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THREESPINE STICKLEBACKS (GASTEROSTEUS ACULEATUS) IN DRIZZLE LAKE AND ITS INLET, QUEEN CHARLOTTE ISLANDS: ECOLOGICAL AND BEHAVIOURAL RELATIONSHIPS AND THEIR RELEVANCE TO REPRODUCTIVE ISOLATION.

University — Université

UNIVERSITY OF ALBERTA

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

MASTER OF SCIENCE

Year this degree conferred — Année d'obtention de ce grade

1983

Name of Supervisor — Nom du directeur de thèse

DR. J.S. NELSON

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by

ELIZABETH M. STINSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING 1983

THE UNIVERSITY OF ALBERTA

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DEGREE FOR WHICH THESIS WAS PRESENTED MASTER OF SCIENCE

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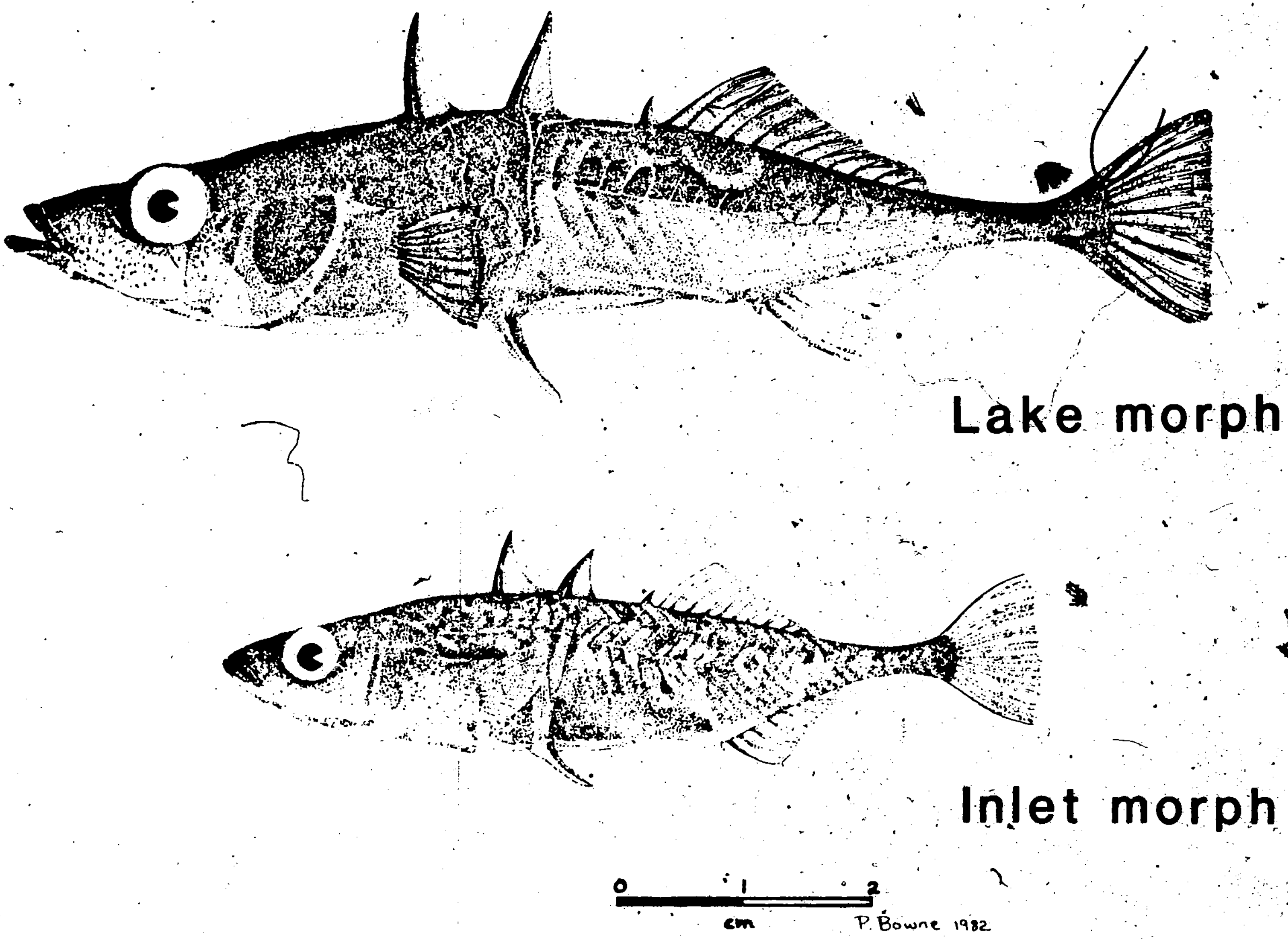
Lake morph
Inlet morph
0
1
2
cm
P. Bowne 1982

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Supervisor

Mark V.H. Wilson

W.G. Evans

Fred C. Zwickel

Date 29 October 1982

Abstract

Two morphological types (morphs) of threespine stickleback (*Gasterosteus aculeatus*), occurring parapatrically in the Drizzle Lake watershed, Queen Charlotte Islands, appear to be reproductively isolated. Only one adult of a visibly intermediate phenotype has been found in the examination of more than 1500 fish near the zone of contact.

Field and experimental work was conducted to determine whether premating reproductive isolating mechanisms (ecological, temporal, and ethological) were in effect. Additionally, a morphological study of inlet fish was made to see if a clinal pattern of variation existed along the length of the stream.

Trapping studies revealed that inlet fish were confined to the lower 450 m of the stream and were most numerous in the region 200 m from the mouth. An increase in movement was observed in inlet subadults in the early fall. Adult lake and inlet fish were rarely found in the other's habitat.

In a morphological comparison of adults and subadults of the two morphs, differences were greatest between lake and inlet adults. Inlet adults more closely resembled lake juveniles in head morphology than did lake adults. The possible retention of juvenile characters in the inlet adults is discussed. Within the inlet morph few significant differences were found between areas of the stream; no strong clines were evident.

Ecological isolation was inferred from the distribution and movement of the two morphs. Experimental testing of habitat preferences indicated that water type and current may serve as cues for the inlet fish; lake fish responded most strongly to different vegetation types, preferring that found in the lake.

Breeding seasons of the two morphs appeared to overlap considerably. Thus, temporal isolation was not in effect.

Positive assortative mating by lake males was demonstrated with males at their nests in the lake. The males approached lake females approximately 70% of the time, and virtually all courtship approaches were to lake females. Whether lake males were using colour pattern or the larger size of the lake females as a cue was tested using epoxy models in the two sizes and painted in the two colour patterns. Colour was shown to be the stronger stimulus.

A model involving disruptive selection and the establishment of habitat selection is presented to explain the divergence of these two groups from a single ancestral population. Alternative historical hypotheses are discussed.

The investigation of premating mechanisms indicates that alone they are not sufficient to maintain the distinctness of the two morphs. The existence of postmating mechanisms is postulated to account for the lack of hybrid individuals.

Acknowledgements

This study stems from a visit I made to Drizzle Lake, the central research site of Dr. T.E. Reimchen, in August, 1979. I thank Dr. Reimchen for his encouragement then and throughout the last two years. His stimulating suggestions, challenging questions, and especially his time were offered freely while I was at Drizzle. I am also indebted to him for the loan of specimens and for permitting me to use his unpublished data.

To my supervisor, Dr. J.S. Nelson, I express my gratitude for his guidance and encouragement; for his interest in this project from the beginning, and particularly for his timely visit to the Charlottes in September, 1981. This manuscript has benefited from his patient review.

My appreciation is also extended to members of my supervisory committee, Dr. W.G. Evans and Dr. M.V.H. Wilson, for their helpful comments and suggestions. Discussions with fellow graduate students, particularly Pat Bowne, helped clarify many ideas. I thank Dr. Ruth Dubin for drawing the model-bottle experiments to my attention. Technical help from Pat Bowne, Gertrude Hutchinson, and Al Lindoe, and assistance with invertebrate identification from Vytenis Gotceitas was most appreciated.

To Sheila Douglas, I extend my thanks for valuable suggestions and for her assistance in identifying plant specimens at Drizzle. I apologize to Mesa and Clea for any inconvenience my presence may have caused.

My field assistants, Karen Romanyk and Hazel Speed, deserve great credit for their good spirits, tolerance, and willingness to work and live under what were occasionally less than ideal conditions.

I thank Barbara Mackay and Joanna Lubberts for their work typing the manuscript and preparing the tables.

Finally, I wish to add a very special thanks to my parents, Dr.and Mrs. R.H. Stinson, for their role in sparking my early interests and for the continuing encouragement I receive as I explore those interests.

Permission to work on the Drizzle Lake Ecological Reserve was granted by Dr. J. Bristol Foster, Director of the Ecological Reserves Unit, Ministry of Lands, Parks, and Housing in the B.C. Provincial Government. For his permission and for the loan of a tent for two summers, I extend my sincere thanks.

This study was conducted while I was the recipient of an NSERC Postgraduate Scholarship. Field research was supported for the most part by a federal government grant-in-aid from the Boreal Institute for Northern Studies at the University of Alberta, and by an NSERC grant to Dr. J.S. Nelson.

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I. INTRODUCTION

The processes responsible for speciation are of fundamental interest in understanding the reasons for the spectacular diversity of living organisms. Speciation is a slow process which cannot be studied in a human lifetime. There are, however, populations in which this process appears to be nearly complete or only recently completed. It is in these groups that the factors responsible for the development of reproductive isolation, the ultimate determinant of a completed speciation event, can be studied.

Threespine stickleback (*Gasterosteus aculeatus*), are nearly circumpolar with both freshwater and anadromous forms being widespread throughout the northern hemisphere (Scott and Crossman 1973). After Hagen (1967) and others, the freshwater form is referred to as *leiurus*, the anadromous form is called *trachurus*. *Leiurus* lives in freshwater streams and ponds throughout its life. It has few lateral plates and is typically olive to brown in colour and mottled (Hagen 1967). *Trachurus* lives in the sea for most of its life, migrating to freshwater to breed in the spring. Adults and young return to the sea in early autumn. *Trachurus* is distinguished from *leiurus* by a complete series of lateral plates and silvery colour (Munzing 1963).

Populations of the freshwater form are thought to have evolved independently numerous times from the anadromous form (Bell 1976 presents a model for this). Many of these freshwater forms typify the *leiurus* form but others are highly divergent in body shape, colour, relative spine length, and number of lateral plates (e.g. McPhail 1969; Moodie 1972a; Reimchen 1980). These different morphological types (morphs) of stickleback have apparently evolved at several localities on the northern Pacific coast of North America. In some cases the morphs are sympatric within a lake, or parapatric with one form occupying the lake and the other(s) living in inlet or outlet streams. Most of the detailed studies of these polymorphic populations have concentrated on differences between elements of a lake population (for example, Larson 1976; Reimchen 1980), or within a stream population (Bell and Richkind 1981; Bell 1982). Moodie (1972a) and to a lesser extent, Moodie and Reimchen (1976a), and Hagen and Gilbertson (1972), have compared fish from lake/stream pairs. These studies included watersheds on the Queen Charlotte Islands. It is in these populations, contiguous yet morphologically and ecologically distinct, that a study of reproductive isolation, or its potential, is particularly exciting.

Three morphs occur in the Drizzle Lake drainage on the Queen Charlotte Islands, British Columbia (Fig. 1) (T.E. Reimchen pers. comm. and personal observation). Each of these is restricted to one part of the watershed comprising Drizzle Lake, its only significant inlet, and its outlet stream, Drizzle Creek. This study included only the "lake" and "inlet" forms.

Adults of the two morphs are readily distinguished. Inlet sticklebacks resemble the typical *leiurus* form (see Hagen 1967 for description) and have a mean standard length in June of 47.85 mm (S.D.= 3.85, n = 82). The lake form is black above with silvery countershading and has a mean standard length in April of 77.93 mm (S.D.= 3.97; n = 100). Longer dorsal and pelvic spines relative to body length and a more terete shape also distinguish the lake form. These and other morphometric differences between the inlet and lake forms had previously been noted by T.E. Reimchen.

Long term observation of the lake morph in the vicinity of the inlet mouth by T.E. Reimchen and the trapping of hundreds of fish in the inlet for this study indicated that virtually no fish of an intermediate phenotype (presumed "hybrids") exists. (One specimen, considered by Dr. Reimchen to be an intermediate form, was caught in the lake near the inlet mouth in July, 1982.). If the morphological differences described here have a genetic basis, and differences in some of the same characters in other populations of *G. aculeatus* have been shown to have a high heritability (Heuts 1947a,b; Munzing 1963; Hagen 1967, 1973), then the lack of intermediates suggests that barriers to successful intermorph crosses are present.

These barriers, termed reproductive isolating mechanisms, isolate one population from another, preventing or restricting gene exchange between them. Mayr (1963) groups intrinsic reproductive isolating mechanisms into premating mechanisms (those that prevent interspecific crosses) and postmating mechanisms (those that reduce the full success of interspecific crosses). Postmating mechanisms (gametic and zygotic mortality, hybrid inviability, and hybrid sterility) were not studied in this project. Three premating mechanisms were considered, namely, temporal, ecological, and ethological isolation.

The purpose of this study was to determine if the lake and inlet populations of the Drizzle Lake system are reproductively isolated by identifying, through observation

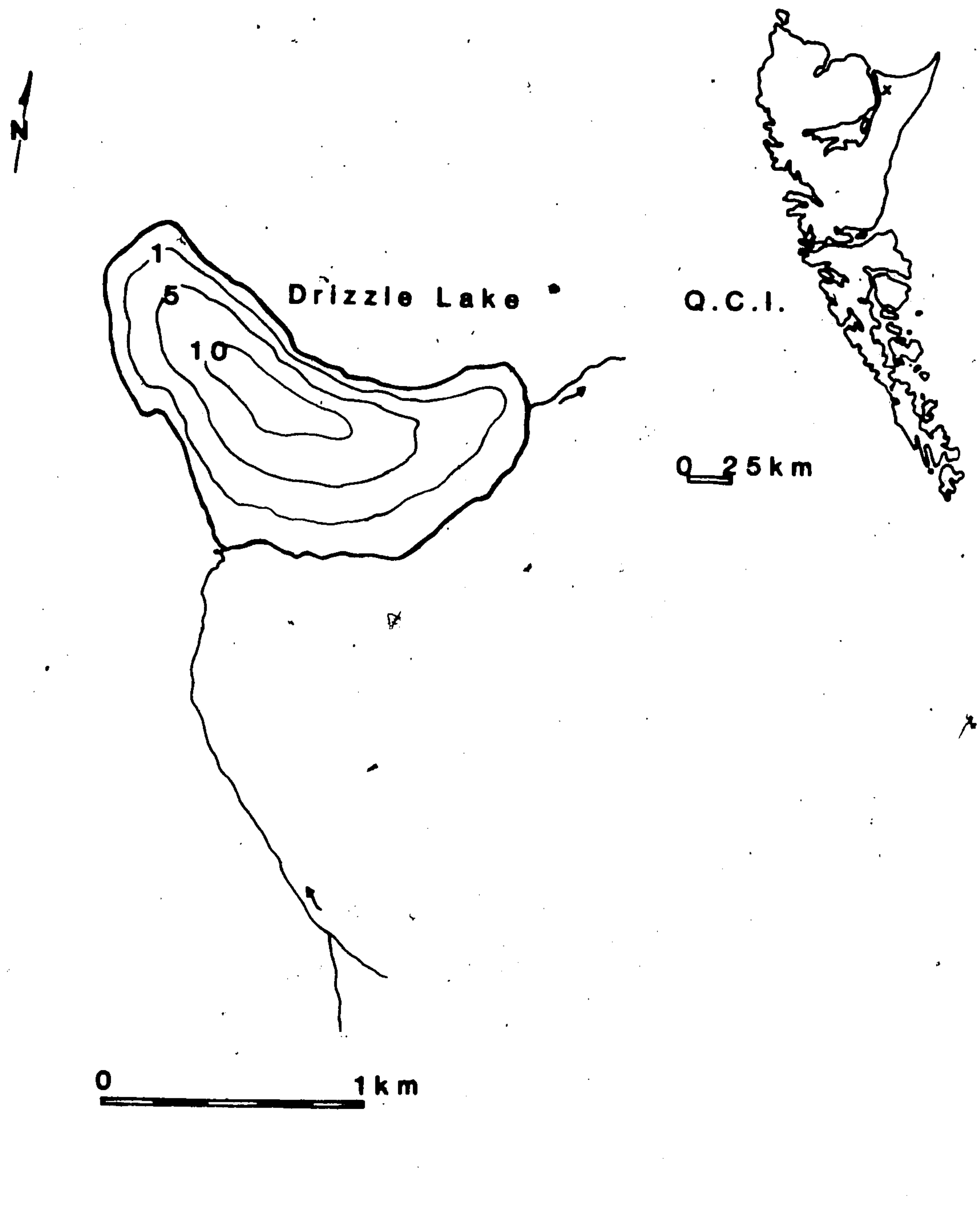


Figure 1. Drizzle Lake, Queen Charlotte Islands. Inlet and outlet streams indicated by arrows. Depth contours are in meters.

and experiment, elements in the life histories of both forms which could serve as isolating mechanisms.

The five lines of investigation which were taken to meet the above objective are as follows:

1. Field study of the inlet population to determine distribution and amount of movement within the stream and between stream and lake.
2. Morphological comparison of the two morphs which continued from the work of Dr. Reimchen and included characters not previously used.
3. Mate preference tests (lake males only) to establish if assortative (non-random) mating occurs.
4. Experimental testing of habitat preference to determine whether any of several variables tested alone serves to restrict the morphs to their respective habitats.
5. Analysis of food preferences of the two morphs.

II. THE STUDY AREA

Drizzle Lake (53 56'N, 132 05'W) lies in the northeast corner of Graham Island, the most northern of the two main islands of the Queen Charlottes (Fig. 1). The lake is approximately 1.75 km long and 0.75 km wide. It drains north to the Pacific through Drizzle Creek and two rivers, the Skonun and the Sangan. A broad littoral zone is found on the western, southern, and eastern sides while on the north side, the bottom drops quickly to a maximum depth of approximately 16m.

The major inlet flows about 4.5 km through a steep-sided channel and enters the lake on the south shore (Fig. 1). The width of the stream varies from less than I m in the upper-reaches to more than I2 m near the mouth. Water levels fluctuate greatly, reaching their maximum during the period of heaviest rainfall from September to December. However, periods of rain during the drier summer months can lead to sudden increases in depth and current, particularly in narrow stretches of the stream. Increases in depth of 30-45 cm within 48 hours were not uncommon in some areas in 1981. Maximum depths of 1.5-1.8 m are reached in the lower 150 m of the stream. During periods of low rainfall the stream is interrupted by moss or grass-covered bridges and in places (upstream) consists of a series of isolated pools.

Except for the lower 150 m and occasional stretches upstream, the inlet is bordered by a narrow band of coniferous forest (*Tsuga heterophylla, Thuja plicata,* and *Picea sitchensis*). Beyond this forested strip are broad, flat areas of muskeg in which *Sphagnum* spp., *Pinus contorta,* and members of the heath family (Ericaceae) are dominant. Runoff from the muskeg reaches the inlet through temporary runnels and subsurface drainage.

The bog habitat abuts the lower reaches of the inlet (below FF2, Fig.2) where the forested band is absent. In this region the stream is conspicuously broader, deeper, and warmer than in areas upstream. Temperatures in the inlet range from 8 - 16°C between early May and early August with those in the forested section typically I - 2°cooler than the temperatures in the nonforested stretch.

Inlet water is dark brown in colour for most of the year, becoming lighter in winters of heavy rainfall (T.E. Reimchen , pers. comm.). Consequently, depth of light penetration changes throughout the year. In midstream the substrate is firm and consists

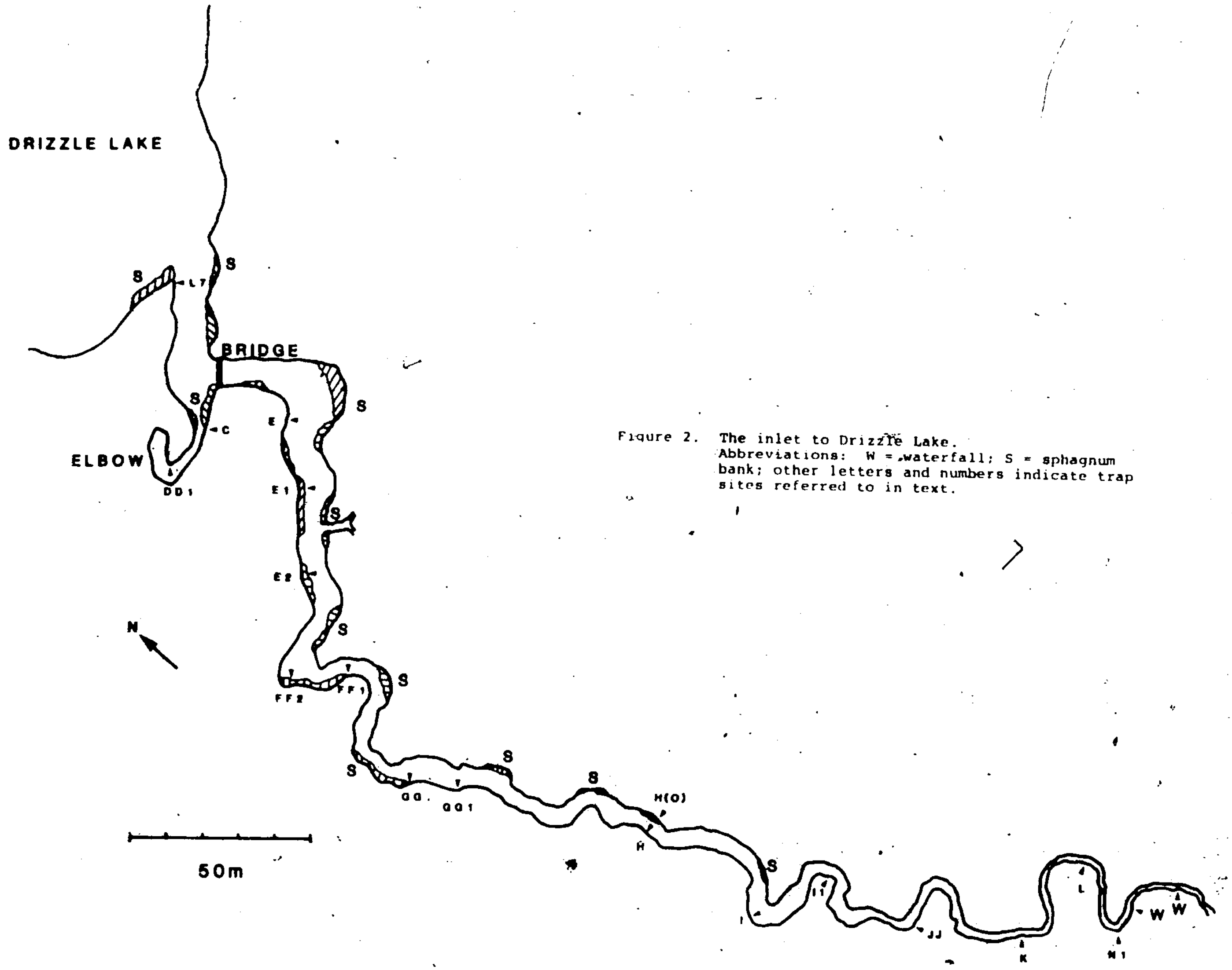


Figure 2. The inlet to Drizzle Lake.
Abbreviations: W = waterfall; S = sphagnum bank; other letters and numbers indicate trap sites referred to in text.

of coarse sand, but areas of soft organic mud are found in backwater areas (DD 1, Fig.2) and at the outside corners of the stream where current is reduced. Submerged logs are found along most of the forested length.

Aquatic vegetation in the stream is limited to occasional banks of *Sphagnum* in areas of low flow. In the open area downstream a filamentous alga, a liverwort (*Scapania* sp.), and a rush, *Juncus oregonensis* are found.

Beyond the inlet mouth an intermediate area extends along the eastern shore of the lake for approximately 50 m. It is bounded on the shore side by a continuation of the steep stream bank and on the other side by a submerged sandbar. Here, water from the inlet mixes with that of the lake and other variables are found in transitional states. At the stream mouth, the water depth decreases from 60 cm in the stream to 35 cm about 10 m beyond the mouth (mid July, 1981 depths). There is an increase in the density and variety of submerged vegetation probably due to the increase in the amount of light reaching the substrate. An unidentified filamentous alga, *Lilaeopsis occidentalis*, the yellow pond lily, (*Nuphar luteum*), and *Juncus* spp. are abundant. The substrate in this region of stream outflow consists of fine white sand overlain with a 3-4 cm layer of dark coloured detritus. Submerged moss-covered logs are common.

The littoral zone in the lake is of variable width. Substrates include organic ooze, fine sand, sand-gravel combinations (often firmly compacted), cobbles with and without sand, and large rocks. Except for the area at the inlet mouth, vegetation is generally sparse and consists primarily of an aquatic liverwort and *Lilaeopsis occidentalis*, *Eleocharis* spp., *Juncus* spp., with *Nuphar luteum* in protected bays.

Temperatures in the littoral zone taken at a depth of 1 m range from 12.5 - 19°C between mid-May and early August. The average differences between these and inlet temperatures during that period were 4.1°C (1981) and 4.3°C (1982).

Water colour in the lake is a deep brown but is lighter than that of the inlet and consequently light penetrates farther.

Table I summarizes major differences in the physical and biotic components of the two habitats. A detailed chemical analysis of water was not made.

The study area was visited from mid-April to mid-October, 1981 and early May to August 1, 1982.

