THE UNIVERSITY OF MANITOBA

A STUDY OF PREY PREFERENCE AND SELECTION BY CREEK CHUB, *SEMOTILUS ATROMACULATUS*, IN THE MINK RIVER, MANITOBA

by

G.E. (BUCK) NEWSOME

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ABSTRACT

In the Mink River adult creek chub are selective feeders consuming mostly brook stickleback in early summer and crayfish in late summer despite the abundance of other species of potential prey. Johnny darters were the most highly preferred species of prey followed by cyprinids (pearl dace and common shiners), brook stickleback, and crayfish. Johnny darters were inaccessable to chub in the presence of a rocky substrate. The presence of vegetation reduced the accessability of brook stickleback. The presence of the cyprinid fright pheromone although detectable by chub had no influence on the preference of chub for cyprinids and did not appear to affect the accessability of cyprinids. An alternate mechanism controlling the accessability of cyprinids is discussed.

ACKNOWLEDGEMENTS

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INTRODUCTION

During summer, adult chub (Semotilus atromaculatus) form schools in deep pools and channels of streams, and are piscivorous (Barber and Minckley 1971; Moshenko and Gee 1973). Barber and Minckley (1971) suggested that chub of their study area were non-selective in their choice of fish prey since they ate mostly common shiner (Notropis cornutus) which was an abundant species cohabiting the stream with chub. However, observations of Moshenko and Gee (1973) indicated that chub may be selective in their choice of fish prey. Adult chub in the population they studied ate brook stickleback (Culaea inconstans) almost exclusively even though this species occurred in lower densities than a number of other potential prey fishes. They postulated that the avoidance of other species, mostly cyprinids, was due to the cyprinid fright pheromone (Pfeiffer 1962; 1963; Reed 1969). The objectives of this study were: 1) to determine the degree to which creek chub prey selectively on fishes and 2) to describe some of the factors that influence selection of prey.

Ivlev (1961) considered selection of prey to be controlled by two major factors: preference of the predator and accessability of the prey. Preference is an inherent characteristic of the predator, determined by physiological properties such as innate inclination, degree of satiation and conditioning. Estimates of a predator's preference can be obtained by maintaining a similarity in the accessability of potential prey. In this situation selection of prey by a predator would reflect preference (Ivlev 1961). Accessability is a more difficult parameter to investigate since it involves attributes of the predator and prey both of which can be further subdivided into contributing factors such as distribution, abundance, size, and behaviour (Ivlev 1961).

PLAN OF INVESTIGATION

An estimation of the degree to which creek chub prey selectively on fish was determined by comparing the proportion of each species of an edible size found in the environment occupied by chub with their proportion in the diet of chub. Significant differences in these proportions for any particular species would indicate selection or rejection. The extent to which accessability of prey influences their selection was determined by relating the frequency of different species in the diet of chub to their ranking in terms of preference. The preference of chub for different species of fish was determined in the laboratory by presenting chub with a number of dead, equally accessable, individuals of a number of species that chub commonly encounter in the Mink River. Experiments were designed to determine the effect of current, substrate, vegetation and abundance of alternate food on the accessability and resulting selection of prey by chub. Observations were also conducted in the field and laboratory to investigate the reaction of chub and potential prey to the cyprinid fright pheromone to determine if this affected accessability and selection of prey.

MATERIALS AND METHODS

Selectivity in Feeding by Chub

Eleven samples of creek chub and potential prey fishes were collected from four sites (500-700 m apart) in the middle zone (Gibbons and Gee 1972; Moshenko and Gee 1973) of the Mink River, Manitoba, during June-August, 1972 (Appendices 1 and 2). To sample fish, a deep channel or sheltered pool (Moshenko and Gee 1973), 15-20 m in length, was quickly enclosed by barrier nets (6.2 m x 1 m, 3.5 mm mesh) to block the possible escape of fishes. Then a two-man seine (1.4 m x 2.0 m, 3.5 mm mesh) was used to capture all fishes. On capture, fishes were placed in containers of water and seining continued in all portions of the site until no further captures were made.

Since fish become a major component of the diet of creek chub approximately 80 mm fork length (Moshenko and Gee 1973), chub greater than 90 mm standard length were kept for analysis of stomach contents. They were killed in MS222, a solution of 10% formalin was injected into the anterior portion of the digestive tract to arrest further digestion, and fixed in 20% formalin. Of the remaining fishes captured, every second one was preserved so that estimates of the densities of potential prey fishes occuring in the same environment as the creek chub could be made. The rest were returned to the stream. Measurements of depth, temperature, flow, and area sampled were recorded upon completion of the sampling procedure (Appendix 1). Samples obtained were returned to the laboratory and washed in water for 24 hours and then preserved in 70 percent isopropyl alcohol.

Fish were identified and standard lengths (S L) measured ($\pm$ 1 mm).

Stomach contents of chub greater than 90 mm S L were removed and classified according to their dominant food item (by weight). Creek chub lack a well defined stomach and therefore the region between the esophagus and the second loop of the digestive tract was considered to be the stomach. Non-cypriniform fishes found in stomach contents of chub were identified by the shape of the opercular bone (Appendix 3) while species-specific differences in pharyngeal bones were used to identify cypriniform species (Appendices 3 and 4).

Creek chub were divided into two groups to simplify analysis of diet: Group 1 (90-129 mm S L), and Group 2 (>129 mm S L).

Size of fish that each group of chub caught and ate was determined from the estimated length of fish found in the stomachs of chub. These estimates were established from regression equations relating the logarithm of the standard length to the logarithm of the length of the anterior margin of the opercular bone for each of the 10 species of fish commonly found with the creek chub in the middle zone of the Mink River (Appendix 5). These measurements were used to correct the observed densities of potential prey fishes so that only densities of fishes that were 'edible' by chub were used in determining the degree of prey selectivity.

Electivity indices (Ivlev 1961) were calculated, where sufficient data were available for each of the fish species consumed by creek chub. This index (E) compares the proportion of food items observed in the diet (r_i) with the proportion of the food item observed in the environment (p_i) in the following manner,

$$E = \frac{r_i - p_i}{r_i + p_i}$$

The index varies between plus and minus one, so that indices greater than zero indicate that food items occur in the diet in a greater proportion than in the environment. Negative indices indicate an avoidance or inaccessability of the potential food item, and indices near zero indicate that the item is chosen in equal proportion to the occurrence of the item in the environment. Potential prey fishes too large to be eaten by chub were not considered in these calculations.

Feeding Preference of Creek Chub

Preference experiments were carried out in a 1200 l round fiberglass tank (1.81 m diam) filled to a depth of 0.6 m and supplied with a constant flow (500 l/h) of water at 12°C and 13 h period of light. The tank was screened with black curtains but observations could be made without disturbing the fish through five plexiglass windows in the sides of the tank below the water surface.

Eight chub (105-140 mm S L) were used as predators in all the experiments. Whenever possible chub from the Mink River were used but due to a winter-kill (1972-1973) in the study area chub became unavailable. In some tests chub from the Norquay Channel, Manitoba, were used. Immediately after collection, chub were placed in a 45 l aquarium at 12°C for 48 h. Both Norquay Channel and Mink River chub had been feeding on crayfish (*Orconectes virilis*) almost exclusively prior to capture (from analysis of feces). Chub were then placed in the experimental tank and acclimated for 5-7 days without food.

Two experiments each consisting of five replicates were performed. The first experiment used Norquay Channel chub and the second Mink River chub as predators. Prey, 10 each of brook stickleback, johnny darter (Etheostoma nigrum), pearl dace (Semotilus margarita), common shiner and crayfish, were killed by freezing. Swimbladders were punctured to ensure that all prey settled to the bottom of the experimental tank. The five prey species were randomly scattered in the tank 5-10 minutes before the beginning of the light period. Food items not consumed were removed and counted 12 h after the beginning of each replicate.

To test the possible effect of the fright pheromone on choice of prey by creek chub a preparation of the pheromone was added to the water during the first preference experiment. The pheromone was prepared by scraping the skin of a large pearl dace and washing in 2 l of water. This solution was added to the experimental tank at a rate of one drop per minute using a Mariotte bottle (Leduc 1966). It was considered that by maintaining a constant presence of the pheromone during this experiment, the effect of the fright reaction on prey choice by creek chub would be reduced or eliminated. This procedure was not followed during the second preference experiment so that any differences in prey selection would either be the result of the presence of the pheromone or of differences in preference between Norquay Channel and Mink River chub. To reduce possible contamination with fright pheromone of the dead cyprinid prey, they were immersed in tap water for 30 min and rinsed with distilled water before being scattered in the experimental tank.

Factors Affecting Selection of Prey

Five selection experiments were performed (Table 1) in the same tank utilizing the same eight predators from the Norquay Channel and Mink River as in the preference experiments. Unlike the preference experiments, chub had access to the 'prey spectrum' for 24 h. All prey were living and were removed, counted, and new prey added each day at the beginning of the light cycle. These experiments were conducted so that comparisons in food selection by creek chub from both live and dead (preference experiments) prey 'spectra' could be made, and to determine the effect of various combinations of current, substrate, vegetation, and high density of crayfish, on the choice of prey by chub.

The first and second experiments were performed immediately following the respective preference experiments in the bare experimental tank to examine selection of live prey by Norquay Channel and Mink River chub. Results of these two experiments were then compared with those of the two preference experiments, which used dead prey, as described above.

The remaining three selection experiments were performed to investigate possible environmental factors which might influence prey selection by creek chub. These experiments were conducted in sequence using Norquay Channel chub following the completion of the first selection experiment described above (Table 1).

The third experiment tested the effect of presence and/or absence of current and substrate on selection of prey. Current (20 cm/sec) was maintained in the circular tank by using two submersible pumps. Substrate consisted of rocks, 3-10 cm diam glued to four removable fiberglass plaques.

TABLE 1. Summary of the conditions used in each test of the five selection experiments conducted using Norquay Channel or Mink River creek chub. Five replicates trials were performed in each test. [STBK=brook stickleback, JD=johnny darter, PD=pearl dace, CS=common shiner, CRFH=crayfish, + present, - absent]

Sel. Exp.	Test No.	Origin of Predators	No. Prey per Replicate	Features investigated		
				current	substrate	vegetation
1	1	Norquay Ch.	10 each of STBK, JD, PD, and CS	-	-	-
2	1	Mink R.	10 each of STBK, JD, PD, CRFH	-	-	-
3	1	Norquay Ch.	10 each of STBK, JD, PD, and CS	+	-	-
3	2	"	"	+	+	-
3	3	"	"	-	+	-
4	1	"	10 each of STBK, JD, CRFH, 5 each of PD and CS	+	+	-
4	2	"	"	+	+	+
4	3	"	"	+	-	+
5	1	"	5 STBK, 3 JD, 5 PD, 1 CS, 26 CRFH	+	+	+

The fourth experiment was designed to investigate the effect of substrate and vegetation on the choice of prey by chub. 'Vegetation' consisted of dark green plastic sheeting cut into strips anchored to a hardware cloth frame and bouyed up by small pieces of styrofoam.

The fifth experiment was conducted to investigate the effect of large numbers of crayfish on the choice of prey by the chub. The relative numbers of prey used closely resembled the occurrence of the same prey in the Mink River during June of 1972. Current, substrate, and vegetation were provided as described above.

A pool was found in the middle zone of the Mink River which afforded excellent conditions for observing behaviour of chub toward potential prey and responses to the cyprinid fright pheromone. A total of approximately 10 hours were spent observing the chub using facemask and snorkel, the longest single observation period being 1.5 hrs.

Field tests to determine the response of chub to the cyprinid fright pheromone and its possible effect on prey selection were performed on June 29, July 9, and July 26, 1972, as follows. Cyprinids (pearl dace, blacknose dace (Rhinichthys atratulus), or common shiners) and non-cyprinids (brook stickleback or johnny darter) were offered separately to a school of creek chub and their behaviour recorded. The offered fish was pithed, squashed slightly, and held tightly between thumb and first finger. The number of attempts to seize it by large creek chub were counted for one minute. The procedure was repeated only four times on any one particular occasion, twice for non-cyprinid and twice for a cyprinid prey in sequence as follows:

1) non-cyprinid prey presented (1 min),

2) recovery period (15 min),

3) cyprinid prey presented (1 min),

4) recovery period (15 min),

5) non-cyprinid prey presented (1 min),

6) recovery period (15 min),

7) cyprinid prey presented (1 min).

Steps 1 and 3 were designated first test results and those at steps 5 and 7 as second test results.

Laboratory experiments were also conducted to observe and quantify the response of creek chub to three sources of the fright pheromone. Five chub (between 100 and 120 mm S L) were placed in a 270 l modified stream tank (Gee and Bartnik 1969) with a running water supply at $12^{o}C$. An extract of the pheromone was prepared as outlined by von Frisch (1941), using a sacrificed cyprinid in three experiments (creek chub, pearl dace, and blacknose dace respectively). The test tank (45 cm x 90 cm x 50 cm) was isolated from external visual stimuli and the extract introduced using an hydraulically-operated remote control syringe. The observation side of the aquarium was marked off in four equal horizontal rows such that the depth position, using the eye of each fish, could be recorded at the end of each minute of observation. Location of chub was recorded at one minute intervals for 15 minutes in the first experiment, and 10 minutes in the second and third experiments, prior to introduction of the fright pheromone. After introduction of the pheromone the location of the chub was recorded for 10 minutes in the first experiment and for 5 minutes in the second and third.

