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THE UNIVERSITY OF ALBERTA
PREDATION AS A MECHANISM IN THE EVOLUTION OF AN UNUSUAL POPULATION OF
STICKLEBACKS IN THE QUEEN CHARLOTTE ISLANDS, CANADA
(PISCES: GASTEROSTEIDAE)

by



G. E. E. MOODIE

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING, 1970

UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read,
and recommend to the Faculty of Graduate Studies for
acceptance, a thesis entitled Predation as a Mechanism
in the Evolution of an Unusual Population of Sticklebacks
in the Queen Charlotte Islands, Canada (Pisces: Gasterosteidae)
submitted by G. E. E. Moodie in partial fulfilment of the
requirements for the degree of Doctor of Philosophy.

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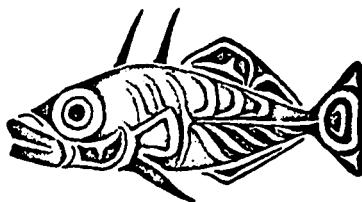
Date. *April 20/70.*

.....

There is also a little Fish called a *Sticklebag*;
a Fish without scales, but hath his body fenc'd
with several prickles. I know not where he dwells
in winter, nor what he is good for in summer, but
onely to make sport for boyes and women-Anglers,
and to feed other Fish that be Fish of prey,
as Trouts in particular, . . .

Izaak Walton, 1676.

The Compleat Angler.



from a design by R. Wilson

ABSTRACT

In Mayer Lake, Queen Charlotte Islands, Canada, an endemic threespine stickleback, termed the Black form, is subject to predation by two species of fish (*Salmo clarki* and *Cottus asper*) and a species of leech (*Haemopsis marmorata*). Parapatric with the Black form is the fresh-water threespine stickleback, *Gasterosteus aculeatus* form *leiurus*, which is not exposed to predation due to its restriction to creeks entering the lake.

The objective was to determine how much of the differentiation of the Black form could be considered a result of selection by predation. The Black form differs from the form *leiurus* in its larger size, darker pigmentation, drab and variable nuptial coloration, longer pelvic spines, streamlined body shape and higher gill raker and meristic counts. Data based on field observations supplemented by some experimental studies suggest most of the differences are indeed a result of selection by predation.

Comparisons were made between sticklebacks of the Black form found in trout stomachs and those obtained by netting. Females of the Black form taken from the stomachs had relatively shorter pelvic spines than did those captured by netting. Fish of both sexes found in the stomachs differed in the frequency distribution of lateral plate number from fish at large in the lake.

Breeding males of the Black form were individually captured while at their nests. Characteristics of the males and the environment around the nests were noted. Analysis showed that the differences in lateral plate number of the males was related to differences in the location of the nest and the behavior of the fish.

Males with and without red throats were exposed to trout in aquaria. Results suggested that non-red breeding colors have been selected for in order to render the male more cryptic while guarding the nest.

Comparison of the red (14 - 18% of the population) and non-red males of the Black form indicates that red males may be more aggressive than non-red males.

The large size of individuals of the Black form is probably a result of selection for several different traits. There is a stronger correlation between the size of prey and the predator size in the case of the sticklebacks than there is in the case of cottids (*C. asper*), a relatively spineless prey. This supports the hypothesis that a large stickleback is theoretically more difficult to swallow than a small one, given the same predator. Females of the Black form prefer as mates the larger of a pair of Black males. This results in an ethological isolating mechanism.

As a result primarily of the marked ecological isolation between the two forms in Mayer Lake, the Black form probably represents a biological species. The phylogeny of the Black form is difficult to assess because of the problem of convergence. However, it seems most likely that it has evolved from the anadromous form of *G. aculeatus*, i.e. *trachurus*.

The differentiation of the Black form of Mayer Lake as well as that of sticklebacks in several nearby lakes is probably best considered as a post-glacial phenomenon.

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My field assistants deserve great credit for persevering under conditions which were often unpleasant. The presence of Mr. T.E. Reimchen was invaluable. His suggestions, observations and help in analysis of the data contributed much to the study.

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INTRODUCTION

Predation is a powerful selective force. This has been clearly demonstrated by the classical studies of balanced polymorphism in the snail, *Cepaea nemoralis* L., transient polymorphism in the moth, *Biston betularia* L., and mimicry (Ford, 1964).

In the Queen Charlotte Islands several populations of threespine stickleback, *Gasterosteus aculeatus* L. have diverged markedly from the typical *G. aculeatus*, in respect to their morphology, ecology, and sometimes behavior. Much of the variation exhibited in these populations appears to have been a result of either predation or its absence. The end product in the two cases is different. This study is concerned with certain facets of the biology of one of these divergent forms.

Threespine sticklebacks may be anadromous or occur in fresh-water for the entire life-span. The fresh-water, partly plated form, termed *leiurus* by European workers, has a life-span of from one to three years. It generally dwells in still or slow-moving waters where vegetation is abundant. The anadromous, fully plated form, termed *trachurus*, enters fresh-water only to breed. In the spring sexually mature males of both forms develop bright nuptial colors, in particular, red throats. These males establish territories in which they construct a nest. Females are courted by means of an elaborate display. After spawning the female is driven from the territory. The male guards and aerates the eggs and protects the fry for several days after hatching. Sticklebacks are weak swimmers and rely upon their cryptic coloration and spines for protection. Further life-history information and details of the reproductive cycle may be found in Greenbank and Nelson (1959) and van Iersel (1953).

In Mayer Lake, the study area, the divergent population is parapatric with the typical *G. aculeatus*, form *leiurus*. In this discussion the divergent form is termed the 'Black' stickleback (life-history notes about this form are given in Appendix I). It must be emphasized that the use of the term 'Black' in this discussion is not intended to imply any relationship or close similarity between the form in Mayer Lake and other populations of black sticklebacks in the Pacific Northwest.

The Black stickleback is known only from Mayer Lake, although a parapatric pair of populations, one of whose members have characteristics similar to the Black form is present in another nearby lake. Black sticklebacks are about three times larger than individuals of *leiurus* in the same lake, and have uniform black sides and back with silver countershading. Specimens of *leiurus*, in contrast, have the usual mottled olive on the sides and back with gold countershading.

The Black form is found in all parts of the lake, except the mouths of the three inlet streams. Individuals of *leiurus* are found only in the streams and stream mouths. Of great importance is the distribution of predator species in the lake and stream habitats. I found no predators in the streams or stream mouths. Members of *Salmo clarki* Richardson, the cutthroat trout, *Cottus asper* Richardson, the prickly sculpin, and *Haemopsis marmorata* (Say), a species of leech, all prey upon the Black form and are common in the rest of the lake.

Preliminary observations suggested that predation might have influenced differentiation of stickleback forms in Mayer Lake. The aim of this research was to test this hypothesis. This approach would serve several purposes:

1. The importance of predation as a selective force in a vertebrate population could be evaluated.
2. The adaptive value of certain characteristics of the genus *Gasterosteus* could be elucidated.
3. In the light of the knowledge of the adaptive value of these characteristics some phylogenetic conclusions might be possible. This is particularly important because Hubbs' (1929) assessment of the sticklebacks as "systematically bewildering" remains true to a considerable extent.
4. Certain features of the population which are typical of insular species could be examined from the standpoint of adaptive function. This is of special interest because the Queen Charlotte Islands are rich in endemic forms (Foster, 1965; Calder and Taylor, 1968), whose evolutionary history has been a controversial subject (Sutherland Brown and Nasmith, 1962).

The general approach to the problem has consisted first of an inter-population comparison of the Black form with *leiurus* and, second, an intra-population examination of variation within the Black form. The bulk of the study has consisted of field observations, supported by experiment where possible.

THE STUDY AREA

The Habitat

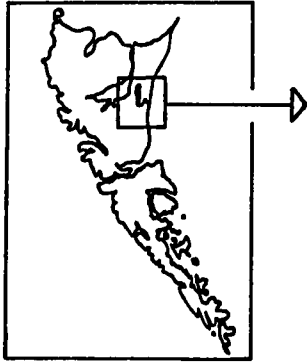
Mayer Lake (Lat. 53°40'N, Long. 132°02'W) is on the eastern side of Graham Island, the largest of the Queen Charlotte Islands, British Columbia. The lake is 74 feet (22.5 m) above sea level and flows into the Pacific Ocean via Mayer River. The lake is seven and one half miles (12.1 km) long and the average width less than one half mile (0.8 km) (Fig. 1). The maximum depth is about 30 feet (9.1 m). The shoreline is wave-swept and vegetation ranges from moderate to scarce. The substrate in the littoral region is generally firm and ideal for collecting and observation. Table 1 summarizes information about the vegetation present, substrate composition, and the bottom gradient.

The stream habitat differs from that of the stream mouth and both differ from the lake. Stream bottoms consist of organic ooze, often as deep as 12 inches (30.5 cm). Stream banks are vertical. The chief vegetation is mats of the moss *Sphagnum* sp. Sticklebacks spawn in shallows created by spring high water. In botanical terms the streams and their surroundings are classified as mires. Temperature in the lake and Gold Creek is similar (of 11 pairs of readings between May 21 and June 25, 1967, the mean difference was 0.6°C). Water color in the streams as in the lake, is brown. Stream mouths have mud bottoms. Populations of the water lily *Nuphar luteum*, emergent grasses, and sometimes *Sphagnum*, predominate.

Fishes present in the lake other than those mentioned above are migratory species. These include small numbers of *Salvelinus malma* (Walbaum), dolly varden, and *Oncorhynchus kisutch* (Walbaum), coho salmon.

Fig. 1. Mayer Lake and the Queen Charlotte Islands (inset), British Columbia. Habitat of *leiurus* form: creeks and creek mouths. Habitat of Black form: lake proper. Model experiments with *S. clarki* indicated by o. Other symbols explained in text.

Mayer Lake



Cott
Creek
11
24



19

n

{ B
C
D

11

8

7

1
mile

1
km

Woodpile
Creek
f

d

4

3

Gold
Creek
I

Mayer
River

Table 1. Littoral environment of Mayer Lake. The available habitat is compared with spawning habitats chosen by Black sticklebacks in 1969. Entire shoreline was sampled at 300 foot (91.4 m) intervals, 248 stations. For details see Appendix I.

		Vegetation				
		none	<i>Fontinalis</i>	<i>Nuphar</i>	<i>Isoetes</i>	n
Littoral environment		49	101	139	81	248
	%	19.8	40.7	56.0	32.7	
Chosen		31	167	238	106	355
	%	8.7	47.0	67.0	29.9	

$\chi^2=17.98$ (2 by 4 contingency table) df=3, $P<0.001$

		Substrate				
		mud	sand	pebbles	rocks	n
Littoral environment		48	130	46	18	242
	%	19.8	53.7	19.0	7.4	
Chosen		58	236	36	25	355
	%	16.3	66.5	10.1	7.0	

$\chi^2=13.14$ (2 by 4 contingency table) df=3, $0.001<P<0.01$

		Gradient			
		steep	medium	gentle	n
Littoral environment		44	56	144	242
	%	18.0	22.9	58.8	
Chosen		34	47	274	355
	%	9.4	13.1	77.5	

$\chi^2=23.45$ (2 by 3 contingency table) df=2, $P<0.001$

Materials and Methods

Five trips were made to the study area: May 25 - June 2, 1966; May 8 - September 2, 1967; March 10 - 15, 1968; May 6 - August 3, 1968; April 27 - August 11, 1969. Laboratory studies were made in Seattle during August, 1968.

Large samples were obtained with 8 foot (2.4 m) by 50 foot (15.2 m) monofilament gillnets of several mesh sizes. Two seines, one 4 feet (1.2 m) by 30 feet (9.1 m) and the other 3 feet (91.4 cm) by 10 feet (3.0 m) were used.

Live fish were transported locally in styrofoam picnic coolers. Live fish were shipped to Edmonton and Seattle in 5 gallon (22.7 l) plastic "jerry-cans".

The distribution of the two stickleback forms is shown in Fig. 1. Samples of *leiurus* for morphological analysis were obtained from locations I and II. Samples of the Black form were obtained from several locations in the southern half of the lake (see Table 2). Observations on the reproductive biology of the Black form were conducted in 1968 and 1969 at locations 3, iB, iC, iD, d, 8, and f. These stations provided a full spectrum of spawning environments utilized by the fish. Standard seine hauls were conducted at station 4. In 1967 trout were sampled between stations f and the outlet, Mayer River. In 1968 trout were sampled between stations j and 19.

Table 2. Partial list of samples, showing sampling location and use of the data.

<u>year</u>	<u>collection number</u>	<u>museum number</u>	<u>locality</u>	<u>figure or table</u>
1929		BC62-8	probably N	Fig. 4
1962		BC60-434	probably 7	" 3
1966	14		area 4-3	" 13
1966	15		area 24	" 3
1966	13		area 24	" 3
1966	8		Gold Cr.	" 3
1966	6		Gold Cr.	" 3
1966	3		Gold Cr.	" 3
1966	4		Gold Cr.	" 3
1967	23		south 1/2	" A2
1967	12		Gold Cr.	" 3
1967	35		area 4-3	" 3
1967	26		area 4-3	" 9
1967	32		area 4-3	" 9
1967	34		south 1/2	" 3
1967	G1-G6		south 1/2	" 11
1967-'68	S1-S16		south 1/2	Table 11
1968, March	unnumbered		south 1/2	Fig. 11
1968	2		south 1/2	" 13
1968	38		area D	" 3
1968	39		area 8	" 3
1968	40		area 4-3	" 3
1968	43		area 4-3	" 11
1968	44		area 4-3	" 9, 12
1968	A-X		south 1/2	" 11
1969	46		south 1/2	" 12
1969	47		area 11	" 4

MORPHOLOGY

Materials and Methods

A number of morphological characters distinguish individuals of the Black form from those of *leiurus*: color and pattern of color, gill raker number, vertebral number, lateral plate number, dorsal and anal fin ray number, relative length of the pelvic spines, relative body depth and standard length at sexual maturity. Other differences in body proportions exist, especially in the head region, but these were not quantified. Counting and measuring procedures were those given by Hubbs and Lagler (1964), with the exception of anal and dorsal fin rays in which case the last two rays which do not divide close to the base were counted as two. All lateral plates were counted, on both sides. In certain cases counts are given only for the left side, otherwise two digits are presented; e.g. 6—7 indicates a fish having six plates on one side and seven plates on the other side. Some of the Black sticklebacks (about 13%, n=845 males) have the first plate fused with or indistinct from the cleithrum. Such "plates" are indicated by a bracketed superscript, e.g.: 7(8)-7(8) means that the first (anterior) plate on each side was closely attached to the cleithrum and was followed by seven normal plates. The difficulty in determining the plate number of these fish, especially in the field, made it seem wisest to exclude them from most comparisons. Plates on preserved specimens were counted using a probe and dissecting microscope. Plates of small specimens were counted after staining with Alizarin Red S in 10% KOH. Adult fish were large enough that plates could be counted in the field by drying off the plated area and checking off the plates with a soft probe.

Body proportions were measured with vernier calipers from the

positions indicated in Fig. 2. Vertebral counts were taken from radiographs. Specimens for morphological analysis were preserved in 10% formalin. These fish are stored in the Museum of Zoology, University of Alberta (UAMZ), except for two samples from the Institute of Animal Resource Ecology (formerly Institute of Fisheries), University of British Columbia (BC). Table 2 lists the major collections, the collecting locality and museum accession numbers. Representative samples are deposited in the National Museum of Natural Sciences, NMC67-718; Museum of Zoology, University of Michigan; Scripps Institute of Oceanography, of the University of California, and the College of Fisheries, University of Washington.

Results

A comparison of the characters measured in the two forms is given in Fig. 3. Body length is the best single means of separating individuals of the two forms, for all other characters overlap. Mean standard length of sexually mature Black females was 89.94 mm, $s=6.464$, $s\bar{x}=0.631$, $n=105$. Mean standard length of nesting Black males was 80.24 mm, $s=4.723$, $s\bar{x}=0.258$, $n=336$. Mean standard length of sexually mature *leiurus* females was 50.68 mm, $s=2.247$, $s\bar{x}=0.490$, $n=21$. Fish of the Black form have relatively larger spines and more streamlined bodies than do specimens of *leiurus*. Fish of the Black form have higher gill raker counts, numbers of vertebrae, lateral plates, and dorsal and anal fin rays than do individuals of the *leiurus* form.

As well as differing in mean value, the variance is less in the Black form in most of the characters measured (Table 3).

Fig. 2. Morphometric co-ordinates used:

A—B: body length (standard length).

C—D: body depth.

E—F: pelvic spine length.

