

## The Trophic Cascade of a Common Stream Environment and Optimal Feeding Behavior in *Notropis cornutus*.

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### ABSTRACT

Working at UNDERC, the property of the University of Notre Dame, we attempted to find differences in both the trophic level orientation and the foraging strategies of freshwater fish. Using field collecting methods and TKN protein analysis, some conclusions were made. The two fish, on different tiers of the food pyramid, exhibited independent fluctuations as the study progressed. Both species examined in the study demonstrated differences in food choice as the summer passed. *Perca flavescens* seemed to change its food source as was necessary to consume the same amount of protein. *Notropis cornutus* does not optimize invertebrate protein ingestion, but instead, feeds on those insects that occur in high frequency in its environment. At the time of observation, *N. cornutus* fed selectively on Trichoptera. Plant ingestion rates showed selective feeding on Ulothrix algae which is both high in protein, and occurs in great abundance in the stream environment. Although, at times of high prey abundance, *N. cornutus* consumed a protein-dominated diet which could be stored as energy reserves for later use; this shows some feeding optimization. This study cannot resolve which is the dominant factor in foraging behavior, for both protein optimization and frequency dependent models can account for some feeding trends.

### INTRODUCTION

The optimality theory and cost/benefit models are proven to be excellent representations for describing animal behavior, fitness maintenance, and survival.

Optimality theory is strongly rooted in biology as an analytical tool to decipher how behavior is shaped. Because many variables can influence how an animal acts, it is important to test each part separately and to eliminate those aspects that either do not effect behavior, or effect behavior only slightly. Although protein content in fish is the focus of this paper, many alternate theories of how and what fish eat have been explored by other experimenters; these theories fall into three main categories:

- 1) Frequency dependent optimization.
- 2) Non-frequency dependent optimization.
- 3) Unselective behavior.

Protein content in food stuffs as a selective influence in foraging falls within the non-frequency dependent model of optimization because under any circumstances, fish will attempt to maximize protein intake despite what the environment may allow. Thus, this model shows that, if true, fish choose to feed on food that has the highest protein content. In fact studies have concluded that bluegill will only eat prey that maximize energy intake (Feder '96).

Although our study attempts to prove or disprove the non-frequency dependent model of protein optimization, the frequency dependent model of foraging has many important components believed to be relative to this experiment. For example, copepods were found too have varied eating behavior for different seasons. This was due to the fact that seasonal variation caused food resources in the water to change. Copepods showed selective feeding in the spring, choosing small, numerous phytoplankton; but in the fall, as the quality and abundance of food decreased, copepods changed their diet to larger, but poor quality foods. Thus, it was concluded that frequency dependent selection based on resource availability within the stream was the key factor in food selection (Demott '95).

Besides the concept of optimal foraging, the examination of the trophic levels in which all species fall into their separate niches can convey additional information

concerning feeding habits. Food webs suggest pathways by which materials are transferred and along which energy flows. Problems which can occur and complicate a study on topological webs are too few species, too few feeding links, too few omnivores or too few cannibalism (Polis '96). Food webs are delicate balances which must be kept in a sort of equilibrium. Changes in foraging strategies can have severe effects on the other links in the web. The effects of top predators can extend throughout the web, all the way to the primary producers (Vanni '96).

Variations in the web of Tenderfoot Creek at UNDERC can yield both qualitative and quantitative results, according to Holt ('96). Qualitative measures include species composition changes, caused by environmental, seasonal, or local colonization and extinction. Quantitative variations include the strength or existence of interactions. By studying these variations and understanding the structure and dynamics, one can expound on the foraging habits of the members of the trophic levels.

When placing organisms into this trophic structure, competitive and predator-prey interactions determine the extent of the different levels. It is important to understand the theory that it is the trophic structure which controls the fraction of energy consumed at each level, rather than energetics controlling trophic structure (Hairston '93). For example, in freshwater pelagic communities, the collective efficiency of herbivorous plankton in consuming primary producers is up to ten times as great as the efficiency of forest herbivores. Conversely, forest predators are about three times as efficient in consuming their food as zooplanktivorous fish are. The presence of an additional level, piscivorous fish accounts for the difference and allows the zooplankton to flourish on the plant matter. Ecological efficiencies do not determine trophic structures; rather, they are its product (Hairston '93).

Lastly, it is possible that fish do not try to maximize their energy intake at all, but merely are opportunistic feeders who are relatively unselective in their feeding behavior. Giske has attempted to show that pelagic fish must weigh the cost of foraging

against the increased risk of being eaten while foraging at the surface. Thus, because the intensity of light can be correlated to an increased mortality rate, the fish will avoid the risk and stay at the bottom of the stream. Because pelagic fish are pushed to the bottom where food resources have dwindled, those that are unselective (i.e., opportunistic feeders) will survive at a high rate than foragers (Giske '95).

The search for optimal foraging strategy inevitably leads to a complex interaction between perceived costs and benefits, actual cost and benefits, and the environment that such decisions are made. This study takes a linear approach to the problem of optimization and attempts to disregard other foraging strategies and the complexities that arise within these interactions. This is done so that protein intake can be assessed and determined if it is relevant to foraging behavior in perch and common shiners.

### RELEVANCE OF STUDY

Fish provide a good model system to test the application of the optimality theory. Second, observing how and what fish eat could lead to a greater understanding of how growth and protein intake are related from a behavioral perspective. Studying trophic interactions can provide models for how energetics are dispersed and controlled at different levels. Lastly, studying foraging behavior as it pertains to protein intake and protein concentration may provide a better knowledge of how to optimize human intake of protein during development and beyond, by comparing the two.

### MATERIALS AND METHODS

Analysis of the protein content and quantitative analysis of gut contents were completed to determine the feeding/foraging behavior in two species of fish: perch, *Perca flavescens*, and the common shiner, *Notropis cornutus*. Collections were made in

Tenderfoot Creek on the University of Notre Dame Environmental Research Center property in Gogebic County, MI. The collection site (Fig. 1) encompassed a 20 m riffle (0.1-0.5 m deep) ending in a deep pool (0.5-3.0 m deep). Three collection dates were established to determine the temporal feeding pattern of the fish that were studied. Collections for *N. cornutus* took place on June 1, 17, and 28, 1996 at 8:30 a.m. Collections for *P. flavescens* took place on June 8-12, 19, and June 30-July 1, 1996 in the evenings.

To collect *N. cornutus*, the riffle was electroshocked. Approximately 20 fish were gathered per collection. To collect *P. flavescens*, two methods of capture were used. In the first collection, subjects were caught in a fyke net over a period of several days. In the second and third collection, subjects were collected by fishing with spinners, live shiners as bait, and worms. Ten *P. flavescens* were caught per collection in the pool just below the riffle.

Once the fish were collected from the stream, measurements of length, mass, and volume were recorded for both species. Volume was assessed by water displacement using two methods. *P. flavescens* were submerged in a Mason jar filled to the brim and the displaced water was collected and transferred to a 200 ml graduated cylinder ( $\pm 0.5$  ml). *N. cornutus* volume was estimated by measuring water displacement through the attachment site on a sidearm Erlenmeyer flask. Flow-through was collected and transferred to a 50 ml titration pipette and measured to  $\pm 0.1$  ml. All catalogued data compiled in Table 1.

