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Variation in Dietary Ecology of Two Invasive American Bullfrog (*Lithobates catesbeianus*) Populations in Southern California

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ABSTRACT: Invasive American Bullfrogs (*Lithobates catesbeianus*) are a threat to native species in riparian ecosystems worldwide. They are indiscriminate predators consuming both vertebrate and invertebrate prey, negatively affecting biodiversity. Documenting the diet and feeding ecology of invasive *L. catesbeianus* can help management agencies identify affected species and facilitate eradication efforts. We present a dietary analysis of two invasive *L. catesbeianus* populations over multiple breeding seasons (2016–2020), elucidating ontogenetic changes in diet and dietary differences between sexes and habitats. This is the first study to analyze dietary variation from contemporary populations of invasive *L. catesbeianus* occupying different watersheds in Southern California, an area where their invasion presents acute conservation challenges. Our analysis of 667 gut contents shows that adult females had more prey in their guts than adult males, even though male and female body size was not significantly different. Adults were more likely than juveniles to consume vertebrate prey, and juveniles were more likely than adults to have empty stomachs. We also found that invasive Red Swamp Crayfish (*Procambarus clarkii*), made up a substantial portion of the diet of adult *L. catesbeianus* at the site where they were present. These results provide an important ecological context for designing mitigation actions that ameliorate the impacts of invasive *L. catesbeianus*.

Key words: Alien predator; Gut content survey; Invasive crayfish; Ontogenetic shift; Prey dynamics; Stomach contents

INVASIVE species are a major factor driving extinctions and biodiversity declines worldwide (Simberloff et al. 2013; Havel et al. 2015). Predatory species that become invasive are often opportunistic generalists that adjust to the novel resources present in invaded ecosystems (Shik and Dussutour 2020), but the impact of invasive predators depends in part on how invaders interact with local conditions. Therefore, it is important to understand how the dietary ecology of invaders is influenced by ontogeny, environmental variation, and inter-specific interactions (Jiménez-Valverde et al. 2011). For example, analyses of the dietary ecology of invasive parakeets (*Myiopsitta* sp.), Red Swamp Crayfish (*Procambarus clarkii*), lionfish (*Pterois* sp.), and rats (*Rattus* sp.) have aided in the creation of habitat restoration plans and other management actions that have sought to remove or control invasive predators (Layman and Allgeier 2012; Jackson et al. 2017; Egeter et al. 2019; Borray-Escalante et al. 2020).

Lithobates catesbeianus are originally native to wetlands in the eastern United States and have been introduced to riparian ecosystems worldwide because of a combination of intentional release for sport and game fishing, the pet trade, and dispersal (Bury and Whelan 1985). Introduced populations of *L. catesbeianus* have had devastating impacts on native fauna (reviewed in Adams et al. 2003) and have contributed to the local extirpation of native amphibians, snakes, and turtles in several watersheds in Southern California (Fisher and Shaffer 1996; Kupferberg 1997; Lawler et al. 1999; Riley et al. 2005; Miller et al. 2012; Thompson et al. 2016; Nicholson et al. 2020). Their success is due in part to high fecundity (up to 50,000 eggs/yr per female), rapid growth rate, adaptable ecology, and reduced predation pressure relative to their native habitat (Bury and Whelan 1985; Adams and Pearl 2007). They are ambush predators that consume a wide range of

invertebrates and small vertebrates including reptiles, amphibians, birds, fish, and rodents. Their voracious appetite, adaptable life history, and ability to serve as vectors for wildlife diseases (e.g., *Batrachochytrium dendrobatidis*; Schloegel et al. 2010; Miaud et al. 2016) all contribute to their negative impacts to native ecosystems (Greenspan et al. 2012).

Throughout the past 50 yr, ecologists have analyzed gut contents of several *L. catesbeianus* populations to better understand their predation pressure on native species (Cross and Gerstenberger 2002; Hirai 2004; Leivas et al. 2012; Liu et al. 2015). However, few studies have incorporated large, comprehensive data from different sites assembled across a long period (but see Jancowski and Orchard 2013; Bissattini et al. 2018, 2019). Most *L. catesbeianus* diet surveys provide a snapshot view of prey items consumed in one population collected over a short time frame (Hirai 2004; Barrasso et al. 2009). Long-term studies that incorporate data from more than one site provide a more comprehensive view of the harm that invasive *L. catesbeianus* inflict on native species, especially those that are rare or only seasonally active.