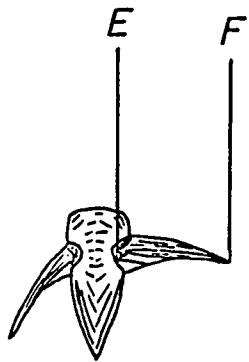
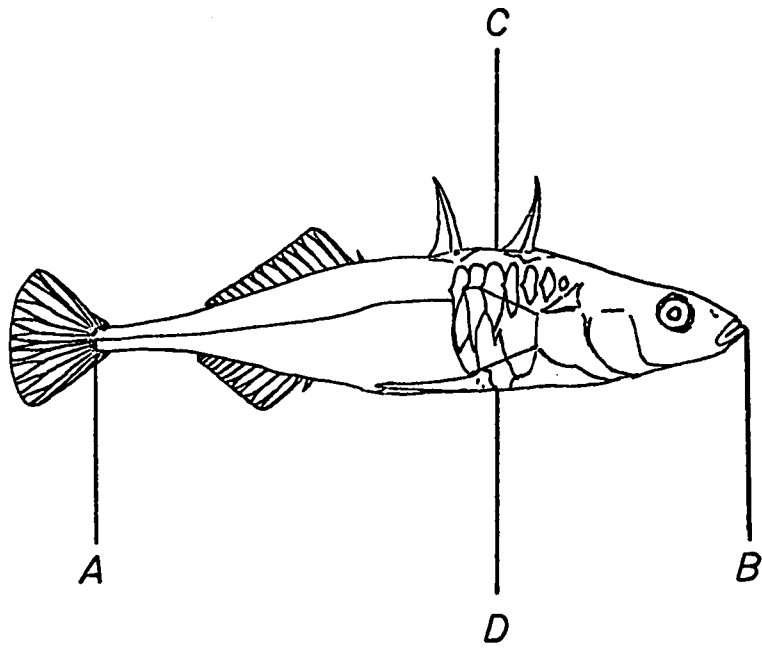


Fig. 3. Some morphological characteristics of *leiurus* (below line) and the Black form (above line). \bar{x} , mean; s , standard deviation; $s\bar{x}$, standard error; n , sample size.

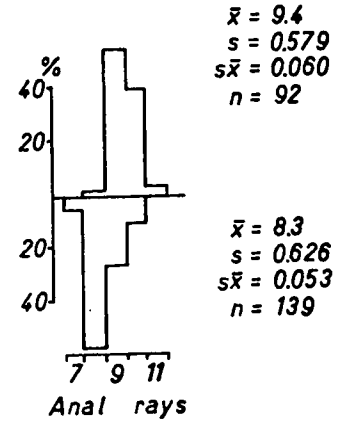
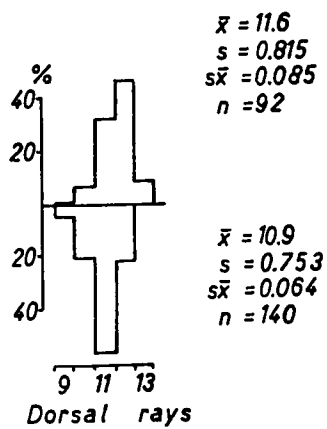
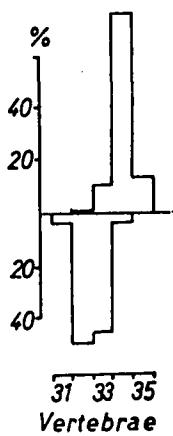
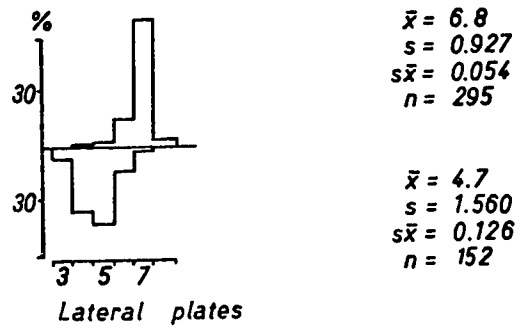
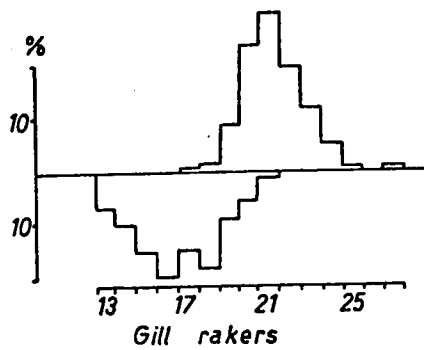
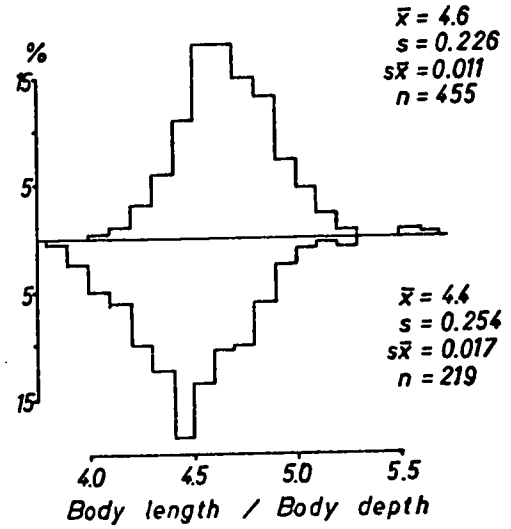
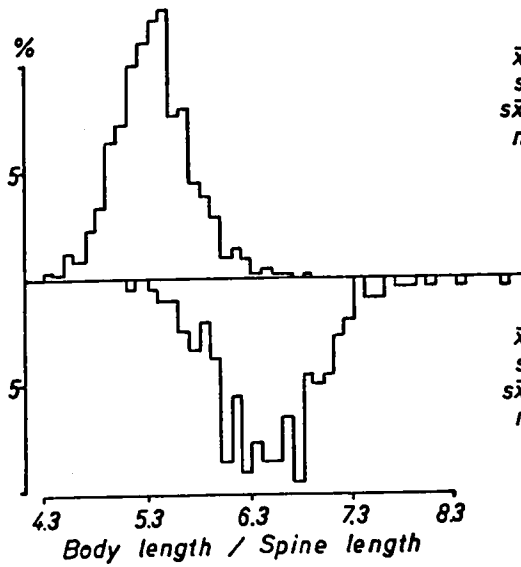
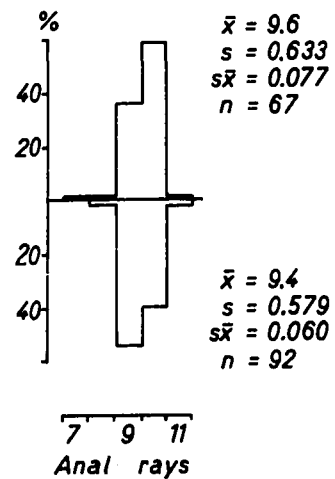
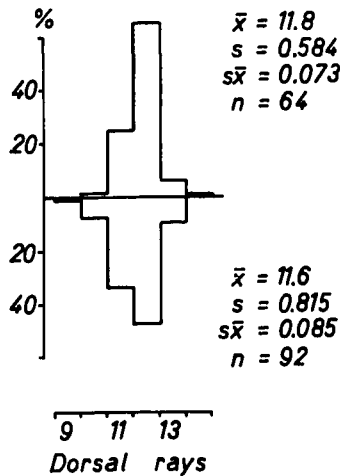
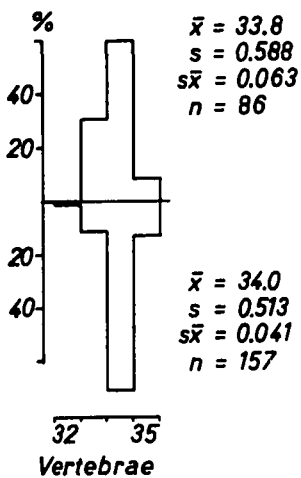
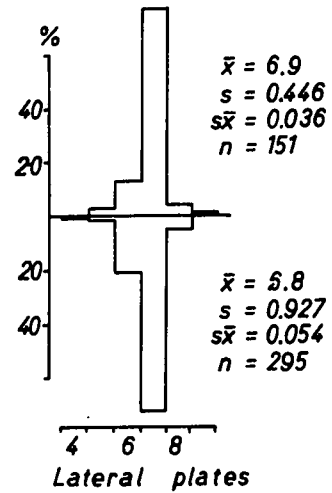
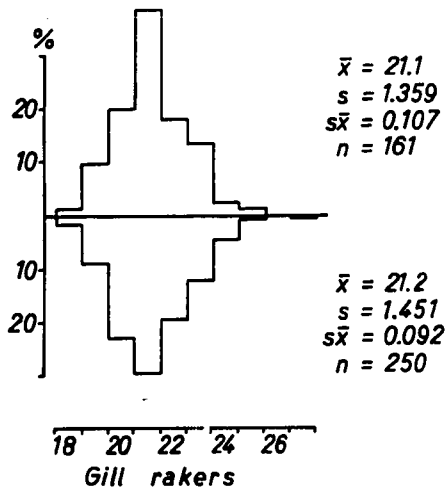
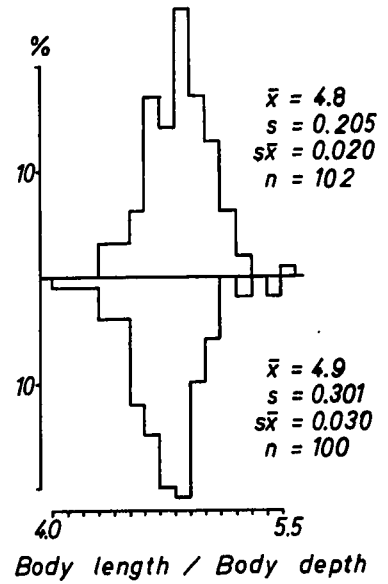
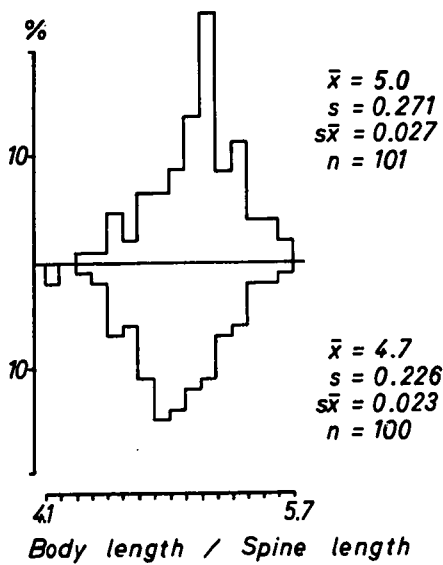


Table 3. Comparison of the Variance in some morphological characters of *leiurus* and the Black form.

character	Variance Black	Variance <i>leiurus</i>	Variance Ratio, F	df	Probability (two-tailed)
standard length: body depth	0.0511	0.065	1.266	218;454	0.05>P>0.025
standard length: spine length	0.0135	0.255	18.945	220;456	0.01>P
gill rakers	2.106	3.730	1.772	204;249	0.01>P
lateral plates	0.865	2.434	2.814	151;294	0.01>P
vertebrae	0.263	0.367	1.395	70;156	0.20>P>0.10
dorsal ray number	0.664	0.568	1.170	91;139	P>0.20
anal ray number	0.335	0.392	1.170	91;138	P>0.20

The phenotype of the Black form appears to have been stable for a 39-year period. Figure 4 shows the frequency distributions of a sample collected in 1929.

Fig. 4. Data illustrating stability of the morphological parameters in the Black form. 1929 sample above line, 1968 sample below line.



PREDATION

STOMACH ANALYSIS OF PREDATORS

Methods

Studies on predation were mainly concerned with the cutthroat trout, which feeds on Black sticklebacks of all ages. The prickly sculpin feeds on eggs of the Black stickleback and to a minor extent on juveniles, while very rarely a small adult is taken.

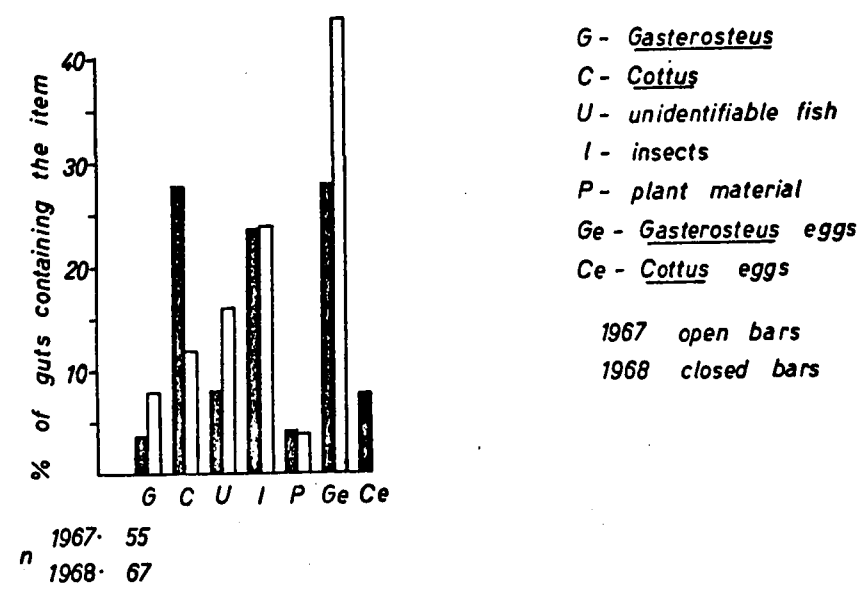
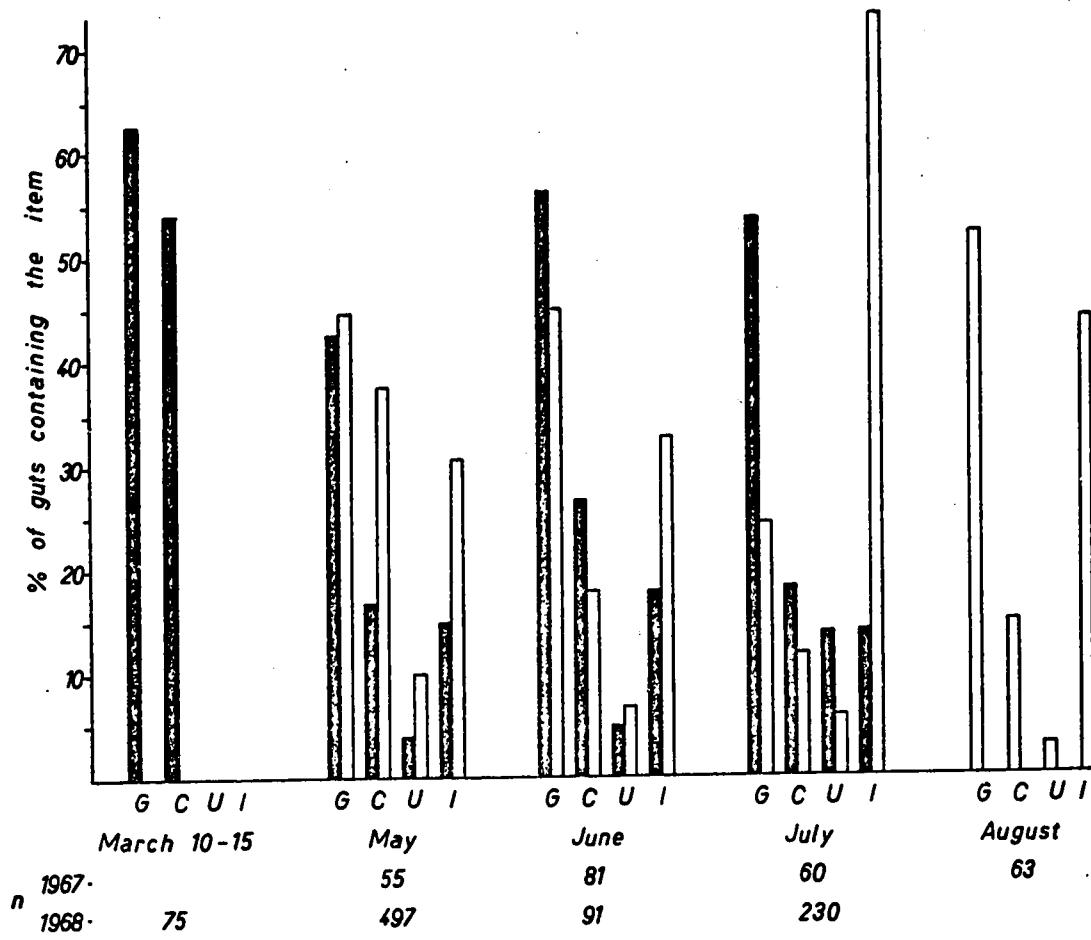
Trout and cottids were captured in gillnets set in deep water adjacent to breeding areas. Occasionally nets were set in among nesting Black sticklebacks and catches confirmed the presence of the predators in the shallow water with the nesting fish. Gillnets were left in the water continuously and checked at least twice daily. As soon as fish were removed from the net, up to one ml of concentrated formalin was placed in the stomach via the pharynx. This served to halt digestion and harden fish remains in the gut. Within an hour or so, the stomachs were opened, the fish length and the contents recorded. Fish remains were labelled and placed in 10% formalin, either in a vial or wrapped in cheesecloth.

Results

Analysis of the stomach contents of trout and cottids (Fig. 5) indicates the importance of the Black stickleback and its eggs in the diet of populations of these species. Sticklebacks are the major food item in the stomachs of the trout in all months but one in which samples were taken.

In the summer months stickleback eggs form an important part of the diet of adult cottids. Stickleback eggs are distinguished from those of the prickly sculpin (which are cannibalized) by their larger size and

Fig. 5. Histograms illustrating diets of *Salmo clarki* (upper) and *Cottus asper* (lower), in Mayer Lake.



gold rather than pink color. Juvenile cottids were seen to eat stickleback fry after the male had been frightened away. However, larval sticklebacks were not found in the stomachs of cottids, perhaps because fry are digested extremely quickly (D.W. Hagen, pers. comm.).

