Using Stable Isotopes to Determine Food Web Effects of Non-Native Sunfish on Native Rainbow Trout in Wildcat Creek

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Introduction

Tilden Regional Park is one of the three oldest parks in the East Bay Regional Park District (EBRPD). Wildcat Creek runs through Tilden Regional Park and historically supported a native steelhead trout (*Oncorhynchus mykiss)* population. The creation of two lakes, Lake Anza and Jewel Lake, prevented upstream fish migration (Leidy *et al*. 2005); but, a resident population of rainbow trout remained in Wildcat Creek until the mid to late 1970's when EBRPD biologists found rainbow trout had nearly vanished from most sections of the stream for unknown reasons. In 1983, EBRPD biologists transplanted 615 rainbow trout from nearby Redwood Creek into Wildcat Creek. Annual monitoring and research suggests that rainbow trout are currently reproducing successfully in Wildcat Creek (Leidy *et al*. 2005) and the population remains fairly robust. However, recent surveys have also documented increasing numbers of non-native green sunfish (*Lepomis cyanellus*) throughout the Wildcat Creek watershed, which may pose as a potential threat to the native rainbow trout population. It is unknown when green sunfish were first introduced in Lake Anza, but recent surveys suggest their population has been rapidly increasing the past several years. With no barrier to stop them from spilling downstream, green sunfish are becoming increasingly established in Wildcat Creek and have been documented throughout the system from Lake Anza to its delta in San Pablo Bay.

The introduction of invasive fish can lead to population instability (Li and Moyle 1981), shifts in habitat use (Brabrand and Faafeng 1993) and negative changes in growth and reproduction (Fraser and Gilliam 1992) among native fishes (Marchetti 1999). A study of the effects of non-native bluegill (*Lepmois macrochirus*), a member of the sunfish family, on

Sacramento perch (*Archoplites interruptus*) showed that the growth of the Sacramento perch decreased due to competitive interactions in the presence of bluegill. Additionally, it was observed that native perch altered their habitat use when non-native bluegill were present (Marchetti 1999). The introduction of non-native sunfish can also alter whole-lake ecosystems. Vander Zanden *et al*. (1999a) found that the presence of several non-native sunfish species caused a decrease in prey fish abundance and caused a trophic shift in native lake trout (*Salvelinus namaycush*). The study concluded that native lake trout were forced to shift their food source towards zooplankton because their main food supply had been depleted by non-native sunfish. In both stream and lake ecosystems, the habitation of invasive fish species can negatively impact the native ecosystem from the individual species level to entire ecosystem function. To date, the present study is the first to investigate the food web interactions between green sunfish and rainbow trout in a California stream.

Materials and Methods

Study Area

From its headwaters at Grizzly Peak in Tilden Regional Park to its outlet at San Pablo bay, Wildcat Creek is approximately ten miles long and its watershed encompasses approximately 4,500 acres. For this study, two separate reaches of Wildcat Creek were chosen: Upper Wildcat Creek (above Lake Anza) and Lower Wildcat Creek (below Lake Anza); both study reaches were approximately 500 meters long. There is a physical barrier which prevents green sunfish migration upstream of Lake Anza making the Upper Wildcat Creek reach our control system, where only native rainbow trout and three-spined stickleback (*Gasterosteus aculeatus*) are found. There is no barrier preventing any fish in Lake Anza from traveling downstream; thus, Lower Wildcat Creek was chosen as a treatment because of the addition of green sunfish to the system.

Food-Web Sampling

Aquatic organisms from multiple trophic levels were sampled in each reach to characterize each food web. A kick net was used to collect aquatic macroinvertebrates which were then identified to family or genus level and preserved in 75% ethanol. An electrofishing backpack was used to collect rainbow trout (RBT; n=21), green sunfish (GSF; n=16), three-spined stickleback (TSSB; n=10) and signal crayfish (*Pacifastacus leniusculus;* CF; n=5). The stomach contents of RBT and GSF were removed and identified in order to compare prey preference between species. All invertebrate and fish tissue samples were dried at 60°C for ~24 hours. In some cases the entire fish was dried or multiple invertebrates were dried together because of their small size in order to get enough tissue sample for analysis.

The dried samples were then ground into a fine powder using a mortar and pestle. Samples were weighed out to 1.0 ± 0.2 mg, placed into tin capsules (5 x 9 mm; Costech Analytical Technologies, Inc., Valencia, CA, USA), and sent to the University of California, Davis, Stable Isotope Facility for isotopic analysis.