RESULTS

Selectivity in Feeding by Chub

Summer diet of creek chub. Fish were the major food of group 1 (90-129 mm S L) chub through most of the summer although there was a general decline in the proportion of stomachs containing them as the season progressed (Table 2). Crayfish were the dominant item in only 16-32% of the stomachs examined although they were the major food item during the last sampling period. 'Other' food items (primarily aquatic insect larvae, but also terrestrial insects, molluscs and leeches) were dominant in 29% or less of the stomachs examined.

Group 2 (> 129 mm S L) chub change their diet very significantly during the summer (Table 2). In early June the dominant food item was fish which were found in 65% of the 31 stomachs examined. Crayfish accounted for 13% and aquatic and terrestrial insects only 6%. By early July, the proportions of fish and crayfish were reversed with little change in the contribution by aquatic and terrestrial insects. Crayfish continued to increase as the dominant food item through July at the expense of fish but by early August a sharp decline was observed.

In the August sample a substantial increase in the proportion of empty stomachs among both groups of creek chub was also observed.

Size of fish consumed by creek chub. Group 1 chub consumed significantly ($p < 0.001$; analysis of variance) smaller prey than did group 2 (Fig. 1). The size range of prey that each group caught and ate were considered to be plus and minus two standard deviations of the mean. Thus creek chub of group 1 and 2 could catch and eat prey

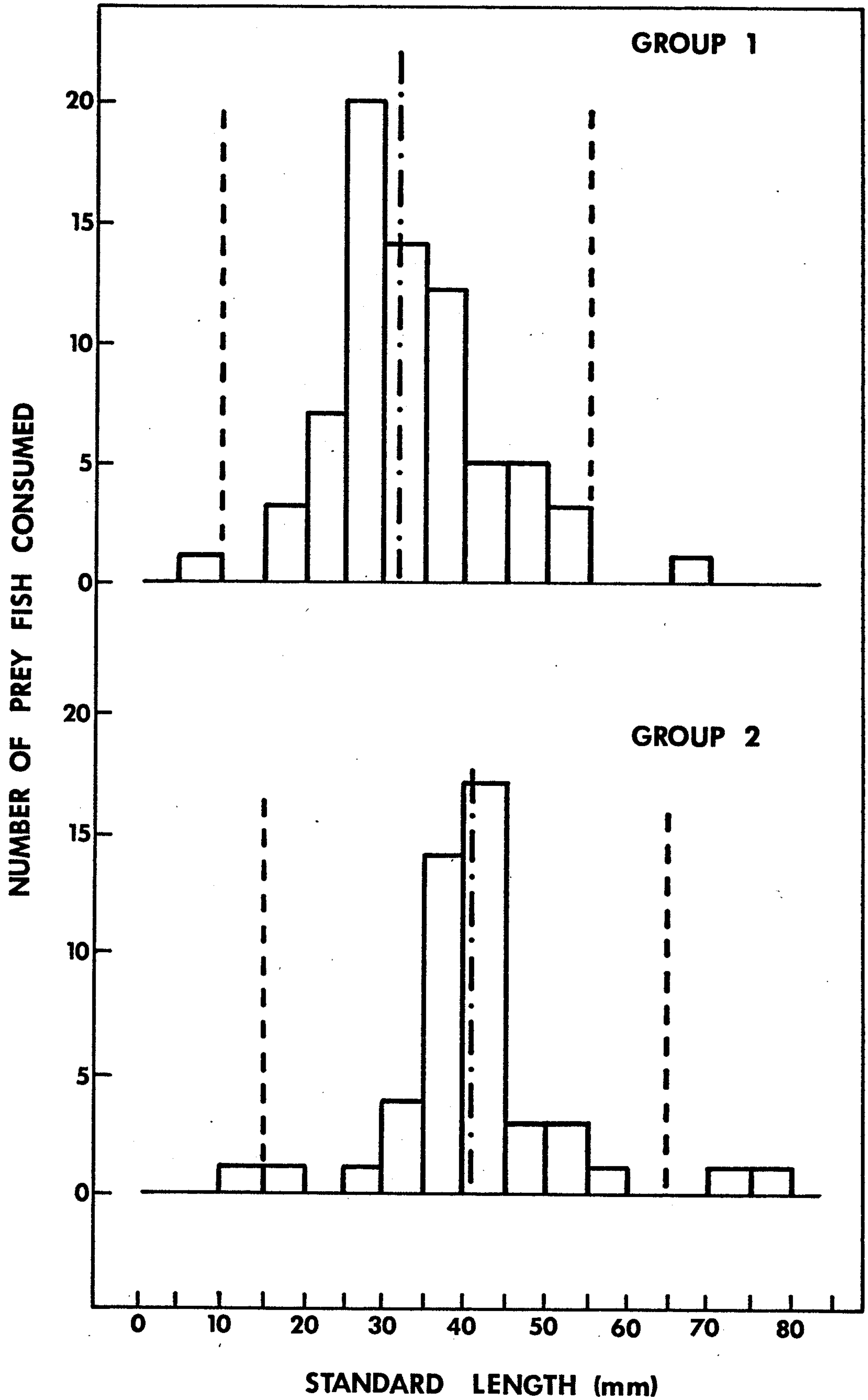
TABLE 2. Major components of the summer diet of creek chub expressed as a percent of the total number of stomachs examined, each of which were classed according to the dominant food item by weight.

Creek chub Group	Date	Percent of stomachs in which the dominant prey were: Fish	Crayfish	'Other'	% of stomachs empty	No. of stomachs examined
1 (90-129 mm S L)	Jun 6-7	56	16	28	0	25
	Jul 7-9	39	32	29	0	38
	Jul 25-26	63	21	8	8	24
	Aug 10	13	29	13	46	24
2 (> 129 mm S L)	Jun 6-7	65	13	6	16	31
	Jul 7-9	26	61	9	4	23
	Jul 25-26	0	82	9	9	11
	Aug 10	12	20	4	64	25

FIGURE 1. Frequency distribution for the estimated standard lengths of prey fish found in the stomachs of size groups 1 and 2 creek chub.

-.- = mean

--- = two standard deviations of the mean, rounded to the nearest class interval.


GROUP 1
GROUP 2
NUMBER OF PREY FISH CONSUMED
STANDARD LENGTH (mm)
0
5
10
15
20
0
10
20
30
40
50
60
70
80

between 10-50 mm and 15-60 mm S L respectively.

Relative frequency of stickleback and other fishes in diet of creek chub and in environment. Although large numbers of non-stickleback prey occur with the creek chub, they contribute very little to the fish diet. This is especially evident among group 2 chub (Fig. 2).

In late summer there is no apparent relation between the proportion of brook stickleback present in the stream and their occurrence in the diet of group 1 chub (Fig. 2). Within almost all size classes of potential prey fish that the chub can handle, non-stickleback fish outnumber the stickleback although the occurrence of non-stickleback fish in the diet is very low.

Electivity indices of prey sëlection by both groups of creek chub, calculated for brook stickleback and 'other' fishes are given in Table 3, based on the data for early and late summer (Appendix 7 and 8). The indices indicate a strong positive sëlection for brook stickleback and an avoidance of 'other' fishes by both groups of chub.

Feeding Preference of Creek Chub

There was no significant difference (goodness-of-fit test, Sokal and Rohlf 1969; see Appendix 9) between Norquay Channel and Mink River creek chub in the proportions of dead prey consumed, shown as follows (refer to Table 1 for the key to symbols used for prey fishes):

Species	STBK	JD	PD	CS	CRFH	Total
No. of prey consumed:						
Expt. 1, Norquay Ch. chub	13	46	22	25	2	108
Expt. 2, Mink R. chub	10	43	26	13	5	97
	23	89	48	38	7	205

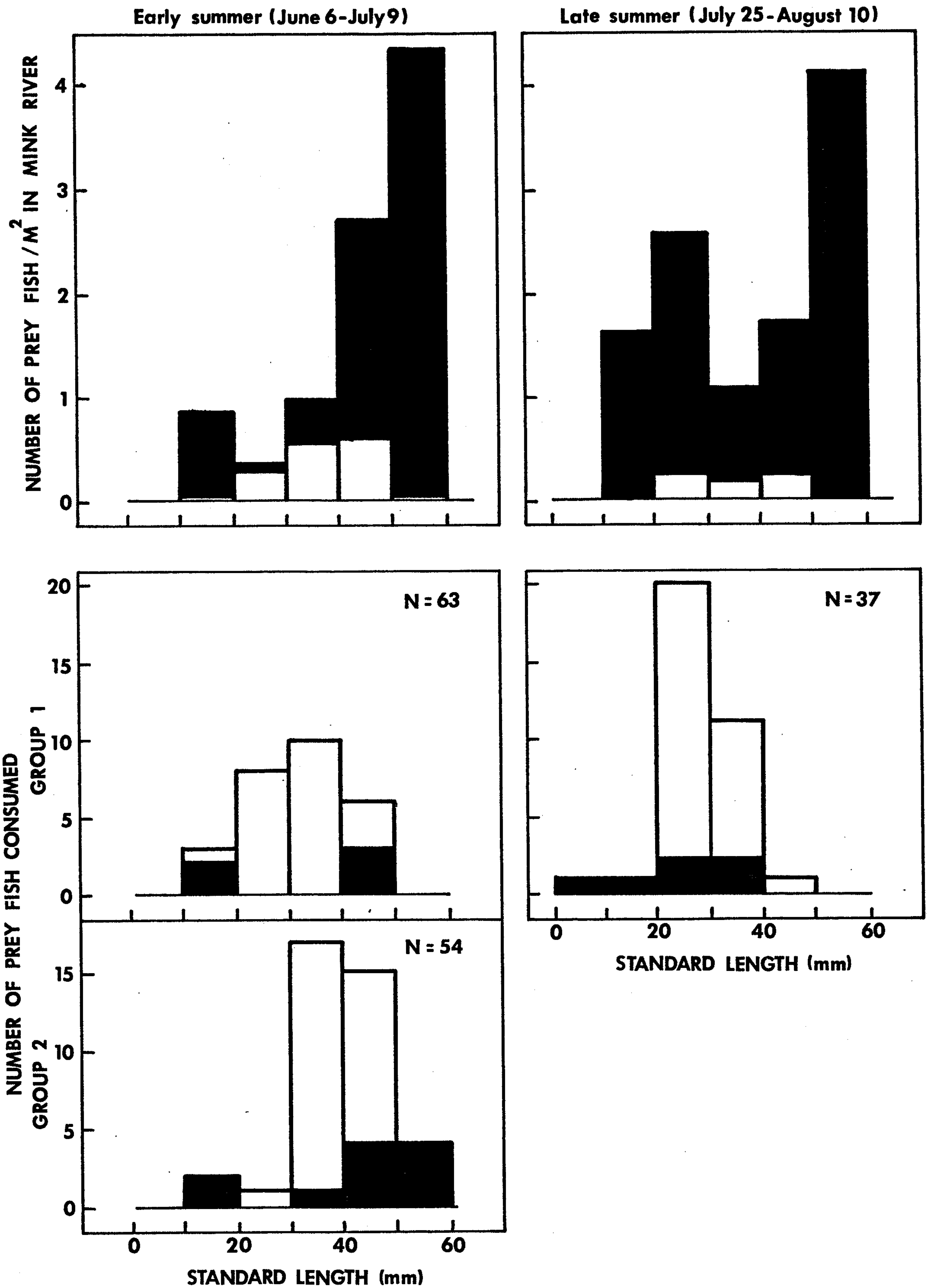
TABLE 3. Electivity indices by both size groups of creek chub, during early and late summer, toward brook stickleback (STBK) and 'other' fishes. The column 'density of fishes present', represents all fishes in the size range 'edible' by creek chub.

Chub Size Group	Time	No. of STBK consumed	Total fishes consumed	Density of STBK present (m^{-2})	Density of fishes present	Electivity STBK	Electivity 'Other'
1	early summer	22	32	1.42	8.22	0.60	-0.45
2	early summer	29	45	1.41	19.68	0.63	-0.41
1	late summer	30	36	0.67	8.59	0.83	-0.69
2	late summer	1	2	0.67	10.43	0.77	-0.30

FIGURE 2. Frequency distribution of prey fish found in stomach contents of creek chub compared with densities of prey fish in the Mink River. A figure for group 2 chub in late summer is not given since only two fish were eaten.

brook stickleback

'other' fishes


Early summer (June 6-July 9)
Late summer (July 25-August 10)
NUMBER OF PREY FISH / M² IN MINK RIVER
0
1
2
3
4
N = 63
N = 37
N = 54
NUMBER OF PREY FISH CONSUMED
GROUP 1
GROUP 2
0
5
10
15
20
0
20
40
60
STANDARD LENGTH (mm)

Pooling the results of the two preference experiments demonstrated that a definite preference was shown by the chub for at least one of the five species of prey ($p < 0.001$). Ivlev's index of electivity was calculated on the total number of prey consumed in both experiments. Johnny darters were highly preferred while brook stickleback and crayfish were strongly rejected, shown as follows:

Species	JD	PD	CS	STBK	CRFH
Electivity index	0.37	0.08	-0.04	-0.28	-0.71

However, inspection of the results (Appendix 9) also showed a slight change in the degree of selection for each prey consumed over the period of five trials (Fig. 3). Electivity for johnny darters changed very little from the first to the fifth trial. Electivity for both pearl dace and common shiners fluctuated more widely about zero, while, the electivity of creek chub toward both stickleback and crayfish decreased with time. The overall effect caused a decline in the total number of prey consumed but preferred items generally rose in their contribution to the chub's diet while non-preferred items declined.

Factors Affecting Prey Selection

<u>Selection of live prey</u>. The pooled results of the two experiments in which Norquay Channel and Mink River creek chub selected food from a 'spectrum' of live prey (Appendix 10) are given as follows:

Species	STBK	JD	PD	CS	CRFH	Total
No. of prey consumed:						
Norquay Ch. chub	0	30	2	3	-	35
Mink R. chub	34	12	18	-	5	69

FIGURE 3. Changes in the electivity of creek chub toward five prey species over five food portions (see text).