I am not certain whether cottids rob nests which are being cared for by the male, or whether they merely take eggs which have already lost, or been abandoned by, the male stickleback and are therefore doomed in any event. In two of three cases where a large cottid was placed in a tank containing a nesting Black stickleback, the cottids did not eat the eggs although they were hungry and spent considerable time at the edge of the territory. Whenever they left cover to approach the nest, they were attacked by the male and driven back into hiding. In the third case, however, a guarded nest with fry disappeared after the cottid was placed in the tank. In one instance, a nest marked in the field the previous evening was gone the following morning while the male still appeared to be present at the nest site. This is limited evidence that cottids on occasion take eggs from guarded nests.

Leeches of the species *Haemopsis marmorata* feed on eggs in the nest, ingesting them whole. The leeches are ubiquitous in the lake and the number of eggs they consume probably depends mainly on the attentiveness with which the male stickleback guards his nest. When a leech is discovered in the territory it is immediately grasped by the guardian male and deposited beyond the territorial boundary. When 10 leeches were allowed to feed on stickleback eggs until satiated (in about one hour) the average consumption was 94 with a range from 57 to 152. The mean number of eggs per nest (or clutch) was 773 for a sample of 133 nests.

The largest observed clutch contained 2001 eggs.

PREDATOR BEHAVIOR

Materials and Methods

Because of the color differences in the breeding males of the Black form, the question of the circadian feeding cycle and color vision in the trout and cottids was considered.

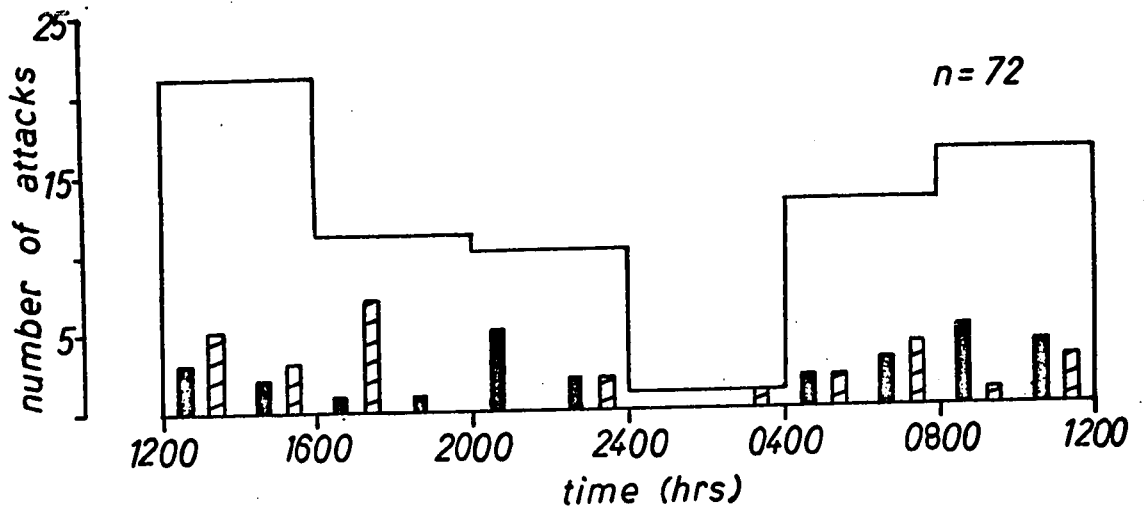
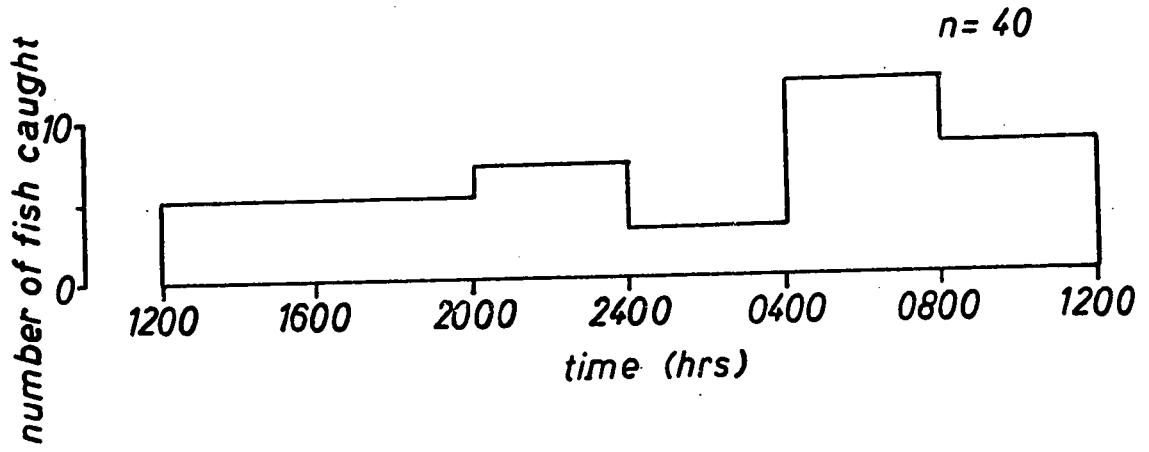
Two methods were utilized to determine the circadian feeding cycle of the trout. In the first method, two 50 foot (15.2 m) gillnets were placed in 4 feet (1.2 m) of water adjacent to stickleback spawning areas (areas 3 and 4) in August, 1967. Fish were removed every 4 hours for an eight-day period. Feeding fish were assumed to be more active and therefore more likely to be caught than non-feeding fish. The second method utilized a clockwork event recorder and stickleback models. When a feeding trout moved the model either up or down, micro-switches activated a pen bearing on a pressure-sensitive paper chart. The drum speed of 1.8 inches (4.6 cm) per hour permitted the time of attack on the model to be determined to within a half hour or less. The device was set on a wooden stand in depths of water from 5 (1.5 m) to 8 feet (2.4 m), from May to September, 1969. That it was trout that were activating the switches and not some other force, e.g. wave action, was verified by the numerous tooth marks which appeared on the models in the course of the summer.

Results

The activity cycle of 40 cutthroat trout is shown in Figure 6. Catches of cottids were too small to provide interpretable data but they showed the same pattern of activity as that of the trout. Figure 7 shows

Fig. 6. Graph illustrating circadian activity cycle of *S. clarki*, Aug. 8-16, 1967. Based on 24 hour gillnet catches.

Fig. 7. Circadian feeding cycle of *S. clarki*, 7 May - 29 Aug., 1969. Solid histograms indicate attacks on red model, striped histograms attacks on non-red models, for 2 hour intervals. Upper line indicates total number of attacks in 4 hour intervals. Simultaneous attacks on both models account for differences between histogram totals and totals indicated by upper line.



the times at which the trout contacted the model sticklebacks. Both sets of data clearly show that cutthroat trout are diurnal feeders. Differences in the peaks (dawn in the gillnet sample, early afternoon in the case of the models) could result from the different locations of the nets and the models, from the seasonal difference, or because different parameters (feeding and activity) were being measured. Of primary importance for this study is that trout are active in daytime and are probably feeding then, and using vision for prey capture.

The evidence for color vision in the cutthroat trout and the prickly sculpin is entirely circumstantial. Both species utilize colors in their courtship behavior. In laboratory experiments both species select prey differing only in color (but brightness was not controlled). Selection of artificially colored food by these species is treated in detail in the section on apostatic selection, page 38. Cone cells are present in the retina of species related to *S. clarki*, i.e., *S. gairdneri*, *S. trutta* (Walls, 1942) and *S. salar* (Ali, 1959).

CONSEQUENCES OF PREDATION ON THE EVOLUTION OF THE BLACK FORM

The nature of the morphological differences between the two forms together with the distribution of the predators in the two habitats suggest that some of the differences are a result of predation.

I examined the effects of predation at two levels, the inter-population (Black-*leiurus*) and the intra-population (within Black).

INTER-POPULATION COMPARISONS

Differences in coloration, spine length (relative to standard length), and lateral plate number between the two forms are the characters most easily related to the difference in selective pressure due to predation. Other characters such as body size, fecundity, and certain aspects of reproductive behavior are also probably related to the effects of predation. Confirmation is more difficult in the case of these characters, for reasons which will be given below.

Coloration

The color of the Black form is probably cryptic in the relatively cover-free lake environment. The uniform black coloration, interrupted only on the operculum and the antero-ventral region by silver, is a typical example of counter-shading. To the human eye, Black sticklebacks are very difficult to see when viewed from underwater or from above, in deep water.

The blotchy, yellow color of *leiurus* seems well-suited to the swamp-like environment occupied by this form. Here the abundance of vegetation makes such a disruptive color pattern appropriate.

Nuptial Color of Males

Less than one fifth of the breeding males of the Black form develop

the characteristic red throat of *leiurus* (Table 4).

It may be very significant that in the buccal cavity of these Black males red pigmentation is usually present, as it is in *leiurus*. This may be functional since it is very noticeable when the fish gape as they frequently do during territorial disputes. The red color in individuals of the Black form does not approach the extensive development seen in typical *leiurus*. Breeding males of the Black form lose their bright silver color and assume a dull, drab appearance. The loss of silver coloration renders the operculum transparent. Frequently, the brightest color is a dull plum area on the operculum, resulting from the gill arches showing through it. In addition to the variable presence of the red and the plum coloration there is great variability in the coloration of other parts of the body of the Black form. The blue iris and the blue and green iridescence of the body, typical of *leiurus*, are not always present. The colors seem to be fairly stable during the reproductive cycle of a given fish, except that, as in other populations, some males become more red on hatching of the eggs (van Iersel, 1953).

In this study I concerned myself solely with the loss of red on the ventral region and its adaptive significance (see intra-population comparisons, page 32).

Spine Size

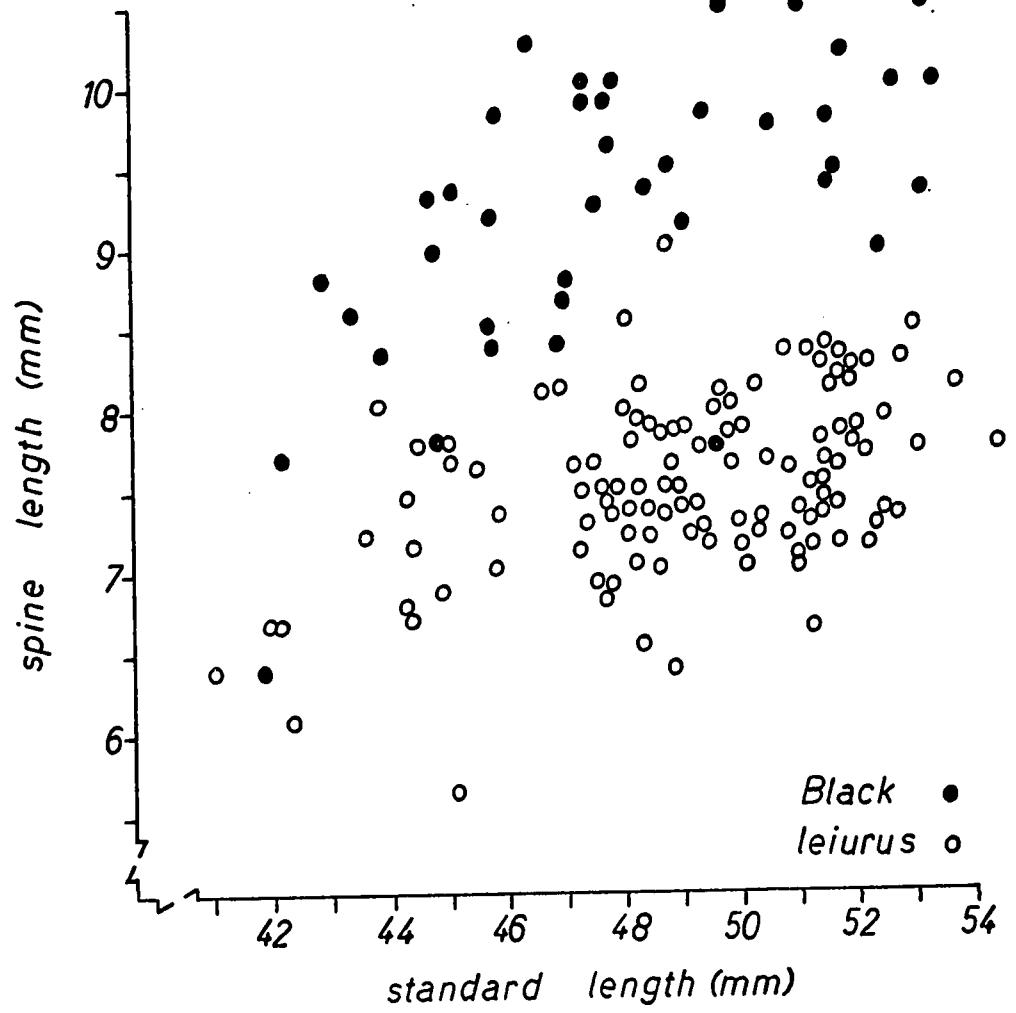
The proportionately greater length of the pelvic spines of the Black form is shown in Figure 3. That this difference is not a function of overall greater size of the Black form, i.e. allometry, is seen when standard length:spine length ratios for fish of the two forms of equal sizes are plotted (Fig. 8). The effect of the spines on the trout is

Table 4. The frequency of red-throated males in the Black population.

	red	non-red	% red
1967	18	80	18.4
1968	57	341	14.3
1969	80	438	18.3

The difference among the three years is not significant,
 $\chi^2=0.69$ (2 by 3 contingency table), $df=2$, $0.975 > P > 0.950$

Fig. 8. Relative length of the pelvic spines in fish of equivalent size. Black form, 43 specimens; *leiurus*, 115 specimens.



probably reflected in their significant preference for swallowing sticklebacks head-first rather than tail-first. Results of the fishes' preferences (determined by examination of stomachs) were recorded in the closed sequential design of Cole (1962), as the trout were caught. Trout showed no such preference in swallowing cottids of a comparable size. The ratio of sticklebacks swallowed head-first:tail first was 20:5, for cottids the ratio was 6:6.

The frequency of predator attacks and/or the efficiency of spines (and other factors) in thwarting attacks is probably reflected in the proportion of fish having damaged spines, 29/892 in the Black form. Spines were classed as broken only if the injury was healed. Specimens of *leiurus* with broken spines were not found in a sample of more than 770 fish.

Plate Number

The relationship between plate number and predation is derived in part from observations in locations other than Mayer Lake. Many west coast populations of *leiurus* have a modal plate count of seven when predatory fish are present (Hagen and McPhail, 1970). In those populations whose members are not in contact with predatory fish, the mode is not seven. In the Queen Charlotte Islands this relationship is found in the 17 populations examined to date (Table 5). Of the populations sympatric with predatory fish in the Queen Charlotte Islands there are perhaps fewer modes at seven than in other areas. However, it is not known that the predators are actually preying on the sticklebacks in all these cases.

Body Length

Probably a number of different selective pressures have contributed to the large body size of the Black form. Because the various correlates

Table 5. Plate number modes and the occurrence of predators in some *G. aculeatus* populations in the Queen Charlotte Islands. Presence of predators (usually *S. clarki*) is indicated by a +.

lake	plate number mode	predators	n
Watt	0	-	24
Skonun	0	-	61
"Tlell"	1	-	11
Loon	3	-	48
Boulton	3	-	48
"Woodpile"	5	-	59
Hickey	5	-	53
Drizzle	4	+?	37
Pure	5	+	54
Yakoun	6	+	21
Kumdis	6	+	24
Skidegate	6	+	39
Ain	6	+	50
Mercer	7	+	22
Mayer	7	+	295
"Leiurus"	7	+	6
Awun	7	+	34

"Woodpile" Lake is the headwater of Woodpile Creek which flows into Mayer Lake. "Leiurus" Lake is on the east coast of Graham Island, near Dead Tree Point. "Tlell" Lake is near the settlement of that name.

of large size cannot be separated, experimental testing is made difficult. Some speculative points may be made, however.

Increased egg production is a common correlate of increase in body length. Data on fecundity of the Black form are compared with that of a sample of *leiurus* and *trachurus* in Figure 9. The Black stickleback produces about two and one half times as many eggs as *leiurus*. The range in egg size in the Black form is similar to that of *leiurus* in Mayer Lake and beyond that reported in the literature for *G. aculeatus* (Table 6).

INTRA-POPULATION COMPARISONS—VARIATION WITHIN THE BLACK FORM

General Methods

Many of the intra-population comparisons of the Black form were obtained by the individual capture of nesting males. Fish were caught with "Lift Nets," consisting of a 12 inch (30.5 cm) diameter metal frame supporting one quarter inch (6.3 mm) nylon netting. Three strings about 24 inches (61.0 cm) long were attached to the frame and were held by the user.

When a male was to be caught, the nest was approached and the lip of the net placed over the nest. Within a short time the owner of the nest returned, and swam over the net in an effort to get to its nest. The net was then quickly lifted. The captured fish was placed in a pail of water and the nest marked with a numbered stake stuck in the substrate close by. The following data were recorded:

- time taken for the fish to return to within six inches (15.2 cm) of its nest. (Timing began after the observer had knelt and positioned the net over the nest.)
- standard length of the fish, measured to the nearest mm.

Fig. 9. Egg production in the forms *leiurus* ($n=40$; $\bar{x}=105$; $Y=-145.9 + 55.3X$), *trachurus* ($n=40$; $\bar{x}=241$; $Y=-312.7 + 94.6X$), and Black ($n=44$; $\bar{x}=257$; $Y=-393.3 + 72.2X$). Data for *leiurus* and *trachurus* from Hagen, 1967.