Once the fish were catalogued, gut content analysis was completed. The fish were opened up and the digestive tract of each was removed and examined. In shiners, the stomach and digestive tract are nearly indistinguishable; therefore, the entire digestive tract was analyzed for contents. *N. cornutus* were dissected with an incision from the anus to just below the operculum. The digestive tract was cut at the neck and the contents were squeezed from the intestine for analysis. Analysis of gut contents were carried out under the dissecting microscope (25X). *P. flavescens* gut contents were

analyzed by dissecting out the stomach separately from the lower intestine and examining both under a dissecting microscope at 25X. Ten randomly selected specimens of *N. cornutus*, including all males if collected, and ten *P. flavescens* were analyzed for gut contents per collection. Once the gut contents were determined, live specimens of the insects and plants found in the guts were collected at the *N. cornutus* collection site through inspection of submerged rocks, the use of kick nets, and by setting a light trap.

Total Kjeldahl Nitrogen of the subjects and samples was found using the TKN procedure (Hach 1989). Two methods were used depending on the sample. For insect, gut contents, and meat samples, 0.1 g (dry weight) of material was added to a heating flask with 4 ml 36N H<sub>2</sub>SO<sub>4</sub>. For the experimental fish and crayfish, a pre digest was used. Each 10 g sample for the pre-digest was weighed out to the nearest thousandth of a gram. *N. cornutus* were sliced into smaller pieces to constitute the sample; if the fish weighed less than 10 g, the maximum mass which could be extracted from an individual fish was used. Each *P. flavescens* was blended in an industrial blender to homogenize it. This solution was subsequently filtered through a 70 µm screen and a random sample was taken from this extract (particle size range: 70 µm -10 mm). Each pre-weighed sample was then placed in an 80:20 ratio of 36N H<sub>2</sub>SO<sub>4</sub>:RO H<sub>2</sub>O. This mixture was placed on a stirring plate for 15 minutes. After the 15 minute pre-digest, 5 ml of the solution was added to a heating flask for the digestion.

Each flask was heated on a Electrothermal Microdigester™ at 468° C. 2 ml H<sub>2</sub>O<sub>2</sub> was added to the flask 4.5 min. after the observation of the reflux line. The H<sub>2</sub>O<sub>2</sub> was then boiled off and the flask was cooled for 15 min. Following the cooling period, the TKN procedure outlined in the Hach methods manual (Hach 1995) was utilized for sample analysis. 1 ml of the digest was used in the sample analysis and the samples were not pH stabilized.

Only fish used in both gut content and TKN analysis were considered in the final

statistical examination. All spreadsheet and statistical work was completed at the research laboratory on the UNDERC property using the program Systat.

## RESULTS

### *N* itrogen Content vs. Trophic Level

We experimentally measured nitrogen in the two fish species to determine a potential relationship across trophic levels. *Notropis cornutus* represented the tier 1 predator/omnivore, while *Perca flavescens* represented the tier 2 predator.

As the summer progressed, the *N. cornutus* nitrogen content (mg/L TKN) increased significantly from collection 1 to collection 2 and 3 (n=29, p=.025). The first collection yielded a value of ~10300 mg/L TKN, which fell to the range of 8250-8500 mg/L TKN in the 2nd two collections (Fig. 2). This constituted a 17% drop from the first collection, using 95% confidence intervals. There was no significant difference between the second and third collection results. There were no significant results upon comparison of the two genders either (#1- n=9, p=.889; #2- n=10, p=.789).

*P. flavescens* displayed an increasing nitrogen content as the summer progressed. The first two collections did not yield a significant difference in the results (n=20, p=.158), but by the third collection the mg/L TKN increased by 14%; these were significant results (n=31, p=.003). The first two collections resulted in mg/L TKN between 8050-8400, while the third collection yielded ~9800 mg/L TKN (Fig. 3). Once again, no significant differences between the genders could be reported (#1- n=10, p=.854; #2- n=10, p=.286).

Upon comparison of the two species, a contrasting trend is obvious. As *N. cornutus* TKN decreased, *P. flavescens* TKN increased. Statistically comparing the mg/L TKN for each separate collection, one gets significant results in collection 1 and

collection 3 (Fig. 4). Collection 1 yielded *P. flavescens* to have a 23% lower TKN reading than *N. cornutus* (n=19, p=.001). Collection 2 contained insignificant results (n=20, p=.60). Collection 3 yielded marginally significant results for this study; *P. flavescens* had 12% greater mg/L TKN than *N. cornutus* (n=20, p=.082).

In a comparison of the nitrogen content of the tail meat of specimens obtained in collection 1, *P. flavescens* demonstrated a TKN reading of 38000 mg/L while *N. cornutus* demonstrated a TKN reading of 33800 mg/L (Fig. 5). This yielded a 11% difference and this result was significant for the two species (n=20, p=.016).

### *Food Selection in the Two Species*

Upon examination of the gut contents of *P. flavescens*, trends can be noted in terms of food selection (Table 2). *P. flavescens* consumed mostly *N. cornutus* (87.5%) throughout the first collection, with exception to a few larger insect larvae. As time passed, the amount of *N. cornutus* selected as a food source decreased by 57% in the second collection and an additional 66% by the third collection. Replacing *N. cornutus* as a food source was the crayfish *Orconectes*. The average number of *Orconectes* per fish in the second collection was  $.300 \pm .153$  ( $\bar{x} \pm 1$  S.E.M.); this raised 62.5% to  $.8 \pm .512$  in the third collection. Both *N. cornutus* and *Orconectes* show similar nitrogen contents, based on the data received from collection 3 (Fig. 6).

*N. cornutus* also demonstrated a varying choice of food as time progressed. Trends include the increase in the amount of green macrophytes, algae consumed, and the decrease in the frequency of certain insects (Table 3). Food selection was varied as was the total nitrogen content accounted for by each insect group (Fig. 7). Percentages of green macrophytes, algae steadily increased from  $5.9\% \pm 1.882$  of the entire gut contents in the first collection, to  $46.2\% \pm 11.836$  ( $\bar{x} \pm 1$  S.E.M.) in the third collection. As algal and macrophytic populations in the riffle increased, so did the "greens" content of

the guts. Simuliidae occurred in the guts in only the first collection, with minor exceptions. *Cheumatopsyche* consumption decreased as the collections progressed. The first collection guts contained  $24.5 \pm 6.486$  individuals per fish, while the third collection decreased 98% to  $.500 \pm .269$  individual insects. In contrast, the amount of *Hydropsyche* slowly increased 94% from the first to third collections. No Odonata were found in the third collection, for they had already emerged by this time. Chironomidae increased from the second to the third collection (47.5% increase), while both Molluska and Trichoptera adults only occurred in a single collection.

#### *Optimal Foraging in N. cornutus*

It was found that Limniphilidae and Gomphidae had the highest individual mg/L TKN, followed by Hydropsychidae (collections 1 and 2) and Heptagenidae. The third highest was Hydropsychidae from collection 3 followed by *Cheumatopsyche* (collection 1) and Trichoptera adults. Because Total Kjeldahl Nitrogen (TKN) provides a good representation of crude protein content in food, TKN measures can be correlated to protein content. Figure 7 shows the protein content found in 1 L of food stuff, while Figure 9 shows the average protein found per individual.

It was found that *Cheumatopsyche*, *Hydropsyche*, and Chironomidae protein decreased over the collection dates. *Cheumatopsyche* protein dropped 34% from collection 1 to collection 2 ( $1034.7 \pm 105.5$  mg/L TKN to  $679.1 \pm 89.9$  mg/L TKN). *Hydropsyche* protein dropped from  $2952.1 \pm 466.7$  mg/L TKN in combined collections 1 and 2, to  $1632.4 \pm 190.5$  mg/L TKN in collection 3 which corresponded to a 45% decrease in protein content. Chironomidae protein dropped only 20% from collection 2 to collection 3 ( $560.3 \pm 64.5$  mg/L TKN to  $447.0 \pm 13.2$  mg/L TKN).