We quantified the body size, sex, and gut contents of 667 *L. catesbeianus* removed from two watersheds in San Diego County, CA, USA as part of local invasive species mitigation efforts. We collected all frogs over 5 yr while conducting habitat restoration in the invaded streams. We analyzed *L. catesbeianus* gut contents to test the hypotheses that the type and quantity of prey taken by frogs would vary by age class (adult versus juveniles), sex, and geographic location. Specifically, we wanted to understand the degree to which native species versus other nonnative species were consumed by *L. catesbeianus*, and whether any of the native species detected are currently of conservation concern. Given that several past studies found that sympatric invasive crayfish (genus *Procambarus*) decrease the relative quantity of native vertebrates in the gut contents of *L. catesbeianus* (Liu et al. 2018; Bissattini et al. 2018, 2019), we also

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TABLE 1.—Models considered if response variables (vertebrates, odonates, crayfish, and other invertebrates) were influenced by site, age, sex, or any of their interactions (ω_i = AICc weight, other invertebrates = total invertebrates other than crayfish and odonates).

Model	Model class	Model distribution	AICc	Δ AICc	ω_i	Dispersion
Vertebrates ~ site + age + sex + (site $\times$ age) + (site $\times$ sex) + (age $\times$ Sex)	Negative binomial	—	552.1	0.00	0.98	1.00
	Zero inflated	Poisson	561.0	8.97	0.01	1.01
	Zero inflated	Negative binomial	566.6	14.56	0.00	1.01
Odonates ~ site + age + sex + (site $\times$ age) + (site $\times$ sex) + (age $\times$ sex)	Zero inflated	Poisson	1128.9	0.00	0.68	0.93
	Zero inflated	Negative binomial	1130.4	1.52	0.32	0.91
	Negative binomial	—	1164.3	35.42	0.00	1.20
Crayfish ~ age + sex + (age $\times$ sex)	Negative binomial	—	471.8	0.00	0.49	0.81
	Zero inflated	Poisson	472.4	0.60	0.36	0.93
	Zero inflated	Negative binomial	474.3	2.44	0.15	0.90
Other invertebrates ~ site + age + sex + (site $\times$ age + (site $\times$ sex) + (age $\times$ sex)	Zero inflated	Poisson	759.7	0.00	0.74	0.78
	Zero inflated	Negative binomial	761.8	2.10	0.26	0.78
	Negative binomial	—	796.7	27.04	0.00	0.71

predicted that adult bullfrog diets would be dominated by invasive Red Swamp Crayfish rather than by vertebrate prey at one of the study sites that was coinvasion by crayfish.

MATERIALS AND METHODS

Frog Collection

We collected data from carcasses of *L. catesbeianus* collected between April and November (the species was inactive in winter months) of 2016–2020. Because we obtained the carcasses through separate eradication efforts, sampling was haphazard with respect to time of year and effort per year. Less than 1% of our records come from 2015, approximately 3% from 2016, 70% from 2017, 15% from 2018, 5% from 2019, and 6% from 2020 (see Supplemental Data S1 for full details on samples, available online). With this large inconsistency across years and seasons, rigorous temporal comparisons were not possible. Instead, we pooled samples across years to analyze differences related to size, sex, and site. Surveys took place between sunset and 0100 h, as frog activity diminished after midnight as temperatures decreased. Technicians avoided consecutive survey nights to reduce *L. catesbeianus* sensitivity to the presence of humans and to allow time for wary frogs to become active again. We obtained specimens using firearms and pole spears and recorded date, time, location, age, and sex for all dispatched individuals.

We collected frog carcasses from two sites, Wheatley Ranch and the Rancho Jamul Ecological Reserve (RJER). Both sites are primarily open-range grassland, actively managed with cattle, and interspersed with closed oak canopy woodland riparian habitat, which provided refugia and breeding pools (that were primarily artificial ponds created by land managers). Wheatley Ranch is located 1040 m above sea level on an upper tributary of the San Dieguito watershed adjacent to Scholder Creek and contains one semipermanent pond and two ephemeral pools, with the semipermanent pond supporting *L. catesbeianus* and invasive Red-eared Sliders (*Trachemys scripta elegans*). RJER is 236 m above sea level within the Otay Watershed and contains a matrix of ponds and creeks with up to seven ephemeral pools and three perennial water bodies. RJER also harbored dense populations of invasive crayfish and African Clawed Frogs (*Xenopus laevis*).