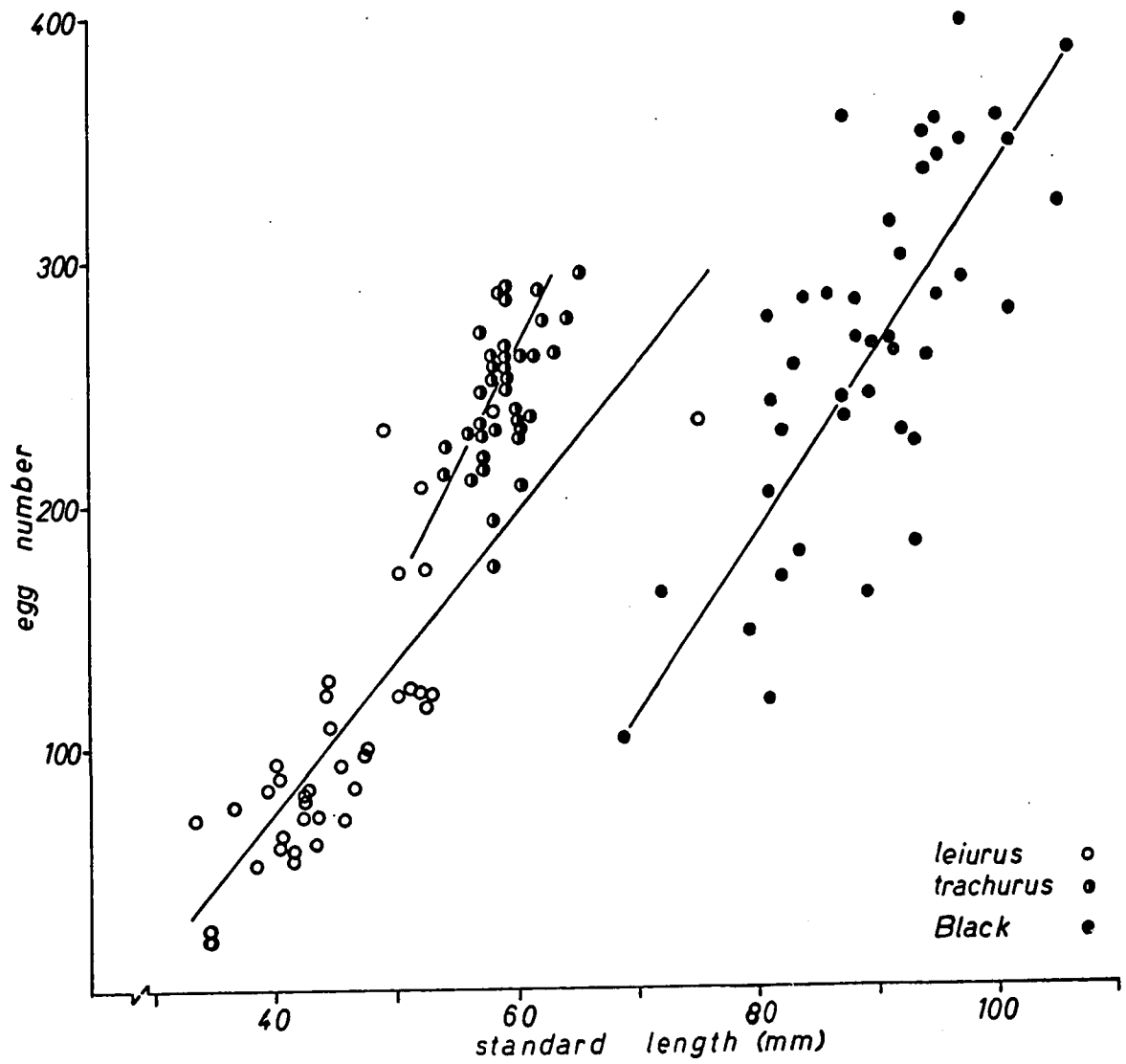


Table 6. Egg Diameter in *G. aculeatus*.

source	size range mm	\bar{x}	type of egg	locality	form
Vrat, 1949	1.2-1.6	1.30	freshly stripped	California	?
	1.1-1.5	1.36	"	"	?
present study	1.7-2.1	1.90	"	Mayer L.	Black
present study	1.5-2.2	1.87	"	"	<i>leiurus</i>
W.H. Van Vliet NMC	1.0-1.8	1.3	preserved ovarian	Newfoundland	<i>leiurus, trachurus</i>
Van den Assem, 1967	1.44-1.52	?	?	Netherlands	"
Swarup, 1958	1.2-1.7	?	?	Britain	?

" , *semi-armatus*

—total number of free lateral plates on each side of the fish.

—color of the fish. Five regions of the body were classified.

In the end, only throat color was used.

—distance from the nest to shelter. Shelter consisted of large rocks, clumps of *Fontinalis* or *Nuphar* or sunken logs and sticks.

—depth of water over the nest.

—stage of the breeding cycle: nest building, nest empty, eggs

in nest, young fry (yolk not absorbed) at nest, old fry at nest.

The fish was then released and usually resumed its normal activities at the nest within a short time.

Nests containing eggs were located to study clutch size. Usually a sample of one or two eggs was carefully removed from the nest. If these eggs were eyed, the entire nest was scooped up and placed in a numbered jar containing 10% formalin. The use of eyed eggs reduced the risk of getting clutches from males which had not finished courting. The nests were eventually picked apart and the eggs counted with the aid of a hand-counter.

In 1968, 402 nests were sampled and clutches were counted from 72 other nests. In 1969, 433 nests were sampled and the clutches of 126 other nests were counted.

A Stepwise Multiple Regression Analysis was applied to the nest and clutch size data using program CS101 from the University of Alberta Computing Science Program Library, and an IBM 360/67 computer. Only four plate categories were used, 6—6, 6—7, 7—7, 7—8. Fish with fused or indistinct anterior plates were excluded from the analysis. There was no *a priori* means of knowing what sequence or order the plate categories would follow, therefore all permutations of sequence were tested.

Unfortunately the nest data of 1968 and 1969 cannot be considered to be replicates. Two factors are responsible. In 1969 an influx of sand and consequent absence of vegetation eliminated a major breeding ground which had provided a large fraction of the 1968 sample. In 1969 it seemed desirable to increase the intensity of sampling and to sample only major spawning areas. The natural loss of the one major breeding area and the deliberate omission of poorly utilized areas resulted in different localities being sampled in the two years. Secondly, the increased intensity of sampling had the effect of reducing the variability in the total sample because fewer fish which were in an advanced stage of the breeding cycle were taken. In terms of the statistical analysis the latter effect was particularly untoward.

Breeding Color

Materials and Methods

A possible explanation for the loss of the red breeding color can be based on differential predation of the color morphs. Three methods were used in testing this theory:

- field observations on nesting males of different colors (above), in 1968 and 1969.
- presentation of red and non-red sticklebacks to cutthroat trout in aquaria at Seattle, August 1968.
- presentation of red and non-red models to unconfined trout at large in Mayer Lake, 1969.

In August, 1968, red and non-red males were presented in pairs to trout. The number of attacks made on each color type was recorded. The trout (usually one, but sometimes four) were kept in a 300 gal. (1400 l)

aquarium. The aquarium was surrounded by a screen of black polyethylene plastic provided with a small peephole. A single 150 watt light bulb centered over the tank provided illumination when required. The trout used were two and three year old *S. clarki* from the Tokal Creek Hatchery near Seattle. The total number of trout used in the experiment was six. Stocks of Black sticklebacks were brought to Seattle from Mayer Lake. Some tests utilized sticklebacks from Wapato Lake, Washington. Like the Black form, the Wapato fish are exposed to heavy predation by trout (*Salmo gairdneri*).

The use of natural nuptial colors was not practicable so all sticklebacks were colored artificially. Sticklebacks to be offered to trout were matched in length to within two mm, in overall color, and in plate number. The throat region was blotted dry and melted lipstick was quickly applied with a fine camel hair brush. Each fish of the pair was so treated, one with red lipstick (Hot Coral, by Revlon Inc., color 5R5/12 in the Munsell System, Anon (1929)), the other with clear lipstick (Lip Gloss, by Bonne Bell, Inc.).

The fish were then placed in the aquarium and observed. Each open-mouthed rush of the trout to within 1 to 2 inches (about 2.5 - 5.0 cm) of the stickleback was recorded as an "attack." Each attack was terminated by turning off (or on) the overhead light just as the trout was about to contact the prey. This was necessary in order to accumulate sufficient data. Permitting the trout to contact the stickleback would eventually result in either avoidance because of the spines, or feeding satiation, depending on the experimental design. Whenever the members of a stickleback pair showed dissimilar behavior, both individuals were removed and discarded.

Often the attacks and escape responses resulted in some of the lipstick flaking off. When this occurred, the pair was removed and the lipstick re-applied, with the colors reversed on each fish.

Three tests were made under the following conditions:

- I— bright light and red coloration comparable to that of typical *leiurus*.
- II— bright light and red coloration reduced to a single 1.25 mm dot on each operculum.
- III— dim light (<10fc) and red coloration comparable to typical *leiurus*.

From May to September, 1969, models and the event recorder discussed previously, tested red (Munsell color 5R4/14) versus non-red preference of Mayer Lake trout.

Results

Field observations in 1968 showed that red-throated males nested within 4 feet (1.2 m) of cover more often than did non-red males (Table 7). These observations also suggest that survival of the fry was positively correlated with proximity of the nest to cover (Table 8). The presence of cover may reduce the visibility of the male and fry to predators and/or it may provide a source of shelter when discovery by a predator does occur. Results of similar comparisons based on the 1969 data were not significant.

Results and details of fish used in the Seattle tests are given in Table 9. A strong tendency to attack the red fish was shown in all cases.

Results of the model presentations in the lake (Fig. 8) do not indicate a color preference. The reason for the lack of preference could

Table 7. Proximity of the nest to cover and the throat color of the owner-male (1968 nest sample).

	throat color	
	red	not red
nest within 48 inches (1.2 m) of cover	38	77
nest more than 48 inches (1.2 m) from cover	7	64

2 by 2 contingency table, adjusted $\chi^2=11.63$, $P<0.005$

Table 8. Proximity of the nest to cover and the age of the offspring (1968 nest sample).

	distance from nest to cover	
	within 48 inches	beyond 48 inches
old fry	28	4
young fry	46	16
eggs	106	59

2 by 3 contingency table, $\chi^2=8.022$, $df.=2$, $0.025>P>0.010$

Table 9. Results of Seattle predation experiments.

number of attacks on:	red	dull	total	χ^2	probability due to chance
I bright light	42	13	55	15.29	0.005>P
II dim light	39	21	60	5.40	0.025>P>0.01
III bright light	40	18	58	8.34	0.005>P

- two male, two female *S. clarki* used in each test (I, II, III).
- Black form used in tests I and II, Wapato Lake fish used in test III.
- red coloration in I and II: from below anterior margin of eye back to rear of operculum (below eye) as well as down the midline of the throat to the isthmus.
- red coloration in III: a single dot 1.25 mm in diameter on the lower hind quarter of each operculum.

result from the mainly ventral position of the red region and the lack of movement of the models, which meant that the ventral area was never directly illuminated as it would be in a living fish. If this explanation is correct, one would expect the red model to be attacked more often at dawn and dusk, when the angle of the sun would illuminate more of the sides of the model and thus some of the red. The data do not illustrate such an effect, however (Fig. 7).

The Significance of Red Males in the Population

Three possible explanations of the adaptive value of red throat color were considered:

- sexual selection
- apostatic selection
- inter-male rivalry

Sexual Selection

The earlier literature (e.g., Tinbergen, 1953) emphasizes the importance of the red throat of the male as a releaser in the spawning behavior of the female. To determine whether red-throated males were more attractive to females than the non-red males, mate preference tests were conducted. Tests were performed in the same manner as those described by Hagen (1967). The dimensions of the test aquaria were 12 inches (30.5 cm) by 36 inches (91.4 cm) by 12 inches (30.5 cm). Only one series of tests was completed, in June, 1968. Analysis followed the procedure of Cole (1962). There was no significant preference for one male over the other, the females selecting the red male nine times and the non-red male seven times.

To determine whether sexual selection was operating in nature, in spite of the experimental results, clutch sizes of red and non-red males were compared in 1967, 1968, and 1969. Multiple regression analysis showed there was no significant correlation between clutch size and color of the male ($t=-0.630$, $df=100$, $P>0.5$).

Apostatic Selection

Individuals having phenotypes which differ visibly from the norm may enjoy a selective advantage when they are exposed to visual predators which form search images. Clarke (1969) termed such selection apostatic.

The biology of the Black form seems to fulfill the criteria suggested by Clarke to favor such selection: the predator feeds visually, sticklebacks are probably slightly distasteful (spines to the trout, attacks of the male to the cottid), and, finally, the colored individuals are rare, both by the seasonal nature of the color and because of its restriction to adult males.

As a preliminary approach in determining the probability of whether apostatic selection was controlling color variation in the males, feeding experiments were conducted with trout and cottids. Individuals of both species were fed for several days on food which had been dyed a particular color (Nutty Club Vegetable Food Coloring). The fish were then offered the same food, but of two colors. If the fish formed search images, a fundamental prerequisite of apostatic selection, they would continue to feed on the color to which they had become accustomed in the previous feedings.

Tests of the trout were conducted in Seattle in 300 gal. (1400 l) aquaria. The fish came from the Tokal Creek Hatchery. Colors tested were

red, green, and yellow. The maximum number of presentations given in attempting to establish the search image was 117. No color-based search images were formed. These results could be due to the experimental conditions and should be considered preliminary.

C. asper from Mayer Lake was similarly tested in Edmonton. The fish used were kept in 16 gal. (73 l) aquaria. A single test specimen was fed 20 frozen brine shrimp for 14 days. Shrimp were stained a bright blue. On the fifteenth day, pairs of shrimp were offered, one blue, one red. As soon as one of the pair was eaten, the remaining one was removed and another pair was offered. Results of this experiment (Table 10) indicate search image formation, and suggest that prickly sculpins possess color vision (recognizing that intensity was not controlled). Two other cottids were tested in search image experiments similar to those described by Popham (1941), but using shrimp as prey. The results again strongly suggest formation of search images, but the differences are not significant.

Inter-Male Rivalry

Tinbergen (1953) emphasized the importance of the red belly in the threat display of male threespine sticklebacks. To determine if the red throat is important in the threat display of the Black form, a simple preliminary test was made.

The experiment consisted of presenting a red and a non-red model to Black sticklebacks nesting in the lake. The model was suspended from two 4 foot (1.2 m) long poles by a 2 foot (61.0 cm) and a 4 foot (1.2 m) long thread. The attitude and direction of the model could thus be controlled. The experimenter approached to within 4 feet (1.2 m) of the nest.

Table 10. Search image formation in *C. asper*

date	number of shrimp eaten		
	red	blue	
Jan. 19	15	0	*
21	11	4	*
22	12	3	*
23	13	2	*
26	17	4	*
29	14	13	

* indicates significance in Cole's design, 1962.

The direction of approach was always from the shore side. After a few minutes the male resumed its normal behavior at the nest. The model was then placed in the water and slowly made to "swim" towards the nest. This usually resulted in the fish attacking the model. Sometimes the stickleback made threat displays prior to attacking. The distance between the nest and the point of attack was recorded along with any displays. Each fish experienced both the red and the non-red models. The interval between presentations was about one hour. One half the fish were shown the red model first and the other half experienced the non-red model first. The mean distance of attack did not differ between the two models in the 49 fish tested.

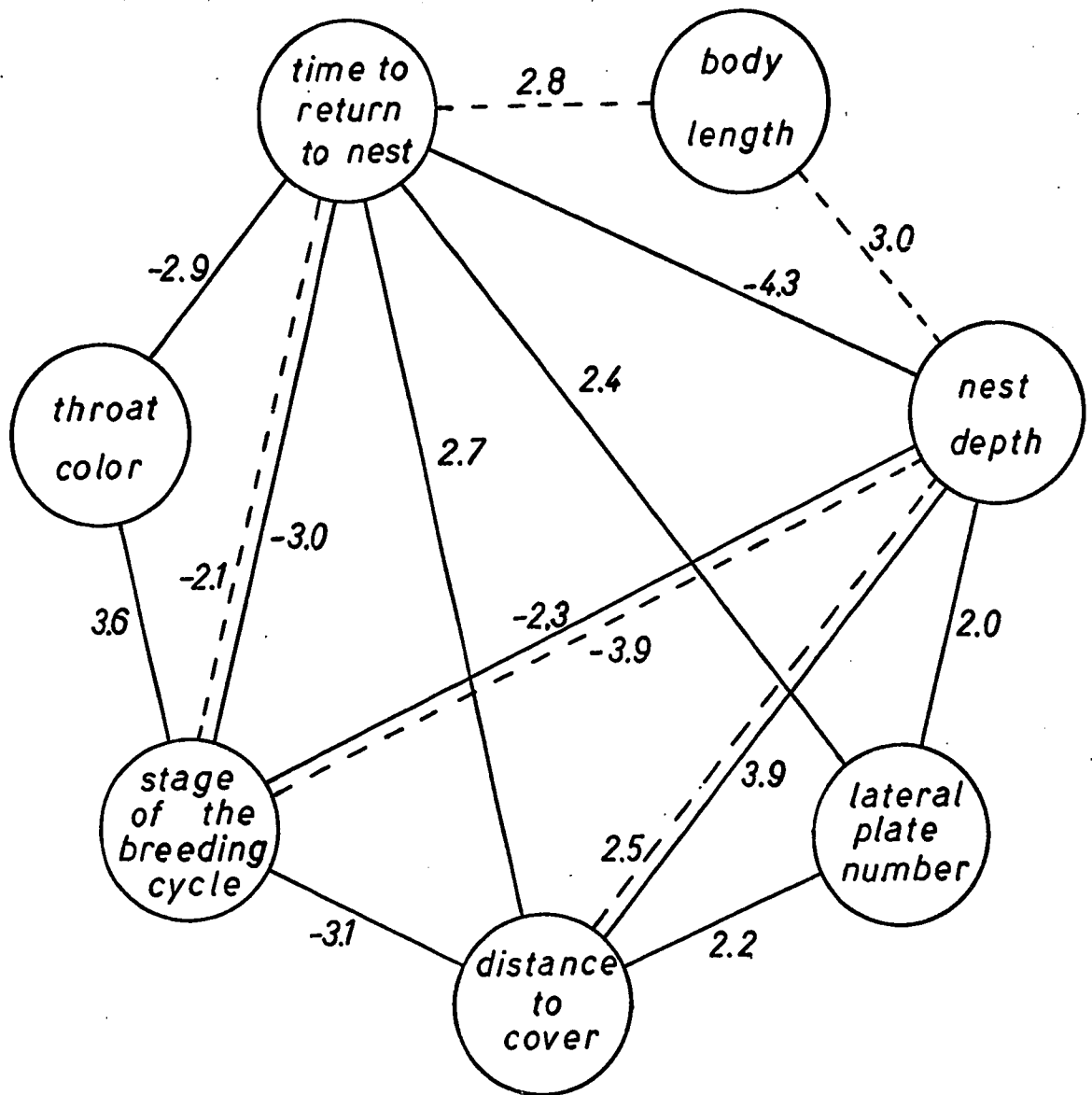
Analysis of the data from males nesting in the lake (Fig. 10) shows that color of the male is correlated with the variable "time to return to the nest;" red males returned to their nests more quickly than did non-red males. The time taken to return to the nest may be an indicator of the level of aggressiveness. This correlation was significant in 1969 but not in 1968.

