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Nutritional physiology of the Santa Ana sucker (*Catostomus santaanae*): A threatened freshwater fish endemic to Southern California

Bao-Quang Nguyen-Phuc[1] | **Carl Demetropoulos[2]** | **Sam Stewart[2]** | **Parsa Saffarinia[3]** | **José Bastian Salgado[4]** | **Elin Hawkins[1]** | **Alyssa R. Frederick[1]** | **Donovan P. German[1]** 🆔

[1]Department of Ecology and Evolutionary Biology, University of California, Irvine, California

[2]Southwest Aquatic & Terrestrial Biology, Thousand Oaks, California

[3]Department of Evolution, Ecology, & Organismal Biology, University of California, Riverside, California

[4]Department of Biology, California Lutheran University, Thousand Oaks, California

Correspondence
Donovan P. German, Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697.
Email: dgerman@uci.edu

Abstract

The herbivorous fish *Catostomus santaanae* is a federally "threatened" freshwater fish species endemic to southern California and is the centre of legal battles over water use. Because little is known about the nutritional ecology of this species, we investigated the nutritional physiology of *C. santaanae* to better understand their dietary and energetic needs with the goal of generating data useful in conservation efforts. Individuals of *C. santaanae* were raised for six weeks on an algal diet in the laboratory. They digested approximately 45%, 55% and 80% of protein, soluble carbohydrate and lipid, respectively, from the algal diet. Their metabolic rate (~0.0024 mg O_2 min^{-1} g^{-1}) suggested they would need to eat more than their body mass per day of an algal diet to thrive. Digestive enzyme activities of the laboratory-reared and wild-caught fish showed patterns typical of a "plug-flow reactor" gut with high intake and rapid gut transit. However, lipase activities remained elevated throughout the gut, and this result, coupled with the lipid digestibility data, and evidence of feeding selectivity on specific diatom taxa in nature, suggests that *C. santaanae* targets diatoms as it grazes and that diatom lipid may be crucial to their survival. Our data set provides parameters that can be used in conservation modelling efforts towards habitat restoration.

KEYWORDS
adaptive modulation hypothesis, digestive enzyme activity, phenotypic plasticity

1 | INTRODUCTION

Some of the most disturbed and imperilled habitats (and by extension, the inhabitants found therein) in North America are the freshwaters of southern California. With its arid climate, increasing drought conditions and large human population, there are no naturally occurring freshwater systems in southern California that are not impacted directly by humans (Richmond, Backlin, Galst-Cavalcante, O'Brien, & Fisher, 2018). One of the most discussed and litigated naturally occurring residents of southern California freshwater systems is the Santa Ana sucker (*Catostomus santaanae*), which is a federally threatened (U.S. Fish and Wldlife Service, 2000) native of the Santa Ana, Los Angeles, San Gabriel and Santa Clara River drainages (Richmond et al., 2018) (Figure 1). *Catostomus santaanae* is an herbivorous fish (Greenfield et al. 1970; Saiki, Martin, Knowles, & Tennant, 2007), and similar to other suckers (family Catostomidae) and minnows (family Cyprinidae) with sub-terminal mouths (Figure 2), *C. santaanae* is a benthic grazer, subsisting on periphyton
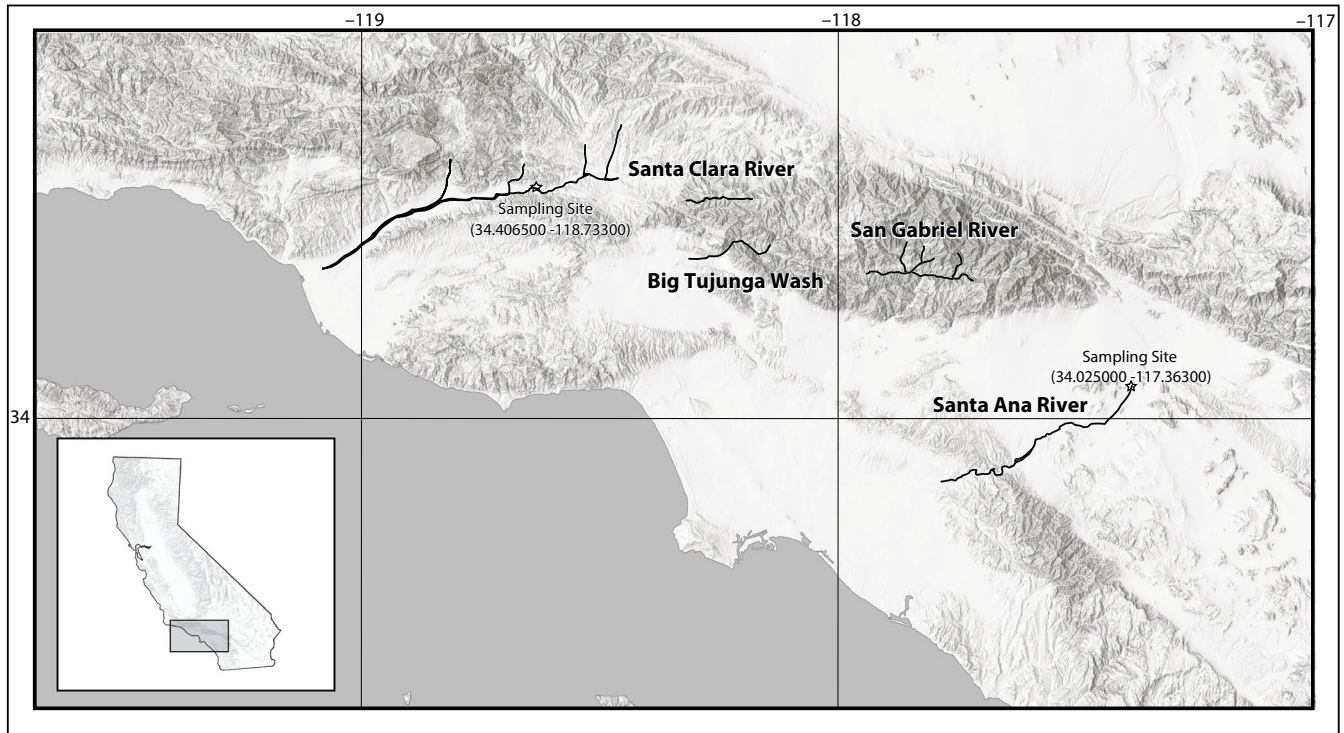
FIGURE 1 Natural watersheds of *Catostomus santaanae* in southern California, USA. Collection sites in the Santa Clara River drainage (Ventura County, California, USA) and the Santa Ana River drainage (Riverside County, CA, USA), are shown [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 2 (a) Seining for *Catostomus santaanae* in a tributary of the Santa Clara River (Ventura County, California, USA). (b) A captive fish consuming formulated algal diet in the laboratory [Colour figure can be viewed at wileyonlinelibrary.com]

within the riverine systems it inhabits (German, 2009a; Greenfield et al., 1970; Saiki et al., 2007).