Gut Content Identification Procedures

After eradication efforts, all the frog carcasses from a given pond on a given night were frozen until we could identify gut

contents in the laboratory. We recorded biometrics and gut contents of thawed frogs including wet mass, snout–vent length (SVL), excised stomach mass, sex (via gonad differentiation), and presence/absence of developed eggs in females. We removed the stomach via severance at the pharynx and pyloric sphincter. We recorded stomach content and lower gut contents if the digested material was still identifiable. We identified gut contents to the lowest taxonomic level possible, and for most vertebrates this was often to species. Invertebrate remains were often less diagnostic at the species level and most invertebrates were categorized into higher-level taxonomic classes.

We analyzed gut content differences between *L. catesbeianus* sexes and age groups. We classified frogs as either juveniles or adults, with adults measuring >100 mm and juveniles measuring <85 mm. When comparing age classes, we omitted individuals between 85 and 100 mm from analysis to reduce the uncertainty of age classification (Bury and Whelan 1985). This buffer concurs with the relative maturity of ovaries that we observed in the necropsies (i.e., no individuals <85 mm were reproductive in the sample).

Statistical Analyses

We calculated the Shannon–Wiener diversity index of each gut to determine the overall diversity of prey in gut contents. We analyzed counts of vertebrates, crayfish, odonates, and other invertebrates (i.e., total invertebrates – [crayfish + odonates]) in the gut, diversity of gut contents (Shannon diversity index), total items in gut, and empty stomachs in a generalized linear modeling (GLM) framework to understand if age, sex, site, or any of their interactions played a role in dietary variation. Some of these response variable counts were zero inflated (number of zeros for vertebrates in gut = 88.5%; odonates in gut = 65.1%; crayfish in gut = 87.3%; other invertebrates = 82.71%). Therefore, we used Akaike information criterion corrected for small sample size (AICc) to compare negative binomial GLMs, zero-inflated Poisson GLMs, and zero-inflated negative binomial GLMs and chose the top model on the basis of AICc values (Table 1). Because no crayfish were present at the Wheatley Ranch site, our models for crayfish did not include site or its interactions as explanatory variables. To understand how age, sex, and site played a role in the diversity of gut contents, total items in gut, and empty stomachs, we used a quasi-Poisson (for diversity of gut contents and total items in gut) or a binomial (for empty stomachs) GLM to model explanatory variables (because data were no longer zero inflated). For all GLM analyses, significance was determined through an analysis of variance type III test. We compared

TABLE 2.—Prey taxa identified in *Lithobates catesbeianus* gut contents at RJER and Wheatley Ranch. Numbers indicate total count of prey items within taxonomic categories; percent indicates proportion of that prey taxa over all prey items consumed within that age/sex class. Sample sizes refer to numbers of bullfrogs within each category. The “Total” column for RJER includes the number of prey items for two adults of unknown sex (not shown).

Site	Taxon	Adult females	Adult males	Juveniles	Total
RJER		<i>n</i> = 99	<i>n</i> = 74	<i>n</i> = 84	<i>n</i> = 259
	Amphibia	13 (4.5%)	4 (2.6%)	0	18 (2.3%)
	Aves	0	0	1 (0.3%)	1 (0.1%)
	Reptilia	5 (1.7%)	3 (1.9%)	1 (0.3%)	10 (1.3%)
	Rodentia	5 (1.7%)	2 (1.3%)	0	7 (0.9%)
	Arachnida	9 (3.1%)	5 (3.2%)	16 (5.4%)	30 (3.9%)
	Clitellata	1 (0.3%)	0	0	1 (0.1%)
	Coleoptera	35 (12.1%)	11 (7.1%)	19 (6.4%)	80 (10.4%)
	Decapoda	87 (30.1%)	76 (48.7%)	15 (5.1%)	178 (23.2%)
	Dermaptera	5 (1.7%)	17 (10.9%)	39 (13.2%)	61 (7.9%)
	Diplopoda	0	1 (0.6%)	0	1 (0.1%)
	Diptera	4 (1.4%)	1 (0.6%)	9 (3.0%)	14 (1.8%)
	Gastropoda	1 (0.3%)	4 (2.6%)	1 (0.3%)	6 (0.8%)
	Hemiptera	0	0	0	0
	Hymenoptera	18 (6.2%)	22 (14.1%)	39 (13.2%)	79 (10.3%)
	Isopoda	61 (21.1%)	1 (0.6%)	117 (39.5%)	181 (23.6%)
	Lepidoptera	0	0	1 (0.3%)	1 (0.1%)
Wheatley	Odonta	30 (10.4%)	3 (1.9%)	27 (9.1%)	61 (7.9%)
	Orthoptera	15 (5.2%)	6 (3.8%)	11 (3.7%)	40 (5.2%)
		<i>n</i> = 7	<i>n</i> = 6	<i>n</i> = 395	<i>n</i> = 408
	Amphibia	11 (47.8%)	0	50 (3.7%)	61 (4.4%)
	Aves	0	1 (6.7%)	1 (0.1%)	2 (0.1%)
	Reptilia	0	0	5 (0.4%)	5 (0.4%)
	Rodentia	0	0	0	0
	Arachnida	1 (4.3%)	0	39 (2.9%)	40 (2.9%)
	Clitellata	0	0	0	0
	Coleoptera	3 (13.0%)	1 (6.7%)	134 (10.0%)	138 (10.0%)
	Decapoda	0	0	0	0
	Dermaptera	0	0	6 (0.4%)	6 (0.4%)
	Diplopoda	0	0	0	0
	Diptera	0	6 (40.0%)	155 (11.6%)	161 (11.7%)
	Gastropoda	2 (8.7%)	2 (13.3%)	115 (8.6%)	119 (8.6%)
	Hemiptera	0	0	7 (0.5%)	7 (0.5%)
	Hymenoptera	1 (4.3%)	0	305 (22.8%)	306 (22.2%)
	Isopoda	0	0	7 (0.5%)	7 (0.5%)
	Lepidoptera	2 (8.7%)	1 (6.7%)	24 (1.8%)	27 (2.0%)
	Odonta	2 (8.7%)	4 (26.4%)	269 (20.1%)	275 (20.0%)
	Orthoptera	1 (4.3%)	0	221 (16.4%)	222 (16.1%)