Table I

A Comparison of Lake and Inlet Habitats During the Breeding Season (May -August), 1982

	Lake	Inlet
Physical		
Temp (°C)	16.98(11.0-19.5)	10.95(5.17-15.3)
pH	4.71(4.55-4.85)	5.66(4.45-6.65)
Water colour	medium tea colour	dark tea colour
Transparency (Secchi disc-cm)	133.07(117.5-142.0)	60.5(40.5-80.5)
Bottom	fine sand, hard-packed sand/gravel	coarse sand, ooze along banks and in backwater
Biotic		
Vegetation	*Juncus* sp., Unidentified, filamentous alga, *Scapania* sp., *Lilaeopsis occidentalis*, *Eleocharis* sp., *Nuphar luteum*, *Sphagnum* spp.	*Sphagnum* spp., *Juncus* sp., Unidentified, filamentous alga,
Vertebrates Fish	*Gasterosteus aculeatus*, *Salmo clarki*, *Salvelinus malma*, *Oncorhynchus kisutch*	*G. aculeatus*, *O. kisutch*, *S. malma*, *S. clarki*
Amphibians	*Bufo boreas*, *Hyla versicolor*	*B. boreas*, *H. versicolor*
Invertebrates Benthic	Chironomidae, larvae of Trichoptera, Lumbriculidae, nymphs of Odonata	Gammaridae, Chironomidae, Mollusca (*Pisidium*), Lumbriculidae, nymphs of Odonata (Aeshnidae, Libellulidae, and Zygopteran), larvae of Trichoptera and other Diptera
Planktonic	Cladocera (Bosminidae, Chydoridae), Copepoda (*Cyclops*), Hydracarina	Rotifers, Chironomidae, Cladocera (Chydoridae, Bosminidae), Copepoda (*Polyphemus, Cyclops*), copepod nauplii, *Chaoborus*
Plankton density (approx.)	3900/m^3	54/m^3

Notes: -Physical characteristics are average conditions with range in brackets.

-Floral and faunal composition listed in order of abundance within each category.

-Area of the lake considered is that bordering the inlet mouth and extending for 125 m. along lakeshore.

III. THE POPULATION

A. METHODS

Knowledge of the distribution, size, and amount of movement of the groups involved is critical in a study of isolating mechanisms to indicate the degree of physical segregation between the morphs. A long term study of the lake population by Dr. T.E. Reimchen is in progress ; therefore, the focus of this work was on the inlet form.

Distribution

Baited minnow traps were set in groups of ten in successive 250 m sections of the stream and left for periods of 5-14 days (length of time increased with distance upstream). The inlet was surveyed to a point approximately 4.5 km upstream where it was underground for short stretches and the water depth was less than 5 cm. The stream could not be seen on aerial photographs beyond this point.

Traps were set downstream from the waterfalls (Fig.2) to sample a variety of habitats. Those set above the falls were placed at sites judged to be close to acceptable stickleback habitat based on successful trap sites downstream. Successful traps (those which caught sticklebacks) were left in place for the remainder of the 1981 season. Additional traps were set on the other side of the stream and all were involved in more detailed aspects of this study.

To determine distribution of sticklebacks relative to the width of the stream, five traps were suspended from a rope stretched across the stream. The traps, numbered 1 to 5, were arranged at each setting so that traps 1 and 5 were within 1 m of the banks and at the surface, trap 3 was midstream and 50 -75 cm from the surface, and traps 2 and 4 were in intermediate positions and 75-100 cm below the surface. Occasionally, traps 2,3, and 4 rested on the bottom. The rope was moved 10 m upstream until the bend at FF2 (Fig.2) was reached. Submerged logs and the progressive narrowing of the stream made it impractical to continue upstream and the rope was returned to the mouth 10 m at a time. Thirty-six trap sets were made.

Size

I assumed that the sticklebacks found from the mouth to the first waterfall in the inlet (450 m upstream) comprised one panmictic population and the estimate of population size was based on this assumption.

In May, 1981 adult fish (entering their third summer) were caught in traps placed in the inhabited portion of the inlet. Approximately 2 mm was clipped from the tip of the first dorsal spine. This method was chosen for its simplicity, especially under adverse conditions, and for its apparent lack of effect on the health of the fish as shown by the survival and unimpaired swimming of clipped fish held in an aquarium. Recaptured fish were clipped on the second dorsal spine. The few double recaptures were clipped on the upper lobe of the caudal fin.

A second attempt to estimate the size of a single age class was made in September, 1981 when a brief increase in numbers of fish in the traps (Fig.3) and an earlier marking study indicated that there was considerable movement of fish in the stream, thus validating one of the assumptions of capture-recapture models. Small squares of orange elastic band were placed on the second dorsal spine of subadult fish (in their second autumn). As noted below, however, this is not an ideal marking technique.

The weighted mean variation of the Peterson index described by Begon (1979) (also referred to as the Schnabel method in Smith (1968)) was used to calculate both the summer and fall estimates. This model has the same assumptions as the Peterson index but it accumulates data over several days, an advantage when only a small number of captures per day are possible.

Movement

The study of movement of adult fish within the stream was carried out in May and July, 1981. Fish were marked with 2 mm pieces of light-coloured elastic band slipped over one of the dorsal spines. Those caught, marked, and released on one side of the stream were banded on the first dorsal fish caught on the other side were banded on the second.

Bands were cut in four shapes (○,□,△,⌓), each representing a naturally delimited section of the stream. Within each section, site specificity was indicated by a variety of waterproof ink marks on the band. For example, rectangular bands were used for the "E" traps in the non-forested lower section. These were marked ◨,▣,⧅ for sites E, E1 and E2 respectively. Thus, when fish were recaptured, a minimum estimate of the distance travelled from their point of release could be made. Twenty-nine fish were

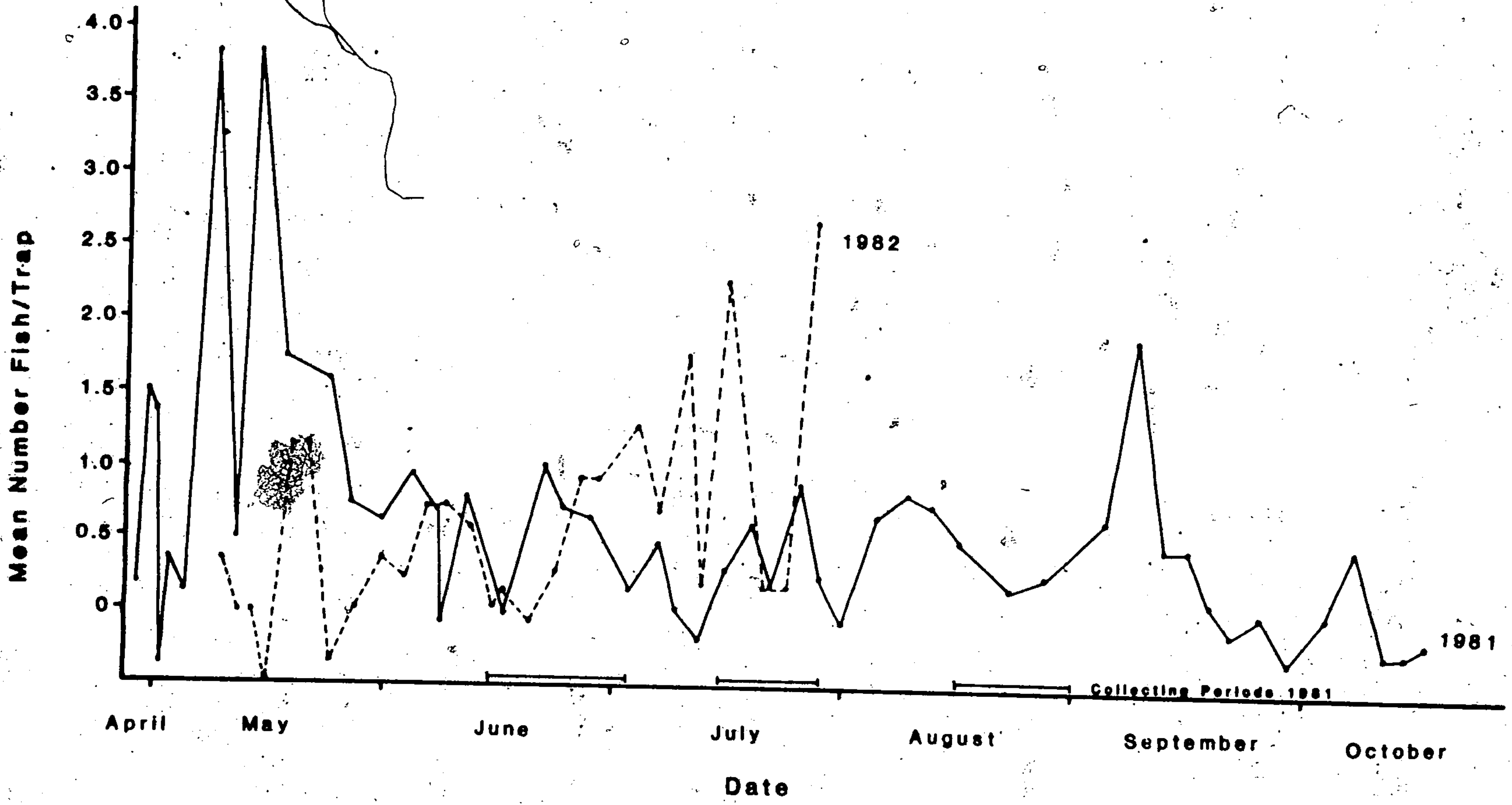


Figure 3. Variation in mean number of fish per trap in 1981 and 1982. Specimens for morphological analysis were collected during three two-week periods in 1981.

banded on May 18 and recaptures were made from May 24 to June 25. On July 12 and 15 an additional 19 fish were banded with recaptures made from July 15 to August 5. Bands were removed on first recapture. All fish were of the same age class and the banding and recapture periods were within the breeding season. I assumed movement patterns were the same throughout that period and the data were pooled.

The banding technique led to the softening and ultimate loss of the affected spine. Accompanying this there was often tissue damage to the musculature surrounding the dorsal plate at the base of the spine. Healing of this area was observed in many fish after the band and spine were removed. In addition to causing injury, the light coloured bands were visible in the dark water and may have increased the liklihood of predation. These sources of mortality should not have influenced the movement of the fish except that perhaps fewer recaptures could be expected.

In August, 1981, a second mark-recapture program was completed to evaluate the amount and range of movement of individuals in the subadult age class. In late June, members of this cohort were large enough to be trapped (28-30 mm S.L.). By August, with a standard length of 35-40 mm, they made up the largest age class in the traps. Fifty-four fish were marked on August 1,5,9, and 12 and recaptures were made from August 9 to October 7. Fish were marked with different patterns of dorsal spine and caudal fin clips, a different combination for each site or adjacent pair of sites. Regrowth of clipped tissue was not fast enough to interfere with mark recognition. Until September 1, recaptured fish (as well as unmarked fish) were removed from the population for morphological analysis. Marked fish captured from September 5 to October 7 were released and none were recaptured.

From July 16 to September 1 four traps were set across the stream mouth approximately 1 m apart in 0.5 m of water to monitor movement of fish in that area. Also, beginning July 9, traps were set along the east shore of the lake for about 100 m from the inlet mouth. This area was checked because the steep bank is an extension of the stream bank and I thought it was the most likely route to be followed by inlet fish leaving the stream.

The presence of inlet fish in the lake beyond the stream mouth was detected in a transect of 13 traps set at various depths from the shore to a point about 300 m out in the bay near the mouth. This transect is set on a continuing basis for a two day period every six weeks from March to October by T.E. Reimchen.

Individuals of the lake morph were noted when caught in inlet traps. Special attention was given to adults in breeding condition.

Movement of lake fish within the lake was not considered.

Feeding Habits

The determination of selective feeding by animals requires information on the ratio of food items being consumed and the ratio of the same group of food items in the habitat of the animal (Ivlev 1961). Stomach contents analysis was used to identify what was being ingested. Two groups of inlet fish were used. One sample of 42 fish representing both sexes, two age classes, and taken from the length of the inhabited portion of the stream was drawn from fish collected for morphological analysis. Fish in this group had been in traps for up to 3 days when collected. The second group comprised 7 fish collected incidentally in the summer of 1981 when dipnetting for invertebrates, and 49 fish seined from the inlet in July, 1981. All inlet fish were placed in 10% formalin within 1 hour of their removal from the stream.

Stomach contents of 29 lake fish were also examined. These fish had been in bottom traps for up to 2 days before being collected and were then held for about 6 hours before being placed in formalin.

The procedure for content analysis was the same for all groups. The stomach was removed, opened and flushed thoroughly with water to empty it. Under a dissecting microscope food items were separated and identified to order and family when possible. Counts of all items were made except when numerous Cladocera were present in which case their number was estimated.

In a thorough, comparative investigation of methods used in studying foods of fishes, Hynes (1950) found that all methods (points, occurrence, dominance, etc.) are likely to give "substantially the same result". I used the occurrence method in this study because of its simplicity. By this method, the number of stomachs in which a food type occurred is expressed as a percentage of the total number of stomachs examined (Hynes 1950).

The food habits of salmonids in the stream were examined in fish collected mostly by gill nets and traps. Gill nets of various mesh sizes (2.5 cm, 5.0 cm, 10.0 cm) were set periodically in the inlet just above the bridge and checked every four to six hours. Stomachs were opened within 30 minutes of removing the fish from the nets, and food types recorded. Numbers of each food item were not counted. Four young coho were also collected during the seining for sticklebacks.

For one week in July, 1981, salmonids were collected daily from traps set between the two waterfalls where sticklebacks are not found. By leaving the fish in the trap for at most one day I hoped to minimize the problem of the stomach contents reflecting only what had been eaten since the fish entered the trap.

Only rough estimates of planktonic and benthic invertebrate densities were sought. It was difficult to collect perfectly comparable samples of benthic organisms as the lake and stream plant communities and substrates are different. I sampled those elements of the two habitats which I expected might shelter high densities of invertebrates on the grounds that these would probably be sources used by the fish. The *Sphagnum* banks and the substrate were sampled in the inlet. Samples of substrate were collected from the littoral zone of the lake.

Two 2 litre samples of *Sphagnum* were taken from a large mass which had been quickly rolled up onto the stream bank. Invertebrates were counted on sight. This technique probably greatly underestimates species that are difficult to see because of small size or cryptic colouring (e.g. chironomid larvae). Samples of substrate in the stream were collected with a dip net (for coarse sand) and an Eckman dredge (for ooze). Subsamples of 20 ml were run through a series of seives (Canada Standard Seive Series #20, #40, #200) to isolate small specimens. The remainder of the sample was screened by a larger mesh (2 mm) for larger organisms.

In the lake, 400 ml samples of sand and ooze were scooped in a jar during transect surveys. These were analyzed in the manner described for inlet substrate samples.

Most densities were calculated as mean number per litre of substrate or *Sphagnum*. Trichopteran and odonate larval densities were estimated per metre square because in the lake they are primarily bottom surface-dwellers and thus occupy in effect, a two dimensional space.

Plankton tows were made in both habitats on three successive days in July, 1982. A net with mouth diameter of 26.3 cm sampled 10 m of water in each horizontal tow. Three tows were made in the lake near a nesting area of lake sticklebacks (depth 1 m). Tows in the inlet were just upstream from the bridge and in the area of FF1–FF2 (Fig.2). Zooplankters (other than rotifers) were identified to family or genus under a dissecting microscope. Total counts were made of inlet samples. Lake collections were first scanned for species composition and then subsampled for counting.

B. RESULTS

Distribution

No sticklebacks were caught above the lower of two small waterfalls (Fig.2) in the series of trap settings between May 3 and July 16, 1981. From July 16 to October 16 1981 and throughout the summer of 1982 one or two traps were set above the falls and again no sticklebacks were found.

Below the falls, population numbers peak at between 195 and 220 m upstream from the mouth (Fig 4). Numbers of males and females differ ($\chi^2 = 15.63$, $P < .001$) above and below this section. More females were caught downstream (171 to 89 males) and more males upstream (118 to 107 females) (Fig.5). More subadult fish were found downstream of the peak area than upstream (208 to 308, $\chi^2 = 14.16$, $P < .001$).

Traps were set in a variety of habitats in the inhabited section of the stream. Few fish were caught at sites with a swift current or where the water depth fluctuated widely in a short period of time. Table II shows the effect of adjacent patches of emergent *Sphagnum* and the presence of an overhanging stream bank on the mean number of fish per trap setting. Even in the broadest sections of the stream, however, the effects of these variables will vary with changing water depth.

Numbers of fish caught in the five traps suspended across the stream peaked in July and declined in the early fall. Mean numbers of fish per trapsetting in each month were: June 1.50 July 5.22 August 3.14, September–2.22, October–1.60. The traps were moved between successive settings so the above differences may reflect a seasonal or spatial pattern or a combination of the two. Fewer are found midstream than

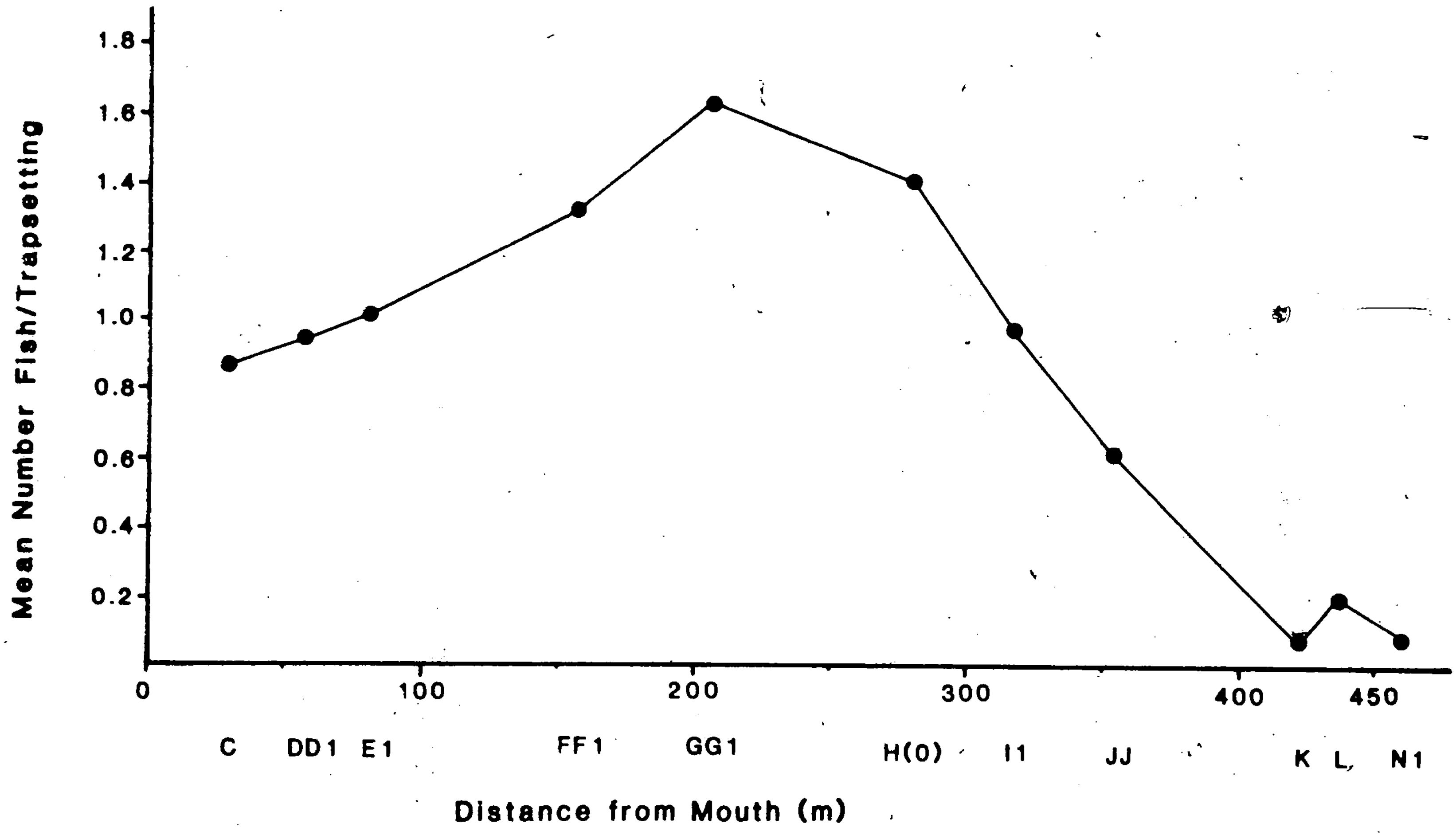


Figure 4. Relationship between mean number fish per trap setting and distance upstream. Letters on horizontal axis refer to nearest trap sites. Values for groups of adjacent sites are combined.

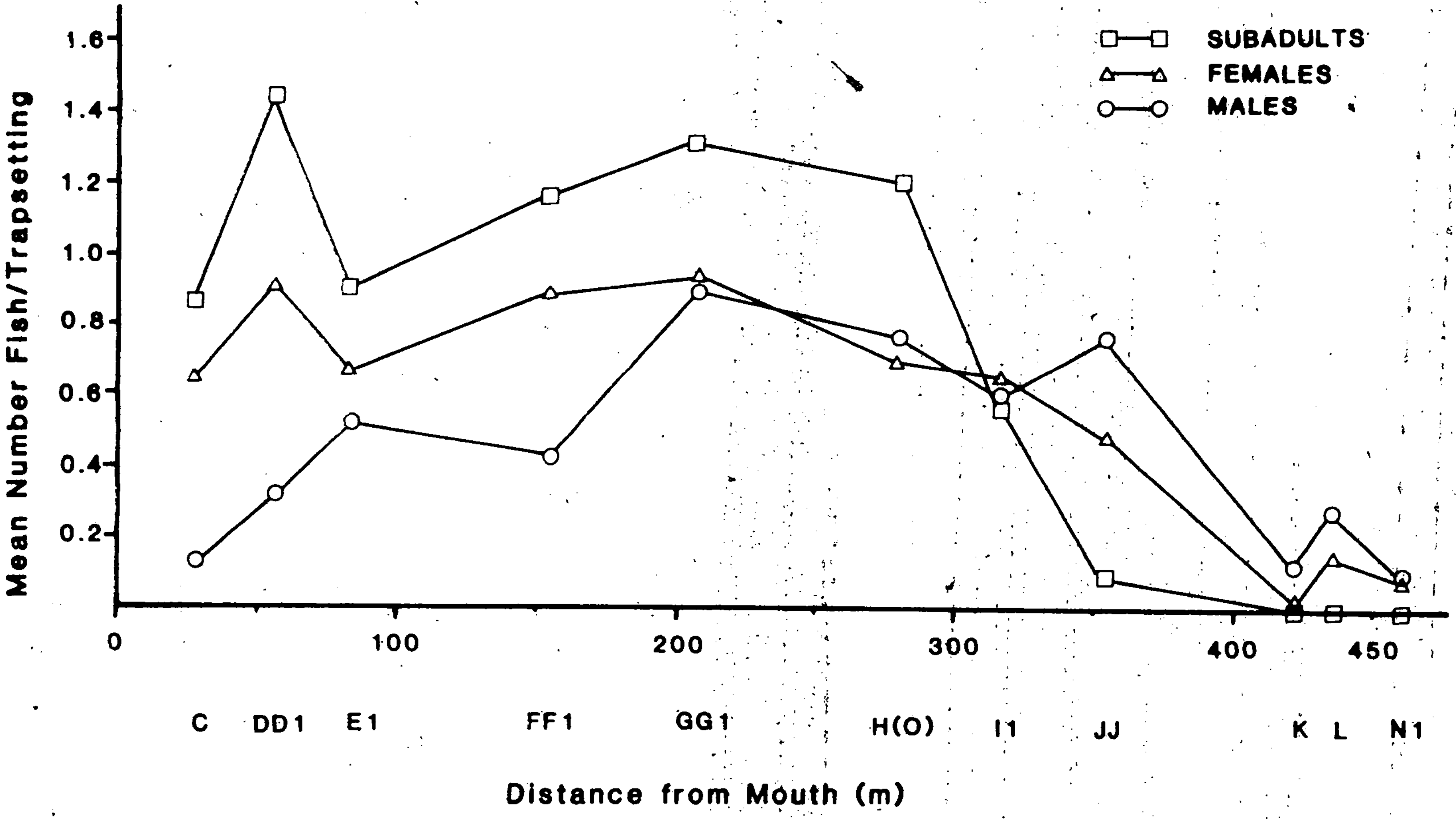


Figure 5. Variation in mean numbers of males, females, and subadults per trap setting (1981) with distance from mouth.

Table II

Mean Number of Fish Per Trap Setting in Relation to Adjacent Vegetation and Streambank Character

	Vegetation		Streambank Character	
	Sphagnum	No *Sphagnum*	Overhang	No Overhang
Number of sites	10	12	8	14
Mean no.fish/trapsetting	1.367	0.843[a]	0.916	1.236[b]

Notes: Traps were in place for at least 30 settings in 1981.

[a] Difference significant, $P < .05$ (Mann-Whitney $U_{(2)}10,12 = 93$).

[b] Difference not significant, $P > .02$ ($T_{(2)}.20 = 1.2032$).