Stable Isotope Analysis

To compare food web structure between reaches we used stable isotope ratios of Carbon (^{13}C:^{12}C, expressed as $\delta^{13}C$) and Nitrogen (^{15}N:^{14}N, expressed as $\delta^{15}N$). $\delta^{13}C$ provides information about the production base (aquatic or terrestrial) of the food web and $\delta^{15}N$ indicates the trophic position of an organism (Cabana and Rasmussen 1996). Used together,

$\delta^{13}C$ and $\delta^{15}N$ reflect temporally integrated data on an individual's trophic niche, which is difficult to compile with stomach data alone (Sepulveda *et al.* 2009). Stable isotope analysis is a useful tool to examine the impacts of invasive species to ecological communities because stable isotopes can describe changes in food web structure, competitive and predatory interactions in the form of trophic niche overlap, and changes in native niche space (Vander Zanden *et al.* 1999a). Continuous flow isotope ratio mass spectrometry (IRMS) (20-20 mass spectrometer, PDZEuropa Scientific, Sandbach, United Kingdom) was used to analyze the samples for carbon. At 1000°C, sample combustion to CO_2 occurred in an inline elemental analyzer (PDZEuropa Scientific, ANCA-GSL). Before going into the IRMS, the gases were separated on a Carbosieve G column (Supelco, Bellefonte, PA, USA) and after were compared to a standard gas. Isotopic signatures (δX) were calculated using the formula δX (‰) = $(R_{sample} / R_{standard} – 1) \times 1000$; δX being the amount (in parts per thousand) of ^{13}C or ^{15}N heavy isotope, $R_{standard}$ being the ratio of heavy to light isotope of the standard, and R_{sample} being the ratio of heavy isotope (13C and 15N) to light isotopes (12C and 14N) of tissue sample (Fry 2006).

Food Web Structure

Isotopic bi-plots were constructed to compare food web structure between the upper and lower reaches of Wildcat Creek. To compare the two bi-plots, the consumer signatures were corrected to account for differences in biogeochemical processes and basal conditions that influence the consumer signature for carbon and nitrogen by converting consumer $\delta^{15}N$ to a trophic position (Vander Zanden and Rasmussen 1999b). This was done using the equation: $TP_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/3.4) + 2$. $\delta^{15}N_{consumer}$ is the consumer's $\delta^{15}N$ and $\delta^{15}N_{baseline}$ was $\delta^{15}N$ for either Gastropoda or Psephenidae for the lower and upper regions, respectively.

3.4 is the amount of trophic level enrichment in $\delta^{15}N$ and 2 is the trophic level position of the primary consumers (Vander Zanden and Rasmussen 1999b). Gastropoda and Psephenidae (algae scrapers) were used in place of periphyton for the baseline because there was very little periphyton observed at both reaches. All invertebrate data were combined based on functional feeding group. Stomach contents were removed from 8 rainbow trout from the upper reach and 3 rainbow trout and 5 green sunfish from the lower reach. Invertebrate prey items were identified to Order.

Results

A total of twenty taxa were sampled the from two study reaches over a two month period (July-August): 17 invertebrate taxa, two native fish species (three-spined stickleback and rainbow trout), and one non-native fish species (green sunfish). In the absence of green sunfish in the upper reach, rainbow trout occupied the highest trophic position (Figure 1). In the lower reach where green sunfish were present, rainbow trout were replaced as the top predator and appear to occupy a lower trophic position than they would in the absence of the non-native fish (Figure 2). The enrichment of δ13C suggests the base of the Wildcat Creek food web is reliant on terrestrial carbon sources; this is consistent with other small, heavily shaded streams. A slight shift in the carbon signatures of rainbow trout was observed in the presence of green sunfish which suggests that trout are shifting their diet in the lower reach and consuming a broader range of food items. Green sunfish and rainbow trout δ13C signatures also overlap, suggesting they consume similar prey items; these results are consistent with the similar diet items found in the stomachs of both species (Figure 3).

