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River Otter (*Lontra canadensis*) colonization of the Merced River in Yosemite Valley sustained by predation on invasive Signal Crayfish (*Pacifastacus leniusculus*)

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Stefan Samu

Committee in charge:

Professor Jonathan Shurin, Chair
Professor Elsa Cleland
Professor Carolyn Kurle

2022

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The Thesis of Stefan Samu is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego
2022

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This master's thesis is coauthored with Grasso, Rob; Li, Stephanie; Steiner, Cynthia and Shurin, Jonathan. The thesis author was the primary author of the thesis.

ABSTRACT OF THE THESIS

River Otter (*Lontra canadensis*) colonization of the Merced River in Yosemite Valley sustained by predation on invasive Signal Crayfish (*Pacifastacus leniusculus*)

by

Stefan Samu

Master of Science in Biology

University of California San Diego, 2022

Professor Jonathan Shurin, Chair

In 2014 North American River Otters expanded up the North Fork of the Merced River into previously and historically unoccupied Yosemite Valley. Their range expansion posed the questions: 1) What are river otters preying upon in Yosemite Valley? 2) Could invasive Signal Crayfish provide a prey subsidy that promotes the upward elevational expansion of river otter populations into Yosemite Valley? To answer these questions, otter scats were collected and prey DNA present in the scats was amplified using a metabarcoding approach. The diet data revealed that invasive Signal Crayfish were present in 100% of scats collected and native Sacramento Sucker was present in only 16% of the scats. These results indicate that invasive Signal Crayfish

are the most important diet species in the river otter diet, and the invasion of the Yosemite Valley by Signal Crayfish may have provided essential nutritional resources that facilitated upward elevational range expansion by river otters from Central Valley drainages. This study adds to the body of literature supporting the use of next-generation sequencing technologies and environmental DNA for addressing the ecology of species and ecosystem dynamics in the natural world.

INTRODUCTION

Over the past two centuries the world has become increasingly connected due to human-mediated dispersal of organisms across biogeographic barriers. For instances, plant communities across continents and fish communities across North America have both become homogenized with significant increases in compositional similarity and shared species among disparate regions (Daru et al., 2021; Rahel, 2000). Many other organisms which were previously bound to their natural ranges by low dispersal capabilities have exploited human means of dispersal to colonize new regions, resulting in a loss of beta diversity or regional distinctiveness (Bailey, 2015).

Invasive species impact native communities through interactions including competition, predation, and a network of indirect effects that can give rise to positive and negative effects on the population growth of native species. The effect of invasion on an ecosystem is determined by the interaction between the invader, the resident community and its physical environment. The impact of invaders on native diversity ranges from negligible to catastrophic. Some invasions have been shown to cause wholesale local extinction of suites of native species; for example the Brown Tree Snake invasion of the Pacific Island of Guam caused the local extinction of nearly all native bird species (Wiles et al., 2003). Invaders can also operate as ecosystem engineers altering available habitats and resource availability. The invasion of the Common Water Hyacinth in the Southeast United States, which depletes oxygen levels throughout the water column and smothers surface waters preventing light penetration, is one example (Villamagna & Murphy, 2010). The effects of these two invasive species dramatically transformed invaded ecosystems, but not all invaders negatively impact native species. In some cases, the presence of an invasive species may provide native species with food, habitat, or other symbiotic associations. For example, in central Pennsylvania native bird population size and native plant

seed dispersal were both positively influenced by the presence of invasive Honeysuckle (Gleditsch & Carlo, 2011). The impacts of invasive species on native diversity and ecosystem services remain a topic of contentious debate.

Invasive crayfish in freshwater ecosystems provide some of the most dramatic examples of transformation of ecosystems by exotic species. Signal Crayfish are one of the most common benthic invaders and can drastically impact river and lake ecosystems they settle in (Nyström & Strand, 1996; Sanders et al., 2021). Crayfish function as ecosystem engineers because of their impact on functions such as benthic primary production and decomposition of detritus in lakes and rivers. Their movement and burrowing behavior increase riverbank erosion and the export of sediment downstream (Sanders et al., 2021). Their feeding habits cause them to greatly reduce biomass and diversity of macrophytes, while also increasing decomposition rates and reducing available detritus material (Nyström & Strand, 1996; Usio, 2000; Wilson et al., 2011). This increase in decomposition rates speeds up the recycling of bioavailable nutrients (McLatchey & Reddy, 1998). The changes to macrophyte and detritus biomass leads to shifts in richness and biomass of native macroinvertebrates and a reduction in cover for juvenile fish (Ercoli et al. 2021; Carvalho et al. 2016; Moorhouse et al. 2014; Wilson et al. 2011; Rozas and Odum 1988; Chick and McIvor 1997). The damage caused by Signal Crayfish invasions in Europe and Japan has sparked management actions for their removal and population management. Unfortunately, Signal Crayfish management and eradication is extremely difficult and, in most cases, impossible (Holdich et al., 2018; Moorhouse et al., 2014; Peay, 2001).