Table III

Estimate of Population Size of Two Age Classes of Inlet Sticklebacks by the Weighted Mean Method

$$(\hat{N} = \frac{\Sigma M_i n_i}{(\Sigma m_i)+1})$$

Date	No. Trap Sets After First Marking	No. Days After 1st Marking	ΣM_i	n_i	$\Sigma M_i n_i$	Σm_i	$\hat{N}$	$P = .95$
May 14 (Adult)	7	13	144	84	7075	32	214	± 75.39
Oct. 14 (Subadult)	8	28	157	107	7049	35	227	± 41.97

Notes: $\hat{N}$ = size estimate, M_i = no. trapped, n_i = no. marked fish in the area, m_i = no. recaptures.

closer to the banks (Fig.6) in the six month period

Size

Table III gives estimates of population size for each of the two age classes which could potentially be caught in minnow traps. The weighted mean method of making such estimates makes several assumptions which are also implicit in the Peterson index (Begon 1979). One of these, that marking does not affect probability of survival, may have been violated in estimating the subadult cohort size. If, as described earlier, the banding technique does lower the probability of survival there would be fewer marked fish than expected in the area (smaller n_i, Table III), and the population size would be overestimated. However, as estimated, the size of this age class is not significantly greater than the estimate for the adult cohort (Table III), and it seems unlikely that there could be fewer subadults than adults if overwinter mortality is considered.

Another assumption, that all individuals have the same chance of being caught, may not have been met in the estimate of adult population size. The estimate was based on data collected during the breeding season of a species well–known for its territorial males and it is possible that not all fish were equally likely to be caught. If traps were placed within territories those males would have been more susceptible to capture and recapture than other males and most females. If this assumption is violated the result is an underestimate of the true population size (Begon 1979). The size of the adult cohort given in Table III may be an underestimate, but again if overwinter mortality is considered it is unlikely that the adult cohort would be larger in number than the subadult group. It is possible that there was a marked difference in year–class strength of the two cohorts

These estimates yield densities of 0.24 adults and 0.25 subadults per linear metre of stream bank (about 900 m including both sides) or about one adult fish for every 4 m of stream bank. This is close to that considered average for other stickleback populations (T.E. Reimchen, pers. comm.) suggesting that the size estimates are reasonable

Movement

Differences in amount of movement between the two time periods (Table IV) are explained by the increased number of "movers" in the fall (80% of recaptures) compared to those in the early summer (40% of recaptures) rather than by fish moving greater

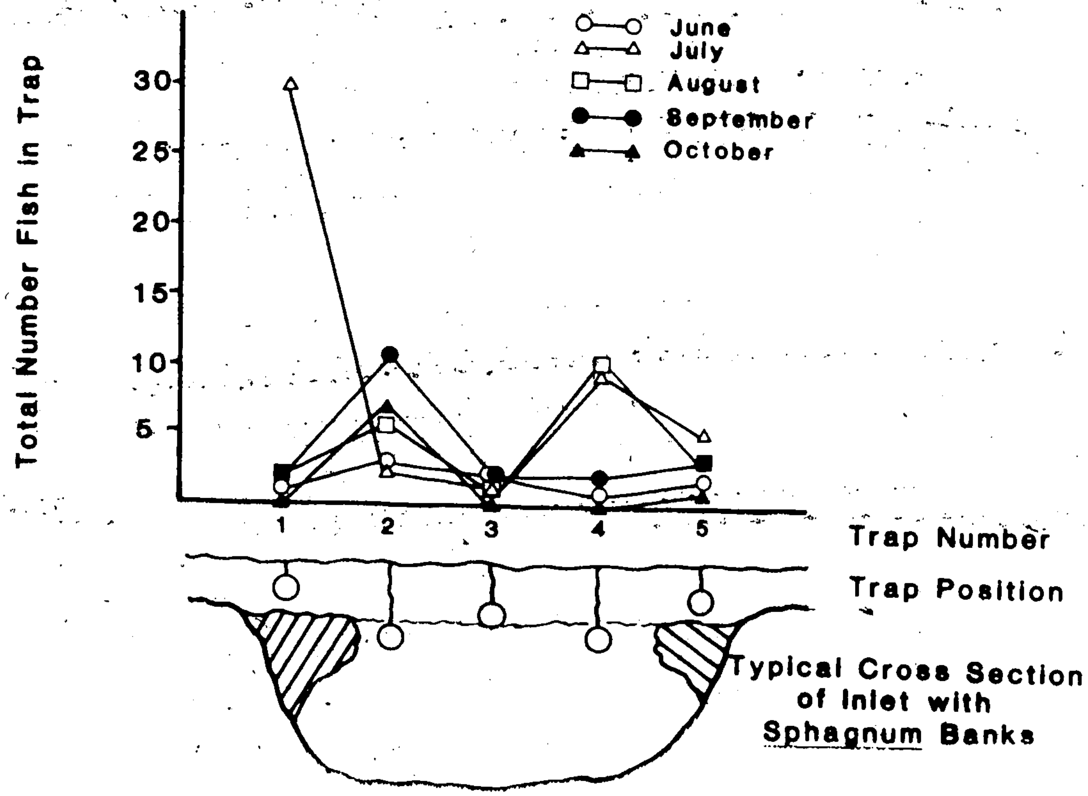


Figure 6. Number of fish caught per month across stream width (1981). Traps 1, 2, 3, 4 were set 36 times, trap 5 was set 33 times.

Table IV

Comparison of Movement of Two Age Classes of Inlet Sticklebacks in 1981

Month	Age Class	No. Banded	No. Recaps(%)	No. Not Moving(%)	No. Moving(%)			Mean Dist. (All Fish)(S.D.)	Mean Dist. (Movers)(S.D.)
					≤50 m	51-99 m	≥100 m		
May & July	Adult	48	30(62.5)	18(60.0)	8(26.7)	3(10.0)	1(3.3)	17.4(31.2)	43.5(36.5)
Aug. & Sept.	Sub Adult	54	30(55.6)	6(20.0)	13(43.3)	6(20.0)	5(16.7)	52.9(54.6)	58.6(49.07)

Notes: -Mean distance travelled by all fish in the two periods are different ($P < .01$, Normal approximation to Mann Whitney, $Z = -2.7868$).
-Mean distances of movers are not significantly different ($P > 0.2$).

Table V

Numbers of Inlet Fish Trapped From Stream Mouth Along Southeast Lakeshore

	Distance From Mouth (Meters)			
1981	0-6	10-15	18-30	75-110
July	16	2	0	0
August	3	0	0	0
September	11	4	6	0
October	0	0	0	0
1982				
May	1	2	0	0
June	11	4	0	0
July	2	3	0	0

distances later in the year. Other indications of increased movement at that time are in the increased mean number of fish per trap in 1981 (Fig.3) and the more than ten-fold increase from summer to fall in the percentage of fish crossing the stream between successive captures (3.3% in May-July, 36.7% in August-September). Also, only 13.3% of fish recaptured in early summer were recovered upstream of their point of release (mean distance moved upstream = 42.0 m). In the early fall, 50% were recaptured upstream at distances of up to 210 m from their release point (mean distance moved upstream = 74.1 m). Downstream movement in the majority of the first group could be attributed to passive drifting, but upstream movement always requires an expenditure of energy by the fish and is a more valid indicator of activity.

Higher numbers of inlet fish were also seen in the lakeshore traps in early fall (1981) and there was an increase in the distance travelled along the lakeshore from the inlet mouth (Table V).

The four traps set across the stream just beyond the mouth yielded eight inlet fish and four lake fish in the six weeks they were set (July 19-September 1, 1981). This is equivalent to 0.18 fish per trapsetting. A corresponding value for the traps set along the bank in the same area (within 15 m of the mouth) at the same time (July-August) was 0.25 fish per trapsetting.

The appearance of inlet fish elsewhere in the lake is a rare event and they have been found only in the bay into which the stream flows. Three males, in breeding coloration, were caught in May, 1981 at depths of 2 and 3.5 m approximately 200 m from the mouth. That only three inlet fish have been taken in over 150 trap settings (yielding approximately 750 sticklebacks) in over two years (T.E. Reimchen, pers. comm.) attests to their restriction to the stream.

Numbers of the lake morph in breeding condition caught in the inlet were approximately the same in the two years (Table VI). In 1981, all were trapped in the area known as the "Elbow" (Fig.2), a region characterized by its relative shallowness (30 cm), warmth and lack of current. Two of these fish were males. In 1982 only one adult, a male, was caught in the "Elbow", three were trapped at the mouth (site L7, Fig.2), two at E, 70 m upstream, and one at site I, about 300 m upstream.

Table VI

Numbers of Individuals of the Lake Morph Trapped in the Inlet During the Breeding Season, May - July

	Adults (Breeding Condition)		Subadults (Non Breeding)	
	1981	1982	1981	1982
May	5	2	0	11
June	1	2	0	68
July	0	3	9	52
Total	6	7	9	131

The difference in the numbers of subadults of the lake morph caught in the inlet in the two years (Table VI) is striking. These are fish in the 35–65 mm length class with the majority (87%) less than 50 mm. In 1981 none of the 9 was caught above the bridge (Fig.2). However, in 1982, 47 subadults (36%) were caught upstream from the bridge and several of these were taken more than 250 m upstream. Though not of the same importance now in the question of reproductive isolation as the adults, the invasion of the inlet by such high numbers may be significant in one year's time when this cohort reaches reproductive age (assuming they remain in the inlet).

Feeding Habits

Figure 7 compares the frequency of occurrence of several classes of food items found in the stomachs of the trapped inlets, the seined (or dip-netted) inlets, and the trapped lake fish.

The same food types (Diptera (chironomid larvae) and cladocerans) dominate the two inlet groups. Differences in absolute frequency between trapped and seined fish may be the result of digestion as every identifiable food type was found more often in the seined group than in those from traps. A higher frequency of unidentified material in stomachs of the trapped inlet fish may also be an outcome of digestion.

Over 40% of the stomachs of the lake fish examined were empty (Fig.7c). Cladocerans dominate the diet occurring in twice as many stomachs as the next most frequent food item, chironomid larvae.

Composition of diets of salmonids occurring sympatrically with sticklebacks is shown in Figure 8. A single different food type is prominent in diets of each of the three fish. Juvenile coho (*Oncorhynchus kisutch*) rely most heavily on Hymenoptera of terrestrial origin. Flying ants were common in the vegetation bordering the inlet and were frequently observed on the surface of the water. Chironomid larvae and pupae made up the second largest identified group but these were found in fewer than half as many stomachs as the ants. More than half the stomachs of Dolly Varden char (*Salvelinus malma*) were empty when examined. However, all other stomachs of this fish contained trichopteran larvae, often with their cases intact. Fish dominate the diet of cutthroat trout (*Salmo clarki*). Young salmonids (probably coho) occurred in 7 stomachs while two sticklebacks were found in one stomach. A tendency for cutthroat to consume relatively

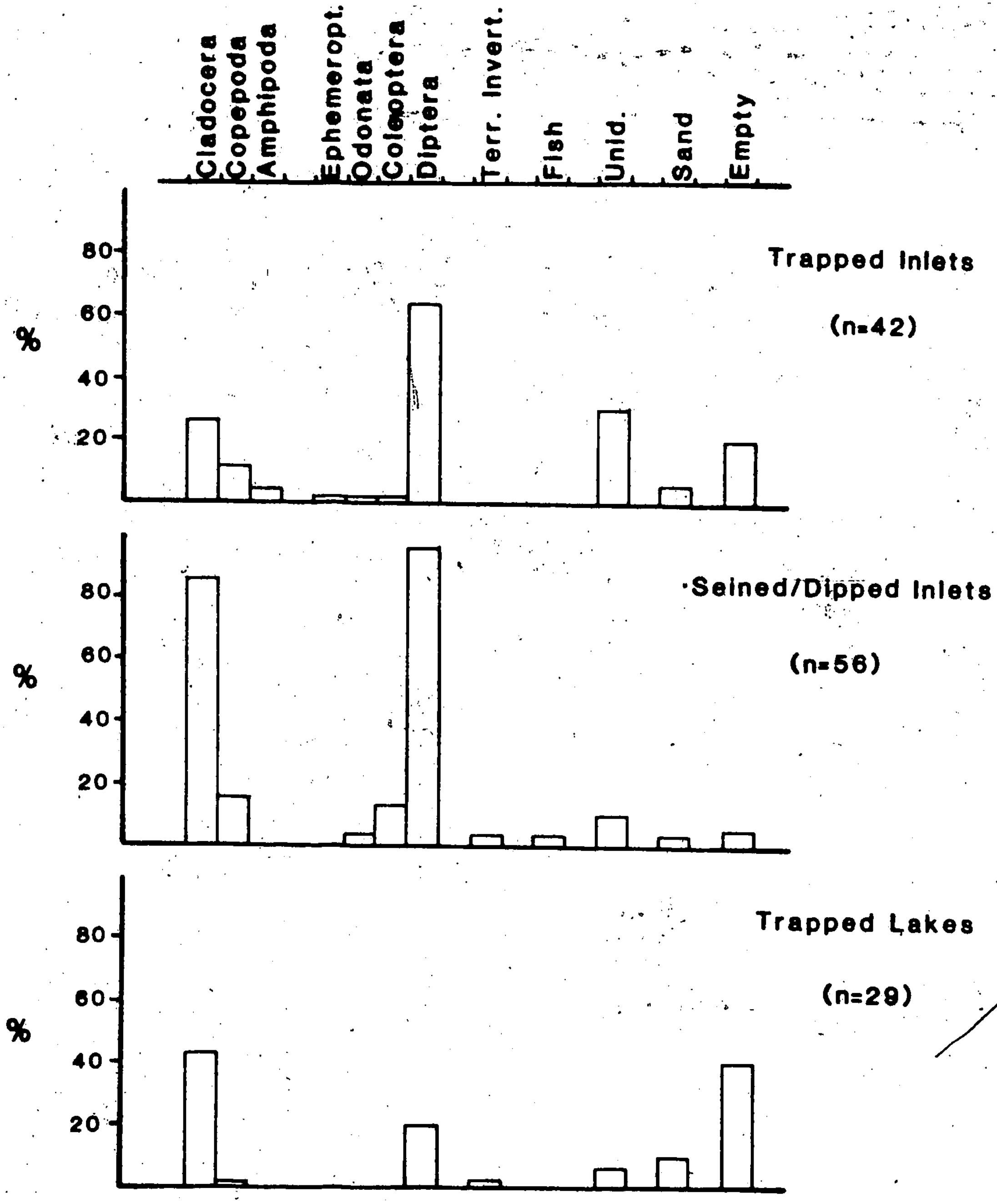


Figure 7. Percent occurrence of food items in stomach contents of three groups of sticklebacks..

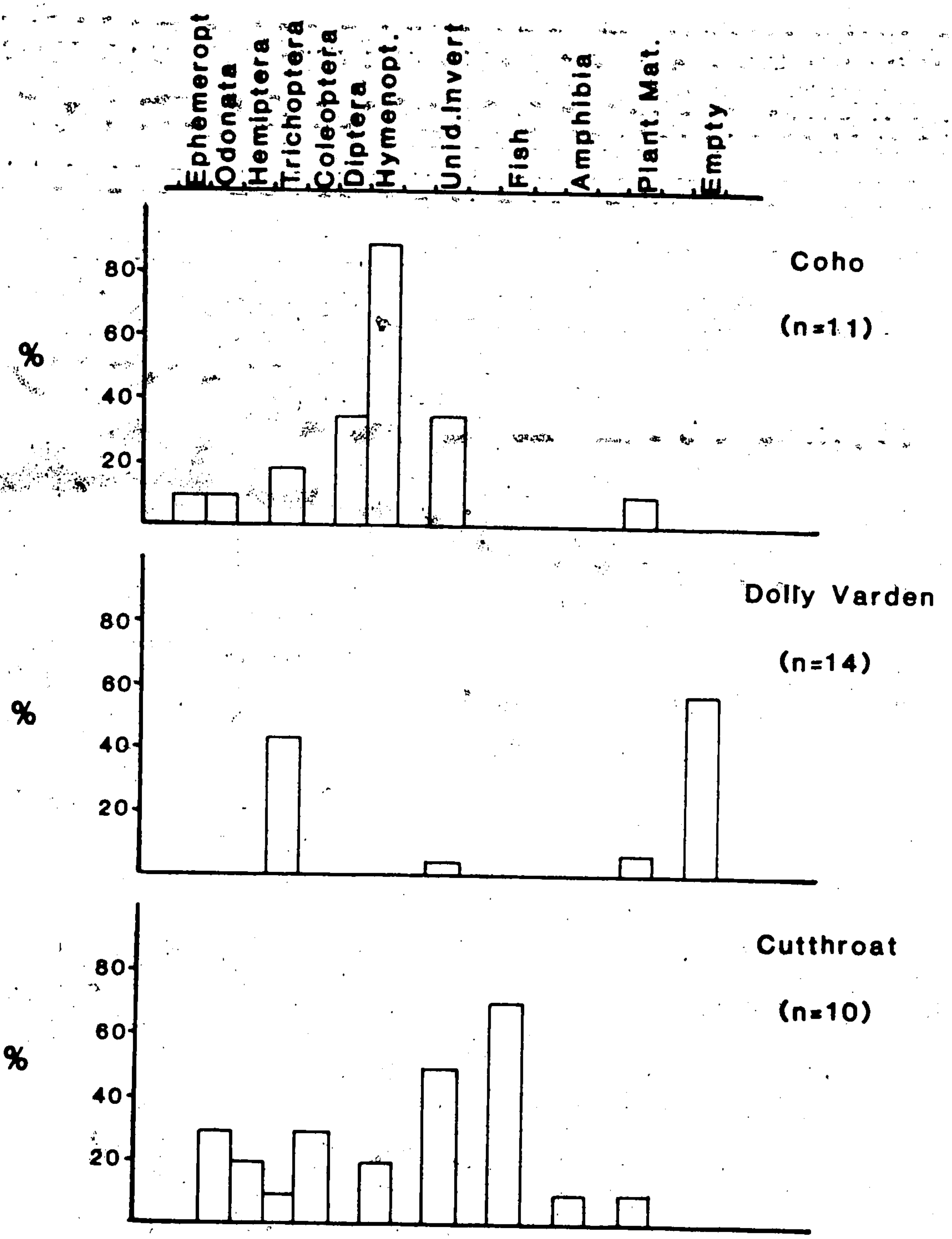


Figure 8. Percent occurrence of food items in stomach contents of salmonids living sympatrically with inlet sticklebacks.

large prey items (odonate nymphs, fish, toads) is apparent in Fig.8 but all stomachs examined were from fish larger than all other salmonids caught.

The rations of coho and Dolly Varden found sympatrically with sticklebacks (Fig.8) can be compared with those of fish caught above the waterfalls where stickleback are not found (Fig. 9). Coho in this upstream region continue to take Hymenoptera but more stomachs contained chironomid larvae than flying ants. Trichopteran larvae were found in half the Dolly Varden stomachs above the falls (though only 4 were examined) but amphipods occurred with equal frequency. Chironomid larvae were found in char above the falls though not in those caught downstream.

Table VII gives estimated densities of those groups of invertebrates collected during the sampling period. It is not an exhaustive list. For example, trichopteran larvae and adults of the family Dytiscidae (Coleoptera) were frequently found in the traps, and are thought to be common in the stream, yet neither appears in the inlet list. Hynes (1950) combined a numerical sampling technique with a full year's collecting to derive a list of about 30 organisms or groups. The two methods were used to overcome the difficulties inherent in short term sampling of these populations.

Chironomid densities are likely underestimated particularly in the inlet where they were difficult to see in the *Sphagnum*. The *Aeshna* density in the lake is probably lower than estimated as 1.5/m^2 is based on one sighting during the sampling period. No *Aeshna* were seen in other snorkelling activities (although they do frequent the shorelines).

A greater variety of planktonic organisms was found in the inlet samples than in those from the lake, but overall density of zooplankton in the lake is much greater. Organisms collected in the inlet tows (with % frequency) included chironomid larvae (50%), cladocerans (24%), the copepod *Polyphemus* (16%), copepod nauplii (6%), and *Cyclops, Chaoborus,* and terrestrial invertebrates all at 1%. Lake tows yielded the cladoceran *Bosmina* (58.4%), *Cyclops* (22%), other cladocerans (16%), water mites (2%), and chironomid larvae (1.5%). Rotifer densities were not estimated. When total zooplankton densities were estimated (excluding rotifers), values of approximately 55/m^3 for the inlet and 3900/m^3 for the lake were obtained.

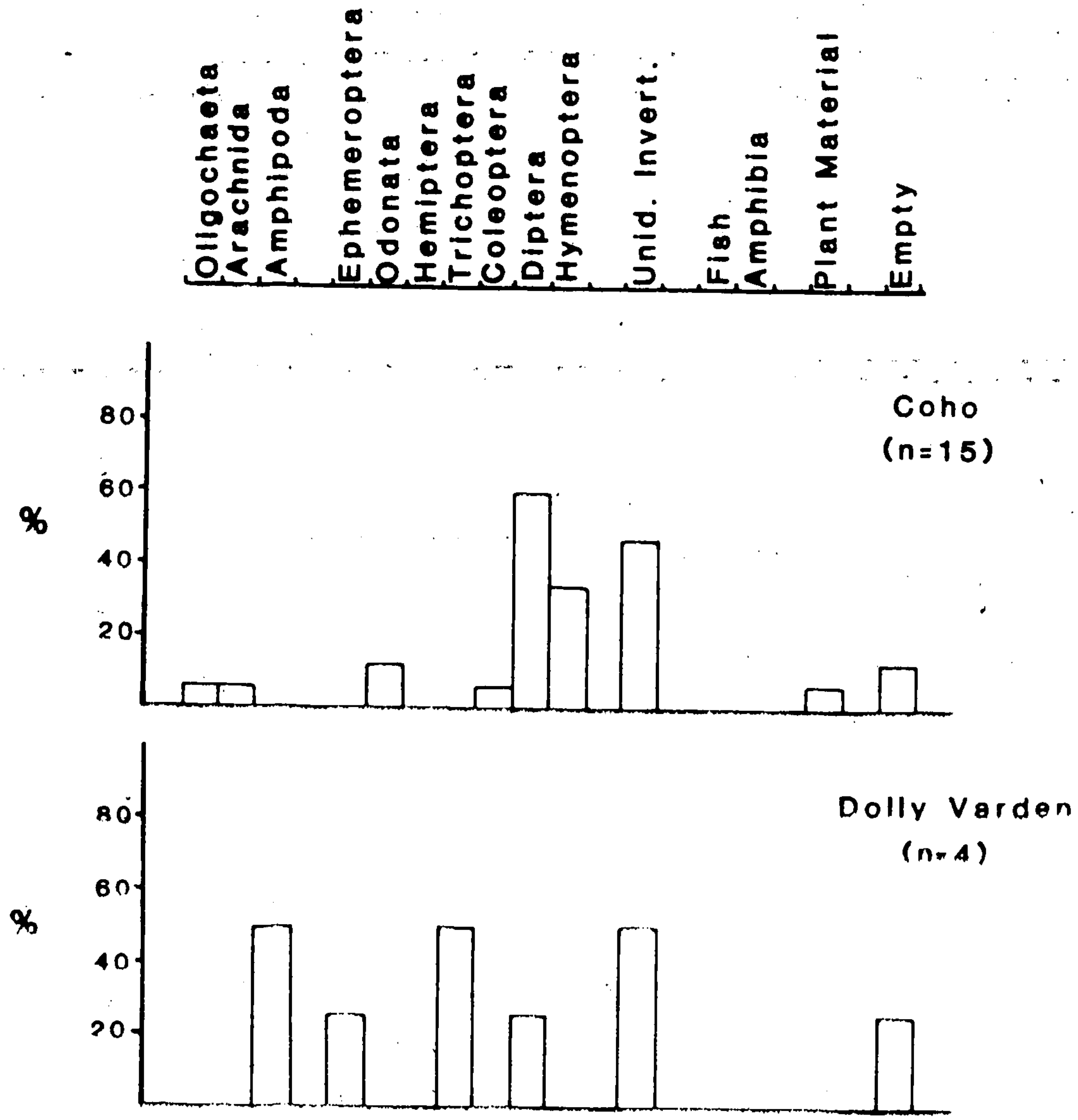


Figure 9. Percent occurrence of food items in stomach contents of salmonids trapped above the waterfalls.

Table VII

Estimated Invertebrate Densities From Samples

	Inlet		Lake	
	Sediment(#/ℓ)	Sphagnum(#/ℓ)	Sediment(#/ℓ)	Bottom Surface(#/m^2)
Amphipoda	10.57	11.25	--	--
Sphaeriidae	10.14	0.75	--	--
Chironomidae	9.04	5.75	45.8	--
Oligochaeta	0.87	3.25	20.75	--
Libellula sp. nymphs	0.47	0.5	--	--
Aeshna sp. nymphs	--	0.25	--	1.5
Zygoptera nymphs	--	0.25	--	1.5
Trichoptera larvae	--	--	--	79.45
Other Diptera larvae	0.07	0.25	--	--

C. DISCUSSION

Factors which may be responsible for limiting inlet fish to the inlet and for their distribution within the stream are discussed below. Preferences for some of these (water type, current, and vegetation) were tested experimentally and are considered in subsequent chapters.

Sticklebacks occurring in the inlet stream of Drizzle Lake are found close to stream banks in areas of reduced current with adjacent patches of emergent *Sphagnum*. The presence of an overhanging bank does not affect numbers of sticklebacks nearby. The value of an overhang for cover may be outweighed by the swifter current present which is responsible for the existence of the undercut bank. Adult fish are not found within the *Sphagnum* beds but appear to occur where the stream bottom meets the edge of the moss. Occasionally, subadult fish were collected by a dip net or seine pulled through the moss but these could have been on the outside edge when first caught. The age composition of the exceptionally high number of fish caught in trap #l in July, 1981 supports this. Twenty-five of thirty fish were caught in a ten-day period when the #1 trap was moved along the upper edge of a single *Sphagnum* bank. Nineteen of these fish were subadults, the remainder were females.

Sphagnum is not continuous along the length of the stream which was surveyed with the suspended traps. Thus, the stream bank traps (#l and #5) were often free of surrounding vegetation. Lower numbers of fish (except #1 in July) in these two traps relative to traps #2 and #4, therefore, reflects both the absence of fish in the moss and the avoidance of the upper 15-20 cm of the water column near the bank (or the avoidance of more visible traps). Other single traps suspended from the bridge or from logs and not resting on the bottom were similarly less successful.