SVL data for adult frogs using nonparametric Wilcoxon rank sum tests, as these data could not be transformed to achieve normality (Shapiro–Wilk test; $P < 0.01$ for all transformation attempts). All analyses were performed in the program R (v4.3.1; R Core Team 2023) using the dplyr and lme4 packages (Bates et al. 2015; Wickham et al. 2015).

RESULTS

Dietary Variation between Sites, Sexes, and Age Classes

Our analyses included 259 *L. catesbeianus* collected from RJER and 408 from Wheatley Ranch. Few stomachs were ruptured, with <1 gut/100 frogs compromised from trauma. We identified 14 invertebrate taxa and 4 vertebrate taxa in the guts of the collected frogs (Table 2). Gut contents were variable, but the most common native invertebrate prey at both sites were odonates, which included both nymph and adult dragonflies, as well as damselflies Order Odonata. The most common nonnative invertebrate prey were European Honeybees (*Apis mellifera*) and invasive crayfish.

We classified 479 *L. catesbeianus* as juveniles (<85 mm SVL) and 188 as adults (>100 mm SVL; 106 adult females; 80 adult males; 2 unknown sex). Adult frogs were significantly larger at RJER than at Wheatley Ranch (Fig. 1; $W = 1631$,

$P < 0.01$), but there were no significant differences in SVL between the sexes ($W = 4293$, $P = 0.36$). There was also no significant difference in diet diversity between sexes (Table 3), indicating that males and females have broadly similar diets (Fig. 2). However, adult females had more items in their gut overall than adult males ($P = 0.012$, Table 3; Fig. 2). Diets also differed between adults and juveniles, with adults consuming more vertebrate prey than juveniles ($P = 0.048$, Table 3; Fig. 2). We found that adults at Wheatley Ranch consumed more vertebrates than adults at RJER ($P = 0.022$; Table 3); however, adults at RJER also consumed a large quantity of crayfish, which were absent at Wheatley Ranch; juveniles at RJER consumed fewer crayfish than adults ($P = 0.005$; Table 3). Juveniles overall had more empty stomachs than adults (2.1% of adults had empty stomachs versus 6.4% of juveniles; $P = 0.003$; Table 3). Juveniles at RJER also had a significantly higher proportion of empty stomachs than juveniles at Wheatley Ranch (11.9% of RJER juveniles had empty stomachs versus 5.3% of Wheatley Ranch juveniles; $P = 0.027$; Table 3).

