THE UNIVERSITY OF MANITOBA

COMPARISON OF POPULATIONS OF BROOK STICKLEBACK *CULAEA INCONSTANS* (KIRTLAND) WITH AND WITHOUT PREDATION BY A PISCIVOROUS FISH

by

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ABSTRACT

Aspects of the morphology, ecology and behaviour were compared between a population of brook stickleback, *Culaea inconstans*, exposed to predation by creek chub, *Semotilus atromaculatus*, and one lacking a piscivorous predator. Differences in morphology and sex ratio were not clearly related to predation. Stickleback exposed to chub had shorter life spans, larger eggs and greater fecundity. Field observations indicated that where chub were present, stickleback were more closely associated with cover and avoided a potential predator at a greater distance. Behavioural experiments in the laboratory with wild caught stickleback suggested that differences observed in the stream were largely phenotypic.

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INTRODUCTION

Although predation is not the only source of mortality for a prey population its effects are often substantial. This study compares aspects of the ecology, morphology and behaviour of two populations of brook stickleback (Culaea inconstans), one exposed to predation by northern creek chub (Semotilus atromaculatus atromaculatus) and the other lacking a piscivorous predator. A comparison of this nature is useful in determining the mechanisms used by the prey to compensate for mortality from predation.

Seghers (1973) compared populations of guppies (Poecilia reticulata) exposed to varying intensities of predation and determined antipredator behaviour to be best developed in association with the highest degree of predation. Krumholz (1963), comparing a predator-free and predator-exposed population of mosquitofish (Gambusia manni), determined lack of predation to be responsible for increased longevity and decreased fecundity.

The objectives of this study are to compare (a) morphological features of the two populations including spine length and number (b) characteristics of the populations including age structure, growth, length-weight relationships, sex ratio and fecundity (c) antipredator behaviour including association with cover and intensity of escape response (reaction distance).

MATERIALS AND METHODS

Description of Study Areas

In the Mink River brook stickleback are exposed to predation by creek chub (Moshenko and Gee 1973) while those in the Drifting River lack a piscivorous predator. Both rivers originate in the Duck Mountains and flow eastwards to Dauphin Lake. The study area in the Mink River was the middle zone (Gibbons 1971) in 1972 and was shifted to the upper zone in 1973 due to winter kill. Creek chub are present in both areas but are at a lower density in the upper zone. Both areas are bounded by deciduous forest alternating with grain and livestock farms. The study area on the Drifting River was located in coniferous forest with no agricultural activity present (see Appendix 1 for further details).

Field Study

I. Population Characteristics and Ecology

To obtain data on age structure, sex ratio, spine length and number, stickleback were collected by seining from Mink and Drifting Rivers monthly from May to August, 1972. Standard length, spine number and sex was recorded for each fish. Length-frequency histograms and probability paper (Cassie 1954) were used to age fish. Fish were separated into 5 mm size groups from each of which individuals were randomly selected for spine measurement. Dorsal, pelvic and anal spines were removed from five-spined individuals, mounted on slides and measured (±0.1 mm) from tip to socket with a stage-mounted micrometer on a binocular microscope.

To compare sizes of stickleback eaten by creek chub with those present in the population, data on stickleback taken from stomachs of chub from the Mink River were obtained from G. Newsome.

To compare fecundity of stickleback from both populations, samples were collected weekly from May 14 - June 20, 1973 by seining. Intact ovaries were removed, weighed (±0.05 gm) and preserved in Gilsons fluid (as modified by Simpson 1951 cited in Bagenal and Braum 1968). The entire fish, minus ovaries, was weighed and maturity index (gonad weight/total body weight) calculated. Fish of equal sexual maturity were used to plot body weight against standard length.

A female was considered sexually mature when the ovaries completely filled the ventral cavity (Kesteven 1960 cited in Bagenal and Braum 1968). Only eggs from females at this stage of development were used for counts and measurement of egg diameter in both populations. Mean egg diameter (±0.1 mm) was obtained by individual measurement of 10 eggs per female with a stage-mounted micrometer on a binocular microscope. Fecundity was determined by actual egg count.

II. Behaviour

Data on association with cover of fish age 1 and older were obtained by underwater observation with a face-plate and snorkel in June and July. The procedure for underwater observation consisted of slow movement upstream until a stickleback was located. Its position, relative to nearest cover, was measured (±5.0 cm) and recorded. Cover was defined as the nearest clump of weeds, deadfall or rock. Each fish was recorded only once.

To describe the response of stickleback to a model predator underwater observations, with a face-plate and snorkel, were made in both rivers in July 1972. Two responses were measured; (a) "reaction distance" or the distance at which a stickleback responds to a model predator by "darting away" using a strong flick of its tail, and (b) "investigatory response" whereby the stickleback orients toward the model and approaches it slowly. The fish stops at some distance from the model and either turns and swims away or moves backward while still oriented toward the model.

The wooden model was painted black dorsally and white ventrally, and mounted on a wire rod. When a stickleback was located the model was moved, by the fully submerged observer, toward the stickleback at a constant rate until it "darted" away. The distance between the model and the stickleback (prior to its response) was measured and recorded by an assistant on shore. Only adult (age 1+) stickleback were tested and each fish recorded only once. If the stickleback moved toward the model as it was being advanced, the response was recorded as "investigatory".

Laboratory Study

Association with cover

To determine if stickleback from the Mink River tended to associate with cover more closely than fish from the Drifting River, an aquarium (115 x 51 x 60 cm) was fitted with a movable bottom marked into 10 x 17 cm sections. This created 3 longitudinal rows 17 cm wide and 10 transverse rows each 10 cm wide (Fig. 1). Sections of "cover" were created by inserting ¼ inch vertical wooden dowels (height 54 cm) into alternating 10 x 17 cm sections (15 dowels per section). The transverse rows were identified by letter (A-K) and the longitudinal rows by numbers (1-3).

Two 122 cm fluorescent lamps, set at 25 cm above the surface of the water, provided equal illumination for the entire tank.

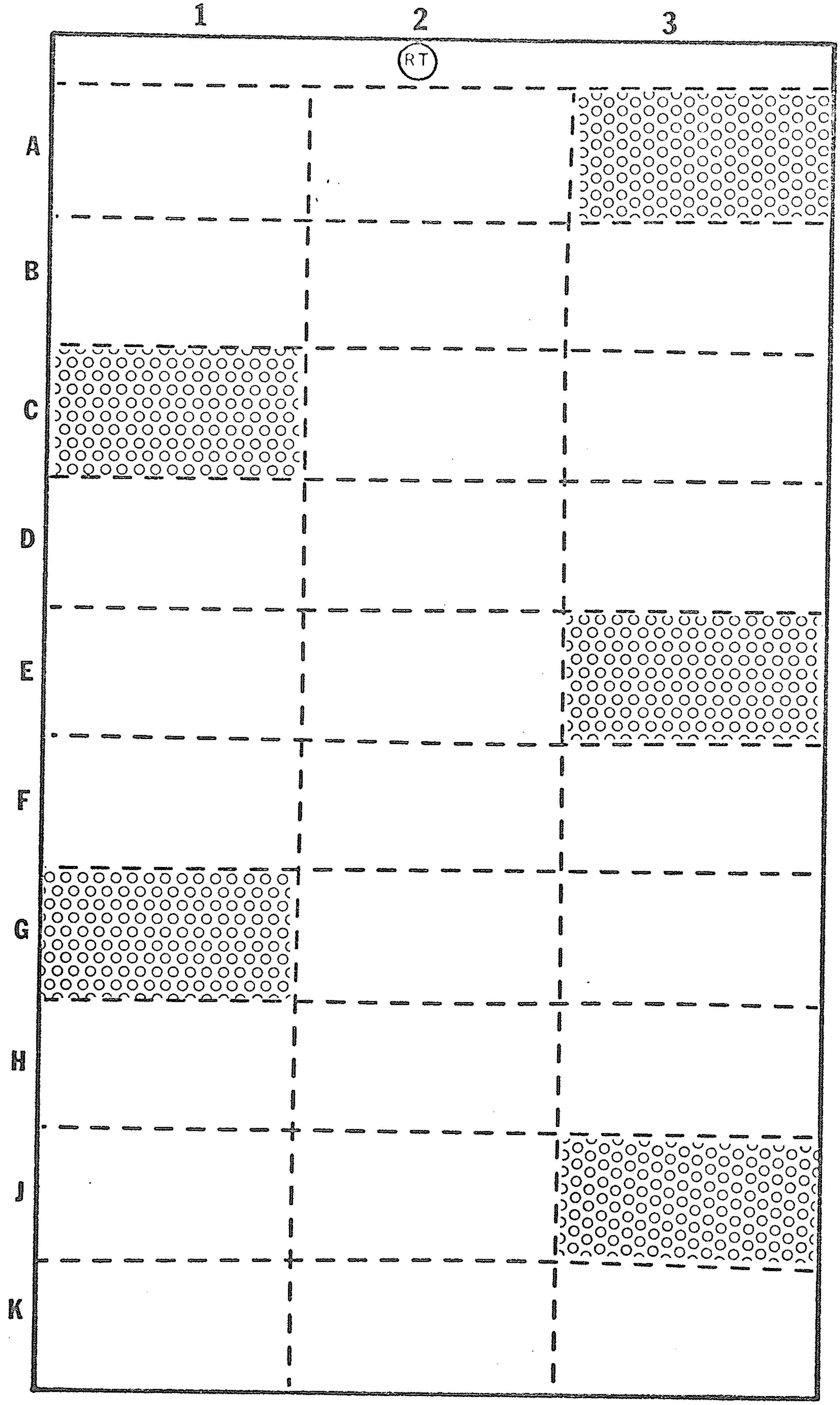
Stickleback, separated into age 0 (fry) and age 1+ (all fish in their second summer and older), were individually placed in an opaque release tube (10 cm diameter x 60 cm) for 15 minutes prior to release. The tube was then raised by pulley and the position of the fish recorded every 10 seconds for 10 minutes.

