Gobiomorus dormitor* from Cousteau's blue hole captured prey much more quickly than *G. dormitor* from Captain Bill's blue hole ($F_{1,8,0} = 8.34$, one-tailed $P = 0.0101$) (see Supporting information, Fig. S7). For all *G. dormitor*, regardless of population, smaller *G. hubbsi* prey required more time for capture ($F_{1,19,89} = 8.27$, $P = 0.0094$). For strike efficiency, *G. dormitor* from Cousteau's required fewer strikes prior to capturing prey, on average ($Z = -1.856$, one-tailed $P = 0.0317$) (see Supporting information, Fig. S7), whereas *G. hubbsi* body size did not affect strike efficiency ($Z = -1.304$, $P = 0.1921$). For both fish-feeding performance measures, trial

effects were weak, revealing no evidence of learning (direction of trend in opposite direction).

DISCUSSION

How predictable are ecological and phenotypic responses to novel environments? We set out to address this question by exploring a relatively simple system, *G. dormitor* populations inhabiting Bahamas blue holes. Importantly for the present study, populations primarily differ along a single axis of environmental variation: the presence versus absence of potential fish prey. In evaluating genetic structure, population demographics, habitat use, diet, morphology, and feeding kinematics and performance between *G. dormitor* populations in the present study, we found clear evidence for populations having diverged between habitats with and without potential fish prey, largely as predicted a priori (Fig. 1).

Especially strong evidence for predictable responses to environmental change in the wild comes from the combination of (1) a match between observations and a priori predictions based on theory, prior empirical study, and natural history of relevant systems, and (2) replicated changes that correspond with predictions observed in multiple, independent populations. Without such evidence, divergence alone might not comprise truly predictable divergence because replicated patterns might emerge for unknown reasons (unpredictable but repeatable divergence) or predictable differences could reflect shared ancestry among

populations inhabiting similar environments (predictable but unrepeated divergence). In the present study, the general agreement between observations and predictions for so many population characteristics (Fig. 1), combined with our population genetic results revealing strong genetic structure independent of the presence of fish prey (see Supporting information, Fig. S3), provides robust support for considerable predictability in ecological and phenotypic responses to prey availability in bigmouth sleepers. This finding is consistent with previous work in Bahamas blue holes on *G. hubbsi* (Langerhans *et al.*, 2007; Langerhans, 2010; Heinen *et al.*, 2013; Heinen-Kay & Langerhans, 2013; Riesch *et al.*, 2013; Martin *et al.*, 2014), and suggests parallel, replicated evolution of convergent phenotypes in *G. dormitor* in blue holes with similar prey regimes (i.e. with or without *G. hubbsi*, or other potential fish prey), assuming observed trait differences at least partially reflect evolved differences (see below).

Studies in evolutionary ecology seldom explore how differences in prey availability between populations affect patterns of demography, despite the potential evolutionary consequences that changes in demography could have on the populations, as well as the ecology and evolution of the broader community (Charlesworth, 1994; Schluter, 2000; Thompson, 2005; Kokko & Rankin, 2006; Knell, 2009; Losos, 2009). In the present study, we predicted that *G. dormitor* populations lacking potential fish prey would be less dense, and comprise fewer juveniles and females without the energy input obtained from piscivory. Although neither density, nor the proportion of juveniles significantly differed between populations with and without fish prey, sex ratio was significantly male skewed in blue holes lacking fish prey, as predicted. Therefore, potentially reduced energy acquisition in blue holes without fish prey apparently did not constrain population density or decrease juvenile survivorship, although it may have resulted in elevated mortality rates for females. If correct, we hypothesize that this may not only derive from the greater energetic requirements of egg production compared to sperm, but also could involve other mechanisms, such as variation in sex-determination factors (e.g. genetic and nongenetic influences on sex are unknown in *G. dormitor*, although it does lack sex chromosome heteromorphism; Maldonado-Monroy *et al.*, 1985), a habitat shift to uncensused regions in females (e.g. increased deep-water use to avoid male aggression) or differences in our ability to capture males versus females, leading to biased estimates of sex ratio. Regardless of the mechanistic cause, if real, observed differences in sex ratio between populations could alter the intensity of sexual selection, leading to even greater differences between prey regimes than