Stream macrophytes and algae were collected and analyzed for protein content as well. Macrophytes all had about the same amount of protein, but algae had nearly

three times the protein that macrophytes had (Fig. 10).

Using the gut content analysis and the average number of individuals found in the stomach, approximations of protein content in the gut were made (Table 5). In collection 1, it was found that protein derived from *Cheumatopsyche* contributed most to overall protein ingestion. *Cheumatopsyche* contributed 65.8% of the overall protein, while Simuliidae pupa contributed 19.4%. Simuliidae adults and Gomphids contributed approximately the same amount of average gut protein; 7.1% and 5.7% respectively. *Hydropsyche* and mollusks contributed to the gut protein as well (Fig. 11a). In collection 2, it was found that *Cheumatopsyche*, *Hydropsyche*, Gomphidae, Limniphilidae, and Trichoptera adults all contributed approximately equal amounts of gut protein and none were significantly different (Table 5). Chironomids contributed less than *Cheumatopsyche*, Chironomids contributed on average 11.5%, while *Cheumatopsyche* contributed 20.7%. Simuliidae larva contributed only a small amount to the average protein found in the gut of shiners; only .8% was Simuliidae larva. In collection 3, *Hydropsyche* contributed on average 65.3% of the gut protein while chironomids contributed 22.4%. *Cheumatopsyche*, Simuliidae adults, and Heptagenidae contributed less to the gut protein and were not significantly different amongst one another (Fig. 11c).

Ingested protein acquired from insects decreased significantly from collection 1 to collections 2 and 3 (Table 5). It was estimated that in collection 1, on an average, 38532 mg/L TKN of protein was found in the guts of shiners. Collections 2 and 3 were not significantly different, and yielded average protein amounts of 10188.4 mg/L TKN and 7999 mg/L TKN respectively. This drop in protein intake corresponded to a decrease in the shiner protein content. (Fig. 2).

Similarly, as protein ingestion due to insect consumption decreased, the amount of plant matter eaten by the shiners increased. In collection 1, average qualitative estimates of plant content was only  $5.9 \pm 1.9$  %. In collection 2, it was  $19.1 \pm 8.2$ % and in

collection 3, it was  $46.2 \pm 11.8\%$  (Fig. 8). However, specialization on Ulothurix algae was observed in all collections. Hence, the increase in consumption of plant matter corresponded to an increase in overall ingestion of Ulothurix algae.

## DISCUSSION

### *Nitrogen content vs. Trophic Level*

Upon examining the results from the comparison between the two species, the first point to note is the lack of significant differences between the two genders. Going into the experiment, we had hypothesized a high dimorphism of the two sexes, with females possessing the larger mg/L TKN of the two. This assumption was based on the fact that the females carry the eggs, thought to be a highly-enriched source of protein. The invalid results could have risen from the lack of replicates for an individual sex in each fish. During collections, male *N. cornutus* were difficult to encounter based on their skittish nature, and the fact that they could have been guarding nests. Female *P. flavescens* seemed to be less likely to hit a baited hook, given that 75% of them were caught in the fyke net. Another possibility for the massive collection of one sex and not the other is that fish school by sex, thus weighing the numbers in one direction.

*N. cornutus* was ultimately placed in the middle level of the trophic pyramid based on its omnivory and the fact that it acted as predator and prey. From figure 2, one can see the drop in mg/L TKN in this species. This drop can be accounted for by several factors. As the summer progressed, the amount of invertebrates in the aquatic environment decreased. More and more emerged and joined the terrestrial world. As this happened, the food source of the fish disappeared. Looking at the raw data in table 2 illustrates this fact; the sheer numbers of individual insects decreased dramatically as time went on. At this time, the aquatic environment also became an ideal medium for

macrophytes and algae (the amounts of phosphates and other necessary nutrients increases throughout the summer). This food source, one of lower quality with respect to protein, replaced the protein-rich nymphs *N. cornutus* originally consumed. Thus, the fish's overall protein content decreased as well.

This was a fact not lost on *P. flavescens*. For as the shiners decreased in overall TKN, *P. flavescens* increased and ultimately passed *N. cornutus* by switching its food source. This predator, placed in the top level on the trophic scale, changed its top food item for another which had a higher protein content: *Orconectes*. To this point, *Orconectes* was ignored as a food source for the predator. But there came a point when the crayfish were more vulnerable to attack by *P. flavescens*. At this later point in the season, we found deposits of calcium in the guts besides that of *Orconectes*. This was theorized to be a gastrolith, the mass of calcium stored by the crayfish as it molts. Once the molting is complete, the calcium is dispersed throughout the exoskeleton again to harden the shell. At this point, with a soft shell, *P. flavescens* was able to take advantage of this weaker prey and increase its protein intake as well. This fact was further solidified by observing the partially-digested crayfish; all were either colored extremely dark (pre-molting), or extremely light (post-molting). In either case, the shell would be soft. One argument against the notion that *P. flavescens* can demonstrate optimal feeding behavior is the fact that no significant results found a difference between the protein content of *Orconectes* or *N. cornutus* (Fig. 6).

Thus it can be seen that *P. flavescens* took advantage of its time-dependent factors, while *N. cornutus* suffered some. *P. flavescens* showed some tendency of consuming larger nymphs such as Gomphids or Tipulids, but the shiner-crayfish interaction was the most significant and interesting. Looking at the comparisons of the mg/L TKN for the two species, one can see the changing of positions by the two fish (Fig. 4). If the experiment had been carried out longer, a fourth collection might have given significant results for the absolute difference in protein between the two; making collections two

and three transitional periods. The feeding habits of *N. cornutus* will be discussed in a later section.

Concerning the analysis done on the meat samples of the tail regions, the larger, more muscular fish possesses the larger TKN value. In overall grinding up of the two fish, *N. cornutus* yielded larger values in the first collection. But from those same experimental fish, *P. flavescens* demonstrated a higher protein content. This was hypothesized to be because of the greater muscle mass and also the fact that the ground up sample of the perch contains a large amount of bones and other protein-free materials, contaminating the overall sample taken from the fish. *N. cornutus* samples often consisted of the entire fish, allowing for all of the high-protein meat to be analyzed.

The qualitative variations in this web existed strictly in the emergence patterns of the aquatic insects, and the subsequent adjusting of the predators in the higher tiers. An environmental variation existed in the form of the ever increasing vegetative population of the stream throughout the study. No local colonization nor extinction was observed during this period. The quantitative variations exist in how much these qualitative effects changed the energetics within the structure. There was a large quantitative variation illustrated, for the two main topics of the study, *N. cornutus* and *P. flavescens* changed positions in the flow of protein in the cascade. These interactions also include the effects of the top tier predator influencing the entire cascade by its behavior. Done on a larger scale, this experiment might have demonstrated the effects the piscivorous perch had on the populations of the invertebrates; less shiners equaled less predators to influence the population of the nymphs.

Results in discussing a trophic level structure does have its problems. With too few feeding links and too few levels, this study, from a food web study's perspective, yields helpful, but not complete data on the interactions between primary producers on up to even a third tier predator such as a bird of prey. Further study can be completed

using similar techniques on a larger scale (more organisms) in learning about the flow of energy from one level to the next.