All stickleback used had been kept in the laboratory 3-4 weeks prior to testing.

<u>Distribution of stickleback before and after visual contact with the predator</u>

To determine if the distribution of stickleback before and after visual contact with creek chub had been established differed between populations, fish were tested in an aquarium (115 x 51 x 30 cm) partitioned to contain both predator and prey. A clear plexiglass sheet divided the tank into sections of 85 and 30 cm in length. The smaller section contained one creek chub (fork length 145 mm). An opaque sheet, attached to a pulley, visually isolated prey from predator. A reference grid of 5 cm intervals was marked on the glass front of the larger section of the tank. Randomly selected age 0 and 1+ stickleback

FIGURE 1. Plan of aquarium used to measure association with cover. Each stippled section indicates vertical "cover"; R.T. = release tube.


1
2
3
RT
A
B
C
D
E
F
G
H
J
K

were individually tested. Each fish was placed in the larger section of the tank and its position recorded every 10 seconds for 10 minutes. The opaque screen was then raised and the stickleback's position recorded in a similar manner. The distance at which each fish saw the predator and the time taken to first notice the predator were also recorded. A reaction to the predator generally consisted of raised dorsal spines and a "tail-down" sigmoid (S-shaped) posture with direct orientation toward the predator.

As differences in predator movement could affect stickleback distribution, a record of the activity of the predator was kept for each fish tested.

Reaction distance

To compare reaction distances between populations, randomly selected stickleback were individually placed in an aquarium (115 x 51 x 55 cm) for a 10 minute acclimation period after which a wooden model, suspended on a wire rod, was moved toward the stickleback at a constant rate. The distance at which the stickleback "darted" away and the distance it moved were measured (± 1.0 cm) using a grid drawn on the front of the tank. All stickleback were recorded only once.

RESULTS

Field Study

I. Population Characteristics and Ecology

Morphological comparison

Spine lengths were averaged for all 5-spined individuals. Each population was divided into 5 mm size groups and the overall mean spine length calculated for each group. No significant differences (t-test) were found between populations within size groups for overall mean dorsal, mean pelvic and anal spine lengths (Appendix 2).

Comparison of the ratio of 4-, 5- and 6-spined fish between populations produced significant ($p < 0.05$) chi-square values for June, July and August (Table 1). For June and July the numbers in the 6-spined category contribute most to the total chi-square, there being more 6-spined fish in the Mink than in the Drifting River. For August the numbers in the 4-spined category contribute most to the total chi-square, there being more 4-spined fish in the Drifting River.

Age structure and longevity

Comparison of length frequency histograms (Fig. 2) indicated that in May the Mink River population lacked very small (<25 mm) and large (>50 mm) stickleback that occurred in the Drifting River. Separation of age groups by probability paper indicated that large (>50 mm) stickleback from the Drifting River in May and June were age 3.

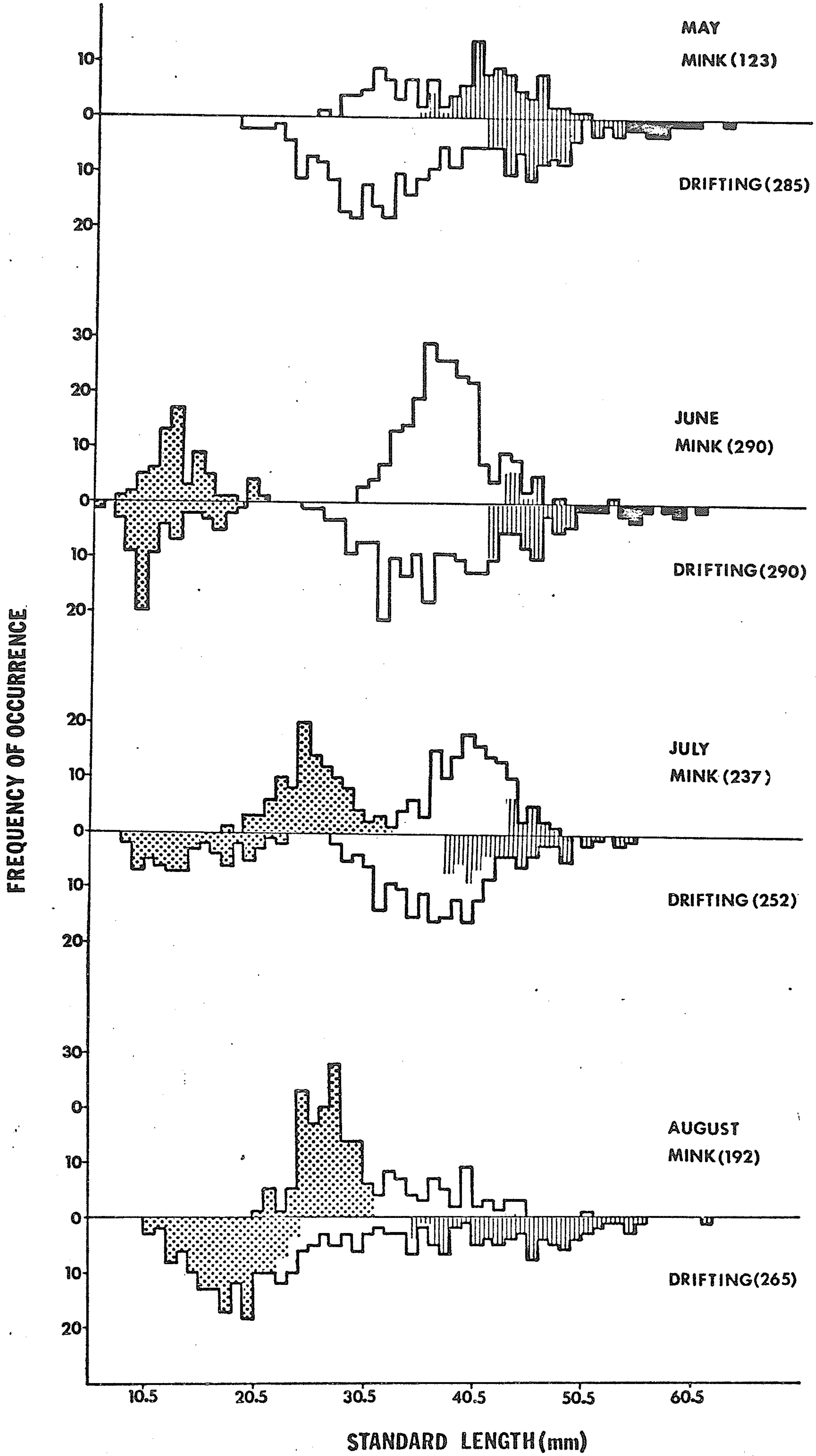
TABLE 1. Comparison of the number of 4-, 5- and 6-spined fish in Mink and Drifting Rivers.