uncovered here. For example, populations with a male-biased sex ratios could harbour stronger female choice, greater male mate guarding, or more male-male aggression (Kvarnemo, Forsgren & Magnhagen, 1995; Kvarnemo & Ahnesjo, 1996; Wacker *et al.*, 2012). *Gobiomorus dormitor* exhibits mate pairing during breeding periods in blue holes, with males closely guarding females and chasing off rival males (R. B. Langerhans, pers. observ.); whether these behaviours or other secondary sexual traits have diverged between prey regimes requires future study.

Gobiomorus dormitor is highly piscivorous throughout its range, typically feeding on fish and large invertebrates; a pattern confirmed in the present study in blue holes where they co-occur with potential fish prey. However, in the two blue holes lacking fish prey, piscivory was quite rare (representing cannibalism in these cases). Instead, bigmouth sleepers primarily preyed upon a wide array of small invertebrates in blue holes lacking other fish species and, interestingly, although habitat breadth and offshore use were unchanged, their use of shallow water increased, where these prey may be more commonly found on the substrate (small crustaceans) and via allochthonous input (small insects).

Dietary differences between prey regimes manifested in differences in both total niche width and individual specialization, matching our a priori predictions that, in the absence of fish prey, increased competition for resources would lead to greater diet specialization by individuals and consequently a greater population diet breadth (Bolnick *et al.*, 2003; Araújo *et al.*, 2011). Our dietary findings suggest that the loss of a major prey item might often facilitate increased individual diet specialization, owing to a combination of (1) an effectively more diverse resource pool, as individuals resort to feeding on an array of less preferred prey items, and (2) increased competition for smaller, and potentially more easily depleted, resources. Competitive release could also lead to enlarged population diet breadth and increased specialization (Bolnick *et al.*, 2010; Araújo *et al.*, 2011) and may possibly contribute to the patterns observed in the present study. However, *G. dormitor* and *G. hubbsi* do not share many diet items, and diet overlap occurs mostly for the smallest size classes of juvenile *G. dormitor* (R. B. Langerhans, unpubl. data). Nevertheless, release from a minor interspecific competitor could facilitate the patterns found in the present study. These causal mechanisms for broadening dietary niches via increased inter-individual diet variation have so far received very little attention (Araújo *et al.*, 2011) but could prove common and deserve future investigation.

A specialized piscivorous diet should impose very different selective pressures than foraging on a broad

invertebrate diet. Therefore, as a consequence of dietary change between blue holes with and without fish prey, we predicted, as informed by prior theory and empirical work (Table 1), that we would see concurrent adaptive changes in locomotor and trophic morphology of *G. dormitor*, resulting in differential feeding kinematics and performance on evasive and non-evasive prey. As we predicted for locomotor morphology, *G. dormitor* populations co-occurring with (and intensively preying upon) prey fish exhibited a body morphology associated with increased S-start performance compared to populations lacking *G. hubbsi*. First, bigmouth sleepers inhabiting blue holes with other fish species have a deeper mid-section and an elongated body in comparison to populations from blue holes lacking potential fish prey (Fig. 2). Second, *G. dormitor* exhibited a posterior shift in body allocation in blue holes with fish prey compared to sleepers in blue holes lacking fish prey. Taken together, these results support the hypothesis that *G. dormitor* has evolved divergent body shapes across blue holes in response to selection for greater thrust generation during predation on elusive fish prey in one case and, in the other, possible selection for greater locomotor endurance for prolonged prey-searching behaviours or through relaxed selection and drift. These divergent body shapes match both biomechanical predictions for enhancing different locomotor modes (acceleration and maneuverability in the presence of fish prey versus steady-swimming endurance in their absence), as well as empirical findings in other fishes experiencing similar selection on locomotor performance (Langerhans, 2010).