Although an animal's diet can be inferred from observing what they eat, through behavioural observation, direct analyses of gut contents or the use of chemical tracers (e.g. stable isotope analyses, fatty acid profiling), understanding an animal's post-ingestive processes (i.e. digestive strategy) is becoming increasingly necessary to grasp how that animal fits within its community (Birnie-Gauvin, Peiman, Raubenheimer, & Cooke, 2017; Clements, German, Piché, Tribollet, & Choat, 2017; Crossman, Choat, & Clements, 2005; German, Sung, Jhaveri, & Agnihotri, 2015; Leigh, Papastamatiou, & German, 2018; Lujan, German, & Winemiller, 2011). This is particularly true in conservation efforts, as there are calls for more physiological and biochemical data on managed species (Birnie-Gauvin et al., 2017; Cooke, Blumstein, & Buchholz,

2014; Leigh et al., 2018; Tracy, Nussear, & Esque, 2006) and predictability for suitable foraging habitat in translocation recovery efforts (C. Demetropoulos, pers. obs.). These facts all ring true for *C. santaanae*, which is increasingly threatened by habitat loss (Richmond et al., 2018) and is at the centre of many legal arguments pertaining to water use in southern California (e.g. Anderson & Floyd, 2016; Busey, 2016; U.S. Fish and Wildlife Service, 2005). For an organism with federal protections, *C. santaanae* is understudied. For example, past reports of diet have been solely observational (Greenfield et al., 1970; Saiki et al., 2007; Thompson et al., 2010). Further, an understanding of *C. santaanae* resource use and food preference is important for managing its conservation in the light of its existence in degraded urban watersheds, such as the Santa Ana River, which can be subject to extreme flow manipulations and impacted habitat

(Thompson et al., 2010). As a result, current efforts to translocate *C. santaanae* to suitable unoccupied habitat include assessment of diatom, periphyton and macroinvertebrate assemblages, and comparison with species-specific autecological information known from occupied habitat with elevated *C. santaanae* population density and condition index (Dudek 2018). A full understanding of superior *C. santaanae* habitat requires knowledge of the nutrients essential for the maintenance of life, growth, the normal functioning of organs and the production of energy. Hence, to better understand what food resources are essential to the survival of the species and how these resources are processed, we investigated the nutritional physiology of *C. santaanae*.

We took a similar approach to some previous investigations in herbivorous fishes that focused on energetics and gut function (Crossman et al., 2005; German, 2009a; German, 2009b; German & Bittong 2009; German et al., 2015; Skea, Mountfort, & Clements, 2005; Skea, Mountfort, & Clements, 2007). First, we reared *C. santaanae* in the laboratory on an artificial algal diet to discern what it could digest from algae. Second, we measured the metabolic rates of these captive fish to better understand their energetic needs with regard to what is viewed as a "low-quality" algal diet (Bowen, Lutz, & Ahlgren, 1995; Horn & Messer, 1992). Finally, we measured the digestive enzyme activities in the gut of *C. santaanae*, in both the fish reared in the laboratory on the artificial algal diet and wild-caught fish consuming their natural food. Based on their long, thin-walled intestine and their periphyton-rich diet, we expected these fish to have a gut that functions as a "plug-flow reactor" (Horn & Messer, 1992; Jumars, 2000; Penry & Jumars, 1987) and enzyme activities that largely decrease moving distally along the intestine (German, 2009a; German et al., 2015). In short, we expected the nutritional physiology of *C. santaanae* to mimic those observed for grazing cyprinids, which features more of a reliance on endogenous digestive processes than enteric microbial symbionts. By learning more about how this fish acquires resources, we hoped to provide data useful for better habitat modelling, preservation and recovery. Moreover, as captive populations of these fishes grow within some agencies, our data set may provide important information on artificial diet formulation that mimics natural diets while maintaining *C. santaanae* vigour and natural foraging selectivity.

2 | MATERIALS AND METHODS

2.1 | Fish capture, maintenance and feeding experiment

Ten *C. santaanae* were captured by seine from the Santa Clara River in Ventura County, CA (34.408°N, 118.745°W; Figure 1), in September 2015. While we recognize the likelihood of some hybridization between *C. santaanae* and *C. fumeiventris* in the lower Santa Clara River, Richmond et al. (2018) showed that genetic input from *C. fumeiventris* is limited to areas downstream of the Piru Gap, where a large, stable, dry section of the river isolates surface flow from sections upstream of the confluence with Piru Creek. The fish sampled for this study were sourced from reaches upstream of the Piru Gap and could be classified as "pure" *C. santaanae* based on microsatellite genotypes. Moreover, the morphology and habitat selection of specimens sampled for this study were consistent with *C. santaanae* (Page & Burr, 2011). The collected fish were held in buckets of aerated river water and transported to University of California, Irvine, where they were transferred to a system of six 75.6-L aquaria connected to common filtration, including a sump, biological, particulate, activated carbon and UV filtration. The system contained deionized water supplemented with appropriate salts, and fish were under a 12L:12D light cycle. The water temperature was maintained at 19°C (the water temperature measured at the collection site) with a submersible heater for the duration of the experiment, and the temperature and chemical conditions (pH and ammonia concentrations) of the tank system were monitored daily to confirm that they did not vary during the experimental period. The tanks were scrubbed, debris and faeces siphoned out, and 20% of the water changed every three days. Because of potential high mortality with captured fish due to handling stress, we did not weigh the individual fish at the start of the experiment.

The fish were fed an artificial algal diet that was made by combining 1% agar with *Nannochloropsis* sp. (Nanno 3,600, Reed Mariculture) in a 1:1 ratio. The algal mixture was supplemented with casein (12% on a mass basis), soybean meal (12%), corn oil (3.3%), cod liver oil (3.3%), menhaden oil (3.4%), vitamin premix (1.4%) and mineral premix (0.6%). The resulting diet was 72.7 ± 3.2% (mean ± standard deviation) organic matter (OM), 16% protein (on a dry mass basis), 1.6% soluble carbohydrate (glucose equivalent on a dry mass basis) and 5% lipid (on a dry mass basis). The fish were fed the diet, which they readily consumed (Figure 2), three times daily to satiation for six weeks.

Faecal collections began after 3 weeks of acclimation. Four fish died during this period, which led to n = 6 for the digestibility studies with each fish housed individually in their own tanks. Prior to each daily feeding, tanks were checked for faecal material, which was different in appearance from uneaten food, and the faecal material was siphoned out of the aquaria with a 25-mL bulb pipette into a weigh boat. Samples of food were saved for analyses. The faeces and food were dried at 60°C for 24 hr, weighed and stored in sealed glass vials until analysed. Because it was difficult to discern the exact amount of food eaten, insoluble ash (Bjorndal, 1985; Galetto & Bellwood 1994) was used as an indirect marker to determine intake.

Protein content of the food and faeces was determined using bicinchoninic acid (Smith PK et al. 1985). Soluble carbohydrate content was determined using the phenol–sulphuric acid method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956), and lipid was determined using the charring method of Marsh and Weinstein (1966), after lipid extraction following Bligh and Dyer (1959). Ash was determined by drying the faeces and diet at 105°C (dry matter) and then combusting them at 550°C for 3 hr. The remaining content was ash; organic matter was determined as the material that was lost through combustion (German, 2009b). Apparent digestibility was determined using the equation [(intake-faeces)/intake X 100], where intake is total grams of organic matter (or a given nutrient class) consumed during the trial and faeces is grams of organic matter (or a given nutrient class) in the faeces produced during the trial. It should be noted that the digestibilities are "apparent digestibilities" because the fish contribute organic waste, such as sloughed intestinal cells, to the faeces (German, 2011).