Month (1972)	Population	Spine Number 4	5	6	No.	P-value
May	Mink	1	105	17	123	
	Drifting	2	242	41	285	
June	Mink	1	248	41	290	$p<0.05$
	Drifting	4	264	22	290	
July	Mink	2	189	46	237	$p<0.05$
	Drifting	1	224	27	252	
August	Mink	2	167	23	192	$p<0.05$
	Drifting	16	232	17	265	

FIGURE 2. Length frequency distribution for Mink and Drifting Rivers 1972. Age groups were separated by probability paper (see Appendix 4) for May - August.

Age 0   Age 1

Age 2   Age 3



MAY
MINK (123)
DRIFTING (285)
JUNE
MINK (290)
DRIFTING (290)
JULY
MINK (237)
DRIFTING (252)
AUGUST
MINK (192)
DRIFTING (265)
FREQUENCY OF OCCURRENCE
10.5
20.5
30.5
40.5
50.5
60.5
STANDARD LENGTH (mm)

Maximum age for Mink River fish was 2 years. Sample size for September was too small for accurate separation of age groups but closely followed the length frequency pattern of May.

Analysis of the size of stickleback eaten by creek chub in June (Fig. 3) indicated that primarily age 1 fish were being captured. No age 0 fish were eaten by the size groups of chub examined (>90 mm fork length) and predation on age 2 stickleback was low. During July predation was most intense on age 0 and 1 stickleback.

Growth

Comparison of mean lengths (Fig. 4) indicated that growth of age 0 and 1 stickleback from the Mink River was more rapid than growth of corresponding age groups from the Drifting River during the summer 1972.

Sex ratio

Monthly and overall pooled sex ratios were tabulated for each age group of stickleback from both populations (Table 2). Chi-square analysis was done on individual monthly ratios only when pooled ratios were heterogeneous.

Analysis of 1972 data for age 0 stickleback from the Mink River indicated that the pooled sex ratio deviated significantly ($p<0.05$) from a 1:1 relationship. All monthly samples of age 0 fish from the Mink River had an excess of females but only the August sample deviated significantly ($p<0.05$) from a 1:1 ratio. Significant excesses of females occurred for age 1 fish (Mink River) in August ($p<0.05$) and age 2 fish (Mink River) in June

FIGURE 3. Comparison of stickleback found in stomachs of creek chub (Mink River) with those present in the stream. Separation of age groups as in Figure 2.

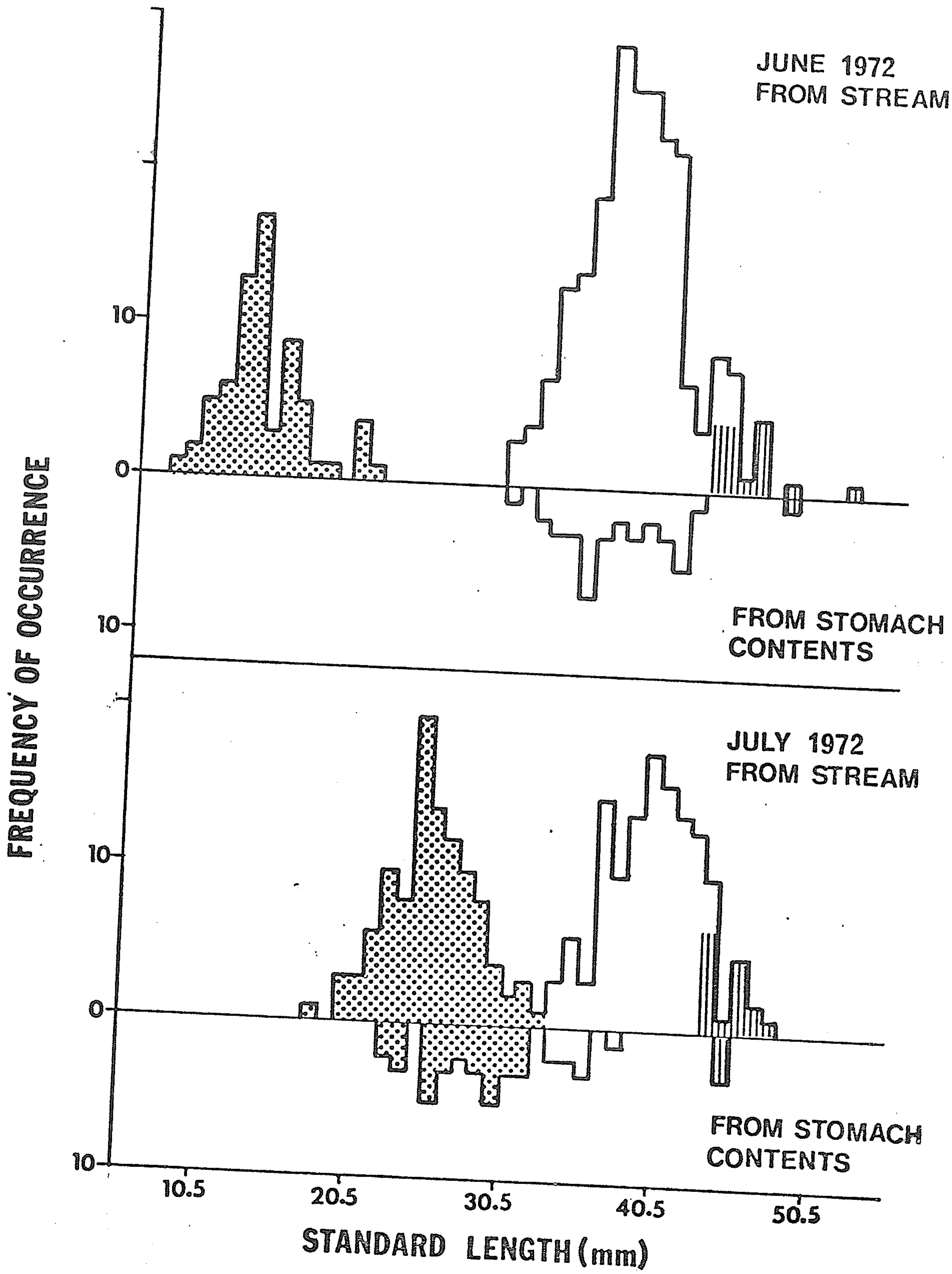
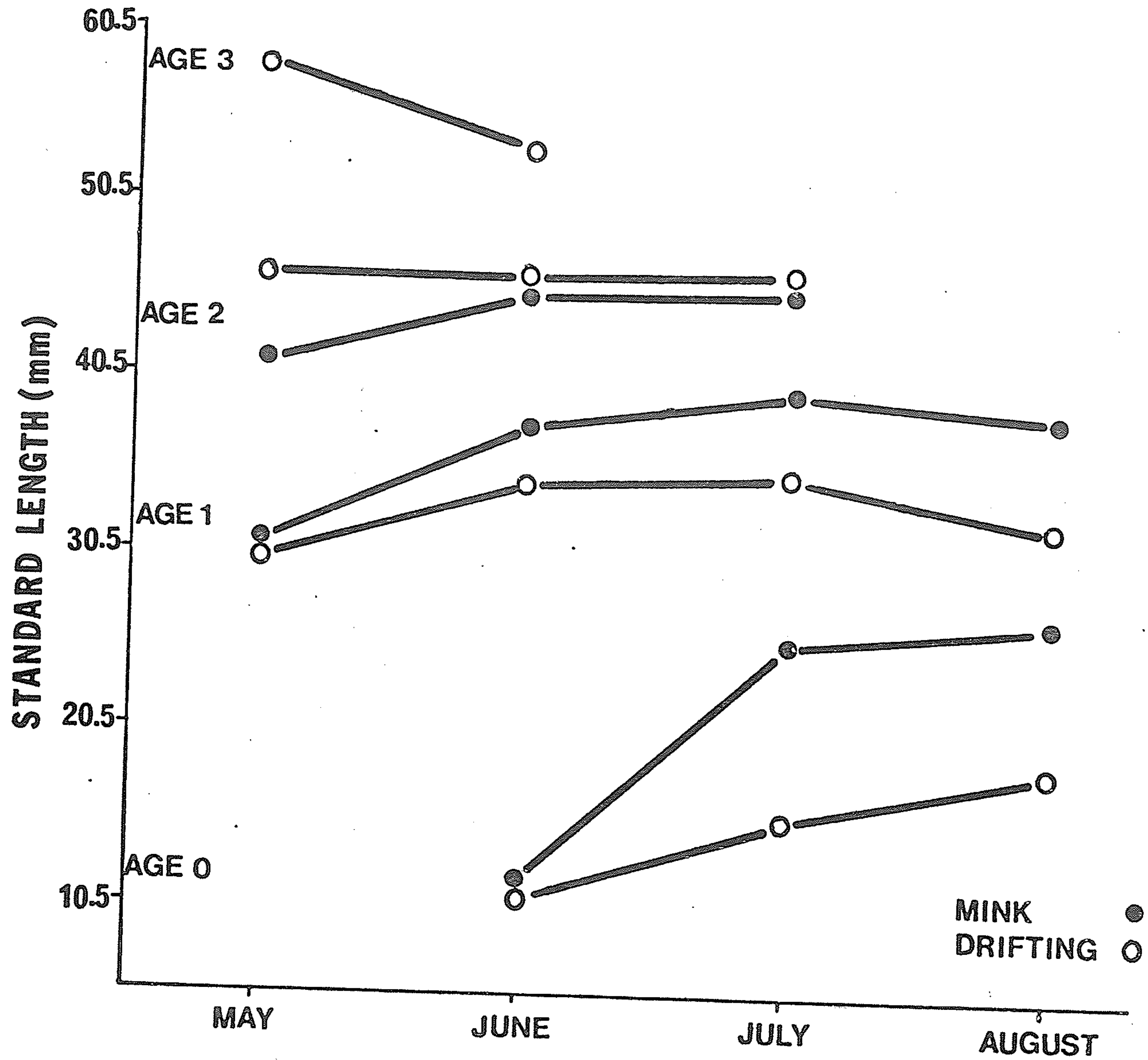