Signal Crayfish invaded the Merced River in Yosemite Valley in 1975, over time their numbers have grown steadily. The impact of Signal Crayfish on the Merced River ecosystem has not been evaluated. However, in 2014 North American River Otters (*Lontra canadensis*) were

observed in Yosemite Valley for the first time. No prior record of the North American River Otter in Yosemite Valley exists although otter populations exist throughout river drainages of the California Central Valley such as the San Joaquin at lower elevations. The North American River Otter is a voracious generalist predator that occupies the top trophic level in many river ecosystems in Western North America. As an endotherm that spends most of its time in the water, otters have a high metabolism and require a large daily calorie intake (Dekar et al., 2010). The range expansion by otters in the North Fork of the Merced River in Yosemite Valley likely resulted in the addition of a trophic level with potential predatory effects on both fish and invertebrates like crayfish. The aquatic community of the Merced River includes both native (Rainbow Trout, *Oncorhynchus mykiss* and Sacramento Sucker, *Catostomus occidentalis*) and invasive (Brown Trout, *Salmo trutta*) fishes in addition to invasive crayfish (Signal Crayfish, *Pacifastacus leniusculus*; Rusty Crayfish, *Orconectes rusticus*; Virile Crayfish, *Faxonius virilis*). However, the dietary preference and predation rate of otters on fish, crayfish and other prey in the Merced River are unknown.

Our project investigated predation by river otters on different prey items in the Merced River using a metabarcoding approach on scat samples collected during the summer of 2019. We conducted the study in the North Fork of the Merced River which flows through Yosemite Valley. Prior to the arrival of crayfish and otters, the river ecosystem in Yosemite Valley contained several native and invasive fish species. We hypothesize that the arrival of crayfish may have facilitated colonization by otters, if crayfish make up a substantial fraction of the otter diet. This is supported by Roemer et al. who showed that the addition of a non-native prey species (feral pigs on the Channel Islands) can function as a trophic subsidy that facilitates range expansion of a native predator (Golden Eagles), providing them with a food source that is not

usually available within the habitat or region (Roemer et al., 2002). The availability of crayfish as a prey species may provide a trophic subsidy for the otter allowing it to thrive in an ecosystem that would otherwise lack a suitable prey base. Invasive Signal Crayfish may be easier to catch than fast-swimming fish like Sacramento Sucker or game fish such as trout and therefore may operate as a crucial trophic link facilitating the range expansion of the North American River Otter. The two main questions we sought out to answer with this study were: 1) How frequently do different prey items occur in the diets of river otters? 2) Could invasive Signal Crayfish provide a prey subsidy that promotes the upward elevational expansion of river otter populations into reaches of the Merced River that were previously unoccupied? To answer these questions, we determined the composition of the river otter diet, allowing us to better understand their relationship with the Signal Crayfish invasion, their reliance on native and invasive prey species, and help determine the expected trophic impacts of otters in the river ecosystem of Yosemite Valley.

METHODS

Sample Collection:

Otters use locations where they repeatedly defecate, referred to as latrines, and the repeated use of these latrines permits collection of scats once a latrine has been located. During our collection period between July 5, 2019 and August 17, 2019, 72 otter scats were collected from two regularly used latrine sites along the bank of the Merced River in Yosemite Valley. One latrine was located at Sentinel Bridge and the other at Yellow Pines Campground. The Sentinel Bridge latrine was sampled weekly, and most of the scats found at this location were collected upon discovery. The latrine site at Yellow Pines Campground was not located until the last day of sample collection due to high water levels in the Merced River, therefore all the scats from that location were collected on August 17, 2019. Gloves were used to place each individual scat into a specimen container, and scats were frozen at -20°C within 12 hours.

Sample Extraction:

Inside of a fume hood, a piece of otter scat from each sample was homogenized using a mortar and pestle along with enough liquid nitrogen to submerge the scat. This procedure ensures that material representing all prey species present within the otter scat would be grinded into a fine powder for DNA extraction. About 20 mg of the powdered scat was placed in two separate microcentrifuge tubes allowing for extraction replicates.

The Qiagen Qiamp DNA Mini Kit (Qiagen, Germantown) was used to extract DNA from the scat replicates according to manufacturer's instructions. DNA extracts were evaluated using a NanoDrop instrument (ThermoFisher, San Diego), and DNA quality and concentration determined

Positive Controls:

The Qiagen DNeasy Blood and Tissue Kit was used to extract DNA for three reference samples representing expected otter prey species: Sacramento Sucker, Eastern Brook Trout, and Louisiana Crayfish. Sacramento Sucker DNA was collected from fin clips provided by the Genetic Variation Lab at UC Davis while Eastern Brook Trout DNA was extracted from muscle tissue prepared for stable isotope analysis. Louisiana Crayfish DNA was extracted from the tissue of a crayfish found in Peñasquitos Creek in San Diego, CA. These DNA extracts were used as positive controls while conducting polymerase chain reactions (PCRs), and for testing primers' efficacy in amplifying fish species.

DNA Mix Creation:

The extracted positive control DNAs were all diluted to 16 ng/ul and then mixed in different proportions to help us identify any primer amplification bias present in our study. The DNA mixes are shown in Table 1.