Vertebrate Prey

Vertebrates represented only a small portion of the total prey records in each population, with vertebrates representing

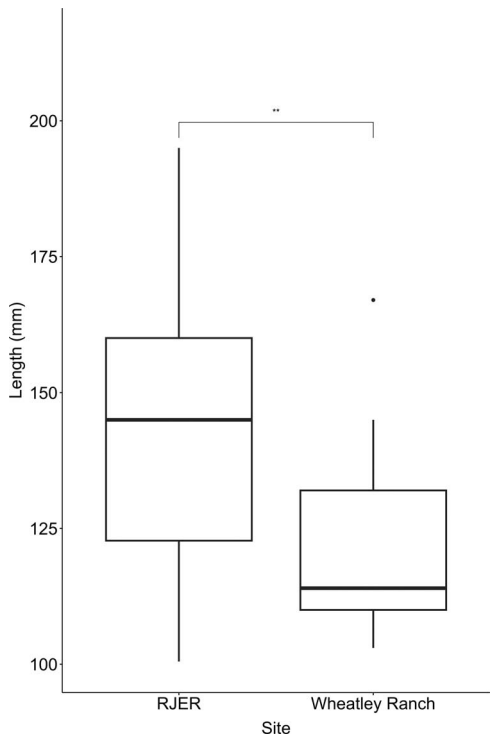


FIG. 1.—Boxplots showing median and interquartile range of body length (SVL) of adult *L. catesbeianus* collected at RJER and Wheatley Ranch. Adult collected at RJER were significantly larger than frogs collected at Wheatley Ranch (Wilcoxon rank sum test, $W = 1631$, $P < 0.01$).

4.9% of prey records at Wheatley and 3.7% at RJER (Table 2). Of the vertebrate prey, native Baja California Treefrogs (*Pseudacris hypochondriaca*) were the most common prey item for both age classes ($n = 69$). Western Toads (*Anaxyrus boreas*) were also found in gut contents at both sites ($n = 5$). Although they are abundant at RJER, we detected only one African Clawed Frog in all the surveyed gut contents and only one Western Spadefoot (*Spea hammondi*). Two-Striped Gartersnakes (*Thamnophis hammondi*) were the most common snake species found in the gut contents across both sites.

DISCUSSION

Invasive *L. catesbeianus* in our two surveyed watersheds in Southern California consumed a wide variety of native and nonnative invertebrate and vertebrate prey. Although

adult females had more prey items in their guts compared with adult males, we found no difference in body size between the sexes. However, adult body size differed across sites, with larger adults captured at RJER than at Wheatley Ranch. The major notable dietary difference between our sites was the abundance of invasive crayfish at RJER, a prey item that was absent from Wheatley Ranch. Below we discuss these patterns in more detail.

Ontogenetic Variation

We detected ontogenetic shifts in the diet of *L. catesbeianus*, with juveniles relying mostly on invertebrates and adults incorporating larger prey into their diet, including more vertebrates and crayfish. Although juveniles consumed some vertebrate prey, these mostly consisted of native *P. hypochondriaca*, which are small enough to be eaten by younger, more gape-limited individuals (Bury and Whelan 1985). Because larger frogs consume a higher variety and quantity of vertebrate animals, adult diets are more likely to include threatened or endangered vertebrate species (e.g., Arroyo Toad [*Anaxyrus californicus*]). This heavier reliance on vertebrate prey is consistent with other *L. catesbeianus* dietary studies documenting ontogenetic expansion of prey type (Bissantini et al. 2019), and this same pattern is recorded in other gape-limited generalist amphibian invasives such as the Cane Toad (*Rhinella marina*; Kidera et al. 2008). Mitigation efforts may therefore benefit from prioritizing the removal of adult *L. catesbeianus* to minimize any negative effects on endangered native vertebrates and reduce the invasive population’s reproductive potential. However, when resources are available for removal efforts, it may be prudent to also target juvenile frogs, as they consume significant quantities of native prey.

We also observed more juveniles with empty stomachs relative to adults, a finding that may reflect competitive exclusion by adults. *Lithobates catesbeianus* have classic type III survivorship, with large numbers of eggs and high juvenile mortality (Turner 1962), and at RJER, juveniles were more often found in closed-canopy creek systems, whereas adult males tended to be in open ponds guarding their territories and calling for mates. Therefore, adults may exclude juveniles from larger, more open ponds with more readily available prey. Alternatively, the incidence of empty stomachs in juveniles could reflect their greater reliance on smaller invertebrates and differential gut-passage time for

TABLE 3.—Estimates and P values for explanatory variables and their interactions for top ranked models.^a P values were generated from an analysis of variance type III test for each explanatory variable and interaction term. Asterisks (*) indicate statistical significance at $P \leq 0.05$.

