THE EFFECT OF TEMPERATURE ON THE DISTRIBUTION OF THE BROOK STICKLEBACK, *CULAEA INCONSTANS* (KIRTLAND) IN THE ROSEAU RIVER, MANITOBA

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ABSTRACT

During spring, brook sticklebacks moved from deep, cold water in the main channel of the Roseau River into shallow, warm water in meltwater ponds and ditches, where they reproduced. Prior to reproduction, adults preferred the warmest available water, but avoided temperatures above 19-22° C. Post-reproductive adults were found in water above 22° C. Pre-reproductive adults moved upstream in current, although movement was influenced by water velocity, temperature and light intensity. Adults were found in deeper and colder water than young after parental behavior had ceased.

The selected temperatures of acclimatized and acclimated brook sticklebacks were determined in the laboratory in a horizontal temperature gradient. Selected temperatures were influenced by reproductive phase, age, thermal history and season. Pre-reproductive adults selected a narrower range of temperatures than post-reproductive adults. Young selected higher temperatures than adults in early summer, but there was little difference in August. In addition young were more resistant to sudden fluctuations of water temperature than adults during early summer.

Observations on the movements of both pre-reproductive adults and young in an experimental stream under varying

conditions showed that upstream movement was greater in light than in darkness and was enhanced over a particular range of temperatures, which was higher for young than adults and was similar to the ranges selected in the laboratory gradient.

Thus, water temperature and current appeared to influence the distribution and movements of brook sticklebacks.

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INTRODUCTION

Andrewartha and Birch (1954) have defined the environment of an individual as anything which influences its chances to survive or reproduce. Browning (1963), modifying an earlier classification by Andrewartha and Birch (1954), divided the environment into five components: (a) weather, (b) resources, (c) other individuals of the same species, (d) other individuals of a different species, (e) hazards. The environment affects the survival and reproduction of an individual and thus determines the distribution and abundance of individuals comprising a population. The purpose of this study was to describe the effect of temperature, part of the component weather, on the distribution of brook sticklebacks, *Culaea inconstans*.

Brook sticklebacks are distributed from eastern British Columbia, across Canada to New Brunswick, and south into Indiana. Recently an isolated population has been found in northeastern New Mexico (Nelson, 1968a). Individuals occupy a wide variety of freshwater environments, but are most abundant in cool, clear, heavily vegetated waters (Gerking, 1945; Hubbs and Lagler, 1947; Trautman, 1957; Miller, 1957; Winn, 1960; Lustick, 1963; Reisman and Cade, 1967). In southern areas of their range, brook sticklebacks are confined to spring fed streams (Trautman, 1957) and deep lakes (Nelson, 1968b). Brook sticklebacks are able to tolerate wider ranges of pH and salinity than most freshwater fish (Woolman, 1895; Cox, 1922; Hankinson, 1929; Trautman, 1957; Nelson, 1968c).

During spring, brook sticklebacks move into shallow water, where they reproduce (Winn, 1960; Reisman and Cade, 1967; Nelson 1968b). Males establish territories, build nests, court females, and guard both eggs and young until offspring are sufficiently mobile to leave the nursery area (Reisman and Cade, 1967). The movement of adults into deeper water or downstream during spring and summer, either prior to or after reproduction, has been related to rates of water flow (Applegate and Brynildson, 1952; Applegate, 1961) and temperature requirements for reproduction (Lamsa, 1963). None of the authors observed upstream movement at any time of the year. Young remain in shallow water during summer (Faber, 1967; Nelson, 1968b).

During 1967 and 1968, a wide variety of environments were sampled in the Roseau River to describe the seasonal distribution of brook sticklebacks. When densities of sticklebacks from different environments were compared, a correlation between water temperature and distribution emerged. Several hypotheses, based on field observations, were tested in the laboratory under different experimental conditions, to investigate the possibility that temperature may affect the distribution of brook sticklebacks.

LITERATURE REVIEW

This review will be concerned with the influence of temperature on fish. The discussion will be divided into two main sections:

(a) the effect of temperature on survival and reproduction of the individual,

(b) the effect of temperature on distribution of the individual.

EFFECT OF TEMPERATURE ON SURVIVAL AND REPRODUCTION

In an ecological sense, adaptations may be defined as adjustments of living systems to one or more factors of their natural environment, which result in an increase in their capacity to survive or reproduce (Kinne, 1963). A distinction is made between non-genetic adaptations, also known as acclimation or acclimatization, and genetic adaptations. Acclimation is the compensatory changes of response mechanisms which occur when environmental variables are altered under controlled laboratory conditions, while acclimatization is a similar change which occurs in nature, where a complex of environmental variables act (Fry, 1964; Hoar, 1966). The adaptations of fishes to the multiple effects of temperature so that the capacity for survival and reproduction is optimal will be discussed in this section. A similar argument was developed by Jones (1964) and the subject matter has been reviewed by Brett (1956), Kinne (1963), Fry (1964; 1967) and Hoar (1967).

Lethal Temperature

Each animal has a genetic capacity to compensate for environmental change in order to tolerate normal fluctuations. When environmental conditions exceed a certain degree of variation, stress is placed on the animal, and although it may resist for a period of time, it will eventually die (Brett, 1958). For each fish, the total range of temperature is divided into an upper and lower zone of resistance and a central zone of tolerance. The zone of tolerance is bounded above and below by the upper and lower incipient lethal temperatures respectively, at which a given percentage (usually 50%) of the tested animals are able to survive for an indefinitely prolonged exposure (Fry, 1964). The previous thermal history of an individual affects its incipient lethal temperatures, through either acclimation or acclimatization. As temperatures in the environment increase, so do the incipient lethal temperatures. Thus death from sudden fluctuations is prevented by adjusting the zone of tolerance to prevailing temperatures. Brett (1944) showed that over a period of time fishes acclimatize to the highest temperature of exposure, rather than the mean. Heath (1963) suggested that stream fishes were adapted to a diurnal temperature cycle, and tend to be more tolerant of sudden change than fishes in a more stable environment. Other factors which influence incipient lethal temperatures are: age (McCauley, 1962; 1963), season (Hoar, 1955; Tyler, 1966) or day length (Hoar and Robertson, 1959) and diet (Fisher, 1958). Other environmental variables may place stress on the fish, and thus affect lethal levels for temperature. Most fishes are genetically adapted to survive in a wider range of temperatures than those normally encountered, and there are few records of mortality in nature from extremes of temperature (Brett, 1956).

Temperature and Function

The functions of an organism may be divided into: (1) metabolism, the total of all processes which use and convert material for maintenance, repair, growth, and reproduction, and which make energy available so that the organism can continue to exist; (2) activity, the total result of integrated metabolism, such as breeding, movement, or aggression (Kinne, 1963). Metabolic rates of poikilotherms generally increase with increasing temperature, while activity rates may either increase as temperature increases or may show a decrease before lethal levels are reached (Fry, 1964).

The scope for activity, or the difference between the metabolism of an active and a resting individual, is a measure of the energy available for activity, and reaches a maximum at a particular temperature, which is dependent on acclimation temperature (Fry, 1957; Brett, 1960). Both above and below this optimum there is a temperature zone in which sufficient energy is available to provide for all essential functions (Brett, 1960). Within this zone, growth (Brown, 1957; Norris, 1963; Fry, 1964; Hurley and Woodall, 1968), cruising speed (Fry, 1947; 1964; Fry and Hart, 1948; Fisher, 1958), survival (Norris, 1963), resistance to other stress factors (Bisset, 1948; Jones, 1964), maximum consumption and maximum efficiency of conversion of food (Brown, 1957; Baldwin, 1957; Kinne, 1963), and reproductive rate (Brett, 1958; 1960) are all optimal. Thus, within this temperature zone, the chances for survival and reproduction are greatest.

The boundries of the optimal temperature zone may be altered by either age (Brett, 1960; Fry, 1964), season (Hart, 1947; Evans et al, 1962) or sexual maturity (Brett, 1960; Kinne, 1963). Several authors (Brett, 1958; 1960; Macan, 1963; Jones, 1964) have suggested that the temperature range, outside of which survival is reduced, is narrow, and that requirements for normal reproduction are even narrower.

EFFECT OF TEMPERATURE ON DISTRIBUTION

The Nature and Sensitivity of Temperature Receptors

If fish are to react to temperature in order to locate and remain within optimal temperature ranges, a sensitive temperature receptor system is required. The conclusions of reviews by Fisher (1958), Murray (1962), and Norris (1963) will be summarized in this section.

The temperature-sensitive receptors of fish consist primarily of diffusely scattered cutaneous receptors (Krause, 1923; Bardach, 1956). Lateral line nerves were also suggested as a second source of thermal information (Dijkgraaf, 1940; Sullivan, 1954), but are temperature-sensitive nociceptors, and incapable of discriminating between different stimuli (such as vibrations or temperature) (Murray, 1962). Dijkgraaf (1940) suggested that temperature changes rather than absolute temperatures acted as stimuli, while Bardach (1956) and Fisher (1958) proposed that fish must be able to respond to absolute temperatures.

Observations on the detection of temperature differentials are from two sources (Harden Jones, 1968): (1) the minimum stimulus fish react to in natural conditions, (2) the minimum stimulus fish can be trained to respond to by conditioning.

Collins (1952) showed that Pomolobus sp reacted to temperature differentials of 0.5° C during migration.

Breder (1951) thought that schools of *Jenkinsia lamprotaenia* reacted sharply to temperature differentials as small as 0.10° C. Powers (1915) found that *Clupea pallasi* reacted to differentials of 0.2° C in a temperature gradient.

Bull (1936) trained 19 species of marine teleosts to swim upstream in a trough when water temperatures changed by 0.03° to 0.07° C. Bardach and Bjorklund (1957) trained 5 species of freshwater teleosts to react to temperature differences of 0.05° C.

Norris (1963) showed that acclimation to a constant temperature reduced the sensitivity of *Girella nigricans* to temperature change, and suggested that a loss of temperature sensitivity might explain the movement of *Pomolobus pseudoharengus* into lethal temperatures during early spring (Graham, 1956), despite an ability to detect small temperature differentials (Collins, 1952). Brett (1956) suggested that the ability of fish to perceive small temperature gradients is only exercised under conditions of either internal drive, such as migration, or stress, such as near zones of resistance.

Behavioural Responses to Temperature

Fish appear to have the sensory capacity to detect and stay within optimal areas. This section will discuss behavioural responses to temperature stimuli which guide individuals to such temperature zones.

Fry (1947) stated that temperature could act as a directive factor in the ecology of an animal. In a temperature gradient in the laboratory, fish will select a temperature range which is related in their distribution in the gradients found in nature. The mean temperature chosen by several individuals in a laboratory gradient is termed either the selected or the preferred temperature. Fry (1958) and Norris (1963) have reviewed experimental procedures, while Brett (1956), Norris (1963) and Fry (1964; 1967) have discussed experimental results.

When selected temperatures are plotted against acclimation temperatures, there is a point at which both are equal; the final preferendum. It is assumed that if fish are left in a gradient for a long period of time they will eventually gravitate towards the final preferendum. The final preferendum has been related to the optimal temperature for: maximum spontaneous movement and growth of trout, *Salvelinus fontinalis* (Baldwin, 1957; Fisher and Sullivan, 1958; McCauley, 1958), maximum cruising speed and growth of goldfish, *Carassius auratus* (Audigé, 1921; Fry and Hart, 1948), maximum distance moved by salmon, *Salmo salar*, and trout, *S. fontinalis*, in response to a stimulus (Fisher and Elson, 1950), and development of resistance to lethal temperatures and growth of guppies, *Lebistes reticulatus* (Gibson, 1954; Gibson and Hurst, 1955; Tsukuda, 1960). Thus fishes, through their behaviour, may select temperatures within their optimal zone for metabolism and activity, where chances for survival and reproduction are optimal.

Several factors may influence the temperatures selected: acclimation and acclimatization to temperature (Brett, 1956; Fisher, 1958; Fry, 1964; 1967), age (Ferguson, 1958; Norris, 1963; Hurley and Woodall, 1968), season (Sullivan and Fisher, 1953), light (Sullivan and Fisher, 1954; Ferguson, 1958), time of day (Heath, 1963), feeding behaviour (Brett, 1952; Pearson, 1952), social behaviour (Pearson, 1952), and degree of starvation (Javid and Anderson, 1967). The effect of these factors is to shift both selected temperatures and the boundries of the optimal temperature zone in the same direction.

There is disagreement on the nature of the response which guides fishes to their temperature preferendum (Fry, 1964). Fisher and Sullivan (1958) reported a peak in spontaneous activity of _S. fontinalis_ at the preferred temperature, with decreasing activity on both sides and a second peak near upper lethal temperatures. Ivlev (1960) found a minimum of spontaneous activity of _S. salar_ and _Cyprinus carpio_ at their selected temperatures. Fry (1964) suggested that differences in experimental technique could explain the contradictory results, but differences between species may also exist. Both results suggest a thermokinetic response. Rozin and Mayer (1961) conditioned goldfish, *C. auratus*, to regulate water temperatures in their aquarium by pressing against a bar. Goldfish preferred a particular temperature, which suggests that temperature selection cannot be explained on the basis of locomotion alone.

Temperature and Distribution in Nature

If temperature may act as an environmental cue to guide fishes to optimal temperature zones, where chances for survival and reproduction are greatest, the distribution and movements of fishes in nature should be related to temperature. This subject has been reviewed by Gunter (1957), Ferguson (1958), Northcote (1962), Norris (1963) and Harden Jones (1968).

A relationship between temperature and distribution has been shown for (1) the migration and movements of the cisco, *Leucichthys artedii* (Fry, 1937); the rainbow trout, *Salmo gairdneri* (Northcote, 1962; 1969); the Atlantic mackeral, *Scomber scombrus* (Sette, 1950); the cod, *Gadus gadus* (Mackenzie, 1956; Templeman and Fleming, 1965; Templeman and May, 1965); the haddock, *Melanogrammus aeglefinus* (McCracken, 1965; Templeman and Hodder, 1965); young opaleye, *Girella nigricans* (Norris, 1963); young pink salmon, *Oncorhynchus gorbuscha* (Hurley and Woodall, 1968). (2) the depth distribution of large mouth bass, *Micropterus salmoides*, walleye, *Stizostedion vitreum*, and sauger, *Stizostedion* *canadense* (Dendy, 1945); lake trout *Salvelinus namaycush* (Rawson, 1961).

This is a brief list, and more complete records can be found in reviews by Gunter (1957), Ferguson (1958) and Norris (1963).

The selected temperatures of fishes in gradients in the laboratory have been related to their observed distribution in nature. Ferguson (1958) compared the temperature preferendum of 19 species of freshwater fishes to the temperature of their midsummer distribution, and found that in only four species was there any similarity. The other 15 species selected a higher temperature in the laboratory than in nature, which was attributed to the use of young fishes in the laboratory, while observations in the field had been made on adults. The distribution of young opaleye (Norris, 1963) and young pink salmon (Hurley and Woodall, 1968) has been closely related to their temperature selection during various phases of growth. Alabaster (1962) found that the behaviour of adult roach, *Rutilus rutilus*, in heated effluents,was related to their selected temperatures, determined in large outdoor tanks.

In summary, temperature may act as a cue, guiding fishes to areas where survival and reproduction are optimal, and thus determining their distribution and affecting their abundance.

DISTRIBUTION OF BROOK STICKLEBACKS IN THE ROSEAU RIVER

STUDY AREA

The Roseau River rises in northern Minnesota, flows northwest through southern Manitoba, and enters the Red River near Dominion City, 97 km south of Winnipeg (Figure 1 A). The river passes through agricultural and marsh land in Manitoba, and in most parts is deep and slow moving throughout the year. Within the study area (Figure 1 B), there is a mean gradient of approximately 0.4 m/km, producing a series of riffles and pools during summer and fall, when the volume of water flowing is low (Figure 2). This results in a wider variety of environments than is found in other sections of the river. A number of stations within the study area were sampled, to describe the distribution of brook sticklebacks.

MATERIALS AND METHODS

During 1967, a wide range of environments was sampled every two weeks from March to October, at five stations within the study area. Fish were caught with a 1 meter seine (5 meshes/cm), preserved in 10% formalin, and later transferred to 40% isopropyl alcohol. The area sampled was

Figure 1:

A Map of southeast Manitoba, showing location of study area on Roseau River.