PCR Amplification:

The PCR protocol was developed using positive controls and otter samples to confirm that otter digestion did not affect our ability to amplify DNA present within the sample. Extracted DNA replicate pairs from 40 scat samples, positive controls, and 3 replicates of each DNA mix were diluted to a concentration of 16ng/ul. Each PCR reaction consisted of 3ul of the forward and reverse primer, 7.5 ul of Kapa Kit, and 2.5 ul of extracted DNA according to the modified PCR protocol suggested by the Illumina sequencing library preparation guide (Part # 15044223 Rev. B). Vertebrate and invertebrate prey species within the otter's diet were amplified using two primer sets targeting different mitochondrial barcode genes: the 12s ribosomal RNA (12s rRNA) and Cytochrome c oxidase subunit I (COIX). Partial sequences from

the COIX gene were amplified using the ZBJ primers (Zeale et al., 2011), and the12s rRNA gene was targeted using the EP primers (Riaz et al., 2011)(Table 2). Illumina adapter overhang nucleotide sequences were added to the gene-specific sequences according to the Illumina sequencing library preparation guide. Forward overhang corresponds to sequence: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG - [locus- specific sequence], and reverse overhang to 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG - [locus-specific sequence].

The two separate primer sets had different annealing temperatures which resulted is different thermal cycling conditions. The ZBJ thermal cycling conditions are as follow: an initial denaturation at 95.0 C° for 3 minutes followed by 30 cycles of a short denaturation 95.0 C° for 30 seconds, annealing at 46.0 C° for 30 seconds, and elongation at 72.0 C° for 30 seconds. This was followed by a final elongation period of 5 minutes at 72.0 C°. For the EP primers, we used a touchdown PCR protocol allowing us to test a range of different annealing temperatures during the PCR process. The EP thermal cycling conditions are: an initial denaturation at 95.0 C° for 5 minutes, followed by 25 cycles of a short denaturation at 94.0 C° for 30 seconds, an annealing temperature that decreased by 0.5 C° every cycle starting at 60.0 C° and ending at 47.5 C°, and elongation at 72.0 C° for 45 seconds. This was followed by another 15 cycles of 94.0 C for 30 seconds, 60.0 C° for 45 seconds, and 72.0 C° for 45 seconds. Lastly, a 10-minute elongation period at 72.0 C°. Following each of the PCR protocols, amplification product was checked on 2% agarose gel.

PCR Cleaning and Indexing:

PCR products were cleaned using an AMPure XP (Beckman Coulter, Indiana) following a standard double-sized bead cleanup protocol to remove any DNA fragments under 100 bp and

over 300 bp. Once cleaning was performed, Nextera indexes and Illumina sequencing adaptors (Nextera XT Index Kit v2; Illumina, San Diego) were added to our amplicons using a short PCR protocol: 95 C° for 3 minutes, followed by 8 cycles of 95 C° for 30 seconds, 55 C° for 30 seconds, and 72 C° for 30 seconds. Lastly, the DNA was held at 72 C° for 5 minutes for a final elongation. An additional cleaning step was conducted to remove unincorporated indexes and sequencing adaptors using a standard one-sided AMPure XP bead cleanup protocol (1.8X bead volume).

Denaturing and Library Pooling:

Genomic libraries were quantified in a Qubit instrument (ThermoFisher, Carlsbad) using a dsDNA high sensitivity assay kit (ThermoFisher, Carlsbad). To estimate molarity of genomic libraries, fragment size was calculated by running samples in a Bioanalyzer (Agilent, Santa Clara, CA) using a High Sensitivity DNA kit. Genomic libraries were diluted to 4 nM and then pooled in equimolar volumes. The pooled library was denatured and subsequently diluted to 20pM and to a final concentration of 10 pM. 25% PhiX control was used to spike-in the low diversity pooled library. The denatured and diluted pooled library was loaded into a MiSeq v2 cartridge for running 300 cycles of 150 paired-end reads.

Sequence Identification using DADA2:

Fastq files were retrieved from the Illumina BaseSpace environment after being demultiplexed according to unique indexes. Reference databases were either created or retrieved for each barcode gene. The reference database for the 12s rRNA gene was retrieved from CALeDNA (REF or website), and the COIX reference database was created using the in-silico PCR feature available on FastPCR (https://ucedna.com/reference-databases-for-metabarcoding). All available CO1X sequences were downloaded from NCBI and input into the general

sequences tab in the in-silico PCR feature in FastPCR (Kalendar, Lee, and Schulman 2014; National Center for Biotechnology Information). The ZBJ primer pair was input into the pre-designed primers tab in the in-silico PCR feature in FastPCR and the annealing temperature for the in-silico PCR was changed to 46 C°, to replicate laboratory PCR conditions. The results of the in-silico PCR were extracted as a text file and formatted into FASTA format for use in the DADA2 pipeline. The DADA2 pipeline (Callahan et al. 2016; https://benjjneb.github.io/dada2/tutorial.html) was used to filter, trim, and clean sequences before assigning them to taxa using the provided reference databases (Fig. 1). The DADA2 tutorial was followed exactly except for changes to the filter step and the minimum bootstrap value used while assigning taxonomies to sequences. For the EP primer set sequences under 70bp in length were filtered out and for the ZBJ primer set sequences under 120 bp were filtered out. DADA2's learnErrors function was used to determine the error rate in our sequence data and then the dada function was used to group unique sequences into true variants of each sequence, according to the learned error rates. After determining the true variants in each sample, the forward and reverse reads were merged. We then removed the chimeras present and the assignTaxanomy function was used to assign sequences to taxonomies present in the reference databases we provided. Following assignment by DADA2, sequences that were attributed to the same species were combined and each sequence's assigned taxonomy was cross referenced on NCBI via Blast (Altschul et al., 1990; Camacho et al., 2009). When multiple prey species shared the same sequence (percentage of identity higher than 98%), knowledge of regional fish species was used to distinguish accurate prey identification.