Model	Site		Age		Sex		Site $\times$ age		Site $\times$ sex		Age $\times$ sex	
	Estimate	P	Estimate	P	Estimate	P	Estimate	P	Estimate	P	Estimate	P
Vertebrates	1.63	0.02*	-2.33	0.05*	-0.89	0.07	0.44	0.72	-0.55	0.62	0.85	0.43
Odonates	-16.83	0.70	-1.12	0.14	0.81	0.31	12.98	0.77	6.92	0.80	-6.67	0.83
Crayfish	—	—	-2.88	<0.01*	0.18	0.40	—	—	—	—	-0.19	0.90
Other invertebrates	1.04	0.43	-0.51	0.69	1.21	0.10	-0.26	0.87	-0.72	0.59	0.56	0.71
Diversity of gut	0.27	0.65	0.56	0.15	-0.10	0.75	0.28	0.67	-0.43	0.32	0.59	0.25
Items in gut	0.11	0.71	0.03	0.89	-0.41	0.01*	-0.03	0.92	-0.14	0.61	0.54	0.05
Empty stomachs	1.25	0.28	0.80	<0.01*	1.22	0.38	1.31	0.03*	0.91	0.46	1.33	0.84

^a Specific model terms are as follows. Vertebrates (negative binomial): vertebrates ~ site + age + sex + site $\times$ age + (site $\times$ sex) + (age $\times$ sex). Odonates (Poisson): odonates ~ site + age + sex + (site $\times$ age) + (site $\times$ sex) + (age $\times$ sex). Crayfish (negative binomial): crayfish ~ age + sex + (age $\times$ sex). Other invertebrates (Poisson): other invertebrates ~ site + age + sex + (site $\times$ age) + (site $\times$ sex) + (age $\times$ sex). Diversity of gut (quasi-Poisson): diversity of gut ~ site + age + sex + (site $\times$ age) + (site $\times$ sex) + (age $\times$ sex). Empty stomachs (binomial): empty stomachs ~ site + age + sex + (site $\times$ age) + (site $\times$ sex) + (age $\times$ sex).