An additional seven *C. santaanae* were collected from the Santa Ana River (34.025°N, 117.363°W) that were mortalities from a shutdown of a water treatment facility that significantly diminishes flow in the Santa Ana River at that site (Richmond et al., 2018). Freshly dead specimens were collected by hand and dissected as described below in the Tissue harvesting, preparation and digestive enzyme analyses section. These wild-caught fish served as representatives of the wild condition in terms of digestive enzyme activities in the guts of *C. santaanae*.

2.2 | Metabolic rate determination

At the conclusion of the feeding trials, the routine metabolic rates of each fish were measured in a respirometer. The fish were held without food overnight (12 hr or more) to ensure they were in a postprandial state. Although we did not measure gut transit times in *C. santaanae*, their feeding habits and gut structure are similar to minnows in the genus *Campostoma*, which have high intake (German, 2009a; German, Nagle, et al., 2010) and rapid gut transit, and do not hold food in their guts through the night (Fowler & Taber 1985). The closed chamber respirometer resembled that described by Reardon and Chapman (2010), featuring a 400-mL chamber that housed the fish, and the system contained a total of 730 ml with a flow rate of 5 L per minute. Decreases in oxygen concentration (% O_2 saturation) were used to estimate the rate of VO_2 of the fish. Oxygen and temperature data were recorded every 30 s during the trial with Ocean Optics FOXY probes and thermistors, respectively. The temperature was maintained at 19°C (±0.2°C) by conducting the metabolic rate measurements in the same chilled room in which the aquarium system was housed, and using a submersible heater. The fish were allowed to acclimate to the chamber for at least 30 min before starting measurements. Once the O_2 concentrations dipped below 90% of saturated, the system was opened, flushed with ambient water for five minutes and then closed again for the next measurement period. Each fish was measured three times. The fickle nature of the fish prevented us from holding them in the system for more than two hours to prevent mortality.

2.3 | Tissue harvesting, preparation and digestive enzyme analyses

At the conclusion of the experiment, individual fish were euthanized in buffered water containing 1g/L tricaine methanesulfonate (MS-222, Argent Chemicals Laboratory, Inc.), measured [standard length (SL) ± 1 mm], weighed [body mass (BM) ± 0.5 g] and dissected on a chilled (~4°C) cutting board. Dissected bodies were kept as voucher specimens and are stored at −80°C. Whole GI tracts were removed by cutting at the oesophagus and at the anus and processed for digestive enzyme activity analyses. For each fish, the whole GI tract was weighed, and the intestine length was measured [intestine length (IL) ± 1 mm]. Relative intestine length (RIL = IL x SL^{-1}) and digestive somatic index (DSI = intestine mass x body $mass^{-1}$) were determined. Following German and Bittong (2009), the intestines were divided into proximal, mid and distal sections of equal length, and gut contents were squeezed from the intestinal tissue and placed in a separate 1.5-mL centrifuge vial from the tissue itself. The intestinal contents and tissues were frozen in liquid nitrogen and stored at −80°C until homogenized (within 1 week).

The intestinal tissues and gut contents of laboratory-fed and wild-caught fish were homogenized on ice following German and Bittong (2009). To ensure the rupture of microbial cells and the complete release of enzymes from the gut contents, the pelleted gut contents were defrosted, diluted 3–5 volumes in 0.025 M Tris-HCl, pH 7.5, sonicated at 5 W output for 3 x 30 s, with 30-s intervals between pulses, and homogenized with using a Polytron Homogenizer (Brinkmann Instruments) with a 12-mm generator for 3 x 30 s at 3,000 rpm. The homogenized pelleted gut contents were then centrifuged at 12,000 x *g* for 10 min at 4ºC, and the resulting supernatant was used for enzyme assays.

Intestinal tissue samples were homogenized according to German, Horn, and Gawlicka (2004). Gut wall sections were defrosted, diluted in 5–10 volumes of 0.025 M Tris-HCl, pH 7.5, homogenized with the Polytron Homogenizer at 112x *g* for 3 x 30 s and centrifuged at 9,400x *g* for 2 min

TABLE 1 Standard length, body mass, relative intestine length and digestibility (%) of organic matter, protein and carbohydrate of *Catostomus santaanae* raised in the laboratory on an artificial algal diet

Standard Length (mm)	Body Mass (g)	Relative Intestine Length (IL × SL⁻¹)	Organic Matter	Protein	Soluble Carbohydrates	Lipid	Metabolic rate (mg O_2 min^{-1} g^{-1})
84.4 ± 2.58	8.56 ± 0.86	2.81 ± 0.21	20.70 ± 2.12	45.03 ± 2.74	54.68 ± 3.92	79.34 ± 1.97	0.0024 ± 0.0008

Note: Values are mean ± *SEM*, n = 6.

at 4°C. Following centrifugation, the supernatants from the gut contents and the intestinal tissue sections were collected and stored in small aliquots (100–200 μl) at –80°C until just before use in spectrophotometric assays of activities of digestive enzymes (within 5 days).

All assays were carried out at 19°C in duplicate or triplicate using a BioTek Synergy H1 Hybrid spectrophotometer/fluorometer equipped with a monochromator (BioTek). All assay protocols generally followed methods detailed in German and Bittong (2009), unless otherwise noted. All pH values listed for buffers were measured at room temperature (22°C), and all reagents were purchased from Sigma-Aldrich Chemical (St. Louis). All reactions were run at saturating substrate concentrations as determined in preliminary assays. Each enzyme activity was measured in each gut region of each individual fish, and blanks consisting of substrate only and homogenate only (in buffer) were conducted simultaneously to account for endogenous substrate and/or product in the tissue homogenates and substrate solutions.

Amylase activity was measured using 1% potato starch dissolved in 25 mM Tris-HCl containing 1 mM $CaCl_2$. The amylase activity was determined from a glucose standard curve and expressed in U (μmol glucose liberated per minute) per gram wet weight of gut tissue.

To measure maltase activity, we used 112 mM maltose dissolved in 200 mM phosphate buffer, pH 7.5. The maltase activity was determined from a glucose standard curve and expressed in U (μmol glucose liberated per minute) per gram wet weight of gut tissue.

Alkaline phosphatase, β-glucosidase and N-acetyl-β-ᴅ-glucosaminidase (NAG) activities were measured following German et al. (2011), using 200 μM solutions of the substrates 4-methylumbelliferyl phosphate, 4-methylumbelliferyl β-ᴅ-glucoside and 4-methylumbelliferyl N-acetyl-β-ᴅ-glucosaminide, respectively, dissolved in 25 mM Tris-HCl (pH 7.5). Briefly, 90 μL of substrate was combined with 10 μl of homogenate in a black microplate and incubated for 30 min. Following incubation, 2.5 μl of 1 M NaOH was added to each microplate well and the fluorescence read immediately at 365 nm excitation and 450 nm emission. Each plate included a standard curve of the product (4-methylumbelliferone), substrate controls and homogenate controls, and enzymatic activity (μmol product released per minute per gram wet weight tissue) was calculated from the MUB standard curve.

