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Freshwater ecosystems are some of the most susceptible to invasive species. It is one of the reasons they face the highest levels of species endangerment. Rusty crayfish Faxonius rusticus are one such invasive species, they fragment food webs and negatively affect native species. However, it seems as though native species are beginning to rely on them as a source of food. Yellow perch Perca flavescens, an important forage and game species, is one such species that is highly affected by these invasions. At smaller sizes yellow perch primarily feed on invertebrates whose populations are degraded by rusty crayfish. Therefore, the objective of this study is to assess the effects of the invasive rusty crayfish on the diets of yellow perch. To accomplish this goal, yellow perch were collected throughout the summer of 2022 in an invaded and non-invaded lake. The stomachs of these fish were dissected, the contents were keyed to lowest possible taxonomic group, and counted by individual. Following that, the contents were dried and weighed. While yellow perch diets naturally vary with the season, rusty crayfish have exacerbated these shifts and altered these food webs permanently. Due to the abundance of crayfish found in Cass Lake, fish of a smaller size shift their diets to rely primarily on them leading to significant differences in the diets of the invaded versus natural system.

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Introduction

The invasion of foreign species is one of many factors that causes freshwater ecosystems to have the highest level of species endangerment and extinction (Ricciardi and Rasmussen 2001). The effects of this do not remain in the water, the loss of aquatic insects is felt by riparian ecosystems which in turn is felt in the next interconnected ecosystem and so on (Polis et al. 1997, 2004). Previous attempts to describe biotic changes have focused primarily on biodiversity and species abundance (Nillson et al. 2012). Trophic flow has proven more difficult and has mostly been ignored (McCann 2007). Those that have studied trophic flow networks have focused on the use of stable isotopes to trace food web structure.

Yellow perch *Perca flavescens*, is a small to medium bodied percid native to the cool temperate lakes of North America (Page and Burr 2011). These fish are a key component in the ecosystems in which they are found. A strong perch hatch can mean the difference between a good year for predator growth and the loss of a year class. Perch are a vital part of a large tourism industry, whether anglers are traveling to target them or the large predatory species that eat them. Yellow perch are an important item in the diets of many fish, such as: walleye *Sander vitreus*, northern pike *Esox lucius*, and muskellunge *Esox masquinongy* (Glade 2021). As a result, it is important to monitor their abundance, growth, and recruitment (Hilborn 2017). Studies have shown that yellow perch are sensitive to the effects of invasive species (Newman 2020).

One invasive species that might be threatening yellow perch is the rusty crayfish Faxonius rusticus. Rusty crayfish are a large bodied benthic omnivore native to the Ohio River valley (Olden et al. 2006). Crayfish often play a vital role in the structuring of lotic and lentic ecosystems (Nilsson et al. 2012). They have been shown to be destructive to their invaded environs often defoliating vegetation, outcompeting native cravfish, and by decoupling littoral and pelagic energy flows (Kreps et al. 2016). Rusty crayfish were first found in Cass Lake in the 1990's (Kennedy 2019), they are quickly becoming a problem in north-temperate lakes (Lodge et al. 2000). In Cass Lake, in Beltrami County Minnesota, the population of rusty crayfish has been increasing, and coinciding with this, a sustained increase in the average size of yellow perch at each age was noticed by area fisheries managers (Kennedy 2019). It is suspected yellow perch were forced away from their normal forage and have begun to feed on rusty crayfish. Therefore, the objective of this study is to analyze the effects of rusty crayfish on the diets of yellow perch.

Methods

To assess the effects of the invasive cravfish. two lakes were selected for sampling. One with rusty crayfish (Cass) and one without (Bemidji). Perch were sampled three times throughout the summer of 2022 (June-August) with four weeks between each sampling event. Fish were sampled via boat electrofishing in shallow water (< 3 m). Many types of habitats were sampled, including shoreline areas and any other shallow structure (ex: bullrush beds, sandbars, etc.). Three length bins were established so that the effects could be seen throughout the age structure, small (0 - 99 mm), medium (100 - 199 mm), and large (200 + mm). To minimize variation, ten diets per length bin per collection event were targeted. Planning for empty stomachs to occur in 30-50% of fish, fifteen fish were collected for each bin. Due to the size of the fish, non-lethal options were limited, so fish were sacrificed in an ice water slurry. Total length was taken to the nearest mm, and wet weights were taken to the nearest gram. Total length, weight, serial number, and date were all recorded on data sheets.