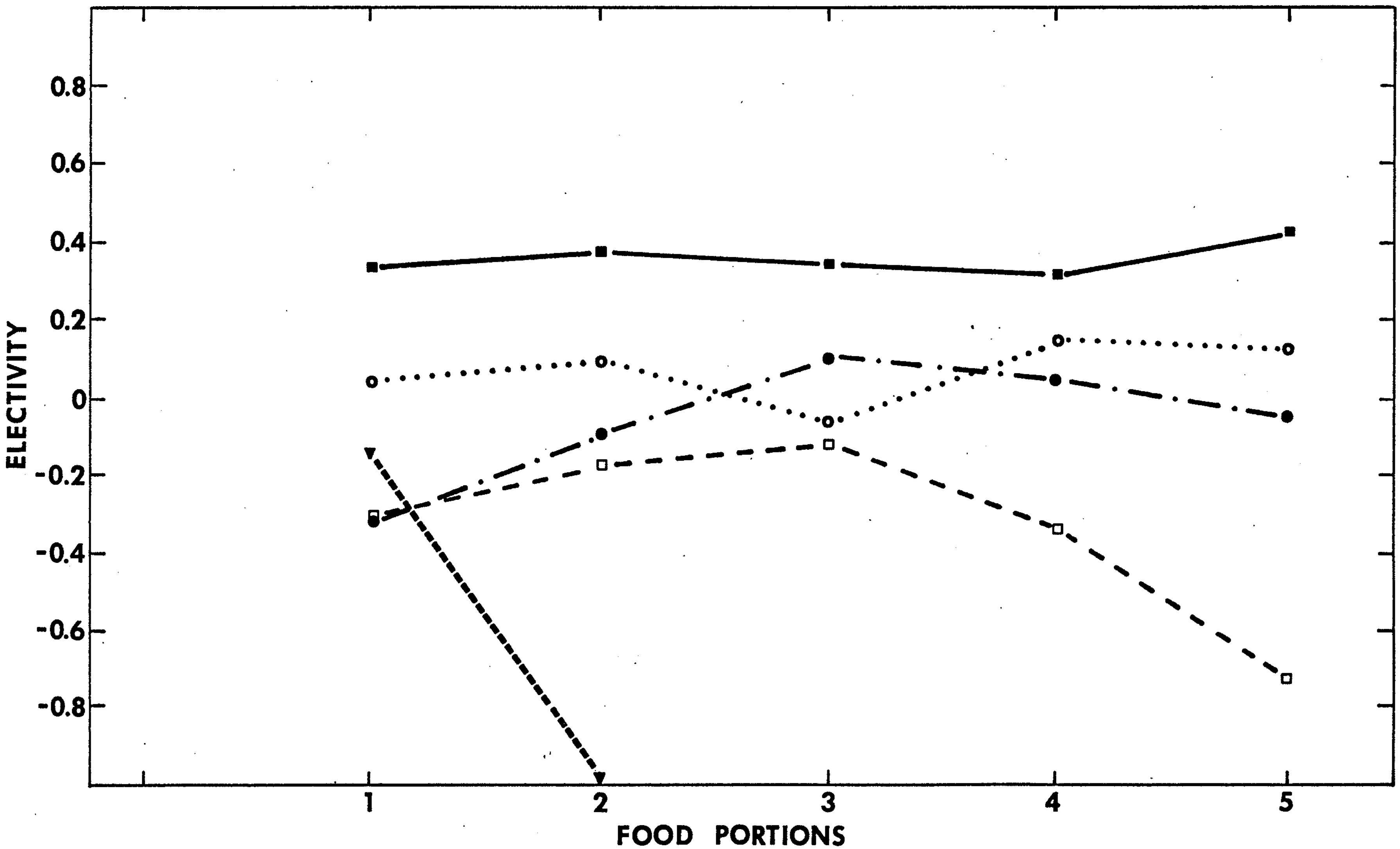
■——■ johnny darter

o······o pearl dace

▼----▼ crayfish

□– – –□ brook stickleback

●—·—● common shiner



0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
ELECTIVITY
1
2
3
4
5
FOOD PORTIONS

Analysis showed a significant ($p < 0.001$, test of independence omitting common shiner and crayfish data) lack of independence in the selection of live prey between Norquay Channel and Mink River chub. Therefore Norquay Channel and Mink River chub select prey differently. Comparison of these results, in a similar manner, with the results of the preference experiments also showed a significant ($p < 0.001$) difference between the selection of live and dead prey by both Norquay Channel and Mink River chub (Appendix 10). However, the reasons for the differences in prey selection were found to be different upon closer examination of the data.

Selection of live prey by Norquay Channel chub showed a disproportionate decrease in the numbers of brook stickleback, pearl dace, and common shiners consumed when compared with their selection from a 'spectrum' of dead prey. However, johnny darters were the dominant selection from both live and dead prey 'spectra' although relatively more live than dead johnny darters were consumed as follows:

Species	JD	Others (STBK + PD + CS)	Total
No. of prey consumed:			
Dead	46	60	106
Live	30	5	35

On the other hand, examination of the selection by Mink River chub from 'spectra' of live and dead prey showed that the dominant item consumed differed. From a 'spectrum' of live prey, the dominant choice was brook stickleback whereas from a 'spectrum' of dead prey Mink River chub chose johnny darters. At the same time, selection of other components from both live and dead prey 'spectra' did not differ significantly as follows:

Species	STBK	JD	Others (PD + CRFH)	Total
No. of prey consumed:				
Dead	10	43	31	84
Live	34	12	23	69

Comparisons of the total numbers of live and dead prey consumed by Norquay Channel and Mink River chub also demonstrated that Norquay Channel chub consumed proportionately fewer ($p < 0.001$, 2x2 test of independence) live than dead prey than did Mink River chub as follows:

Prey condition	Dead	Live	Total
No. of prey consumed:			
Norquay Ch. chub	108	35	143
Mink River chub	97	69	166
Total	205	104	309

<u>Effects of current, substrate, and vegetation on prey selection.</u>

Analysis of experiments (Appendix 11) comparing selection by chub from a 'spectrum' of live prey in the presence and absence of current and a rock substrate demonstrated that current had no significant éffect on prey choice as follows:

Species	STBK	JD	PD	CS	Total
No. of prey consumed:					
current absent	15	38	3	5	61
current present	10	34	3	4	51

However, in the presence of a rock substrate, a significant ($p < 0.001$) change in selection of prey was observed as follows:

Species	STBK	JD	PD	CS	Total
No. of prey consumed:					
substrate absent	3	57	3	6	69
substrate present	22	15	3	3	43

When a rock substrate was provided, chub chose brook stickleback as their dominant prey while in its absence the johnny darter was selected. Also, the presence of the rock substrate significantly ($p < 0.025$, analysis of variance) reduced the total numbers of prey consumed.

The presence/absence of substrate and vegetation affected the selection of live prey by chub (Appendix 12) as follows:

Cover		No. of prey consumed				
Substrate	Vegetation	STBK	JD	CYP	CRFH	Total
present	absent	45	27	7	9	88
present	present	11	10	6	0	27
absent	present	17	39	4	6	66

Analysis demonstrated a significant ($p < 0.001$, test of independence) difference in the selection of prey between the three combinations of environmental protection offered. When a rock substrate was the only form of protection present, brook stickleback were the dominant prey selected by the chub whereas when only simulated vegetation was present johnny darters dominated. When both forms of protection were included in the test brook stickleback and johnny darters were selected in similar numbers. The numbers of cyprinids (CYP) and crayfish consumed by the chub were not significantly affected by the combinations of cover provided.

High abundance of an alternate prey. The results of the experiment investigating prey selection by chub when crayfish dominated the 'spectrum' of prey presented (Appendix 13) showed that the numbers of each prey species consumed were proportional to the numbers presented as follows:

Prey species		Total No. presented	Number consumed	
			Observed	Expected
STBK		25	1	2.1
JD		15	2	1.3
CYP		30	1	2.5
CRFH		130	13	11.1
	Total	200	17	17.0

Clearly, when crayfish, a low preference food of creek chub, were abundant, they formed the dominant item selected as prey.

To demonstrate that the predators, in particular the Norquay Channel creek chub, did not become conditioned during each of the selection experiments, tests of homogeneity were performed. In all but one of the experimental tests, the replicated results were homogeneous. In the one experimental test which showed some heterogeneity (Appendix 12), examination of the results indicated that the cause was due to a greater proportion of crayfish and brook stickleback being consumed in the first and second replicates respectively, and not because of any conditioning of the predator to consume proportionally greater numbers of one prey as testing proceeded.

Field and laboratory observations of cyprinid fright reaction. The pool (Fig. 4) was inhabited by 6-10 chub (100-200 mm S L). The dominant landmark of the pool was a large boulder, approximately 1 m diam,

FIGURE 4. Diagrams of observation pool in the Mink River to show the relative distributions of fish species.

A. Surface view.

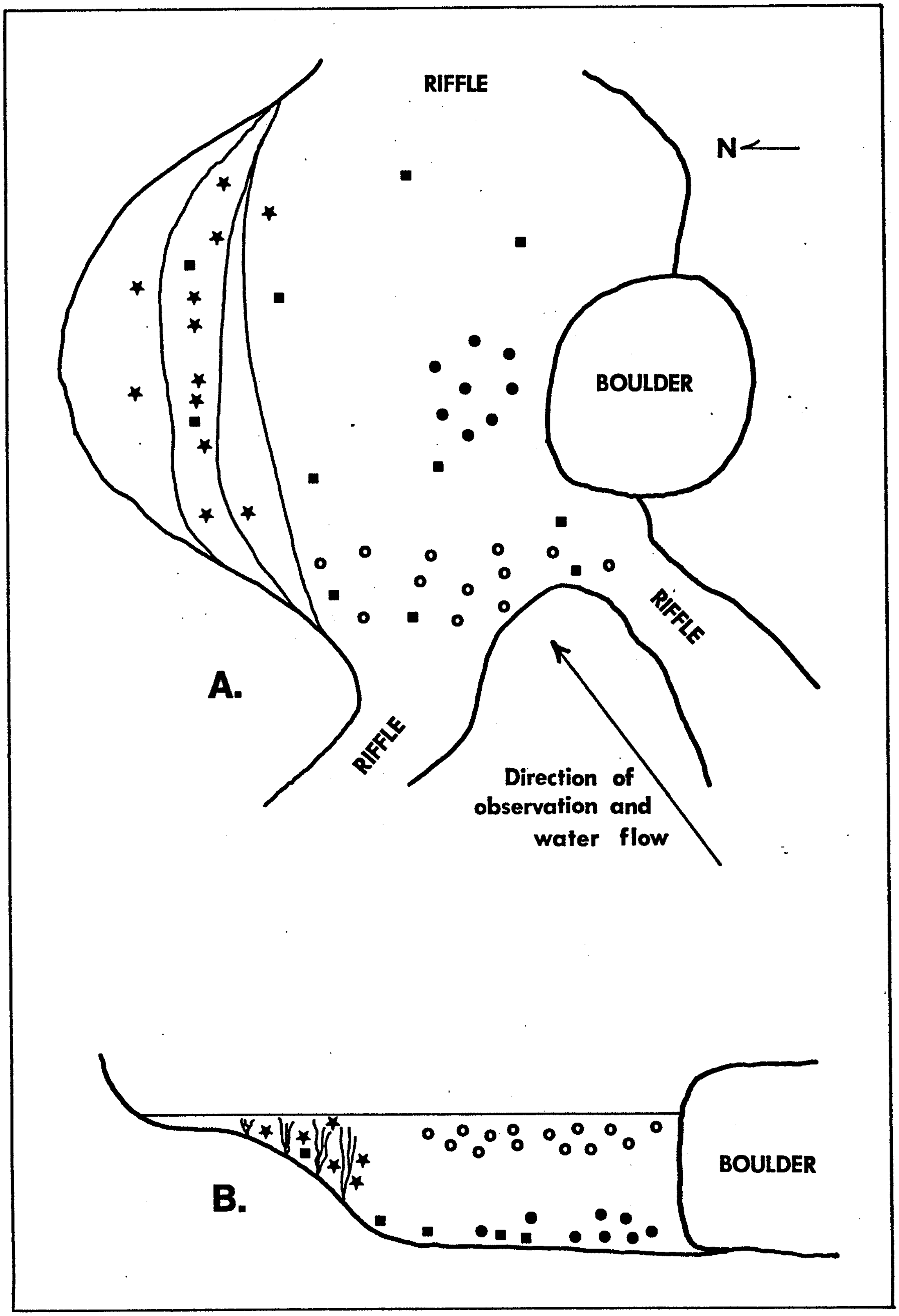
B. Profile

● large creek chub

✱ brook stickleback

■ 'bottom species' (longnose dace, blacknose dace, johnny darter, common sucker)

o 'mid-water schooling species' (pearl dace, small creek chub, brassy minnow, common shiner)



RIFFLE
N
BOULDER
RIFFLE
A.
RIFFLE
Direction of observation and water flow
BOULDER
B.

located in the south bank and projecting into the pool. Chub spent most of their time in close proximity to this boulder, just above (10-20 cm) the stream bottom. Brook stickleback were most abundant in shallow regions of vegetation in the pool along the north side of the stream. They seldom ventured near the surface except when pursued by chub. Bottom fish, such as blacknose dace (Rhinichthys atratulus), johnny darter, and common suckers were observed scattered throughout the pool but generally avoided the region occupied by the large creek chub. The bottom of the pool was scattered with rocks (2-20 cm diam). The shallow region, however, had a smooth, silt, clay and sand bottom. Small common suckers were generally more common in the sand and clay bottom region and the darters and dace in the rock bottom region. Mid-water schooling species, pearl dace, smaller creek chub (0+ and 1+ year classes), common shiners and brassy minnows (Hypognathus hankinsoni), were generally found together below the inlet riffles of the pool. They occurred approximately 5-30 cm below the surface and appeared to feed on drift organisms and detritus. Large chub were observed, on many occasions, to pursue individuals of this group that drifted backwards above the region where the large chub were located.