Data Quality:

Two steps were taken to ensure quality after receiving the identified sequences from the DADA2 pipeline. First, a prey species was considered a diet item if 1% of the reads returned for a scat sample were attributed to that prey species (McInnes et al., 2017). Second, only prey taxa that were detected in both replicate samples from the same scat were included in our diet reconstructions. Our sequence data detected Tui Chub (Siphaletes bicolor) in 35% of our scat samples (24 samples), but in the majority, 14, of those cases Tui Chub sequences were present in only one of two DNA extraction replicates, indicating that its presence is likely a result of cross contamination during the extraction step. Tui Chub are studied in the lab where extractions were performed and were stored in the same lab freezer as the otter scats. Additionally, Tui Chub are native to the Eastern Sierras and not present on the western side of the Sierras, therefore their presence in the otter diet within Yosemite Valley is extremely unlikely, and their reads were removed from final analysis.

Table 1. DNA Mixes. Percent of DNA contributed by each prey species to the DNA mixes created to assess primer amplification bias.

DNA Mixes	*Percentage Trout DNA*	*Percentage Sucker DNA*	*Percentage Crayfish DNA*
1:1:1	33	33	33
1:1:2	25	25	50
1:2:1	25	50	25
2:1:1	50	25	25

Table 2. Primer Information.

Primer Name	*Target Organisms*	*Target Amplicon*	*Primer Sequence (5' to 3')*	*Annealing Temp*	*Sequence Length (BP)*
ZBJ-Art-F1c	Arthropoda	CO1X	AACWTTATATTTTATTTTTGG	46 C	160 BP
ZBJ-Art-R2c	Arthropoda	CO1X	AGGATTTGGWAATTGATTAG	46 C	~
EP-F	Vertebrates	MT-R1N1 (12s)	TAGAACAGGCTCCTCTAG	47.5 C – 60 C	134 BP
EP-R	Vertebrates	MT-R1N1 (12s)	TTAGATACCCCACTATGC	47.5 C – 60 C	~

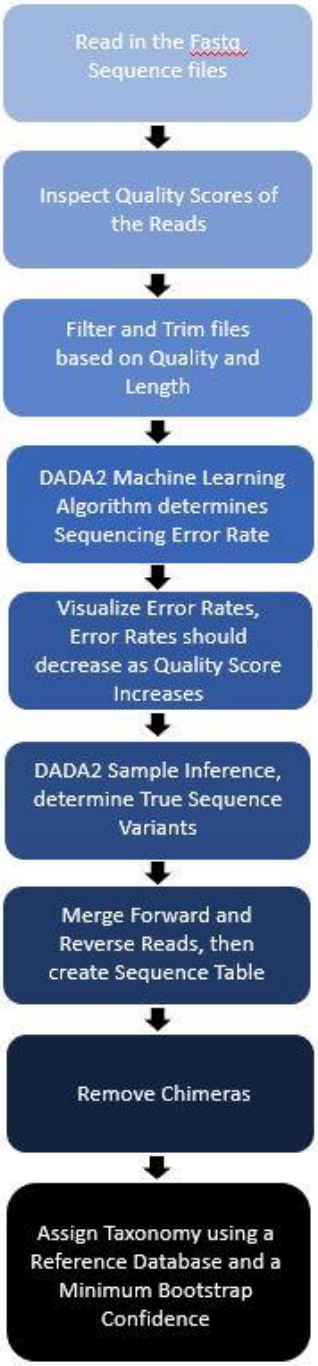

Figure 1. DADA2 Pipeline. Pipeline used to process Illumina sequence data.

RESULTS

Primer Bias:

The DNA mixes created to assess the primer bias included DNA extracted from Brook Trout and Sacramento Suckers. The results showed considerable primer bias in the 12s rRNA vertebrate primers, favoring the amplification of the Sacramento Sucker over Brook Trout. The bias was consistent between mix replicates. The bias also increased as the amount of total DNA within the DNA mix decreased (Mixes 1 and 2). Lastly, the bias decreased as the proportion of Sacramento Sucker increased relative to Brook Trout (Mixes 1, 3, 4) (Fig. 2). The primers response to changes in the total amount of DNA and changes in proportion of DNA within DNA mixes indicates that the number of reads detected is not a reliable indicator of the prevalence (amount) of prey consumed in the diet of otters. As a result, the diet data was interpretated using a presence and absence approach.

Frequency of Prey Occurrences in Otter Diets:

The otter scat data revealed that 5.9% of otter scats contained Brown Trout, 14.7% contained Sacramento Sucker, and 100% of otter scats contained Signal Crayfish (Fig. 3). Indicating that Signal Crayfish is the most frequently consumed prey species within the North American River Otter Diet in Yosemite Valley, and the only diet present in all otter scats. Although the river otter is a generalist, their diet breadth seems to be small within Yosemite Valley, feeding exclusively on three species.

We assessed our sampling efforts effectiveness by a species accumulation curve to ensure that we captured the entirety of the North American River Otter diet (Fig. 4). The results show that the number of samples collected was adequate to detect the species present in the scat samples.

Table 3. Vertebrate DNA content in DNA. Used to determine 12s rRNA primer bias.