### *Optimal Foraging in N. cornutus*

The main hypothesis studied was to what extent does protein optimization determine what foods *Notropis cornutus* will eat. Optimization theory states that organisms will try to maximize their intake of foods while minimizing the risk associated with foraging. Hence, under highly dangerous situations, fish will be unselective feeders, while in times of relative safety, they will maximize their intake of food by specializing on a protein rich food sources. In the stream environment chosen for the study, the fish were isolated from the rest of the river basin by shallows both upstream and downstream; hence, one would assume that shiners are specialized feeders that optimize protein ingestion. However, this is not the case.

Within a very broad context, one can see that protein maximization may play a role in the feeding behavior of *N. cornutus*, but certain restraints are placed on what can be eaten. For example, it was found that Limnephilidae and Gomphidae yielded large mg/L TKN protein readings per individual insect. However, Limnephilidae live in protective cases firmly attached to rocks; thus, they would be very hard to find, let alone consume. Gomphidae are very hard to digest due to the thick exoskeleton; hence, ingestion and digestion would take much more time and effort. So, although insects may be high in protein, other constraints, including digestibility, size, protective devices, life styles, and frequency in the environment, may play an active role (or major role) in determining which foods will be eaten.

Stream-living *Notropis cornutus* specialized on a diet rich in Trichoptera. In collection 1, 67% of the gut content protein was derived from Trichoptera; it was 53% in collection 2 and 70% in collection 3 also. Although it may seem that Trichoptera were

actively sought out as a prey source, actual ingestion rates of individual Trichoptera decreased over time from 25,948 mg/L TKN in collection 1, to 5,356 mg/L TKN and 5,562 mg/L TKN in collections 2 and 3 respectively. This overall decrease in ingestion rates could show that frequency dependent selection is actually a stronger driving force in foraging behavior than is the drive to optimize protein intake.

It was observed that over time, the relative abundance of Trichoptera larvae decreased over time, probably due to emergence and predation. Based on collection rates, although both *Cheumatopsyche* and *Hydropsyche* numbers decreased over time, *Cheumatopsyche* were in greater abundance than *Hydropsyche* in collection one. However, by collection 3, *Hydropsyche* was in the greater abundance of the two. The ingestion rates of these two types of caddisflies correlate very well with this trend, leading one to believe that frequency dependence may have played a deciding role in feeding preference (Fig. 11). If protein ingestion was the key determinate factor of predation, *Hydropsyche* should have been eaten in greater numbers initially. Then, as their numbers decreased due to emergence and predation, *Cheumatopsyche* would be eaten in greater numbers. This trend was not observed; which leads one to conclude that protein ingestion is not the ultimate determinant of predation.

Similarly, if protein intake alone were the key determinate factor of feeding behavior, *N. cornutus* would be strongly specialized for one food source and would eat more high protein foods and less low protein foods. Instead, *N. cornutus* ate large amounts of low-protein simuliidae larvae, while eating small abundances of high-protein Trichoptera during collection 1 (Table 5). Hence, *N. cornutus* does not eat more protein-rich prey than low-protein prey. This directly negates the theory that *N. cornutus* selectively forages on the basis of protein. Again, the trend to eat large numbers of simuliidae larvae can be explained by a frequency dependent model, for large numbers of simuliidae larvae existed in the stream during the first collection.

Not only was frequency dependence observed in the consumption of insects, but

it was also seen in the algae ingestion rates. Qualitative measures of percent of gut containing plant matter showed an increase from 5.9% to 19.1% to 46.2%. Similarly, more plants were observed in the stream over time. Hence, the greening of the river led to an increase in ingestion of that food source. In contrast to this frequency dependent model, *N. cornutus* was found to specialize on Ulothurix algae in all collections. This algae was found to have nearly three times as much protein as other plants in the stream environment. Hence, on this level, one can conclude that vegetative selection is based on protein optimization. It is difficult to observe which aspect played a greater role in plant foraging: the frequency of the plant in the environment, or the high protein that it contained. In any event, *N. cornutus* actively selected to feed on Ulothurix.

Although frequency dependent selection was observed throughout the five week collection period, it is hard to determine if the numbers of individuals ingested correlated greatly with their relative abundance in the river system. No quantitative numbers for density of food source were collected. Hence, if protein was an underlying requirement for ingestion, one would observe an increased percentage of ingested insects relative to the percentage of abundance in the river environment. Therefore, a study correlating this data to protein per insect would better answer the question: To what extent do fish selectively feed on high protein foods?

The second hypothesis was that fish forage to optimize a specific amount of protein in the gut and, once reached, foraging no longer is profitable. For example, the optimal range would be one that maintained life sustaining activities while excess energy would not be wasted on foraging. Instead, it was found that fish eat large amounts of protein when present and store it away for later use. In collection 1, the protein ingestion per fish was high, as was the total protein of the fish. In collection 2, the protein ingestion per fish dropped significantly, as did the overall protein of *N. cornutus* (Fig. 2 and Table 5). Ingestion rates of insect matter dropped even when

insect protein dropped over the season. Because the frequency of finding insects decreased with time, presumably due to emergence, and because protein per insect decreased over time, *N. cornutus* feeding behavior as well as overall protein content reflects these trends. *N. cornutus* must store energy reserves in times of high prey abundance. Thus, the hypothesis that fish will eat only to an optimal level and then will feed no longer is incorrect.

In conclusion, *N. cornutus* foraging behavior is strongly based on frequency dependence, while the extent of selectivity based on protein is inconclusive. Whatever the case may be, it is obvious that *N. cornutus* behavior is not based on unselective feeding behavior since they tend to prefer caddisflies and algae. Instead, the complex inter-relationships between protein ingestion and frequency dependence can not be resolved from this study. Algae ingestion shows a strong basis toward optimal foraging based on protein as well as frequency dependence. Zoufal discovered a significant positive correlation between the energy content of the algae and the feeding activity of herbivorous fish ('90); similar results were observed in our study. In contrast, insect foraging shows only the frequency dependent aspect of selection. Similar conclusions were arrived at by Bres, who determined that rainbow trout diet selection is strongly influenced by the relative abundance of prey types rather than the caloric content of prey items (Bres 1986). Further study is needed to determine to what extent protein optimization plays in foraging.

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	<i>Notropis cornutus</i>	<i>Perca flavescens</i>
Sex	6 males/24 females	26 males/4 females
Weight	13.089 ± 1.829	107.014 ± 7.179
Volume	13.362 ± 2.126	112.717 ± 7.370
Length #1	10.570 ± 0.347	20.613 ± 0.441
Length #2	8.543 ± 0.279	17.390 ± 0.373

**Table 1:** Average measurements ( $x \pm 1$  S.E.M.) of experimental (gut contents and TKN analysis) *N. Cornutus* and *P. flavescens*. Units for measurement: weight (g), volume (ml), length (cm). Length #1 represents the measurement from the nose to base of the tail. Length #2 represents the measurement from the nose to the end of the tail fin.

	Collection #1	Collection #2	Collection #3
Chordata			
Osteichthyes			
Cyprinidae			
<i>Notropis cornutus</i>	1.400 ± 0.306	0.600 ± 0.340	0.200 ± 0.200
Arthropoda			
Crustacea			
Decapoda			
Cambaridae			
<i>Orconectes sp.</i>	∅	0.300 ± 0.153	0.800 ± 0.512
Insecta			
Odonata Anisoptera			
Gomphidae			
Unknown	0.100 ± 0.100	0.100 ± 0.100	0.400 ± 0.2
Diptera			
Tipulidae			
Unknown	0.100 ± 0.100	∅	∅
Trichoptera (Adult)			
Unknown	∅	0.100 ± 0.100	∅

**Table 2:** Average number of each individual food item ( $x \pm 1$  S.E.M.) found in *P. flavescens* gut contents. *N. cornutus* were found in various stages of digestion, from the whole form, to only the spine remaining. Crayfish were always found with a gastrolith (Ca<sup>2+</sup> deposit). Gomphids in the first two collections were larvae, while half of those in the third were adults.