Results of the three field experiments performed to investigate the response of creek chub to non-cyprinid and cyprinid prey (Appendix 14), are pooled as shown in Table 4. Significantly ($p < 0.001$) more attacks per minute were made by chub on non-cyprinid prey than on cyprinid prey. Also, the number of attacks during the second tests were considerably reduced.

When a stickleback or johnny darter was injured and offered to the chub, attacks began immediately by both the large chub and also

TABLE 4. Pooled results of field tests investigating the response, measured in number of attacks/min, of creek chub to non-cyprinid and cyprinid prey.

Test No.	Number of attacks on prey offered for one minute: Non-cyprinid	Number of attacks on prey offered for one minute: Cyprinid	Total
1	50	5	55
2	13	1	14
Total	63	6	69

other smaller species of fish pesent. However, when a dead small cyprinid was offered to the fish present, and the skin rubbed between the fingers, instantly the small fishes in the immediate surroundings vanished. The reaction was so rapid that tracking responses of individual fish was impossible. Non-cyprinid species also responded but the effect may have been caused by the flight of the cyprinids. Johnny darter and stickleback were the first species to return to the region, usually within 3-4 minutes. Cyprinids returned very slowly. Generally between 10 and 15 minutes was required for the numbers to return to the average before the next test was conducted.

The response of large chub to the fright pheromone was quite different to that of the smaller cyprinids, including the smaller creek chub. Instead of darting for cover they slowly drifted back and down toward the bottom of the pool. They gathered in a compact school, with their pectoral fins touching the rocks on the bottom, in the darkest part of the pool beside the large rock. This response lasted for 5-10 min. All fins were kept erect and frequent 'yawning' was observed by all members of the school.

At the end of the minute test period when the killed cyprinid prey was released to drift downstream, the large chub darted after it. One chub would consume the prey and all quickly returned to their 'hiding place' which they had sought after presentation of the injured cyprinid had first been made.

The pooled results of the three laboratory experiments (Appendix 15) conducted to investigate the reaction of creek chub to three sources of the cyprinid fright pheromone are shown as follows:

Depth (cm)	Frequency of Occurrence: Before introduction	After introduction
0 - 9	47	3
10 - 19	21	3
20 - 29	40	29
30 - 39	67	65

Chi-square tests of homogeneity between the results of the three experiments, before introduction of the pheromone, and after introduction, showed no significant difference. However, a Chi-square test of the hypothesis that the distribution of creek chub was the same after introduction of the pheromone as before introduction was rejected ($p < 0.001$). These results clearly show that chub are capable of detecting the presence of the pheromone at least from the three sources used: creek chub (Expt. 1), pearl dace (Expt. 2), and black-nose dace (Expt. 3). The response of the chub to the pheromone was very similar to that observed in the field. The chub glided to the bottom of the test tank with fins erect. Frequent 'yawning' by the chub was also observed.

DISCUSSION

In the Mink River during summer large creek chub (>90 mm S L) were selective feeders toward brook stickleback despite the presence of other, more abundant, fishes in the same environment. Given equal accessability, the order of preference of chub was; johnny darter, cyprinids (pearl dace and common shiners), brook stickleback and crayfish. A rock substrate reduced the accessability of johnny darters while vegetation reduced the accessability of brook stickleback. Consumption of crayfish increased in proportion to an increase in their density. The existence of a fright reaction (von Frisch 1938) among creek chub was confirmed but had no apparent effect on prey selection.

The observed diet of creek chub from the Mink River generally agrees with observations made by previous investigators (Forbes 1888; Hankinson 1910; Leonard 1927; Greeley 1930; Dinsmore 1962; Barber and Minckley 1971; Moshenko and Gee 1973). Barber and Minckley (1971) concluded that chub inhabiting the headwaters of the Mississippi River were non-selective in reference to their choice of prey fishes since common shiners were the dominant prey fish consumed by the chub and were also the most abundant species of the region studied. The present study and the observations of Moshenko and Gee (1973) demonstrated that Mink River creek chub were selective feeders, since proportionally more brook stickleback were consumed than were present in the environment.

Determining the 'preference order' of a predator is an important consideration since if a first preference prey becomes unavailable or inaccessable, then the second preference prey will become the dominant selection if it is available and accessable (Ivlev 1961).

Experiments showed that chub from Norquay Channel and Mink River exhibited similar strong preferences for prey other than the stickleback, which may in fact be general to creek chub.

The field study of prey selection by Mink River chub showed a strong selection for brook stickleback during early summer, switching especially among the larger chub, to crayfish by late summer. However, the preferred prey of both Norquay Channel and Mink River creek chub was the johnny darter and during early summer they were as abundant in the Mink River as brook stickleback. Clearly, johnny darters were inaccessable.

Accessibility of a prey to a predator may be reduced by a number of different physical and biological features, such as distribution, abundance, behaviour, use of shelter, and constitutional protection (including possession of armament, size, appearance, toxicity, etc.) (Ivlev 1961; Mauck and Coble 1971). Field and laboratory observations eliminated possession of armament, size, distribution, and relative abundance as being responsible for the reduced accessibility of johnny darters. Of those remaining, behaviour, constitutional protection, and use of shelter, the latter seemed most likely.

Johnny darters, lacking a swim bladder, remain in close proximity to the substrate. In an environmental situation such as the Mink River, a relatively clear stream with a bed well covered with rocks and boulders, they would be quite inaccessable to the creek chub. However, the selection experiments performed to examine this possibility, using both Norquay Channel and Mink River creek chub as predators provided with equally abundant live prey but without any type of 'environmental protection', gave contradictory results. Norquay Channel chub continued to select johnny darters while the Mink River chub switched to brook stickleback. In experiments employing a rocky substrate, Norquay Channel chub switched to the brook stickleback as the major diet item. These rather large differences in selective responses between Mink River and Norquay Channel creek chub to live prey, in the absence of environmental protection, lead to some interesting speculations as to possible causes.

The differences in the physical environments between the Mink River and Norquay Channel are striking although they are of similar size. The Mink River is a moderately fast-flowing stream with relatively clear water. The bottom is covered with rocks of all sizes. The Norquay Channel, on the other hand, is essentially a drainage ditch with slow-flowing, very turbid water. The bottom is heavily silted. The differences in turbidity may be important as well as differences in the potential prey fish populations. Previous studies indicate that creek chub can be considered sight feeders. Kuehne (MS 1958) was able to demonstrate that schooling behavior among blind chub and chub confined to aquaria with lighting levels less than 1.35 luxes, completely disappeared. Similarly, Barber (MS 1968) showed that feeding was reduced during hours of darkness. The high turbidity of the Norquay Channel probably reduces the chances of the creek chub locating and catching fish, forcing them to rely entirely on crayfish and other bottom organisms as a source of food. Fecal examinations of the creek chub collected from the Norquay Channel revealed that they had been feeding almost exclusively on crayfish. The situation in the Mink River is quite different where, although crayfish also contribute significantly to the chub's diet, small fish such as the brook stickleback are visible and at times (especially in early June) dominate as the major source of food.

The difference in turbidity, between the two streams suggests that Mink River chub develop not only a 'search image' (Tinbergen 1960) for crayfish but also for fish, particularly brook stickleback. Norquay Channel chub, on the other hand, may never develop a 'search image' for fish. Dietary conditioning is well documented in the literature (Ivlev 1961). This explanation also accounts for the large differences in the numbers of live and dead prey consumed by Mink River and Norquay Channel chub during the preference and selection experiments. The differences in response of Mink River chub in the number of live and dead prey consumed were not significant while the responses of Norquay Channel chub were highly significant. If Norquay Channel chub are conditioned to bottom feeding solely, while Mink River chub are used to searching the bottom and the water column (for fish) it would be expected that Norquay Channel chub would consume more dead than live prey. Similarly, one would not expect a large difference in the number of live and dead prey consumed by Mink River chub since they would be conditioned to searching for food located either on the bottom or in the water column. This explanation may also account for the continued selection of johnny darters by the Norquay Channel chub in the absence of a rock substrate since they are restricted to the bottom where the chub are conditioned to search for food. Mink River chub, on the other hand, may have developed a strong 'search image' for brook stickleback and thus chose them in the experiments using live prey without any form of environmental protection. The fact that the stickleback were chosen over both pearl dace and common shiners when the johnny darters became relatively inaccessable suggests that they are protected either behaviorally or because of their appearance as will be discussed below.

Experiments in which Norquay Channel chub were presented live prey provided with substrate in the form of rocks, clearly demonstrate that the johnny darter is environmentally protected, since the chub switched to the brook stickleback. This was an unexpected result in light of experiments conducted by Ivlev (1961). He found that when a preferred prey became inaccessable, the predator generally switched to the second preference prey. In experiments of this study, once the johnny darters were protected by the presence of a rocky substrate, pearl dace and/or common shiners should have been selected since in both experiments they ranked equally second on the preference list of the chub. During experiment in which both types of cover, substrate and vegetation, were provided, selection of johnny darters and brook stickleback by the creek chub were equalized since vegetation provided a shelter for the brook stickleback. However, the inaccessability of the stickleback and johnny darters had no effect on the use of cyprinids, or crayfish, as a food resource by the chub. Crayfish disappeared from the diet altogether while cyprinids were consumed in approximately the same quantities as observed in previous experiments. Clearly the crayfish used both types of environmental shelter to escape the chub while the pearl dace and common shiner used neither. These results again suggest that cyprinids are inaccessable to creek chub because of behavioural or consititutional protection.

Moshenko and Gee (1973) speculated that the cyprinid fright reaction (Pfeiffer 1962; 1963; Reed 1969) may be responsible for the apparent avoidance of cyprinid potential prey by Mink River creek chub. Kuehne (MS 1958) felt that the creek chub lacked the ability to detect the presence of the pheromone. However, results of the laboratory preference experiments, discussed below, and field observations conducted during the present study, revealed that creek chub possess the fright pheromone and are able to detect its presence. The nature of the chub's fright reaction probably lead Kuehne (MS 1958) to an erroneous conclusion.

Because the creek chub exhibits the cyprinid fright reaction does not necessarily support the hypothesis of Moshenko and Gee (1973), regarding the avoidance of potential cyprinid prey. Pfeiffer (1962; 1963) showed that the piscivorous northern squawfish (Ptychocheilus oregonense, Cyprinidae) relied heavily on another cyprinid, the redside shiner (Richardsonius balteatus) as a food resource. Young squawfish, however, exhibit the typical ostariophysan fright reaction. Pfeiffer (1963) demonstrated that first attempts, by maturing squawfish, to consume cyprinids generally resulted in the predator scaring itself but with repeated attempts a decline in the reaction was observed. A similar situation may indeed occur among the creek chub and experiments conducted to confirm this may prove interesting. A number of authors have shown that cyprinids are not infrequent food items of creek chub and cannibalism is not altogether unknown (Greeley 1930; Minckley 1963; Noble 1965; Barber and Minckley 1971). Whether in fact, the presence of the fright reaction among the chub's potential cyprinid prey reduces their accessability to the chub by 'warning' the cyprinids of an attack on an individual of their school and thereby increasing their wariness, remains to be tested. However, the identical results of the two preference experiments in which Norquay Channel chub selected dead prey in the continual presence of the fright pheromone and Mink River chub in the absence of the pheromone, suggest that it had limited or no effect on preference. Similarly, the results of the selection experiment in which Norquay Channel chub selected live prey in the absence of 'environmental protection' showed that cyprinids continued to rank second in the prey chosen. Although the experimental results do not appear to greatly favour the mechanism proposed by Moshenko and Gee (1973), another, possibly more important cause for the low vulnerability of the cyprinids may lie in behavioural protection.

Throughout the field observations made in the Mink River, during the summer of 1972, it was noted that the cyprinids, especially the pearl dace and common shiners cohabiting the pool with the creek chub, formed a heterogeneous school below the inlet riffle. This school generally remained between approximately 10 and 30 cm. from the surface. Although many attacks on individuals of this school by the creek chub were observed, none were successful. Many similar observations were made while conducting selection experiments in the laboratory experimental tank. Many of the attacks made on cyprinids, either pearl dace or common shiners, resulted in misses, the chub always falling short of the apparently intended target. It appeared that the predator was attacking the image of the prey reflected from the under surface of the water.

This phenomenon in relation to the importance of counter-shading (Marshall 1965) in protecting surface schooling fish requires further study. Both the common shiner and pearl dace are counter-shaded, dark dorsally and white to silver ventrally. When viewed from below by a predator, they may be very difficult to perceive. If the prey remain close to, but not at the surface, predators some distance away and below the prey would obtain an image of a dark object at the surface. This image would always be between the predator and the real prey. If the prey remains motionless and the surface of the water is slightly agitated, the image received by the predator is one of a moving object inviting attack. However, such a system of protection would only continue to operate as long as the predator is periodically rewarded with success in attacking a surface object. That such is the case in many stream situations inhabited by creek chub is revealed by the numbers of terrestrial insects, plant material, and amphibians, found in the stomach contents of creek chub (Barber and Minckley 1971; Moshenko and Gee 1973; the present study and other).