B Map of study area, showing location of stations sampled during 1967-68. Substations of station 5 (51-56) are also shown.

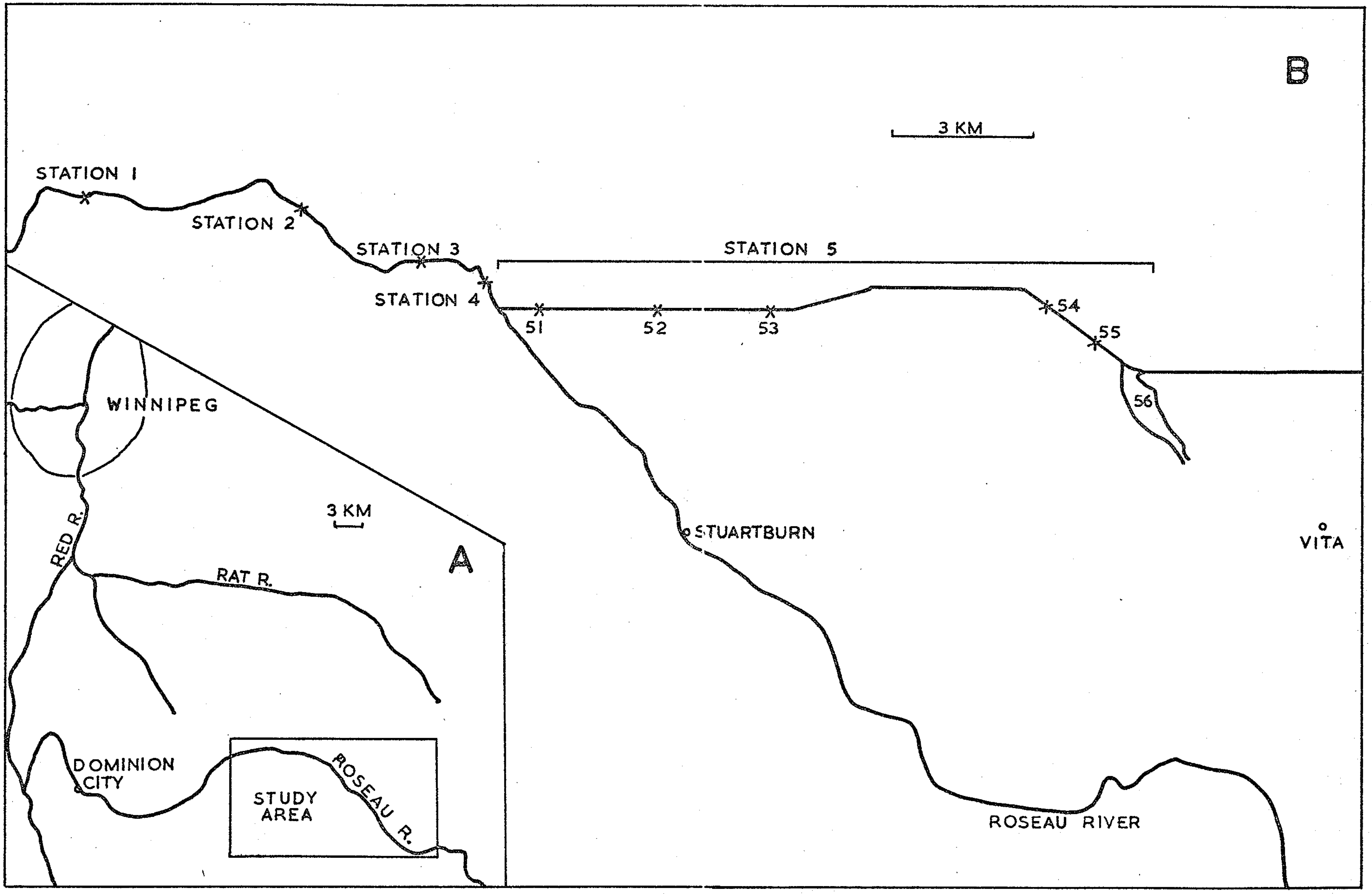
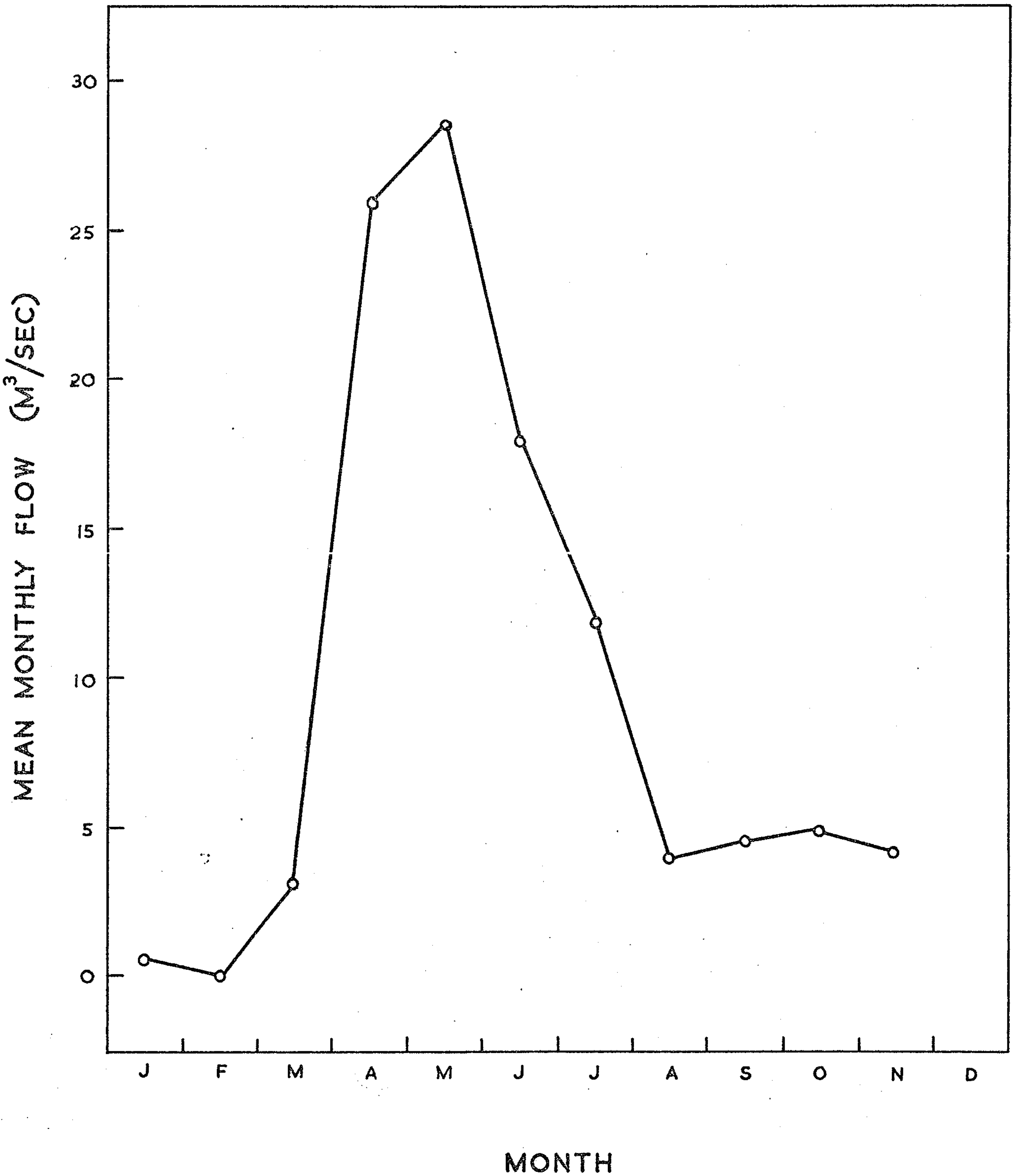

B
3 KM
STATION 1
STATION 2
STATION 3
STATION 4
STATION 5
51
52
53
54
55
56
STUARTBURN
VITA
ROSEAU RIVER
WINNIPEG
3 KM
A
RED R.
RAT R.
DOMINION CITY
STUDY AREA
ROSEAU R.

Figure 2: Long term mean monthly flows for Roseau River. Adapted from Province of Manitoba, Department of Mines and Natural Resources, Water Control and Conservation, Major Streams in Manitoba: Roseau River.


MEAN MONTHLY FLOW (M³/SEC)
30
25
20
15
10
5
0
J
F
M
A
M
J
J
A
S
O
N
D
MONTH

measured so that densities of each species could be calculated in numbers per unit area. To investigate the influence of environmental factors on the distribution of brook sticklebacks a number of variables were measured with each collection. Water velocity was measured on the surface by timing the movement of a floating object over a known distance. Water temperature was measured near the substrate with a variable resistance thermometer. Substrate, depth, turbidity, and vegetation were also measured. The area seined in each collection was as homogeneous as possible with respect to each of the above environmental variables.

During 1968, field studies were concentrated on the effect of the environment on the distribution and movements of a local population of brook sticklebacks in a pond and ditch at substation 56 (Figure 3). Ten sites along the ditch (560 to 569) were seined, to describe the distribution of sticklebacks. The method of seining and measuring environmental variables was the same as in 1967, except that fish captured were not preserved, but were counted and released.

An upstream-downstream trap (Figure 4) was placed at site 567 in the mouth of a shallow side channel, where the side channel flowed into the main ditch. The trap, constructed from plywood and screening (3 meshes/cm), was 45.7 cm long, 38.1 cm wide and 30.5 cm deep. Fish moving either

Figure 3: Map of substation 56, showing location of sampling sites (560 to 569) and position of upstream - downstream trap at junction of side channel and main ditch.

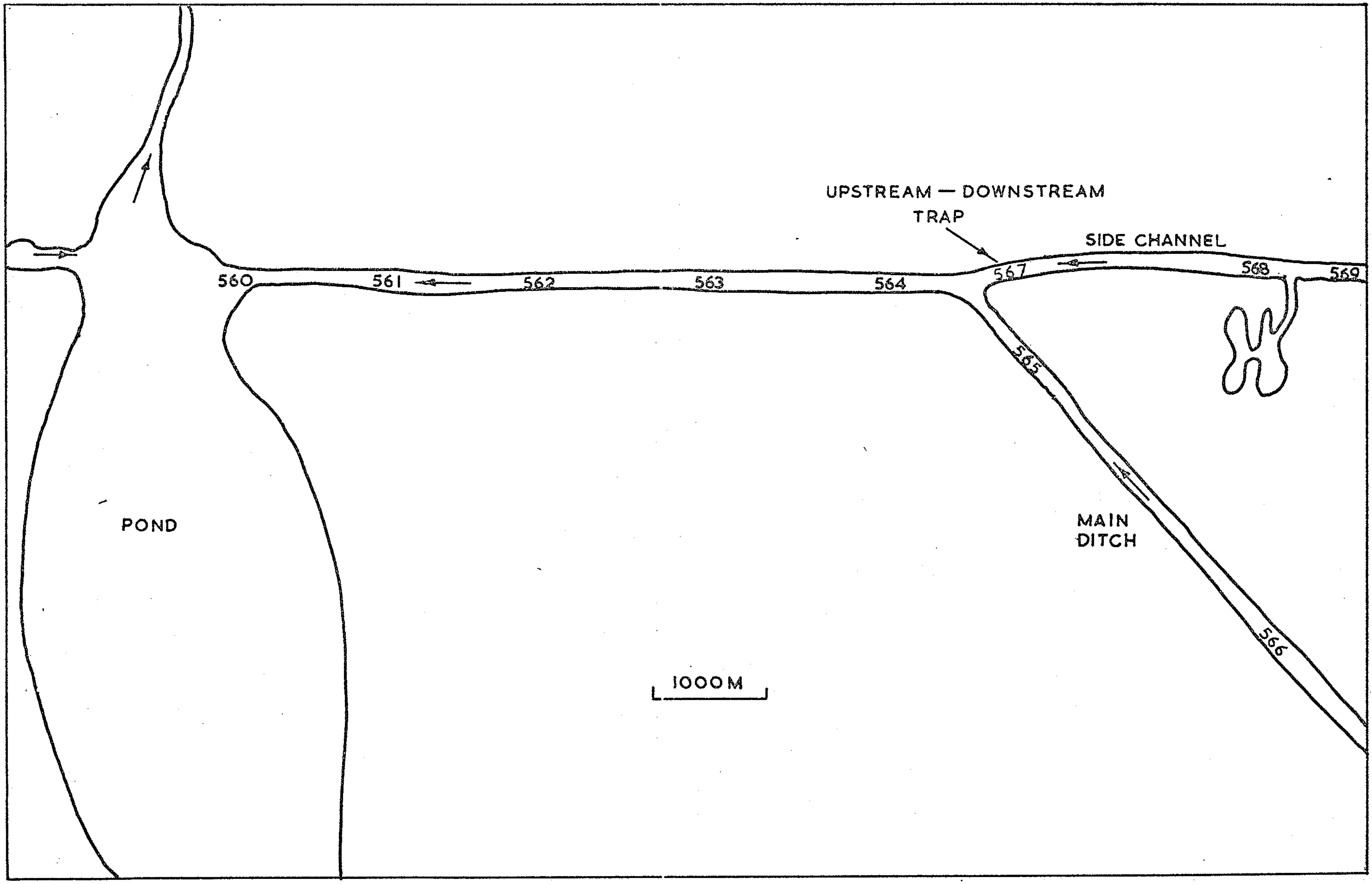
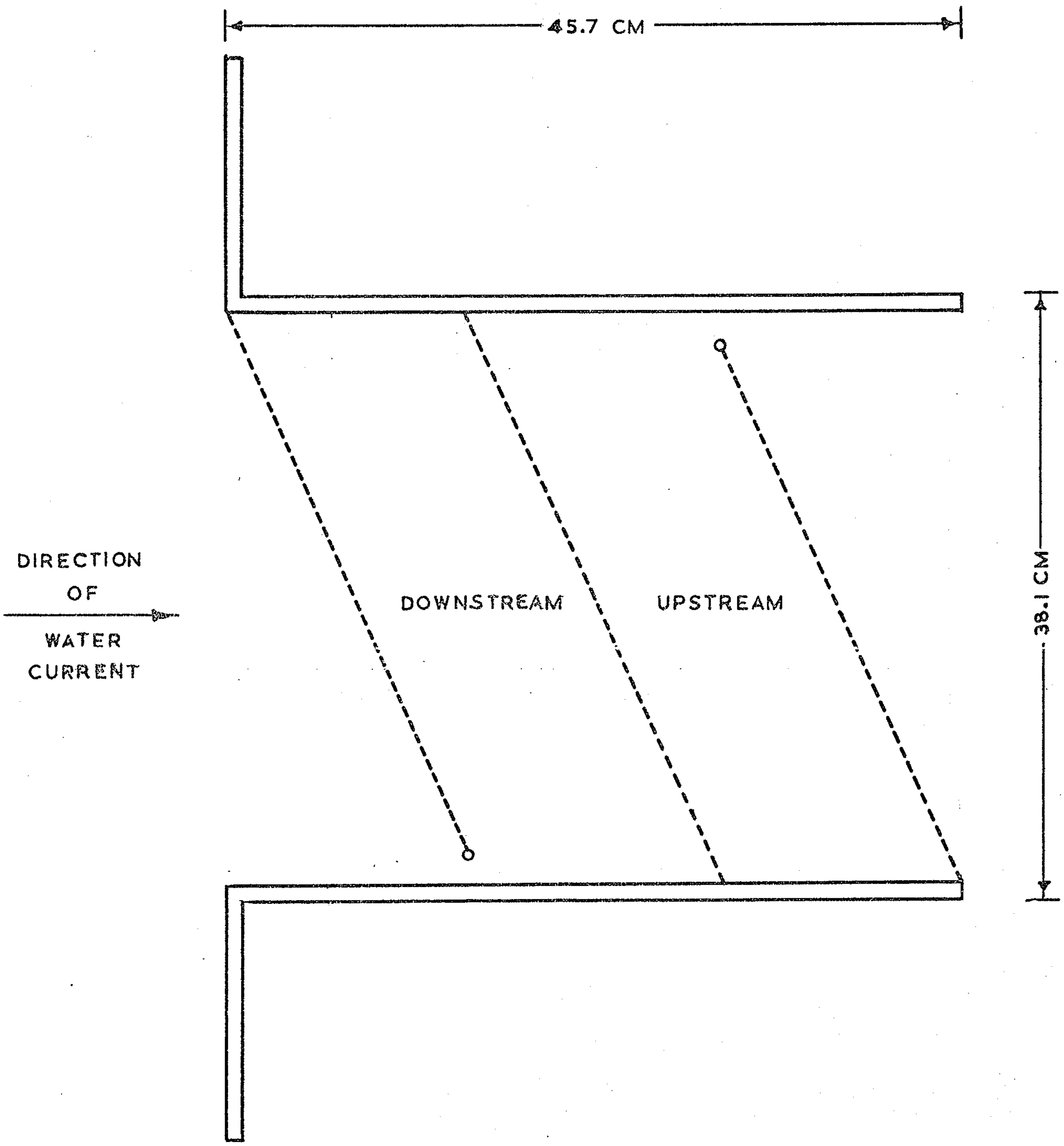

UPSTREAM — DOWNSTREAM
TRAP
SIDE CHANNEL
560
561
562
563
564
565
566
567
568
569
MAIN
DITCH
POND
1000M

Figure 4: Upstream - downstream trap used to record movements of brook sticklebacks in current. Dotted lines indicate screening.