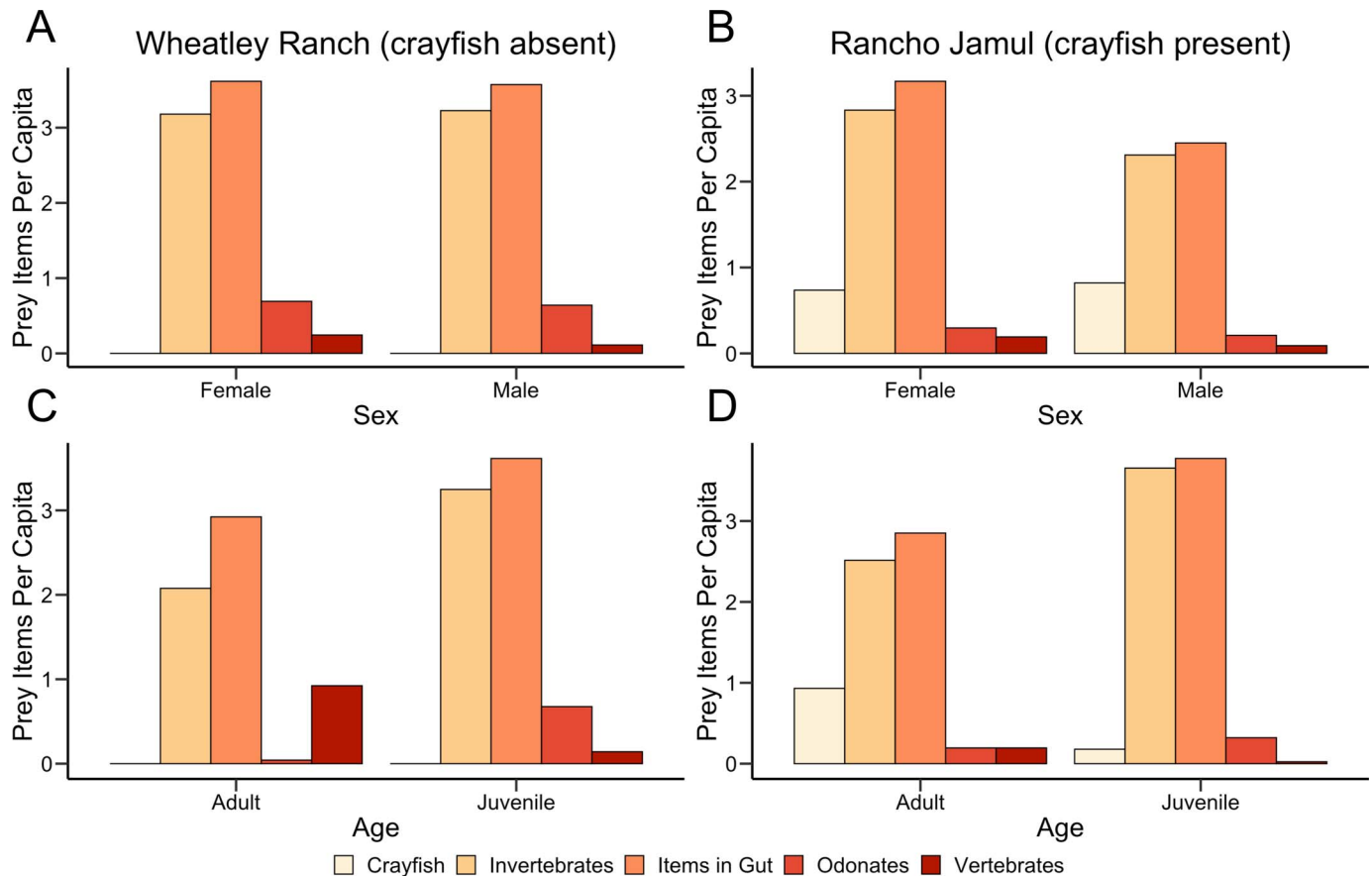


FIG. 2.—Per capita prey items (crayfish, invertebrates [including crayfish and odonates], total items in gut [including plant material], odonates, and vertebrates) consumed by *Lithobates catesbeianus* at each site categorized by sex and age at Wheatley Ranch (A, C) and Rancho Jamul Ecological Reserve (B, D).

our prey categories. It is possible that material from vertebrates and crayfish is retained longer than material from smaller invertebrates, leading to fewer adults with empty stomachs. To address this hypothesis, future studies could quantify gut passage time of different prey classes using experimental approaches.

Sex Differences

When estimating body size, we used SVL as a proxy for body size, rather than mass, as mass varies throughout the year in relation to breeding cycles. At our sites *L. catesbeianus* exhibited no sexual dimorphism in SVL, despite evidence of sexual size dimorphism at other sites (reviewed in Bury and Whelan 1985—however, note that Bissattini et al. 2018 also found no difference in SVL between sexes). We also detected no significant difference of prey diversity between sexes, indicating that adult male and female frogs share broadly similar diets at these sites (Table 3; Fig. 2). However, female adult frogs had more items in their guts overall versus adult males ($P = 0.012$; Table 3; Fig. 2), a difference that might be explained by reproductive behavior. *Lithobates catesbeianus* in Southern California exhibit a prolonged breeding season, with females producing up to 20,000–50,000 eggs/yr, a reproductive output that requires sustained energetic resources (Bury and Whelan 1985). Males are territorial during the breeding season and dedicate significant time to calling for females and defending oviposition sites—activities that largely

preclude foraging (Emlen 1968). In contrast, adult females tracked in telemetry studies had larger home-range sizes than males during the active breeding seasons (Austin et al. 2003; Cooper 2017).

Invasive Species

Our data corroborate other studies indicating that *L. catesbeianus* populations sympatric with invasive crayfish exhibit larger body sizes relative to populations at sites without crayfish (Bissattini et al. 2018; Liu et al. 2018). Our data are limited to two populations, making comparisons preliminary (many other factors also differ between these two sites), but the pattern follows that described by Bissattini et al. (2018), who reviewed the literature and found that *L. catesbeianus* diet is dominated by crayfish (and includes fewer amphibians) in sites where the crayfish and bullfrogs are coinvasors. In line with this pattern, we found that adult *L. catesbeianus* in our site with crayfish (RJER) were approximately 10% larger than in the site without crayfish (Wheatley Ranch), and adult *L. catesbeianus* at Wheatley Ranch were more likely to contain vertebrate prey in their gut contents than adults at RJER. Although crayfish might in part replace vertebrates in the diet of *L. catesbeianus*, this subsidy also appears to increase *L. catesbeianus* population density (Li et al. 2011; Bissattini et al. 2018; Liu et al. 2018) and may also increase fecundity by allowing individuals to grow larger, which could in turn increase the long-term impact on

native species. This could potentially affect nearby habitats (such as ephemeral water bodies adjacent to streams) that do not contain crayfish yet are affected by the overland movements of bullfrogs dispersing from crayfish-laden streams. At RJER, some of the potentially affected species are protected, including juvenile Southwestern Pond Turtles (*Actinemys pallida*), Arroyo Toads (*A. californicus*), Western Spadefoots (*S. hammondi*), and Two-Striped Gartersnakes (*T. hammondi*; Miller et al. 2012; Nicholson et al. 2020). Understanding the interactions between invasive frogs and crayfish at sites where they co-occur is important for guiding management efforts and attempts at removing *L. catesbeianus* alone may be less productive than those that target both species (Bissattini et al. 2018).