The laboratory results of this investigation do not entirely account for the greater utilization of brook stickleback by Mink River creek chub since vegetative cover which stickleback use as shelter is relatively abundant. Also, the mechanism described above for protecting surface schooling cyprinids such as the pearl dace and common shiner seems to be at variance with the observations made by Barber and Minckley (1971). The creek chub in their study area, the upper Mississippi River, used common shiners as a major food resource. The ultimate solution to these widely variant observations quite likely lies in a functional response (Solomon 1949; Holling 1966; 1969) by the predator to the changing densities of accessable and preferable prey. In the Mink River the pearl dace were occasionally present in densities greater than the brook stickleback, in the size range available to creek chub, while common shiners rarely were more abundant than the stickleback. Johnny darters were generally more abundant than the stickleback but the environment afforded them ample protection. An additional reason for the apparent greater vulnerability of brook stickleback to predation by the creek chub in the Mink River may lie in the fact that they are consumed in large numbers in June when the stickleback are at the peak of reproductive activity. The preoccupation with spawning and resultant displays, as well as a darker colouration especially among males, may increase their vulnerability to the chub and is a source of future experimentation and observation. Also during early summer the amount of aquatic vegetation is considerably less than in late summer.

That chub have an influence on the number of stickleback has been demonstrated by Weselowski (MS 1974). He noted a considerable rise in the density of brook stickleback in the Mink River following the severe winter conditions between 1972 and 1973, which greatly reduced the numbers of large, piscivorous creek chub.

The field observations of the diet of both Mink River and Norquay Channel creek chub demonstrated that crayfish were a major food resource while laboratory experiments clearly showed a strong avoidance of them. Although quantitative estimates of the densities of crayfish in the Mink River were not made, qualitative observations showed that they were clearly the dominant potential prey in most regions of the stream examined. Estimates of between five and 30 crayfish per square meter seem reasonable especially during the months of July and August. These observations suggested that density was a major factor controlling the utilization of crayfish as a food supply by creek chub and the laboratory experiment conducted using high densities of them support this conclusion. That creek chub consumed crayfish in proportion to their occurrance during the experiment in which crayfish were the most abundant prey available was unexpected since they are strongly rejected when in equal abundance with alternate prey. This suggests some other phenomenon increased the chub's selectivity toward the crayfish. One possible explanation is that the chub develop a 'search image' for crayfish (Tinbergen 1960) or at least become conditioned to handling them. Royama's theory (1970) suggests another; that the chub become conditioned to hunting where crayfish occur, that is, on the bottom, at the expense of hunting elsewhere in the environment. However, an alternate explanation, based on observations while conducting these experiments, can be proposed. At greater densities, the amount of suitable shelter for crayfish may not meet their requirements and probably results in greater aggressive interaction which further increases their vulnerability. Although further experimentation is required, it seems reasonable that an additional component should be added to the six components proposed by Holling (1966) involving the functional responses of a predator to prey density. This component could be referred to as prey interference.

The reason that crayfish rank so low on the preference scale of creek chub may lie in the fact that they represent a considerably lower net gain in calories per gram of dry weight than do fish. Crayfish on the average represent, in total calories per gram of dry weight, approximately 2000 calories including the indigestible exoskeleton while fish represent between 4000 and 5000 calories per gram of dry weight (Cummins and Wuycheck 1971).

The present investigation into selective feeding by Mink River creek chub has shown that although johnny darters and cyprinids rank higher on the 'preference scale' of the chub and are present in relatively large numbers, they are inaccessable because of environmental and behavioural or constitutional protection. Faced with this situation the chub first select third preference brook stickleback as a food resouce during early summer, but switch to crayfish which become very abundant and possibly more vulnerable during the latter part of the season.

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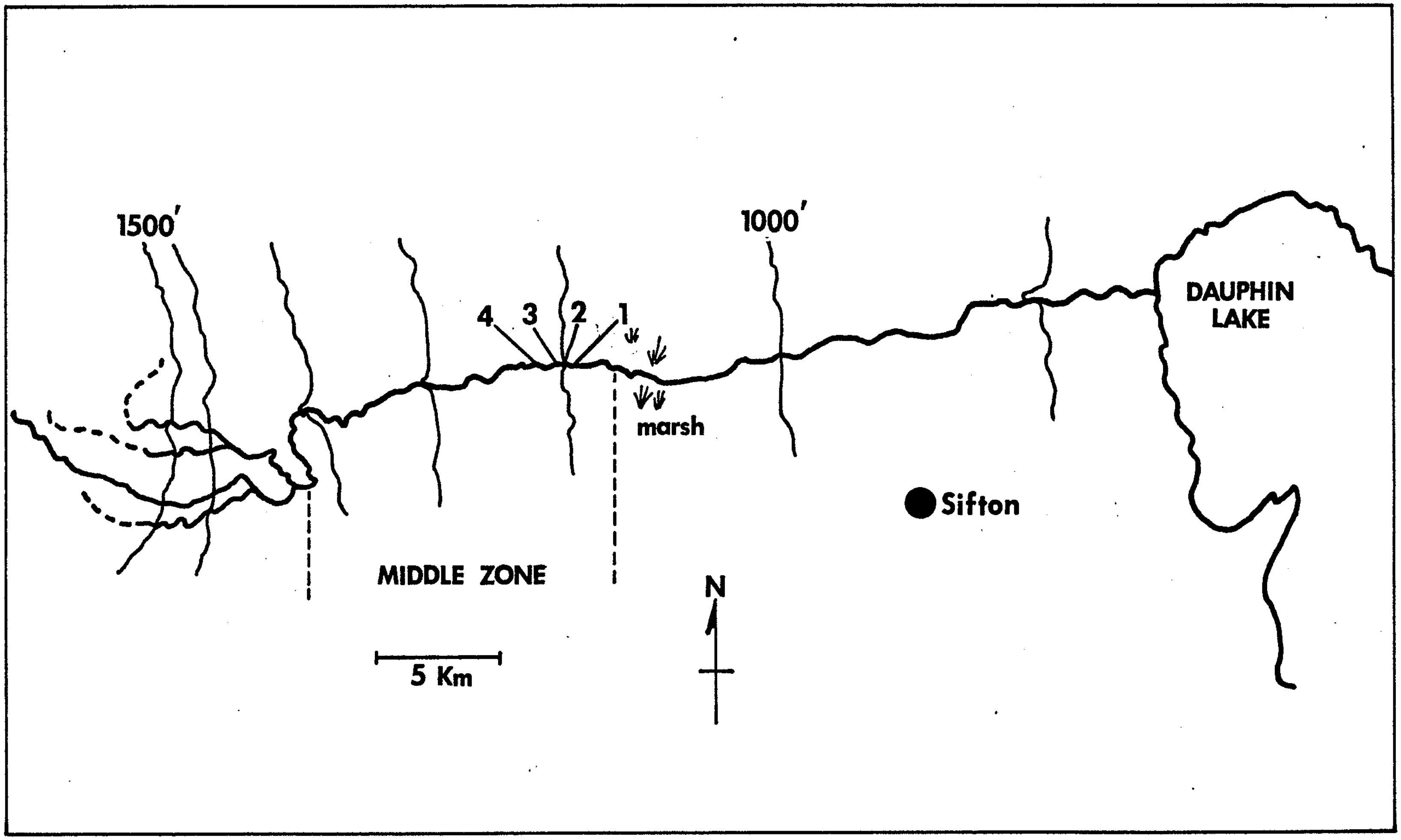
APPENDICES

APPENDIX 1. Dates and location of sampling and certain physical parameters of the middle zone of the Mink River, Manitoba, summer 1972.

Date 1972	Site (c.f. map, Appendix 2)	Water Temp. °C.	Surface Rate Water flow (cm/sec)	Area Sampled (sq.m)	Mean Depth (m)
June 6	3	23	6	58.5	0.58
June 7	2	22	14	94.1	0.44
June 7	2	26	9	41.2	0.43
June 7	3	18	10	62.0	0.39
July 8	1	18	11	39.0	0.37
July 8	2	20	9	35.1	0.34
July 9	4	16	7	40.4	0.35
July 25	3	16	15	62.1	0.34
July 26	4	16	5	16.8	0.34
August 10	3	19	5	54.7	0.34
August 11	3	20	11	41.8	0.33

APPENDIX 2. Map of the Mink River showing the middle zone, elevation in feet above sea level, and the sampling stations used during this study.



1500′
1000′
4
3
2
1
marsh
DAUPHIN
LAKE
Sifton
MIDDLE ZONE
N
5 Km

APPENDIX 3

USE OF OPERCULAR BONES TO IDENTIFY AND ESTIMATE PREY LENGTH

INTRODUCTION

To establish a relationship between the length of creek chub and the length of the prey fishes they consumed, a method was needed which would identify and estimate the sizes of often well-digested fish remains taken from the stomach contents of the predator. The opercular bone was considered a possible source of most of the desired information since a body of literature exists indicating a strong relationship between opercular measurements and sizes (lengths) of fish (Le Cren 1947; Menon 1949; McConnell 1952; Grimaldi MS 1968; Grimaldi and Leduc 1973).

MATERIALS AND METHODS

Calibration correlations were established for each of the ten species of fishes inhabiting the middle zone of the Mink River, relating the size of the operculum to a number of body measurements, standard length, pectoral body depth, pectoral girth, and total wet weight. The data were obtained from sub-samples of the fishes taken from the Mink River during the summer of 1972, as described above. Each fish was measured and the opercular bone removed from the left side. Opercula from the right side were substituted in cases where the left had been damaged. McConnell (1952) demonstrated that no difference existed between the left and right opercula in determining age and growth of <u>Cyprinus carpio</u>.

The opercula were immersed in an enzyme solution (0.4 gm papain powder, 0.4 gm enzyme detergent (Bioad), and 45 gm of any detergent powder, made up to 4.5 liters of warm water; H.E. Drescher, personal communication) at 40°C, for one to two days to clean them of remaining tissue and were mounted, dry between two microscope slides taped together. The anterior margin of the opercula were measured (to nearest 0.1 mm) under a compound microscope, at a magnification of 40 times, using a stage micrometer.

An experiment was also conducted to determine if passage through the digestive tract of the creek chub had any effect on the accuracy of predictions of standard lengths of prey made from measurements of their opercular bones. This was accomplished by feeding five chub (S L between 200 and 260 mm) brook stickleback of known standard lengths. Each chub was held in a 45 l aquarium at a water temperature of 12°C until they had voided. The feces were collected and the opercula removed and measured. The estimated standard length and measured standard length distributions were then compared.

RESULTS

Appendix 5 lists the species of potential prey fish inhabiting the middle zone of the Mink River, Manitoba, along with the regression statistics relating log. standard length to the log. of the anterior margin of the opercular bone. The relationships were all found to be highly significant permitting estimates of standard length to be made with 95% confidence limits of approximately plus or minus six millimeters or less.

Observations and comparisons of the opercular bones from each of the species (c.f. photographs A to J) showed, however, that they are of limited use as a means of identification of the species from which they were obtained. The brook stickleback and johnny darter are readily identified from the shape of their opercular bones, the former being somewhat triangular with the posterior and ventral corners slightly rounded and the latter, quite triangular and possessing a well developed spine. The opercula of the remaining eight species, all cypriniforms, are of the same general shape (square to rhomboid). It is difficult to distinguish between them with the possible exception of the common sucker which possesses opercular bones with relatively longer foci (point of attachment with the hyomandibular) than the other cypriniform species (all Cyprinidae).

In order to identify the cypriniform species found in stomach contents of chub a collection of pharyngeal bones was made for comparison. This technique proved successful and whenever a cypriniform fish was encountered during analysis of stomach contents, both the opercular bones and pharyngeal bones were removed to facilitate identification and length estimation. Drawings of representative pharyngeal bones are shown in Appendix 4 for the eight cypriniform species encountered in the Mink River.

The results of an experiment to determine what effect digestion would have on the accuracy of standard length estimates made from opercular bones are shown graphically in Appendix 6. Analysis of variance between the two distributions showed no significant difference between the mean measured standard length and estimated standard length of brook stickleback indicating that digestion had little if any effect on the accuracy of the technique.

Photographs of representative opercula of fishes inhabiting the middle zone of the Mink River, Manitoba.