45.7 CM
DIRECTION
OF
WATER
CURRENT
DOWNSTREAM
UPSTREAM
38.1 CM

TOP VIEW

upstream into the side channel or downstream into the ditch were caught in the trap. Fish were removed from the trap and counted at 6 a.m. and 7 p.m. each day. Those trapped in the downstream portion of the trap were replaced just downstream, while those in the upstream portion were replaced several yards upstream from the trap. Water temperatures in the side channel were measured with a maximum-minimum thermometer placed just below the trap.

Observations of movements of brook sticklebacks at the junction of the side channel and the main ditch were made on April 27 from 10 a.m. to 6 p.m., while water temperatures in both areas were monitored with a variable resistance thermometer. Notes were made of the distribution of sticklebacks at substation 56 during late spring and summer.

RESULTS

Although several environmental variables were measured, the results of the field study presented here will concentrate on the effect of water temperature and current on the distribution of brook sticklebacks. Other variables are not dismissed, and may affect distribution, but will not be described. The results of the field study will be presented separately for 1967 and 1968.

Distribution of Sticklebacks at Stations 1 - 5, 1967

The distribution of brook sticklebacks in the Roseau River was not random during the spring of 1967. Sticklebacks were abundant at stations 1, 3 and 5, but were rare or absent from stations 2 and 4 (Table I). Higher densities occurred near runoffs, which drained meltwater ditches and ponds in nearby fields into the river. Runoffs were present at stations 1, 3 and 5, but not at stations 2 and 4.

The runoffs usually discharged water warmer than that in the river. The shallow meltwater ponds and ditches, drained by the runoffs during spring, were more influenced by fluctuations of air temperature than the river, and thus tended to be warmer, especially during the day. Sticklebacks appeared to be abundant in the runoffs only when water temperatures of the runoffs were higher than those of the river. Few sticklebacks were found at substations 51 - 53 of station 5, a large drainage ditch or runoff which emptied meltwater ponds into the river, on May 5, 11 and 18 (densities from 0 to 0.3 $/m^2$), when water temperatures in the ditch were either lower than or nearly equal to those in the river (Table II A). Sticklebacks were abundant in the same area on May 23 and 26 (14.3 and 25.1 $/m^2$, respectively), when water temperatures in the ditch were higher than those in the river. On June 5, temperatures in the ditch (22° C) were again higher than those in the river but no sticklebacks were caught in the ditch. Sticklebacks were found in a runoff which drained a series of roadside ditches into the river at station 3 on April 7 and May 23, when temperatures in the runoff were

Table I: Monthly density of brook sticklebacks collected from stations 1 to 5 during Spring 1967. Densities are calculated from the total area sampled and the total number of sticklebacks caught during the month.

Month	Station 1		Station 2		Station 3		Station 4		Station 5	
	Area Seined (m^2)	Stickle-back Density (Number/m^2)	Area Seined (m^2)	Stickle-back Density (Number/m^2)	Area Seined (m^2)	Stickle-back Density (Number/m^2)	Area Seined (m^2)	Stickle-back Density (Number/m^2)	Area Seined (m^2)	Stickle-back Density (Number/m^2)
March	0	--	0	--	0	--	18.7	0.1	0	--
April	20.1	13.3	3.3	0	17.9	66.7	0	--	0	--
May	18.9	10.8	14.5	0	36.9	0.1	35.7	0	123.1	2.3

Table II: Relationship of mean density of sticklebacks in runoffs at stations 5 (A) and 3 (B) to water temperatures in the runoff and the river. Mean densities are calculated from the total area sampled and the total number of sticklebacks caught. For station 5, results from substations 51 - 53 were combined.

A

Date	Area Seined (m^2)	Stickleback Density (Number/m^2)	Temperature ° C	
			Runoff	Roseau River
May 5	9.75	0.1	5.0	5.6
May 11	21.98	0.3	6.1	7.2
May 18	17.66	0	12.2	11.7
May 23	9.57	14.3	15.0	11.7
May 26	63.66	25.1	18.3	13.3
June 5	21.93	0	22.2	18.3

B

April 7	7.16	52.4	6.1	0
April 23	1.86	0	2.2	2.2
May 5	9.01	0	5.0	5.0
May 23	11.15	0.3	10.0	7.2

higher than those in the river (Table II B). No sticklebacks were caught in the runoff on April 23 and May 5, when temperatures in the runoff and the river were the same. Thus, the movement of brook sticklebacks from the river into the runoff appeared to be related to higher water temperatures in the runoff than in the river, although extremely warm water may be avoided, as on June 5 at station 5.

Densities of sticklebacks in different areas of the runoffs at stations 1, 3 and 5 appeared to be correlated with water temperature. Results at each station will be presented separately.

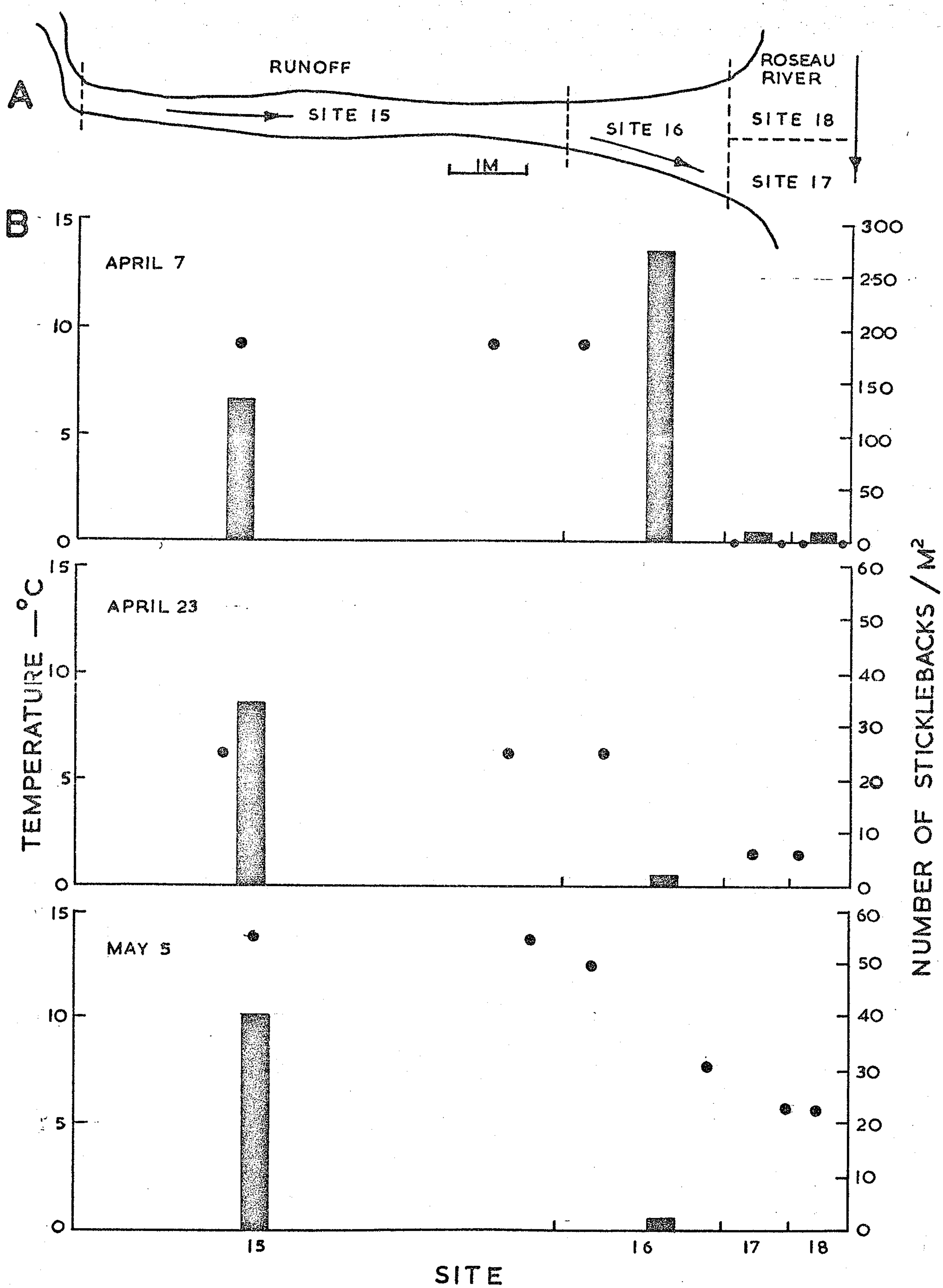
Station 1

A series of meltwater ponds drained through a narrow runoff into the river at station 1. Water velocity in the runoff was uniform and less than 8 cm/sec. On April 7, the highest density of sticklebacks (250.7 $/m^2$) was found at the mouth of the runoff (site 16), where runoff and river water mixed, so that water temperatures were intermediate between those in the runoff (7.2° C) and the river 0° C) (Figure 5, Table A-I of Appendix A). Large numbers of sticklebacks (119 $/m^2$) were also found in the runoff (site 15), but few were caught in the river, either downstream (site 17, 0.6 $/m^2$) or upstream (site 18, 0.2 $/m^2$) from the runoff. The ponds which drained into the runoff were carefully searched, but no sticklebacks were seen. On April 23, the

Figure 5:

A Diagram of runoff at station 1, showing sites 15 to 18.

B The relationship between density of sticklebacks and water temperature at sites 15 - 18 of station 1 on April 7, April 23, and May 5, 1967. Temperature, represented by solid circles, was measured for each seine haul. Mean density for each site is represented by vertical bars.



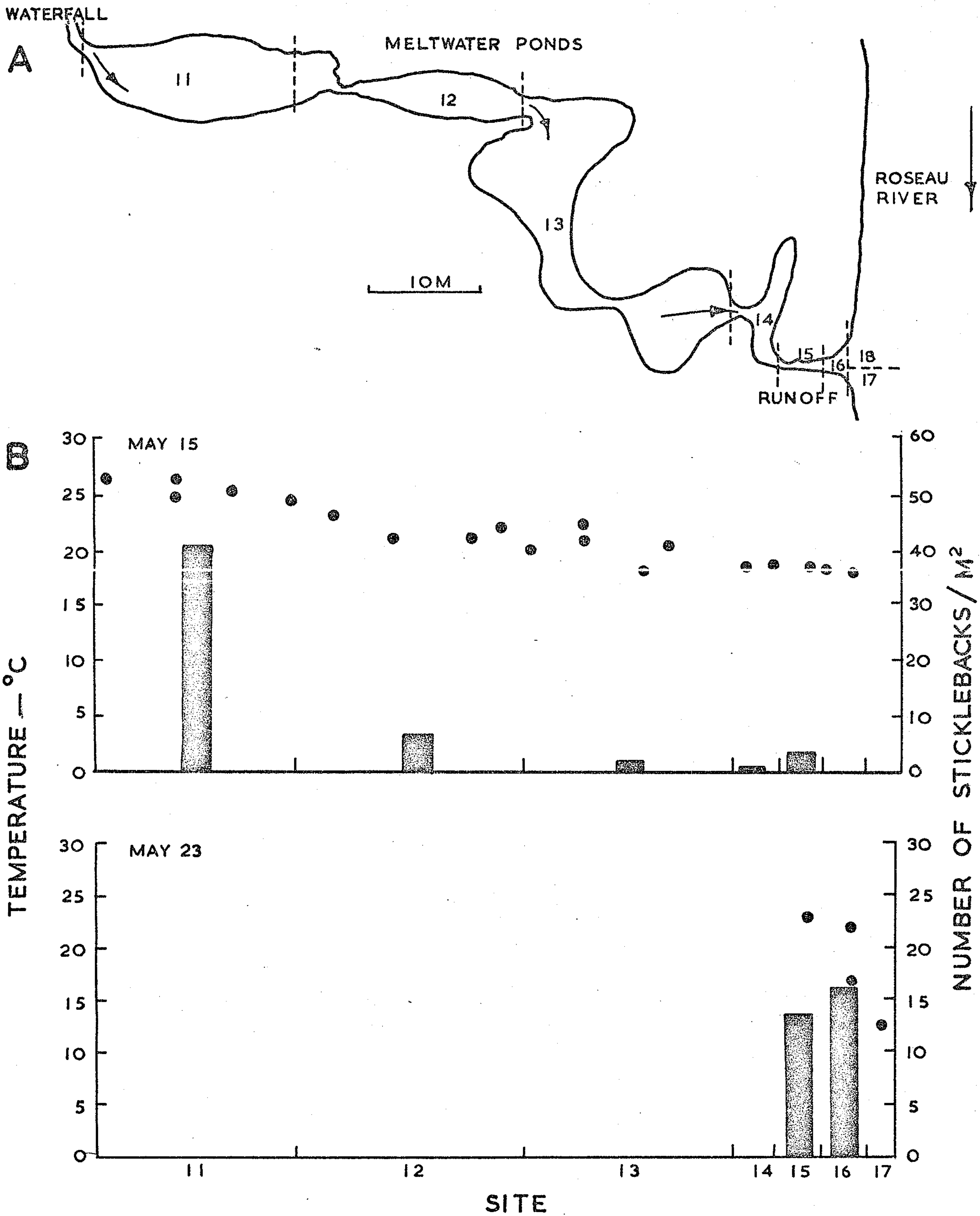
A
RUNOFF
ROSEAU RIVER
SITE 15
SITE 16
SITE 18
SITE 17
1M
B
APRIL 7
APRIL 23
MAY 5
TEMPERATURE — °C
NUMBER OF STICKLEBACKS / M²
SITE
15
16
17
18

highest density of sticklebacks (32.1 /m^2) was found in the runoff (6.1° C), few (0.2 /m^2) were caught in the mouth of the runoff, and none were collected in the river (1.7° C). On May 5, sticklebacks were again concentrated (37.7 /m^2) in the runoff (14.4° C), while few (1.3 /m^2) were found at the mouth of the runoff and none were taken from the river (5.6° C). On May 14, large numbers of sticklebacks were observed in the meltwater ponds upstream from the runoff for the first time, and the ponds were extensively seined the next day. Water flowed into the pond farthest from the runoff (site 11) from a small waterfall, which acted as a barrier to fish (Figure 6 A). Water velocities varied from 0 cm/sec in large areas of the ponds to 1 cm/sec in the shallow areas connecting each pond. Water temperatures increased gradually from the runoff to the waterfall, and the highest density of sticklebacks was found in the warmest water (Figure 6 B). Nests containing eggs were also found. On May 23, the runoff was seined and the highest density (16.1 /m^2) was found in the mouth of the runoff. Considerable numbers were still found in the runoff (22.8° C), but none were caught in the river (13.3° C). Both adults and young were taken from the ponds in late July, while the runoff had dried up earlier that month. Thus at station 1, brook sticklebacks moved upstream from the river into the meltwater ponds, where they reproduced. During this movement,

Figure 6:

A Diagram of meltwater ponds and runoff at station 1, showing sites 11 to 18.

B The relationship between density of sticklebacks and water temperature at sites 11 - 17 on May 15 and sites 15 - 17 on May 23, 1967. Temperature, represented by solid circles, was measured for each seine haul. Mean density for each site is represented by vertical bars.