The midstream trap of the five was often resting on the bottom because of the varying topography of the stream bed. Faster current and maximum distance from cover may make this the least suitable region of the stream width for these fish.

Absence of areas of reduced current and *Sphagnum* in the stream above the small waterfall at N1 (Fig.2) may account for the restriction of sticklebacks to the lower reaches. When water levels are high these falls are replaced by a short stretch of very fast water. This occurs in early fall and coincides with the greatest upstream movement of the sticklebacks. If they can overcome these barriers, and this is considered quite likely (J.S. Nelson, pers. comm.), one would expect to find them upstream. Specific reasons for their absence cannot be given; however, the habitat may be unsuitable for a permanent population. This upstream region of the falls is typically narrow (0.5–2.0 m) with deeply undercut banks on both sides, an indication of the scouring effect of the stream at high water levels. Riffles are also present. Areas of slow water with submerged vegetation, like those frequented by sticklebacks downstream, are rare. If such areas are necessary for feeding or as nest sites then the fish could be excluded from this area. Hagen (1967) found that a similar stream form always built nests in areas of no detectable current. A strong preference for nesting in vegetated habitat (60/64 fish tested) was reported by Kynard (1979) for lake and stream-dwelling sticklebacks. Appropriate nesting habitat above the falls in the Drizzle Lake inlet is found in isolated areas and lasts for unpredictable periods of time. Sticklebacks nesting or attempting to nest would not likely succeed. Thus, a strong selection pressure, nest success, may be acting to confine these fish to the lower, more stable, 450 m. Unfortunately, no inlet stickleback nests were found in the course of the present study to corroborate earlier findings.

Alternative hypotheses for the limited upstream distribution of sticklebacks include competition or predation from young coho salmon (*Oncorhynchus kisutch*) and Dolly Varden char (*Salvelinus malma*).

Downstream, the diets of the three fish species do not overlap to a great extent. Stickleback feed principally on chironomid larvae and zooplankton. Dolly Varden prey most heavily on trichopteran larvae and young coho take adult flying ants. Above the falls, the two salmonids' food intake is modified somewhat. Coho feed on chironomid larvae as well as flying ants and the char include amphipods in their diet. Both of these are primarily benthic organisms and may have been taken while the fish were in the trap. If they were not, however, competition for food above the falls may restrict sticklebacks to the lower reaches. Plankton tows were not made in the upstream area but it is unlikely that cladocerans would occur in the swift flowing water (Reid and Wood 1976). Thus, sticklebacks would be forced to rely more heavily on chironomids and face competition from the larger and perhaps more abundant coho parr.

A different set of limiting factors is apparently in effect at the mouth of the stream. Of all variables, water depth (and its consequences: temperature, light penetration, and vegetation) undergoes the most rapid change. Inlet fish appear to favour a deep and, consequently dark environment. No similar habitat exists until the deeper sections of the lake (greater than 1.5 m) are reached about 250 m from the mouth. Inlet fish are more likely to be found close to the banked shore of the lake than offshore in that area, but not beyond a point approximately 30 m from the mouth. This suggests that factors other than depth alone may act to limit movement into the lake. Intolerable but unknown differences in water chemistry may be present. In a series of measurements (July 10, 1982) the pH ranged from 4.75 in the lake about 100 m from the inlet, through 4.9 at 50 m, 5.1 at 30 m, 5.95 at 15 m, 6.35 at 7.5 m, to an average pH in the stream of 6.5. These readings were taken near the end of a seven-week period with no major rainfall (i.e., enough to saturate the bog and raise the stream level). Four days later, July 14, after two days of very heavy rain, the average inlet pH had fallen to 4.6. Lake pH values remained in the 4.5–4.8 range throughout the summer, almost within the range experienced by the inlet fish. Hynes (1970) states that most species of fish have a wide tolerance to pH. It is unlikely that periodic differences in pH confine the inlet fish to the stream.

Higher predation on inlet sticklebacks than on other fish in the lake could also result in the restriction of this group to the stream. Cutthroat trout (*Salmo clarki*) and avian predators (particularly *Gavia immer*, *Gavia stellata*, *Podiceps grisegene*, and *Podiceps auritus*) are present in greater numbers in the lake than in the inlet and all are known to prey on sticklebacks (Carl et al. 1977; Reimchen and Douglas 1980; Terres 1980). Lacking the strong countershading and longer spines characteristic of lake fish, the inlet fish may be more vulnerable to predation in the lake.

Inlet fish, particularly subadults (which made up the majority of the seined sample), include a high proportion of zooplankton in their diet. If the plankton density in the lake is not limiting, subadult inlet fish entering the lake would not be in competition with lake sticklebacks. Adult inlets rely more heavily on chironomid larvae which occur in both the sediments and *Sphagnum* in the stream. They are, however, five to eight times more abundant in the lake sediments. It appears that this food source too would not be limiting. However, accessibility of the prey item must be considered (Ivlev 1961). Inlet sticklebacks may prey on chironomids in the *Sphagnum* in the stream, rather than on those from the sediments. Or, they could be taken from the water column. Chironomid larvae were rare in the lake plankton and those living below the surface may not be available to the sticklebacks (lake or inlet). The question of differential food utilization by these two groups of fish requires further investigation and a large sample of fish seined from each habitat for the stomach contents analysis.

Within the stream, more females are found in the downstream section which is broader, slower, and unshaded. In a lake population on the Queen Charlotte Islands, Reimchen (1980) found an excess of females occupying open water during the reproductive season. Schooling of females between spawnings in areas away from the littoral nesting sites is well known (Wooton 1976). The lower reaches of the stream offers the closest approximation to a limnetic habitat of any section. The greater number of females inhabiting this region suggests that a similar association to that seen in lakes may exist in this stream.

The reduction in movement of the adult cohort in the reproductive season is not unusual in a territorial species. Although no nests were found in the inlet, the region where nest density should be highest can be inferred from the location of the highest percentage of breeding males. Of 53 males displaying breeding coloration, 31 (58.6%) were taken between sites E and GG1 (Fig 2), a section 70 to 210 m upstream from the mouth. Lake males' nests closest to the inlet were approximately 50 m from the mouth. Thus, a minimum distance of 120 m separates the closest males of the two morphs during the reproductive season. This is within the cruising range of both as indicated by the few adult lake fish caught more than 100 m upstream and those adult inlet males trapped 200 m offshore in the lake. However, these appear to be isolated cases and their trapping locations are not indicative of the range limits of the two groups.

Movement of juvenile inlet fish in the fall suggests that a dispersal phase may be part of the life history of this group. If site-specific homing does not occur prior to the onset of their first reproductive cycle, this dispersal may act to reduce inbreeding among offspring of the same male. Inbreeding can result in a decline in viability or fecundity and does reduce the level of heterozygosity in a population (Futuyma 1979). A higher proportion of deleterious alleles would be exposed to selection as the frequency of homozygous loci increased, lowering the fitness of the individuals involved. Higher levels of homozygosity render the population less flexible in dealing with environmental change. The inlet is a habitat of widely fluctuating variables over both short and long term. Selection may act to maximize heterozygosity by favouring a dispersal trait in juveniles.

IV. MORPHOLOGY

A. METHODS

Lake and inlet sticklebacks can be distinguished by a number of morphological characters. Standard length, gill raker number, vertebral number, relative body depth, and relative length of pelvic spines have been used by Moodie (1972a and b) to separate forms similar to these. The same characters were used in this study. Colour and colour pattern also distinguish the two forms but were not quantified. In another lake on the Charlottes with lake and stream forms, Moodie (1970) recognized that other differences in proportions, especially in the head region, exist. I observed similar differences in the Drizzle Lake sticklebacks. Head length, snout length, snout to first dorsal spine, snout to second dorsal spine, eye diameter, cheek height, width of gape, and length of upper jaw were measured. Head measurements (other than head length) are expressed as a proportion of the head length for comparisons between morphs and between age classes. Head length is expressed as a proportion of standard length.

Samples of inlet fish for the general morphological analysis were collected over two week periods in June, July and August 1981 during regular trap checks. Fish were preserved in 10% formalin. Measurements of standard length and the head characters followed Hubbs and Lagler (1964). Body depth and spine lengths were measured as indicated in Moodie (1972a). All dorsal and anal fin ray elements were counted, including the last two rays in each fin which do not divide close to the base. Snout to first and second dorsal spine measurements were made from the anterior end of the upper lip to the point of articulation between the spine and dorsal plate. Dial calipers calibrated to 0.05 mm were used for all measurements. A sample of 42 inlet fish representing both sexes, three collection periods, and the length of the inlet was drawn from the general sample. These fish were stained with alizarin red and cleared using the method of Taylor (1967) to facilitate counting the gill rakers and vertebrae. Gill rakers on the anterior arch on both sides were counted. The hypural plate and its centrum were counted as one in the vertebral count.

Morphometric data on the lake morph for the general morphological comparison were provided by T.E. Reimchen.

A sample of 100 adult lake fish and 29 juvenile lake fish from the 1981 collection of T.E. Reimchen were used in the comparison of head morphology. The remaining 40 juvenile lake fish were collected by myself in July, 1982.

Ratios to standard length or head length (determined arithmetically), and meristic characters were used in most comparisons. Two methods were used to compare means of head measurement ratios. Pairwise t-tests compared means of lake and inlet adults, lake and inlet juveniles, lake adults and juveniles, inlet adults and juveniles, and inlet adults and lake juveniles. Similarity coefficients based on the Manhattan distance formula of Sneath and Sokal (1973, p. 125) were calculated. The latter method permitted a ranking of pairs on the basis of similarity.

Pairwise t-tests were used in preference to a multisample testing procedure (eg. ANOVA) because it was the difference between only two samples (i.e., two morphs or two age classes or one of each) that was of interest. Approximate t-tests (t') were used when variances of the two samples were significantly different (Snedecor and Cochrane 1980). This approximation allows fewer degrees of freedom for a given t value. Therefore, the calculated t must be greater than could be acceptable using the usual formula for degrees of freedom $(n_1 + n_2 - 2)$.

B. RESULTS

The mean values for gill raker number, vertebral number, dorsal fin ray number and anal fin ray number are significantly different for the two morphs ($P < .001$). Other means (Table VIII) were not compared statistically because variance values for the lake morph were not available. Sample sizes for both morphs for these characters, however, are large and the differences between means substantial, so it is likely they too are significant.

Standard length, though not a taxonomic character, is probably the best single means of separating adults of the two groups (Fig. 10); there is no overlap between the morphs in adult lengths. This is also true for the Mayer Lake sticklebacks (Moodie 1972a,b). Fish of the inlet morph are shorter, deeper bodied and possess fewer gill rakers, dorsal fin rays, and anal fin rays. Pelvic spine length relative to body length is shorter than that of the lake morph. Tables IX and X indicate that the inlet fish are

Table VIII

Comparison of Some Morphological Characteristics of the Lake and Inlet Morphs

Character		Inlet	n	Lake	n	Source of Lake Data
Max. S.L.	♀	56.75	33	85.2	164	Moodie & Reimchen, 1976b
	♂	56.30	29	73.6	144	"
Mean S.L.		46.43(8.4)	31	76.6	117	T.E.Reimchen, unpubl.
Standard length / body depth	♀	4.461(.242)	180	5.34	164	Moodie & Reimchen, 1976b
	♂	4.410(.211)	118	5.26	144	"
Gill rakers	♀	17.91(1.306)	22	20.7(1.24)	164	"
	♂	17.70(0.801)	20	21.3(1.22)	144	"
Vertebrae		31.9(0.617)	42	33.0(0.58)	308	"
Dorsal rays		11.21(0.696)	302	12.0(0.59)	117	T.E.Reimchen, unpubl.
Anal rays		8.27(0.692)	302	8.7(0.62)	117	"
Standard length / left pelvic spine length		6.64(0.661)	303	5.9	308	Moodie & Reimchen, 1976b

Notes: -Data are means and one standard deviation in parenthesis (when available).
-Characters 1,2 for inlet morph based on June, 1981 sample only. For lengths only, fish > 40 mm. considered adult (see Fig. 7).

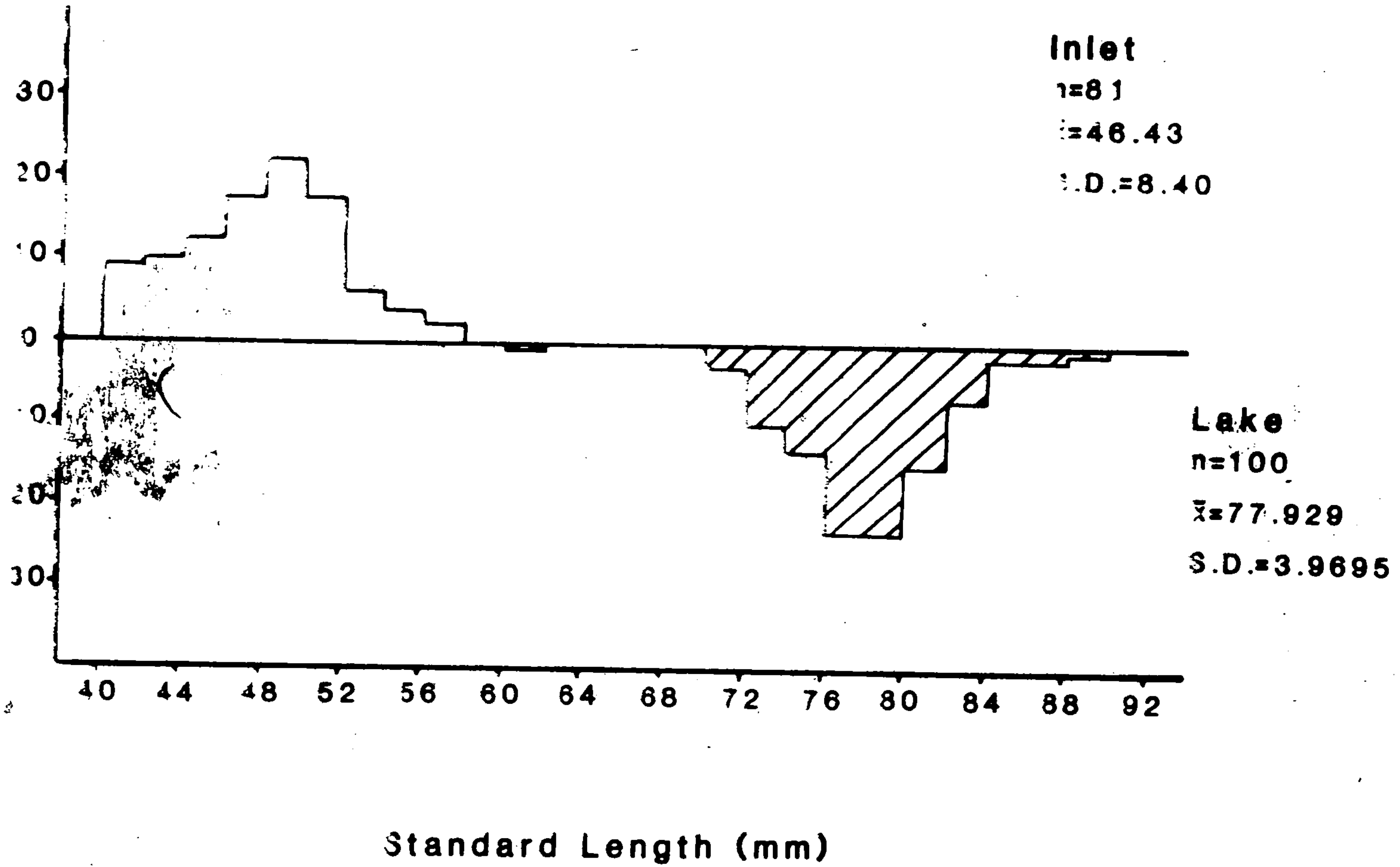


Figure 10. Range of standard lengths of adult inlet and lake sticklebacks. Inlet fish collected in June; lake fish collected in April.

Table IX

Head Character Ratios for Lake and Inlet Fish in Two Classes

	SL/HL	SL/SnDI	SL/SnDII	HL/SnL	HL/ED	HL/CH	HL/IO	HL/G	HL/UJ	n
Inlet Adults	3.484 (.143)	2.715 (.059)	2.192 (.050)	3.708 (.218)	3.353 (.197)	6.136 (.574)	4.843 (.648)	4.466 (.318)	4.171 (.529)	70
Lake Adults	3.706 (.163)	2.890 (.086)	2.282 (.058)	3.334 (.136)	3.501 (.201)	5.656 (.354)	5.085 (.369)	4.057 (.271)	4.019 (.261)	100
Inlet Juvs	3.633 (.169)	2.767 (.086)	2.226 (.056)	3.790 (.344)	3.233 (.200)	2.095 (.797)	5.169 (.715)	4.530 (.356)	4.942 (.592)	33
Lake Juvs	3.734 (.132)	2.779 (.079)	2.112 (.480)	3.575 (.233)	3.311 (.241)	7.070 (1.054)	5.377 (.802)	4.256 (.300)	4.198 (.678)	69

Notes: -Abbreviations: SL = Standard length; HL = Head length; SnDI = Snout to first dorsal spine length; SnDII = snout to second dorsal spine length; SnL = snout length; ED = Eye diameter; CH = Cheek height; IO = Interorbital distance; G = Width of gape; UJ = Upper jaw length.

-Data are means (standard deviations).

Table X

Absolute Differences and Significance Levels Between Pairs of Head Character Ratios

	SL/HL	SL/SnDI	SL/SnDII	HL/SnL	HL/ED	HL/CH	HL/IO	HL/G	HL/UJ	Sum
IA-LA	.222 ***$_1$	.175 ***$_2$	.090 ***$_1$	.374 ***$_2$	.148 ***$_1$	.480 ***$_2$	.242 ***$_2$	.409 ***$_1$	.152 *$_2$	2.292
IA-IJ	.149 ***$_1$	.052 **$_2$	.034 **$_1$	.082 NS$_2$	.120 *$_1$	.959 ***$_1$	.326 *$_1$	.064 NS$_1$	.771 ***$_1$	2.557
IA-LJ	.250 ***$_1$	.064 ***$_1$	.080 NS$_2$	.133 ***$_1$	.042 NS$_1$	.934 ***$_2$	.534 ***$_1$	.210 ***$_1$	.027 NS$_1$	2.274
LA-IJ	.073 *$_1$	.123 ***$_1$	.056 ***$_1$	.456 ***$_2$	.268 ***$_1$	1.439 ***$_2$	.084 NS$_2$	.473 ***$_1$	.923 ***$_2$	3.895
LA-LJ	.028 NS$_1$	.111 ***$_1$	.170 **$_2$	.241 ***$_2$	.190 ***$_1$	1.414 ***$_2$	.292 **$_2$	.199 ***$_1$	.179 *$_2$	2.824
IJ-LJ	.101 NS$_1$	.012 NS$_1$	.114 NS$_2$	.215 **$_2$	.078 NS$_1$	.025 NS$_1$	.208 NS$_1$	.274 ***$_1$	.744 ***$_1$	1.771

Notes: -Mean values compared using student's t-test or approximate t(t') test following F test for homoscedasticity.

-^{a}P-levels with subscript 1 determined using Student's t-test.

-^{b}P-levels with subscript 2 determined using approximate t-test.

- Abbreviations: IA = Inlet adult; LA = Lake adult; IJ = Inlet juvenile; LJ = Lake juvenile; NS = Not significant.

* P < .05.
** P < .01.
*** P < .001.

proportionately longer in the anterior half of the body. Head length, and the two snout to spine lengths relative to the length of the fish are longer in the inlet morph than in the lake morph. Within the head region, adult inlet fish have larger eyes, shorter snouts, a deeper cheek area, and a wider interorbital distance ($P < .001$ in all cases) than their lake counterparts. Upper jaw length and width of the gape are smaller relative to head length than those of the lake fish ($P < .05$, $P < .001$ respectively). Again, there is considerable overlap between morphs in all head characters (Fig. 11).

Adults of the two morphs were compared with juveniles to test the hypothesis that proportions in the head region of the inlet morph are the result of paedomorphosis, the retention of juvenile characters in the adult (Gould 1977). If juvenilization in the inlet fish contributes to the difference between lake and inlet morphs, and if inlet and lake juveniles are alike, then inlet adults will be more similar to the inlet juvenile and to the lake juvenile than they are to the lake adult. Table XI shows that the two juveniles are the least different pair (see also Table X for number of characters where means are not significantly different), and the inlet adults do resemble lake juveniles more than lake adults. As measured by Manhattan distance, inlet adults were more similar to lake adults than to inlet juveniles which seems to contradict the other results. However, Manhattan distance calculations use only absolute differences between means and do not consider the statistical significance of those differences (i.e., the amount of variation about the mean). As a second method of ranking pairs which would take this into account, I assigned points on the basis of P values (Table X). Those characters for which the differences were more significant were therefore more heavily weighted. For $P < .001$ a pair received 3 points, for $P < .01$, 2 points, for $P < .05$, 1 point, and for nonsignificant differences 0 points were assigned. Points and rank are given in Table XI. Under this system, lake and inlet adults are more different than any other pair and inlet adults are almost as similar to lake juveniles as they are to inlet juveniles.

Within the inlet population, beginning in mid-May, three age classes were present. Young-of-the-year were too small to be caught in minnow traps and were not included in the 1981 samples. The bimodal distribution in the frequencies of standard lengths of fish collected in June (Fig. 12A) indicates that two age classes of fish were being caught. It is unlikely that the June bimodality represents two distinct size classes of one age. No

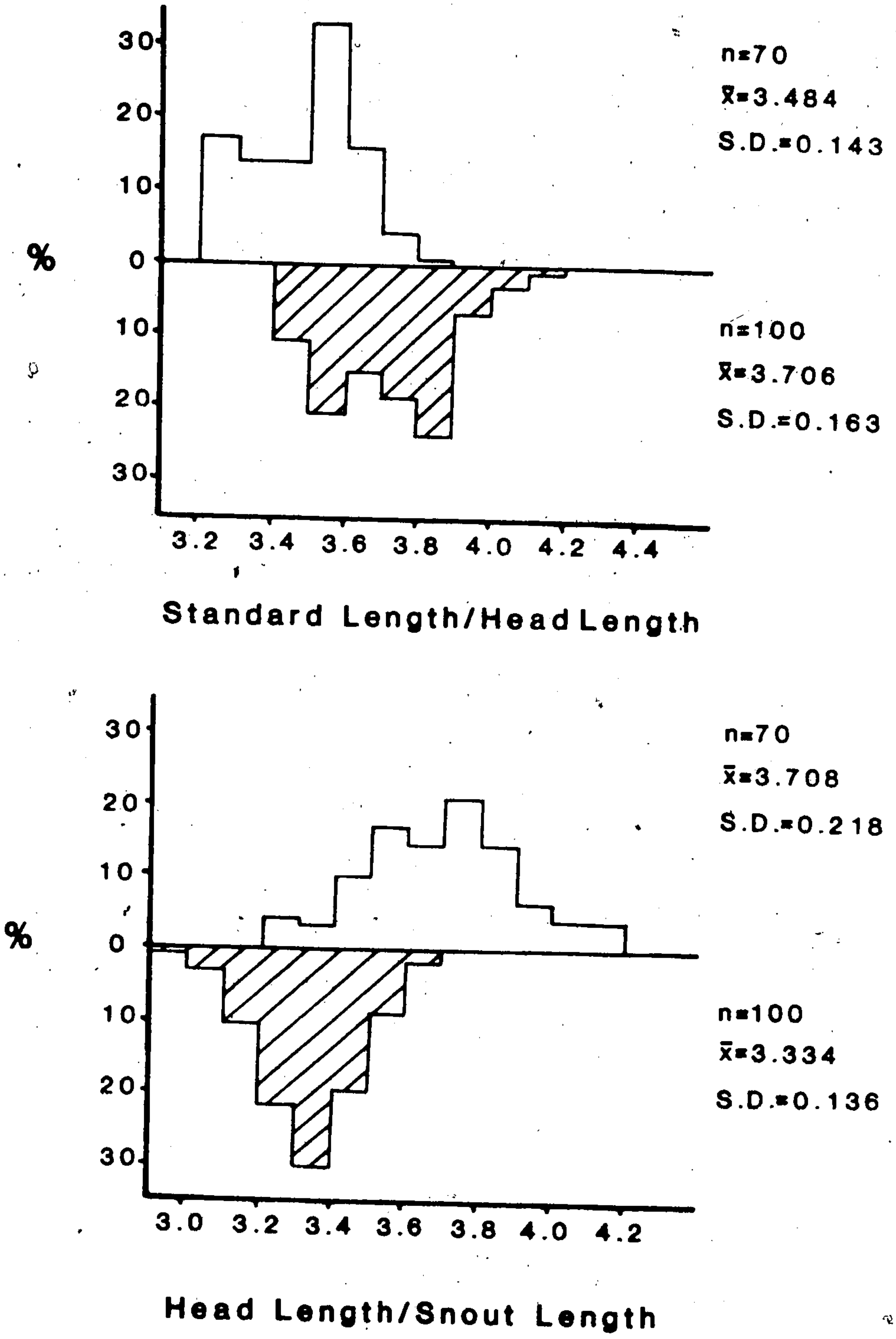
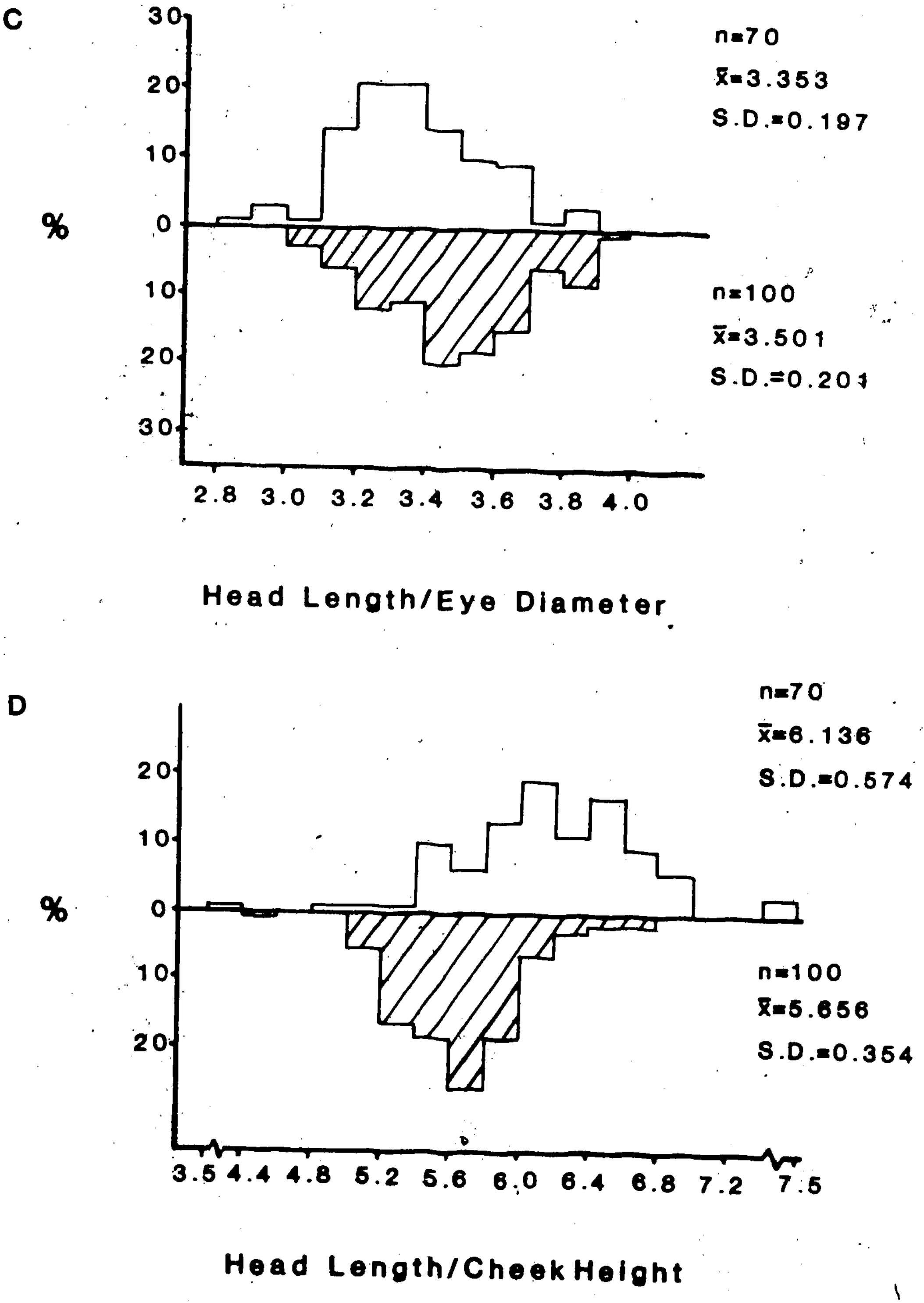


Figure 11. Some morphological characteristics (head region) of inlet (above the line) and lake (below the line) sticklebacks. Abbreviations: n = sample size; $\bar{x}$ = mean; S.D. = standard deviation.