In the lab, fish were then dissected and had their stomachs removed and placed in Whirl-Pak bags which were labeled with a unique serial number indicating which lake and fish the stomach came from. Finally, the stomachs were preserved in ethanol for later examination. After the end of the field season, stomachs were examined in the lab and all prey items were identified to the lowest possible taxonomic group, with special attention being given to differentiating native and invasive crayfishes. Various dichotomous keys and structures were used to identify stomach contents. The prey items were then grouped by lowest possible taxonomic category and counted to determine proportion of diet items. After each dissection was complete, stomach contents were placed in trays and baked at 60 °C for 24 hours (Tipton and Bell 1988).

Using program R, an index of relative importance (IRI, Pinkas 1971; Martin 1996) was calculated for each prey taxon:

IRI=% $F \times (\% N + \% M)$

using three standard dietary metrics, frequency of occurrence as F, prey number as N, and prey mass as M (West et al. 2003; Glade 2021). The IRI value that results indicates importance for each prey category while reducing any bias that may be caused by large and rare or abundant and small prey items. The IRI value was then translated into a percentage to allow comparison within groups and lakes.

Non-metric multi-dimensional (NMDS) ordinations were used to further visualize diet overlap between the invaded and non-invaded lakes. Some diets were removed due to exceedingly rare diet items. NMDS uses number of prey consumed in each category. The created ordinations display average diet (centroid) and 95% confidence interval for each lake.

Table 1. Common names and abbreviations used to						
represent	20	different	prey	taxon	categories	
observed i	n the	diets of ve	ellow p	erch.		

observed in the diets of	yellow perch.	
Prey Taxon	Abbreviation	
Amphipoda	AMP	
Annelida	ANN	
Bivalvia	BIV	
Coleoptera	COL	
Daphnia	DAP	
Decapoda	DEC	
Diptera	DIP	
Ephemeroptera	EPH	
Fathead Minnow	FHM	
Gastropoda	GAS	
Hemiptera	HEM	
Mimic Shiner	MMS	
Northern Crayfish	NCF	
Odonata	ODO	
Plecoptera	PLC	
Rusty Crayfish	RCF	
Spottail Shiner	SPO	
Trichoptera	TRI	
Unknown Crayfish	UNKC	
Unknown Fish	UNKF	
Yellow Perch	YEP	

Results

Between the two study lakes 130 yellow perch (37 mm – 308 mm) diets were sampled. In total, 18 different prey taxon categories were encountered in diets (Table 1). The most prey items found in a single individual was 306 *Daphnia* sp. Crayfish were an important diet item in both systems; however, they were more important in the invaded system. Invertebrates made up most of the diet items, with the first vertebrate (yellow perch) coming in as the 7th and 11th most important in Cass and Bemidji, respectively.

According to percent IRI calculations perch diets in Lake Bemidji consisted of crayfish (42%), dipterans (29%), amphipods (24%). In the invaded system yellow perch diets were made up of crayfish (59%), amphipods (20%), dipterans (9%) (Figure 1). Total length was a significant factor influencing the diets of perch (Figure 3). In Lake Bemidji small and medium fish relied on crayfish little; their diets were compromised of > 1% and 6%, respectively, while large fish relied on them heavily (91%). Like Lake Bemidji, small fish in Cass Lake relied on crayfish > 1%, however, the diets of medium fish were 68% crayfish and large were 93%. The IRI values for crayfish also changed between the June and July collections (Figure 4). In Lake Bemidji the diets of the fish collected in June consisted of 36% crayfish and the July fish were 41%. In Cass Lake the percent IRI value increased 10% from June (49%) to July (59%).

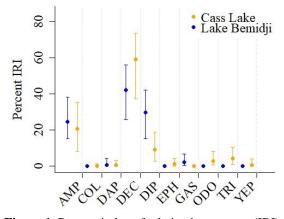


Figure 1. Percent index of relative importance (IRI) for common prey taxon in the diets of yellow perch. Error bars represent 95% confidence intervals calculated using bootstrapping methods.

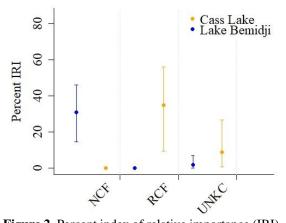


Figure 2. Percent index of relative importance (IRI) for northern crayfish (NCF), rusty crayfish (RCF), and unidentifiable crayfish (UNKC) from the diets of yellow perch. Error bars represent 95% confidence intervals calculated using bootstrapping methods.

NMDS ordinations suggest some overlap between the diets of perch in both systems (Figure 5). Applying vectors showed that both length and season are factors in diet selection. As the summer progresses and as total length increases perch become more likely to prey on crayfish and vertebrate prey and move away from smaller invertebrate prey.