Species	*Mix 1*	*Mix 2*	*Mix 3*	*Mix 4*
Brook Trout	6.66 ng	5.00 ng	10.00 ng	5.00 ng
Sacramento Sucker	6.66 ng	5.00 ng	5.00 ng	10.00 ng

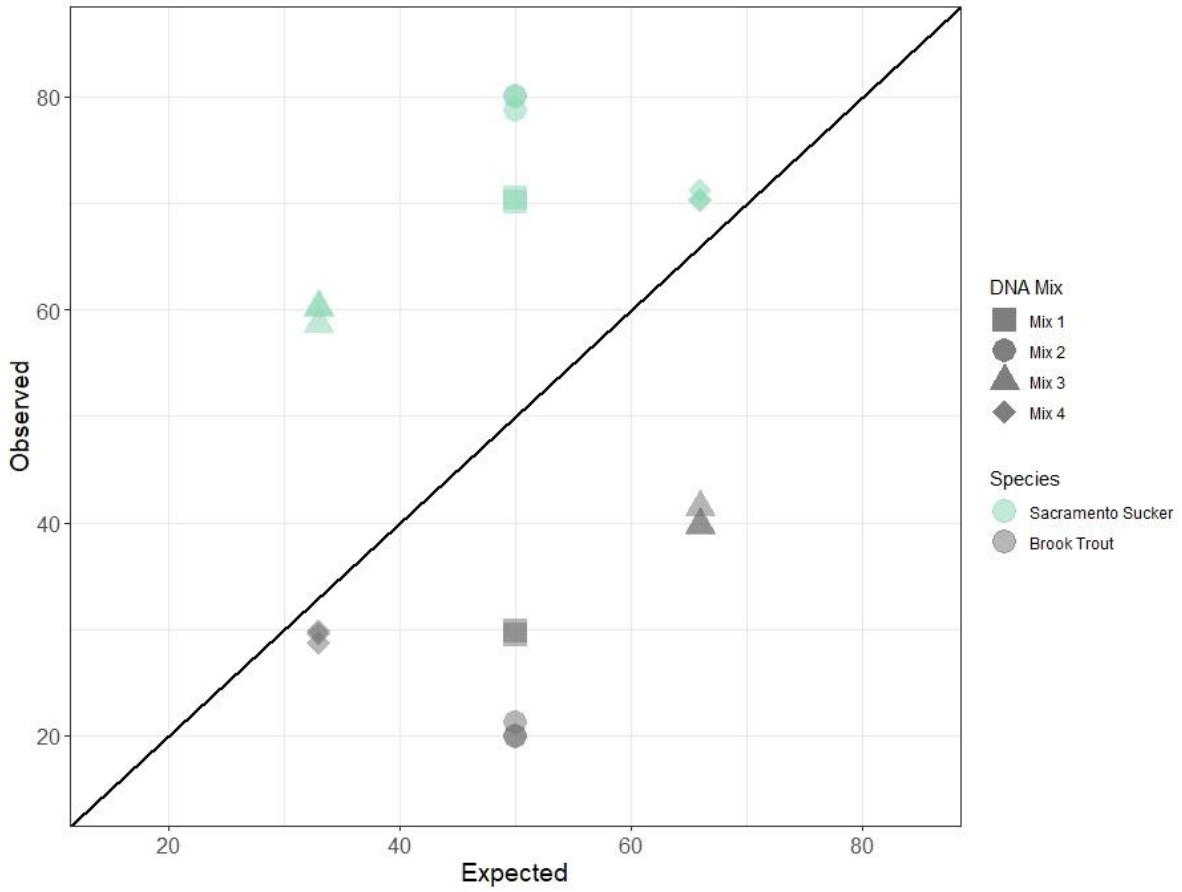

Figure 2. Percentage of Reads Expected and Observed for each DNA Mix Replicate. Proportion of reads expected is equal to the starting DNA proportions within each mix. The observed proportion of reads refers to the proportion of reads post-amplification using 12s rRNA primer. X=Y line indicates expected results if there was no primer bias.