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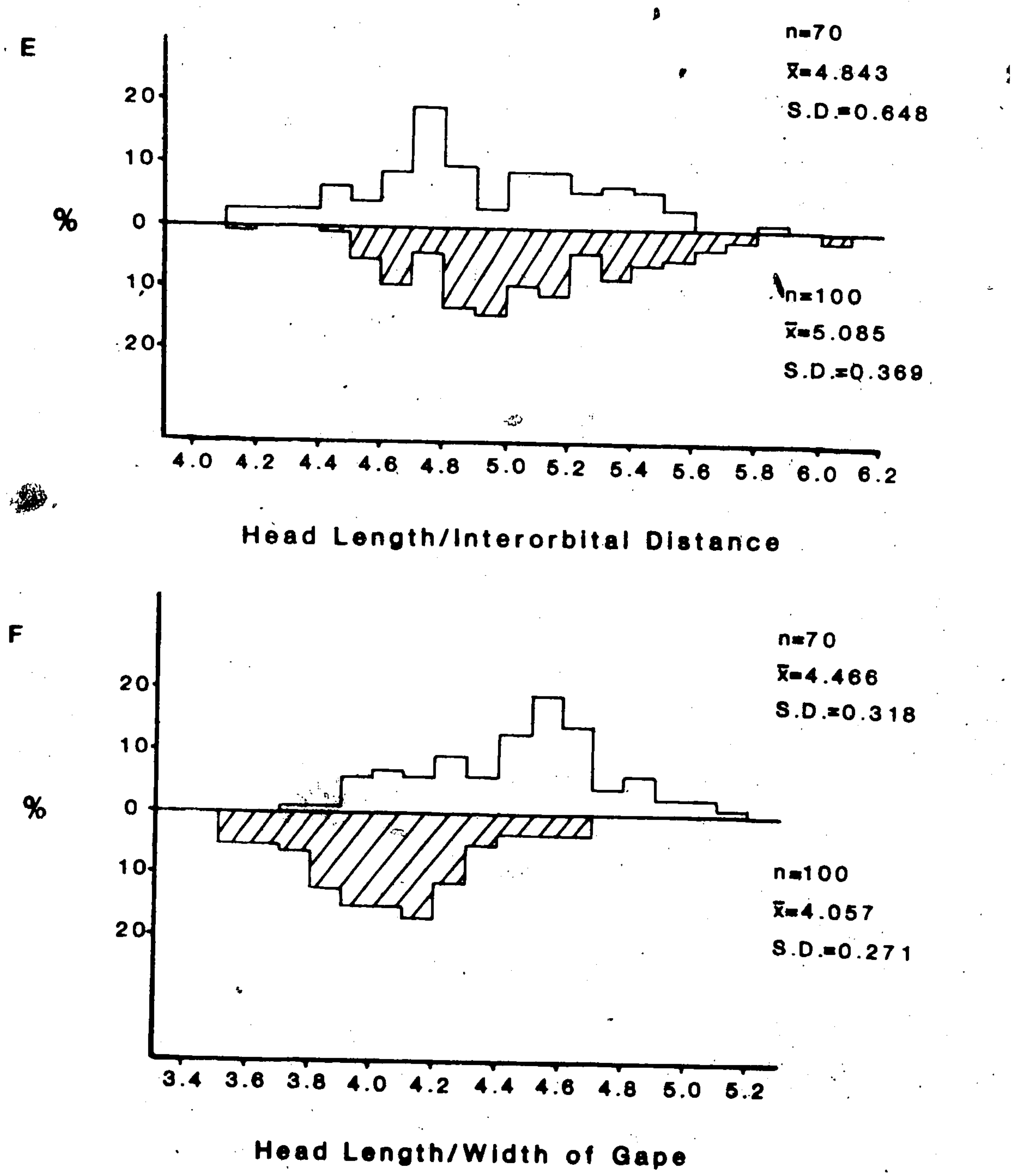

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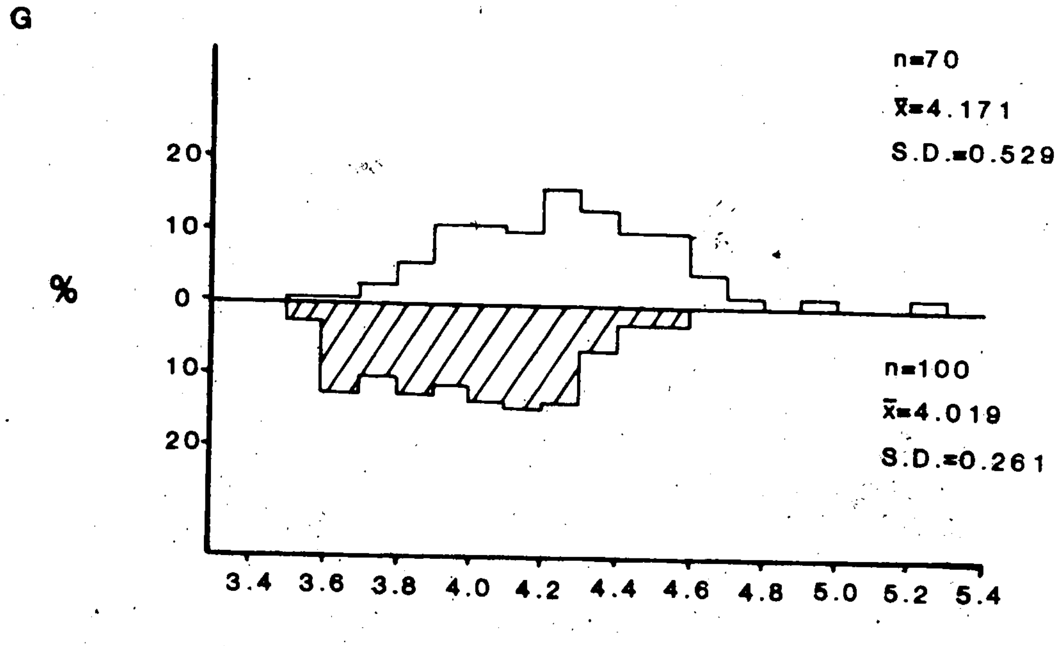

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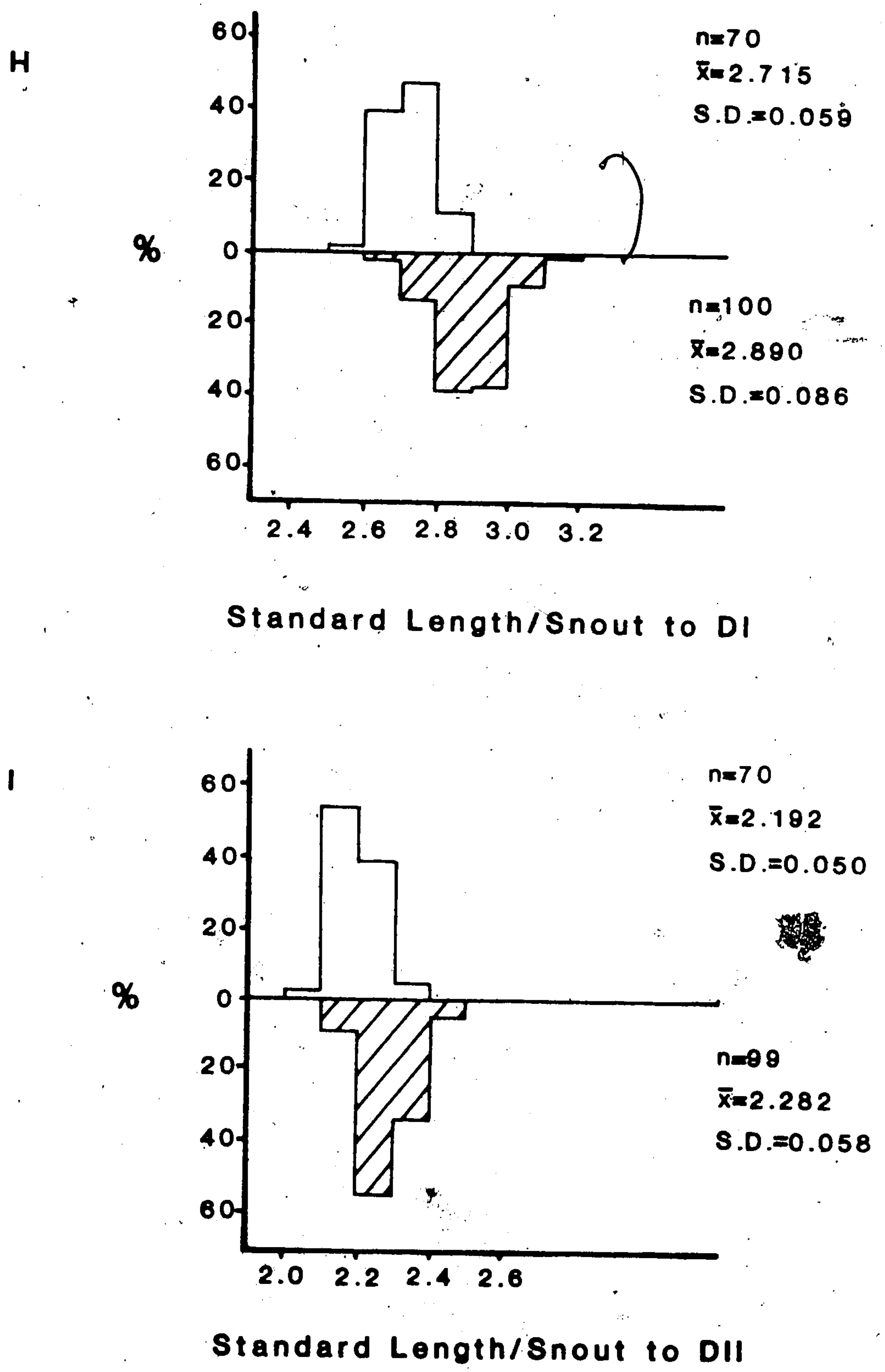

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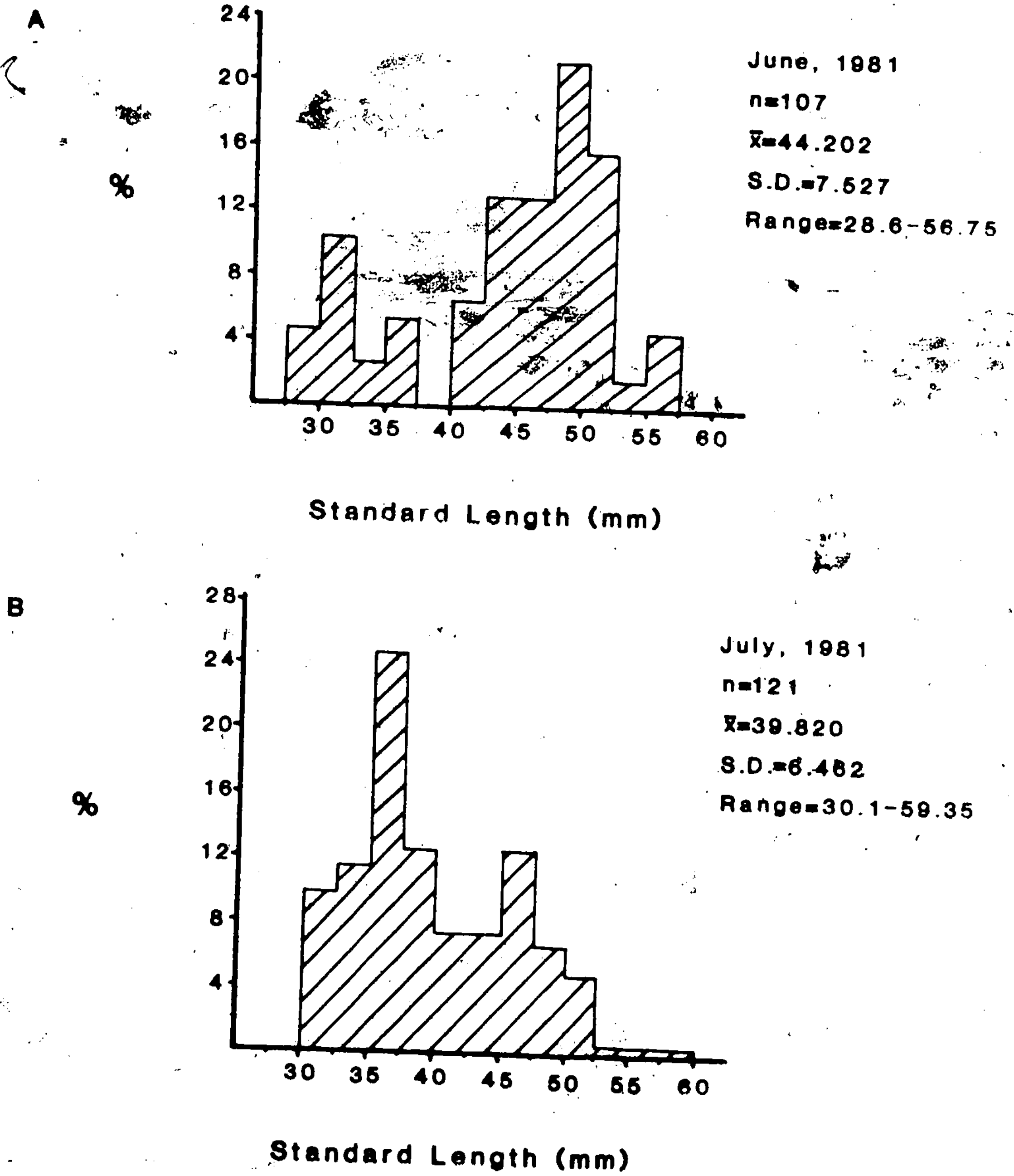


Figure 12. Length-frequency relationship in inlet fish sampled through the summer, 1981.

Table XI

Amount of Difference Between Pairs as Indicated by Manhattan Distance (M.D.) and Points System (Points Assignment Described in Text)

Pair	M.D.	Rank	Points	Rank
IA-LA	2.292	4	25	1
IA-IJ	2.557	3	15	5
IA-LJ	2.274	5	18	4
LA-IJ	3.895	1	22	2
LA-LJ	2.824	2	20	3
IJ-LJ	1.771	6	8	6

Notes: -Abbreviations as in Table IX.
-Pairs are ranked most (1) to least (6) different.

fish less than 40 mm in length were caught in breeding condition (nuptial colours in males, signs of gravidness in females), nor was it apparent that members of this cohort entered reproductive condition before the end of the spawning season (early August). Fish less than 40 mm S.L. were in their second summer. Those greater than 40 mm were in their third summer. Figure 12 (A, B, C) reflects the increasing dominance in trap catches of second year or subadult fish.

Few characters of inlet fish change significantly along the length of the inlet. Figure 13 indicates a difference in standard length ($P < .01$) between fish from the lowest trap sites (C to F) and those from farthest upstream (Model 1 ANOVA: $F=0.004$, $N=301$, $df=3,297$).

Regression analyses of several morphometric and meristic characters with distance from the lake revealed few significant correlations (Table XII). These characters were selected because fish of both size classes could be included. Among males there are declines in mean number of anal and right pectoral fin rays with distance upstream. Increases in number of caudal fin rays and in the relative length of the second dorsal spine are also evident in males (Table XII). Gill raker numbers are the only characters in which a significant correlation with distance is found in females (Fig 14). When all fish are combined, a slight increase in relative body depth and an equally small decrease in relative length of the left pelvic spine are evident. With the exception of those of female gill raker number, all significant correlation coefficients are small ($|.3|$).

DISCUSSION

Variation Between Lake and Inlet Morph

Differences in meristic and morphometric characters between lake and inlet stream forms in the Drizzle Lake drainage are not unlike those found for a comparable pair in Mayer Lake, 50 km to the south (Moodie 1972a). In that study and in work on yet another population of "black" sticklebacks in the Chehalis River in Washington (McPhail 1969), predation was considered the major selective force responsible for the development of the black form. A predator group similar to that of Mayer Lake is found in Drizzle, and it is reasonable to assume that the lake form described here evolved in response to similar pressures.

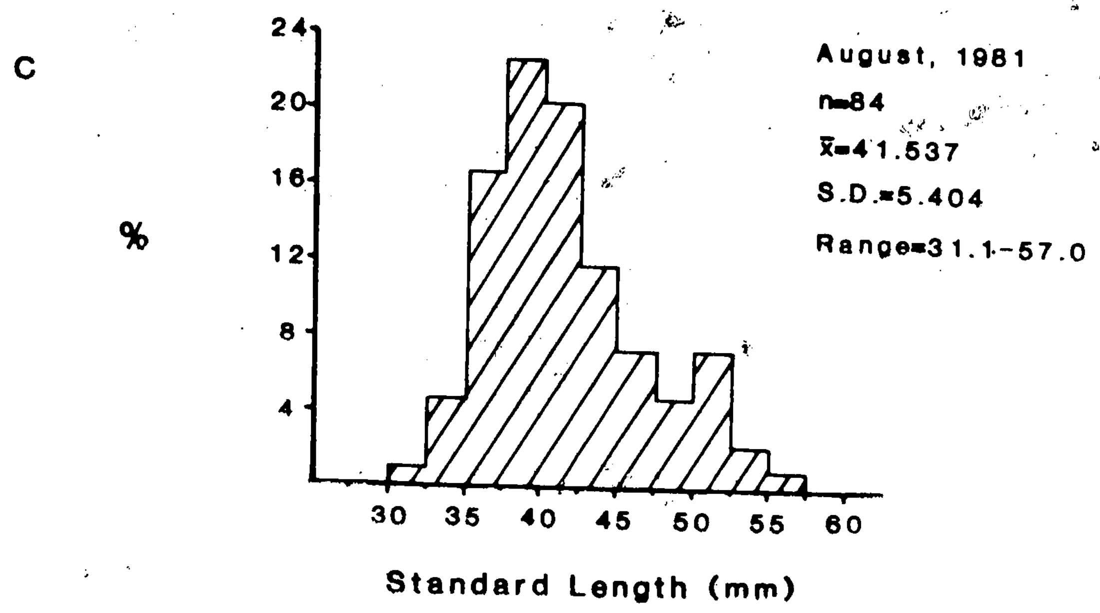


Figure 12 (cont'd).

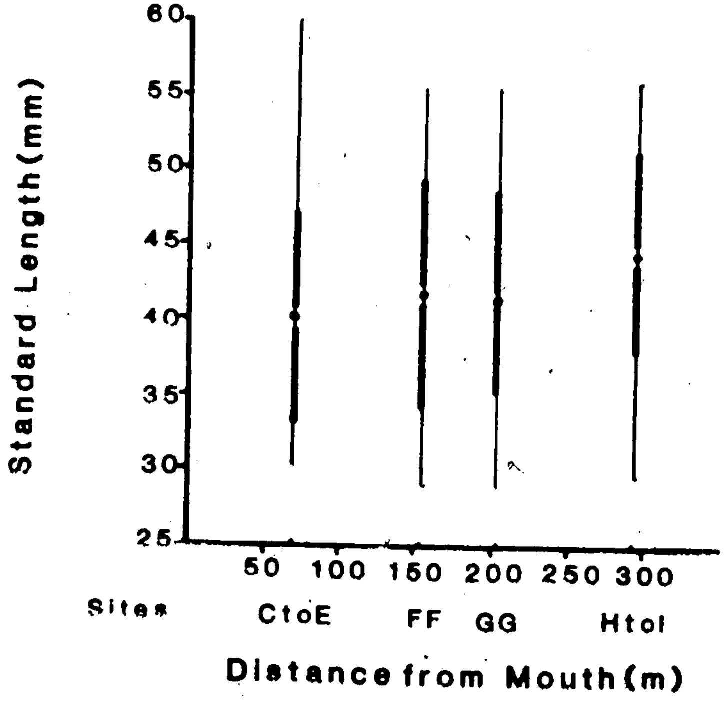


Figure 13 Average length of inlet sticklebacks. Samples from three months combined. Trap sites grouped and mean distance calculated. Point = mean; thick vertical line = 1 standard deviation; thin vertical line = range. Sample sizes: C to E = 108; FF = 60; GG = 66; H to I = 67.

Table XII

Relationship of Morphometric and Meristic Characters of Inlet Fish With Distance From Lake as Expressed by Correlation Coefficients

Character	MALE			FEMALE			COMBINED		
	n	r	P	n	r	P	n	r	P
No. Vertebrae	20	.061	NS	22	-.060	NS	42	.010	NS
No. L.Gillrakers	20	.118	NS	22	-.452	<.05	42	-.238	NS
No. R.Gillrakers	20	-.026	NS	22	-.496	<.02	42	-.258	NS
No. Dorsal rays	118	-.033	NS	179	.011	NS	297	.004	NS
No. Anal rays	118	-.204	<.05	179	-.076	NS	297	-.098	NS
No. Caudal rays	118	.192	<.05	178	.026	NS	296	.096	NS
No. L. Pectoral rays	118	-.048	NS	180	.049	NS	298	.014	NS
No. R. Pectoral rays	118	-.188	<.05	180	-.028	NS	298	-.087	NS
SL/BD	118	-.155	NS	180	-.105	NS	298	-.134	<.05
SL/DII	105	.304	<.001	148	.055	NS	253	.181	NS
SL/LP	118	.160	NS	180	.107	NS	298	.131	<.05

Notes: Abbreviations: No. = number; L = left; R = right; SL = standard length; BD = body depth; DII = second dorsal spine; LP = left pelvic spine; NS = not significant (P > .05).