JUNE 1972
FROM STREAM
FROM STOMACH CONTENTS
JULY 1972
FROM STREAM
FROM STOMACH CONTENTS
FREQUENCY OF OCCURRENCE
10
0
10
10
0
10
10.5
20.5
30.5
40.5
50.5
STANDARD LENGTH (mm)

FIGURE 4. Growth in standard length of separate age groups of *Culaea inconstans* during the summer 1972.



60.5
50.5
40.5
30.5
20.5
10.5
STANDARD LENGTH (mm)
AGE 3
AGE 2
AGE 1
AGE 0
MAY
JUNE
JULY
AUGUST
MINK
DRIFTING

TABLE 2. The sex ratios of brook stickleback (5-spined individuals) from Mink and Drifting Rivers (1972, 1973). Asterisks under the ratios indicate levels of significance (*p<0.05, **p<0.01).

Source	Collection Date	Age 0 ♂	0 ♀	1 ♂	1 ♀	2 ♂	2 ♀	3 ♂	3 ♀	Total Sample Size
	1972									
	May			19	30	36	32			117
MINK	June	17	29	107	92	3*	13			261
RIVER	July	45	56	61	57	10	3			232
	August	46**	88	19*	38					191
	Pooled ratio	108*	173	206	217	49	48			801
	1973									
	July	227	193	308	334	11	10			1083
	August	113	123	58	64					358
	Pooled ratio	340	316	366	398	11	10			1441
	1972									
	May			82	98	28	42	9	11	250
DRIFTING	June	12	8	88	72	24	18	5	6	233
RIVER	July	32	22	65	78	32*	9			238
	August	72	64	19	25	26	28			234
	Pooled ratio	116	94	254	273	110	97	14	17	955

($p<0.05$). Analysis of 1973 data for July and August indicated no significant deviation from a 1:1 ratio for any age group.

Pooled sex ratios for age 0, 1 and 3 stickleback from the Drifting River did not deviate significantly from a 1:1 ratio. Analysis of monthly sex ratios for age 2 fish indicated an excess of males in July 1972.

Sexual maturity

Comparison of the number of gravid females between the two populations indicated that there was a significantly ($p<0.01$) lower proportion of sexually mature females in the Drifting River. No age 3 females from the Drifting River were considered sufficiently gravid to deposit eggs and none were determined to be in spent condition. The number of sexually mature females in both populations is as follows (total number of females sampled in parenthesis):

Population	Mink River	Drifting River
Number of sexually mature females	37(203)	13(212)

Length-weight relationship

Regression lines for net body weight (minus gonads) relative to standard length indicated similar slopes but different intercepts as follows: Mink River, log net weight = -11.15 + 2.97 log standard length (N=66) Drifting River, log net weight = -10.65 + 2.78 log standard length (N=36). Analysis of covariance indicated that female stickleback of similar length were significantly ($p<0.05$) heavier in the Mink than the Drifting River as follows:

Population	Mink River	Drifting River
Adjusted mean weight (gm)	0.94	0.76

Fecundity

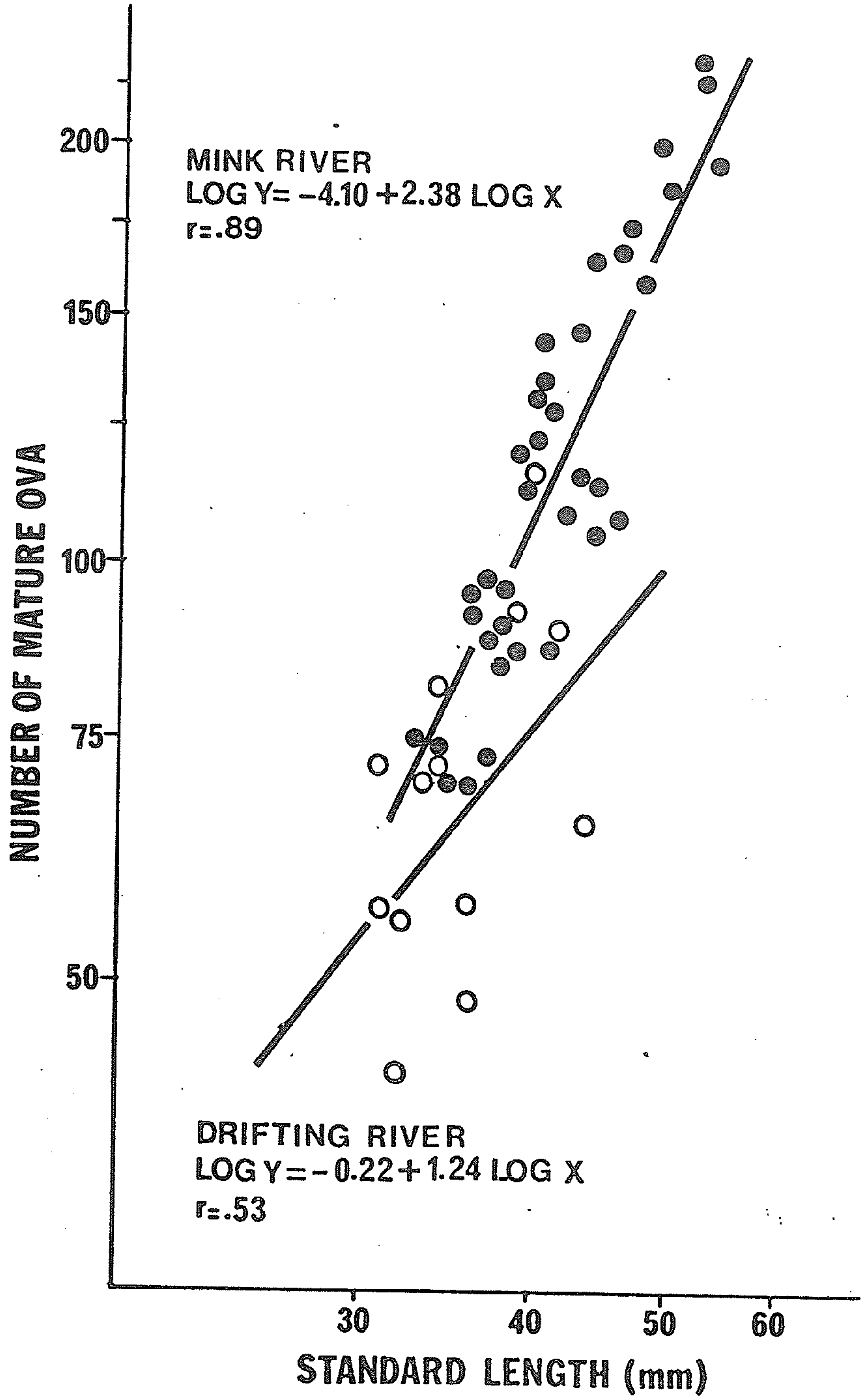
Analysis of covariance indicated that female stickleback of similar length produced significantly ($p<0.01$) more eggs in the Mink than Drifting River (Fig. 5). The mean number of eggs adjusted for fish length, is shown as follows (number of fish used for analysis in parenthesis):