	Collection #1	Collection #2	Collection #3
Arthropoda			
Insecta			
Diptera			
Chironomidae			
Unknown	Ø	2.100 ± 0.674	4.000 ± 1.265
Simuliidae			
Unknown			
(Adult)	19.900 ± 11.037	Ø	1.400 ± 0.806
(Pupae)	38.900 ± 12.384	Ø	Ø
(Larvae)	Ø	0.100 ± 0.100	Ø
Odonata			
Gomphidae			
Unknown	0.300 ± 0.213	0.300 ± 0.213	Ø
Trichoptera			
Hydropsychidae			
<i>Cheumatopsyche</i> sp.	24.500 ± 6.486	3.100 ± 0.706	0.500 ± 0.269
<i>Hydropsyche</i> sp.	0.200 ± 0.133	0.600 ± 0.221	3.200 ± 1.236
Adult (unknown)	Ø	1.600 ± 0.542	Ø
Ephemeroptera			
Heptageniidae			
Unknown	Ø	0.600 ± 0.340	0.200 ± 0.200
Mollusca	0.300 ± 0.213	Ø	Ø
Algae/Macrophytes	5.9% ± 1.882	19.1% ± 8.185	46.2% ± 11.836

**Table 3:** Average number of each individual food item ( $\bar{x} \pm 1$  S.E.M.) found in *Notropis cornutus* gut contents. Simuliidae adults found in collection three are unidentifiable Diptera members placed in this category for statistical work. Trichoptera adult also includes pupae cases found in the guts of the fish. The final row is an average of the estimated percentage that plants and algae made up of the gut.

**Figure 1:** Location of the collection sites on the UNDERC property. An X marks the site of the riffle (*N. cornutus*), an O marks the *P. flavescens* fishing sites, while a F marks the site of the fyke net.

**Figure 2:** The nitrogen content (mg/L TKN) of *N. cornutus* for the three collections. Error bars represent  $\bar{x} \pm 1$  S.E.M. (n=29, p=.025).

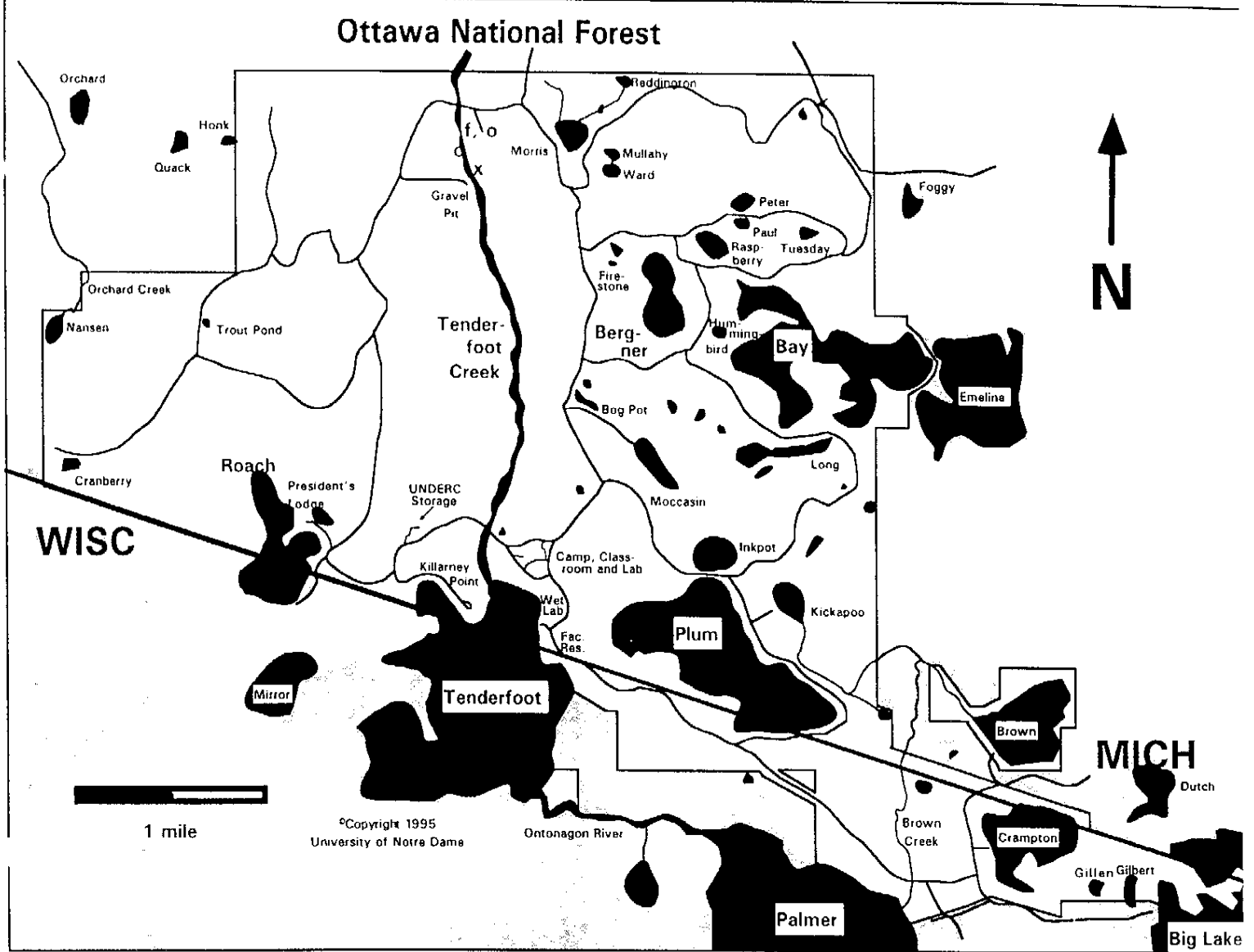
**Figure 3:** The nitrogen content (mg/L TKN) of *P. flavescens* for the three collections. Error bars represent  $\bar{x} \pm 1$  S.E.M. (n=31, p=.003).

**Figure 4:** Comparisons between the two species for each collection, starting with the first on the top. Error bars represent  $\bar{x} \pm 1$  S.E.M. Collection 1: n=19, p=.001. Collection 2: n=20, p=.600. Collection 3: n=20, p=.082.

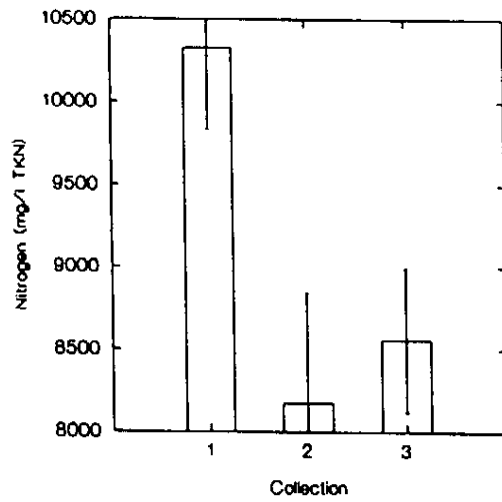
**Figure 5:** Comparison of nitrogen content (mg/L TKN) in meat samples of *N. cornutus* and *P. flavescens*. Error bars represent  $\bar{x} \pm 1$  S.E.M. (n=20, p=.016)

**Figure 6:** Comparison between the nitrogen contents (mg/L TKN) of the two main food sources in *P. flavescens*, *N. cornutus*, and *Orconectes*. The figure shows the two to be similar. This data was taken from organisms collected in the third collection.