Trypsin activity was assayed using a modified version of the method designed by Erlanger, Kokowsky, and Cohen (1961). The substrate, 2 mM Nα-benzoyl-ʟ-arginine-p-nitroanilide hydrochloride (BAPNA), was dissolved in 100 mM Tris-HCl buffer (pH 7.5). Trypsin activity was determined with a p-nitroaniline standard curve and expressed in U (μmol p-nitroaniline liberated per minute) per gram wet weight of gut tissue.

Aminopeptidase activity was measured using 2.04 mM L-alanine-p-nitroanilide HCl dissolved in 200 mM sodium phosphate buffer (pH 7.5) and determined with a p-nitroaniline standard curve. Activity was expressed in U (μmol p-nitroaniline liberated per minute) per gram wet weight of gut tissue.

Lipase (nonspecific bile salt-activated) activity was assayed using 0.55 mM p-nitrophenyl myristate (in ethanol) in the presence of 5.2 mM sodium cholate dissolved in 25 mM Tris-HCl (pH 7.5). Lipase activity was determined with a p-nitrophenol standard curve and expressed in U (μmol p-nitrophenol liberated per minute) per gram wet weight of gut tissue.

2.4 | Statistics

Prior to all significance tests, Levene's and Bartlett's tests for equal variances were performed to ensure the appropriateness of the data for parametric analyses, and any data sets that did not meet the assumptions of ANOVA (including homoscedasticity) were transformed using a Box-Cox transformation. All tests were run using R (version 3.5.1). Comparisons of enzymatic activities among the gut regions were made separately for tissue and contents with ANOVA followed by a Tukey's HSD with a family error rate of $p = .05$. Wild-caught and laboratory-reared fish were analysed separately in this regard. For each gut region, the activity of each enzyme was compared among the wild-caught and laboratory-fed fish with t test, using a Bonferroni correction. Tissue and content enzymatic activities were compared among the wild-caught and laboratory-fed fish separately.

3 | RESULTS

The fish reared in the laboratory had average body lengths of 84.4 ± 2.58 mm (SL) and average body masses of 8.56 ± 0.86 g (Table 1). Because members of the family Catostomidae lack a gastric stomach (Wilson & Castro 2011), their entire gut is essentially a long intestine (Figure 3). The relative intestine length of *C. santaanaae* was greater than twice their body length. The digestibility of organic matter ($20.7 \pm 2.74\%$), protein ($45.03 \pm 2.74\%$), soluble carbohydrates ($54.68 \pm 3.92\%$) and lipid ($79.34 \pm 1.97\%$) showed that the fish could access nutrients in the food (Table 1). The metabolic rates measured at the end of the experiment revealed average oxygen consumptions of 0.0024 ± 0.0008 mg O_2 mg^{-1} min^{-1}. These metabolic rates translate to about 618 Joules per day. Given the digestible caloric content of the artificial algal diet was approximately 53.6 Joules/g, the fish would need to consume about 11.5 g of the artificial algal diet per day to meet their energetic requirements. That is, they need to consume more than their body mass of the artificial algal diet each day to meet their energetic requirements.

The digestive enzyme activity data showed some different patterns among wild-caught fish and those reared on the artificial algal diet in the laboratory (Table 2, Figures 3 and 4, Table S1, Figure S1). Both categories of fish showed patterns common for a plug-flow reactor gut, with activities that generally decrease moving distally along the intestine. However, amylase showed little change in activity along the gut, and lipase showed a pattern of increasing activity (albeit not significantly so) moving distally along the intestine in laboratory-reared fishes (Figure 3). Generally, the enzyme activities in the intestinal contents of the wild-caught fish were higher than those of the laboratory-reared fish, with statistical significance detected for trypsin (Figure 3), alkaline phosphatase and NAG (Figure 4) and aminopeptidase (Figure S1).

4 | DISCUSSION

We successfully reared *C. santaanae* on an artificial algal diet in the laboratory for six weeks. We found the fish digested carbohydrates and proteins with moderate efficiency from this laboratory diet, but that they were best at digesting lipids. Consistent with other freshwater fishes with similar diets, we found the *C. santaanae* gut to function within the expectations of a plug-flow reactor (Horn & Messer 1992), indicating that this species has high intake and likely rapid transit of material through their gut. Coupled with the food quality of periphyton, their metabolic rates strongly suggest that these fish need to maintain a high intake of food to sustain reproductive health and fecundity. Their long, thin-walled intestine supports this contention (German, 2011).

The metabolic rates we measured for *C. santaanae* are consistent with other similarly sized ectotherms measured at 20°C (Fu et al., 2009; Gillooly, Brown, West, Savage, & Charnov, 2001; Jung, Brix, & Brauner, 2019; Ling, Fu, & Zeng, 2019); we measured our fish at 19°C Periphyton is typically defined as a loose assemblage of bacteria, cyanobacteria, filamentous green algae, diatoms and detritus that grows on hard substrates in aquatic systems (van Dam, Beveridge, Azim, & Verdegem, 2002; Hoagland, Roemer, & Rosowski, 1982; Klock, Wieland, Seifert, & Michaelis, 2007). Given the relatively low-quality (i.e. low-protein) aspects of a periphyton diet (Bowen et al., 1995; German, Nagle, et al., 2010), it follows that this fish species must eat roughly its own body mass in food on a daily basis to meet protein and energy demands. This is not uncommon for other freshwater fishes, like the grass carp (*Ctenopharyngodon idella*) and detritivorous catfishes (family Loricariidae), which have high intake (Fu et al., 2009; German, Neuberger, Callahan, Lizardo, & Evans, 2010; Stevens et al. 1998). However, *C. santaanae* may be more of a diatom specialist than other grazers (like grazing