Spine Length

The difference in spine length between specimens of the Black form and those of *leiurus*, the difference in the variance of this character in the two forms, and the presence of fish with broken spines in the Black population suggests selection for long spines. This was further investigated by comparing the body length:spine length ratio of Blacks taken from stomachs of trout with a seine-captured sample of equivalent body length and sex.

Females taken from the stomachs were found to have mean pelvic

Fig. 10. Significant correlations among variables as determined by stepwise multiple regression analysis. Based on nest samples of the Black form. Numerals represent t -values. For 0.05 and 0.01 probability levels t -values are 1.980 and 2.617 respectively. Degrees of freedom are 277 for 1968 (———) and 261 for 1969 (- - - -).



spine lengths significantly shorter than those of females taken by seining (Table 11). Males from the stomachs also had shorter pelvic spines than netted males but the difference was not significant. I do not think that the results were biased by the effects of digestion, although it is difficult to be certain. The null hypothesis would be favored if digestion took place, because the head and tail would be reduced before the bony spines, therefore leading to the conclusion that the spine length was relatively greater than was the case. All fish measured were recently swallowed, with the skin intact at head and tail. Bias in the measuring process was checked by having the fish measured by an individual unaware of the purpose of the determination.

Lateral Plate Number

Materials and Methods

The distinctly different distribution of plate counts in the Black form and *leiurus* raises the question of the adaptive nature of the bony plates typical of the genus *Gasterosteus*.

Experimental work by J. D. McPhail (pers. comm.) with *leiurus* suggested a correlation between plate number and survival in the face of predation. In the present study plate counts of prey fish taken from trout stomachs were compared with those of seine-caught fish. Counts on prey fish were made only if the skin was intact or the plate series indicated no plates had been lost due to digestion. When a choice was possible, the count on the left side was used to establish the frequency distribution. Seine-caught fish matched the prey fish in year of capture, size, and sex (however, the last two could not always be determined).

Seine-caught fish were also used to study plate number distribution

Table 11. The body length:spine length ratio of females of the Black form taken from stomachs of the trout, *S. clarki*, and compared to the body length:spine length ratio of fish of equal size taken in seine hauls.

fish from stomachs			fish from seine hauls		
body length	spine length	<u>body length</u> <u>spine length</u>	body length	spine length	<u>body length</u> <u>spine length</u>
80.85	14.05	5.75	79.90	16.20	4.93
81.10	15.05	5.39	81.80	14.10	5.80
87.75	15.10	5.81	87.85	15.20	5.78
77.75	13.60	5.72	79.05	16.10	4.91
86.60	14.40	6.01	86.50	16.25	5.32
84.25	15.20	5.50	83.15	17.25	5.74
99.85	17.75	5.62	99.65	17.05	5.84
80.10	15.30	5.23	80.80	15.70	5.15
75.15	13.55	5.55	77.55	13.65	5.68
87.30	16.10	5.41	87.30	16.30	5.35
102.10	16.60	6.15	102.45	18.30	5.60
91.55	15.25	6.00	92.15	16.85	5.47
90.45	16.25	5.57	90.20	15.90	5.67
84.00	15.30	5.49	83.50	17.10	4.88
74.55	12.15	6.13	76.75	13.85	5.54
84.25	14.30	5.89	83.25	17.30	4.81
73.30	13.25	5.53	71.00	12.55	5.66
68.55	13.65	5.02			

mean body length:spine length
ratio = 5.653

mean body length:spine length
ratio = 5.419

$t=2.0837$, $df=33$

$0.025 > P > 0.01$ with one tail

The difference in the mean ratios is equal to about 0.5 to 0.6 mm in actual spine length difference.

between the sexes and the different age classes.

Results

Figure 11 shows the frequency distributions of lateral plates for [trout and seine-caught] sticklebacks of the Black form. The difference is highly significant even though the modes are the same. These results demonstrate in nature the differential survival of fish with various plate numbers.

The distribution of plate numbers among sexually mature females is significantly different from that of sexually mature males although the modes are the same (Fig. 12). This may be a consequence of differential predation on the two sexes (see below, body size, page 50).

Differences in plate number among the Black males appear to be associated with several behavioral traits involved in reproduction:

Behavior on the nest. Multiple regression analysis based on the nest sampling data of 1968 showed plate number to be significantly correlated with three variables:

—time to return to the nest ($t=2.45$, $R=0.183$, $df=277$, $0.05>P>0.01$).

—depth of water in which nest was built ($t=2.03$, $R=0.175$, $df=277$, $0.05>P>0.01$).

—distance from nest to cover ($t=2.20$, $R=0.161$, $df=277$, $0.05>P>0.01$).

The different plate types were related to the above variables as follows:

time:	7—7 long	6—6	6—7	7—8 short
depth:	6—6 shallow	7—8	7—7	6—7 deep
distance:	6—7 close	7—7	6—6	7—8 far

In 1969 differences in these variables (time, depth, distance) were not

Fig. 11. Plate number frequencies in fish from *S. clarki* guts compared with frequencies of seine-caught (lake) fish, Black form.

Fig. 12. Plate number frequencies of males and females, Black form.

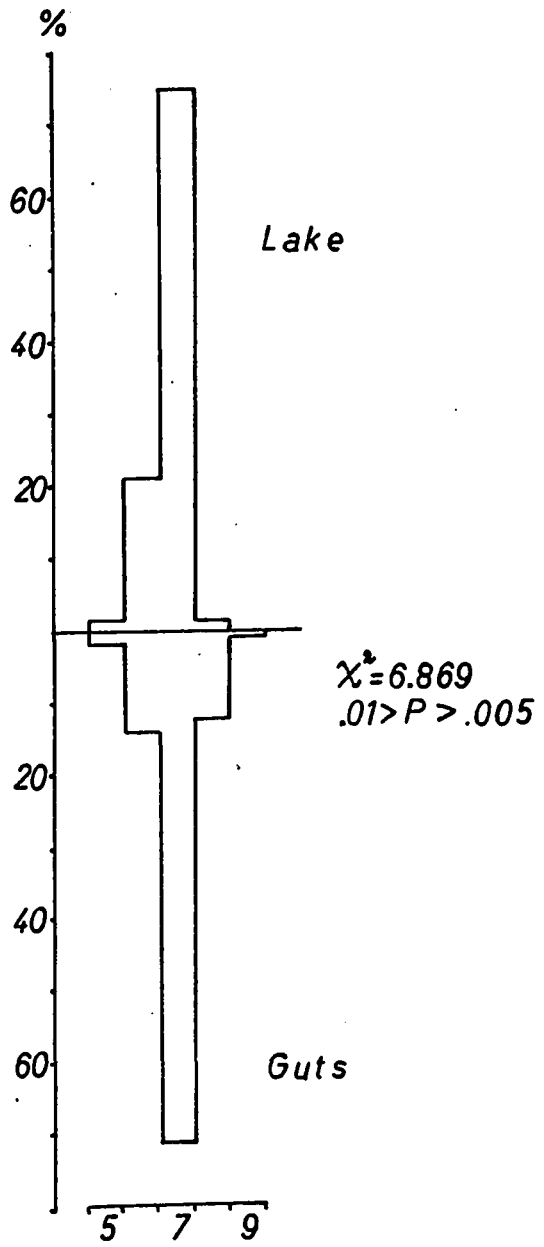


FIGURE 11.

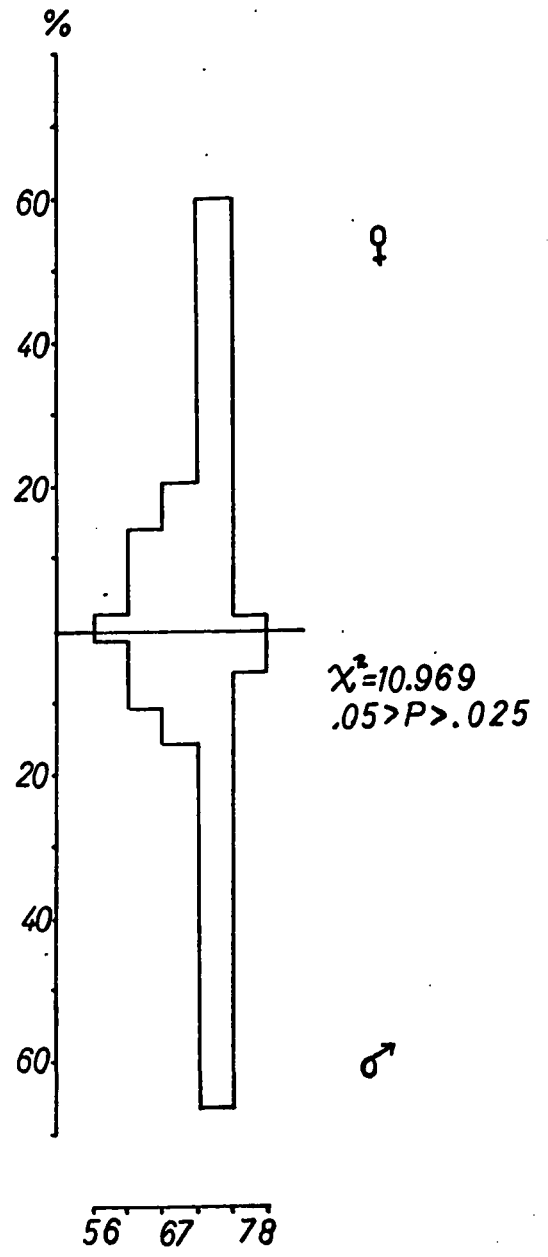


FIGURE 12.

significantly related to differences in plate number. Two explanations are possible for the inconsistency. The first is that the 1968 correlations are not representative, perhaps a result of sampling bias or chance. Second, because of the narrow range of variation in the stage of the breeding cycle in 1969, relationships simply could not be detected. Figure 10 shows that stage of the breeding cycle is a major independent variable, significantly correlated with "time to return" and "nest depth". Concentrated sampling of a single stage in the breeding cycle can only have a corresponding effect on the other variables correlated with age. Thus other real, but weakly correlated, variables will not appear as significant because of the loss of the full range of variability for several characters.

The first possibility, sampling bias, seems unlikely because of the order in which the variables were recorded and because usually one did not know which fish belonged to a given nest until the data had been recorded. In addition to these safeguards, there were no *a priori* expectations as to the nature of the relationships between the variables.

The only definitive answer will come from an experimental analysis of the plate question. Unfortunately such an approach failed in 1969. Fish of various plate numbers were placed in large 35 foot (10.7 m) by 30 foot (9.1 m) pens with artificially matched substrates. The experiment used fish interrupted in the middle of their breeding cycles; these fish proved unable to initiate new cycles when placed in the pens.

Spawning locality. The nest sample was collected from several different areas in the lake. The fish from these areas were compared on the basis of their plate numbers.

In 1968, locations iC and iB (Fig. 1) were sampled throughout the summer. One locality, iC, consisted of fine, clean sand. Cover was scarce and consisted chiefly of scattered *Nuphar* and the occasional piece of sunken driftwood. The extent of this area was about 300 feet (91.4 m) in length, with nests extending in a band about 40 feet (12.2 m) wide, about 10 feet (3.0 m) offshore. Adjoining this sandy area was locality iB, having a rocky bottom lacking any sand and with no cover of any sort. The nests in this area were found in a strip measuring 300 feet (91.4 m) by 20 feet (6.1 m) where the bottom gradient was steeper. The shallowest nests were 10 to 20 feet (3.0 to 6.1 m) from shore. In this area there were significantly fewer seven-plated fish nesting than there were in the area with a shallow gradient and sand and cover (Table 12). Such a difference could either represent a preference on the part of the different types of fish, or one type of fish could be displacing the other from a habitat desired by both kinds.

In 1969 locality iB could not be adequately sampled owing to a scarcity of spawning fish. None of the breeding areas sampled in 1969 (3, d, iC, j) showed any significant differences in plate number frequency. However, the differences between these environments were less than those between iC and iB.

Average clutch size was determined in areas iC, iB, and iD. The clutch sample from area iB was too small to be of use. The sample from the sandy area, iC, could be compared with a sample taken immediately to the south, area iD, however. Area iD was intermediate between iC and iB in that the bottom consisted of mixed sand and rocks with considerable cover provided by moss of the genus *Fontinalis* and large boulders. The

Table 12. Frequency of plate numbers in two adjacent habitats.

habitat	7—7	plate number all counts but 7—7
area iC (sandy cover, shallow)	48	13
area iB (rocky, no cover, deep)	15	14

2 by 2 contingency table, adjusted $\chi^2=5.58$, $0.025 > P > 0.01$

Table 13. Influence of habitat on clutch size.

area iC (sandy, cover moderate)	-	$\bar{x}=947$ eggs/nest, n=10
area iD (rocky, cover abundant)	-	$\bar{x}=687$ eggs/nest, n=21

"Test of the difference in mean level between two groups of observations,"

Quenouille (1959), $P < 0.01$

bottom gradient was the same as in area iC. The clutch size in the two regions, iC and iD, was significantly different (Table 13).

The differences was probably due in a large part to the higher density of males in area iD, which would tend to reduce the number of females per male. There was no difference in plate number frequencies in these two regions.

Stabilizing selection for lateral plate number was not obvious (Fig. 13). The samples used were not ideal for such a study, however. It was not possible to compare fish from the same year class. Perhaps more serious is the fact that the "fry" were about two months old (the earliest at which plates could be counted) when captured. The fish had therefore undergone considerable selection before the plates were fully formed and this selection may have been for characters correlated with plate number.

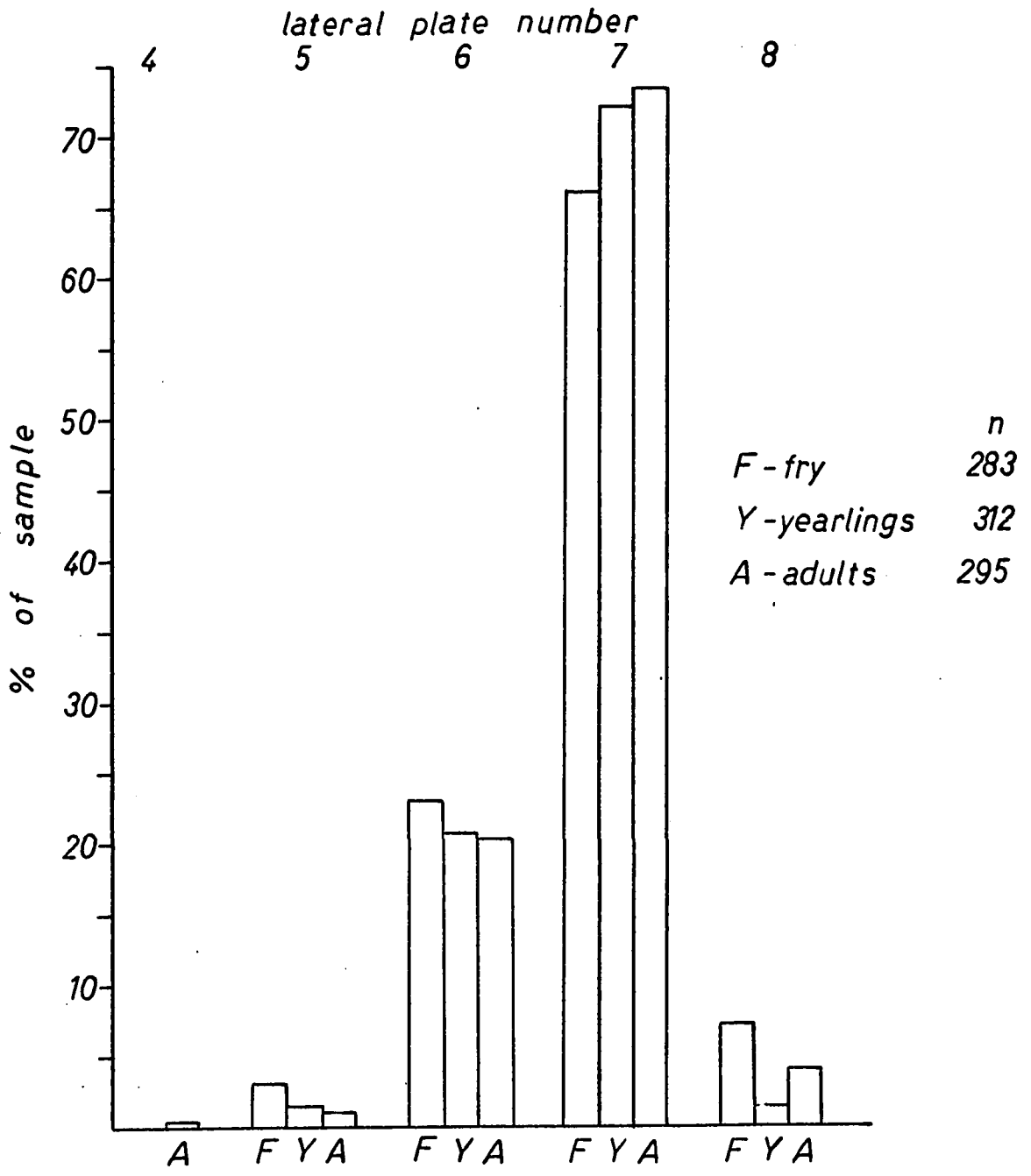
Body Size

Materials and Methods

Although large size is a major character distinguishing the Black form, intra-population variation in body length was not investigated in detail. Most of the information gathered in this respect was obtained incidentally in the course of other studies.