A
WATERFALL
MELTWATER PONDS
11
12
13
14
15
16
17
18
ROSEAU RIVER
10M
RUNOFF
B
MAY 15
MAY 23
TEMPERATURE — °C
NUMBER OF STICKLEBACKS / M²
SITE

sticklebacks appeared to prefer the warmest available water, although the collections on May 23 suggested that high temperatures may be avoided prior to reproduction.

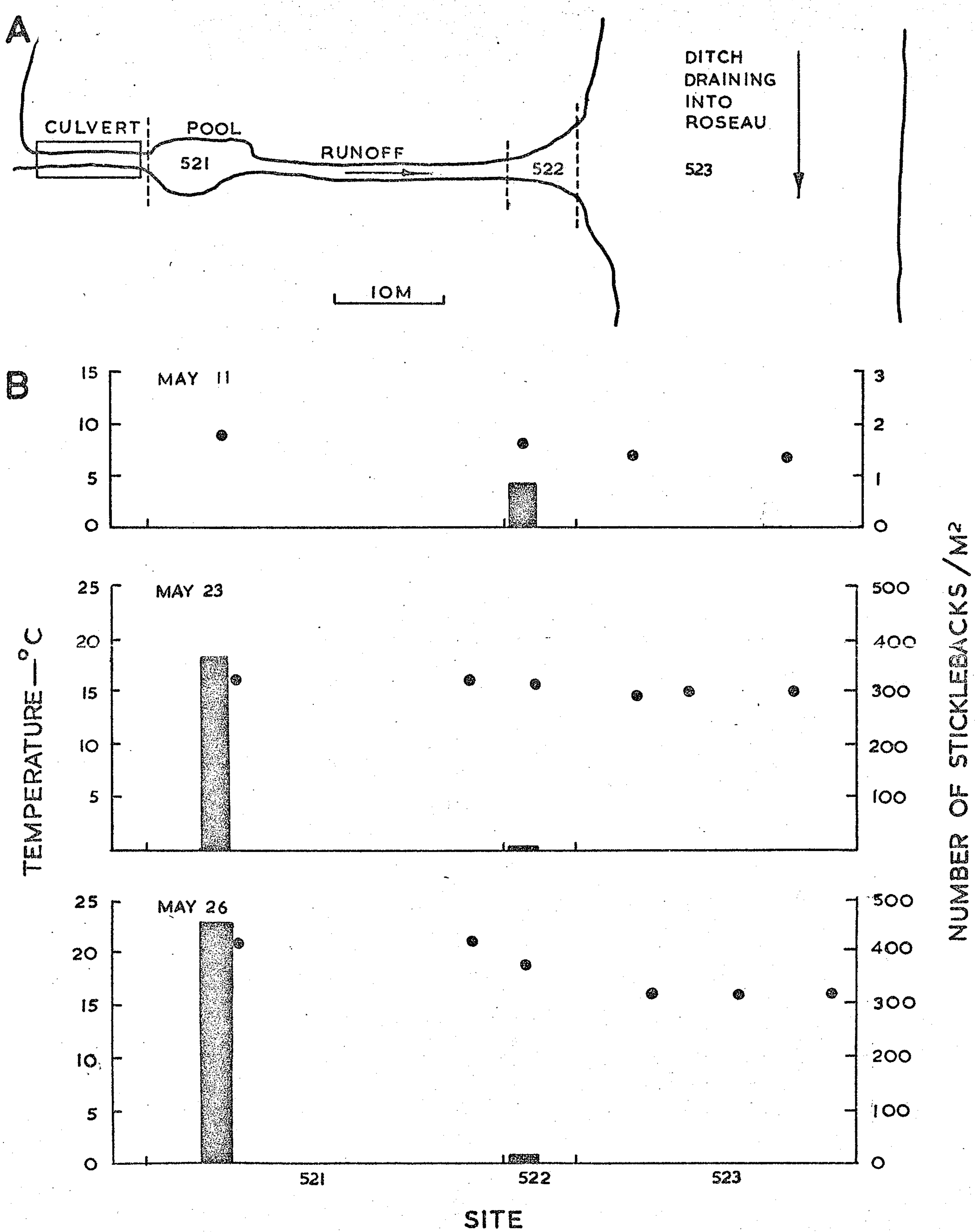
Station 5

A small runoff emptied meltwater ponds in nearby fields into station 5 at substation 52 (Figure 7 A). Water velocity was uniform in the runoff and was less than 8 cm/sec. On May 11, a few sticklebacks (0.7 /m^2) were found at the mouth of the runoff (site 522), where runoff (8.3° C) and ditch water (6.1° C) mixed, but none were found in either the runoff (site 521) or the ditch (site 523) (Figure 7, Table A-II of Appendix A). On May 23, a high density of brook sticklebacks (344.2 /m^2) was found in a pool in the runoff (16.7° C), while few (0.7 /m^2) were found at the mouth of the runoff and none were caught in the ditch (15.0° C). Water flowed into the pool through a culvert, which was 15 cm higher than the level of water in the pool. The resulting waterfall was a barrier to upstream movement of sticklebacks. On May 26, sticklebacks were again concentrated (449.2 /m^2) in the pool (21.1° C), and none were caught in the ditch, but considerable numbers (36.1 /m^2) were caught at the mouth of the runoff. Thus brook sticklebacks appeared to move upstream from the cold ditch into the warm pool in the runoff.

Figure 7:

A Diagram of runoff at substation 52, showing sites 521 to 523.

B The relationship between density of sticklebacks and water temperature at sites 521 - 523 on May 11, May 23 and May 26, 1967. Temperature, represented by solid circles, was measured for each seine haul. Mean density for each site is represented by vertical bars.



A
CULVERT
POOL
521
RUNOFF
522
DITCH
DRAINING
INTO
ROSEAU
523
10M
B
MAY 11
MAY 23
MAY 26
TEMPERATURE — °C
NUMBER OF STICKLEBACKS /M²
0
5
10
15
20
25
1
2
3
100
200
300
400
500
521
522
523
SITE

Station 3

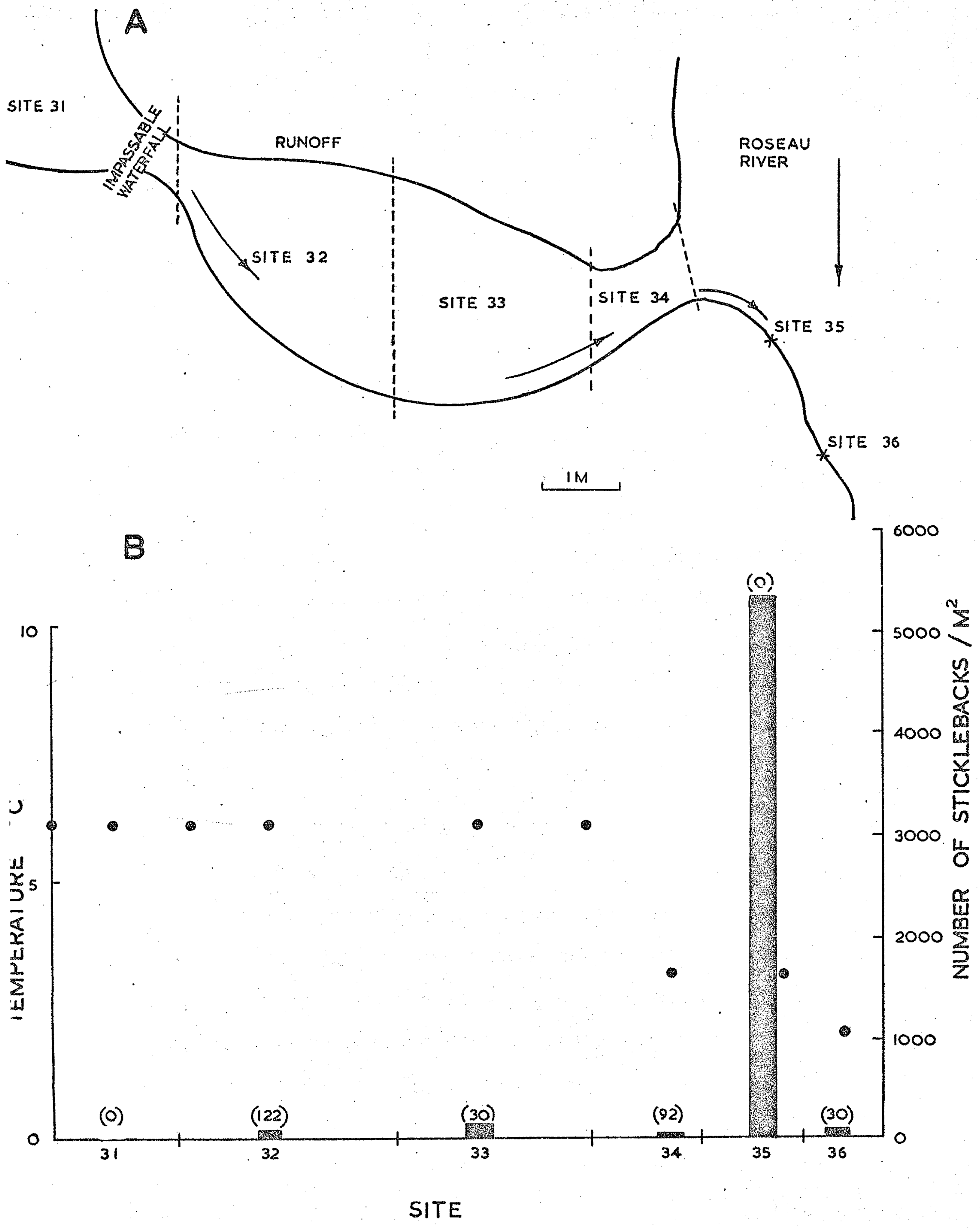
On April 7, the greatest density of brook sticklebacks (5207.8 /m^2) at station 3 (Figure 8) was found near the mouth of the runoff (site 35), where water from the runoff (6.1° C) and the river (0° C) mixed. Considerable numbers (a mean of 45.6 /m^2) were found in the runoff (sites 32 and 33), but few were caught at sites 34 and 36 (1.2 /m^2 and 7.2 /m^2, respectively) in the mixing area. No sticklebacks were caught in the ditch which drained into the runoff (site 31) on either April 7 or subsequent dates (April 23, May 5 and May 23). A waterfall separating the ditch and the runoff appeared to prevent upstream movement of sticklebacks from the runoff, although observations at the base of the waterfall suggested that sticklebacks were attempting to move upstream from the runoff into the meltwater ditch. No sticklebacks were observed along the river bank upstream from the runoff, but seining was impossible because of the steep river banks.

Water velocity may have affected the distribution of brook sticklebacks at station 3. In the runoff, a higher density of sticklebacks was found at site 33 (61.1 /m^2) in a velocity of 30 cm/sec than at site 32 (30.1 /m^2), where water velocity was 122 cm/sec. A large school of sticklebacks was observed in a still pool in the runoff, but seining was impossible because of the depth of the pool. In the mixing area, a higher density of sticklebacks (5207.8/m^2)

Figure 8:

A Diagram of runoff at station 3, showing sites 31 to 36.

B The relationship between density of sticklebacks, water temperature, and velocity of current at sites 31 to 36 on April 7, 1967. Temperature, represented by solid circles, was measured for each seine haul. Water velocity, in cm/sec, is given by numbers in brackets. Mean density for each site is represented by vertical bars.



A
SITE 31
IMPASSABLE WATERFALL
RUNOFF
ROSEAU RIVER
SITE 32
SITE 33
SITE 34
SITE 35
SITE 36
1M
B
TEMPERATURE °C
0
5
10
NUMBER OF STICKLEBACKS / M²
0
1000
2000
3000
4000
5000
6000
(0)
(122)
(30)
(92)
(0)
(30)
31
32
33
34
35
36
SITE

was found at site 35 in 0 cm/sec than at site 34 (1.2 /m^2) where water velocity was 92 cm/sec. Thus on April 7 at station 3, brook sticklebacks appeared to be attempting to move from the cold river into a warm meltwater ditch. Their distribution in the runoff appeared to be influenced by water velocity, as greater densities were found in the lowest velocity.

Results from the field study in 1967 indicate that, during spring, adult brook sticklebacks move from the cold river into warm meltwater ponds, where they reproduce. Sticklebacks appear to prefer the warmest available water, although they may avoid temperatures above approximately 20° C, prior to reproduction. Sticklebacks were found with fertilized eggs in nests at temperatures above 25° C. Brook sticklebacks appear to move upstream in runoffs and current in the meltwater ponds, although upstream movement may be influenced by water velocity.

Analysis of Collections of 1967

(1) Age Structure

The age structure of the pre-reproductive population was determined from a length-frequency graph of 902 sticklebacks, collected from stations 1 and 3 on April 7, 1967. Standard length, defined as the distance from the tip of the snout to the origin of the central caudal fin rays, was used as the sole indicator of age in brook sticklebacks (Mullem and Vlught, 1964).

The length-frequency graph (Figure 9) suggests that the population was composed mainly of one year old individuals (2.0 to 3.9 cm in length), born during the spring of 1966, and a smaller percentage of age two plus (4.0 to 5.2 cm in length), born prior to the spring of 1966. The age groups were separated arbitrarily.

(2) Length and Distribution

Length-frequencies and mean lengths of collections from different environments at each station were compared to determine if size influenced the distribution of sticklebacks. Size differences did appear to exist between the sticklebacks from the runoffs and those from the meltwater ponds. On May 23 at station 1, 22 sticklebacks collected from the runoff had a mean length of 2.76 cm, while 43 sticklebacks collected from the ponds had a mean length of 3.74 cm (Figure 10). On May 26 at substation 52, the mean length of 84 sticklebacks collected from the mouth of the runoff was 3.11 cm, while 169 sticklebacks in a pool along the runoff had a mean length of 3.40 cm. These data suggests that larger fish may move upstream from the runoffs into meltwater ponds before smaller individuals.

(3) Sex-Ratios and Distribution

Sex-ratios of sticklebacks collected from different environments at the same station were tested in 2 x 2 contingency tables, to determine if distribution was related

Figure 9: Length-frequency of 902 brook sticklebacks collected from various stations on April 7, 1967.