Invasive African Clawed Frogs (*X. laevis*) are also present throughout the RJER water system, but we found only one individual in the *L. catesbeianus* gut contents. The lack of predation was surprising, as *X. laevis* occurs at high density and occupies similar microhabitat. This lack of predation warrants further investigation, as *X. laevis* could use microhabitats that make them less likely to encounter *L. catesbeianus* (e.g., deeper water in the middle of the pond rather than along the edges) or possess other antipredator adaptations that make them unsuitable as prey.

Native Species

Some rare and protected native species were found in the guts of *L. catesbeianus*, including *S. hammondi* and *T. hammondi*. These species have declined throughout their historic range and are a California state-listed Species of Special Concern, with *L. catesbeianus* cited as one of the many drivers of their decline. Although our data set did not allow us to quantify population impacts on these species, *L. catesbeianus* predation has been implicated as a major obstacle for the protection or restoration of *A. californicus*, *A. pallida*, and California Red Legged Frog (*Rana draytonii*) in Southern California (Miller et al. 2012; Thompson et al. 2016; Nicholson et al. 2020). Future studies could use our data to help parameterize models designed to formally assess population-level impacts of bullfrogs on some of these species.

The absence of juvenile or hatchling turtles in gut surveys at these two sites is likely due to the lack of juvenile turtles present at these sites, as both sites are currently being restored for *A. pallida* and did not have documented recruitment during the *L. catesbeianus* harvest period. Other studies have shown a direct correlation between the presence of *L. catesbeianus* in a riparian system and absence of juvenile pond turtles (*Actinemys* sp.), even though adults occur at the same sites (Nicholson et al. 2020). Turtles have been found in bullfrog guts at other study sites in Southern California (Nicholson et al. 2020). *Lithobates catesbeianus* represent a significant predatory threat for juvenile *A. pallida* even in the most remote and well-protected tributaries of Southern California and might be the primary factor limiting recruitment in affected stream systems (Nicholson et al. 2020).

Insects constituted the largest category of *L. catesbeianus* prey by number across all life stages, and Odonates were the most consumed native taxa. Nymph and adult dragonflies (both were common in gut contents) are significant predators of larval and adult mosquitos, respectively. Odonates are important to humans as controls for disease vectors

because of their high consumption of dipterans (mosquitos). Previous studies have shown that invasive crayfish can suppress odonate populations and have an indirect positive impact on mosquito populations (Bucciarelli et al. 2019). Given that invasive *L. catesbeianus* also consume high quantities of both aquatic nymph and adult odonates, they might similarly negatively affect mosquito predators and thus have a positive indirect effect on mosquito populations.

Future Directions

Our dietary analysis revealed important patterns of intra-specific variation in diet, with adult females consuming more prey compared with adult males, and a higher likelihood of adults consuming vertebrate prey compared with juveniles. Furthermore, the presence of invasive crayfish at RJER contributed substantially to the diet of adult *L. catesbeianus*, suggesting potential interactions between these species in invaded freshwater habitats (Vannini et al. 2015; Bissattini et al. 2018; Liu et al. 2018). As ongoing efforts continue to mitigate the negative impacts of these invasive species, several factors should be investigated to better understand invasion dynamics and synergistic interactions between *L. catesbeianus* and their sympatric invaders. As scavengers, crayfish convert benthic detritus into palatable biomass for *L. catesbeianus*, and represent a large, easily accessible food source that might increase frog fecundity (Klose and Cooper 2013). In any restoration plan, several environmental concerns must be triaged relative to the resources available and the projected impact of chosen actions. With a better understanding of the interaction between *L. catesbeianus* and crayfish, land managers and habitat restoration planners can make more educated decisions when choosing which species and sites to address first and how much effort to dedicate toward extirpation. Data on feeding ecology and stomach contents can be a key parameter in models designed to determine the extent to which invasive species mitigation may aid in the conservation of target species, and thus provide valuable guidance for managers tasked with balancing multiple demands on limited resources.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at <https://doi.org/10.1655/Herpetologica-D-23-00057.S1>.

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