Population	Mink River	Drifting River
Adjusted mean egg number	108 (37)	86 (13)

Egg size

Egg diameter was found to be linearly related to standard length and net body weight in the Mink but not the Drifting River population. To compare egg size, mean egg diameter were grouped according to fish length and the overall means compared. The average diameter of Mink River eggs exceed that of Drifting River eggs in each size group. The overall means showed a significant ($p<0.05$) difference as follows (number of fish examined in parenthesis):

FIGURE 5. Mean egg number relative to standard length for Mink and Drifting Rivers 1973.



MINK RIVER
LOG Y= −4.10 +2.38 LOG X
r=.89
DRIFTING RIVER
LOG Y=−0.22+1.24 LOG X
r=.53
NUMBER OF MATURE OVA
200
150
100
75
50
30
40
50
60
STANDARD LENGTH (mm)

Size group(mm)	Mink River		Drifting River	
30 - 34.9	1.11	(4)	1.07	(6)
35 - 39.9	1.13	(13)	1.09	(3)
40 - 44.9	1.19	(11)	1.06	(4)
Overall mean egg size (mm)	1.15	(28)	1.06	(13)

II Behaviour

Association with cover

Underwater observation for June and July indicated that stickleback in the Mink River were found significantly ($p<0.01$, t-test) closer to cover than stickleback in the Drifting River shown as follows (number of fish observed in parenthesis):

	Mean distance to cover (cm) ± 1 standard deviation			
Population	Mink River		Drifting River	
June	10.90 ± 0.95	(56)	17.80 ± 1.40	(64)
July	5.10 ± 1.00	(41)	17.90 ± 2.80	(52)

Reaction distance

Stickleback from the Mink River reacted to the model predator at a significantly ($p<0.05$, t-test) greater distance than stickleback from the Drifting River shown as follows (number of fish tested in parenthesis):

Population	Mink River	Drifting River
Mean reaction distance ±1 standard deviation	14.70 ± 1.50 (22)	9.10 ± 0.90 (28)

Investigatory response

When confronted with the model predator a significantly ($p<0.01$, chi-square) higher proportion of stickleback from the Drifting River exhibited an investigatory response as follows (number of fish tested in parenthesis):

Population	Mink River	Drifting River
Number of fish exhibiting investigatory response	1 (22)	9 (28)

Laboratory Study

Association with cover and "freezing" response

When the acclimation tube was raised the stickleback either (a) sank to the bottom and remained immobile, (b) began slow exploration of the tank, or (c) darted rapidly to cover.

The mean time after release required by age 0 and 1+ stickleback to reach cover did not differ (Mann-Whitney U-test, Siegel 1956) between populations shown as follows (number of fish tested in parenthesis):

Population	Mink River		Drifting River	
Age group	0	1+	0	1+
Mean time to reach cover (sec)	85 (24)	155 (37)	107 (24)	127 (37)

Comparison of the mean time spent in cover indicated no difference (Mann-Whitney U-test) between populations or age groups shown as follows:

Population	Mink River		Drifting River	
Age group	0	1+	0	1+
Mean time in cover(sec) /fish/test period	442	420	446	434

Individuals remaining immobile during the entire 10 minute test period were considered to exhibit the "freezing response" (Benzie 1965) and were treated separately. The "freezing response" occurs with Gasterosteus and Pungitius when a predator is encountered very suddenly or when the stickleback is subjected to a new environment such as a test aquarium. The fish becomes very still with reduced opercular beat-rate and little fin movement. It either sinks to the bottom or rises to the surface presenting a minimal stimulus to release attack by the predator. With Culaea the "freezing response" was often preceded by erratic swimming and always terminated with the fish sinking to the bottom. Both populations exhibited this response to a similar extent although significantly ($p<0.01$, chi-square) more age 1+ fish responded in this manner as follows (number of fish tested in parenthesis):

Population	Mink River		Drifting River	
Age group	0	1+	0	1+
Number of fish showing "freezing response"	1 (24)	12 (37)	1 (24)	10 (37)

Distribution before and visual contact with the predator

No differences in the activity of the predator were observed at any time during the experiment.

Distribution of stickleback before and after visual contact with the predator did not differ significantly (chi-square test) between populations (Table 3).

Time taken to react and distance from predator reaction occurs

The mean time and distance at which each group of fish reacted to the predator are shown as follows:

Population	Mink River		Drifting River	
Age group	0	1+	0	1+
Mean time for reaction (sec)	148	64	111	75
Mean distance at which reaction occurs (cm)	30	30	20	25

No significant difference occurred between populations in the time required for age 1+ stickleback to react or in the distance at which they reacted to the predator. Age 0 fish from the Mink River took significantly ($p<0.05$, Mann-Whitney U-test) longer to react and reacted at a significantly ($p<0.05$) greater

Table 3. Distribution of <u>Culaca</u> before and after visual contact with the predator. B=before A=after; number of fish tested in parenthesis.

Age Group	Population		Distance from predator 0-20	25-40	45-60	65-85
			number observations per fish per 10 min			
0	Mink (24)	B	33	7	6	15
		A	22	6	6	27
	Drifting (18)	B	33	6	6	16
		A	27	5	5	20
1+	Mink (35)	B	27	4	3	11
		A	25	5	4	12
	Drifting (30)	B	24	6	5	11
		A	25	4	4	11

distance than age 0 fish from the Drifting River.

Reaction distance to model predator

Reaction distances and distance moved after reacting are shown as follows (number of fish tested in parenthesis):

Population	Mink River		Drifting River	
Age group	0	1+	0	1+
Mean reaction distance (cm) ± 1 standard deviation	6.60±0.75 (16)	7.20±0.80 (49)	4.60±0.40 (19)	6.10±0.60 (51)
mean distance moved (cm) ± 1 standard deviation	10.60±2.30 (16)	41.90±3.50 (49)	14.70±2.40 (19)	24.80±2.70 (51)

Age 0 fish from the Mink River reacted to the model at a significantly ($p<0.05$, t-test) greater distance than age 0 fish from the Drifting River. No difference in reaction distance was found for age 1+ fish between populations. Age 1+ fish from the Mink River moved significantly ($p<0.05$) further than age 1+ fish from the Drifting River during their escape response. No difference was found between populations for age 0 fish.