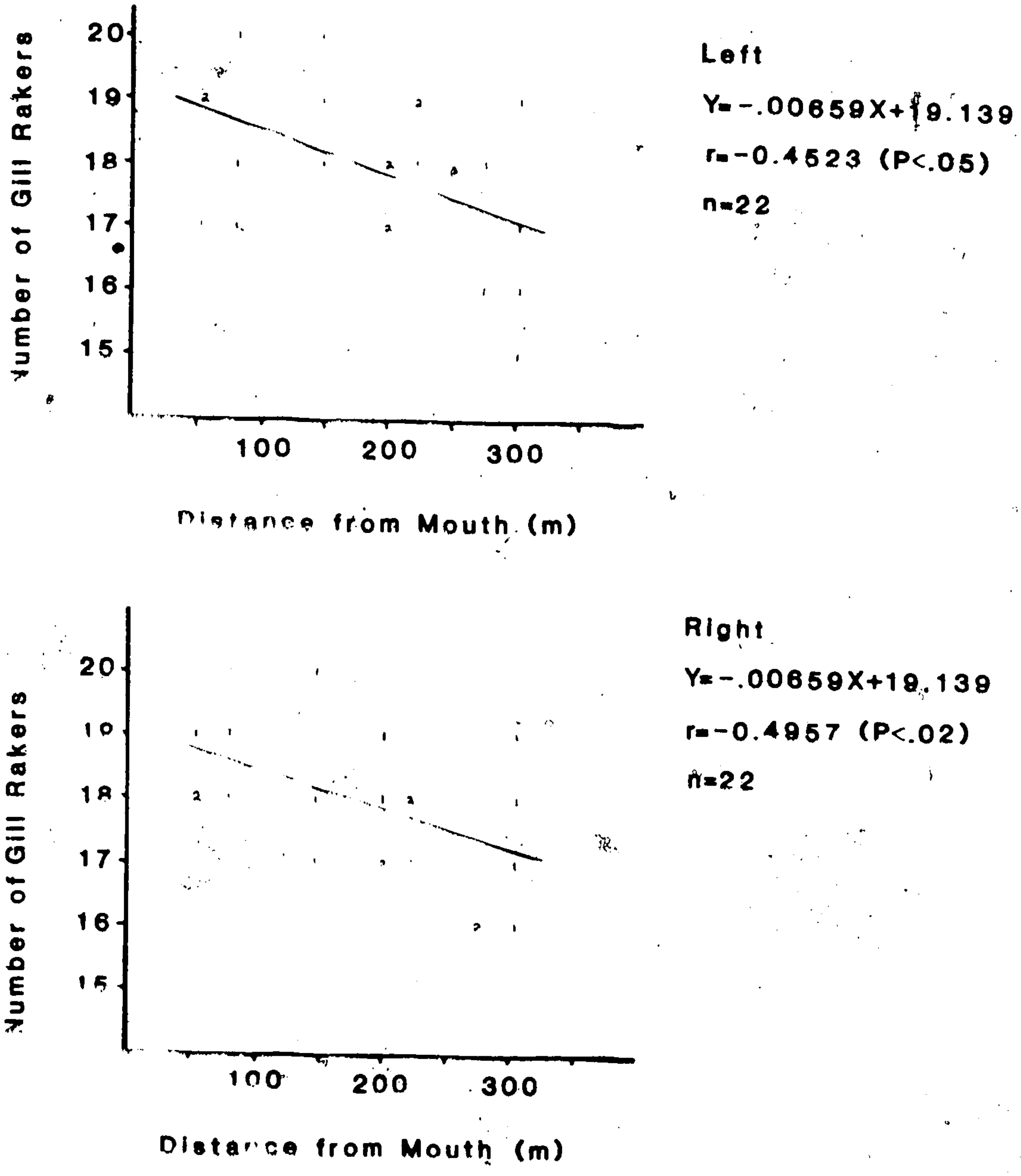


Figure 14. Variation in gill raker number in female inlet sticklebacks with distance upstream. Numbers on graph represent number of observations at that point.

An explanation for the characteristics of the stream-dwelling sticklebacks is perhaps less straightforward. The mottled brown-beige colour pattern would have a disruptive effect in the heterogeneous stream habitat. A greater body depth (less terete shape) has been reported for other stream populations of sticklebacks (Hagen and Gilbertson 1972; Gross 1979) when compared to lake populations. This relative difference is perhaps best explained by selection for a more streamlined form in the lake.

Gill raker number is related to the size of food items taken by the fish (Hildebrand, in Brooks and Dodson 1965; Larson 1976; Lagler, et al 1977, p.142). Higher numbers of rakers, as are found in the lake morph, characterize plankton feeders. Species which prey on larger organisms generally have fewer rakers spaced farther apart. Moodie and Reimchen (1976a) and Hagen and Gilbertson (1972) found lower gill raker numbers in stream populations than in lake populations. Similar differences have also been noted between lake and stream-dwelling populations of the ninespine stickleback, *Pungitius pungitius* (Coad and Power 1973; Gross 1979). This relationship between gill raker number and differences in availability of food types is apparent in this study also. Average plankton densities were considerably higher in the lake (3600 organisms/m^3), than in the inlet (54 organisms/m^3). Relative densities of benthic invertebrates are less easily determined but there is a greater variety of such organisms in the inlet than in the lake.

The higher vertebral number in lake fish, which has also been noted in other lake populations (Hagen and Gilbertson 1972), may have a genetic or non-genetic basis. Lindsey (1962) found that within what was considered one genotype of stickleback, vertebral number varied with temperature during the time of egg development. The relationship between the two variables tended to be V or W-shaped. Differences between genotypes were visible as shifts in the apex of the V to a lower or higher temperature. It is possible that the difference of one vertebra between the two Drizzle Lake morphs may be related to a temperature difference of about 4°C during the rearing period. However, the direct relationship seen in this case (higher vertebral number in warmer water) is the opposite of that observed by Lindsey (1962) for water of this temperature (less than 20°C). It may be that an underlying genetic difference has an overriding effect on the phenotype, or that in these populations the minimum number of vertebrae would occur at a lower temperature (10°C., for example). If there is a strong genetic component, the higher number of vertebrae in lake fish combined with a lower mean for body depth may be the outcome of selection for reduced drag (Alexander 1967), an advantage in escaping from fast swimming predators. Vertebral number may also be related to fish size; the lake fish are larger, and larger fish tend to have more vertebrae (Lindsey 1962).

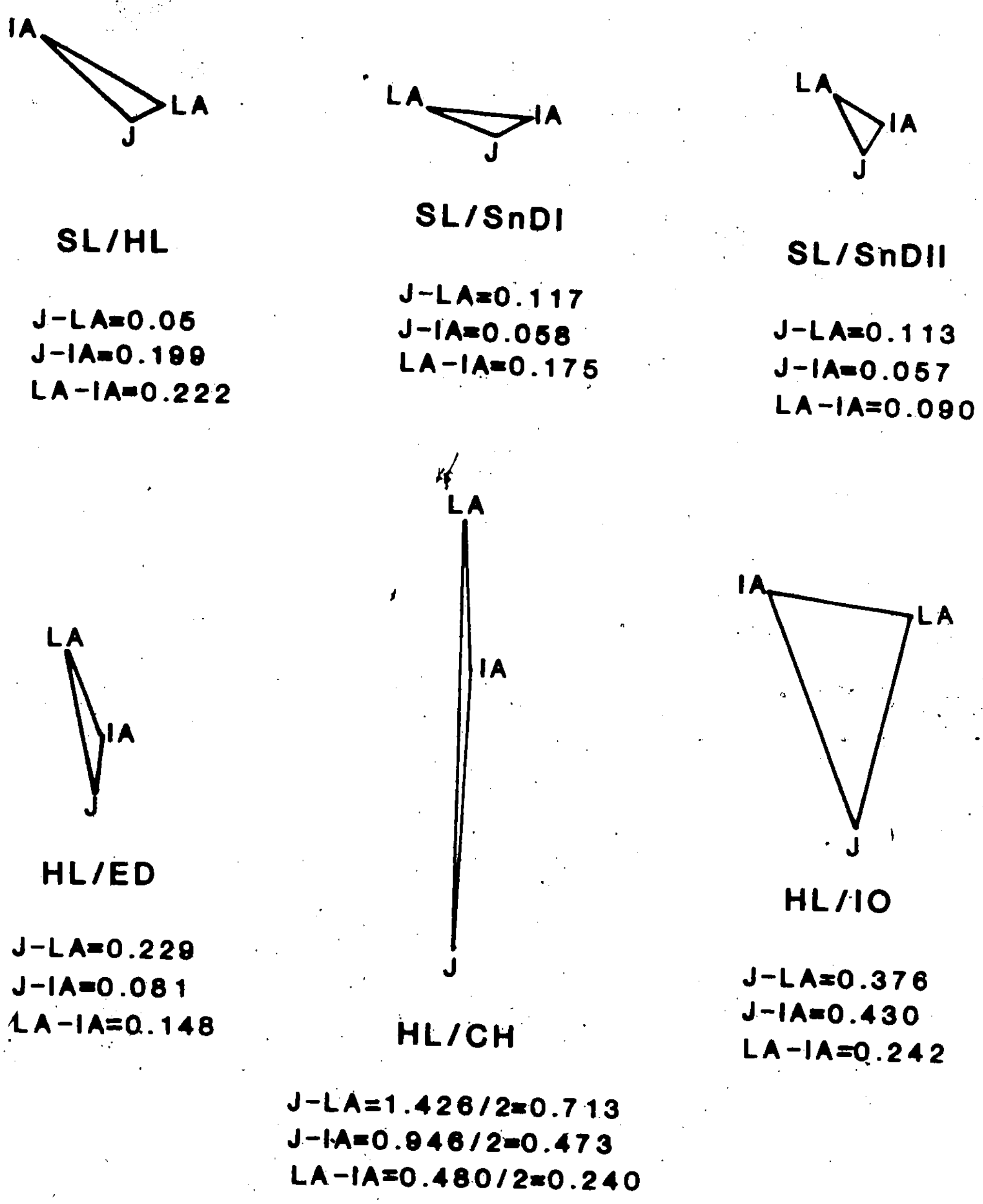
Inlet fish are also characterized by smaller and presumably weaker spines. Although only data for relative pelvic spine lengths are given, a similar situation (lake vs inlet) exists for the two prominent dorsal spines. Reimchen (1980), in a study of Boulton Lake sticklebacks (15 km south of Drizzle Lake), found a positive correlation between the density of macroinvertebrates, particularly dragonfly nymphs and the frequency of the "spineless" phenotype. In Drizzle, aeshnid nymphs are more abundant in the inlet than in the lake. The presence of stickleback vertebrae and otoliths in the fecal pellets of nymphs dip-netted from the stream confirmed this source of mortality on young-of-the-year fish in the inlet. Selection acting on this cohort could favour delayed emergence of spines which would result in shorter-spined adults. Fish with short spines in the subadult age class may be at an advantage if long spines improve the grip of the nymph on the fish (Reimchen 1980). In an experiment to test for differences between morphs in vulnerability to nymph predation, I observed nymphs capturing young fry (20 mm) in aquaria. When captured, the fish were held by the labial palps which pierced the body. Of 11 attacks, 5 were directed at the opercular region, 3 to the body below the dorsal spines and fin, and 3 to the caudal peduncle. Manipulation prior to ingestion did not involve the forelegs as was seen when larger fish (30 mm) were captured (Reimchen 1980). The presence of spines in small fry did not appear to deter the nymphs.

Bell (1974, 1976), in studies on plateless sticklebacks in southern California, and Moodie and Reimchen (1976a) suggest that the regression of defensive structures (spines and lateral plates) is related to the reduction of predation. Inlet stickleback share the stream with juvenile *Oncorhynchus kisutch* and *Salvelinus malma* which are abundant, and the less numerous *Salmo clarki*. Only *S. clarki* is known to prey on inlet sticklebacks. Analysis of stomach contents of 10 trout yielded young sticklebacks in only one, although none of the stomachs were empty. Thus, in the summer months and early fall, predation on sticklebacks by other fishes appears to be light. However, in a population of less than 300 breeding adults, predation by a few trout, or even a single grebe, could have a significant effect. In addition, stomachs of two adult male sticklebacks which had been dipnetted from the stream were full of young sticklebacks (S.L. 20 mm; 7 in one, 11 in the other). More information on frequency of cannibalism in this population is needed.

Variation in fin ray number has been shown to be at least partly under environmental control (Heuts 1949; Lindsey 1962; Hagen 1967) and to vary with individual fish size (Lindsey 1962). Fewer dorsal and anal rays in the inlet morph may be explained by the environmental differences between the two habitats (Table I), or may be the result of having a smaller fin area to support.

The adaptive significance of the differences in head morphology may be tied to feeding strategies of the two morphs which, in turn, are influenced by food types available in the two habitats. The stream is a darker environment than the lake with a very low density of planktonic organisms and a diverse macroinvertebrate fauna. Inlet fish may have to spend more time actively searching for food items. For this purpose, larger eyes positioned closer to the front of the head may improve visual acuity. Baerends, Bennema, and Vogelsang (1960; cited in Moodie 1970) demonstrated an increase in visual acuity in cichlids which was related to an increase in diameter of retinal cells. There is no mention of a concommitant increase in retinal surface area or overall eye size, but these are inferred. A larger eye, as seen in inlet fish, would presumably have a larger retina made of more cells or of cells with a larger diameter. Lower cheek height and a broader interorbital width are likely related to the three-dimensional increase in eye size.

The juveniles are not distinguishable with respect to eye diameter (Table X). Adults of both morphs have diverged from this common background, but in the lake adult the process has been carried further. Amount of change from the juvenile stage varies among characters (Tables X and XI). Figure 15 illustrates relative amounts of divergence of the two adults from the common juvenile for six characters where juveniles are indistinguishable. In general, the lake form has diverged more than the inlet form. Assuming these characters (which reflect osteological characters) have a genetic basis, an adaptive explanation can be sought for what may be the retention of juvenile characters



SL/HL

J-LA=0.05
J-IA=0.199
LA-IA=0.222

SL/SnDI

J-LA=0.117
J-IA=0.058
LA-IA=0.175

SL/SnDII

J-LA=0.113
J-IA=0.057
LA-IA=0.090

HL/ED

J-LA=0.229
J-IA=0.081
LA-IA=0.148

HL/CH

J-LA=1.426/2=0.713
J-IA=0.946/2=0.473
LA-IA=0.480/2=0.240

HL/IO

J-LA=0.376
J-IA=0.430
LA-IA=0.242

Figure 15. Geometric representations of divergence of adults of lake (LA) and inlet (IA) morph from common juvenile. Only characters where difference between juveniles not significant are shown. All figures are drawn to the same scale (.01 "ratio units" = 1 mm) except HL/CH (.01 "ratio units" = 0.5 mm).

$$\text{J-LA} = \frac{\text{IJ-LA} + \text{LJ-LA}}{2} \qquad \text{J-IA} = \frac{\text{IJ-IA} + \text{LJ-IA}}{2}$$

by the inlet form. Those in the head region (eye diameter, snout length etc.) are involved in one or more aspects of feeding (search, pursuit, capture, ingestion). If they improve the fish's efficiency in one of these areas when dealing with benthic invertebrates in a dark habitat, or if they render it a more generalized feeder, they will be retained and spread through the population. The shifting of characters from an earlier part of the ontogeny ("paedomorphosis", Dobzhansky, et al 1977; Balon 1979; "progenesis", Gould 1977) may not require major changes in structural genes (the number of necessary mutations would probably make it an unlikely occurrence), but instead could follow from a "repatterning" of regulatory genes (Dobzhansky, et al 1977).

An alternative to the differential retention of juvenile characters in the two forms is the possibility that the inlet form more closely resembles the ancestral form and it is the lake morph which is the more divergent.

Freshwater populations of *Gasterosteus aculeatus* are thought to have arisen independently from the anadromous, fully plated form (Munzing 1963; Bell 1976). In the Drizzle Lake watershed, the invasion into freshwater by this form likely occurred via two rivers and Drizzle Creek, the present outlet stream. In a preliminary comparison of inlet, lake, and outlet forms, results in Table XIII were obtained (T.E. Reimchen, unpublished data). The inlet and outlet morphs are more alike in seven of nine characters than are lake and outlet morphs. Outlet fish are also closer to inlets in colour, being predominantly brown or beige. When compared to the descendants of what may have been the ancestral form, the anadromous *trachurus* form, the lake morph is more similar in fin ray and gill raker number while the inlet is more similar in standard length and relative body depth (*trachurus* data in Hagen 1967).

The present differences between lake and inlet morph cannot be explained totally as the retention of the most likely ancestral form by one morph and the evolution of the other. Several authors have emphasized the importance of local selection, particularly in populations in western North America (Hagen 1967; McPhail 1969; Hagen and McPhail 1970; Hagen and Gilbertson 1972; Moodie 1972b; Bell 1976; Moodie and Reimchen 1976a; Reimchen 1980). Selection pressure from predatory birds, fish, and aquatic insects, and differing food availabilities may have played significant roles in the evolution of differences between lake and inlet morphs.

Table XIII

Morphometrics of *Gasterosteus* from Drizzle Lake Watershed (T.E. Reimchen, Unpublished)

	Lake (117)	Inlet (69)	Outlet (64)
Adult length (mm)	76.6	52.5	53.6
Body depth (%SL)	21.3(1.2)	22.1(1.4)	22.7(1.5)
Scutes (number-left)	4.7(1.1)	4.0(1.0)	4.8(0.93)
Gillrakers (anterior left arch)	20.6(1.1)	17.6(1.4)	17.7(1.4)
Dorsal spine (%SL)	10.6(1.0)	7.7(0.87)	8.3(0.96)
Dorsal rays	12.0(0.59)	11.1(0.85)	10.9(0.55)
Anal rays	8.7(0.62)	8.1(0.57)	7.8(0.57)
Ascending Process (W/H)	33.7(6.2)	23.4(5.1)	29.1(5.9)
Ascending Process (% forked)	47.4	18.4	18.2

Notes:- Data show mean and one standard deviation.

- Bracketed numbers beside headings are sample sizes.

Variation Within the Inlet Morph

A regression analysis of morphometric characters with distance from the lake was made because of the linear nature of the inlet and the absence of a barrier at the mouth. Two possible clines were considered but there was no *a priori* expectation regarding their existence.

A cline is a gradient in a measurable character (Huxley 1938, in Endler 1977). Endler recognizes six "structural patterns" of geographic variation, one or more of which may be exhibited by a given species. One of these, conjunction, describes the lake/ inlet situation, that is, "...distinct but contiguous groups of populations (that) are not separated by hybrid zones." (Endler 1977). It was another pattern, gradation, that was considered possible along the length of the stream. If there was, or had been, successful interbreeding between lake and inlet morph in their zone of contact (but not enough to swamp the inlet population), a higher frequency of lake or intermediate characteristics would be found near the mouth and gradually decrease upstream. The opposite pattern, a reverse cline, in which fish closest to the zone of contact resemble the lake morph less than those farther away, (character displacement, Brown and Wilson 1956), was the second possibility.

Of the few characters in which a significant correlation with distance was found, female gill raker number, relative length of second dorsal spine in males, relative pelvic spine length, and body depth are those with a strong genetic basis (Wooton 1976). The decline in mean right gill raker number with distance from the mouth seen in females in this study is similar in magnitude to that found in a southern California stream (Bell 1982). A 9.5% decline (18.9-17.1, 2 rakers) in number of rakers was found over the 450 inhabited meters of the Drizzle Lake inlet. Bell (1982) noted an 8.2% decline (21.7-19.8, 2 rakers) along the 6500 m of Brush Creek. No explanation was put forward for the decline in the latter. Whether a difference of 1-2 rakers/arch is enough to affect an individuals ability to ingest different food items is not known. The higher number of gill rakers in females in the lower reaches of the inlet may be explained as an adaptation to presumably higher plankton densities in that area (as higher gill raker number and the importance of plankton in the diet are directly related).

Although significant, correlations for the other variables mentioned are so low (<| .3|) that their biological significance is questionable.

The apparent positive trend in standard length with distance from the mouth (Fig. 13) is explained by the lower frequency of subadults in the upper reaches (Fig.5). That is, standard length appears to increase because younger fish are not found upstream.

Thus, no clines which cannot be explained as a result of local adaptations to existing environmental conditions, rather than as a result of gene flow, exist in the characters used. There is no indication of character displacement in any character and no need to seek explanations for intramorph variation beyond the inlet itself.

V. TIMING OF REPRODUCTION

If the breeding seasons of two sympatric groups do not overlap to some degree then the two forms will be reproductively isolated (Mayr 1963).

In previous studies of reproductive isolation between two forms of threespine stickleback (Hagen 1967), and between two congeneric species of dace (Bartnick 1970), temporal or seasonal isolation were found to be only partially developed. Nelson (1968) found that it was not a factor in the isolation of two congeneric species of sucker

A. METHODS

Females were used in this study because their reproductive condition was easier to assess than that of the males. For lake fish, three minnow traps were set in 1 m of water on the edge of a known nesting area for periods of two (1981) or three (1982) days each week. Sexes were separated on the basis of spine morphology and the females were initially grouped into six categories (0–5) determined by the degree of abdominal swelling. These categories were correlated with degree of ripeness of eggs removed from two females in each category. Occasionally, eggs were released spontaneously as females classified as stage 5 (prior to the eggs being released) were being examined. Numbers of fish trapped were too small to treat the categories separately and only two categories were used for the analysis: 1) no sign of abdominal swelling (including females that had spawned recently), and 2) some indication of abdominal enlargement.

A similar method of collecting inlet females was tried but a single 3-day trapping period seldom yielded enough fish to include a large number of females. Instead, every female caught in the traps set continuously in the stream was categorized in the same manner as the lake females. These data were grouped by week and provided sample sizes comparable to lake samples. This study continued from early May to mid-August in 1981, and from mid-May to late July in 1982.

Numbers of females of each morph with visibly swollen abdomens are referred to as gravid and expressed as a percentage of total number of females caught. Gravid females could be distinguished from those harbouring the cestode *Schistocephalus solidus* by the colour and position of the swelling. Infected fish had shiny white abdomens (the colour of the parasite), and the swelling associated with the infection extended anteriorly beyond the pelvic plate.

With the exception of an early nest survey on May 18-19, 1981, nests of lake males were monitored from early June to late July in both summers.

B. RESULTS

As indicated in Figure 16, the percentage of gravid females of both morphs peaked in May, 1981 and continued at a relatively high rate until mid-July of that year. In 1982, the greatest proportion of gravid females was reached about two and a half weeks later (Fig. 17) than in the previous year. The 100% values shown in mid-July, 1982 (Fig 17) are based on samples of only one or two fish.

Two nests of lake males were found in May, 1981. In early June, however, several were located. Some of those contained fry almost ready to leave the nest area (free-swimming, outside the nest itself). The time between hatch and nest-leaving was estimated to be 4.5 days (S.D.=1.8, n=10). Eggs in water of 16-18°C could be expected to hatch in 7 to 9 days (Wooton 1976). Back calculation places the start of the nesting season at about May 22-24 (and May 31 1982).

C. DISCUSSION

For the inlet morph, the delay in 1982 may be related to lower stream temperatures that year (Fig. 17). Average daily water temperatures of 9°C were reached by May 8-16 in 1981 but not until the end of May in 1982. These dates correspond to the dates of peak numbers of potentially breeding females. The same relationship is not seen in the lake. Until late July, lake temperatures were higher in 1982 than 1981 (Figs. 16, 17). Temperatures of 18-19°C reached in June, 1982 were not reached in 1981 until mid-July. Yet, the maximum breeding activity was in May, 1981 and June, 1982. Temperature data are not available for May, 1982 but it is possible that there was a sudden increase beginning May 25 (the last day of steady rain until July). Reduced runoff and full sun after that date led to higher temperatures in lake and inlet. Lake temperatures may have been cooler in early May, 1982 than in 1981 (as occurred in the inlet), thereby delaying the onset and peak of breeding.

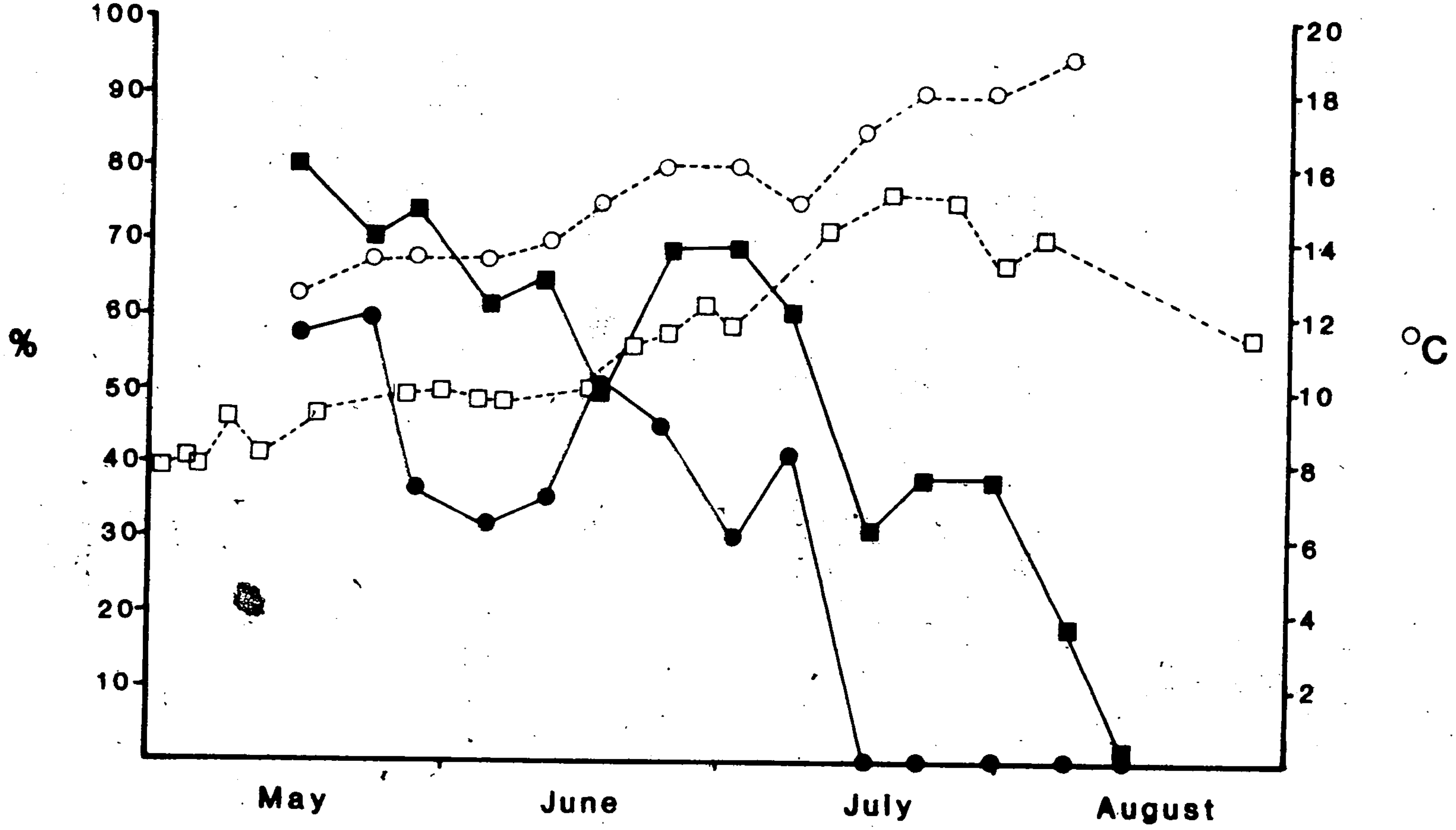


Figure 16. Proportion of females displaying indication of gravidity, and water temperatures, 1981. ■—■ = inlet females; ●—● = lake females; □--□ = inlet temp.; ○--○ = lake temp.