A. Johnny darter, *Etheostoma nigrum*, 12X.

B. Brook stickleback, *Culaea inconstans*, 12X.

C. Fathead minnow, *Pimephales promelas*, 8X.

D. Brassy minnow, *Hypognathus hankinsoni*, 8X.

E. Blacknose dace, *Rhinichthys atratulus*, 9X.

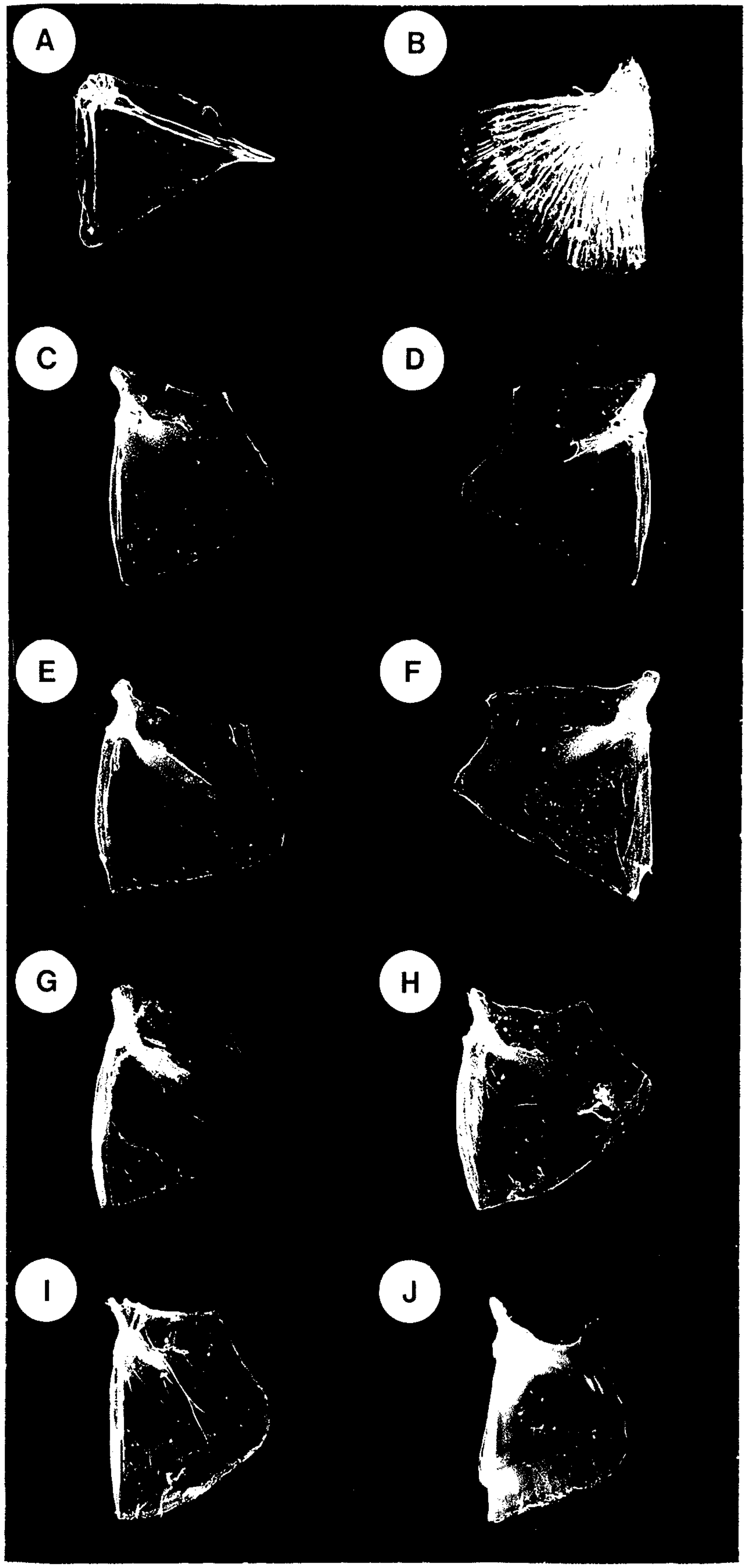
F. Longnose dace, *Rhinichthys cataractae*, 9X.

G. Creek chub, *Semotilus atromaculatus*, 5X.

H. Pearl dace, *Semotilus margarita*, 8X.

I. Common shiner, *Notropis cornutus*, 6X.

J. Common sucker, *Catostomus commersoni*, 5X.


A
B
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DISCUSSION

The use of bones as a means of estimating the lengths of prey fish consumed by a piscivor is a very useful technique, although rarely employed in North America, for investigations involving size selectivity. Russian investigators have used a number of skeletal elements to this end. Duskin and Serezhkina (1966) have demonstrated a good correlation between size of pharyngeal teeth and length for the cyprinid Abramis brama. Kovaler (1959) used basihyal and dentary bones to estimate weights and body lengths of five species of fishes cohabiting the Volga delta. Similarly, Pikhu and Shatshneyder (1966), and Pikhu and Pikhu (1970) have demonstrated a technique of fish size estimation involving use of fragments of the vertebral column. The use in archaeological studies of fish skeletal remains to identify and estimate body size is well documented (c.f. eg. Follet 1970).

In the present investigation of prey size-selection by creek chub, the use of the opercular bone was considered since a body of literature exists indicating a strong relationship between opercular measurements and lengths of fish (Le Cren 1947; Menon 1949; McConnell 1952; Grimaldi MS 1968). To my knowledge, the use of opercular bones as an aid to identification of well-digested remains of prey fish has not been attempted nor has the effect of digestion on the accuracy of the size estimation technique been evaluated.

Experiments conducted during this study demonstrated that digestion has little, if any effect on the accuracy of the technique employing opercular bones to estimate the lengths of prey fish removed from the stomach contents of piscivorous creek chub. However, use of opercular bones was found to be of limited use as an aid to identification of prey fish, although it may be used to distinguish broad taxonomic groups of fishes. In the present study it was found that opercular bones readily separated the four families of fishes (Gasterosteidae, Percidae, Cyprinidae, and Catostomidae; Greenwood, et al. 1966) cohabiting the middle zone of the Mink River. Pharyngeal bones were used to identify the seven cyprinid species occurring in the study stream.

The use of the opercular bone, in situations where information concerning the identity and size of a predator's prey fish is desired, is recommended since it can be easily recognized, and readily removed from stomach samples. Also, the technique gives reasonably accurate estimates of prey lengths once calibration correlations have been established.

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APPENDIX 4. Drawings of representative pharyngeal bones of the cypriniform fishes inhabiting the middle zone of the Mink River, Manitoba. (Mag. approx. 40X)

A. Creek chub, _Semotilus atromaculatus_

B. Pearl dace, _Semotilus margarita_

C. Brassy minnow, _Hypognathus hankinsoni_

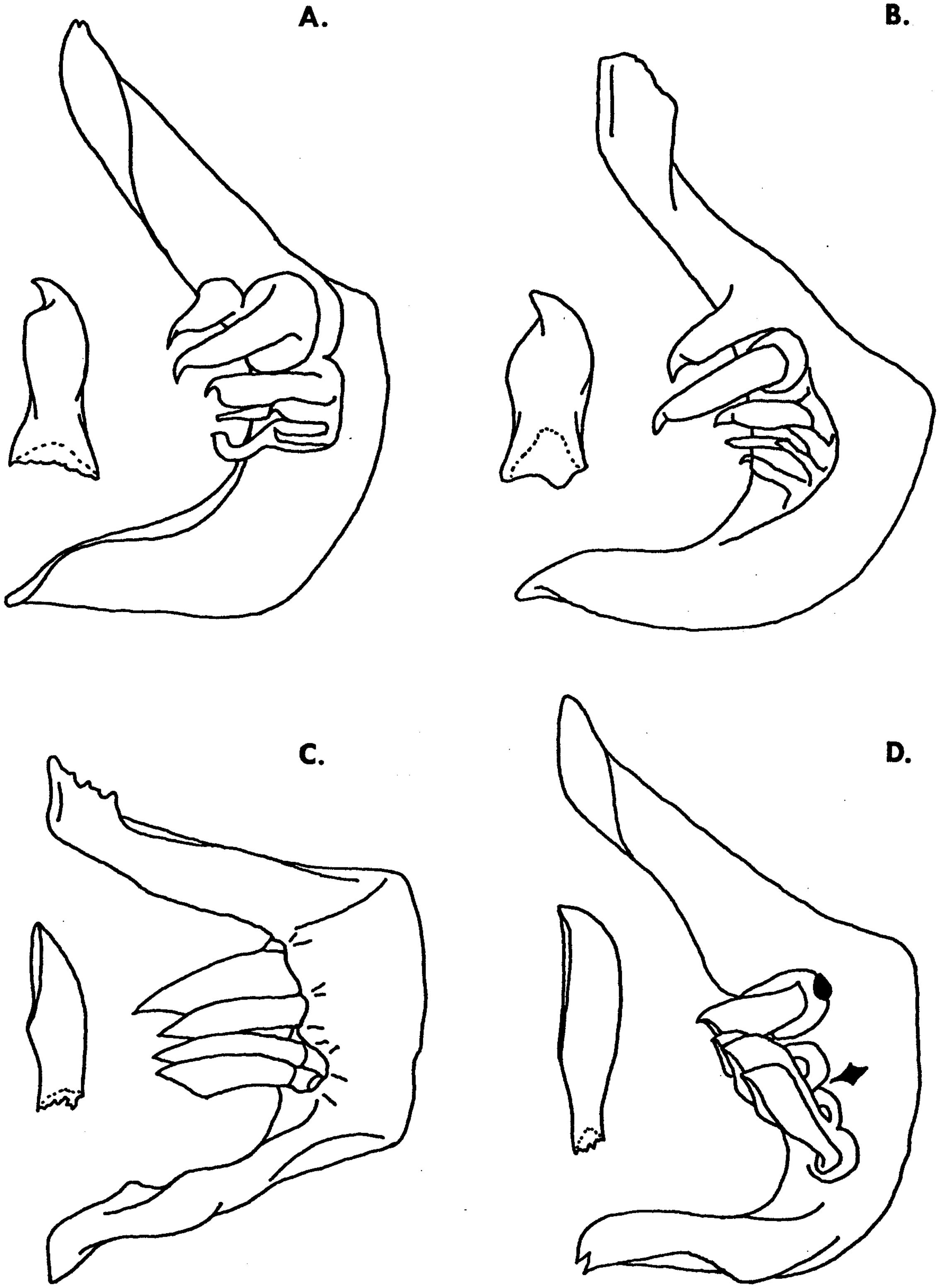
D. Fathead minnow, _Pimephales promelas_

E. Blacknose dace, _Rhinichthys atratulus_

F. Longnose dace, _Rhinichthys cataractae_

G. Common shiner, _Notropis cornutus_

H. Common sucker, _Catostomus commersoni_


A.
B.
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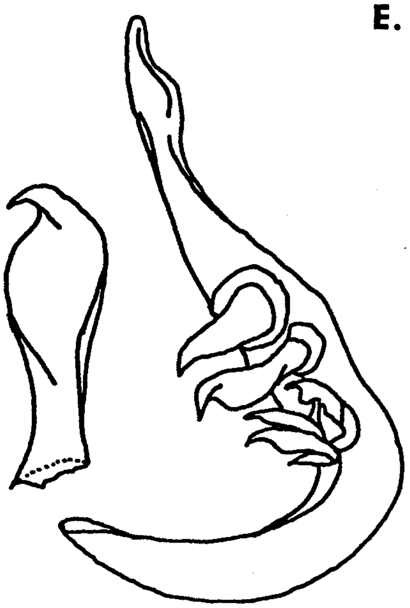

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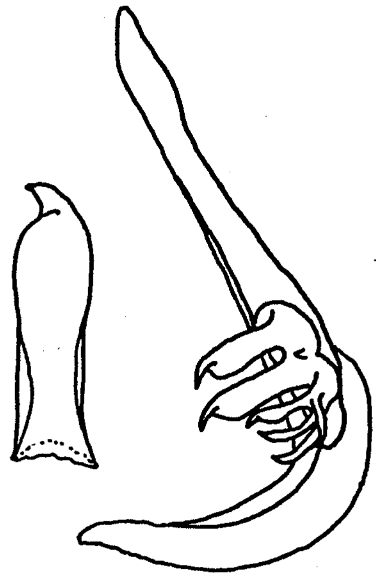

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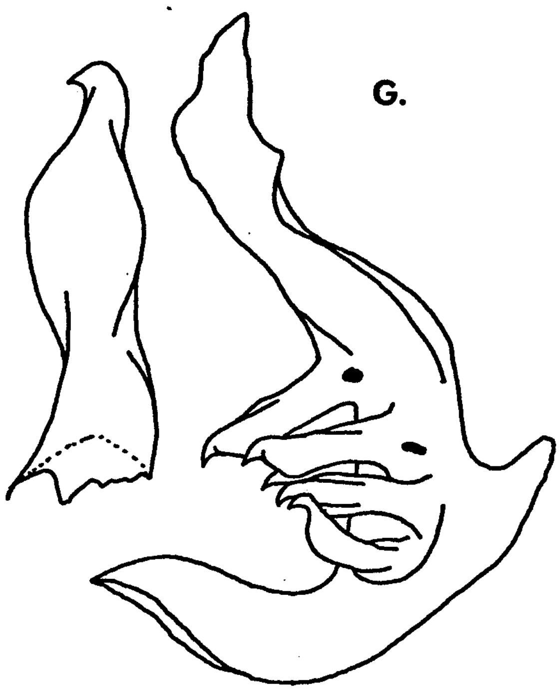