DISCUSSION

Morphology

Mean spine length

Spiny fin-rays are one of the adaptations that protect prey species against predation (Popova 1967). All members of the Gasterosteidae possess well-developed dorsal, pelvic and anal spines. Their effectiveness as antipredator mechanisms has been demonstrated both for Gasterosteus aculeatus and Pungitius pungitius against small pike and perch (Hoogland et al., 1956). The longer spines of Gasterosteus were more effective against the predators than the relatively short spines of Pungitius.

Intraspecific differences in spine length could result from selective pressures within populations. If the spines of Culaea are an effective antipredator adaptation against creek chub, individuals possessing the longest spines would be better protected when exposed to predation. In such a population individuals with the longest spines would predominate. A population of stickleback lacking a piscivorous predator would not be exposed to selection for spine length and would have shorter spines. Comparison of spine length between two such populations should reflect these selective pressures.

The lack of significant differences in spine length between populations with (Mink River) and without (Drifting River) a piscivorous predator suggests that spine length is of minor importance in determining the survival of Culaea. In addition, analysis of the sizes of stickleback eaten by small (90-130 mm) and large (>130 mm) creek chub indicated that the greatest variation in the length of stickleback eaten occurred among the small chub. Small chub were capable of eating the largest stickleback available to them in the stream (G. Newsome, pers. comm.). As the effectiveness of spines decreases with increasing predator size (Frost 1954) the ability of small chub to consume very large stickleback indicates that spine length is not an important antipredator adaptation against the size range of chub examined.

Dorsal spine number

Dorsal spine number, for Culaea, varies from four to seven with counts of five and six predominating. Geographical variations in dorsal spine number have been documented (Lawler 1958) but the causal factors are not yet clear. The higher proportion of six-spined fish in the Mink River (predator-exposed) suggests that dorsal spine number might be associated with predation. Differences in the proportions of six-spined individuals could be associated with behavioural differences just as variation in lateral plate count in Gasterosteus is linked with antipredator behaviour.

Age, Growth and Longevity

Determination of maximum longevity for the Gasterosteidae has produced highly variable results. Jones and Hynes concluded that Gasterosteus and Pungitius could exceed age 3. Maximum life span for Culaea was found to be age 1 (Winn 1960) and age 2 (Acere 1971) but survival to age 2 was low.

The greater longevity in the Drifting River (to age 3) has two possible causes. Slower growth rate for age 0 fish might tend to increase longevity (Gerking 1957 cited in Alm 1959). However, Alm (1959) and Krumholz (1963) determined that growth rate did not increase longevity but maximum life span could be attained in the absence of predation. In the present study the lack of piscivorous predation in the Drifting River probably influences life span to a greater extent than the slower growth rate of age 0 stickleback.

Predation - the mechanism limiting longevity in the Mink River

Creek chub prey most heavily on Culaea during June and July in the Mink River. Predation is directed against the most abundant age groups, age 1 in June and age 0 and 1 in July while predation upon age 2 fish is low for both months. In addition, physiological stress due to spawning could cause mortality directly or render stickleback more susceptible to being caught by chub. Consistent reduction of numbers of stickleback would severely reduce all age groups but would increase the proportion of young in the population (Cole 1954). Age 2 fish were eliminated from the samples by late summer. A possible sequence of exploitation of age groups is shown in Appendix 3.

Sex Ratio

In most species the two sexes are usually produced in approximately equal numbers (Hamilton 1967). Deviation from a 1:1 Mendelian sex ratio suggests differential mortality or unequal production of the sexes and warrants investigation of the causes.

Krumholz (1963) determined that unbalanced sex ratios for *Gambusia* were due primarily to the shorter physiological life span of males. Differential mortality can also be caused by predation. Seghers (1973) concluded that male *Poecilia reticulata* were less adept than females at avoiding capture thus producing significant excesses of female fish.

In the present study significant deviations from a 1:1 ratio occurred in the predator-exposed population with females consistently in excess. The significant overall excess of age 0 females in the Mink River (1972) was due primarily to the August sample. Age 1 females were also in excess during August. The excess of females from both age groups during the same month suggests a common cause.

Creek chub prey heavily on age 1 stickleback during June and July but age 0 fish are not recruited into the size range eaten by chub until July. Thus the significant excess of age 0 females in August could reflect differential mortality occurring in July. The absence of a significant deviation in the age 1 sex ratio until August could represent a lag in the effect of differential predation upon the age group.

Behavioural dimorphism between the sexes occurs during reproduction (Reisman and Cade 1967) but differences in behaviour outside this period have not been determined. The single excess of age 2 females after reproduction (June) is not sufficient evidence to support differential mortality due to predation based on behavioural dimorphism.

Subsequent sampling in 1973 indicated no deviations from a 1:1 sex ratio in the Mink River. Biotic conditions in the stream had changed in that density of creek chub had decreased and density of stickleback had increased (G. Newsome, pers. comm.). Population density, acting through changes in the quantity and quality of the food supply, has been determined to cause alteration in the sex ratio of fish (Nikolskii 1969 cited in Seghers 1973). Seghers found no apparent correlation of density and food supply with sex ratio but concluded that sex ratios of Poecilia approached 1:1 in streams exposed to low densities of a predator, Rivulus hartii. In the present study a decreased density of the predator and a 1:1 sex ratio in the Mink River (1973) suggests a similar relationship.

If stickleback assort themselves into microhabitats with respect to sex, distorted sex ratios may be caused by inadequate sampling. To eliminate this possibility sampling procedure was designed to include all habitat types and extensive areas of each type. Another source of error might be differences between the sexes in the ability to avoid capture. No information was available to substantiate this possibility.

Fecundity

The ability of a population to survive exploitation by a predator is determined by its compensatory response to increased mortality. A population may increase its fecundity, increase the number of spawnings per season, reproduce at an earlier age or minimize wastage of environmental resources on sterile members of the population (Cole 1954). All age groups, excluding age 0, in the Mink River (predator-exposed) were reproductive thereby eliminating environmental resource wastage that occurred in the Drifting River (no piscivorous predator). Increased longevity enabled female stickleback in the Drifting River to reach age 3 creating a non-reproductive segment of the population. The greater fecundity of stickleback from the Mink River could be either a genetic or phenotypic response to the environment. Fish culturists have shown that high fecundity can be selectively propagated. In a population experiencing high mortality, females producing the greatest number of eggs would contribute more individuals to successive generations and this genotype would be selected over those contributing fewer offspring. No breeding experiments were done to confirm the hypothesis. Alternately the compensatory response to increased mortality could be phenotypic in that reduction in density of stickleback due to predation would make additional resources available to the survivors thus increasing their fecundity. Increased food supply has been determined to increase fecundity in some species of fish (Scott 1962, McFadden et al 1965, Wootton 1973a). The relationship between food supply and increased fecundity in the Mink River is complicated by the apparent unequal fertility of the two streams (Appendix 1b). Greater weight (Ellis and Gowing 1957) and a higher proportion of maturing females (Wootton 1973b) can result from increased food level in a stream. Both parameters did occur in the Mink River, relative to the Drifting River, in the present study. Although no quantitative estimates of productivity were available, the possibility of unequal stream fertility contributing to differences in fecundity is present.

Egg Size

The direct cause of larger eggs could be food supply. Egg size can increase in response to increased quality or quantity of food (Schoener 1971), or remain stable (Scott 1962, Wootton 1973a). The direct relationship between egg diameter and stickleback weight in the Mink River indicates that if food quantity controls body weight, egg diameter is also influenced by food supply. The difference in egg diameter between the two populations could be due to reduced intraspecific competition for resources as a result of mortality caused by predation in the Mink River or to the difference in stream fertility. The lack of a similar direct relationship between egg diameter and fish weight in the Drifting River could be due to the small sample size.