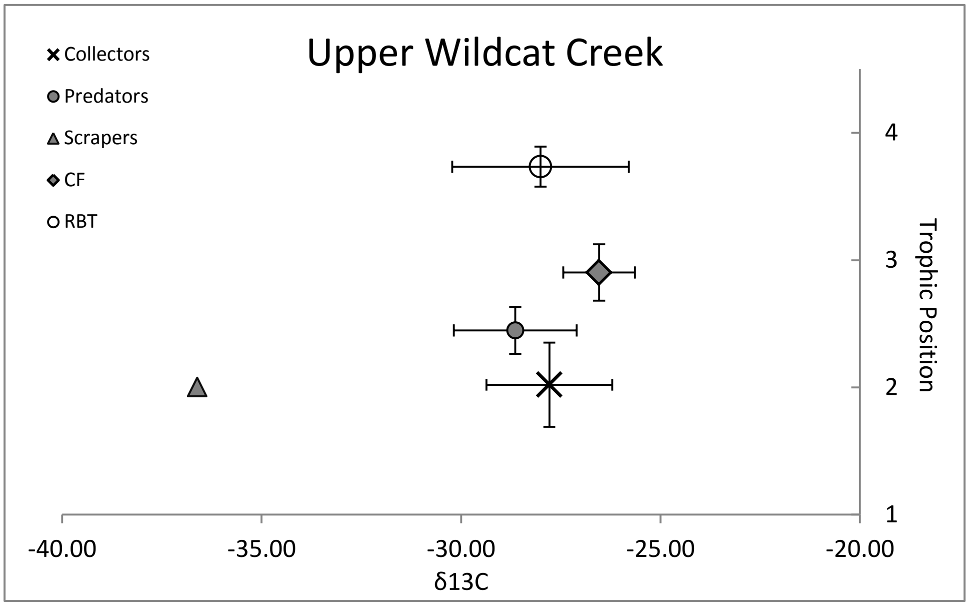

Figure 1: Isotopic bi-plot for Upper Wildcat Creek including δC13 and trophic position (± standard deviation) values for collectors (black cross), predators (gray circle), scrapers (gray triangle), crayfish (gray diamond), and rainbow trout (white circle).

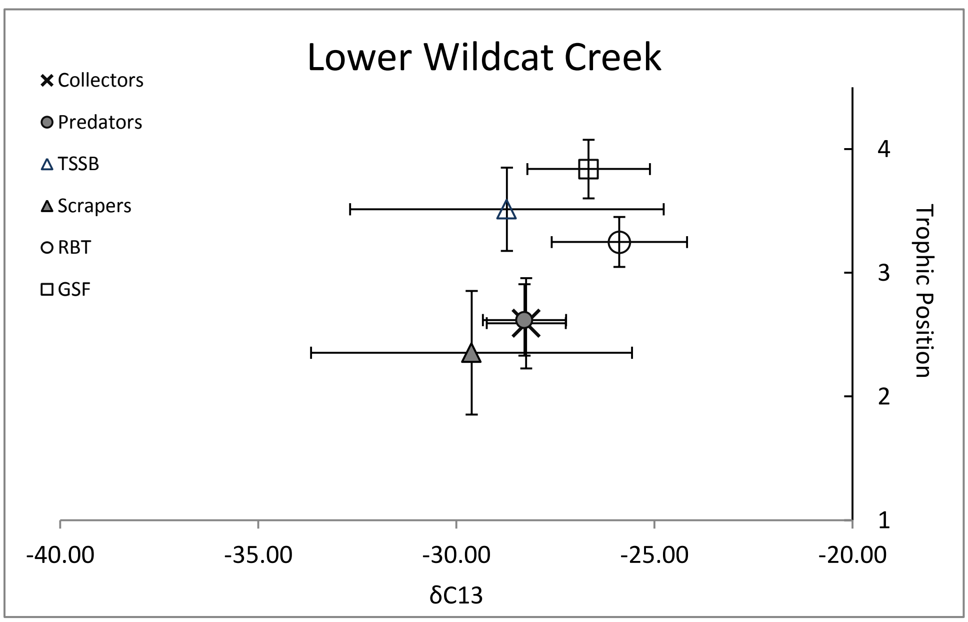

Figure 2: Isotopic bi-plot for Lower Wildcat Creek including δC13 and trophic position (± standard deviation) values for collectors (black cross), predators (gray circle), scrapers (gray triangle), stickleback (white triangle), rainbow trout (white circle), and green sunfish (white square).

a) b)