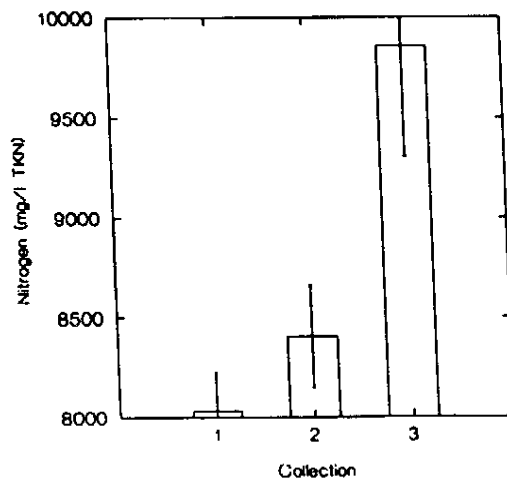
**Figure 7:** A comparison in nitrogen content (mg/L TKN) of all insects consumed by *P. flavescens* and *N. cornutus*. Chironomidae 2 and the Tipulidae adult used as comparisons and were not actually consumed.



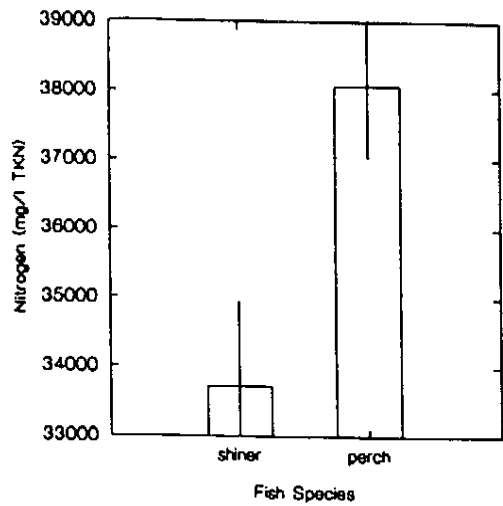
**Figure 1:** Location of the collection sites on the UNDERC property. An X marks the site of the riffle (*N. cornutus*), an O marks the *P. flavescens* fishing sites, while a F marks the site of the fyke net.



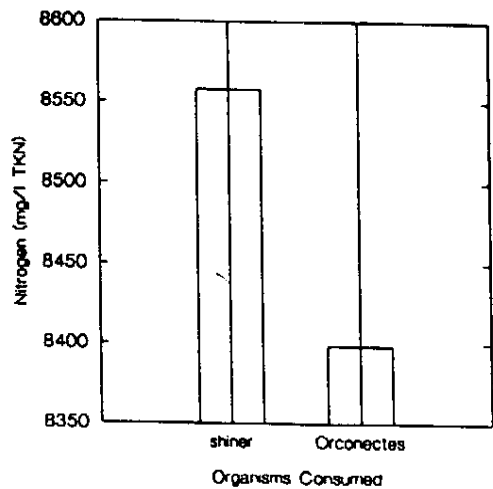
**Figure 2:** The nitrogen content (mg/L TKN) of *N. cornutus* for the three collections. Error bars represent  $\pm 1$  S.E.M. (n=29, p=.025).



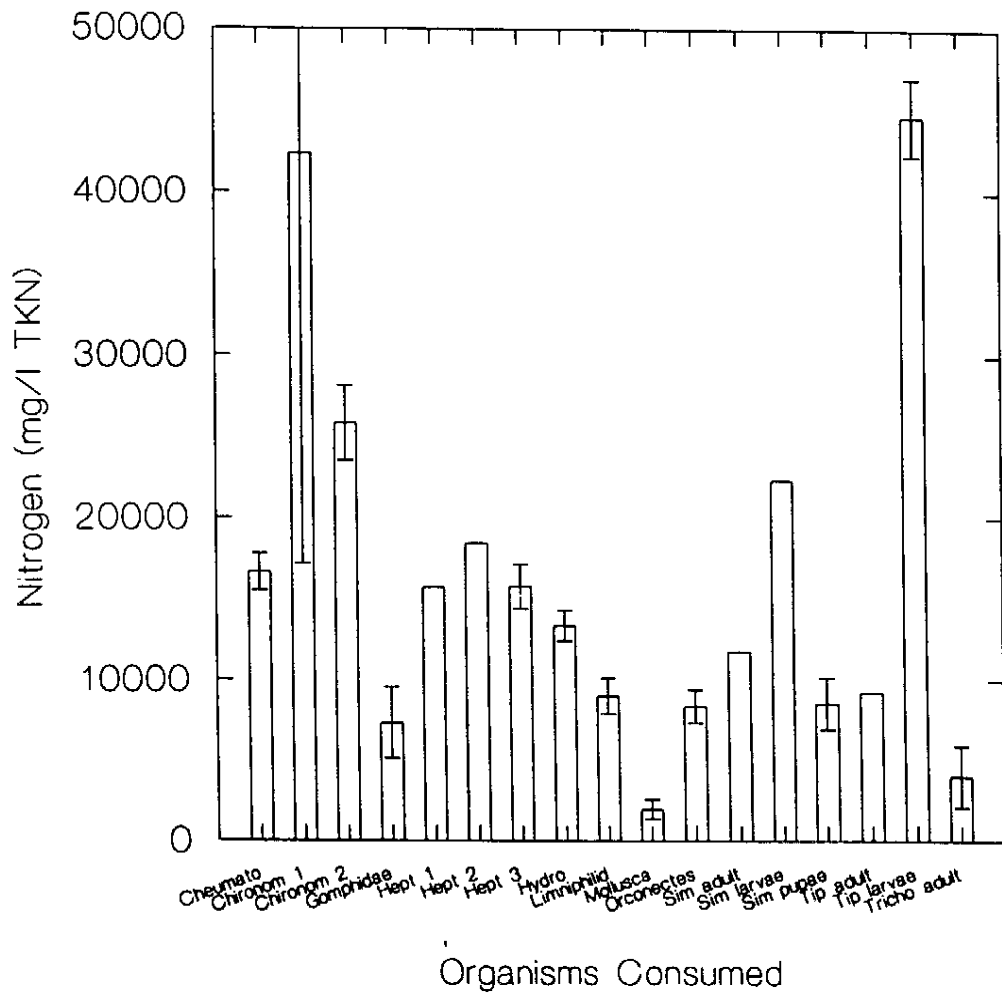
**Figure 3:** The nitrogen content (mg/L TKN) of *P. flavescens* for the three collections. Error bars represent  $\pm 1$  S.E.M. (n=31, p=.003).