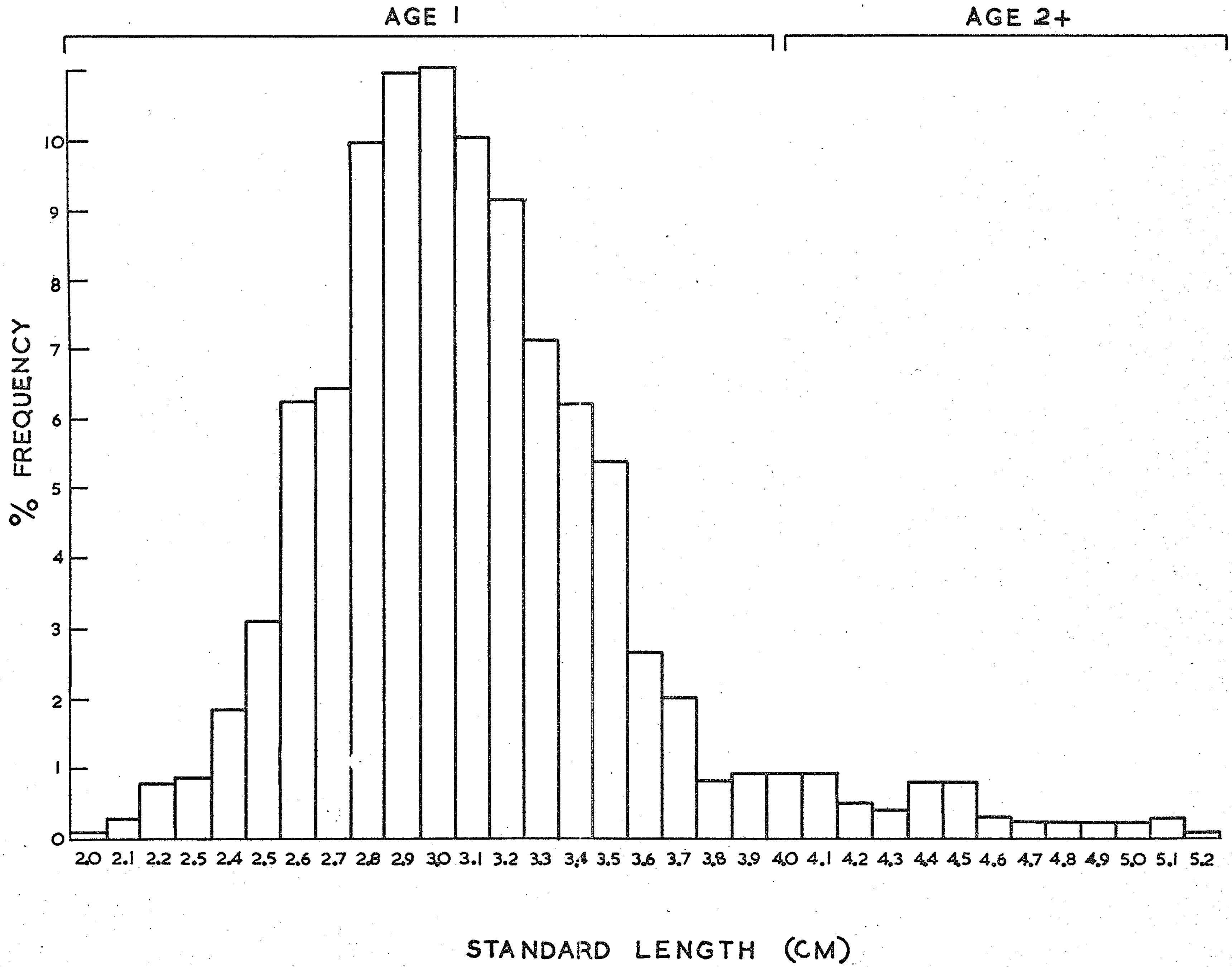
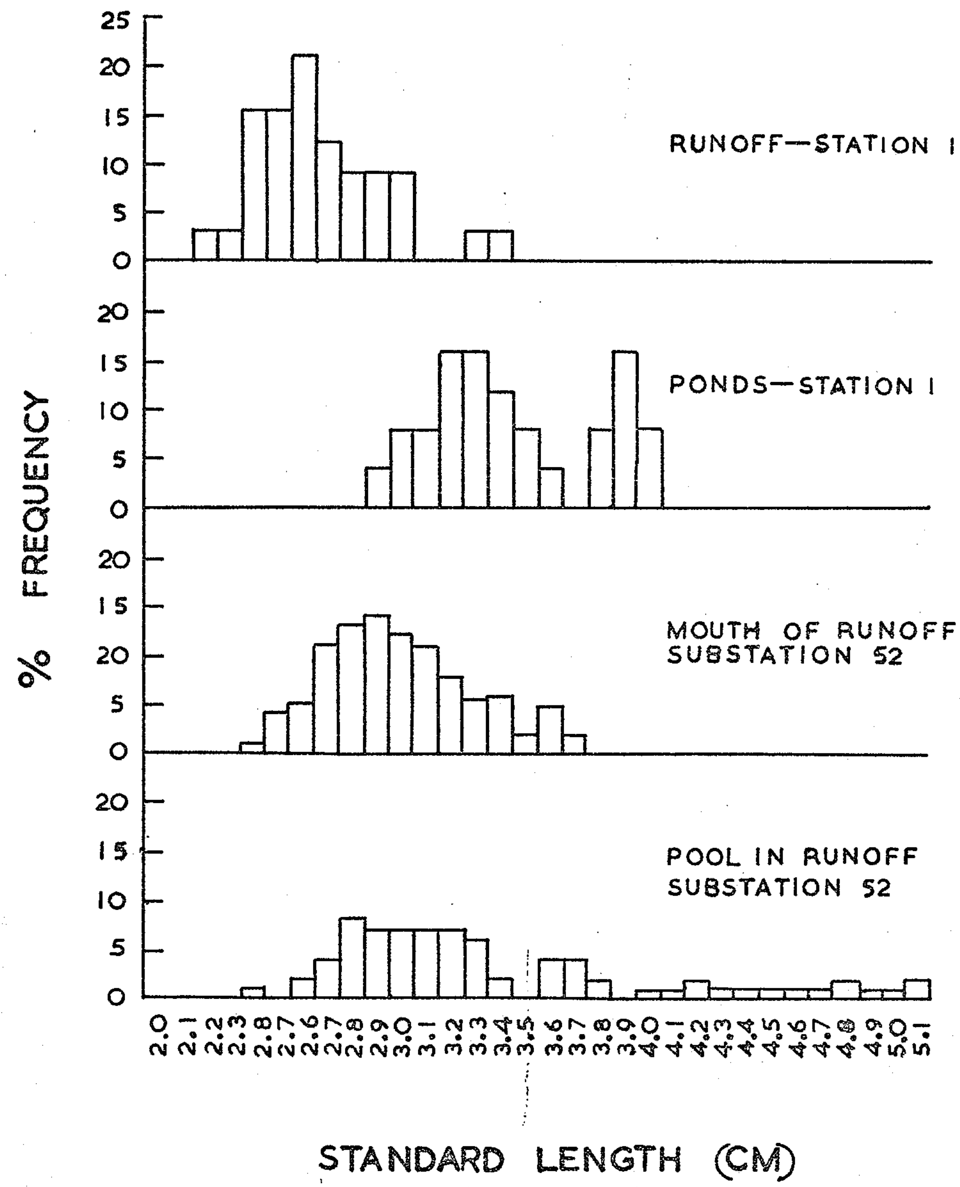

AGE 1
AGE 2+
% FREQUENCY
0
1
2
3
4
5
6
7
8
9
10
2.0 2.1 2.2 2.5 2.4 2.5 2.6 2.7 2.8 2.9 3.0 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0 4.1 4.2 4.3 4.4 4.5 4.6 4.7 4.8 4.9 5.0 5.1 5.2
STANDARD LENGTH (CM)

Figure 10: Length-frequencies and mean lengths of brook sticklebacks collected from runoff and ponds at station 1 and substation 52 on May 23 and May 26, 1967.



DATE	NUMBER OF STICKLEBACKS	MEAN STANDARD LENGTH (CM)
MAY 23	22	2.76
MAY 23	43	3.74
MAY 26	84	3.11
MAY 26	169	3.40

to sex. Several authors (Seal, 1932; van Iersel, 1954; Sevenster, 1961) have observed segregation of the sexes during the pre-reproductive movements of other species of sticklebacks. The sex of an individual was determined by examining a smear of a small section of the gonad with a microscope.

Sex-ratios of sticklebacks collected from the runoff at station 1 on April 7 and May 23 were homogeneous ($x^2 = 0.08$, $p < 0.90$), suggesting that both sexes may move from the river into the runoffs during spring at the same time, although sexual segregation may have occurred in movement prior to April 7 (Table III). Ratios from the ponds and the runoff at station 1 were also homogeneous ($x^2 = 0.33$, $p < 0.75$), as were ratios of sticklebacks from the pool and the mouth of the runoff at substation 52 on May 26 ($x^2 = 1.96$, $p < 0.25$) (Table III), suggesting that males and females move from the runoffs into the meltwater ponds upstream at the same time.

None of the sex-ratios tested differed significantly from a 1:1 ratio. Of a total of 285 adults examined, 147 were males and 138 were females, suggesting that the sex-ratio of the pre-reproductive population may not differ from a 1:1 ratio.

Distribution and Movements of Sticklebacks of Substation 56, 1968

Table III: Summary of 2 x 2 contingency tests on homogeneity of sex-ratios of brook sticklebacks in different environments.

Station	Date	Site	Tempera- ture ° C	Sex Ratio		x^2	p
				Males	Females		
1	April 7	15	7.2° C	13	12		
						0.08	<0.90
	May 23	15	21.1	13	12		
						0.33	<0.75
		11	26.7	16	9		
52	May 26	521	21.1	28	22		
						1.96	<0.25
		522	18.3	20	30		

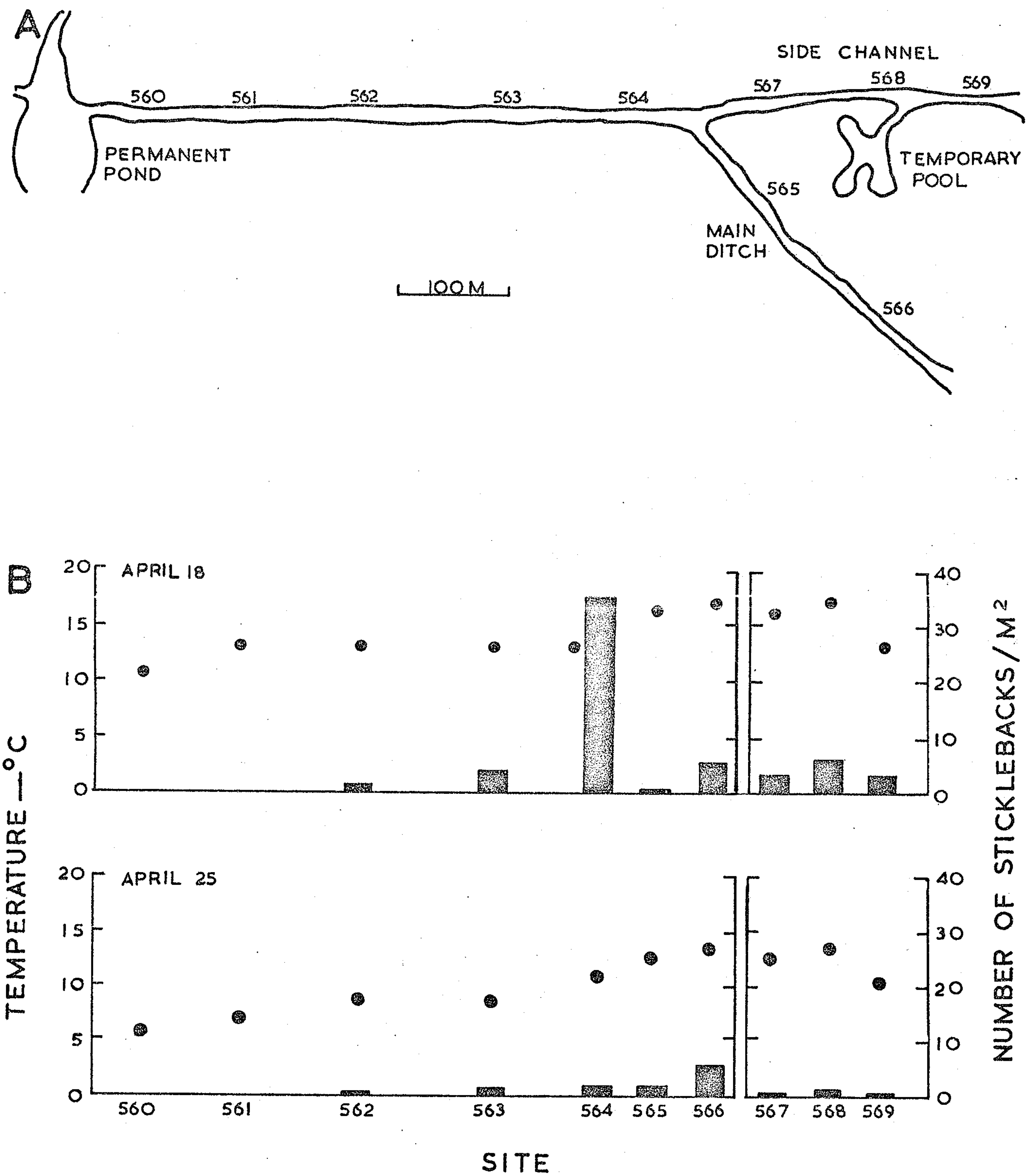
In 1968, at substation 56, a large roadside ditch drained several temporary meltwater pools, which had formed in nearby fields, into a deep permanent pond. During the summer and fall of 1967, the ditch and temporary pools were dry. The ditch was carefully searched on April 8, 1968, but no sticklebacks were seen. On April 17, large schools of sticklebacks were observed in the ditch, and they appeared to be moving upstream from the permanent pond.

Seven sites (560 - 566) along the main ditch and three sites along a shallow side channel (567 - 569) which drained into the main ditch (Figure 11 A), were seined on April 18 and April 25 to investigate the relationship between the distribution of sticklebacks and the environment. On April 18, few sticklebacks (densities from 0 to 0.6 $/m^2$) were caught in the ditch near the permanent pond (sites 560 to 562), while sticklebacks were more abundant (densities from 0.6 to 35.5 $/m^2$) further upstream in the ditch and side channel (sites 563 to 568) (Figure 11 B, Table A-IV of Appendix A). Sticklebacks appeared to be moving upstream along the ditch. The highest density was found at site 564, where large schools of sticklebacks (>20 individuals) were observed while moving upstream near the sides of the ditch, under the protection of overhanging willows. Seining was efficient and not entirely random, so that estimates of density at site 564 may be slightly high. In contrast,

Figure 11:

A Diagram of substation 56, showing sites 560 to 569.

B The relationship between density of sticklebacks and water temperature at sites 560 to 569 on April 18 and April 25, 1967. Temperature, represented by solid circles, was measured for each seine haul. Mean density for each site is represented by vertical bars.


A
SIDE CHANNEL
560
561
562
563
564
567
568
569
PERMANENT POND
565
TEMPORARY POOL
MAIN DITCH
566
100 M
B
APRIL 18
APRIL 25
TEMPERATURE —°C
NUMBER OF STICKLEBACKS/M²
0
5
10
15
20
0
10
20
30
40
560
561
562
563
564
565
566
567
568
569
SITE

sticklebacks were dispersed through the reeds and rushes of sites 567 and 568, so that seining was inefficient, and estimates of density may be erroneously low. When startled, brook sticklebacks darted away swiftly and sought protection under rocks or in vegetation. If such protection was unavailable, or if suddenly startled, sticklebacks swam in a zig-zag pattern along the bottom, towards the seine. This behavior tended to increase the efficiency of seining and density estimates in non-vegetated areas such as at site 564, and to decrease the efficiency of seining and density estimates in heavily vegetated areas such as at sites 567 and 568.

On April 25, sticklebacks were again more abundant at sites 563 - 568 (densities from 0.2 to 5.3 $/m^2$) than at sites 560 - 562 (densities from 0 to 0.1 $/m^2$).

Water temperatures in the ditch were higher than those in the permanent pond. Water from the ditch influenced the water temperatures in a large area of the pond. A temperature gradient was found to exist along the ditch, so that water temperatures increased gradually from the mouth of the ditch (site 560) to a pool in the main ditch (site 566) and the temporary pools (site 568). The movement of brook sticklebacks into and along the ditch appears to be correlated with water temperature.

An upstream--downstream trap caught fish moving into and out of the side channel, but the trap did not appear to capture all individuals moving upstream. Sticklebacks were observed to approach the screening, but seemed to be unable to locate the narrow entrance of the upstream portion of the trap, and were swept downstream.

Counts of sticklebacks in the upstream and downstream portions of the trap were related to water temperature and intensity of light (day or night). Figure 12 suggests that upstream catches are higher in the day and after an increase of water temperatures (as on April 27 and 29). Downstream catches appeared to increase at night and after water temperatures reached approximately 22° C (as on April 27, 29 and 30).

On April 27, observations were made of the upstream movements of brook sticklebacks at the junction of the side channel and the main ditch (Figure 13). Movements were related to water temperatures and time of the day. The side channel was shallower than the main ditch and tended to be warmer, so that fish moving upstream from below the junction were exposed to two water temperatures. Sticklebacks moving upstream appeared to prefer the warmer water of the side channel until water temperatures of the side channel reached 19-20° C (Table III). Sticklebacks then avoided the side channel and moved upstream along the main ditch, until water temperatures in the ditch reached 19-20° C,

Figure 12:

A Water temperatures in the side channel at substation 56 from April 24 to April 30, 1968. Vertical lines represent the range of temperatures observed during day (dotted lines) and night (solid lines). Circles and dots represent the mean of the maximum and minimum temperatures observed during day and night, respectively.

B Numbers of sticklebacks moving upstream and downstream through the trap from April 24 to April 30, 1968. Blank vertical bars represent numbers trapped during the day, while dark vertical bars represent those trapped at night.