It seems certain that large fish, aside from any of the other attributes of large size, would be more difficult for a given predator to swallow than would a small fish. Relevant data were available from samples of trout stomachs. Only sexually mature female Black sticklebacks taken from trout stomachs were considered because estimates of a mean population length of juveniles would be highly dependent upon sampling location and

Fig. 13. Stabilizing selection on lateral plate number, Black form.



time. Males were not considered because their parental behavior (i.e. territoriality) complicates their susceptibility to predation and because of the difficulty in determining sexual maturity of males from stomachs of trout.

Mate size preference of females was tested by the methods previously described for color preference tests. Body length was one of the variables considered in the analysis of the nest and clutch data.

Results

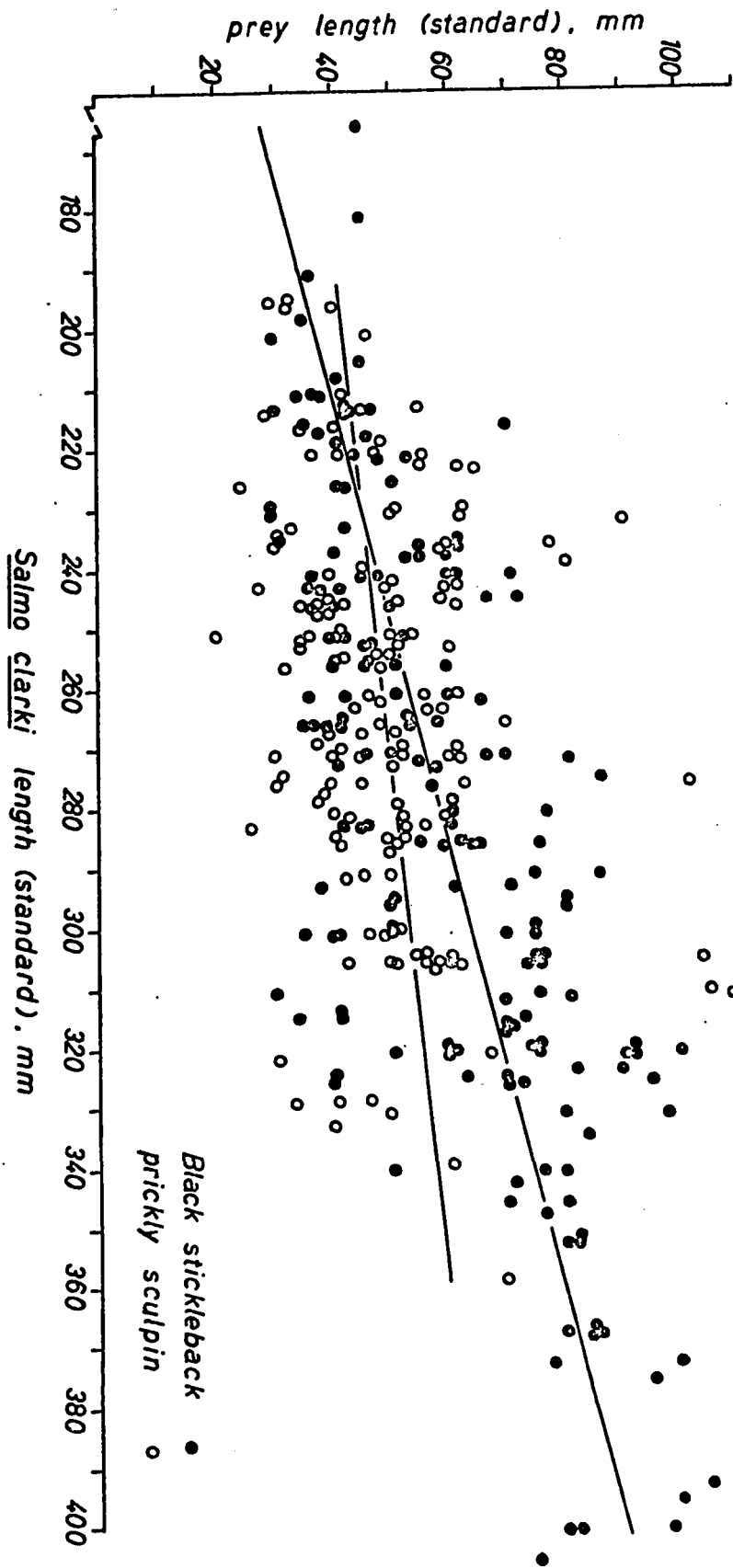
The mean length of sexually mature females in the lake was 89.9 mm, while in the trout stomachs the mean was 87.0 mm. This difference is not significant, however.

The difference of the slope of the regression line of predator length (trout) on prey length (Black sticklebacks and prickly sculpin) (Fig. 14) suggests that the relationship between stickleback length and trout length is more direct than that between cottid length and trout length. This would tend to confirm the supposition that the size of the stickleback is of importance to the predator. The difference in the two slopes is significant ($t=3.41$, $df=306$, $P<0.001$; Steel and Torrie, 1960).

Females showed a significant preference for the larger of a pair of males differing only in size (0 small:14 large). This apparent advantage was not reflected in the clutch sizes of large males in the lake, however, for their clutches were no larger than those of small males. Similarly there was no difference in the sizes of males which had no eggs in their nests compared to those which did.

In 1969 length of breeding males in the lake was positively correlated with "time to return to the nest" ($t=2.8$, $P<0.01$), and "depth

Fig. 14. Prey (Black form and *C. asper*) length as a function of predator (*S. clarki*) length. For the Black form $Y = -19.95 + 0.275X$; $r = 0.721$; $n = 176$; $\bar{x} = 279.7$ mm; $\bar{y} = 57.0$ mm. For *C. asper*, $Y = 8.63 + 0.150X$; $r = 0.314$; $n = 134$; $\bar{x} = 263.8$ mm; $\bar{y} = 48.2$ mm.



of water in which the nest was built" ($t=3.0$, $P<0.01$). However, neither correlation appeared in the 1968 data. The pooled 1968-1969 clutch data showed length of the male to be correlated with plate number ($t=3.0$, $P<0.01$) and that large males tended to be non-red more often than red ($t=-2.0$, $P<0.05$), (Fig. 10).

DISCUSSION

CHARACTERISTICS OF THE BLACK FORM

Breeding Color

It seems certain that individuals of *S. clarki*, and perhaps *C. asper*, select Black form sticklebacks having red throats as prey. The color red not only makes its possessor more conspicuous to feeding trout, but is also a sign stimulus in the agonistic displays of *S. clarki*. Red may thus be particularly inappropriate for the stickleback if it releases attack in the trout as well.

The reason for the existence of red in a minority of the breeding males of the Black form is not clear. Whether the variation can be considered polymorphic is also uncertain, for the genetic factor is unknown and the variation cannot be readily classified into distinct morphs. It is convenient, however, to consider the maintenance of color variations as analogous to that which occurs in cases of balanced polymorphism. Transient polymorphism seems unlikely for two reasons: the transition period from one genotype to another is brief in terms of the evolutionary history of the species, so that we are unlikely to encounter such situations. Ford (1964) states that the melanic form of *Biston betularia* was established in the short time of about 50 years. Secondly, in all aspects of the phenotype but nuptial color, the Black form appears to be quite stable (see below). One would not expect selection to have acted any more slowly on a component of the individual related to a facet of its life-history as crucial as reproduction. Two selective forces which could maintain the red throat color are female mate preference and inter-male rivalry. In assessing the importance of female mate preference (with regard to throat color) the adult

sex ratio may be of significance. If females outnumber the males, the color of the males may lose its importance. No data on sex ratio of the Black population are available, but there is reason to believe that females are in excess. Males, because of their territorial behavior, are probably more liable to predation while guarding the nest. Of anecdotal interest is the frequent observation of courtship by females, directed towards unreceptive males. The mean fecundity of females and the mean number of eggs per nest indicates that the Black males mate with about three or four females per cycle, and sometimes many more. This seems to suggest that females are not in short supply. It may be a mistake to expect strong mate preference in the females of the Gasterosteidae—mate preference may be more pronounced in the males, which bear the burden of reproductive duties. In some (non-Gasterosteid) species this is known to be the case (Williams, 1966).

Although the total clutch size of the males does not indicate female preference for red, a more sensitive experimental procedure, e.g. that used by D. Hay (pers. comm.) in which females are able to enter the nest of the preferred male, could reveal a difference.

However, there are several clues which suggest that red throat color functions in inter-male rivalry rather than in courtship. The original nuptial color was frequently lost or altered when breeding males were taken from their nest in the lake and allowed to nest in the aquaria. Muckensturm (1969) showed that the nuptial color pattern in *leiurus* is strongly influenced by the social order existing in a group of sexually mature fish. The frequent intensification of the red coloration after hatching of the eggs (noted also by van Iersel, 1953) is hard to explain

in terms of sexual selection. It becomes understandable as a component of territorial defence. Aggression, defined in this study as time taken to return to the nest, is greatest following hatching and during the fry stage. This observation differs from that of Sevenster (1961) but is supported by those of Hagen (pers. comm.).

Spine Length

The effectiveness of the spines of individuals of *G. aculeatus* in deterring predators was demonstrated long ago by Hoogland, Morris and Tinbergen (1956). Their work was entirely experimental and they did not deal with the relative length of the spines in a refined manner. That selection for fish with long pelvic spines is occurring among the Black sticklebacks seems reasonably certain. Males sampled outside the breeding season might be expected to show the phenomenon as well, as might juveniles sampled in sufficiently large numbers. The upper limit on pelvic spine length is probably set by the structure of the fish. The length must be stabilized at a size which prevents interference with the cloaca.

A puzzling aspect of the morphology of the pelvic spines and also dorsal spines I and II is the amount of variation they exhibit. Forked and antler-like spines are not uncommon. Many of these are a result of regeneration following injury, but in some cases previous injury is not apparent. Such variability of the phenotype is not usual when a structure is subject to strong selection as seems to be the case with spines.

Lateral Plate Number

Lateral plates of individuals of *G. aculeatus* have previously been correlated with physiological responses to salinity and temperature (Heuts, 1956). Hagen (1967) first noticed that plate number was also correlated

with an ethological trait, time of spawning. He found that early breeding individuals of *trachurus* had significantly higher plate counts than did late breeders. McPhail (pers. comm.) then began experimental work on the relative susceptibility to predation of fish with different numbers of plates.

The present study confirms in nature the differential survival of certain plate types exposed to selection in the form of predation. The nature of the disadvantage conferred by the possession of a particular number of plates is at present unknown. Sticklebacks sometimes escape from the mouth of the predator before being swallowed (Hoogland, Morris and Tinbergen, 1956; J.D. McPhail, pers. comm.). There are thus two points at which differential escape could occur: prior to attack and/or during contact. The plate number of fish with broken spines (fish which had been attacked but not swallowed) was not different from the plate number of fish with normal spines, i.e. fish which had perhaps escaped attack. This suggests that following contact with the predator, plate number has no bearing on the chances of escape. The difference in likelihood of capture could then be a result of differences such as swimming speed or behavior, occurring prior to contact. If a slower swimming speed results from high plate number, it is odd that the population mode is at seven rather than a lower number. If the plates reduce the flexibility of the body, one would expect plates to be few or absent. A more probable explanation may be that a behavioral trait is linked with or is pleiotropic with a plate number of seven. The trait could be one promoting crypsis or possibly a result of the nature of the lateral line organs of the plated region of the body. McPhail's study (pers. comm.) of a population with a mode at

seven in which predation is solely upon larvae, prior to formation of the plates, is the most conclusive evidence that a behavioral factor is probably responsible for the differential survival of fish with various plate numbers.

The differences in nesting behavior associated with variation in plate number are difficult to assess because of the poor replication in the two sampling seasons. To the extent that they are real, they offer an important mechanism for explaining the retention of plate numbers other than the modal value of seven. If fish with different plate counts nest in different microhabitats and differ in their timidity while on the nest, they will be exposed to selection pressures which may favor plate counts other than seven. These opposing selective pressures will then maintain variability in plate number within the population. In the case of time taken to return to the nest, it should be noted that plate asymmetry rather than number could be responsible for the difference observed. Asymmetry is known to be under genetic control in several organisms (Van Valen, 1962). These differences in behavior correlated with differences in a genetic character, plate number (see below), illustrate a commonly forgotten fact, i.e., the amount of genetic variability in a population and its lability to selective processes. Recognition of this dynamic interaction between organism and environment can lead to a greater effort to relate observed genetic variation, e.g., polymorphisms, to particular selective forces. In ecological studies genetic variation is often not considered. Ecological genetics, rather than becoming an agent of introgression between the two fields of ecology and genetics, has unfortunately become a third discipline.

Body Size

An increase in the size of an organism has several independent consequences. The biology of the Black form suggests several selective pressures which would favor large size.

Increased fecundity is indicative of greater reproductive effort, provided egg mass increases and the number of matings per lifetime does not decrease. The egg mass, defined here by the egg diameter, is at least equal to that of other recorded *G. aculeatus* populations. In most threespine stickleback populations individuals probably have a single breeding season in their lifetime. Accurate information on the number of matings per season is not available. Most authors refer to "several" nesting cycles, in each of which the male mates "several" times. The estimates given for the Black population (see Appendix I) are within these broad limits. Until the latter parameter is quantified in various populations, it will be difficult to be sure that the increased fecundity of the Black form in fact indicates a greater reproductive effort.

The larger size of individuals of the Black form relative to those of *leiurus* has very likely resulted in a greater swimming speed in the former. Bainbridge (1958) estimates that the swimming speed of *Leuciscus leuciscus* and *Carassius auratus* increases in proportion to the 0.65 power of body length. Applying this value to the difference in mean length of the two forms, one would expect the Black form to have a speed 1.4 times that of *leiurus* individuals. The actual difference could be greater because of the more streamlined body shape (Fig. 3, body length;body depth) of the Black form. An increased swimming speed could have several advantages. Feeding on free-swimming zooplankters (see Appendix I) may require a

greater speed than would be necessary in feeding on the littoral zone on larger organisms where manouverability may be of more importance.

Quick bursts of speed may be vital in enabling individuals of the Black form to secure shelter when threatened by a predator. In the words of Roeder (1967), 'milliseconds may distinguish the quick from the dead'. Two very vulnerable phases of the life-cycle are spent in the littoral zone where the ability to reach shelter when attacked may be critical, these periods being immediately after hatching and again during reproduction.

Baerends, Bennema, and Vogelzang (1960) demonstrated in cichlids an increase in visual acuity with increase in body length. This increase was related to an increase in the diameter of the retinal cells. The distance between centers of the cells remained constant over a wide range of body sizes. The relationship might be expected to be general in fishes. Improved visual acuity might be selected for in the Black stickleback for the same reasons that increased swimming speed is probably adaptive.

The observation that the larger of a pair of Black males, and similarly, a Black rather than a *leiurus* male, is preferred by Black females indicates the existence of at least partial ethological reproductive isolation between the two populations. This is probably an incidental by-product of selection for some of the concomitants of large size discussed above (and likely others not discussed). Muller's (1940) suggestion that reproductive isolation is merely an outcome of genetic divergence following selection for features of no direct importance to reproductive isolation *per se* seems likely in this instance.

Stability of the Phenotype

It is apparent that in the morphological comparisons made, stabilizing selection is greater in individuals of the Black form, with the exception of anal and dorsal fin ray number which is discussed below. Lindsey (1962) and Hagen (1967) considered the genetic control of some of these characters. Lindsey, using *leiurus* individuals, and Hagen using those of *leiurus* and *trachurus*, observed genetic control of lateral plate number. Hagen (pers. comm.) reports that heritability of lateral plate number is unusually high. Lindsey found evidence of genetic control of vertebral number and Hagen found the same for gill raker number, spine length, and body color. Both workers agreed in finding genetic control of dorsal and anal fin ray number to be very low.