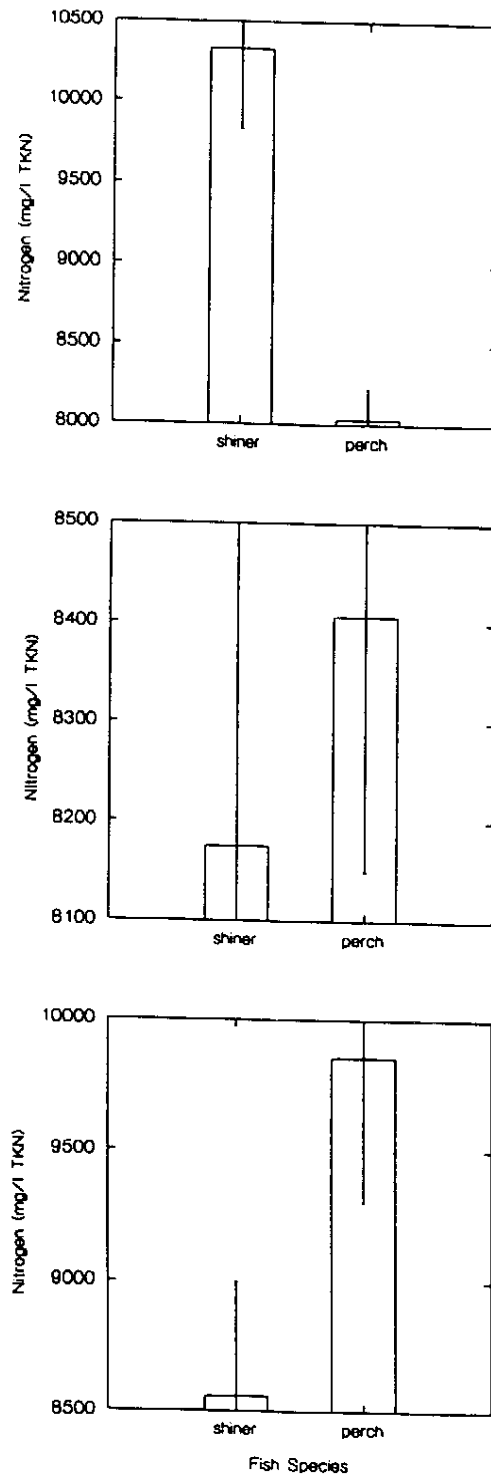
**Figure 5:** Comparison of nitrogen content (mg/L TKN) in meat samples of *N. cornutus* and *P. flavescens*. Error bars represent  $\bar{x} \pm 1$  S.E.M. ( $n=20$ ,  $p=.016$ )



**Figure 6:** Comparison between the nitrogen contents (mg/L TKN) of the two main food sources in *P. flavescens*, *N. cornutus* and *Orconectes*. The figure shows the two to be similar. This data was taken from organisms collected in the third collection.

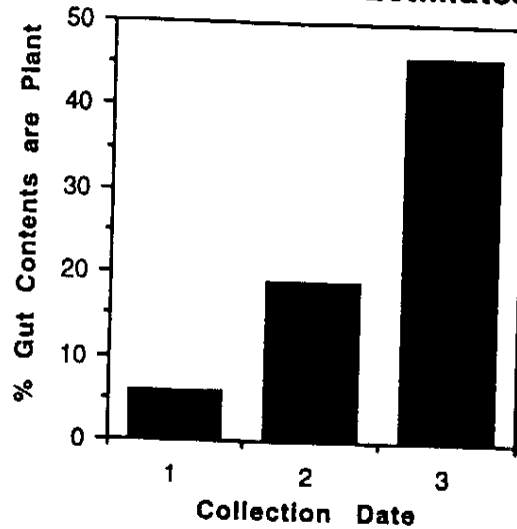


**Figure 7:** A comparison in nitrogen content (mg/L TKN) of all insects consumed by *P. flavescens* and *N. cornutus*. Chironomidae 2 and the Tipulidae adult used as comparisons and were not actually consumed.



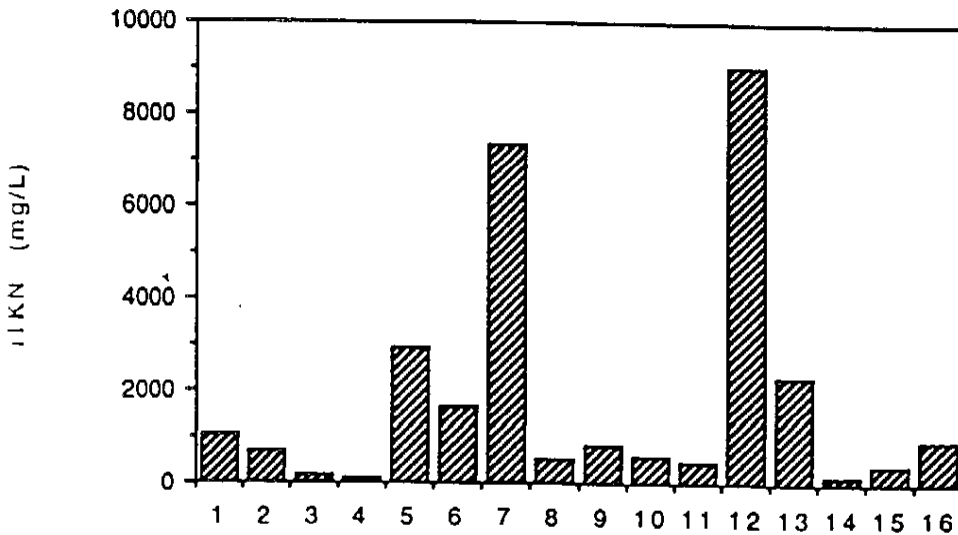
**Figure 4:** Comparisons between the two species for each collection, starting with the first on the top. Error bars represent  $\pm 1$  S.E.M. Collection 1:  $n=19$ ,  $p=.001$ . Collection 2:  $n=20$ ,  $p=.600$ . Collection 3:  $n=20$ ,  $p=.082$ .

**% of Plant Contained in the Gut:  
Qualitative Estimates**



**Figure 8.** Trends of plant content in *Notropis cornutus* gut by percentage. The plant percentages were qualitative estimates taken during observation and identification of the gut contents. Error bars are not present. Collection 1 had  $5.9 \pm 1.882$  % plant in the gut while collection 2 had  $19.1 \pm 8.185$  % and collection 3 had  $46.2 \pm 11.836$ %.

**TKN per Individual Insect**

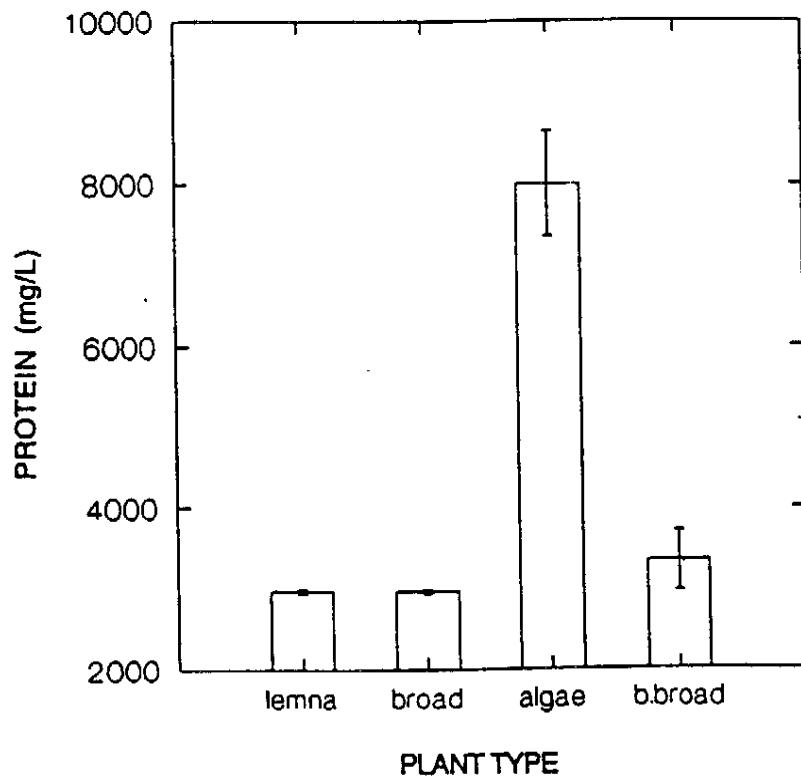


**KEY**

- 1 Chematopsyche (Collection 1)
- 2 Chematopsyche (Collection 2)
- 3 Simuliidae Pupa
- 4 Simuliidae Adult
- 5 Hydropsyche (Collection 1,2)
- 6 Hydropsyche (Collection 3)
- 7 Gomphidae
- 8 Molluska
- 9 Simuliidae Larva
- 10 Chironomidae (Collection 2)
- 11 Chironomidae (Collection 3)
- 12 Limnephilidae
- 13 Heptageniidae
- 14 Midge
- 15 Tipulidae Adult
- 16 Trichoptera Adult