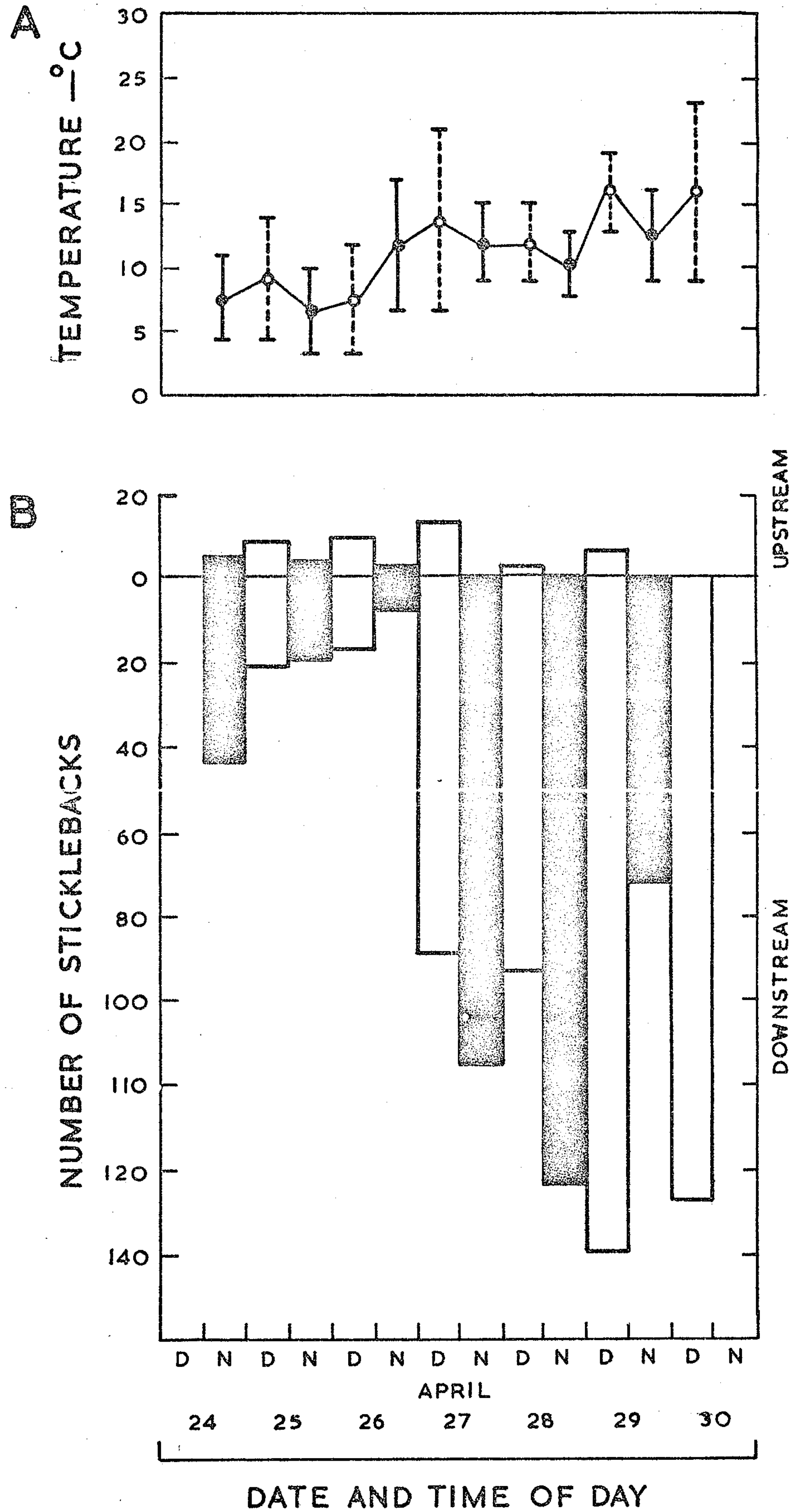

A
TEMPERATURE —°C
30
25
20
15
10
5
0
B
NUMBER OF STICKLEBACKS
20
0
20
40
60
80
100
110
120
140
UPSTREAM
DOWNSTREAM
D N D N D N D N D N D N D N
APRIL
24 25 26 27 28 29 30
DATE AND TIME OF DAY

Figure 13: Diagram of junction of side channel and main ditch at substation 56, showing position of upstream--downstream trap and the observer on April 27, 1968.

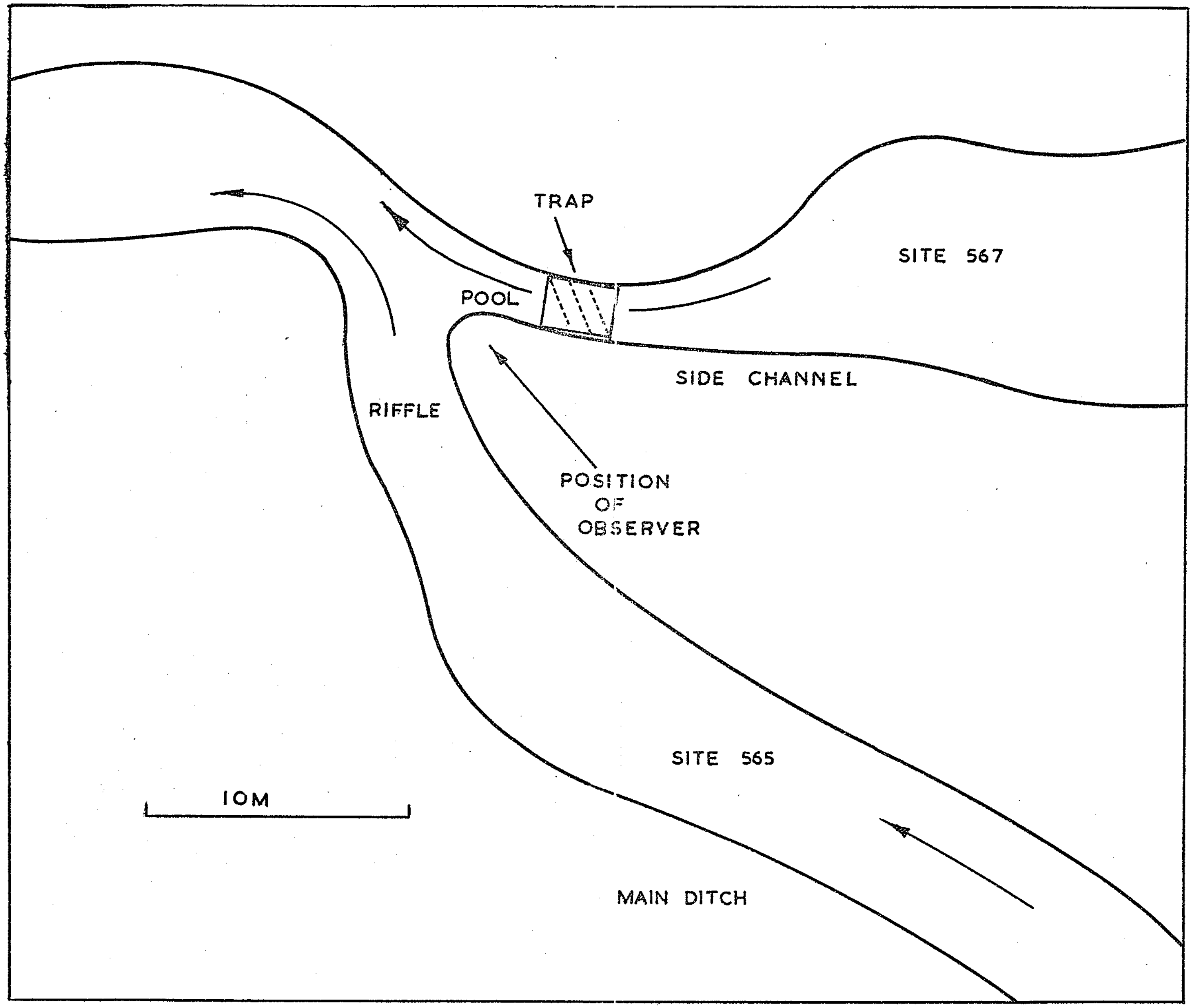

TRAP
SITE 567
POOL
SIDE CHANNEL
RIFFLE
POSITION
OF
OBSERVER
SITE 565
10M
MAIN DITCH

Table IV: Summary of observations at junction of side channel and main ditch on April 27, 1968, relating movements of sticklebacks to water temperatures and time of the day.

Time	Temperature (° C) Side Channel 565	Main Ditch 568	Observations
10:00 a.m.	8.9	7.2	No sticklebacks observed in either station
11:00 a.m.	11.1	8.3	Sticklebacks moved upstream into pool below trap on side channel
12:30 p.m.	14.4	10.6	Large numbers of sticklebacks in pool inside channel. Schools of 10-11 individuals formed before moving into current at mouth of trap --a few entered-- repeated by 6-7 schools, often by same individuals
2:00 p.m.	19.4	13.3	Sticklebacks moved out of pool and formed schools below riffle at 568 before moving upstream
4:00 p.m.	21.1	16.7	Those moving upstream avoided water from side channel--moved along main ditch
6:00 p.m.	19.4	19.4	No upstream movement. Large schools moving down side channel into trap

when upstream movement ceased.

Twenty-eight nests of brook sticklebacks were found in the temporary pools at site 568 during late May and early June. Nests were initially built in water 15 to 20 cm deep, but were built in deeper water, from 30 to 45 cm, later in the spring. Several nests were found in the ditch at sites 563-564 in mid-June. All nests were built of filamentous green algae on an isolated stalk of grass. Sexual segregation was observed both in the pools and the ditch, as males were found at the nests while females were dispersed in deeper water in the center of the pools and the ditch.

Young hatched after one week to ten days in the fluctuating water temperatures of the temporary pools, and spent the first 2-3 days close to the algae of the nest. Later, schools of young ventured farther from the nest. This appeared to be related to the increasing size and mobility of young and the disappearance of the males, who had guarded the eggs and newly hatched young. A few males were later seen in deep areas of the temporary pools, but the majority were never found and may have moved downstream into the permanent pond. Young were collected from dense vegetation in the temporary pools during July and August. No adults were observed or caught during seining at this time. Water flowed from the temporary pools into the side channel during the summer.

SUMMARY OF FIELD STUDY

Observations at Stations 1-5, 1967	Observations at Substation 56, 1968
A. Distribution of Pre-Reproductive Adults During Spring	
Brook sticklebacks were found near runoffs draining meltwater ponds into the river. At station 1 sticklebacks moved from the river, through the runoff and into the meltwater ponds, where they reproduced. At stations 3 and 52, sticklebacks also appeared to attempt to move from runoffs into meltwater ponds and ditches.	Brook sticklebacks moved from a deep, cold, permanent pond, along a ditch and into shallow warm temporary pools, where they reproduced.

Conclusions: During spring, adult brook sticklebacks appeared to move from deep cold water into warm, shallow water, where they reproduced.

B. Effect of Temperature on Distribution of Pre-Reproductive Adults

1967

Sticklebacks were found in runoffs only when runoff temperatures were higher than those in the river. At stations 1 and 52, stickleback density was correlated with water temperature, and the greatest density was generally found in the warmest area. Sticklebacks may have avoided water temperatures in excess of 20-22° C.

1968

Movement of sticklebacks into and along ditch at substation 56 appeared to be correlated with a gradient of water temperatures. Observations of upstream movement of adults suggested that they preferred the warmest available water, but avoided temperatures above 19-20° C, by moving downstream. Nests were built in deeper water as the spawning season progressed.

Conclusions: Distribution and movements of adult brook sticklebacks during spring, prior to reproduction, appeared to be influenced by water temperature. Sticklebacks appeared to prefer the warmest available water, but avoided water temperatures above 19-22° C.

C. Effect of Current on Distribution of Pre-Reproductive Adults

1967	1968
Sticklebacks appeared to move upstream in the water currents of the runoffs and meltwater ponds. At station 1, sticklebacks moved through a series of ponds to the warmest upstream area. At station 3, sticklebacks appeared to be attempting to move upstream in the current of the runoff. Sticklebacks were densest at low water velocities, so that water velocity may also affect movement upstream. At station 5, sticklebacks moved upstream in the current of the drainage ditch and the runoff.	Sticklebacks moved upstream in the water current of the ditch. From counts in the upstream--downstream trap, movement in current appeared to be influenced by water temperature and intensity of light. Upstream movement was greater during the day and after a temperature rise. Downstream movement increased at night and in water temperatures above 20-22° C.

Conclusions: Distribution and movements of adult brook sticklebacks during spring, prior to reproduction, appeared to be influenced by water current. Sticklebacks appeared to move upstream in current, suggesting that they may be positively rheotactic at this time. Movement upstream appeared to be influenced by water velocity, temperature and light intensity.

D. Distribution of Adults and Young After Reproduction

	1967	1968
(a) Adults	Adults and nests with fertilized eggs were usually found in water temperatures above 25° C.	Following reproduction, adults did not appear to avoid water temperatures above 19-22° C, but remained at nest sites for 10-14 days. They may have moved then into deeper and colder water after nesting and parental behavior ceased.
(b) Young		Young remained in vicinity of nest site for several days after hatching. Then they were observed in large schools in the nesting area. They remained in warmer water than adults during

summer.

Conclusions: Adults and young appear to occupy different environments during summer. Adults were usually in colder and deeper water than young after parental behavior has ceased.

LABORATORY EXPERIMENTS

SELECTED TEMPERATURES

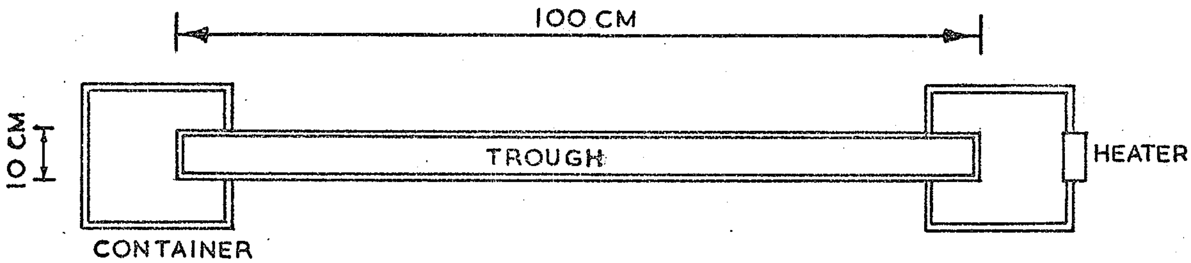
Data collected from the field study suggested that the distribution of brook sticklebacks may be influenced by water temperature. Experiments were conducted to test the hypothesis that the distribution of brook sticklebacks observed in the field was determined by the preferred temperatures of the sticklebacks.

MATERIALS AND METHODS

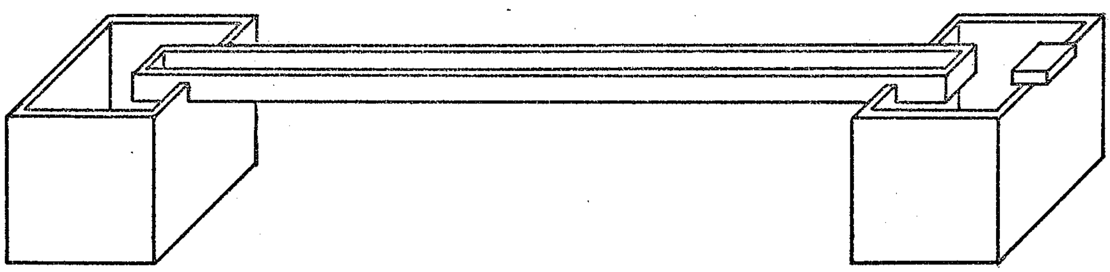
A horizontal temperature gradient was produced in a copper trough, 100 x 10 x 5 cms (Figure 14). The ends of the trough were supported in styrofoam containers, so that water in the containers enveloped the ends and a portion of the sides of the trough. One container was warmed with a heater and the other was chilled with ice, to produce a stable, constantly increasing, gradient of water temperatures from 0° C to 29.4° C along the length of the trough.

Precautions were taken to reduce the effect of other environmental factors on the distribution of sticklebacks in the trough. Light from a 100-watt bulb, placed at the center of the trough, was reflected off a white ceiling so that illumination along the trough was approximately equal. The trough was coated with a non-corrosive and non-toxic paint

Figure 14: Experimental trough used to determine the temperatures selected by brook sticklebacks in a temperature gradient.