FIGURE 3 Digestive enzyme activities in intestinal tissue (left column) and intestinal contents (right column) of *Catostomus santaanae* collected directly from the wild or reared on an algal diet in the laboratory for 6 weeks. Values are mean ± standard error. Note the different y-axis scales for amylase and trypsin intestinal tissue vs. intestinal content. Activities were compared among intestinal sections with ANOVA for wild and laboratory-fed individuals separately. For the wild fish, intestinal sections with a different capital letter for a given enzyme and intestinal fraction (tissue or content) are statistically significantly different, whereas statistical difference is indicated with lower-case letters for the laboratory-fed fish. Significant differences among wild and laboratory-fed fish for a given intestinal section are indicated with an asterisk. The *C. santaanae* gut is shown beneath the x-axes to illustrate the long, thin-walled nature of their digestive tract [Colour figure can be viewed at wileyonlinelibrary.com]
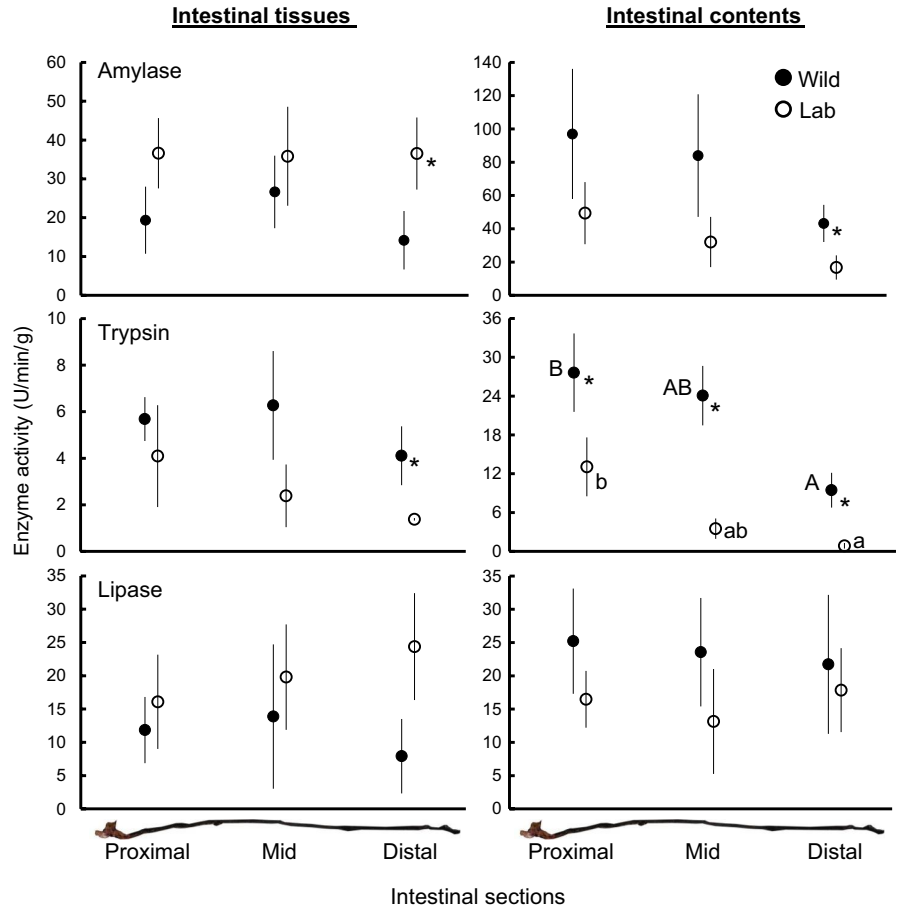

TABLE 2 Summary of ANOVA statistics for comparisons of digestive enzyme activities among different regions of the intestine in *Catostomus santaanae* reared in the laboratory on an artificial algal diet or wild-caught individuals

| Enzyme | Artificial Algal Diet | | Wild-Caught | |
	Intestinal Tissue	Intestinal Contents	Intestinal Tissue	Intestinal Contents
Amylase	$F_{2,12} = 0.002$	$F_{2,12} = 1.276$	$F_{2,15} = 0.537$	$F_{2,12} = 1.204$
	$p = .998$	$p = .314$	$p = .595$	$p = .327$
Maltase	$F_{2,12} = 0.186$	N/A	$F_{2,15} = 4.087$	$F_{2,15} = 1.374$
	$p = .832$		$\mathbf{p = .038}$	$p = .283$
Trypsin	$F_{2,12} = 0.860$	$F_{2,12} = 5.512$	$F_{2,15} = 0.473$	$F_{2,15} = 4.271$
	$p = .448$	$\mathbf{p = .020}$	$p = .632$	$\mathbf{p = .034}$
Aminopeptidase	$F_{2,12} = 0.455$	$F_{2,12} = 2.872$	$F_{2,15} = 3.744$	$F_{2,15} = 1.512$
	$p = .645$	$\mathbf{p = .096}$	$\mathbf{p = .048}$	$p = .252$
Lipase	$F_{2,12} = 0.292$	$F_{2,12} = 0.146$	$F_{2,15} = 0.690$	$F_{2,15} = 0.272$
	$p = .752$	$p = .865$	$p = .517$	$p = .765$
Alkaline phosphatase	$F_{2,12} = 0.038$	$F_{2,12} = 4.551$	$F_{2,15} = 0.523$	$F_{2,15} = 2.854$
	$p = .963$	$\mathbf{p = .034}$	$p = .603$	$\mathbf{p = .089}$
β-glucosidase	$F_{2,12} = 2.895$	$F_{2,12} = 3.658$	$F_{2,15} = 1.759$	$F_{2,15} = 2.490$
	$p = .094$	$\mathbf{p = .058}$	$p = .206$	$p = .116$
N-acetyl-β-D-glucosaminidase	$F_{2,12} = 0.197$	$F_{2,12} = 1.709$	$F_{2,15} = 1.168$	$F_{2,15} = 0.550$
	$p = .824$	$p = .222$	$p = .338$	$p = .588$

Note: Actual enzyme activity data are presented in Figure 3 for amylase, trypsin and lipase; Figure 4 for alkaline phosphatase, β-glucosidase and *N*-acetyl-β-D-glucosaminidase; and 1 (see online version) for maltase and aminopeptidase. bolded p-values indicate statistical significance at the p = 0.10 level.

minnows; German, Nagle, et al., 2010), as diatoms appear to make up the bulk of their gut contents, with specific diatom genera (e.g. *Achnanthidium sp.*) producing the healthiest fish, and being disproportionately concentrated in *C. santaanae* faeces in comparison with diatom relative abundance on substrates in the Santa Clara River drainage (BonTerra Psomas, 2015; Dudek 2018; C. Demetropoulos pers. obs.). Interestingly, *Achnanthidium* are fast-growing, pioneer diatom species found on benthic substrates and thrive in turbulent, well-oxygenated, high-quality, clean flowing water (Round et al., 1990). It is a type of diatom expected to occur and regrow rapidly in riffle habitat where Santa Ana sucker have been observed to aggressively graze (BonTerra Psomas, 2015; Dudek 2018). Moreover, BonTerra Psomas (2015) found relatively high-energy diatoms, such as *Amphora*, were abundant in Santa Ana sucker habitat and diet; *Amphora* species are known for being rich in lipid (Round et al., 1990; C. Demetropoulos, pers. obs.), and thus, it should provide energy for both rapidly growing young and mature Santa Ana sucker preparing for spawning. Like their brethren in the Santa Clara River drainage, *C. santaanae* in the Santa Ana River appear to select specific diatoms from microhabitats such as riffle complexes, with faecal relative abundance of genera such as *Achnanthidium*, *Amphora* and *Fragilaria* outpacing the relative abundance of these genera in the environment (Figure S2; BonTerra Psomas, 2015; Dudek 2018).

Some of the predictions of a plug-flow model are that pancreatic enzyme activities (e.g. amylase, trypsin, lipase) would generally decrease in activity moving distally along the intestine (Day et al., 2011; German et al., 2015; Horn & Messer 1992). This makes sense as the substrates for these enzymes (polymers such as starches, proteins and fats) are higher in concentration as they are ingested into the proximal intestine, and polymer concentrations decline as they are digested moving along the gut (German, 2009a; German et al., 2015). We saw support for this model for the protease trypsin, but pancreatic enzymes amylase and lipase had more variable activities moving along the gut.