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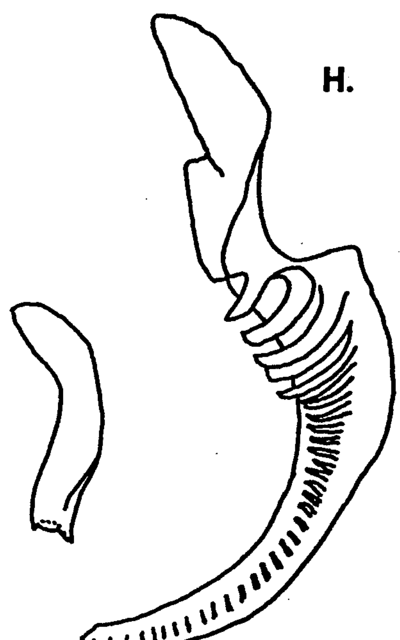
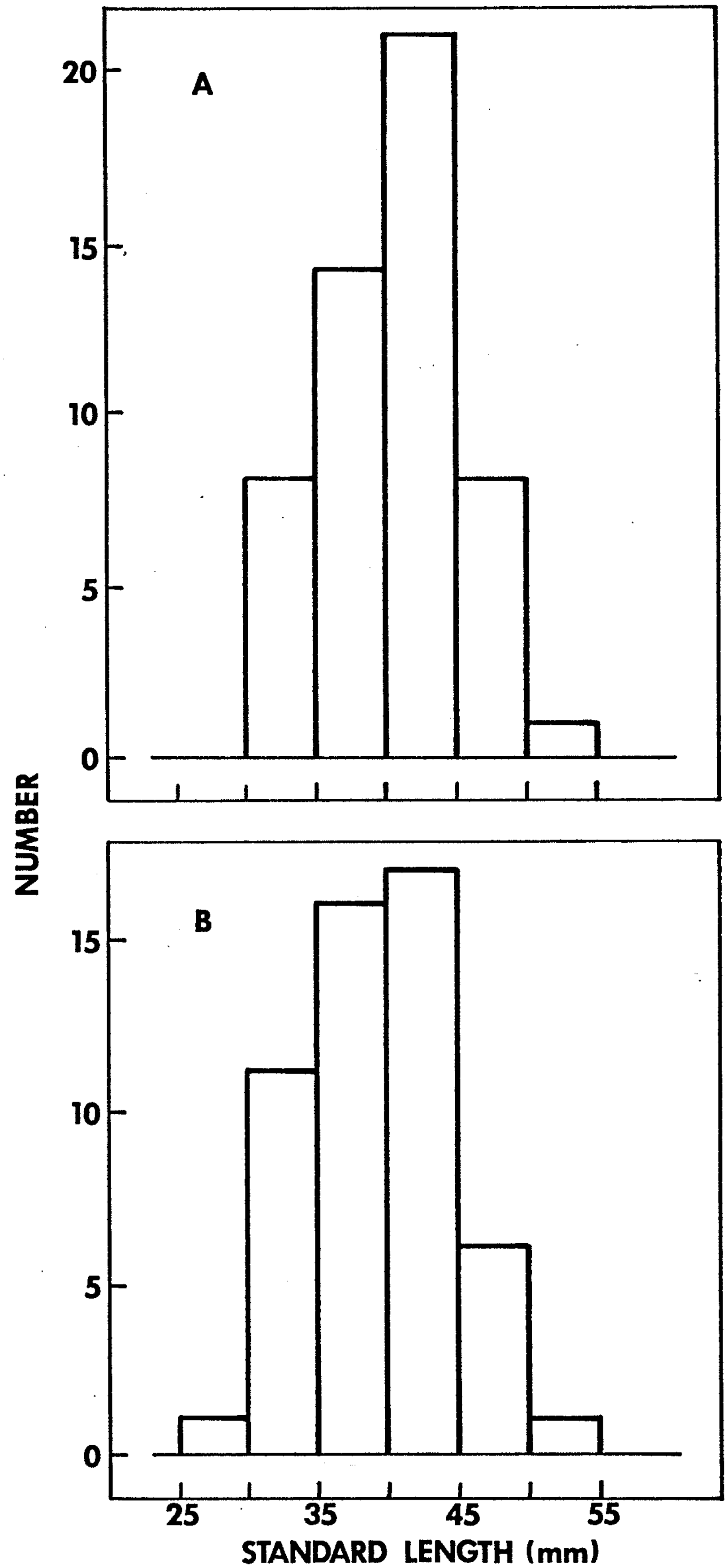


APPENDIX 5. Sampling sizes and regression statistics relating log. lengths of the anterior margins of opercular bones (X) and the log. of the standard lengths (Y) for the ten species of fishes inhabiting the middle zone of the Mink River. [log Y = a + b log X; notation of Sokal and Rohlf 1969]

Species	N	Range of Opercular lengths (mm)	a	b	$\bar{X}$	Σx^2	$S^2_{y.x}$	r^2
Brook stickleback	102	1.63 - 3.96	2.555	0.959	0.962	5.323	0.008	0.860
Johnny darter	47	1.04 - 4.50	2.668	0.977	0.902	6.446	0.007	0.948
Creek chub	50	2.20 - 9.12	2.493	0.917	1.482	8.386	0.002	0.987
Pearl dace	49	3.04 - 7.10	2.489	0.995	1.541	2.495	0.002	0.959
Blacknose dace	47	2.32 - 5.92	2.741	0.911	1.536	2.208	0.004	0.906
Longnose dace	9	2.22 - 5.38	2.754	0.944	1.174	0.555	0.003	0.955
Fathead minnow	23	2.97 - 6.15	2.812	0.689	1.409	1.104	0.007	0.777
Brassy minnow	60	2.62 - 5.70	2.308	1.100	1.463	2.571	0.003	0.952
Common shiner	39	3.00 -16.11	2.651	0.811	1.688	4.173	0.003	0.955
Common sucker	42	1.80 -14.93	2.187	0.980	1.923	9.553	0.002	0.993

APPENDIX 6. Histograms of the distributions of measured standard lengths (A) and standard lengths estimated from opercular bones of brook stickleback after passage through the digestive tracts of creek chub (B).


A
B
20
15
10
5
0
NUMBER
25
35
45
55
STANDARD LENGTH (mm)

APPENDIX 7. Densities, in standard length classes (mm), of potential prey fishes available to Mink River creek chub at four sampling times during the summer of 1972. The values have been adjusted by including fishes found in the stomach contents of creek chub greater than 90 mm S L.

A. Pooled data collected June 6-7 in three samples from a total area of 193.8 m^2.

B. Pooled data collected July 7-9 in four samples from a total area of 176.4 m^2.

C. Pooled data collected July 25-26 in two samples from a total area of 78.9 m^2.

D. Pooled data collected August 10 in two samples from a total area of 96.5 m^2.

STBK = Brook stickleback
PD = Pearl dace
CC = Creek chub
LND = Longnose dace
BM = Brassy minnow

JD = Johnny darter
CS = Common shiner
BND = Blacknose dace
S = Common sucker
FHM = Fathead minnow

APPENDIX 7A.

S L Classes	STBK	JD	PD	CS	CC	BND	LND	S	BM	FHM	TOTAL
10 - 14	0	0	0	0	0	0	0	0	0	0	0
15 - 19	0	0	0	0	0	0	0	0	0	0	0
20 - 24	0	0	0	0	0	0	0	0	0	0	0
25 - 29	0	0.03	0	0.01	0	0.01	0	0	0	0	0.05
30 - 34	0.15	0.06	0.06	0.05	0.14	0.03	0	0.01	0.01	0	0.51
35 - 39	0.63	0.09	0.32	0.07	0.31	0.01	0	0.02	0.08	0.05	1.58
40 - 44	0.40	0.10	0.92	0.12	0.23	0.03	0.03	0.09	0.13	0.01	2.06
45 - 49	0.06	0.10	0.50	0.06	0.03	0.06	0.02	0.04	0.07	0	0.94
50 - 54	0	0.10	1.04	0.13	0.05	0.09	0.01	0.09	0.11	0	1.62
55 - 59	0	0	1.14	0.13	0.04	0.09	0	0.05	0.08	0.02	1.55

APPENDIX 7B.

S L Classes	STBK	JD	PD	CS	CC	BND	LND	S	BM	FHM	TOTAL
10 - 14	0	0.52	0	0	0	0.09	0	0.01	0	0	0.62
15 - 19	0.02	0.56	0.02	0	0.01	0.34	0	0.18	0	0	1.22
20 - 24	0.18	0.03	0.02	0	0.22	0	0.01	0.22	0	0	0.68
25 - 29	0.40	0	0.02	0	0.03	0.01	0	0.06	0	0	0.52
30 - 34	0.09	0	0.03	0.03	0.03	0	0	0	0	0	0.18
35 - 39	0.20	0.10	0.11	0.08	0.15	0.01	0	0	0	0	0.65
40 - 44	0.49	0.31	0.41	0.14	0.34	0.01	0	0	0	0.01	1.71
45 - 49	0.20	0.35	0.74	0.10	0.23	0.01	0	0.01	0.01	0.03	1.77
50 - 54	0.02	0.25	0.70	0.08	0.12	0.05	0	0.09	1.16	0.01	2.48
55 - 59	0	0.02	0.63	0.28	0.05	0.05	0	0.10	2.04	0.01	3.18

APPENDIX 7C.

S L Classes	STBK	JD	PD	CS	CC	BND	LND	S	BM	FHM	TOTAL
10 - 14	0	0.24	0	0.08	0	0	0	0	0	0	0.32
15 - 19	0	0.77	0.03	0.10	0.25	0.05	0	0	0	0	2.20
20 - 24	0.05	0.35	0.05	0	0.33	0.20	0	0.58	0	0	1.56
25 - 29	0.29	0.05	0.05	0	0.53	0.08	0	0.44	0.03	0	1.47
30 - 34	0.13	0	0	0	0.29	0	0	0.13	0.06	0.03	0.64
35 - 39	0.06	0.10	0.03	0.05	0.03	0	0	0	0	0	0.27
40 - 44	0.30	0.41	0.18	0.03	0.15	0.03	0	0	0	0	1.10
45 - 49	0.19	0.33	0.43	0.10	0.23	0.03	0	0	0	0	1.31
50 - 54	0	0.18	0.33	0.13	0.10	0.03	0	0.05	0.56	0.03	1.41
55 - 59	0	0	0.46	0.23	0.08	0.03	0	0.25	4.77	0.03	5.85

APPENDIX 7D.

S L Classes	STBK	JD	PD	CS	CC	BND	LND	S	BM	FHM	TOTAL
10 - 14	0	0.02	0	0	0	0.02	0	0	0	0	0.04
15 - 19	0	0.44	0	0.08	0.02	0.31	0	0	0	0	0.85
20 - 24	0	0.29	0	0	0.19	0.48	0.04	0	0	0	1.00
25 - 29	0.18	0.36	0.17	0.15	0.48	0.19	0.02	0.06	0	0	1.61
30 - 34	0.15	0.08	0.10	0.02	0.23	0.02	0.04	0.31	0	0.08	1.03
35 - 39	0.02	0.04	0.10	0.01	0.06	0	0	0.12	0.02	0.04	0.41
40 - 44	0.03	0.25	0.04	0.12	0.10	0	0	0	0	0.02	0.56
45 - 49	0	0.44	0.21	0.12	0.23	0	0	0	0	0.02	1.02
50 - 54	0	0.15	0.27	0.08	0.08	0.02	0	0.02	0.09	0	0.71
55 - 59	0	0	0.25	0.10	0.02	0	0	0.04	0.33	0	0.74

APPENDIX 8. Electivity indices (E) for groups 1 and 2 creek chub towards potential prey fishes inhabiting the Mink River at four sampling times during the summer of 1972. [AD 1 and 2 are calculated adjusted densities of potential prey in size (S L) ranges available to groups 1 and 2 chub respectively; p = ratio of availability, n = number consumed, r = ratio of consumption]

A. Electivity indices calculated from data collected June 6-7.

B. Electivity indices calculated from data collected July 7-9.

C. Electivity indices calculated from data collected July 25-26.

D. Electivity indices calculated from data collected August 10.

STBK = Brook stickleback

PD = Pearl dace

CC = Creek chub

LND = Longnose dace

BM = Brassy minnow

UNK = Unknown

JD = Johnny darter

CS = Common shiner

BND = Blacknose dace

S = Common sucker

FHM = Fathead minnow

APPENDIX 8A.

	STBK	JD	PD	CS	CC	BND	LND	S	BM	FHM	TOTAL
A D 1	1.24	0.38	1.80	0.31	0.71	0.14	0.05	0.16	0.29	0.06	5.14
p	0.24	0.07	0.35	0.06	0.14	0.03	0.01	0.03	0.06	0.01	1.00
n	10	0	0	0	1	1	0	1	3	0	16
r	0.63	0	0	0	0.06	0.06	0	0.06	0.19	0	1.00
E	0.45	-	-	-	-0.40	0.33	-	0.33	0.52	-	-
A D 2	1.24	0.48	3.98	0.57	0.80	0.32	0.06	0.30	0.48	0.08	8.31
p	0.15	0.06	0.48	0.07	0.10	0.04	0.01	0.04	0.06	0.01	1.02
n	27	0	5	1	2	1	1	0	0	0	37
r	0.73	0	0.14	0.03	0.05	0.03	0.03	0	0	0	1.01
E	0.66	-	-0.55	-0.40	-0.33	-0.14	0.50	-	-	-	-

APPENDIX 8B.

	STBK	JD	PD	CS	CC	BND	LND	S	BM	FHM	UNK	TOTAL
A D 1	1.58	1.87	1.35	0.35	1.10	0.47	0.01	0.48	0.10	0.04	0	7.35
p	0.21	0.25	0.18	0.05	0.15	0.06	0	0.07	0.01	0.01	0	0.99
n	12	0	2	0	0	0	0	1	0	0	1	16
r	0.75	0	0.13	0	0	0	0	0.06	0	0	0.06	1.00
E	0.56	-	-0.16	-	-	-	-	-0.08	-	-	-	-
A D 2	1.60	1.62	2.68	0.71	1.27	0.48	0.01	0.66	3.30	0.06	0	12.39
p	0.13	0.13	0.22	0.06	0.10	0.04	0	0.05	0.27	0	0	1.00
n	2	2	0	0	0	1	0	1	0	0	2	8
r	0.25	0.25	0	0	0	0.13	0	0.13	0	0	0.25	1.01
E	0.003	0.003	-	-	-	0.53	-	0.44	-	-	-	-

APPENDIX 8C.

	STBK	JD	PD	CS	CC	BND	LND	S	BM	FHM	TOTAL
A D 1	1.02	3.25	0.77	0.36	1.81	0.39	0	1.15	0.09	0.03	8.87
p	0.11	0.37	0.09	0.04	0.20	0.04	0	0.13	0.01	0.01	1.00
n	29	2	0	0	0	0	0	1	1	0	33
r	0.88	0.06	0	0	0	0	0	0.03	0.03	0	1.00
E	0.78	-0.72	-	-	-	-	-	-0.63	0.50	-	-

APPENDIX 8D.