TOP VIEW



SIDE VIEW

to prevent the effect of copper ions on the fish. A black cloth, with slits for viewing, separated the observer from the trough. Sticklebacks were tested singly, to eliminate the effects of social behaviour, such as schooling and aggression, which had been observed in the aquaria in which sticklebacks were held prior to testing.

Sticklebacks were collected from various sites in the study area and brought to the laboratory in styrofoam coolers, at a temperature similar to that in which they had been caught. Three experiments were conducted:

Experiment 1: Aim: to determine, as a control, if temperature was the only variable influencing the distribution of sticklebacks in the trough. Ten young sticklebacks (age 0) which were collected from site 568 on June 11, 1968 were tested. The experimental procedure was identical to that used in all other experiments, except that a temperature gradient was not established in the trough.

Experiment 2: Aim: to determine the selected temperatures of acclimatized sticklebacks. Groups of adults (age 1 +) and young (age 0) were brought into the laboratory at various times of the year and tested within 24 hours of capture;

(a) on April 20, 1968, ten pre-reproductive adults, collected from a water temperature of 10.0° C at substation 56, were tested.

(b) on May 28, 1968, ten post-reproductive adults, collected from a water temperature of 14.4° C in the temporary pools at site 568, were tested.

(c) on June 13 and August 20, 1968, ten young sticklebacks collected from water temperatures of 16.1° and 14.4° C, respectively, at site 568, were tested.

(d) on October 31, 1967, a group of 15 adults (age 1+) and young (age 0), collected from a water temperature of 2.2° C at substation 55, were tested. Young and adults were separable when caught in the large isolated pond at substation 55 in early August, but by October the age classes were inseparable on the basis of length.

Experiment 3: Aim: to determine the influence of thermal history on the selected temperatures of sticklebacks. Groups of young and adults were acclimated, for at least ten days, to one of a series of temperatures before being tested.

(a) in late July, 1967, groups of ten adults and ten young were collected from a water temperature of 14° C and one group was acclimated to each of the following water temperatures: 21.1°, 12.8° and 4.4° C.

(b) in early November, groups of 15 sticklebacks, including both adults and young, were collected from a water temperature of 8° C and one group was acclimated to each of the following water temperatures: 21.1°, 12.8°, 4.4° and 1.1° C.

During the period of acclimation, sticklebacks were kept in large aquaria, which were maintained to within 0.3° C of the acclimation temperature by Thermistemp heaters. Photoperiod was ten hours during August tests and six hours during November tests. Frozen brine shrimp were fed to the sticklebacks each day.

A standard experimental procedure was followed for each test. First the temperature gradient was established and then the trough was drained. The trough was refilled to a depth of 3 cm with water at a temperature similar to that at which the stickleback was held after capture. After six to eight minutes the gradient was established. Thirty minutes after the fish was introduced, either 5 or 10 observations were made of the position of the individual at 30 second intervals. Positions of the individual fish were recorded and temperatures at the observed positions were measured with a variable resistance thermometer, before the stickleback was removed. The trough was drained and the procedure was repeated, until all individuals in the group were tested.

The selected temperature of an individual was the mean of the temperatures at which it was observed, and the selected temperature of the group was the mean of the selected temperatures of all individuals in the group. The selected temperature of a group was based on a minimum of 50 observations (10 fish x 5 observations/fish).

RESULTS

Experiment 1

The length of the trough was divided into ten equal sections, and the number of fish observed in each section was determined. A Chi-square test (Table V A) showed that, when there was no temperature gradient, the observed distribution of sticklebacks did not differ significantly from an expected distribution based on equal numbers of fish in each section ($x^2 = 10.2$, $p<0.50$). Thus the observed distribution was random. A slight tendency for sticklebacks to be found near the ends of the trough may occur, as 50% of the observations were made in sections 1 - 2 and 9 - 10.

Table V:

A Distribution of 10 young brook sticklebacks in the trough when there was no temperature gradient.

B Distribution of 10 young brook sticklebacks in the trough when there was a temperature gradient.

Ten observations of the position of each fish were made at 30 second intervals, following their introduction into the trough. Sticklebacks were tested singly.

A

Section	1	2	3	4	5	6	7	8	9	10
Temperature, ° C	16	16	16	16	16	16	16	16	16	16
Observed Number	16	9	5	10	8	7	9	11	15	10
Expected Number	10	10	10	10	10	10	10	10	10	10
x^2	3.6	0.1	2.5	0	0.4	0.4	0.1	0.1	2.5	0

Total x^2 = 10.2, 9df, $p<0.5$

B

Section	1	2	3	4	5	6	7	8	9	10
Temperature, ° C	0	3	6	9	12	15	18	21	24	27
Observed Number	0	0	0	0	0	2	2	53	27	16
Expected Number	10	10	10	10	10	10	10	10	10	10

In comparison, the distribution of ten acclimatized young sticklebacks tested in a temperature gradient two days later (June 13), was non-random (Figure V B). They appeared to prefer a restricted range of temperature. Thus, appears to be the only variable which influenced the distribution of sticklebacks in the trough.

Experiment 2

Pre-reproductive adults selected a mean of 17.3° C with a range from 14.9° to 20.2° C (Table VI). Post-reproductive adults selected a mean of 16.3° C with a range from 8.9° to 25.6° C. While there appears to be little difference between the mean temperatures selected, adults appear to select a narrower range of temperatures prior to reproduction than subsequent to reproduction. The Coefficient of Variability, a relative measure of variation, is 11.1% for pre-reproductive adults and 26.4% for post-reproductive adults.

In June, young sticklebacks selected a mean of 22.6° C with a range from 21.1° to 25.2° C. In August, young selected a mean of 16.9° C with a range from 14.5 to 19.8° C. In early summer, young brook sticklebacks appear to select a much higher mean temperature than post-reproductive adults, but by late summer there appeared to be little difference.

The mixed age group test in late October selected a mean of 10.9° C with a range from 6.7° to 17.6° C, much lower than the temperatures selected by either adults or

Table VI: Summary of experiments on selected temperatures of acclimatized brook sticklebacks (Experiment 2).

Date of Experiment	Age of Fish	Temperature at Capture ° C	Number of Sticklebacks Tested	Number of Observations per Individual	Selected Temperatures° C	Range of Temperatures Selected ° C	Standard Deviation ° C
April 20 1968	1 + Pre-Reproductive	10.0	10	10	17.3	14.9-20.2	1.92
May 28 1968	1 + Post-Reproductive	14.4	10	10	16.3	8.9-25.6	4.30
June 13 1968	0	16.1	10	10	22.6	21.1-25.2	1.27
August 20 1968	0	14.4	10	10	16.9	14.5-19.8	1.60
October 31 1968	0 and 1 +	2.2	15	5	10.9	6.7-17.6	2.09

young at other times of the year. To determine if the temperature selected was related to size and possibly age, the mean temperature selected by individuals of the mixed age group was plotted against standard length (Figure 15) and a correlation coefficient (Pearson's Coefficient) was calculated. No relationship between the two variables could be detected (r = -0.23).

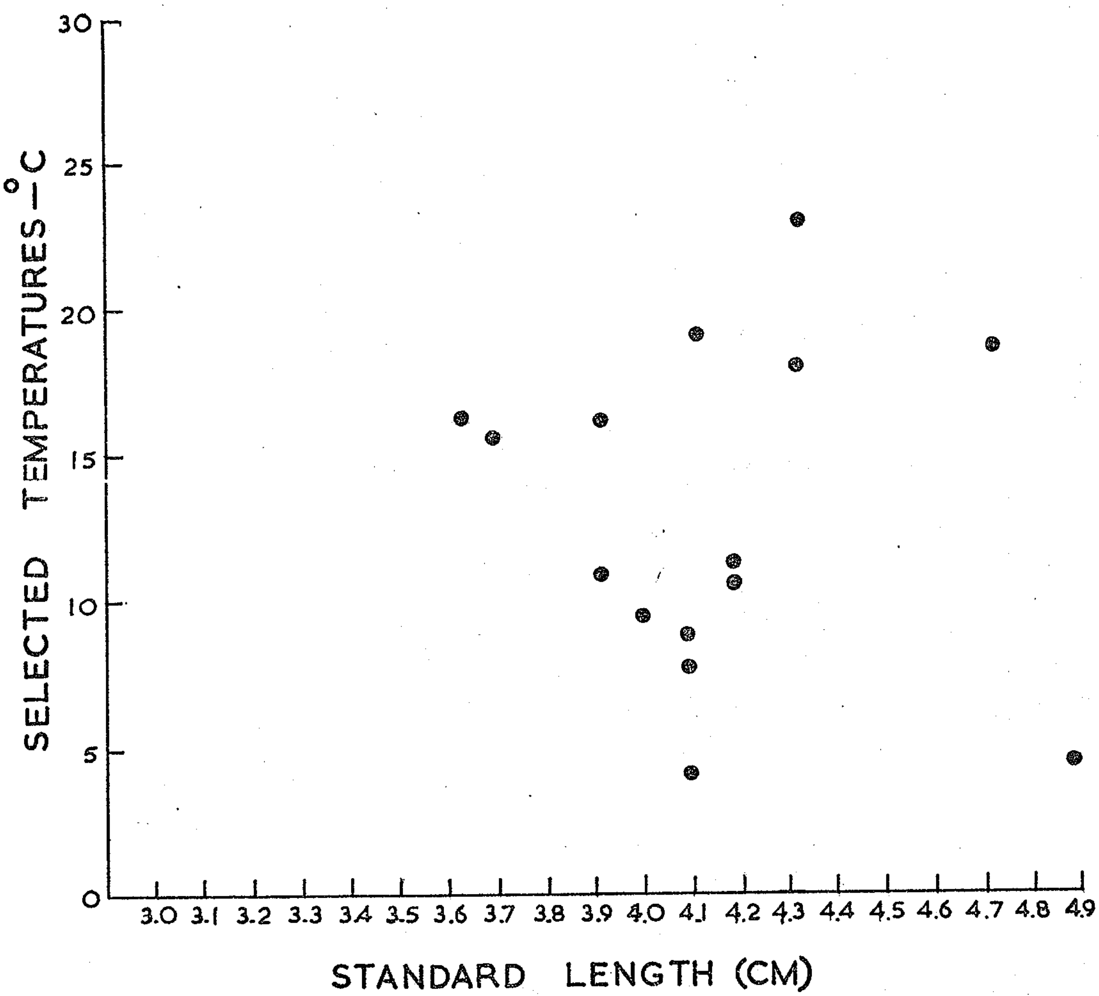
Two explanations are plausible for the differences observed both between age groups at any one time and within age groups at different times of the year. Figure 16 suggests that a correlation may exist between the acclimatization temperature, or the temperature at which the sticklebacks were collected, and the temperatures selected in the gradient. Alternately, differences in the temperatures preferred may be due to changes in temperature requirements related to either reproductive phase, age or season or to a combination of these factors.

Experiment 3

Experiments were conducted on the selected temperatures of acclimated sticklebacks, to determine if the above differences, observed in Experiment 2, occurred independant of the effects of thermal history.

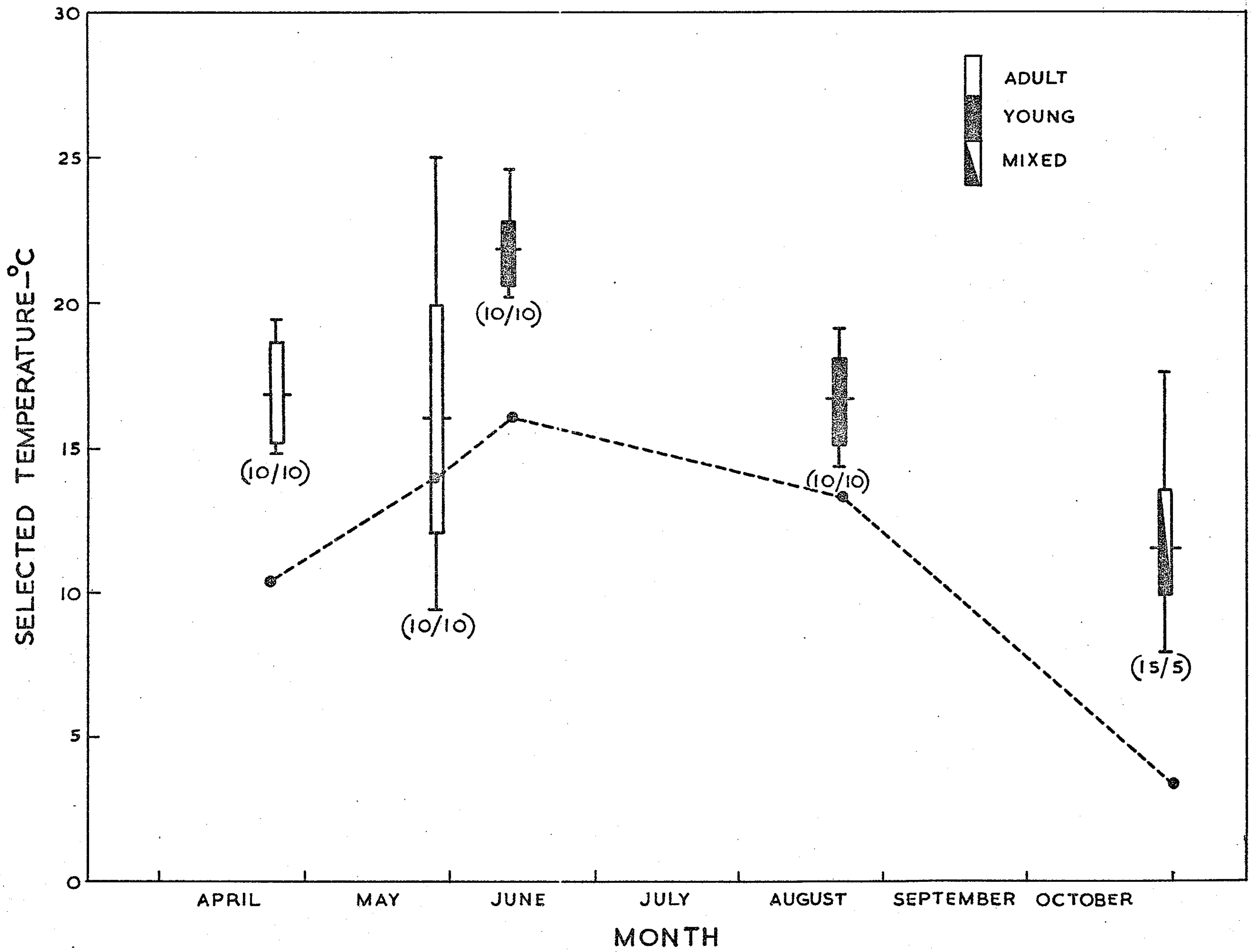
In experiment 2, young sticklebacks appeared to select a higher mean temperature than adults in early summer. To determine whether the apparent difference was

Figure 15: The relationship between standard length and the temperature selected by 15 acclimatized sticklebacks, October, 1967. Each point represents the mean of five observations per individual in the temperature gradient. (Pearson's Coefficient, r = -0.23).



SELECTED TEMPERATURES — °C
30
25
20
15
10
5
0
3.0 3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0 4.1 4.2 4.3 4.4 4.5 4.6 4.7 4.8 4.9
STANDARD LENGTH (CM)

Figure 16: Temperatures selected by acclimatized brook sticklebacks (Experiment 2). Vertical lines represent the range of temperatures selected. The vertical bars indicate the standard deviation of the mean. Horizontal lines bisecting the standard deviations indicate the selected temperatures. The numbers in brackets = (numbers of fish tested/number of observations per individual. Dots represent the temperature at which the sticklebacks were collected in the field.



ADULT
YOUNG
MIXED
SELECTED TEMPERATURE-°C
30
25
20
15
10
5
0
(10/10)
(10/10)
(10/10)
(10/10)
(15/5)
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
MONTH

due to either thermal history or age, adults and young were acclimated to a series of temperatures before their temperature preferences were tested.

Temperatures selected by adults and young at each acclimation temperature (Table VII) were compared in a two (age groups) by three (acclimation temperatures) factorial analysis of variance (Table VIII). The analysis shows that there were differences between the main effects of age groups and temperature of acclimation ($p<0.005$). In addition, the interaction between the effects of age and temperature was also significant ($p<0.01$). Thus age and acclimation temperature significantly affected the temperature selected, but the differences between age groups were dependent on acclimation temperature.