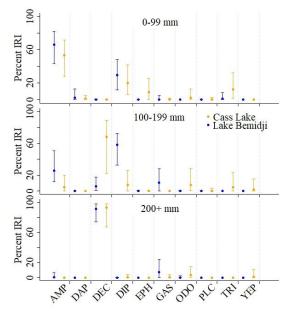


Figure 3. Percent index of relative importance (IRI) for common prey taxon in the diets of yellow perch split between the three study length groups. Error bars represent 95% confidence intervals calculated using bootstrapping methods.

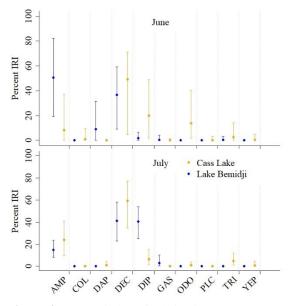


Figure 4. Percent index of relative importance (IRI) for common prey taxon in the diets of yellow perch split between the three study months. Error bars represent 95% confidence intervals calculated using bootstrapping methods.

Discussion

Yellow perch in this study consumed a variety of prey, however, their diets varied between the different lake statuses. Crayfish accounted for 59% of dietary items in Cass Lake and only 42% in Lake Bemidji. This finding is consistent with other studies. For example, Nillson et al. (2012) showed a significant increase in fish benthivory in lakes with a significant population of rusty crayfish. Our data provides evidence to suggest as yellow perch grow, they shift from favoring amphipods and other small invertebrates to crayfish and vertebrate prey. This shift happens significantly earlier in lakes afflicted with rusty crayfish. It is suspected this shift is result of the depletion of other native food sources.

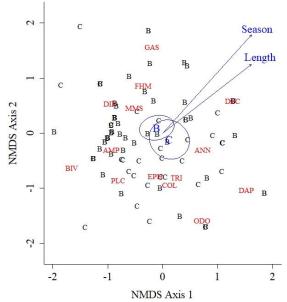


Figure 5. Non-metric multi-dimensional scaling (NMDS) ordinations based on number of items in each prey taxon for yellow perch from Bemidji (B) and Cass (C) lakes. Letters represent the centroid of each species diet in each lake, and ellipses represent 95% confidence intervals.

In Cass Lake yellow perch more commonly ate vertebrate prey, with three of the top ten prey items being fishes. On the other hand, in Bemidji vertebrate prey was quite uncommon and did not appear in the top ten most important prey items. As one model showed, zebra mussels caused Leucisid populations to decline by nearly 50% (Kumar et al. 2016). It also predicted the increase in population of yellow perch (and other species) that corresponded with crayfish beginning to use zebra mussels as a food source. The higher rate of cannibalism (and vertebrate consumption) could be due to a population increase caused by the interactions of the invasive species.

Zebra mussels were a species of tertiary interest to this study. They are present in both systems but are a more recent addition to Lake Bemidji. Zebra mussels are shown to increase reliance on littoral food webs (McEachran et al. 2019). During our time in the lab nine bivalves were found, seven were fingernail clams and two were zebra mussels. One of the zebra mussels found appeared to be a small remnant shell that had become part of a trichopteran casing, the second was an adult mussel. Members of the public have questioned whether the fish may have begun eating zebra mussels, but this does not appear to be the case in either of these systems.

As the season progressed the diets of yellow perch shifted away from other invertebrate prey and towards crayfish. In June, diets were quite varied with ten groups being represented. However, moving into July only eight groups were documented in diets. These results hold true with existing literature, Keast (1978) showed that differing would-be prey items reach their peak at varying times causing seasonality to affect the diets of fish. However, in this study throughout all months observed, crayfish remained an important energy source for yellow perch.

A significant difference was observed in the diets of fish between the three length groups. Smaller perch were more similar in both systems, however, once they broached the 100 mm mark they diverged rapidly. Amphipods remained the most popular diet item in the second length group in Bemidji, but in Cass they were outpaced by crayfish. Schmitt et al. (1984) suggests that dietary composition is determined by a mixture of size selectivity and what can most reliably and effectively be found. A population estimate was not calculated for crayfish on either water body, but anecdotally crayfish are far more abundant in Cass Lake. The selection by smaller fish for crayfish should corroborate that statement.

Our results suggest the invasion of rusty crayfish significantly affects the diets of yellow perch. Which, in turn, might be a possible explanation for the apparent difference in growth rates between the two lakes. When fish are more commonly able to find larger nutrient rich food, they will often change their primary food item. However, it should be noted that this study cannot determine causality as there are secondary effects that might be associated with the diet shift. Future studies should explore the effects of rusty crayfish invasions on other species that might prey on or compete with yellow perch and couple it with macroinvertebrate densities.

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