	STBK	JD	PD	CS	CC	BND	LND	S	BM	FHM	UNK	TOTAL
A D 1	0.38	1.92	0.62	0.50	1.31	1.02	0.10	0.49	0.02	0.16	0	6.52
p	0.06	0.29	0.10	0.08	0.20	0.16	0.02	0.08	0	0.02	0	1.01
n	1	1	0	1	0	0	0	0	0	0	0	3
r	0.33	0.33	0	0.33	0	0	0	0	0	0	0	0.99
E	0.69	0.06	-	0.61	-	-	-	-	-	-	-	-
A D 2	0.38	2.05	1.14	0.68	1.41	1.02	1.10	0.55	0.44	0.16	0	7.93
p	0.05	0.26	0.14	0.09	0.18	0.13	0.01	0.07	0.06	0.02	0	1.01
n	1	0	0	0	0	0	0	0	0	0	1	2
r	0.50	0	0	0	0	0	0	0	0	0	0.50	1.00
E	0.81	-	-	-	-	-	-	-	-	-	-	-

APPENDIX 9. Results and analysis of preference experiments 1 and 2 comparing prey selection by Norquay Channel and Mink River creek chub.
[STBK = brook stickleback, JD = johnny darter, PD = pearl dace, CS = common shiner, CRFH = crayfish]

RESULTS

		Prey Selection No. of each prey consumed					
Predator	Replicate	STBK	JD	PD	CS	CRFH	Total
Norquay Channel chub	1	3	10	6	5	2	26
	2	4	9	6	5	0	24
	3	3	10	3	7	0	23
	4	2	9	5	6	0	22
	5	1	8	2	2	0	13
	Total	13	46	22	25	2	108
Mink River chub	1	2	9	4	0	5	20
	2	2	10	4	2	0	18
	3	4	9	5	4	0	22
	4	2	7	7	3	0	19
	5	0	8	6	4	0	18
	Total	10	43	26	13	5	97

APPENDIX 9 continued:

ANALYSIS

A replicated goodness-of-fit test (Sokal and Rohlf 1969) was used to test the hypothesis that all prey were selected equally. The results were pooled and treated as ten replicated tests.

Tests	d.f.	G	P
Pooled	4	95.980	< 0.001
Heterogeneity	36	45.352	$0.1 < p < 0.5$
Total	40	141.332	< 0.001

Conclusion: The results of all ten tests are homogeneous and the hypothesis is rejected. Johnny darters are clearly the dominant choice of both Norquay Channel and Mink River creek chub.

APPENDIX 10. Results and analysis of selection experiments 1 and 2 comparing prey selection by Norquay Channel and Mink River creek chub.
[STBK = brook stickleback, JD = johnny darter, PD = pearl dace, CS = common shiner, CRFH = crayfish]

RESULTS

Expt. No.	Predator	Replicate	Prey Selection No. of prey consumed STBK	JD	PD	CS	Total
1	Norquay Channel chub	1	0	6	0	2	8
		2	0	6	1	1	8
		3	0	6	1	0	7
		4	0	5	0	0	5
		5	0	7	0	0	7
		Total	0	30	2	3	35
			STBK	JD	PD	CRFH	
2	Mink River chub	1	9	5	7	3	24
		2	5	2	3	1	11
		3	7	2	4	1	14
		4	7	2	3	0	12
		5	6	1	1	0	8
		Total	34	12	18	5	69

APPENDIX 10 continued:

ANALYSIS

1. A G-test of independence was used to compare the selection of prey by Norquay Channel and Mink River creek chub omitting the data for common shiner and crayfish, experiments 1 and 2 respectively. This demonstrated a significant ($G = 58.950$, 2 d.f.; $p < 0.001$) lack of independence in the selection of live prey by Norquay Channel and Mink River chub.

2. Two replicated goodness-of-fit G-tests were also performed to test the hypothesis that Norquay Channel and Mink River chub selected live prey equally.

Experiment No.	Tests	d.f.	G	p
	Pooled	3	65.104	$p < 0.001$
1	Heterogeneity	12	4.465	$0.9 < p < 0.975$
	Total	15	69.569	$p < 0.001$
	Pooled	3	35.994	$p < 0.001$
2	Heterogeneity	12	3.388	$0.975 < p < 0.995$
	Total	15	39.332	$0.001 < p < 0.005$

Conclusion: The results for both experiments showed homogeneity over the five replicates and a significant selection of prey. Norquay Channel selected johnny darters as their dominant prey while Mink River chub chose brook stickleback.

APPENDIX 10 continued:

3. The selection of live and dead prey by Norquay Channel and Mink River chub was also investigated using two G-tests of independence (selection of crayfish and common shiners were omitted from the tests of Mink River and Norquay Channel chub respectively).

Source of data	Prey condition	Prey Selection STBK	JD	PD	CS	CRFH	Total
Norquay Channel chub							
Appendix 9	Dead	13	46	22	25	2	108
Appendix 10	Live	0	30	2	3	-	35
	Total	13	76	24	28	2	143
Mink River chub							
Appendix 9	Dead	10	43	26	13	5	97
Appendix 10	Live	34	12	18	-	5	69
	Total	44	55	44	13	10	166

Norquay Channel chub: $G = 49.924$, 3 d.f.; $p < 0.001$
Mink River chub: $G = 32.366$, 3 d.f.; $p < 0.001$

Conclusion: Both Norquay Channel and Mink River creek chub select live and dead prey differently. Norquay Channel chub select johnny darters more strongly from a live spectrum of prey while Mink River chub switch from johnny darters to brook stickleback when presented a dead and live prey spectrum respectively.

4. A 2 x 2 G-test of the hypothesis that Norquay Channel and Mink River chub consume similar numbers of live and dead prey was rejected ($G = 10.196$, 1 d.f.; $p < 0.001$).

APPENDIX 11. Results and analysis of selection experiment 3 performed to examine the effect of two environmental features, presence and absence of current and rock substrate on the selection of prey by Norquay Channel creek chub.
[STBK = brook stickleback, JD = johnny darter, PD = pearl dace, CS = common shiner, + = present, - = absent]

RESULTS

Test No.	Environmental Features		Replicate	Prey Selection No. of prey consumed				Total
	Current	Substrate		STBK	JD	PD	CS	
1	+	-	1	1	3	0	2	6
			2	1	7	1	0	9
			3	1	8	0	1	10
			4	0	4	0	0	4
			5	0	5	0	0	5
			Total	3	27	1	3	34
2	+	+	1	1	1	0	0	2
			2	2	2	0	0	4
			3	0	0	0	1	1
			4	2	2	1	0	5
			5	2	2	1	0	5
			Total	7	7	2	1	17
3	-	+	1	4	1	1	0	6
			2	2	3	0	0	5
			3	3	0	0	1	4
			4	2	2	0	1	5
			5	4	2	0	0	6
			Total	15	8	1	2	26

APPENDIX 11 continued:

ANALYSIS

1. Three separate G-tests of homogeneity showed that, within each test conducted in Selection Experiment 3, the selection of prey was homogeneous between replicates as follows:

Test Number	d.f.	G	p
1	12	11.406	$0.1 < p < 0.5$
2	12	9.656	$0.5 < p < 0.9$
3	12	12.306	$0.1 < p < 0.5$

2. To examine the effects of current and substrate on prey selection the results of Selection Experiment 3 were combined with the results of Selection Experiment 1 (Appendix 10) and compared using a three-way test of independence (Sokal and Rohlf 1969) as follows:

Prey species	Current		Substrate present	Substrate absent	Total
Brook stickleback	present		7	3	10
	absent		15	0	15
		Total	22	3	25
Johnny darter	present		7	27	34
	absent		8	30	38
		Total	15	57	72
Pearl dace	present		2	1	3
	absent		1	2	3
		Total	3	3	6
Common shiner	present		1	3	4
	absent		2	3	5
		Total	3	6	9

APPENDIX 11 continued:

Hypothesis tested	d.f.	G	p
Prey species x current independence	3	0.446	$0.9 < p < 0.975$
Prey species x substrate independence	3	37.364	$p < 0.001$
Current x substrate independence	1	1.020	$0.1 < p < 0.5$
Interaction	3	6.018	$0.1 < p < 0.5$
Prey species x current x substrate independence	10	44.848	$p < 0.001$

Conclusion: Current had no effect on prey selection by Norquay Channel creek chub but the presence of a rock substrate significantly changed selection. In the presence of a substrate brook stickleback are the dominant prey and in its absence the johnny darter.

APPENDIX 12. Results and analysis of Selection Experiment 4 investigating changes in the selective response of creek chub under three combinations of two types of 'environmental protection'; rock substrate and vegetation.
[STBK = brook stickleback, JD = johnny darter, CYP = cyprinids, pearl dace and common shiner, CRFH = crayfish; + = present, - = absent]

RESULTS

Test No.	Environmental Features		Replicate	Prey Selection No. of prey consumed				Total
	Substrate	Vegetation		STBK	JD	CYP	CRFH	
1	+	-	1	9	6	0	6	21
			2	10	4	2	0	16
			3	9	6	4	1	20
			4	9	5	1	0	15
			5	8	6	0	2	16
			Total	45	27	7	9	88
2	+	+	1	5	3	1	0	9
			2	2	2	2	0	6
			3	0	0	1	0	1
			4	1	3	1	0	5
			5	3	2	1	0	6
			Total	11	10	6	0	27
3	-	+	1	7	10	2	2	21
			2	4	8	0	2	14
			3	3	7	1	1	12
			4	3	8	0	1	12
			5	0	6	1	0	7
			Total	17	39	4	6	66

APPENDIX 12 continued:

ANALYSIS

1. Three separate G-tests of homogeneity showed that, within tests 2 and 3 of Selection Experiment 4, the consumption of prey was homogeneous between each replicate. However, heterogeneity was observed within Test 1 as follows:

Test Number	d.f.	G	p
1	12	22.058	$0.025 < p < 0.05$
2	12	5.978	$0.9 < p < 0.975$
3	12	11.244	$0.5 < p < 0.9$

Analysis by Standard Test Procedure (Sokal and Rohlf 1969) showed that replicates 1 and 2 of Test 1 exhibited the greatest heterogeneity due to excessive consumption of crayfish and brook stickleback than expected in replicates 1 and 2 respectively. Tests 3 through 5 were homogeneous.

2. The heterogeneity exhibited in Test 1 was not considered excessive and the three tests were compared by a G-test of independence which demonstrated a significant ($G = 22.938$, 6 d.f.; $p < 0.001$) difference in prey selection between the three combinations of environmental protection examined.

APPENDIX 13. Results of Selection Experiment 5 investigating the effect of large numbers of crayfish on prey selection by creek chub.
[STBK = brook stickleback, JD = johnny darter, CYP = cyprinids, pearl dace and common shiners, CRFH = crayfish]

Replicate	Prey Selection No. of prey consumed				Total
	STBK	JD	CYP	CRFH	
1	0	0	0	3	3
2	0	0	0	2	2
3	0	0	1	3	4
4	1	1	0	2	4
5	0	1	0	3	4
Total	1	2	1	13	17

APPENDIX 14. Results and analysis of field tests of creek chub responses to the fright pheromone (schreckstoff, von Frisch 1938). The data represent the numbers of attacks, in one minute, made by a school of creek chub on presented non-cyprinid and cyprinid prey.

RESULTS

Date	Test No.	Prey	No. of attacks on prey offered for one minute: Non-cyprinid	Cyprinid	Total
Jun 29	1	JD/PD	15	4	19
	2	JD/BND	8	1	9
			Total 23	5	28
Jul 9	1	STBK/CS	23	0	23
	2	JD/BND	2	0	2
			Total 25	0	25
Jul 26	1	JD/PD	12	1	13
	2	JD/PD	3	0	3
			Total 15	1	16
			Grand Total 63	6	69

APPENDIX 14 continued:

ANALYSIS

The results were pooled and analysed by a replicated goodness-of-fit test of the hypothesis that the number of attacks in one minute on a non-cyprinid prey were the same as for a cyprinid prey as follows:

Tests	d.f.	G	p
Pooled	1	54.884	$p < 0.001$
Heterogeneity	5	7.886	$0.1 < p < 0.5$
Total	6	62.770	$p < 0.001$

Conclusion: Significantly fewer attacks were made on cyprinid than on non-cyprinid prey.

APPENDIX 15. Results and analysis of three laboratory tests of the response of creek chub to three sources of the fright pheromone.

RESULTS

Source of pheromone	Depth (cm) location	Frequency of Location Before	After	Total
Creek chub	0 - 9	16	2	18
	10 - 19	11	1	12
	20 - 29	17	15	32
	30 - 39	31	32	63
	Total	75	50	125
Pearl dace	0 - 9	15	0	15
	10 - 19	4	0	4
	20 - 29	11	7	18
	30 - 39	20	18	38
	Total	50	25	75
Blacknose dace	0 - 9	16	1	17
	10 - 19	6	2	8
	20 - 29	12	7	19
	30 - 39	16	15	31
	Total	50	25	75

APPENDIX 15 continued:

ANALYSIS

1. G-test of homogeneity of results obtained in the three tests.

Test	d.f.	G	p
Before introduction of pheromone	6	3.540	$0.5 < p < 0.9$
After introduction of pheromone	6	5.160	$0.5 < p < 0.9$

2 G-test of the hypothesis that the depth location of the creek chub after introduction of the fright pheromone was the same as before introduction. Expected frequencies were calculated from the proportional occurrence of chub at each depth before introduction of the pheromone.

Depth (cm)		Frequency of location	
		Observed	Expected
0 - 9		3	26.9
10 - 19		3	12.0
20 - 29		29	22.8
30 - 39		65	38.3
	Total	100	100.0
		$G = 92.581$, 3 d.f.; ($p < 0.001$)	