Variable pancreatic digestive enzyme activities along the gut have several potential explanations: (a) the enzymes are not being degraded along the intestine, so they remain active, even towards the distal intestine; (b) there is more axial mixing of digesta along the gut, allowing activities to be more homogenous; (c) pancreatic tissue is diffuse along the gut, and thus, pancreatic enzymes are not only secreted into the proximal intestine, but along the length of the intestine; or (d) the pancreatic enzymes are decreasing in activity, but microbially derived enzymes in the hindgut eliminate typical patterns observed for these enzymes along the intestine. None of these are mutually exclusive and could all be contributing to the patterns seen in the *C. santaanae* gut. Although we did not measure the concentrations of short-chain fatty acids (SCFA), which are the by-products of microbial fermentation

that typically accumulate in the distal intestines of fish that are reliant on microbial fermentation to digest plant material (Clements & Choat 1995; Clements et al., 2017; German et al., 2015; Stevens & Hume 1998), based on gut morphology and diet, we assume that little fermentation occurs in the *C. santaanae* intestine (e.g. German, 2009a; German & Bittong 2009; German, Nagle, et al., 2010; Hao et al., 2017). In fact, no grazing or browsing freshwater fishes have been observed to have high levels of fermentation occurring in their guts (German, 2009a; German & Bittong 2009; German, Nagle, et al., 2010; Hao et al., 2017; Smith et al. 1996).

The lack of fermentative digestion occurring in the hindgut of freshwater herbivorous fishes, like *C. santaanae*, matters because microbial populations that contribute to the digestive process under anaerobic conditions do so with enzymes, like β-glucosidase, which digests components of cellulose degradation, and N-acetyl-β-D-glucosaminidase, which digests components of chitin degradation (Leigh et al., 2018). Neither of these typically microbially derived enzymes increased in activity in the distal intestines of the *C. santaanae* (Figure 4). Furthermore, other than amylase and lipase, most other enzymes showed decreasing patterns of activity moving distally along the intestine (Figure 4, Figure S1). Hence, it is unlikely that a hindgut microbial population is solely contributing to amylase and lipase activities, and not other enzymes. It is the decreasing pattern for most enzymatic activity that supports a plug-flow reactor gut for *C. santaanae* (German et al., 2015; Horn & Messer 1992).

When comparing the digestive enzyme activities of wild-caught vs. laboratory-fed *C. santaanae*, we observed more enzyme activity in the gut contents for fish consuming periphyton (Figures 3 and 4; Figure S1). Although it is unclear what this means, the data suggest there is greater nutritional value for living food (natural periphyton) compared to an artificial diet fed to *C. santaanae* in the laboratory. The greater enzyme activities in the contents of the wild-caught fish can indicate greater enzymatic production by the fish themselves, enzymes that are inherent in the ingested food (since periphyton is living), or represent enzymes produced by intestinal microbes not inherent in our laboratory-fed fish (German & Bittong 2009; German et al., 2015; Skea et al., 2005). The wild-caught fish from the Santa Ana River and those reared in the laboratory (from the Santa Clara River) also come from different genetic backgrounds (Richmond et al., 2018), which may also contribute to this discrepancy. Regardless, living food with greater digestive capacity (i.e. elevated digestive enzyme activities) likely has greater nutritional value for this species. It should be noted that enzyme activities in intestinal contents are not always higher than the tissues in fishes. For example, grazing loricariid catfishes tended towards equal activities in intestinal contents and tissues (German & Bittong 2009), whereas prickleback fishes of varying diets generally showed greater activity in
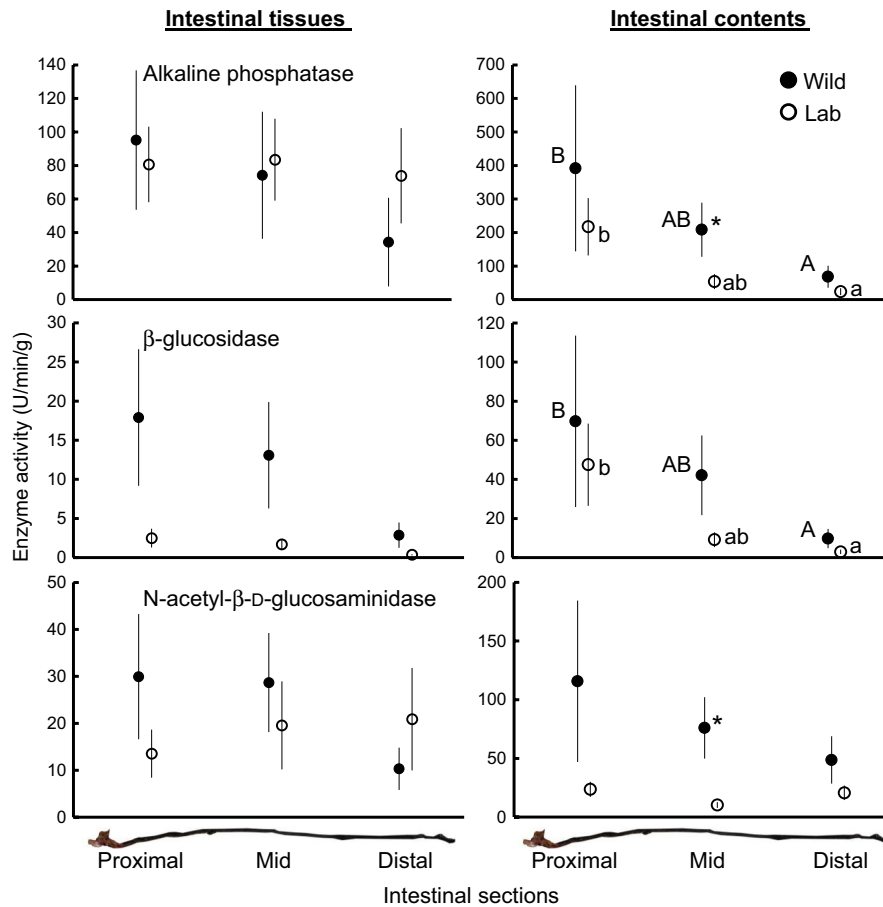
FIGURE 4 Digestive enzyme activities in intestinal tissue (left column) and intestinal contents (right column) of *Catostomus santaanae* collected directly from the wild or reared on an algal diet in the laboratory for 6 weeks. Values are mean ± standard error. Note the different y-axis scales for intestinal tissue vs. intestinal content. Activities were compared among intestinal sections with ANOVA for wild and laboratory-fed individuals separately. For the wild fish, intestinal sections with a different capital letter for a given enzyme and intestinal fraction (tissue or content) are statistically significantly different, whereas statistical difference is indicated with lower-case letters for the laboratory-fed fish. Significant differences among wild and laboratory-fed fish for a given intestinal section are indicated with an asterisk. See Table 2 and Table S2 for more statistical detail. The *C. santaanae* gut is presented just below the x-axes to show the long, thin-walled nature of their digestive tract [Colour figure can be viewed at wileyonlinelibrary.com]

intestinal tissues by an order of magnitude over those in gut contents (German et al., 2015).