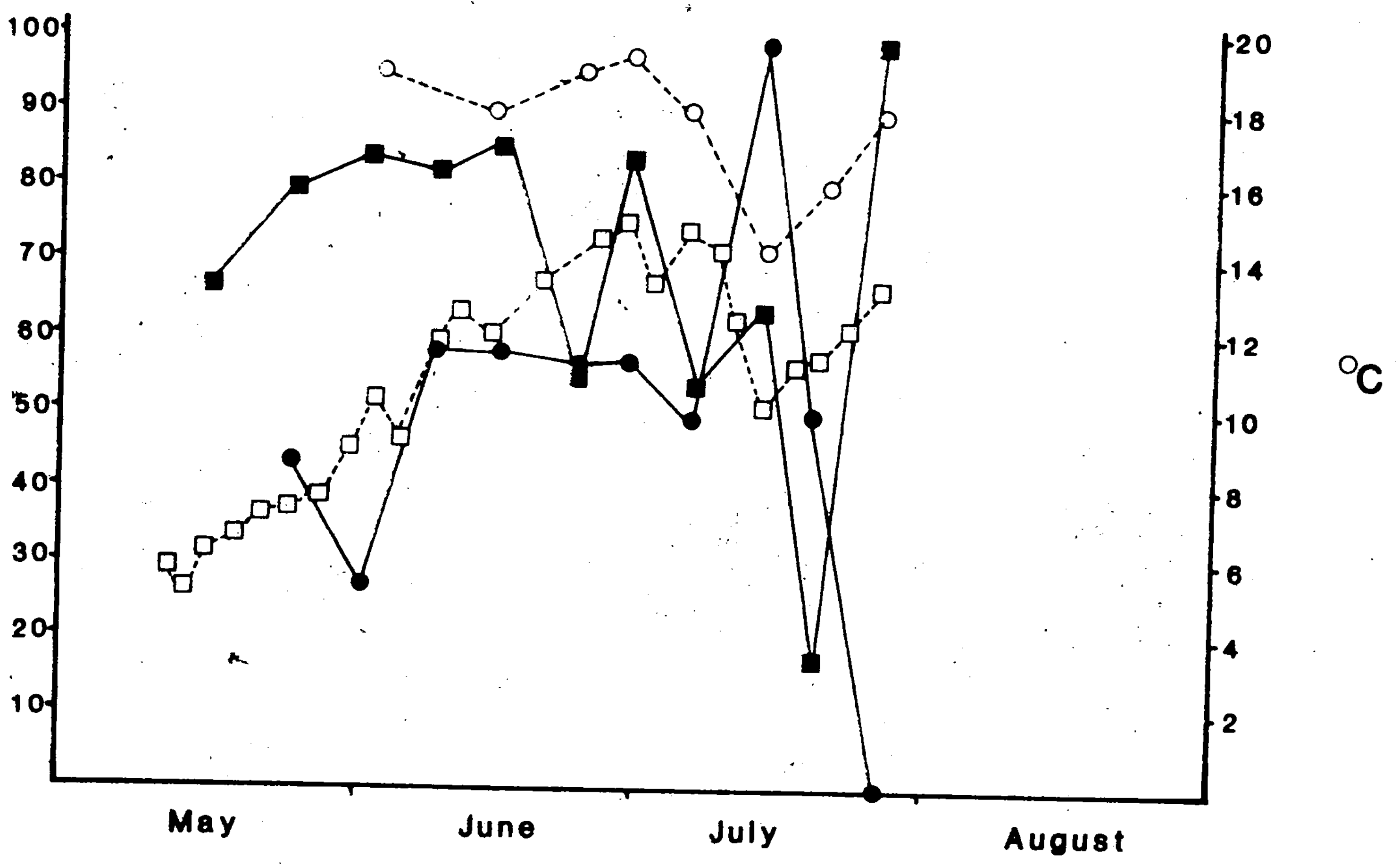


Figure 17. Proportion of females displaying indications of gravidity, and water temperatures, 1982. ■—■ = inlet females; ●—● = lake females; □--□ = inlet temp.; ○--○ = lake temp.

Summarizing several studies, Wooton (1976) states that the onset of breeding in the female depends primarily on changes in the photoperiod, permissive temperatures, and on an adequate food supply. Temperature and food supply may be correlated if threshold temperatures for spawning approximate what is optimal for stimulating reproduction in planktonic and macroinvertebrate prey faunas. Cladocerans, for example, do not actively reproduce until water temperatures reach 6-12°C (Pennak 1978).

Experimental studies in which females are subjected to photoperiod and temperature manipulations tend to employ sudden changes in one or both variables (eg. 8L 16D to 16L 8D and/or 4°C to 20°C) to induce maturation (eg. Baggerman 1972). It is difficult to relate such drastic shifts to natural conditions.

In male preference tests females are typically kept in 16L 8D photoperiod regimes with water temperatures at about 18°C (Hagen 1967; Hay and McPhail 1975) or continuous light and 16-20°C (Semler 1971). Moodie (1972a) related peaks in numbers of breeding adults to water temperature of 15-18°C in Mayer Lake(25 km south of Drizzle Lake). Numbers of potentially breeding females in the Drizzle Lake system during this study peaked at temperatures of around 10°C for the inlet and 14°C for the lake.

Estimates of dates of the start of nest building are in general agreement with those of the peaks in the proportion of gravid females.

Peaks in numbers of potentially breeding females occurred at approximately the same time for the two morphs in both 1981 and 1982. The spawning season of the inlet morph in 1981 extended beyond that of the lake (Fig. 16), but lake fish proved much harder to catch beginning in mid-July. The 0% figures are based on very small samples. If the breeding season for lakes ends earlier than that for the inlets it may also begin earlier as the lake water warms more quickly early in the spring. However, the overlap of the two reproductive seasons is virtually complete, eliminating the possibility that seasonal isolation is involved in maintaining the separation of the two morphs.

During three years of observation of the Mayer Lake population, Moodie (1972a) noted that the reproductive season began between April 30 and May 20 and ended between July 30 and August 15. Data from the present study also indicate that the breeding season lasts for about three months in Drizzle Lake for both morphs.

VI. MATE PREFERENCE

A. LIVE FEMALES

The lack of intermediate forms between lake and inlet morphs suggests that no interbreeding is taking place at present. This may be because breeding adults of the two forms seldom come into contact (as indicated by the trapping data). The question remained whether those few adults which do meet could potentially interbreed. To test the hypothesis that the lack of hybrids is an outcome of ethological isolation, I conducted male-choice mate preference tests to indicate the potential of selective mating with some females and not others.

Methods

Mate preference experiments using threespine sticklebacks are usually conducted in aquaria (Hagen 1967; McPhail 1969; Semler 1971; Hay and McPhail 1975) in which one or two males have built nests and established territories. In the present study I conducted *in situ* tests for two reasons: 1) the need to control environmental variables was eliminated as was the necessity of maintaining males in breeding condition in captivity. 2) to see whether results from the aquarium studies of others are borne out in the natural habitat.

Lake nests were easily located on snorkelling surveys. Two nesting areas were used in 1981, one in 1982. Unfortunately, no inlet nests were found in either year because of the limited visibility in the inlet. Furthermore, inlet males in breeding coloration when placed in darkened aquaria with vegetation showed no signs of nest-building activity other than sand-spitting.

Because male sticklebacks initiate the courtship sequence by approaching a female (Wooton 1976) and therefore are first to make a choice, male-choice experiments were conducted. Also, as males bear the "burden of reproductive duties" male-preference may be more likely (Moodie 1970; Wilson 1980, p.162). Female-choice experiments could not be done in the natural setting because males of two morphs do not build their nests in close proximity.

Nests containing pre-eyed eggs or newly built nests with no eggs were located and staked. A variation of the "model-bottle" method of Thresher (1979) was used to bring females close to the nests. Two females, one of each morph and at similar stages of gravidity, were each placed in a 1400 ml glass jar filled with lake water. Females to be used for the tests were kept in holding pens in their respective habitats. At the beginning of each test a male on a territory was given three minutes to resume nest-directed activity (carrying sand, fanning, etc.). Activities at the nest were observed by a diver with snorkelling gear.

The jars were placed, lid down, on the lake bottom approximately 50 cm from the nest and 40-50 cm apart. Nests used for these tests were in 30-80 cm of water. The number of approaches made by the male to each jar was counted. In July 1981, approaches were identified as aggressive or nonaggressive. They were considered aggressive if they ended in vigorous butting or biting at the jar. Very little aggressive behaviour of this type was noticed in 1982 (in only 3 of 11 males tested) and the approaches were not differentiated. An "approach" was defined as a directed movement by the males toward a jar usually ending within 5 cm of the jar. Most approaches were made from the vicinity of the nest. Each test ran for 10 minutes and usually two tests were run with a single male on one day. The position of the jars was reversed between tests to eliminate the effects of any left/right bias.

Results

In 1981 (Table XIV) the difference in total numbers of approaches to the two morphs is significant (χ^2=4.298, $P < .05$) with the lake females receiving 61.9% of all approaches. There is also a difference in the proportion of approaches that were aggressively directed at the two females (2 x 2 contingency χ^2 =8.6078 ($P < .005$). Inlet females were approached aggressively more frequently than lake females. The 1982 results were similarly significant (Table XV).

When mean number of approaches per test are compared in the 1982 data, the results support those given above. Lake females were approached an average of 14.59 times and inlet females an average of 4.48 times. Although high variances accompany these means (S.D.= 12.43 and 4.65 respectively) they were significantly different (normal approximation to the Mann-Whitney Test, Z = 7.76, $P < .05$, Zar 1974).

Table XIV

Total Number of Approaches by Lake Males, 1981

No. Males	No. Tests	To Lake Females (%)			To Inlet Females (%)		
		AGG.	NONAGG.	n.	AGG.	NONAGG.	n.
4	18	17(32.7)	35(67.3)	52	21(65.6)	11(34.4)	32

Notes: $\chi^2 = 4.298$ (χ^2 goodness of fit on totals only).

$P < .05$.

Table XV

Total Number of Approaches by Lake Males, 1982

No. Males	No. Tests	To Lake Females (%)	To Inlet Females	N
11	29	473(77.5)	137(22.5)	610

Notes: $\chi^2 = 185.08$.

$P \ll .001$.

B. MODEL FEMALES

A difference in numbers of approaches to lake and inlet females by lake males prompted the question as to what aspect of the visible differences between females of the two morphs served as a cue for the lake males. I assumed that the cue(s) must be visual, either morphological or behavioural, because any olfactory and (presumably) auditory stimuli were cut off by the use of glass jars. No obvious inter-morph behavioural differences had been observed in the females. Their response to the males appeared to be mediated by their reproductive condition. When females in a fully gravid state were used, I was more likely to see a "heads up" response to the male or vigorous swimming up the inside of the jar by both females. Those that were slightly less gravid did not respond in such a vigorous manner.

Methods

Female size and colour pattern were selected as the two most striking differences (to me) between the two morphs. Inlet females are light brown and usually mottled above, with a bronze sheen on the ventral surface, particularly on the abdomen. Mean standard length of females collected in June, 1981 was 48.16 mm (S.D. = 3.604, n=52). Lake females are grey/black with silvery countershading. Mean standard length of adult lake females collected in April, 1981 was 79.50 mm (S.D. = 3.425, n = 58).

Models have been used successfully in studies on male aggression (Tinbergen 1951; Peeke 1969) and male reproductive behaviour (Wilz 1970) in the threespine sticklebacks. In this study silicone rubber molds were made from preserved specimens of gravid females. One female of each morph was selected with standard lengths of 79.9 mm (lake female) and 46.1 (inlet female). Models were cast in tinted epoxy and painted to resemble the alternative colour patterns. Each colour pattern (black = lake, brown = inlet) was available in both sizes (big = lake, little = inlet).

Lake males were again tested on their territories by a snorkelling diver. Only males with newly constructed nests (no eggs) or with early pre-eyed eggs (white-pale gold, no evidence of embryo) in the nest were used. Models were presented in pairs such that only one of the two variables was studied, that is, two bigs, two blacks, two littles, or two browns. They were suspended from sticks by thread in a slight head up position.

Approaches to each model in a five minute period were counted, the positions of models were reversed, and a second count was made. Each double five minute session constituted one test. After each test the models were withdrawn and replaced for the next test. When presented to the male, the models hung 20–25 cm above the bottom and were positioned 30–50 cm from the nest. Moving models usually elicited a stronger response than stationary ones. Wave action supplied the motion needed in most tests although occasionally during calm periods I moved them gently in unison. Twelve males were tested in June and July, 1982.

Results

Response of the males varied considerably (Table XVI). Males whose nests or eggs appeared to be at the same stage may or may not have responded in a similar manner. The reactions of males 4b and 9c (Table XVI) were aggressive with approaches often ending in bites directed at the caudal peduncle, abdomen, and operculum of the models. The "ferocity" of these attacks was greater when directed at large-sized or black-coloured models. As indicated by the numbers of zig-zags, males 5c and 1d actively courted the models. The zig-zag is characteristic of the opening round of the courtship sequence of the threespine stickleback (Wooton 1976). Male 5c zig-zagged to the large black model when the alternative was the large brown or the little black. Male 1d zig-zagged to all the models but in each test (with one pair of models) either the black model (when size was controlled) or the big model (when colour was controlled) was zig-zagged to more often than was the alternative.

A test of heterogeneity on Table XVII prevented the data being pooled (heterogeneity $\chi^2=43.773$, $P < .001$). Those data need not be pooled, however, to provide evidence that total number of approaches to each of the four models by one male were not randomly distributed. In only one case, nest 2b, is the observed distribution not different from the expected although the calculated χ^2 is very close to the critical value ($\chi^2=7.815$).

From Table XVII the "order of preference" determined from the percentages of the overall total number of approaches (n = 713) directed toward each model is: big black (48.1%), little black (27.9%), big brown (15.0%), and little brown (9.0%). This suggests that the colour and/or the colour pattern of the female are more important as cues than

Table XVI

Response of Lake Males to Pairs of Models

		Bigs			Blacks			Littles			Browns		
Nest	Date	Blk.	Brn.	Diff.	Big	Lit.	Diff.	Blk.	Brn.	Diff.	Big	Lit.	Diff.
6a	29/06	19	5	**	11	0	**	8	0	*	9	3	NS
11	30/06	8	3	NS	2	4	NS	3	1	NS	0	0	--
1c	30/06	7	1	NS	3	1	NS	0	0	--	1	0	NS
2b	04/07	5	3	NS	2	2	--	1	0	NS	1	0	NS
3b	04/07	6	0	*	6	2	NS	Nest raid - tests cancelled					
4b	06/07	30	9	**	38	18	*	25	15	NS	23	9	*
9c	06/07	28	10	**	45	28	NS	35	9	***	5	2	NS
5c	12/07	8(6)	1	*	14(4)	0	***	2	0	NS	1	1	--
1d	13/07	28(3)	4	***	13(4)	10(3)	NS	17(9)	4(1)	**	14(4)	4	*
2c	17/07	13	3	*	12	4	NS	12	3	*	2	1	NS
7b	20/07	8	0	***	5	2	NS	6	1	NS	3	0	NS
9d	21/07	14	1	**	30	12	**	9	7	NS	8	4	NS

Notes:- Bracketed figures are number of "zig-zags."
- All dates 1982.
- Corrected χ^2 used for comparisons.
- Significance of difference from expected indicated by: NS = not significant; * = $P < .05$; ** = $P < .01$; *** = $P \leq .001$.
- Abbreviations: Blk = Black (lake colour); Brn = Brown (inlet colour); Lit = little.

Table XVII

Total Number of Approaches to Each Model When Results of Four Model Pairs are Combined

Nest	Big Black	Big Brown	Little Black	Little Brown	n	Fi	χ^2	df	P
6a	30	14	8	3	55	13.75	30.014	3	***
11	10	3	7	1	21	5.25	9.285	3	**
1c	10	2	1	0	13	3.25	19.308	3	***
2b	7	4	3	0	14	3.5	7.142	3	NS
⁄4b	68	32	43	24	167	41.75	26.364	3	***
9c	73	15	63	11	162	40.5	76.125	3	***
5c	22	2	2	1	27	6.75	46.032	3	***
1d	41	18	27	8	94	23.5	25.061	3	***
2c	25	5	16	4	50	12.5	23.76	3	***
7b	[illegible]3	3	8	1	35	8.75	33.92	3	***
9d	34	9	21	11	75	18.75	20.94	3	***
Total %	343(48.1)	107(15)	199(27.9)	64(9)	713				

Notes: ⁄ Nest 3b omitted from this table because only two pairs of models were tested.

* P < .05.
** P < .01.
*** P < .001.

size.

This conclusion is supported when results for just the two big models (black vs brown) are compared. These data can be pooled (heterogeneity $\chi^2 = 1.305$, $P > .999$) and the resulting $\chi^2 = 122.72$ ($P < .001$) clearly shows a preference existed for the large lake-coloured female over the large inlet-coloured female.

C. DISCUSSION

Most mate preference studies conducted on threespine sticklebacks have been of the female-choice type (McPhail 1969; Semler 1971; Moodie 1972b). McPhail (1969) reasoned that female mate preference is a potential isolating mechanism because breeding males court female sticklebacks and male nuptial colouring serves as an epigamic signal. A subsequent investigation of a different pair of apparently reproductively isolated populations (Hay and McPhail 1975) revealed the "active male role in mate selection." In the present study, male-choice experiments were performed *in situ* partly because it is the male which takes the initiative in the courtship sequence (Wooton 1976). Males making heterogamic choices from among non-selective females, or males whose size (for example) gives them the qualities of a super-normal stimulus (Manning 1972), and therefore "acceptable" to most females, will be at a disadvantage if selection acts against hybrids for any reason.

In male choice experiments conducted with fish of the typical freshwater *leiurus* form and the anadromous *trachurus* form, Hagen (1967) found no difference in the choice of females by males of both types. The conclusion of random mating was also reached in female choice tests. Hay and McPhail (1975) reached a different conclusion in another series of tests with fish from the same populations used by Hagen (1967). Males of the *leiurus* type chose *leiurus* females 65% of the time, and *trachurus* males chose *trachurus* females 62% of the time. The involvement of males in mate selection in threespine sticklebacks is emphasized by these results.

Manning (1972) points out that the selective advantage in choosing a conspecific (or "conmorphic") mate is particularly strong for females. While this is the case (as Manning indicates) if the relative investment of females is higher than that of males (eg. if they mate only once or by virtue of their greater energy investment in egg production), it would not apply to *Gasterosteus aculeatus* where the high cost of egg production by the female is at least balanced by long periods (two–three weeks) of parental care by the male. Therefore, the correlation between the fitness of males and the preference of females, and the correlation between female fitness and male preference (Pianka 1978) are likely both being selected for in this species. The importance of male choice cannot be underestimated.

I obtained similar results using different criteria of choice than were used in the studies described above. In this case the "conspecific" female received 62% (1981) and 77.5% (1982) of the approaches. Occasionally, males being tested approached the jars doing the zig–zag dance characteristic of their courtship phase. In every case the zig–zag terminated at the jar of the lake female even though both females may have been responding with the "head up" posture. A lower frequency of aggressive approaches to the conspecific female also supports the hypothesis of mate selectivity by the lake male. By itself, this selectivity may not be enough to reduce gene flow sufficiently. However, in conjunction with other mechanisms (which assortative mating will, in turn, reinforce) this ethological mechanism serves to isolate the two forms.

Earlier studies on mate preference in sticklebacks have shown that the typical red colour of breeding males is subject to sexual selection by females (McPhail 1969; Semler 1971). Colour, therefore, can serve as a releaser of courtship behaviour in females. Results of this study indicate that colour (and size to a lesser extent) are involved in releasing the initial approach of the male. Black models received 76% of the approaches; big models were approached 63% of the time.

The variability in the types of responses to the models (eg: courting vs aggressive) may be explained by the "state" of the males at the time of their tests. Once a male has entered the courtship phase he remains in that state until a clutch of eggs has been deposited in the nest (Wooton 1976). Following fertilization the male enters an aggressive phase and will not court another female for about one hour while he attends to his nest. This may be repeated up to seven times (Wooton 1976). It is not possible to determine exactly in which phase a male is, unless he is watched continuously from the beginning of nest building (not practical in this study). Regardless, results from fish at the two extremes (aggressive vs courtship) differ in absolute numbers but not in relative differences between models.

Another explanation for the decline in number of approaches, from big black through to little brown, is habituation. Models were presented in the following order: two bigs, two blacks, two littles, two browns. As defined by Manning (1972) "... if an animal is repeatedly given a stimulus which is not associated with any reward or punishment it ceases to respond" (pg. 177). This may explain differences between tests but does not account for the differences within tests (Table XVI). Nor does it negatively affect the conclusion that colour is the more important variable. Perhaps the strongest support for that conclusion came from the results of the first test in the sequence, big black vs big brown. In 75% of the trials the lake-coloured fish was approached significantly more often than the inlet-coloured model. In the remaining three cases the same trend is evident although differences are not significant ($P > .05$).

VII. HABITAT PREFERENCE

In order to identify aspects of the environment which may serve as cues to inlet and lake fish in their choice of habitats, two sets of experiments were performed using ~~two~~ age classes of fish. The preferences of young-of-the-year fish of both morphs were tested with respect to light, the presence of a dark vertical surface, and proximity of the substrate. Adult fish (in their third summer) were given the choice of water types (lake vs inlet), current, and vegetation.

A. FRY EXPERIMENTS, METHODS AND RESULTS

General Methods

Fry (15-25 mm S.L.) were collected from the inlet (mostly near *Sphagnum*) and from the littoral zone of the lake with dipnets. Fish were kept in shallow plastic holding trays (40 x 60 x 10 cm) floating in the stream for at least three days before each experiment was run. For all experimental situations fry of each type were placed in 45.5 litre aquaria, three at a time (i.e., three lakes or three inlets), and given 30 minutes to adjust to the tanks. Thereafter, the locations of the three fish were recorded every five minutes for 50 minutes providing ten readings for each trial. Ten trials were run for each of the two morphs. All experiments were conducted outdoors under conditions of ambient light, temperature, and rainfall.

Light/Dark

The aquarium for this experiment was wrapped in black plastic for half its length. All sides, top, and bottom at that end were covered.

As shown in Table XVIII the difference between the time spent in light and dark by the two morphs is significant. However, both forms clearly have a preference for the dark end of the aquarium. Lake fry are more strongly dark-oriented than their inlet counterparts. This was not the predicted outcome as I perceived the inlet to be a darker habitat, a result of its darker water colour, overhanging banks, shade cast by the trees and greater depth (compared to the littoral area of the lake).

Dark Vertical Surface

Only one end of an aquarium was covered with black plastic. No plastic extended across the top of the tank. In this experiment I predicted inlet fry would orient toward

Table XVIII

Response of Fry to Light/Dark Choice

	Times in Light End	Times in Dark End	Total
Lake	13	287	300
Inlet	86	214	300
Total	99	501	600

Notes: Contingency $\chi^2 = 61.46$; df = 1; $P < .001$.

Table XIX

Response of Fry to Presence of Dark Vertical Surface

	Quadrant				
	1	2	3	4	Total
Lake	118(39)	78(26)	52(17)	52(17)	300
Inlet	95(32)	55(18)	61(20)	89(30)	300
Total	213(36)	133(22)	113(19)	141(24)	600

Notes:- Contingency $\chi^2_3 = 16.88 \cdot P < .001$.

Quadrant 1 adjacent to darkened surface.
Bracketed figures are percentages.

Table XX

Orientation of Fry With Respect to Substrate

	Near the Substrate	Away from the Substrate	Total
Lake	251	49	300
Inlet	141	159	300
Total	392	208	600

Notes: Contingency $\chi^2_1 = 87.429$; $P < .001$.

the darkened surface as a protected area more strongly than lake fry because such surfaces, i.e. stream banks, are a part of their environment.

The aquarium was divided vertically into four equal quadrants. Quadrant 1 (Table XIX) is that closest to the dark end. Again, differences between the two morphs are highly significant. Contrary to the predicted response, lake fish showed a stronger tendency to stay near the darkened end than inlet fish. The latter appears to prefer the end walls to the middle area (Table XIX) but there is no difference between numbers of fish positioned at the ends ($\chi^2 = 0.136$, $P > .05$).