When a species increases its fecundity egg size should decrease as space within the body cavity is limited (Svärdson 1949). The fact that egg size increases as fecundity increases suggests that the stress imposed upon the female stickleback by increasing ovary volume is secondary to the advantage gained by producing larger eggs. Larger eggs produce larger fry which would have a selective advantage when competition (Bagenal 1969) and faster growth rate (Svärdson 1949) are important. If stickleback fry had to compete with other species for a food resource, larger fry would be competitively superior thereby increasing their chances of survival. Increased survival would ensure an adequate population density to accommodate mortality due to predation. The significance of faster growth in the presence of predators is not yet apparent.

Comparison of Antipredator Behaviour of Stickleback from Mink and Drifting Rivers

Field Study

Efficient utilization of available cover would increase a prey's chances of survival when sharing the environment with a predator. Seghers (1973) showed that guppies (*Poecilia reticulata*) remained closer to cover when exposed to predation. Similar results were obtained in the present study as stickleback exposed to predation (Mink River) maintained a closer association with cover than stickleback lacking a piscivorous predator (Drifting River).

Stickleback display a variety of responses when confronted by a predator. The most intense escape response for *Gasterosteus and Pungitius* was termed "jumping away and fast swimming" (Benzie 1965) and is analogous to "darting away" for *Culaea*. The greater distance at which stickleback from the Mink River (predator-exposed) "darted away" from the model predator is similar to reactions exhibited by guppies exposed to varying degrees of predation. The guppies exposed to the most intense predation had the greatest reaction distance (Seghers 1973).

The main survival value of approach and investigation is the opportunity to reduce the chance of being caught unaware and the chance to learn the characteristics of potential danger (Marler and Hamilton 1967). Both populations exhibited the investigatory response but the Mink River fish to a lesser extent when presented with similar stimuli. Benzie (1965) observed that Pungitius, which depends primarily on behaviour to escape predation as does Culaea, did not utilize the investigatory response when sharing the environment with a predator. Variation in the intensity of the investigatory response by Culaea, as well as the association with cover and reactivity to predators, ensures that no wastage of energy occurs when maximum stimuli are not present. A population of stickleback can vary the intensity of its responses to match the degree of stimulation present. The process of avoiding predation can thus be described as an adjustment of fixed patterns of behaviour to changes in the environment.

Laboratory Study

A new situation for a small animal is a potentially dangerous one (Benzie 1965). Slow exploration of a new environment, such as a test aquarium, combined with maximum utilization of cover would produce few stimuli to release attacks by predators. Laboratory experiments failed to substantiate this hypothesis and produced few differences in behaviour between the populations.

An individual animal's previous experience will affect exploration (Berlyne 1960). Based on this stickleback from the Mink River should display more intense antipredator behaviour than stickleback from the Drifting River. Only age 0 fish from the Mink River reacted to predators at a greater distance than their Drifting River counterparts. While age 1+ fish did not differ in reaction distances, Mink River stickleback swam further after reacting. No other behavioural differences were observed.

Although not observed in the field the "freezing response" was exhibited by both Mink and Drifting River fish in the laboratory. The response could occur in the stream after the stickleback has darted into cover and thus not be seen by an observer. The difference between age 0 and 1+ stickleback showing the "freezing response" is of interest as it indicates that behavioural changes accompany ageing, older fish responding more intensely to potentially dangerous situations.

Differences in phenotype may be traceable to differences in genotype or may reflect non-heritable effects imposed on the individuals by differences in their environment (Mather 1973). Intraspecific differences in antipredator behaviour can have a genetic basis (McPhail 1969, Seghers 1973) but only breeding and rearing of animals in captivity can substantiate it. No such experiments were done in the present study.

The presence of genetic differences may be masked by environmental effects (Mather 1973). Genetic differences may not be expressed unless environmental conditions are suitable. Lack of experimental evidence to conclusively support field data may result from inadequacies in the experimental environment.

Alternately, differences in the intensity of behaviour observed in the stream may be entirely phenotypic. Behavioural plasticity, which is a form of phenotypic plasticity, enables individuals to adapt to sudden changes in the environment (Birch

1960). Intense predation occurs in the Mink River for June and July only. If this caused selection for genotypes with low thresholds of response (very responsive), a population which was very sensitive to a wide range of potentially dangerous stimuli would result. Such sensitivity, if completely automatic and unvarying in intensity, would make life impossible for the animal (Thorpe 1956). The selection for phenotypic plasticity (learning) would prevent needless wastage of energy which would occur if maximum response was induced by sub-maximal stimuli during months when predation was not intense. Since stickleback were maintained in the laboratory for relatively long periods before testing, lack of reinforcing stimuli (attacks by chub) could have induced waning of the antipredator responses in stickleback from the Mink River. Stickleback from the Drifting River, which probably possess reaction patterns of lower intensity, would not have been affected to the same extent.

CONCLUSIONS

The comparison of a predator-exposed population of brook stickleback with one lacking a piscivorous predator determined some of the consequences of predation and some of the strategies used by prey to survive exploitation. While adaptive morphological changes and sex ratio differences between populations were not clearly defined, stickleback exposed to predation had shorter life spans and greater fecundity than stickleback lacking a piscivorous predator. The observed difference in fecundity could be due either to genetic differences, or environmental effects. If environmentally induced, the role of predation is complicated by unequal fertility of the two streams. While the difference in egg size could be genetic, environmental effects are strongly implicated. The association of larger eggs with predation is not clear. Behavioural differences observed in the field demonstrate the adaptive response of the prey population to predation but the relative contributions of genetics and the environment to these differences can only be speculated. The lack of population differences in behaviour in laboratory experiments suggests that the differences observed in the field are phenotypic and were lost in the laboratory through lack of reinforcement.

The differences between the populations have been expressed as the result of predation but other factors such as species diversity and density, temperature regime and water chemistry could contribute to the observed differences.

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APPENDICES

APPENDIX 1a. Details of study areas.

		Mink River	Drifting River
Location	Middle	51°25'N; 100°20'W	51°25'N; 100°45'W
	Upper	51°25'N; 100°30'W	
Mean Elevation	Middle	335 m	686 m
	Upper	396 m	
Length of Study area	Middle	5 km	3 Km
	Upper	2 km	
Mean gradient (m/Km)	Middle	7	10
	Upper	2	
Mean width of stream	Middle	4 m	2 m
	Upper	2 m	
Substrate	Middle	sand-rubble	mud-silt
	Upper	mud-silt	
Vegetation			
(a) Terrestrial			
	Middle	Dense, high stands of willow and poplar	Coniferous growth, dense willow and tall grasses
	Upper	willow and tall grasses	
(b) Aquatic			
	Middle	*Carex*, *Typha* along margin of stream	*Carex* along margin, dense algae and deadfall on bottom.
	Upper	Some *Typha*	
Beaver ponds			
	Middle	Few ponds, stream consists mainly of alternating pools and riffles	Study area a series of ponds (>50 cm) and deep channels (20-50 cm).
	Upper	Mainly deep channels (20-50 cm)	

APPENDIX 1a (continued)

	Mink River	Drifting River
pH	9.5	9.5
Hardness (mg/l $CaCO_3$)	683	232
Mean temperature (C) 1972	middle zone only	
May 3	8	2
16	15	12
30	17	15
June 20	20	19
July 18	18	16
August 21	15	17
September 30	5	6
Species present (designated by X)	middle zone only	
Catostomus commersoni	X	
Culaea inconstans	X	X
Etheostoma nigrum	X	
Hybognathus hankinsoni	X	
Notropis cornutus	X	
Pimephales promelas	X	X
Rhinichthys atratulus	X	
R. cataractae	X	
Semotilus atromaculatus	X	
S. margarita	X	

APPENDIX 1b. Comparison of fertility between Mink and Drifting Rivers.

Although no quantitative determinations of nutrient content (phosphate, nitrate) in the streams were available, the following inferences were made: (a) the presence of agricultural activity on the Mink River meant input of animal waste and run-off containing phosphate and nitrate residue from fertilizer, (b) the absence of agricultural activity on the Drifting River meant no such input. Input of commercial land fertilizers and domestic sewage have been determined as important factors in the increased biological fertility of trout streams (Smith 1959, Ellis and Gowing 1957). Based on this fertility in both middle and upper zones of the Mink River was considered to be greater than in the Drifting River.