The digestibility of protein and carbohydrate from the artificial algal diet is moderate and consistent with what is known as a rate-maximization strategy to digestion: high intake, rapid transit of material through the gut and, hence, relatively moderate digestibility (German, 2011). However, the animal compensates for this moderate digestibility by simply eating more (German et al., 2015). This is a common digestive strategy of herbivores in the animal kingdom with a rate-maximization strategy (Karasov & Martínez del Rio 2007), like pandas (Dierenfeld, Hintz, Robertson, Van Soest, & Oftedal, 1982), and many fishes (German, 2009b; Stevens & Hume 1998). Interestingly, the elevated lipid digestibility exhibited by *C. santaanae* fits with them specializing on diatoms among the available periphyton matrix on which they graze (Figure S2). In fact, the elevated lipolytic activity along the *C. santaanae* intestine is consistent with the grazing *Pterygoplichthys disjunctivus*,

which consumes a large load of diatoms, and may also scavenge lipids in its distal intestine (German, Neuberger, et al., 2010). Lipid digestibility and utilization is a definite area on which to focus on *C. santaanae* biology moving forward. Furthermore, the digestibility of laminarin, the storage polysaccharide of diatoms (German, Nagle, et al., 2010; Painter, 1983), should be investigated.

In conclusion, our results show that *C. santaanae* likely functions similar to other grazing fishes in the families Catostomidae and Cyprinidae. The species appears to have high intake of periphyton that it preferentially selects from its habitat, suggesting this parameter should be considered in spatial models of habitat conservation, particularly when translocating *C. santaanae* (Richmond et al., 2018). Given the position *C. santaanae* plays in legal battles over water use in southern California (e.g. U.S. Fish and Wildlife Service 2014), this type of approach has real conservation utility (Birnie-Gauvin et al., 2017; Cooke et al., 2014; Leigh et al., 2018; Tracy et al., 2006).

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ORCID

Donovan P. German https://orcid.org/0000-0002-7916-6569

REFERENCES

Anderson, I., & Floyd, K. (2016). Lawsuit launched over California cities' killing of threatened Santa Ana Suckers: Colton, San Bernardino halted water releases imperiling rare fish. Center for Biological Diversity. Retrieved from: https://www.biologicaldiversity.org/news/press_releases/2016/santa-ana-sucker-08-22-2016.html

Birnie-Gauvin, K., Peiman, K. S., Raubenheimer, D., & Cooke, S. J. (2017). Nutritional physiology and ecology of wildlife in a changing world. *Conservation Physiology*, 1–18.

Bjorndal, K. (1985). Use of ash as an indigestible dietary marker. *Bulletin of Marine Science*, 36, 224–230.

Bligh, E., & Dyer, W. (1959). A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911–917.

Bowen, S. H., Lutz, E. V., & Ahlgren, M. O. (1995). Dietary protein and energy as determinants of food quality: Trophic strategies compared. *Ecology*, 76, 899–907.

Busey, J. (2016). U.S. Supreme Court allows habitat protections to stand for rare southern California fish: decision ends decade-long fight to protect Santa Ana Sucker habitat. Center for Biological Diversity. Retrieved from: https://www.biologicaldiversity.org/news/press_releases/2016/santa-ana-sucker-01-11-2016.html

Clements, K. D., & Choat, J. H. (1995). Fermentation in tropical marine herbivorous fishes. *Physiological and Biochemical Zoology*, 68, 355–378.

Clements, K. D., German, D. P., Piché, J., Tribollet, A., & Choat, J. H. (2017). Integrating ecological roles and trophic diversification on coral reefs: Multiple lines of evidence identify parrotfishes as microphages. *Biological Journal of the Linnean Society*, 120, 729–751.

Cooke, S. J., Blumstein, D. T., Buchholz, R. et al (2014). Physiology, behavior, and conservation. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, 87:1–14.

Crossman, D. J., Choat, J. H., & Clements, K. D. (2005). Nutritional ecology of nominally herbivorous fishes on coral reefs. -. *Marine Ecology Progress Series*, 296, 129–142.

Day, R. D., German, D. P., Manjakasy, J. M., Farr, I., Hansen, J., & Tibbetts, I. R. (2011). Enzymatic digestion in stomachless fishes: How a simple gut accommodates both herbivory and carnivory. *Journal of Comparative Physiology B*, 181, 603–613.

BonTerra Psomas(2015). *Santa Ana Sucker Habitat Suitability Survey Results and Sixth Annual Santa Ana Sucker and Benthic Macroinvertebrate Survey Results: Big Tujunga Creek, Los Angeles County, California*. Prepared for Los Angeles County Department of Public Works, Water Resources Division-Dams; Alhambra, California, July 23, 2015.

Dierenfeld, E. S., Hintz, H. F., Robertson, J. B., Van Soest, P. J., & Oftedal, O. T. (1982). Utilization of bamboo by the giant panda. *Journal of Nutrition*, 112, 636–641.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350–356.

Dudek 2018. Santa Ana Sucker Translocation Plan. Prepared for the San Bernardino Valley Water District. Retrieved from: https://static1.squarespace.com/static/53920f34e4b05366f07d971c/t/5bb666380d92971daf00501

Erlanger, B. F., Kokowsky, N., & Cohen, W. (1961). The preparation and properties of two new chromogenic substrates of trypsin. *Archives of Biochemistry and Biophysics*, 95, 271–278.

Fowler, J. F., & Taber, C. A. (1985). Food habits and feeding periodicity in two sympatric stonerollers (Cyprinidae). *The American Midland Naturalist*, 135, 217–224.

Fu, S. J., Zeng, L. Q., Li, X. M., Pang, X., Cao, Z. D., Peng, J. L., & Wang, Y. X. (2009). The behavioural, digestive and metabolic characteristics of fishes with different foraging strategies. *Journal of Experimental Biology*, 212, 2296–2302.

Galetto, M. J., & Bellwood, D. R. (1994). Digestion of algae by *Stegastes nigicans* amd *Amphiprion akindynos* (Pisces: Pomacentridae) with an evaluation of methods used in digestibility studies. *Journal of Fish Biology*, 44, 415–428.

German, D. P. (2009a). Do herbivorous minnows have "plug-flow reactor" guts? Evidence from digestive enzyme activities, gastrointestinal fermentation, and luminal nutrient concentrations. *Journal of Comparative Physiology B*, 179, 759–771.

German, D. P. (2009b). Inside the guts of wood-eating catfishes: Can they digest wood? *Journal of Comparative Physiology B*, 179, 1011–1023.

German, D. P. (2011). Digestive efficiency. In A. P. Farrel (Ed.), *Encyclopedia of fish physiology: From genome to environment*, Vol. 3 (pp. 1596–1607). San Diego, CA: Academic Press.

German, D. P., & Bittong, R. A. (2009). Digestive enzyme activities and gastrointestinal fermentation in wood-eating catfishes. *Journal of Comparative Physiology B*, 179, 1025–1042.

German, D. P., Horn, M. H., & Gawlicka, A. (2004). Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): Ontogenetic, dietary, and phylogenetic effects. *Physiological and Biochemical Zoology*, 77, 789–804.

German, D. P., Nagle, B. C., Villeda, J. M., Ruiz, A. M., Thomson, A. W., Balderas, A. C., & Evans, D. H. (2010). Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): Effects on gut size and digestive physiology. *Physiological and Biochemical Zoology*, 83, 1–18.

German, D. P., Neuberger, D. T., Callahan, M. N., Lizardo, N. R., & Evans, D. H. (2010). Feast to famine: The effects of dietary quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae). *Comparative Biochemistry and Physiology Part A*, 155, 281–293.