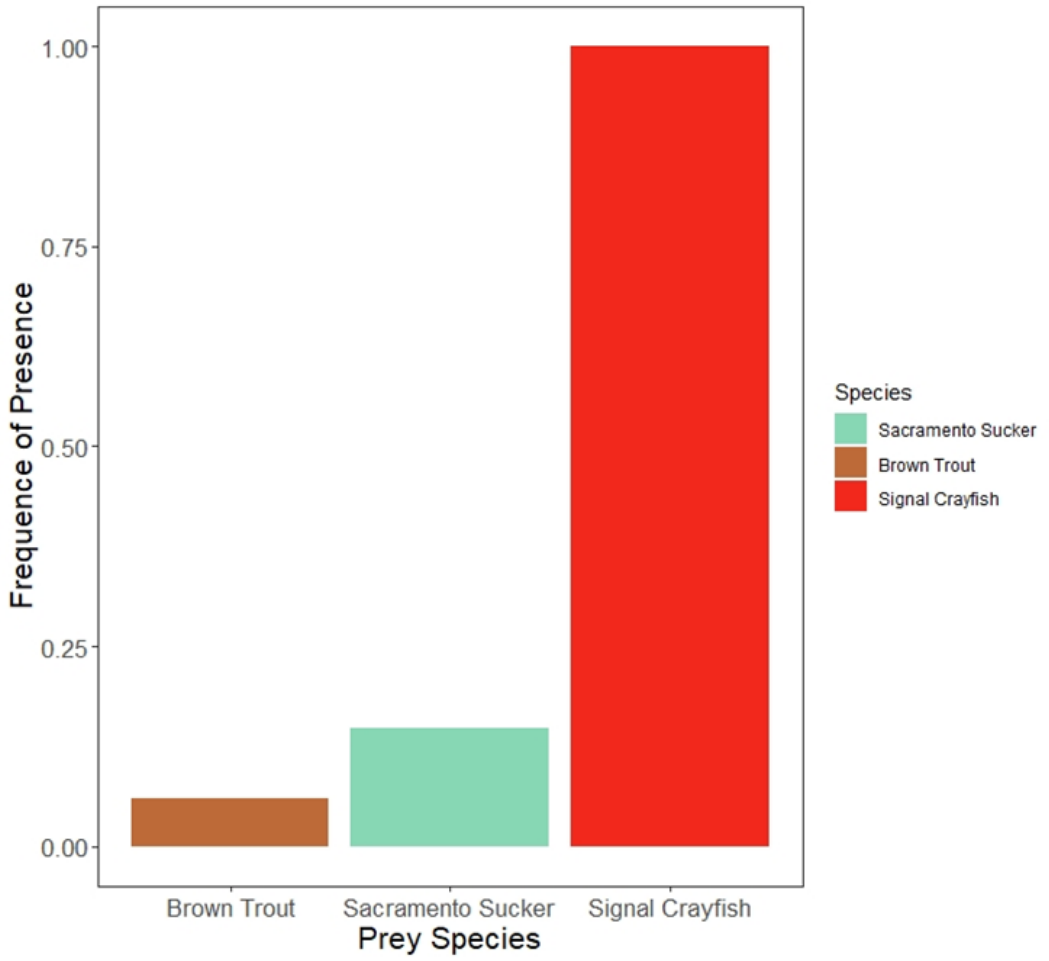

Figure 3. Frequency of Presence in Otter Scat for Each Prey Species. Percentage of the otter scats that included each of the prey species present within the North American River Otter diet.

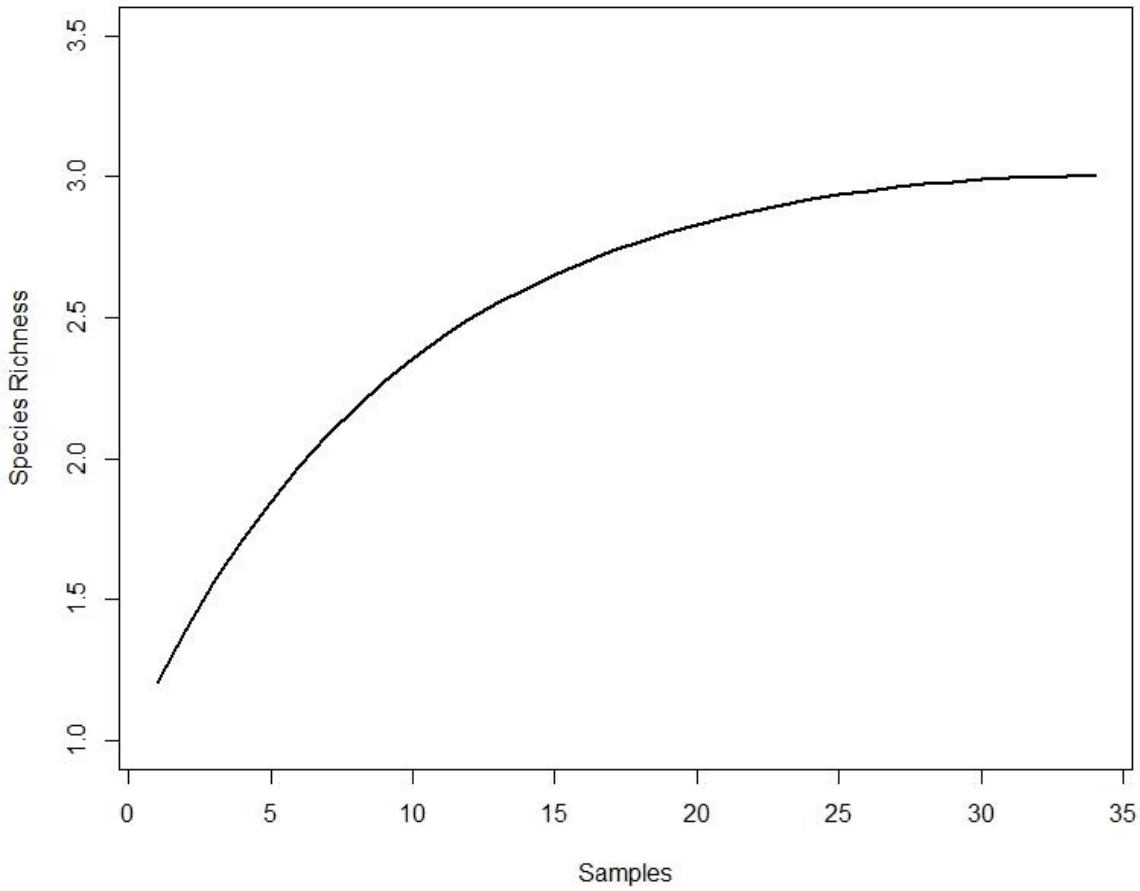

Figure 4. Species Accumulation Curve. Shows the mean expected species accumulation curve using sample-based rarefaction

DISCUSSION

Range Expansion and Diet:

Range expansion or changes in a species distribution can result from a number of processes including climate change, habitat destruction, and the introduction of invasive species which may decrease or increase a species' range depending on the interaction. River otters tolerate a wide range of temperatures and the snowmelt that supplies the rivers they inhabit helps mitigate the warming they experience from climate change; therefore, it is unlikely that climate change is causing their range expansion into Yosemite Valley (DeNeve Weeks, 2020). Additionally, their continued presence in the Lower Merced and Sacramento-San Joaquin River delta indicate that habitat destruction is an unlikely cause for their range expansion.