We further predicted that *G. dormitor* inhabiting blue holes with fish prey would show a reduced frontal profile because a smaller frontal profile would be less visible and therefore allow sleepers to approach closer to evasive prey before initiating a strike. Although the difference in frontal profile between prey regimes did not meet statistical significance, we did observe a suggestive trend in the direction predicted, and all *G. dormitor* populations examined that co-occur with fish prey exhibited a smaller average frontal profile area than the two populations lacking fish prey. Thus, our results suggest that selection may indeed favour a smaller frontal profile area in the presence of fish prey, although counteracting selection for a deepened mid-body to enhance acceleration may constrain the response to such selection.

Divergence in trophic morphology between blue holes differing in the presence of fish prey is consistent with an adaptation to piscivory when coexisting with fish prey and to feeding on small invertebrates in the absence of fish prey. In particular, bigmouth sleepers in the absence of fish prey possessed smaller

mouths, and tended to exhibit deeper and shorter heads with a more terminal mouth position, matching our predictions. These morphological differences suggest that sleepers from blue holes lacking fish prey create greater suction forces when feeding, resulting in increased feeding performance on small invertebrates; at the same time, sleepers from blue holes harbouring fish prey are predicted to create a suction flow with greater reach, increasing feeding performance on larger, evasive prey such as *G. hubbsi*. We examined these morphological implications by estimating suction force generation and suction-feeding performance in the present study.

Our estimation of suction index values from the measurement of external morphology indicated that bigmouth sleepers from populations lacking fish prey can generate greater suction forces than individuals from populations coexisting with fish prey. Although the high-speed video trials lacked population-level replication within prey regimes, our calculations of suction-feeding performance on non-evasive and evasive prey using the SIFF model for these two representative populations provide further evidence supporting that: (1) *G. dormitor* living in the absence of fish prey exert greater force on non-evasive prey, whereas (2) *G. dormitor* coexisting with fish prey can successfully capture a much more evasive *G. hubbsi* (more than twice the evasive force) than their counterparts from fishless blue holes. Thus, our findings clearly suggest that *G. dormitor* from different prey regimes exhibit divergent feeding abilities: sleepers from blue holes lacking fish prey show high feeding performance on non-evasive prey, whereas *G. dormitor* from blue holes with fish prey show high performance on evasive prey.

Differences in feeding performance derive not only from morphological differences, but also from kinematic differences during predatory strikes. We found that *G. dormitor* in a blue hole with fish prey initiate strikes farther away from their prey with a slower gape speed, faster attack speed, greater maximum gape, and greater hyoid depression than *G. dormitor* in a blue hole without fish prey (Fig. 3). These differences matched our a priori predictions, and reflect multivariate kinematic differentiation between prey regimes, where a suite of kinematic variables related to suction-feeding performance have apparently changed in concert to enhance feeding performance on divergent prey items. All of these kinematic characters match a classic example of biomechanical divergence between non-evasive and evasive prey specialists, bluegill sunfish (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*), revealing that intraspecific functional divergence can mirror patterns of divergence across macroevolutionary timescales (Wainwright & Shaw, 1999; Higham *et al.*,

2006a, 2006b; Wainwright *et al.*, 2007; McGee, Schluter & Wainwright, 2013).

We directly measured fish-feeding performance using feeding trials with *G. hubbsi*, and demonstrated that *G. dormitor* co-occurring with *G. hubbsi* embody much more effective fish predators than sleepers that do not co-occur with any potential fish prey. *Gobiomorus dormitor* that have coevolved with *G. hubbsi* outperformed sleepers from a blue hole lacking *G. hubbsi* with respect to both the time required to capture *G. hubbsi* prey and also the efficiency of their predatory strikes. Because we found no evidence of learning (i.e. increased performance over time), and as a result of the range of observed morphological and kinematic differences consistent with such performance differences, these feeding-performance differences unlikely solely reflect learned behavioural differences between populations. Although future work should examine feeding performance in more detail for additional populations, altogether, we have revealed strong evidence for divergent foraging and feeding adaptations in *G. dormitor* between blue holes with and without fish prey.