APPENDIX 2a. Anal spine length (mm) for each size interval.

Standard Length (mm)	Mink River		Drifting River	
	Anal Spine Length (mm) ± st. dev.	No.	Anal Spine Length (mm) ± st. dev.	No.
10-14.9	0.80 ± 0.20	5	1.04 ± 0.16	8
15-19.9	1.24 ± 0.18	8	1.41 ± 0.14	19
20-24.9	1.56 ± 0.18	14	1.72 ± 0.14	13
25-19.9	1.90 ± 0.26	18	2.15 ± 0.16	11
30-34.9	2.17 ± 0.18	10	2.23 ± 0.28	4
35-39.9	2.28 ± 0.08	6	2.56 ± 0.14	9
40-44.9	2.24 ± 0.16	7	2.39 ± 0.14	7
45-49.9			2.42 ± 0.12	9
50-54.9	2.60	1	2.24 ± 0.22	5
55-59.9			2.50	1
60-64.9			2.40	1

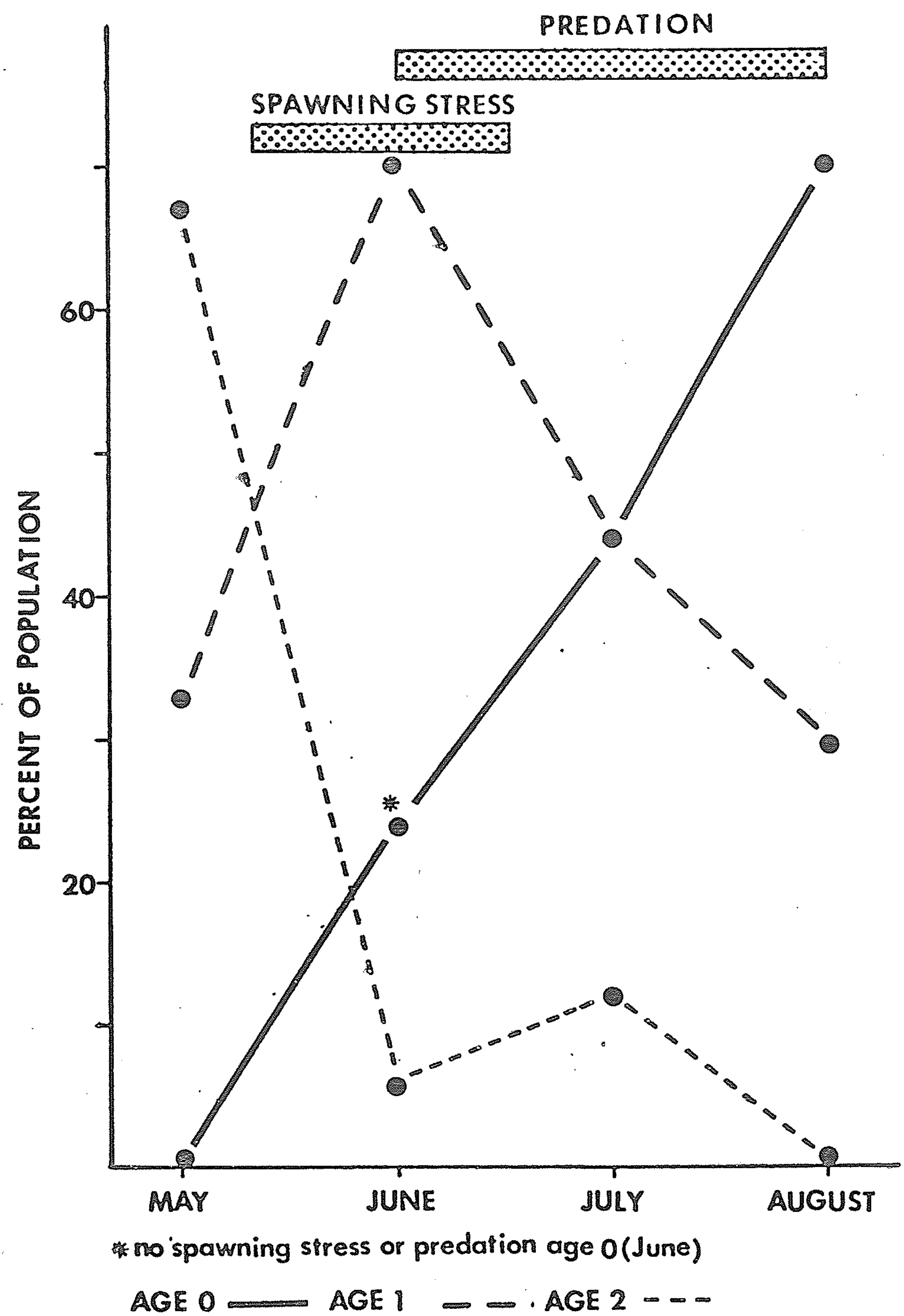
APPENDIX 2b. Overall mean pelvic spine length (mm) for each size interval.

Standard Length (mm)	Mink River		Drifting River	
	Mean Pelvic Spine Length(mm) ± st. dev.	No.	Mean Pelvic Spine Length(mm) ± st. dev.	No.
10-14.9			0.50	1
15-19.9	0.88 ± 0.30	5	1.01 ± 0.18	18
20-24.9	1.42 ± 0.18	16	1.32 ± 0.14	15
25-29.9	1.82 ± 0.16	22	1.93 ± 0.20	11
30-34.9	2.10 ± 0.24	10	2.02 ± 0.30	5
35-39.9	2.13 ± 0.10	7	2.29 ± 0.18	10
40-44.9	2.34 ± 0.32	8	2.45 ± 0.14	8
45-49.9			2.56 ± 0.28	10
50-54.9			2.25 ± 0.18	6
55-59.9			2.70	1
60-64.9			2.60	1

APPENDIX 2c. Overall mean dorsal spine length(mm) for each size interval.

Standard Length (mm)	Mink River		Drifting River	
	Mean Dorsal Spine Length(mm) ± st. dev.	No.	Mean Dorsal Spine Length(mm) ± st. dev.	No.
10-14.9	0.47 ± 0.06	6	0.65 ± 0.06	8
15-19.9	0.80 ± 0.14	10	1.03 ± 0.10	21
20-24.9	1.19 ± 0.12	16	1.29 ± 0.10	15
25-29.9	1.49 ± 0.14	22	1.64 ± 0.10	11
30-34.9	1.70 ± 0.14	11	1.72 ± 0.08	5
35-39.9	1.70 ± 0.10	7	1.69 ± 0.14	8
40-44.9	1.81 ± 0.14	8	1.76 ± 0.20	7
45-49.9			1.89 ± 0.38	8
50-54.9			1.68 ± 0.14	6
55-59.9			2.00	1
60-64.9			2.00	1

APPENDIX 3. Predation - the mechanism limiting longevity in the Mink River.



APPENDIX 4. Separation of stickleback from Mink and Drifting Rivers into age groups by probability paper.

Collection Date	Age Class	Mink River: Mean Standard Length(mm) ± st. dev.	Mink River: No.	Drifting River: Mean Standard Length(mm) ± st. dev.	Drifting River: No
May 1972	0				
	1	31.5 ± 6.0	41	30.5 ± 9.0	196
	2	41.5 ± 7.0	82	46.5 ± 7.0	70
	3			58.5 ± 4.0	19
June 1972	0	12.5 ± 5.0	68	11.5 ± 4.0	67
	1	37.5 ± 7.0	204	34.5 ± 7.0	159
	2	45.5 ± 3.0	19	45.5 ± 3.0	43
	3			53.5 ± 3.0	13
July 1972	0	25.5 ± 6.0	103	15.5 ± 7.0	63
	1	39.5 ± 6.0	108	35.5 ± 8.0	144
	2	46.5 ± 3.0	23	45.5 ± 8.0	42
	3				
August 1972	0	26.5 ± 5.0	156	18.5 ± 7.0	109
	1	38.5 ± 7.0	52	32.5 ± 9.0	96
	2			48.5 ± 8.0	59
	3				