The phenotype of the Black form, as measured here, has apparently been stable for at least 39 years. Although no samples are available for the intervening period it is unlikely that the two samples would show such close correspondence in all seven characters if these were fluctuating during the interval. The stabilization is comparable to that found in the butterfly, *Maniola jurtina* L., by Ford (1964) and colleagues. Such long term stabilizations, which are not often available for study when they are most needed, are valuable illustrations of the continuous action of natural selection. Their resistance to change indicates not relaxation of selection, but rather the converse. "Stabilization is the reflection of a dynamic, multidimensional equilibrium. . . . the selective forces maintaining the equilibrium are powerful ones" (Dowdeswell, Ford, and McWhirter, 1960).

Rate of Differentiation of the Black Form and its Insular Characters

The biology of the Gasterosteidae embodies certain features generally considered to accelerate evolution.

The threespine stickleback has a broad ecological tolerance. This has permitted the species to colonize successfully very diverse habitats. In North America *G. aculeatus* is found as far south as the Mexican-California border (Miller and Hubbs, 1969), and as far north as the Hudson Bay drainage (Carl, Clemens and Lindsey, 1967). It is present under conditions ranging from large, clear lakes, e.g. Lake Chelan, Washington, to shallow, swamp-like ponds (as in the Queen Charlotte Islands) and in rivers and streams. Local populations show strong habitat preferences (Hagen, 1967, and present study) which tend to promote isolation among populations. These two characteristics, together with the great genetic variability (Lindsey, 1962), promote differentiation.

Parental care reduces random (i.e., non-selective) mortality during the immature stages (Dobzhansky, 1951:283). Survival then depends much more on the individual's genotype and intraspecific competition. The role of predation in the differentiation of fish populations is unsettled. Mayr (1963:574) argues that the effect of predation is inhibitory. Fryer (1959) reaches the opposite conclusion on the basis of his study of the fishes of some African lakes. Certainly in Mayer Lake differentiation has been intimately correlated with predation. In other nearby lakes, however, the opposite appears to have been the case—the absence of predators has made possible the loss of protective adaptations such as spines and plates (Table 5). One must conclude that at least in the threespine stickleback, predation either by its existence

or its absence is a most significant selective force.

The statement of Van den Assem (1967) that adult threespine sticklebacks may have few aquatic predators, in part because of their spines, is contradicted by field data from many parts of the world (Frost, 1954; Greenbank and Nelson, 1959; Clemens and Wilby, 1961; D.W. Hagen, pers. comm.; and the present study).

The time interval in which the differentiation of the Black stickleback has occurred can be given an upper limit of about 10,000 years. Prior to this all of the Queen Charlotte Islands but the tops of the highest mountains were completely glacier-covered (Sutherland Brown and Nasmith, 1962). The adaptive value of the distinguishing characters of the Black form clearly fit the population to the ecosystem formed by Mayer Lake. Similarly, three other lakes, all within 4 miles (6.4 km) of Mayer Lake, each have distinctly different stickleback populations. It seems unlikely that these populations could have differentiated in and moved from refugia elsewhere. The problem of envisaging stable, moderately large lakes in refugia on the island are apparent. It thus seems most plausible to think of the evolution of the Black sticklebacks as a post-glacial phenomenon.

One is tempted, nevertheless, by the variety of endemic forms in so many unrelated groups in the Queen Charlotte Islands (birds, mammals, fungi, invertebrates, vascular plants and bryophytes) to seek a single underlying cause for all the differentiation. The refugium concept would be such an explanation. Despite their appeal, single factor explanations are not always the correct ones. It would appear wisest to first judge each group separately. In the case of the sticklebacks there seems no

logical way in which the Black form and also some other populations could have evolved anywhere but in their present locations. These locations are said to have been simultaneously glaciated (Sutherland Brown and Nasmith, 1962). In the case of some of the endemic plants, a refugium explanation may indeed be correct (Calder and Taylor, 1969). What should be avoided in reaching a decision is (a) equating degree of differentiation to duration of isolation, when in fact nothing is known about the particular evolutionary rate of differentiation, and (b) citing a distinctive flora and fauna as evidence for a common cause, when in fact several causes, e.g. endemism, rapid evolution, and relictism, could be involved.

Much of the work on insular organisms is essentially biogeographical. Although many insular species are well known morphologically, usually little is known of their ecology and behavior. Perhaps this is the reason that the unique aspects of these species are commonly explained by untested mechanisms, such as genetic drift or founder effect (Gosline, 1968).

Consideration of two of the characteristics of the Black form which are said to be typical of insular vertebrates is of interest. Melanic and gigantic species have been described from many islands of the world (Carlquist, 1965). In the Black stickleback it would appear that these two features can be adequately and simply explained by natural selection. To suggest that competition and predation is reduced on islands (Carlquist, 1965:5) seems most unwise; in fact the opposite can also occur. The study of the sticklebacks in Mayer Lake indicates that in the Black form predation is intense, while in the *leiurus* form this is not so.

If insular species were more often subjected to the standard

ecological and behavioral analysis applied to continental species, much of the ignorance surrounding insular characters might be dispelled.

Phylogenetic Considerations

The Black population seems to have evolved from either of the forms *leiurus* or *trachurus*. A comparison of the similarities and differences among the forms *leiurus*, *trachurus*, and Black leads to the conclusion that the Black form is most like *trachurus* (see Hagen, 1967, for a description of the morphology of eastern Pacific *trachurus*). The similarity in plate number between individuals of *leiurus* and the Black form must be discounted because preliminary experimental crosses indicate that the genetic control of plate number differs in the two forms.

One wonders, however, whether such comparisons are very meaningful in a case such as this. The genetic variability of the genus *Gasterosteus* is very high (Lindsey, 1962). None of the characters of the Black form seem far beyond the limits of variation of *leiurus* or *trachurus* individuals, because it is largely the character complex of the Black form rather than the characters themselves which set it apart. It seems that the similarities could result from convergence with either form as easily as by descent from a particular form (Cain, 1959). It will be difficult to decide whether the Black form evolved from *leiurus*, from *trachurus* or from a predecessor of both, until more is known of the nature of variation and selection in *Gasterosteus* in many different environments.

For the present one can look to historical factors and to the ecology of the three forms to decide whether the form *trachurus* or *leiurus* is the most likely ancestor. On this basis one would expect Mayer Lake to have been oligotrophic immediately following de-glaciation. Such a lake

would have a poorly developed littoral zone. Limnetic zooplankters would probably be among the early colonizers which would be useable as food for sticklebacks. The form *trachurus* would then be the most suitable immigrant and could subsequently give rise to the Black form. *Trachurus*, because of its larger spines and plated body, may also be better adapted to the effects of coexistence with predators than is *leiurus*.

Taxonomic Status of the Black Form

The Black form differs from the parapatric *leiurus* form in several aspects of its morphology, ecology, and behavior. Hybridization between the two populations seems limited. Fish showing morphological features intermediate between the two forms are occasionally found in the lake, usually where the two populations abut, i.e. near the stream mouths, in particular at Cott Creek. There is reason to believe that at least in the habitat of the Black form, hybrids would be strongly selected against. The stability of the Black phenotype over a long period of time suggests that little introgression is taking place.

Studies of isolating mechanisms are incomplete, but on the basis of evidence available at present, the Black population appears to meet the criteria of a biological species definition.

The Black form does not come into contact with the anadromous form, *trachurus*. The estuary of Mayer River, 10 miles (16.1 km) from the lake, is unstable and lacks vegetation. It is clearly unsuitable as a breeding area for the form *trachurus*. Because of its strong habitat preferences, the Black form is not found near the lake outlet. Presumably it never enters the river.

CONCLUSIONS

1. In Mayer Lake sticklebacks of the Black form are subject to predation by two species of fish, *S. clarki* and *C. asper*, and a leech of the species *H. marmorata*.
2. Sticklebacks of the Black form differ morphologically, ecologically and behaviorally from the parapatric form *leiurus* which is not exposed to predation. The morphological differences include general body color, nuptial color, spine size, body shape, body length, and lateral plate number. Ecological and behavioral differences are a result of the Black form occupying an open water, lake habitat and the *leiurus* form living in vegetation choked streams. The phenotype of the Black form has been stable for at least 39 years.
3. Morphological characteristics of the Black form were studied by the comparison of variation within the population. Most of the differentiation appears to be a result of selection in the form of predation. Selection for crypsis is probably responsible for the loss of the red throat color in many breeding males. The individuals which develop the red throat may be favored in territorial disputes with non-red males. Females with relatively short pelvic spines appear to be selected by cutthroat trout as do fish with lateral plate numbers other than seven. Males with different plate numbers may differ in the time they take to return to the nest, in the depth at which the nest is built and in the proximity of the nest to cover. Predation may have selected for large body size for several reasons, among them because large sticklebacks must be more difficult for a given predator to swallow. Large fish can also swim more quickly than small ones. Large males may be more

- reproductively successful, as are large females, at least in terms of fecundity.
4. The differentiation of the Black form, as well as other Queen Charlotte Island sticklebacks, has probably occurred since the last glaciation.
 5. The phylogeny of the Black form is difficult to determine. Of the probable ancestors, the forms *trachurus* and *leiurus*, the former seems most likely.
 6. The Black form appears to meet the criteria of a biological species on the basis of the evidence at hand.

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APPENDIX I

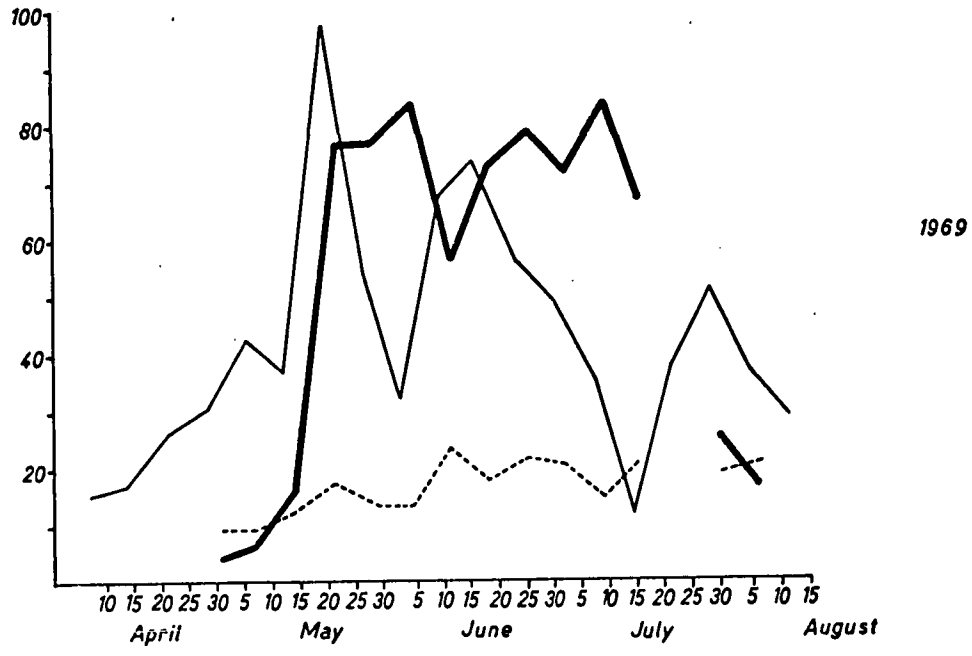
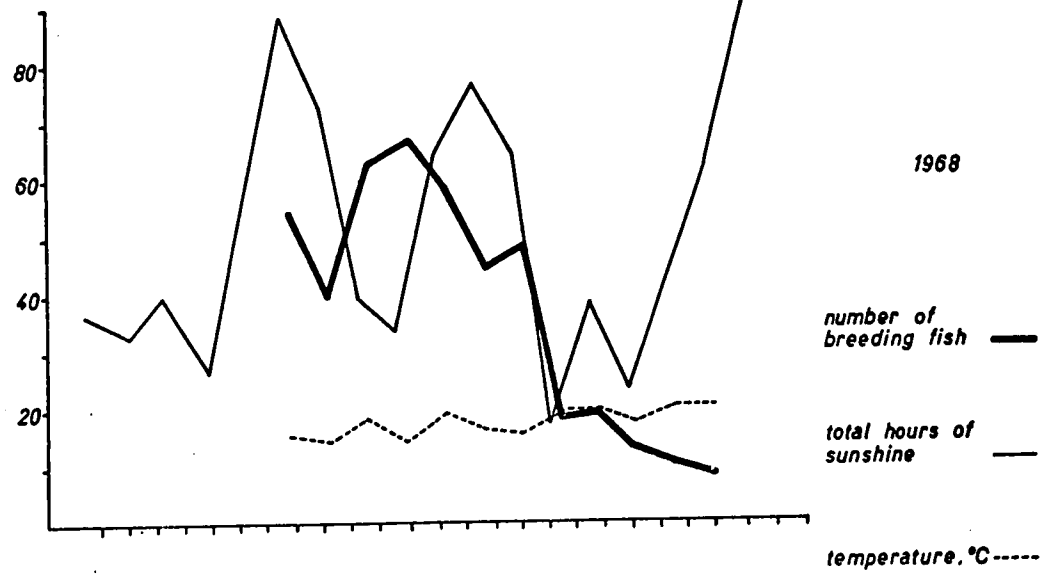
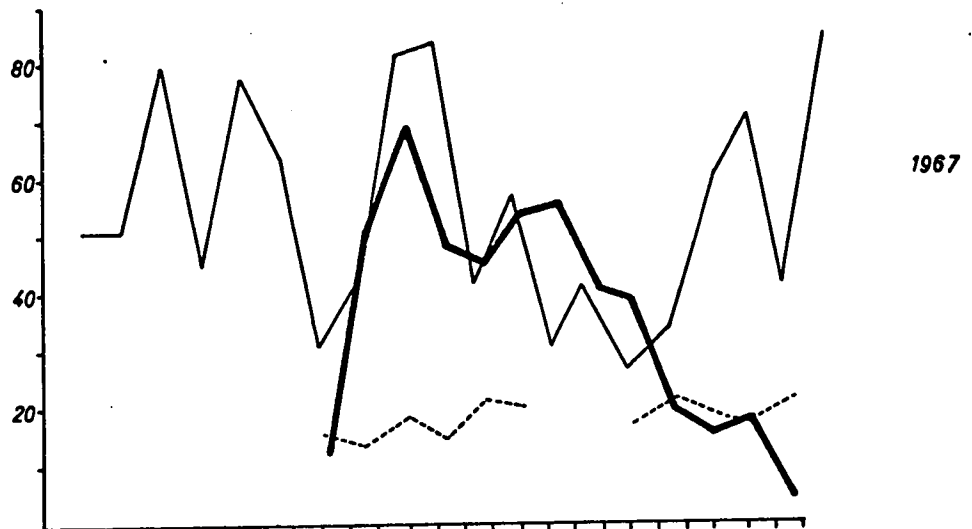
LIFE-HISTORY NOTES

Certain aspects of the life-history of the Black form are described here because of their general relevance to predation.

—the niche of the Black form. I believe, on somewhat poor evidence, that the Black form is an open-water, limnetic zone population. Supporting morphological evidence comes from the high gill raker count, typical of planktivorous species, and the terete or streamlined body shape. I found it difficult to document the limnetic occurrence of the Black form because most of the observation period took place during the breeding season when the fish were inshore. At the start of the season, however, in early May, adult and young Black sticklebacks were caught in the center of the lake. The numbers decreased during the summer, but some were always present. Females were caught in the limnetic zone throughout the summer. On several occasions each summer, schools of juveniles were seen swimming at the surface in the center of the lake. Continuous netting for five days in March, 1968, failed to yield the expected numbers of sticklebacks in the center of the lake, or anywhere else. This inability to catch sticklebacks in a lake during the winter is not unusual (D.W. Hagen, pers. comm.).

—reproduction. The duration of the spawning season in the Black form was estimated from standardized seine hauls. Each week 15 seine hauls were made over the same 100 foot (30.5m) section of typical spawning ground (area 4, Fig. 1). The results of this survey for the three summers, 1967, 1968 and 1969, are shown in Figure A1. Values are based on the total number of males in nuptial coloration and gravid females caught. The explanation for the increase in fish caught in 1969 relative to the two

Fig. A1. Spawning season of sticklebacks of the Black form, with weekly sunshine totals at Sandspit airport and water temperature on the day of sampling.



previous years is unclear. Two possibilities can be suggested: the winter and spring of 1969 were unusually severe, which may have delayed early spawners. Perhaps the more likely reason is that changes in the available breeding grounds from year to year render different areas more or less suitable, through changes in substrate type and vegetation. Fish may thus be forced to make varying use of a given area from summer to summer.

The similarity in the timing of the spawning peak each year raises the question of what are the environmental cues involved. Photoperiod and temperature are respectively the important stimuli known to induce spawning in the threespine stickleback (van Iersel, 1953). Weekly sunshine totals recorded at the Sandspit airport, 30 miles (48.3 km) south (Dept. of Transport, Meteorological Branch, Toronto), and water temperature on the day of sampling are shown in Figure A1.