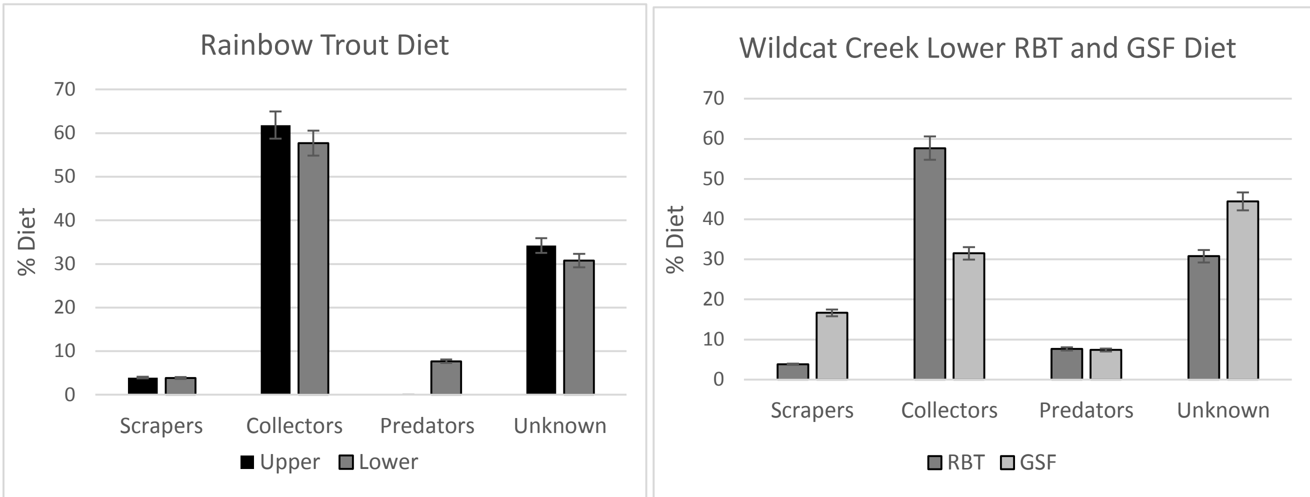

Figure 3: a) Percent diet of rainbow trout in the upper (black) and lower (dark grey) regions of Wildcat Creek. b) % diet of rainbow trout (dark grey) and green sunfish (light grey) in lower Wildcat Creek

Species	**Sample Size**		**δC13**		**δN15**	
Fish	Upper	Lower	Upper	Lower	Upper	Lower
Oncorhynchus mykiss	13	8	-28.01±2.21	-25.89±1.71	10.46±0.53	8.83±0.68
Lepomis cyanellus		16		-26.66±1.54		10.84±0.81
Gasterosteus aculeatus		9		-28.73±4.0		9.73±1.14
Inverts						
Pacifastacus leniusculus	6		-26.54±0.90		7.63±0.76	
Amphipoda			-27.01		5.14	
Anisoptera			-28.37		5.69	
Gastropoda				-26.75		4.59
Gerridae			-27.02	-27.31	5.19	5.30
Glossosomatidae				-32.48		6.99
Heptagenidae			-30.01		5.39	
Hydropsychidae				-28.76		7.40
Lepitostomatidae			-29.25		3.32	
Leptophlebidae			-28.71	-28.86	4.54	7.23
Notonectidae			-31.2	-29.76	6.71	7.48
Oligochaeta			-25.73	-27.09	5.81	5.17
Polycentripodidae			-28.23	-27.93	6.51	6.70
Psephenidae			-36.61		4.56	
Simuliidae			-26.39		5.36	
Tipulidae			-27.41		2.87	
Zygoptera			-28.36	-28.12	6.31	7.27

Figure 4: Average C and N values for all taxa (± standard deviation) and known sample sizes.

Discussion

The food web in Wildcat Creek appears to be relatively simple with only a few trophic levels. There also appears to be a strong reliance on allochthonous (terrestrial) carbon sources which is characteristic of small, cool, shaded streams. Results from this study suggest that in the presence of non-native green sunfish, rainbow trout shifted to a lower trophic position and utilized a broader range of prey items. Thus, non-native green sunfish could be impacting the native food web via competitive and possibly even predatory interactions with native trout. Shifts in food web structure, as seen in Wildcat Creek, are indicative of ecosystems with high levels of invasion and biotic disturbance (Vander Zanden *et al.* 1999a). The level of these biotic disturbances was not investigated in this study. However, previous studies have shown that interspecific competition between native and non-native species could cause a decline in native fish populations (Vander Zanden *et al*. 1999a). Coupled with the observed overlap in prey preference from our stomach content data, the results from this study suggest that competitive interactions are occurring between green sunfish and rainbow trout. Further studies are needed to estimate the degree at which this dietary overlap may be occurring. Although we found no evidence of predation by green sunfish, our results indicate they are feeding at a higher trophic level than rainbow trout, indicating that they are the top aquatic predator in the lower study reach.

This study was the first effort to investigate the food web-based interactions among green sunfish and rainbow trout. Our results suggest that green sunfish are causing a trophic shift in the native food web; the implications of these findings should not be overlooked. As such, there is a need to gain a better understanding of how the green sunfish are affecting not only native trout, but the entire function of Wildcat Creek's ecosystem. Because non-native sunfish are typically more aggressive than native fish (Marchetti, 1999), it would be beneficial to observe the behavior of green sunfish and rainbow trout in a lab or field setting. Such a study could help explain the trophic shifts observed in this study. A more detailed comparative study of fish diets would also lend to the questions remaining whether green sunfish are directly competing with rainbow trout and also whether larger green sunfish are indeed preying on smaller rainbow trout. Over the last several years, EBRPD Fisheries staff have been actively removing green sunfish from Wildcat Creek during annual surveys. Unfortunately, the sunfish population in Lake Anza continues to increase exponentially, creating a constant source of aquatic invaders downstream. Without eliminating the source, Wildcat Creek will continue to be the sink for invasive green sunfish. Thus, complete eradication of non-native fish should be considered for the long term preservation of native species in Wildcat Creek.

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