Substrate Orientation

The vertical surface experiment described above also presented the fish with a surface/bottom choice and the recorded fry positions were analyzed with respect to being in the top half or the bottom half of the aquarium.

The highly significant deviation from the expected values (Table XX) is accounted for almost entirely by the results for the lake fish. These were expected because the lake fry were collected from the shallows (< 0.5 m) in the lake, where they were never far above the sandy bottom. Inlet fry showed no particular orientation with respect to the substrate ($\chi^2 = 0.963$, $0.5 > P > .25$).

B. ADULT EXPERIMENTS, METHODS AND RESULTS

Water Type

Methods

A Y-shaped tank (10 cm deep, 10 cm wide, each arm 50 cm long) of plexiglass was fitted with needletype polyethylene valves (bore diam.= 3 mm) in the ends of the upper arms. A baffle at the junction of the upper arms and extending 7 cm toward the vertical arm was added to reduce turbulence at the junction. Drainage was provided by a 1-2 cm hole drilled in the floor at the lower end of the vertical arm. This was fitted with a cork stopper into which I carved channels to produce appropriate flow rates. Plastic tubing (1 cm) carried water from 22.7 litre buckets with plastic spouts at the bottom to the upper arms where flow could be controlled with the valves. Inlet water filled one bucket, lake water the other. Temperatures in the two buckets were kept within 1.5°C.

The Y-tank was adjusted to produce a gradient of 1.48 cm/m. Rate of flow was kept as low as possible (0.28 cm/sec), sufficient to maintain the difference between the two upper arms but not strong enough to produce a strong current. The two "waters" mixed in the vertical arm about 8 cm below the baffle. No mixing of water back into the arms was noted when tested with food colouring. Thus two distinct choices were offered. A similar apparatus was used by Brannon (1972) to test response to water source (creek vs lake) in sockeye salmon fry.

A fish placed in the lower end of the vertical arm was given 30 minutes to adjust to the tank. Its position and orientation were recorded at the end of that period, and every 5 minutes for 45 minutes. In addition, total time spent in each arm was noted. Only data from fish that had spent time in all three arms were analyzed. This was to ensure that only fish that were "aware" of the alternatives were included.

Fourteen inlet fish were tested, and all met the "awareness" criterion. However, 33 lake fish were tried before 14 were found that explored the whole tank. Later, an additional seven inlet fish were tested and five of these were satisfactory.

Results

Table XXI shows the differences in times recorded in each arm for the two morphs when results from all runs are totalled. Inlet fish were found in inlet water 50% of the time and spent less than 15% of the time in lake water. Lake fish frequented the inlet arm approximately one-third of the time, as would be expected if positions were determined randomly. The "preference" of the lake fish was for the vertical arm. However, lake fish, even those which did explore the entire tank, often remained still for several minutes in the vertical arm. Inlet fish typically were more active from beginning to end of any experimental period.

Both morphs spent more time in the inlet water than in the lake water (Table XXII). Differences are not significant for the lake fish ($t = 1.799$, $.05 < P < .1$) but the inlet fish did spend significantly more time in its "home" water (Mann Whitney $U = 317$, $P < .01$).

Table XXI

Response of Inlet and Lake Adults to Water Type

	Times in Inlet Water	Times in Lake Water	Times in Vertical Arm	Total
Lake	[illegible]	32	63	140
Inlet	95	28	67	190
Total	[illegible]	60	130	330

Notes: Contingency $\chi_2^2 =$ [illegible].910; P <.01.

Table XXII

Mean Amount of Time Spent in Each Water Type (Minutes)

	n	Time in Inlet Water	Time in Lake Water
Lake	14	16.75(10.76)	9.70(9.97)
Inlet	19	19.84(11.11)	6.86(4.98)

Note: Bracketed figure is one standard deviation.

Current

Methods

Although current in the stream is reduced during the summer when these tests were conducted it increases substantially in September and generally remains swift until May (T.E. Reimchen, pers. comm.).

The Y-tank as described above was used with a few modifications. The needle valves were not used; water entered over the top of the upper end of one arm directly from the plastic tubing leading from the bucket. The stopper in the drain was carved to allow a steady flow of 1.64 cm/sec with a depth of about 4.1 cm (volume of flow = 68.9 cm /s). Water flowed from the top of the left arm to the lower end of the vertical arm; the right arm offered the still water choice. Thus, twice the volume of flowing water was present. Rate of flow was determined using food colouring. Fish were introduced individually into the top of the still water arm and given 30 minutes to adjust to the tank. Again, their position was noted every five minutes for 45 minutes from the end of the adjustment period. The total time spent in still water over the experimental period was calculated. Only results from fish that had spent time in both moving and still water are included in calculations. Fifteen fish of each morph were acceptable.

Results

Expected frequencies are based on the 2:1 flowing to still volume relationship. Table XXIII indicates that a positive rheotaxis is present in inlet fish ($\chi^2=37.8$, $P < .001$). Several individuals were observed " "riding" the current near the water entry point. Lake fish apparently distributed themselves randomly in the tank with respect to the current.

Similar results are evident when observed mean time spent in flowing and still water are compared with the expected values for the two morphs (Table XXIV). Again the difference is highly significant for the inlet ($\chi^2=786.76$, $P < .01$), while that for the lake fish is not significant at the 5% level ($\chi^2=3.4594$).

Table XXIII

Response of Adults to Presence of Flowing Water

	Times in Flowing Water	Times in Still Water	Total
Lake	101	49	150
Inlet	136	14	150
Total	237	63	300

Notes: Contingency $\chi^2 = 37.8$; $P < .001$.

Table XXIV

Time Spent in Flowing and Still Water

	Time in Flowing Water (seconds)	Time in Still Water (seconds)
Lake	1852.62(1037)	847.38(1037)
Inlet	2492.82(332)	207.18(332)

Notes:- Data are mean values and one standard deviation.
- Expected values are 1807.34 seconds in flowing water and 892.66 seconds in still water.

Vegetation

Marked differences between vegetative cover in the inlet and the littoral area of the lake prompted an investigation of the importance of vegetation as a cue for distinguishing habitats.

Methods

A plexiglass tank (120 cm x 30 cm x 30 cm) was planted in two types of vegetation typical of the lake and inlet habitats. One half was a low (3–6 cm), sparse (1 plant/56 cm) planting of *Lilaeopsis occidentalis* and *Eleocharis* sp., two plants representative of the nesting area of lake sticklebacks. The other half of the tank was planted with *Sphagnum* sp. densely enough that the substrate was obscured. Heights ranged from 8 to 12 cm. The mixture of lake and rain water was kept at a depth of 24 cm (1 cm).

Ten fish of one morph were placed in the tank and again given 30 minutes to adjust. At the end of that period and every five minutes for 90 minutes the number of fish in each of four areas (two ends, upper and lower half of water column) were recorded. Four replicates with lake fish and two with inlet fish were completed.

Results

Data from lake replicates 1,2, and 4 (Table XXV) show a significant preference by the lake morph for the low, sparse "lake" vegetation (χ^2=25.2, 45.12, and 53.0 respectively, all $P < .001$). Test for heterogeneity showed that the results could be pooled (heterogeneity χ^2= .92, $.75 > P > .5$). Pooled χ^2=122.4 ($P < .001$) indicates that some aspect of the differences between the two vegetation types affects distribution of this morph in the tank. During test 3, where no preference is evident (χ^2=.005, $P > .9$), five fish dove into the *Sphagnum* immediately on introduction. All remained there for half the 90 minute test period and three were not seen at all during that period.

Table XXV

Number of Adult Fish in Two Vegetation Types
(Totals After 20 Readings)

	Replicate	*Eleocharis*	*Sphagnum*	χ^2	P
Lake	1	136	64	25.2	<.001
	2	148	52	45.12	<.001
	3	99	101	0.005	NS
	4	152	48	53.0	<.001
Inlet	1	62	138	28.125	<.001
	2	89	111	2.205	NS

Table XXVI

Substrate Visibility
Number of Lake Fish in Two Vegetation Types Offering Approximately Equal Visibility of the Bottom
(Totals After 20 Readings)

Replicate	*Eleocharis*	*Sphagnum*	χ^2	P
1	134	66	22.445	<.001
2	102	98	0.045	NS
3	142	58	66.125	<.001

Data from inlet trials are less conclusive (Table XXV). In both replicates there is a tendency toward spending more time on the *Sphagnum* side. However, only in the first trial were the results significant ($\chi^2 = 28.125$, $P < .001$).

Substrate Visibility

Several variables were combined in the vegetation preference experiment. Not only were plant species different, but heights and densities were also different. It is possible that when the lake fish appeared to be avoiding the *Sphagnum* side they were instead having a negative response to the invisibility of the substrate. A similar condition exists at the boundary between the two habitats where, because of the sudden increase in depth in the inlet, the bottom is no longer visible from the surface.

Methods

To test this hypothesis, the same tank was used as in the vegetation experiment but the *Sphagnum* was replanted to offer approximately the same amount of visible substrate as was present on the other side. This meant using fewer *Sphagnum* plants than *Eleocharis*/*Lilaeopsis* plants because of the greater coverage provided by individual *Sphagnum* plants. Data collection was as described for the vegetation preference. Three replicates of the test were conducted. As I was interested in what was keeping most lake adults out of the inlet, only lake fish were tested.

Results

Results of replicates one and three (Table XXVI) are very similar to those of the vegetation preference test (Table XXV) for the lake fish. This suggests that a variable other than density or substrate visibility, but still related to the vegetation, may be responsible for their preference for low, sparse vegetation (or their avoidance of *Sphagnum*).

C. DISCUSSION

"Every species population has its own particular habitat requirements and width of habitat tolerance – and hence its own zone of optimal importance" (Pianka 1978). Pianka was referring to distribution of plant species along moisture gradients but the above generalization could apply to any species and any appropriate environmental variable. A positive response to a particular variable or combination of variables (habitat selection) has the effect of placing the animal in an environment which imposes a particular selective regime on the organisms within it (Partridge 1978). Two similar animals selecting different habitats are subject to different suites of selective forces.

Habitat selection may depend on the genotype of the individual or on the environment in which it was raised (Maynard Smith 1962). Mayr (1963), Maynard Smith (1966), and Endler (1977) emphasize the importance of "birthplace philopatry" citing examples of migratory birds, mammals, and fish. Whether the underlying cause for differential habitat selection is under genetic control or the result of early experiences, the outcome for two populations from different niches will be the same – the two groups will be largely isolated (Maynard Smith 1966).

Habitat or ecological isolation, which may be an outcome of habitat selection, is usually first on a list of premating isolating mechanisms (Mayr 1963; Dobzhansky 1970; Merrell 1981). It is perhaps the most obvious – groups of related organisms which do not come into contact on a regular basis because of adaptations to different niches will not have the opportunity to interbreed.

In Drizzle Lake, habitat selection was tested by offering sticklebacks of both morphs samples of the two habitats in an artificial setting. This provides some information as to which variables may be important, but, as Partridge (1978) points out, not necessarily which cues the animal actually uses.

Fry Experiments

When results from the three experiments are combined, lake fish show a strongly negative phototactic response coupled with a positive bottom orientation. Inlet fry are less dark-oriented and displayed a neutral response to the bottom. These differences, though not the ones predicted, can be explained by comparing the habitats of the young-of-the-year. Lake fry school in the shallow (.5 m) littoral zone of the lake where darkness (=cover) is available as submerged stumps and logs, depressions, rocks and vegetation. While snorkelling in the lake I frequently saw fry swimming close to the bottom where their mottled colouring was effective camouflage against the sandy substrate. Inlet fry were taken from deeper water (.5–1.5 m) where the bottom is not visible through the heavily stained water. They were most easily caught near floating patches of *Juncus* or at the edge of banks of *Sphagnum*. Against such a visually diverse background their mottled colouring would have a disruptive effect. Their less pronounced response (relative to lake fry) to darkness may be the result of conflicting avoidance behaviour patterns. On one hand is the intuitively obvious search for cover to elude predatory fish and birds which may be found midstream (or the avoidance of swifter flowing water, although the number near midstream *Juncus* plants contradicts this). At the same time there could be an avoidance of the darker areas (stream banks) which are favoured by dragonfly larvae, known predators of young sticklebacks. Relative pressures exerted by the different predator groups are not known but this interpretation suggests they may be approximately equal.

Adult Experiments

All tests were conducted using fish in their third summer during the breeding season, and several were obviously in breeding condition. Results of preference tests for the most part, agree with field observations in support of ecological isolation.

Inlet fish showed a clear preference for their own water type. Since temperature, depth, current, and colour were controlled, it is likely that some difference in the water chemistry between the lake and the inlet permitted the fish to discriminate between the two. A difference in response to lake and creek water was seen in fry of sockeye salmon (*Oncorhynchus nerka*) from the Fraser River drainage in British Columbia (Brannon 1972). Emerging alevins, fry, and fingerlings all displayed a preference for lake water. Nursery sites for sockeye are lakes regardless of their incubation site (lake or stream), and this preference for lake water ensures that young salmon reach the nursery area. A follow-up experiment with the salmon fry in which the olfactory capsules were sealed, showed that such treated fry cannot select a water source, although their response to current was not affected (Brannon 1972).

In the present study, lake fish showed no preference for water type and there was a tendency for them to spend more time in the inlet water. Hagen (1967), in a water type preference test, found that both *trachurus* and *leiurus* morphs preferred the clear water of the *trachurus* habitat. Although the water types were designated by colour, presumably other chemical differences existed. In Hagen's study and this one, response of fish to water type alone would not be enough to keep the two forms apart.

The positive rheotaxis of the inlet fish is that expected of a stream dwelling fish (Allen 1969; Hynes 1970). To maintain position in a stream a fish must be able to swim against a current as well as take advantage of quiet areas to escape the fastest flowing water. This ability was shown early in the life history as subadults were more likely to move upstream than adults in the same year. Their movement was noted in September when the current had increased with the onset of heavy rainfall. In an aquarium study, Hagen (1967) observed that the *leiurus* (stream) form "rarely moved into the half of the aquarium with current." Fish used for that experiment were collected in an area of a river described as "marshy with mud bottom and dense aquatic vegetation that chokes sections of the stream in the summer" (Hagen 1967, p. 1640). Such a habitat may be virtually still for most of the year and there would be no advantage to reacting positively to flowing water. However, if staying in the vicinity of a prey fauna to which a fish is adapted requires such a response those fish which possess it will be at an advantage.

Results for the lake fish indicate a neutral response, that is, they are as likely to be attracted to an area of flowing water as they are to avoid it. Thus, current may explain the restriction of inlet fish to the stream, but it apparently does not act to keep lake fish out of the stream.

Lake fish showed a strong preference for their "own" type of vegetation in three of four trials when offered a choice. It was not density of the plants, nor was it likely to be the height. In the substrate visibility tests where degree of coverage was about the same, heights of the plants on both sides were also almost the same. At low density the *Sphagnum* plants were recumbent, not floating. In the littoral zone, which is their nesting habitat, lake males often build nests near *Eleocharis*, *Lilaeopsis*, and young *Juncus*, plants which provide very little cover, but by their leafing patterns (long and narrow) and short stature allow for the sticklebacks maximum visibility of their own and adjacent territories.

In this habitat a male could more easily see newly hatched fry or eggs which may become dislodged from the nest pit, as well as trichopteran larvae which were picked up and carried away from the nest whenever they came within 5 to 10 cm. The presence of *Sphagnum*, even in low density, would reduce visibility at the substrate level in the vicinity of the nest.

The trend in inlet fish to favour *Sphagnum* is not strongly supported but is expected in a fish for which *Sphagnum* banks may represent cover and a source of the invertebrate prey species. Additional inlet fish were not available in order to repeat these experiments.

Of the variables tested, only vegetation evoked markedly different responses for the two morphs, each one selecting the type appropriate to its habitat.

Other variables which differ between lake and inlet may play significant roles in keeping adult lake fish in the lake. Temperature, for example, may be implicated because there is an average difference of 4.2°C over the breeding season. However, as Figures 16 and 17 indicate, there are times when lake and inlet temperatures may be almost 10°C apart (early June, 1982). Under the prevailing southeast winds of April–June there is a plume of cooler water emanating from the inlet and extending 30–50 m into the lake. During nest surveys in that bay (Figs. 1, 2), lake nests were found within the area of the plume only under the following conditions: a) when on a sandbar where the water was shallower and warmer than surrounding water, or b) after a prolonged period of northwest wind during which the plume is masked by the "piling up" of warm surface water in the southeast (inlet) corner. This suggests that minimum temperature for nest-building, egg deposition and/or development may be higher for the lake fish than for the inlet.

The effect of wave action in that area could also be investigated as there are areas of otherwise apparently suitable nesting habitat for lake fish which are not used. Three lake nests further down the shore where mate preference tests were conducted were completely covered with sand after a day of particularly strong winds in July, 1981. Only one of these nests was seen again with a male nearby. The littoral area immediately beyond the mouth of the inlet was subject to wave action for one month periods during the breeding seasons in both 1981 and 1982. Lake males which happened to nest in this area would be more likely to contact inlet females than males nesting elsewhere. However, their nests would be more vulnerable to destruction and they would therefore be at a selective disadvantage.

VIII. GENERAL DISCUSSION

Morphological differences between lake and inlet forms can be explained in terms of different selective regimes in the two habitats. The lake morph resembles the "Black" morph of Mayer Lake described by Moodie (1970; 1972a,b) who gave evidence for predation as the agent of selection. In the inlet form, head characteristics are likely adaptations to a largely benthic prey population living in darkly stained water.

Geological studies reviewed by Sutherland Brown and Nasmith (1962) provide evidence that the Queen Charlotte Islands were buried by glacial ice during the Wisconsin period and Moodie (1970) and Moodie and Reimchen (1976b) consider it unlikely that isolated refugia existed. Moodie and Reimchen (1976b) believe that sticklebacks on these islands have undergone rapid post glacial evolution to their present diverse forms. If true, the variation in the Drizzle Lake watershed is the product of 8000 to 10,000 years of evolution.

Although it cannot be ruled out that the Drizzle inlet form did not originate elsewhere and be the result of a separate invasion, I argue that the two resulted from divergence within Drizzle Lake. A model that may explain how this divergence came about combines views for disruptive selection of Mather (1955) and Maynard Smith (1962, 1966). Following the isolation in the new freshwater habitat of a *trachurus*-like ancestor, elements of the Drizzle Lake population would be found in areas eventually to become the inlet stream, outlet stream, and the lake itself. These three areas would become more clearly defined (i.e., a more streamlike, less marshy inlet, and a narrower outlet), with the rebound of the land mass and consequent lowering of inland water levels combined with a reduced volume of glacial meltwater. Differences among the three habitats would intensify. For example, with the gradual buildup of peat, pH values, particularly in the inlet, would drop. Optimal phenotypes in the three habitats would begin to differentiate as effects of different selection pressures in the three areas were "felt". Individuals will be adapted to the niche in which they were raised because they themselves survived in that niche. Those passing on the tendency to raise young in the "home niche" will have a higher fitness (Maynard Smith 1966). The result would be the same if the inherited trait is an actual preference for that niche, or the tendency to return (at some point in the life cycle prior to breeding) to the habitat in which the individual was raised (Partridge 1978).

Once differential habitat selection was in effect, the fitness of heterozygotes (from inter-morph crossing) would be less than that of either homozygote and assortative mating would be selected for (Maynard Smith 1962).

Maynard Smith (1966) offered three alternatives for the genetic mechanism leading to assortative mating. Halliburton and Gall (1981) felt that the second of these,

> "...modifiers [at a second locus] might cause mating preferences based on the phenotype determined by the first locus [where alternative alleles cause the homozygotes to be adapted to two different niches]",

explained their experimental production of assortative mating in *Tribolium castaneum*. It is also a reasonable explanation for the situation in Drizzle Lake sticklebacks. Two alleles for body colour and/or colour pattern could make up the hypothetical first locus. The phenotypic expression of these may adapt the two morphs to their respective niches by making them less vulnerable to predation. At the same time, assortative mating (at least by the lake male), resulting from the action of the modifiers, is based at least in part on the same phenotypic difference (colour). The simplicity of the above is for ease of description as traits involved with assortative mating (and coloration) are not likely to be determined by alleles at a single locus (Hartl 1981).

The other two alternatives of Maynard Smith's (1966) are 1) pleiotropism, where the colour alleles themselves would cause assortative mating, and 2) that genes exist which themselves cause assortative mating regardless of the genotype at (in this example) the colour locus. Maynard Smith himself considered pleiotropism "very unlikely". The second option is less parsimonious than the one included in the model above. Selection would have to act to maintain two separate gene complexes (one for assortative mating, one for colour) in order that both could increase in frequency. If modifiers are present and increase the fitness of the optimal (eg. lake-coloured) phenotype then they will themselves be favoured (Endler 1977).

Other explanations for the existence of the two (or more) morphs are possible. The present situation may reflect secondary contact following a period of geographic isolation. However, as inlet streams drain downhill into lakes, it is unlikely that the two groups were once cut off, particularly if historic stream levels and water volumes were much higher than at present.

The presence of three forms in a sequence of discrete water bodies (outlet, lake, inlet) suggests that a series of invasions occurred. They may have been separated by time intervals long enough that the first group diverged sufficiently from the ancestral form that it was reproductively isolated from subsequent invaders. With three groups involved, two periods of time would be needed (inlet and outlet forms do not appear to be in contact). In evolutionary time, 9000 years is a short period for the completion of one speciation event (as indicated by the establishment of reproductive isolation). It is even less likely that two such events could occur in that period. A combination of serial invasions and disruptive selection would reduce the time required for the morphological divergence and establishment of reproductive isolation found in Drizzle Lake.

The hypothesis of multiple invasions could be tested by electrophoretic analysis. This technique has shown in another study (Withler 1980) that a high degree of polymorphism and heterozygosity exists in freshwater populations of threespine sticklebacks on the Pacific coast. Differences were found between morphs within a watershed. If three invasions are postulated, comparison of the genetic variation in each of the three morphs with descendants of the presumed ancestral stock (anadromous *trachurus*) should reveal three degrees of difference. More allelic differences will accumulate in those groups separated from the ancestral population for a longer period of time for reasons of time alone. Thus, the group which had been geographically isolated from the parent population for the longest period of time would be least like the ancestral form genetically. The most recent break-away group would be most like the anadromous form and the descendants of fish involved in the second invasion would be intermediate in degree of difference. However, if all present populations are the result of disruptive selection acting on a single original population, amount of difference between each of the three groups and the presumed ancestral form should be similar.

This study presents evidence for two reproductive isolating mechanisms, ecological and ethological. Both are premating mechanisms (Mayr 1963) and thus prevent gametic wastage. Neither, however, is perfect. A few males of both morphs in breeding condition have been trapped in the "wrong" habitat. Lake males did approach inlet females (albeit at a much lower frequency), and on more than one occasion the inlet female responded with a "head-up". Whether that pair could have completed a courtship-egg deposition sequence successfully is unknown. Inter-morph matings, even if only by chance, are possible.

In the summer of 1981, the effective breeding population (number of adults leaving surviving progeny, Dobzhansky et al 1977) of the inlet morph was approximately 125-150 individuals (based on the arbitrary assumption that 1/2 to 2/3 of the total adult population will leave surviving offspring). The importance of genetic drift as a determinant of gene frequencies increases as population size decreases. If the population is small enough, frequencies of alleles with differential effects on fitness will change from generation to generation mostly by random drift (Dobzhansky et al 1977). Alleles become fixed or lost by chance in fewer generations in a small population than in a large one (Futuyma 1979).

Alleles introduced into the inlet population by the interbreeding of even a few lake and inlet sticklebacks would alter allelic frequencies throughout the inlet group in relatively few generations. The introgression of the lake morph would, by genetic drift, swamp the inlet genotype resulting in the disappearance of the inlet phenotype. Presumably, the first indication of this process would be the presence of hybrids near the zone of contact. As stated previously, only one adult fish has been found which resembles what one would expect such a hybrid to look like. There must, therefore, be a barrier in effect, other than the imperfect premating mechanisms investigated in this study, that allows the inlet morph to maintain its genetic identity.

Narrow hybrid zones indicate the existence of postmating but not premating barriers (Futuyma 1979). Selection then acts to reinforce premating mechanisms to prevent gamete wastage. The liklihood of this reinforcement is directly related to the degree to which postmating barriers exist (Futuyma 1979). In Drizzle Lake, at least one premating barrier may be selected for inasmuch as the black countershaded coloration of the lake females may reduce predation.

Postmating mechanisms were not investigated in this study. Three of the four mechanisms listed earlier are possible in this situation, namely, gametic or zygotic mortality, or hybrid inviability. Sterility of adult hybrids is not considered because hybrids, if they do exist, do not survive to be recognizable.

The final determination of the mode of reproductive isolation in Drizzle Lake sticklebacks awaits an analysis of these postmating mechanisms.

IX. CONCLUSIONS

1. Threespine sticklebacks found in the inlet to Drizzle Lake differ morphologically, ecologically, and behaviourally from those in the lake itself.
2. Inlet sticklebacks are shorter and deeper bodied with relatively shorter spines and larger heads. Eye diameter of inlet fish is greater. Differences in other head characters between morphs may be related to the eye diameter, but selection favouring the retention of juvenile characters in the inlet morph may be involved.
3. There is little contact between breeding individuals of the two morphs. Ecological isolation may result from differences in habitat preference.
4. Temporal isolation is not a factor in reducing gene flow between the two morphs.
5. Ethological isolation, in the form of assortative mating by lake males, is in effect.
6. Lake males appear to use the black countershaded colour pattern (and to a lesser extent, the larger size) of lake females as a cue for selective mating.
7. Ecological and ethological isolating mechanisms are not likely sufficient to restrict gene flow to the extent noted (i.e., adult hybrids have not been found). One or more postmating isolating mechanisms are presumed to be operating.

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