Yosemite National Park is home to a number of invasive and introduced species that are common prey items in river otter diets across North America (Day et al., 2015; Reid et al., 2011). Prior to the 2014 range expansion of river otters, Signal Crayfish invaded Yosemite Valley in 1975 and various trout were stocked in the lakes and rivers of Yosemite National Park starting in 1877 and ending in 1991, due to their negative impact on native amphibian populations (Knapp, 1996). The addition of multiple prey species, including the recent invasion of slow-moving Signal Crayfish led us to believe that the presence of invasive species may have facilitated the range expansion of the river otter. My diet reconstruction based on metabarcoding indicates that Signal Crayfish are the most frequently consumed prey of river otters, occurring in 100% of scats, while the only native fish observed, Sacramento Sucker, occurred in only 15% of scats. These results indicate that invasion of the Yosemite Valley by Signal Crayfish may have provided essential nutritional resources that facilitated upward elevational range expansion by river otters from Central Valley drainages.

North American River Otters are generalist predators that feed on primarily fish and crustaceans, they select prey in proportion to abundance and inverse proportion to swimming ability (Day et al., 2015; Reid et al., 2011; Wayne E. Melquist, P.J. Polechela, 2003). Our diet data indicates that river otters in Yosemite Valley are feeding in accordance with previous research, favoring the slow-moving Sacramento Sucker which is present in 15% of scats over the fast-moving Brown Trout present in only 6% of scats. The river otter diet in Yosemite Valley has low taxonomic diet breadth when compared to other river otter diet studies in North America, this could be attributed to lower prey richness within the region or high prey abundance for the three prey species compared to other possible prey species in Yosemite Valley (Day et al., 2015; Reid et al., 2011).

Although the diet analysis indicates Signal Crayfish are the primary diet item for the North American River Otter in Yosemite Valley, the sampling for this study only took place in the summer months between June and August. Due to the North American River Otter's generalist feeding strategy, changes in prey availability or activity could cause the North American River Otter to shift its preferred prey (Dekar et al., 2010; Wayne E. Melquist, P.J. Polechela, 2003). Dekar showed that river otters in Arkansas preferred to feed on crayfish during summer and fall months but fed on fish and crayfish equally during winter and spring (Dekar et al., 2010). Therefore, it is important that future research efforts collect scats during each season to get a more complete understanding of the seasonal variation present in the river otter diet in Yosemite Valley.

Future Impact:

The potential impact of North American River Otter range expansion into Yosemite Valley is difficult to predict. However, we can apply food web dynamics and our understanding

of their current diet to determine expected changes to the river ecosystem. Their two main prey, fish and crayfish, have both been shown to have dramatic effects on river food webs through cascading trophic interactions and impacts on habitat forming aquatic plants and decomposition of terrestrial detritus (Nyström & Strand, 1996; Power, 1990; Sanders et al., 2021; Wootton & Power, 1993). They could continue to eat primarily Signal Crayfish, helping to manage and limit the negative effects of the crayfish invasion. Alternatively, their diet could shift to favor native fishes as crayfish abundance decreases, this shift could eventually cause changes to food web dynamics (Roemer et al., 2002; Wootton & Power, 1993). Whether River Otters continue to feed on mostly crayfish or shift their diet to include more fishes, their colonization of Yosemite Valley may have extensive indirect effects on taxa not included in their diet.

If the North American River Otter continues to feed on mostly Signal Crayfish, the direct consumption of Signal Crayfish may trigger cascading effects felt throughout the river and terrestrial food web. Crayfish operate as ecosystem engineers effecting the ecosystems they invade through biotic interactions but also by altering the physical habitat. Signal Crayfish spend nearly all their time in the benthic zone, their movement and burrowing behavior increases riverbank erosion and the export of sediment downstream (Sanders et al., 2021). Additionally, crayfish feed on macrophytes decreasing their richness and biomass, which reduces important habitat for juvenile fish (Nyström & Strand, 1996; Rozas & Odum, 1988; Wilson et al., 2004). Signal Crayfish are omnivorous and feed on many different trophic levels, their diet items include detritus, periphyton, macrophytes, macroinvertebrates, and in some cases juvenile fish and crayfish (Ercoli et al., 2021; Guan & Wiles, 1998). Studies show that the riverine ecosystem can be impacted directly through crayfish consumption of macroinvertebrates and indirectly by crayfish consumption of the macroinvertebrate's main food sources, periphyton and detritus (Fig.