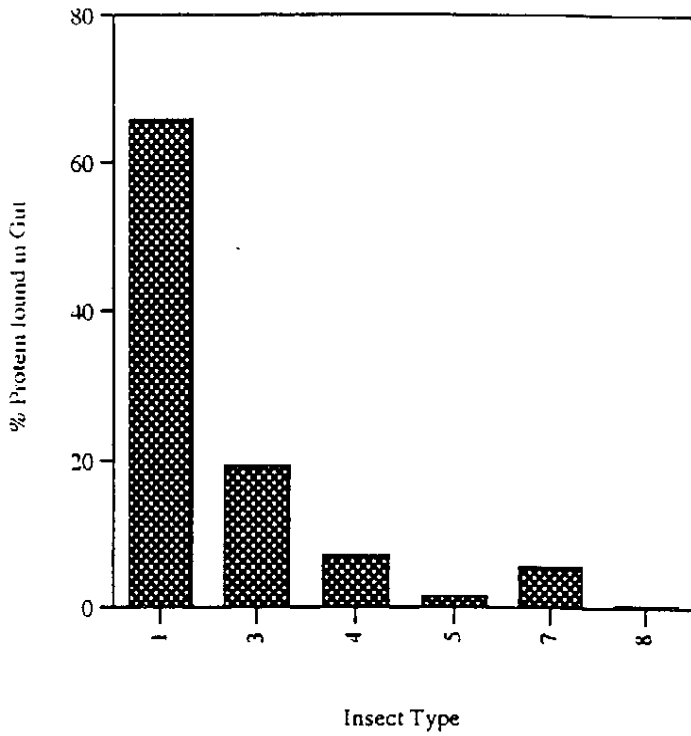
**Figure 9.** Average protein per individual insect. Simuliidae pupa protein numbers were generated using the TKN number divided by the number of individuals. However, in the stream many pupa cases were found without insects in them; therefore, the actual protein ingested by the fish would actually be lower than  $192.4 \text{ mg/l}$

## Plant Protein

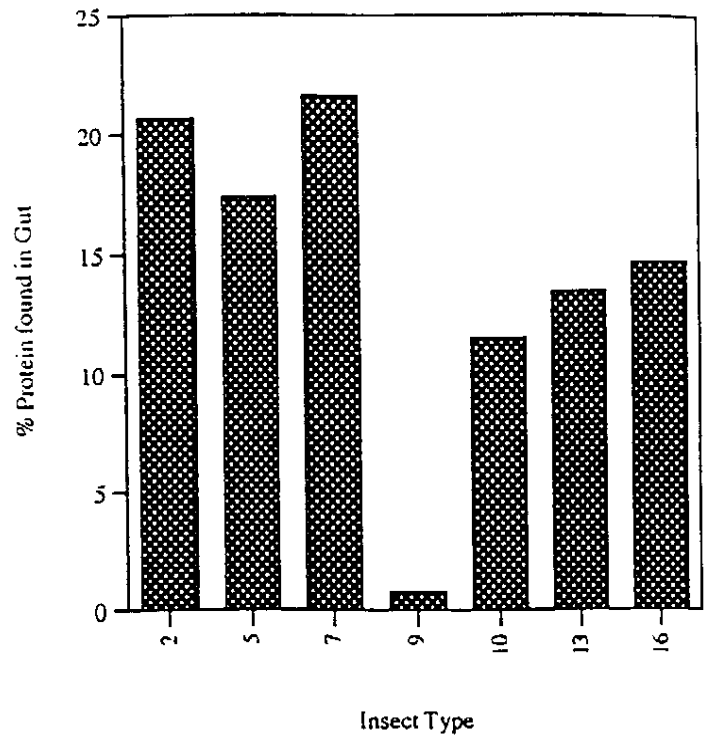


**Figure 10.** Protein in plant matter. Algae has nearly three times the protein that stream macrophytes have. Macrophytes have approximately the same protein content as one another. All types of plants were eaten, but algae was eaten in greatest abundance.

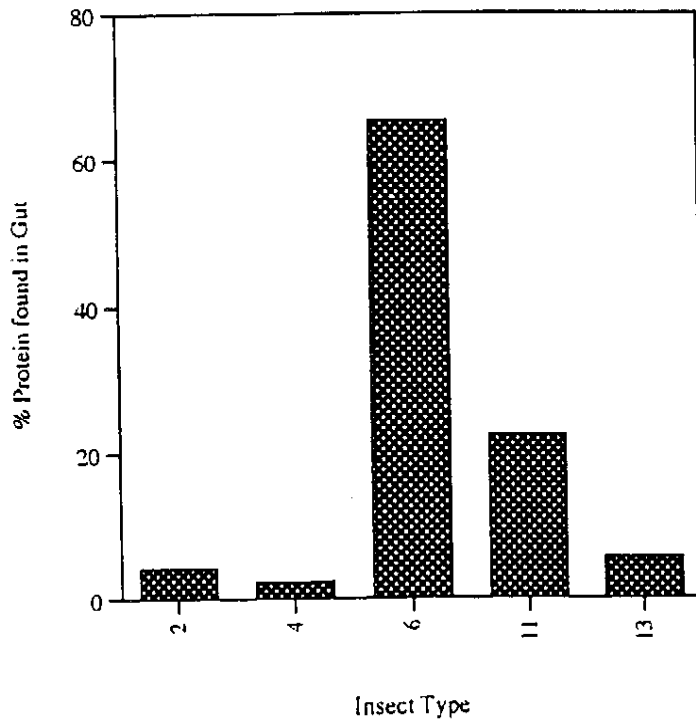
Percent Protein of Gut in  
Collection 1 Shiners



Percent Protein of Gut in  
Collection 2 Shiners



Percent Protein of Gut in  
Collection 3 Shiners



**KEY**

- 1 Chematopsyche (Collection 1)
- 2 Chematopsyche (Collection 2)
- 3 Simuliidae Pupa
- 4 Simuliidae Adult
- 5 Hydropsyche (Collection 1,2)
- 6 Hydropsyche (Collection 3)
- 7 Gomphidae
- 8 Molluska
- 9 Simuliidae Larva
- 10 Chironomidae (Collection 2)
- 11 Chironomidae (Collection 3)
- 12 Limnephilidae
- 13 Heptageniidae
- 14 Midge
- 15 Tipulidae Adult
- 16 Trichoptera Adult

**Figure 11.** Percent protein of gut by collection. Hydropsyche protein contribution increased while Chematopsyche protein contribution decreased with time.