Phenotypic differentiation between *G. dormitor* populations could arise from fixed genetic differences or some degree of environmentally induced differences (i.e. phenotypic plasticity), either of which could reflect responses to divergent natural selection (West-Eberhard, 2003; DeWitt & Scheiner, 2004; Pfennig *et al.*, 2010). Considering the temporal stability of blue hole environments and the strong isolation among populations, likely reflecting thousands of years of blue hole occupation and isolation representing circumstances not conducive for phenotypic plasticity (Fusco & Minelli, 2010), combined with prior work demonstrating many evolved phenotypic differences among blue holes in *G. hubbsi* (Langerhans, 2009a; Heinen-Kay & Langerhans, 2013; Riesch *et al.*, 2013; Martin *et al.*, 2014), the observed differences in *G. dormitor* morphology, feeding kinematics, and feeding performance may largely reflect genetic divergence. Future work should employ common-garden experiments to uncover the underlying source of phenotypic differentiation (genetic, environmental, or both) between *G. dormitor* populations in blue holes with and without fish prey. However, regardless of the source, phenotypic changes *per se* are of focal interest for assessing our ability to predict population responses to environmental change. Because it is through traits that all ecological changes occur (e.g. population-, community-, and ecosystem-level), we need to understand the phenotypic consequences of environmental variation. Armed with this understanding, we might better mitigate negative ecological consequences of human-altered environments.

Although our findings should be generalized and interpreted with reasonable caution given the small number of *G. dormitor* populations that occur without fish prey (those described here are the only such populations known to exist) and our lack of population replication for the high-speed kinematic and fish-feeding performance trials, the present study provides a test and example of our ability to study population differentiation across numerous trait types and disciplines (e.g. ecology, population genetics, functional morphology, behaviour). Previous research has generally lacked a broad predictive framework that addresses multiple types of population responses, usually centering instead on only one or two aspects of morphology. Moreover, although prior work has demonstrated associations between fish morphology and resource use within species and among closely related species (Robinson & Wilson, 1994; Schluter, 1994; Jonsson & Jonsson, 2001; Svanbäck & Eklöv, 2002; Weese, Ferguson & Robinson, 2012), this previous work has primarily focused on nonpiscivorous fishes at high latitudes inhabiting two types of lake habitats: near-shore, littoral zones (benthic) and off-shore, open-water regions (limnetic). We have uncovered a broad range of population characteristics that predictably differed between fish-prey environments in a tropical fish (Fig. 1). Thus, in cases where we have clear a priori predictions based on reasonable assumptions derived from previous work, we might accurately predict many critical aspects of population differentiation. Our findings imply that, in our rapidly changing world, with strong human-induced environmental impacts becoming ubiquitous in many systems, we may sometimes be able to predict, and potentially mitigate, population responses to changing environments.

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SHARED DATA

Data deposited in the Dryad digital repository (Martin, McGee & Langerhans, 2015).

SUPPORTING INFORMATION

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

Figure S1. Location of blue hole study sites.

Figure S2. Illustration of landmarks for geometric morphometric analysis.

Figure S3. Mitochondrial DNA haplotype network.

Figure S4. Differences between prey regimes in ecological parameters and diet.

Figure S5. Summary of differences between prey regimes in prey composition.

Figure S6. Differences between prey regimes and sexes in functional morphology.

Figure S7. Feeding performance of *Gobiomorus dormitor* from divergent prey regimes.

Table S1. Principal components analysis of kinematic variables.

Table S2. Pianka diet overlap values.

Doc S1. Supplemental Methods A: Testing for environmental heterogeneity among blue holes. Supplemental Methods B: Obtaining molecular genetic data for population genetic analysis. Supplemental Methods C: Obtaining stomach contents for diet analyses. Supplemental Methods D: Body size and condition across prey regimes. Supplemental Methods E: Measurement of suction index. Supplemental Methods F: High-speed video feeding trial methods. Supplemental Methods G: Measurement of suction-induced force-field. Supplemental Methods H: Fish-feeding performance trials.