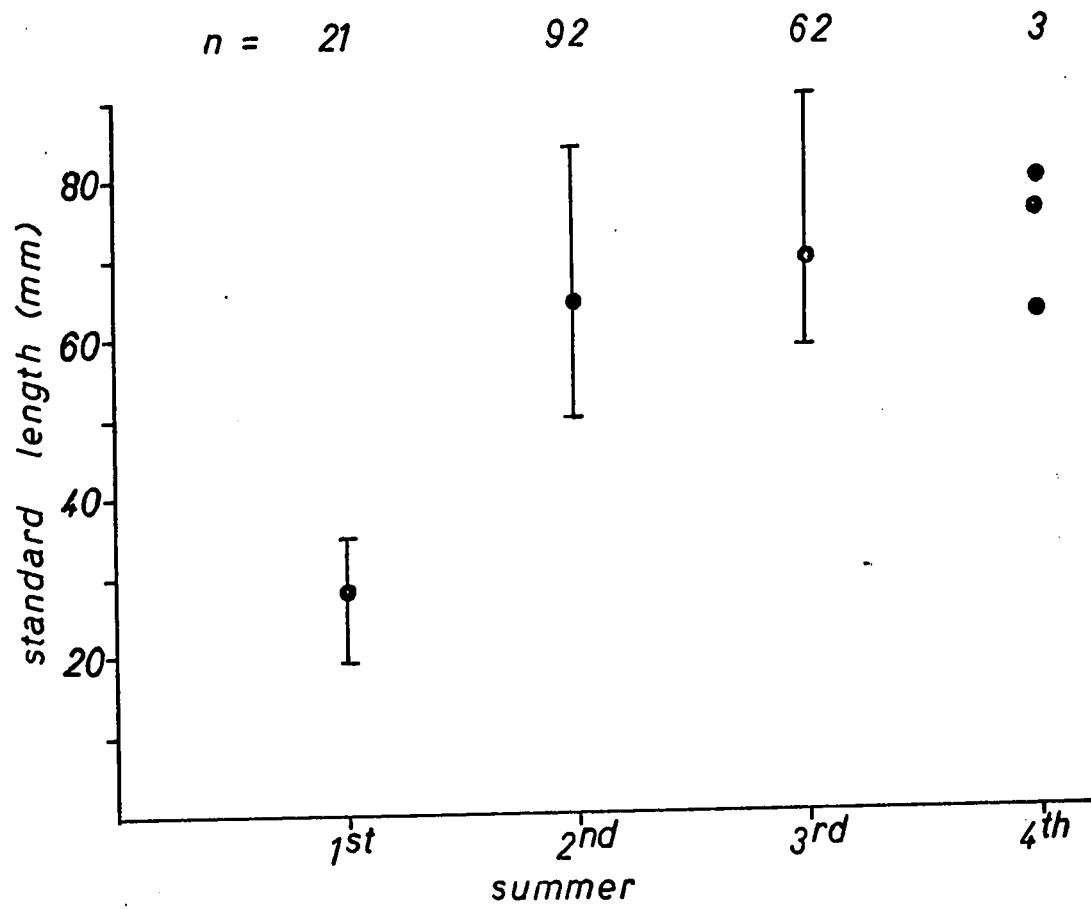
Comparison of the breeding season at Mayer Lake with those reported in the literature for one point to the south, Lat. 48° (Hagen, 1967) and one location to the north, Lat. 57° (Greenbank and Nelson, 1959), suggests that the length of the season is probably typical for sticklebacks in such an environment. The length of the season for *leiurus* in Gold Creek is very similar to that of the Black form in Mayer Lake. Thus there does not seem to have been any appreciable compression of the breeding season as a result of predation.

—Spawning habit preferences. In 1969 I surveyed the entire margin of the lake to determine the breeding habitats available to the Black sticklebacks and their nesting habitat preferences if any. The sampling was conducted by making a five minute visual examination of the

lake bottom 10 feet (3.0 m) from shore at 300 foot (91.4 m) intervals. Vegetation was recorded in terms of presence or absence of the three most important species. Substrate was roughly characterized as being composed of mud, sand, pebbles or rocks. Bottom gradient was classified as steep, moderate or gentle. Number of breeding fish present at each location was decided by totalling the number of nests, adults and nests with males. Care was taken to avoid over-counting of fish and nests subsequently seen together. Crude as the whole procedure was, the methods were necessitated by the length of the shoreline and the time available. The results (Table 1) show a highly significant (χ^2 contingency tables, $P < 0.01$) tendency for fish to nest near stems of *Nuphar* and/or clumps of *Fontinalis*, on a gentle gradient, of sand.

—Breeding effort. The discussion of breeding effort in terms of egg number and egg size is incomplete without knowledge of the number of breeding cycles per season and the number of breeding seasons per lifetime. In 1967, 187 Black sticklebacks were caught for the purpose of age determination. The fish were preserved by freezing. Otoliths were prepared after the methods of Greenbank and Nelson (1959). Results are shown in Figure A2. The repeatability of given determinations was not good. The data shown are thus not ideal. The results suggest that breeding males were always at least two years (three summers) old. This agrees with the observation that large numbers of non-breeding fish in their second summer are present each year. (These are the fish which are the smallest individuals to be found in the spring, prior to the onset of breeding.) Only three fish appeared to be three years old. All the sexually mature fish aged were males however. The life span of *leiurus* may also be about two years (Greenbank and Nelson, 1959) and observations on size classes

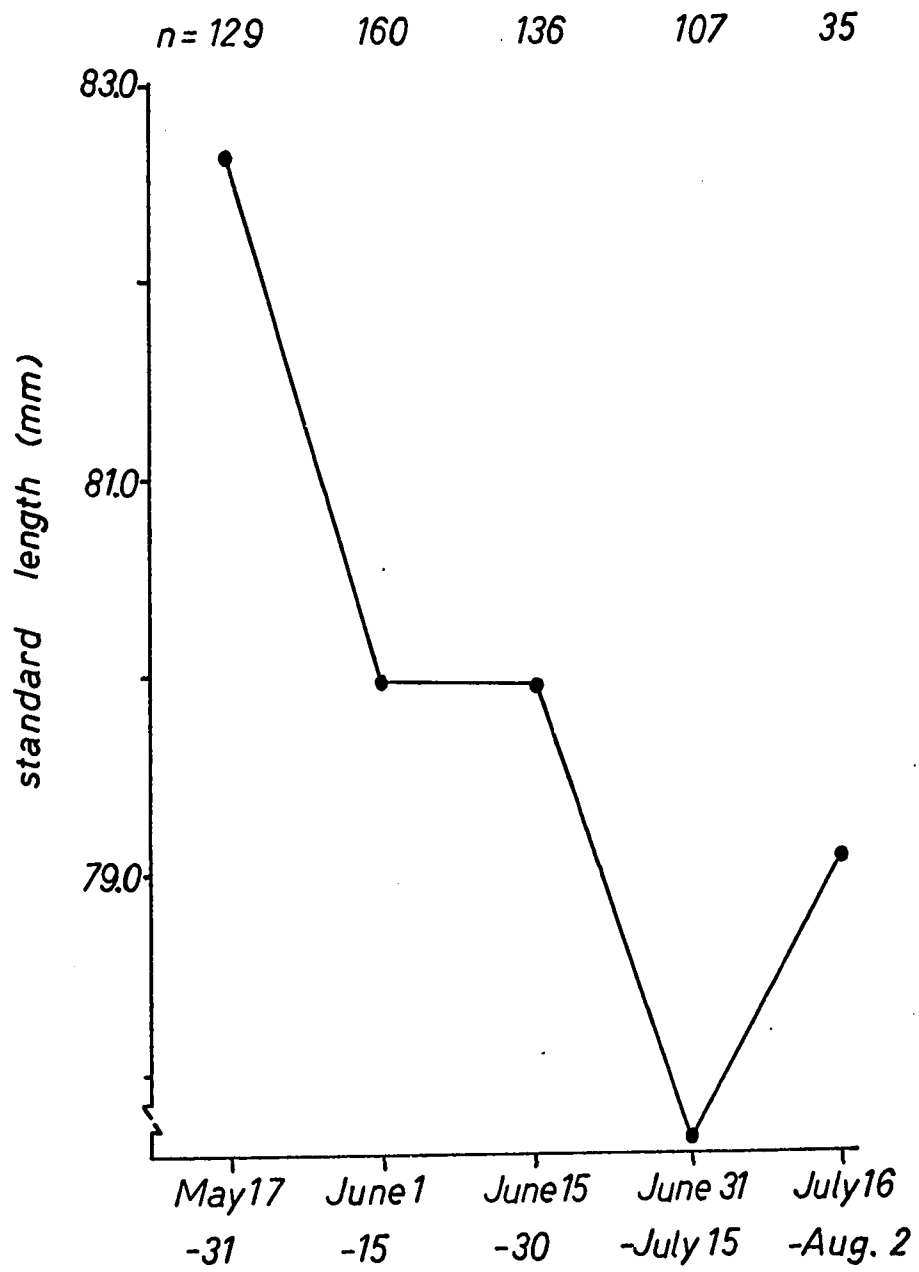
Fig. A2. Age plotted against standard length, for sticklebacks of the Black form. All sexually mature fish were males. Sample collected 28 July, 1967.



and sexual maturity in Gold Creek conform with their estimate.

The number of breeding cycles undertaken by an individual during the course of the breeding season is not well established, although most authors assume several. The total number of cycles possible per season in Mayer Lake must be about five or less. This is based on a season of 90 days, 18 days per cycle, and each cycle consisting of one day to build and court, nine days of incubation and eight days' care of the fry. Measurement of the mean length of breeding males in 1969 (Fig.A3) shows a significant decrease in mean length from May to August (randomized block design, with modification of Tukey's w -test, Steel and Torrie, 1960). This shift in mean length of breeding males suggests that individuals are not breeding throughout the season, so that the number of cycles completed by a given fish should then be less than five.

Fig. A3. Shift in mean length of breeding males during the spawning season. Differences are significant at the 0.05% except for the period June 1-15 and June 15-30, which is not significantly different.



APPENDIX II

ORIGINAL MORPHOLOGICAL DATA ON WHICH FIGURES

3, 4, 11, 12, AND 13 ARE BASED

[Figures 3 and 4]

	lateral plate number							
	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>n</u>
<i>leiurus</i>	9	55	65	21	3			152
Black, 1968		1	4	61	216	13		295
Black, 1929			4	21	118	7	1	151

$\frac{\text{body length}}{\text{body depth}}$ ratio	Black, 1968 Fig. 3	<i>leiurus</i> Fig. 3	Black, 1929 Fig. 4*	Black, 1968 Fig. 4
3.80 - 3.89		1		
3.90 - 3.99		5		
4.00 - 4.09	2	11		1
4.10 - 4.19	4	13		1
4.20 - 4.29	13	22		1
4.30 - 4.39	28	27	3	4
4.40 - 4.49	50	40	3	4
4.50 - 4.59	82	31	6	12
4.60 - 4.69	82	23	16	15
4.70 - 4.79	65	22	13	20
4.80 - 4.89	59	13	14	21
4.90 - 4.99	35	6	16	10
5.00 - 5.09	20	2	12	6
5.10 - 5.19	10	1	6	1
5.20 - 5.29	4	2	2	2
5.30 - 5.39				
5.40 - 5.49				2
5.50 - 5.59	2		1	
5.60 - 5.69	1			
n =	<u>455</u>	<u>219</u>	<u>102</u>	<u>100</u>

* The body proportions shown in Fig. 4 differ from those of the Black form shown in Fig. 3 because the 1929 sample consisted of and was compared with sub-adults. Fish shown in Fig. 3 were sexually mature.

body length spine length ratio	Black, 1968 Fig. 3	<i>leiurus</i> Fig. 3	Black, 1929 Fig. 4*	Black, 1968 Fig. 4
4.10 - 4.19				2
4.20 - 4.29				1
4.30 - 4.39	2		1	2
4.40 - 4.49	1		1	7
4.50 - 4.59	6		5	6
4.60 - 4.69	4		2	10
4.70 - 4.79	10		7	16
4.80 - 4.89	15		7	14
4.90 - 4.99	29		9	12
5.00 - 5.09	33		14	11
5.10 - 5.19	46	1	24	7
5.20 - 5.29	50		9	6
5.30 - 5.39	55	1	12	2
5.40 - 5.49	58	2	4	2
5.50 - 5.59	58	2	4	1
5.60 - 5.69	35	2	2	1
5.70 - 5.79	37	5		
5.80 - 5.89	21	7		
5.90 - 5.99	18	4		
6.00 - 6.09	13	8		
6.10 - 6.19	4	19		
6.20 - 6.29	7	12		
6.30 - 6.39	5	20		
6.40 - 6.49	1	17		
6.50 - 6.59	2	19		
6.60 - 6.69	1	19		
6.70 - 6.79	1	14		
6.80 - 6.89		21		
6.90 - 6.99		10		
7.00 - 7.09		11		
7.10 - 7.19		10		
7.20 - 7.29		6		
7.30 - 7.39		4		
7.40 - 7.49		2		
7.50 - 7.59		2		
7.60 - 7.69				
7.70 - 7.79		1		
7.80 - 7.89		1		
7.90 - 7.99				
8.00 - 8.09		1		
8.10 - 8.19				
8.20 - 8.29				
8.30 - 8.39		1		
8.40 - 8.49				
8.50 - 8.59				
8.60 - 8.69				
8.70 - 8.79	1			
n =	457	221	101	100

* See footnote on previous page.

[Figures 3 and 4]

	anal fin ray number					
	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>n</u>
<i>leiurus</i>	9	86	40	4		139
Black, 1968		2	51	37	2	92
Black, 1929	1	1	25	39	1	67

	dorsal fin ray number					
	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>n</u>
<i>leiurus</i>	6	28	78	28		140
Black, 1968	1	7	31	44	9	92
Black, 1929		1	17	42	4	64

	vertebrae number					
	<u>31</u>	<u>32</u>	<u>33</u>	<u>34</u>	<u>35</u>	<u>n</u>
<i>leiurus</i>	2	35	32	2		71
Black, 1968		1	17	119	20	157
Black, 1929			27	52	7	86

	gill raker number															
	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>n</u>
<i>leiurus</i>	13	20	31	41	30	37	19	12	2							205
Black, 1968					1	3	22	57	74	48	30	12	2		1	250
Black, 1929						2	16	32	54	29	22	4	2			161

[Figure 11]

	lateral plate number					
	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>n</u>
from net catches	2	22	78	2		104
from stomachs	2	15	76	13	1	107

[Figure 12]

	lateral plate number					<u>n</u>
	<u>5-6</u>	<u>6-6</u>	<u>6-7</u>	<u>7-7</u>	<u>7-8</u>	
females	6	32	47	135	5	225
males	8	82	110	464	38	702

[Figure 13]

	lateral plate number										<u>n</u>	
	<u>5-4</u>	<u>5-5</u>	<u>5-6</u>	<u>6-6</u>	<u>6-7</u>	<u>7-7</u>	<u>7-8</u>	<u>8-8</u>	<u>7-5</u>	<u>7-9</u>		<u>8-6</u>
fry		4	8	31	58	153	18	8	2		1	283
yearling		3	5	45	68	175	10	3	2	1		312
adult	2	1	2	31	49	187	19	3	1			295

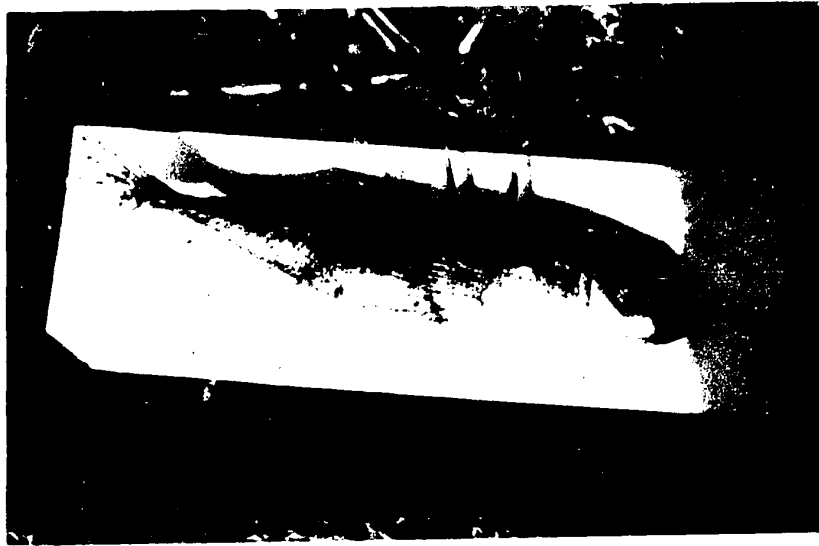
APPENDIX III

COLORATION OF SOME MAYER LAKE STICKLEBACKS

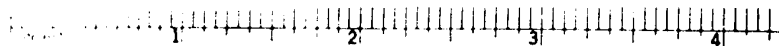
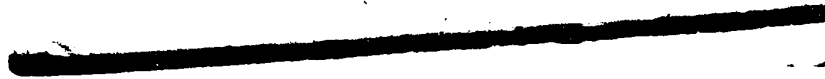
Female of the *leiurus* form.

Gravid female of the Black form.

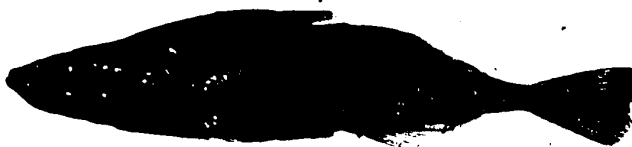
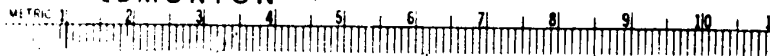
Breeding male of the Black form. The most common color pattern.



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Buccal cavity of a breeding male of the Black form. This fish showed no red pigment elsewhere.

Breeding male of the Black form. The red-throat type.

Breeding male of the Black form. A type with gold coloration.



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