5) (Ercoli et al. 2021; Carvalho et al. 2016). Their impact on macroinvertebrate biomass and richness may affect emergent insect biomass and richness, effecting the terrestrial ecosystem that surrounds the river they inhabit (Nakano & Murakami, 2001). The arrival of river otters and the top-down control they are exerting on the Signal Crayfish population in Yosemite Valley will likely help to mitigate the effects of crayfish invasion. Signal Crayfish population control by the North American River Otter may promote aquatic habitat restoration, a valuable ecosystem service because crayfish invasions are nearly impossible to manage with human intervention and the efforts can be very costly (Francesca Gherardi, 1999; Moorhouse et al., 2014; Peay, 2001).

Currently crayfish make up most of the river otter diet in Yosemite Valley, but the proportion of any prey species in a generalist predator's diet is likely to change according to abundance (Wayne E. Melquist, P.J. Polechela, 2003). If crayfish numbers decrease the river otter will increase its top-down control on the other prey species in the ecosystem. Food web dynamics can help us determine how this shift in diet preference and the prolonged presence of river otters in Yosemite Valley will change the river ecosystem. The addition of a top predator to a river ecosystem causes a trophic cascade effecting the biomass of lower trophic levels in the food web (Wootton & Power, 1993). For example, Wootton used experimental stream setups to show that if a river ecosystem with 3 trophic levels adds a top predator and now has 4 trophic levels, we can expect increased predation on trophic level 3 to cause a decrease in biomass at level 3, an increase in biomass at level 2, and a decrease in biomass at level 1 (Power, 1990; Wootton & Power, 1993). If we apply this to the river ecosystem in Yosemite Valley, as crayfish abundance decreases and river otter predation on fish increases, we expect a decrease in trout and sucker biomass, followed by an increase in macroinvertebrate biomass. Future research should

be done to confirm or deny our expected food web response to prolonged river otter presence in the Yosemite Valley.

In conclusion, river otters in Yosemite Valley feed primarily on invasive Signal Crayfish and only occasionally on native and invasive fish species. The 1975 invasion of Signal Crayfish likely facilitated the 2014 range expansion of North American River Otters into Yosemite Valley. Although primer amplification bias caused experimental limitation for use of metabarcoding to quantify the percentage of a diet attributed to each prey species, metabarcoding is still useful for reconstructing the diet of species in their natural habitat using non-invasive genetic samples. This study adds to the body of literature supporting the use of next-generation sequencing technologies and environmental DNA for addressing the ecology of species and ecosystem dynamics in the natural world.

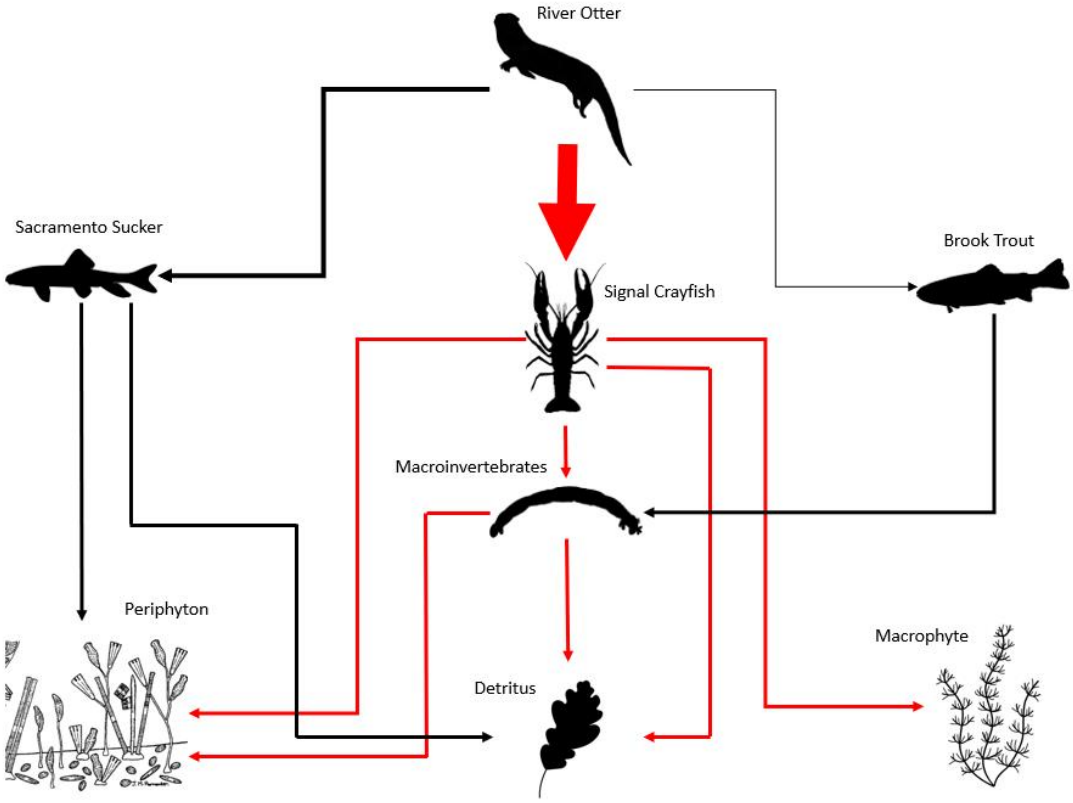

Figure 5. Food Web. Arrows radiating from the river otter scale with increased predation levels according to presence and absence data. Red arrows highlight the interactions expected to change the most due to the range expansion of the river otter according to its current diet.

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