German, D. P., Sung, A., Jhaveri, P. K., & Agnihotri, A. (2015). More than one way to be an herbivore: Convergent evolution of herbivory

using different digestive strategies in prickleback fishes (family Stichaeidae). *Zoology*, *118*, 161–170.

German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., & Allison, S. D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry*, *43*, 1387–1397.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*, 2248–2251.

Greenfield, D. W., Ross, S. T., & Deckert, G. D. (1970). Some aspects of the life history of the Santa Ana sucker, Catostomus (Pantosteus) santaanae (Snyder). *California Fish and Game*, *56*, 166–179.

Hao, Y. T., Wu, S. G., Jakovlić, I., Zou, H., Li, W. X., & Wang, G. T. (2017). Impacts of diet on hindgut microbiota and short-chain fatty acids in grass carp (*Ctenopharyngodon idellus*). *Aquaculture Research*, *48*, 5595–5605.

Hoagland, K., Roemer, S., & Rosowski, J. (1982). Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). *American Journal of Botany*, *69*, 188–213.

Horn, M. H., & Messer, K. S. (1992). Fish guts as chemical reactors: A model for the alimentary canals of marine herbivorous fishes. *Marine Biology*, *113*, 527–535.

Jumars, P. A. (2000). Animal guts as ideal chemical reactors: Maximizing absorption rates. *The American Naturalist*, *155*, 527–543.

Jung, E. H., Brix, K. V., & Brauner, C. J. (2019). The effect of temperature acclimation on thermal tolerance, hypoxia tolerance and aerobic scope in two subspecies of sheepshead minnow; *Cyprinodon variegatus* variegatus and *Cyprinodon variegatus* hubbsi. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *232*, 28–33.

Karasov, W. H., & Martínez del Rio, C. (2007). *Physiological ecology: How animals process energy, nutrients, and toxins*. Princeton, NJ: Princeton University Press.

Klock, J. H., Wieland, A., Seifert, R., & Michaelis, W. (2007). Extracellular polymeric substances (EPS) from cyanobacterial mats: Characterisation and isolation method optimisation. *Marine Biology*, *152*, 1077–1085.

Leigh, S. C., Papastamatiou, Y. P., & German, D. P. (2018). Seagrass digestion by a notorious "carnivore". *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20181583.

Ling, H., Fu, S. J., & Zeng, L. Q. (2019). Predator stress decreases standard metabolic rate and growth in juvenile crucian carp under changing food availability. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *231*, 149–157.

Lujan, N. K., German, D. P., & Winemiller, K. O. (2011). Do wood grazing fishes partition their niche? Morphological and isotopic evidence for trophic segregation in Neotropical Loricariidae. *Functional Ecology*, *25*, 1327–1338.

Marsh, J. B., & Weinstein, D. B. (1966). Simple charring method for the determination of lipids. *Journal of Lipid Research*, *7*, 574–576.

Page, L. M., & Burr, B. M. (2011). *Peterson field guide to freshwater fishes of North America north of Mexico*, 2nd ed. Boston, MA: Houghton Mifflin Harcourt.

Painter, T. J. (1983). Algal polysaccharides. In G. O. Aspinall (Ed.), *The polysaccharides*, Volume *2* (pp. 196–285). New York, NY: Academic Press Inc.

Penry, D. L., & Jumars, P. A. (1987). Modeling animal guts as chemical reactors. *The American Naturalist*, *129*, 69–96.

Reardon, E. E., & Chapman, L. J. (2010). Energetics of hypoxia in a mouth-brooding cichlid: Evidence for interdemic and developmental effects. *Physiological and Biochemical Zoology*, *83*, 414–423.

Richmond, J. Q., Backlin, A. R., Galst-Cavalcante, C., O'Brien, J. W., & Fisher, R. N. (2018). Loss of dendritic connectivity in southern California's urban riverscape facilitates decline of an endemic freshwater fish. *Molecular Ecology*, *27*, 369–386.

Round, F. E., Crawford, R. M., & Mann, D. G. (1990). *The Diatoms. Biology and morphology of the Genera* (pp. 747). Cambridge, UK: Cambridge University Press.

Saiki, M. K., Martin, B. A., Knowles, G. W., & Tennant, P. W. (2007). Life history and ecological characteristics of the Santa Ana sucker, *Catostomus santaanae*. *California Fish and Game*, *93*, 87–101.

Skea, G., Mountfort, D., & Clements, K. D. (2005). Gut carbohydrases from the New Zealand marine herbivorous fishes *Kyphosus sydneyanus* (Kyphosidae), *Aplodactylus arctidens* (Aplodactylidae), and *Odax pullus* (Labridae). *Comparative Biochemistry and Physiology Part B*, *140*, 259–269.

Skea, G., Mountfort, D., & Clements, K. D. (2007). Contrasting digestive strategies in four New Zealand herbivorous fishes as reflected by carbohydrase activity profiles. *Comparative Biochemistry and Physiology Part B*, *146*, 63–70.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., … Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry*, *150*, 76–85.

Smith, T., Wahl, D., & Mackie, R. (1996). Volatile fatty acids and anaerobic fermentation in temperate piscivorous and omnivorous freshwater fish. *Journal of Fish Biology*, *48*, 829–841.

Stevens, C. E., & Hume, I. D. (1998). Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiological Reviews*, *78*, 393–427.

Thompson, A. R., Baskin, J. N., Swift, C. C., Haglund, T. R., & Nagel, R. J. (2010). Influence of habitat dynamics on the distribution and abundance of the federally threatened Santa Ana Sucker, *Catostomus santaanae*, in the Santa Ana River. *Environmental Biology of Fishes*, *87*, 321–332.

Tracy, C. R., Nussear, K. E., Esque, T. C. et al (2006). The importance of physiological ecology in conservation biology. *Integrative and Comparative Biology*, *46*, 1191–1205.

. United States Fish and Wildlife Service (2005). Endangered and threatened wildlife and plants final rule to designate critical habitat for the Santa Ana Sucker (*Catostomus santaanae*); Final rule. *Federal Register*, *70*, 426. Retrieved from: https://www.govinfo.com/content/pkg/FR-2005-01-04/html/04-28286.htm

United States Fish and Wildlife Service (2000). Endangered and threatened wildlife and plants; threatened status for the Santa Ana sucker. *Federal Register*, *71*, 19686–19698.

United States Fish and Wildlife Service (2014). Draft recovery plan for the Santa Ana Sucker. Sacramento, CA: U.S. Fish and Wildlife Service, Pacific Southwest Region. 61 pp. Retrieved from: https://www.fws.gov/carlsbad/TEspecies/Recovery/documents/Draft%20Recovery%20Plan%20for%20the%20Santa%20Ana%20Sucker.pdf

van Dam, A., Beveridge, M., Azim, M., & Verdegem, M. (2002). The potential of fish production based on periphyton. *Reviews in Fish Biology and Fisheries*, *12*, 1–31.

Wilson, J. M., & Castro, L. F. C. (2011). Morphological diversity of the gastrointestinal tract in fishes. In M. Grosell, A. P. Farrell, & C. J. Brauner (Eds.), *The multifunctional gut of fish*, Vol. *30* (pp. 1–55). San Diego, CA: Elsevier.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.