The mean temperatures selected by adults and young at different acclimation temperatures were then compared, to determine the significant differences between them (Table IX). Young acclimated to 21.1° C selected a mean of 22.0° C, while adults, acclimated to the same temperature, selected a significantly lower mean of 13.8° C ($p<0.05$). Young acclimated to 12.8° and 4.4° C also selected higher mean temperatures than adults acclimated to the same temperatures, but differences were not significant. Thus, young selected higher temperatures than adults, but the difference between the two age groups depended on acclimation temperature.

Table VII: The temperatures selected by young and adult sticklebacks acclimated to different temperatures during August 1967. Selected temperatures are based on a mean of 5 observations on each of 10 sticklebacks.

Date of Experiment	Age of Fish	Acclimation Temperature ° C	Selected Temperature ° C	Range of Temperatures Selected ° C	Standard Deviation ° C
August 10	0	21.1	22.0	18.0-26.0	2.57
August 11	1+	21.1	13.8	10.1-17.9	2.53
August 12	0	12.8	16.2	7.6-22.2	4.96
August 13	1+	12.8	15.6	8.7-22.9	4.07
August 14	0	4.4	21.8	17.2-25.3	3.28
August 15	1+	4.4	18.7	13.1-28.5	4.12

Table VIII: Table of analysis of variances on mean temperatures selected by acclimated brook sticklebacks during August 1967.

Source of Variation	df	S.S.	M S	F	p
Age Group	1	756.9	756.9	16.49	<.005
Acclimation Temperature	2	608.6	304.3	6.63	<.005
Interaction	2	462.5	231.2	5.04	<0.01
Error	54	2476.3	45.9		
Total	59	4304.3	72.9		

Table IX: Analysis of variance on acclimated brook sticklebacks, August, 1967. Selected temperatures indicated by * differ significantly from those indicated by arrows ← ($p<0.05$, Duncans New Multiple Range Test).

Age Group	Acclimation Temperature ° C	Mean Temperature Selected ° C	Duncan's New Multiple Range Test
	21.1	22.0	
Young	12.8	16.2	*
	4.4	21.8	
	21.1	13.8	*
Adults	12.8	15.6	*
	4.4	18.7	*

Both adults and young acclimated to temperature, so that there were significant differences in the mean temperatures selected by groups of the same age acclimated to different temperatures. Adults acclimated to 4.4° C selected a significantly higher mean than adults acclimated to 21.1° C. Young acclimated to 12.8° C selected a significantly lower mean than young acclimated to either 4.4° or 21.1° C.

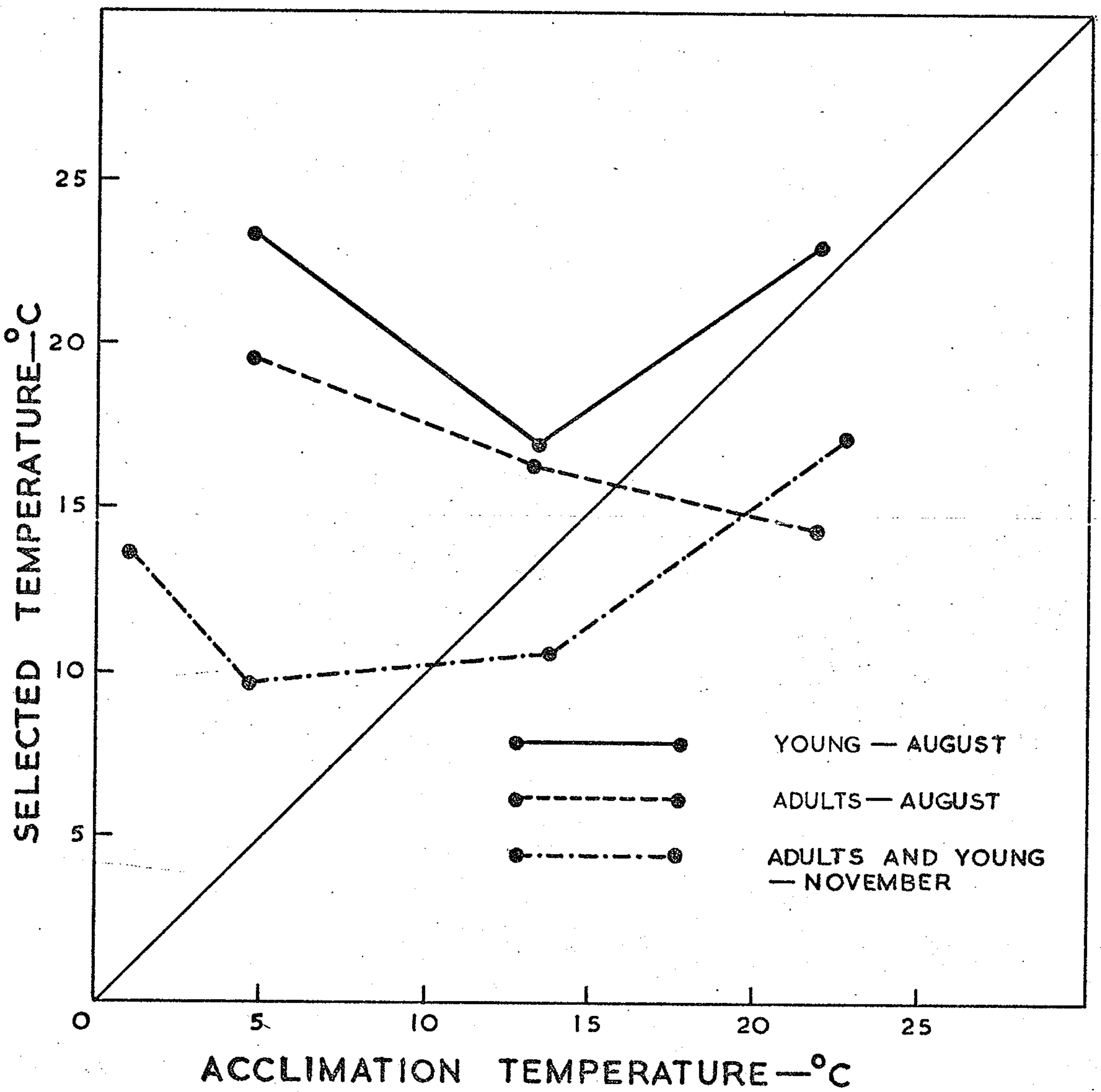
In experiment 2, acclimatized sticklebacks appeared to select lower temperatures in winter than at other times of the year. To determine whether differences were due to either a decrease in acclimatization temperature or a seasonal change independant of thermal history, groups of 15 sticklebacks, both adults and young, were acclimated to a series of temperatures during early November before their temperature preferences were tested. Results were compared to the results from tests on acclimated adults and young during August.

With one exception, to be discussed later, sticklebacks selected much lower mean temperatures in November than in August, at each acclimation temperature (Table X, Figure 17). At an acclimation temperature of 12.8°, sticklebacks selected a mean of 10.2° C, while adults and young selected means of 15.6 and 16.2°, respectively, in August. At 4.4° C, sticklebacks selected a mean of 9.2° C in November, while adults and young selected means of 18.7° and

Table X: The temperatures selected by brook sticklebacks acclimated to different temperatures during November 1967. Selected temperatures are based on a mean of 5 observations on each of 15 sticklebacks.

Date of Experiment	Age of Fish	Acclimation Temperature ° C	Selected Temperature ° C	Range of Temperatures Selected ° C	Standard Deviation ° C
November 12	0,1+	21.1	15.9	1.7-22.6	2.75
November 13	0,1+	12.8	10.2	3.9-16.1	5.08
November 14	0,1+	4.4	9.2	4.0-16.1	3.50
November 15	0,1+	1.1	12.9	4.4-23.5	5.58

Figure 17: Summary of mean temperatures selected by acclimated brook sticklebacks (Experiment 3). Each point represents the mean of a minimum of 50 observations (5 observations x 10 sticklebacks).



SELECTED TEMPERATURE—°C
25
20
15
10
5
0
5
10
15
20
25
ACCLIMATION TEMPERATURE—°C
YOUNG — AUGUST
ADULTS — AUGUST
ADULTS AND YOUNG — NOVEMBER

21.8° C, respectively, in August.

The exception to this pattern was the mean of 15.9° C selected by sticklebacks acclimated to 21.1° C in November. Adults and young acclimated to 21.1° C in August selected means of 13.8° and 22.0° C, respectively. During acclimation for November tests, 10 of the 15 sticklebacks developed secondary sexual characteristics. Thus, seasonal changes in selected temperatures occur independent of the effects of thermal history, but the drop which occurs during fall may be reversed by exposure to high temperatures.

Thus the age of the individual and the season appear to cause changes in the temperatures selected by sticklebacks, independent of the effects of thermal history.

TEMPERATURE RESISTANCE

During summer, young sticklebacks were found in shallow warm pools, while adults appeared to move into deep cold ponds. Young would be exposed to rapid fluctuations of water temperature, while the environment of adults would be relatively stable. The hypothesis tested was that young have a greater resistance than adults to sudden changes of water temperature.

MATERIALS AND METHODS

Four water baths were regulated to 25.6°, 27.8°, 30.0° and 32.2° C (± 0.15° C). Eight plastic containers, 12 x 12 x 15 cm, were filled with water from the area in which the sticklebacks had been captured. A total of 25 individuals, divided between two containers, was placed in each pre-heated bath. The containers were covered and supplied with compressed air, which was bubbled through an air diffusor stone. Water temperatures in the container and the bath were equal after 10 to 15 minutes (time 0). Counts of the survivors were made at 30 minute intervals for eight hours. Individuals that did not respond to a mechanical stimulus were considered dead and were removed. The cumulative percentage of sticklebacks that died over eight hours was plotted against test temperature. The temperature at which 50% of the sticklebacks tested would survive for eight hours (LT50) was used to compare the resistance of the following three groups to sudden increases in water temperature:

(a) 100 adults, collected on June 14, 1968

(b) 100 young, collected on June 16, 1968

(c) 100 young, collected on August 19, 1968

Sticklebacks were collected from site 568 and held for less than 24 hours before testing in water from site 568. Maximum and minimum temperatures were measured at site 568 for three days prior to the collection of each test group. All tests were started at approximately 10 a.m.

RESULTS

Because of the distance between the points in the plot of cumulative percent mortality against test temperature (Figure 18), the derived LT50 values will be considered approximate, and used for comparative purposes only.

The LT50 of adults tested in June was 28.0° C. For young, tested two days later, the LT50 was 30.7° C. Both groups were taken from the same area, and no difference was observed in the maximum and minimum temperatures in that area for three days prior to collection (Table XI).

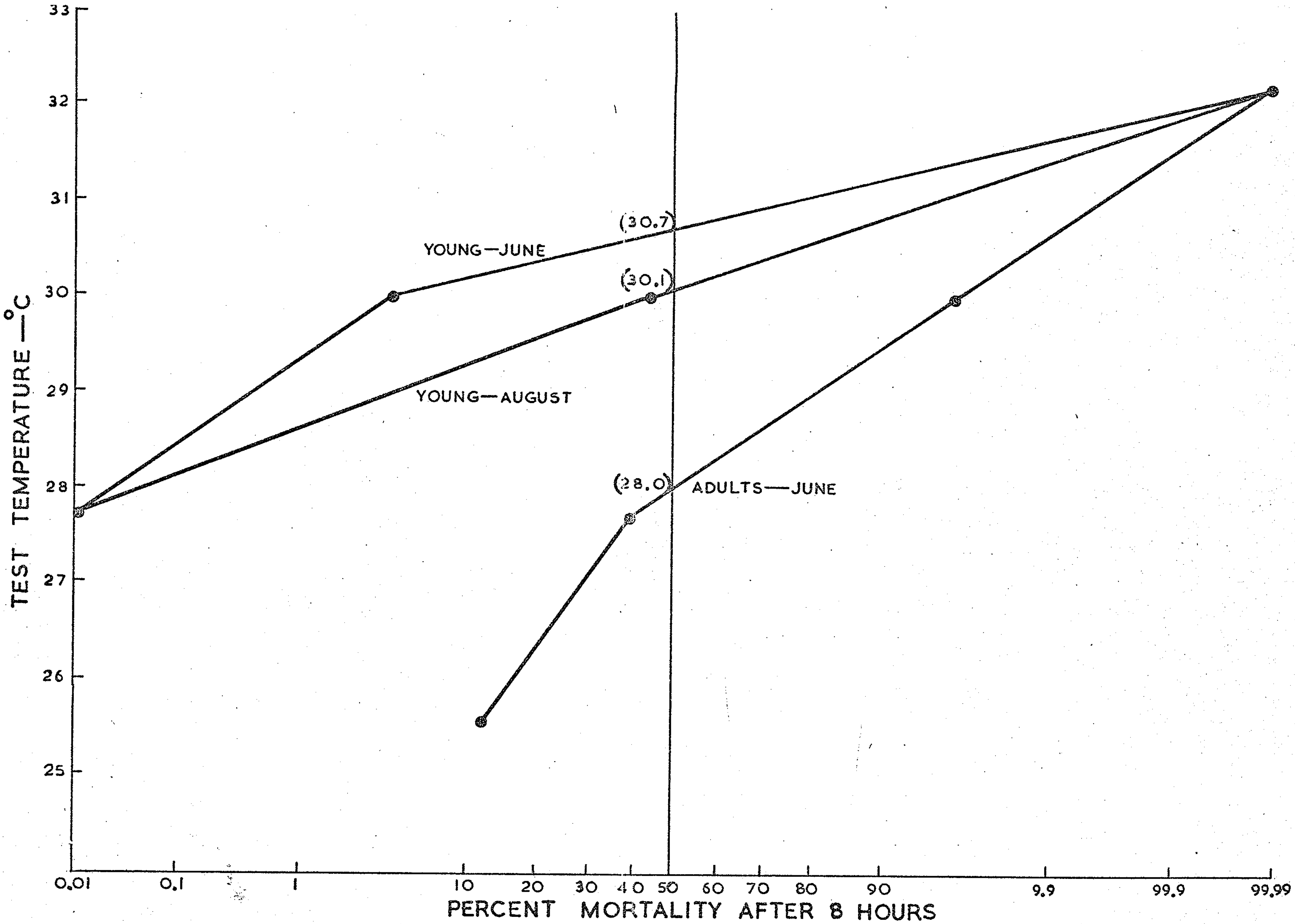
The LT50 of young tested in August was 30.1° C, slightly lower than that for young in June. Maximum and minimum temperatures prior to testing in August were higher than those in June. These results suggest that the resistance of young sticklebacks to fluctuations of water temperature may have decreased during the summer.

Thus, young sticklebacks appear to have a greater resistance than adults to high water temperature in early summer, but later in the summer the capacity of young for resistance may decrease.

EXPERIMENTS IN ARTIFICIAL STREAM

In the field, adult sticklebacks were observed to move upstream in current in spring, prior to reproduction, suggesting that they were positively rheotactic at this time. Movement upstream appeared to be influenced by light

Figure 18: Plot of cumulative percentage of sticklebacks dead after eight hours against test temperature. Groups of 25 sticklebacks were exposed to four test temperatures (25.6°, 27.8°, 30.0° and 32.2° C) for eight hours. The LT50 was the temperature at which 50% of the individuals tested would survive eight hours, and LT50 values for each group are given in brackets.



TEST TEMPERATURE —°C
33
32
31
30
29
28
27
26
25
YOUNG—JUNE
YOUNG—AUGUST
ADULTS—JUNE
(30.7)
(30.1)
(28.0)
0.01
0.1
1
10
20
30
40
50
60
70
80
90
9.9
99.9
99.99
PERCENT MORTALITY AFTER 8 HOURS

Table XI: Summary of experiments on temperature resistance of brook sticklebacks, 1968. Groups of sticklebacks were exposed to lethal temperatures for eight hours. LT50's were derived from a plot of cumulative percent mortality vs. test temperature and represents the temperature at which 50% of the sticklebacks tested could survive for eight hours.

Date of Test	Age of Fish	Temperature Range For 3 Days Prior To Capture, ° C	Temperature at which 50% Will Survive For 8 Hours LT50, ° C
June 15, 1968	1+	5.6-14.4	28.0
June 17, 1968	0	5.6-14.4	30.7
August 19, 1968	0	10.0-20.3	30.1

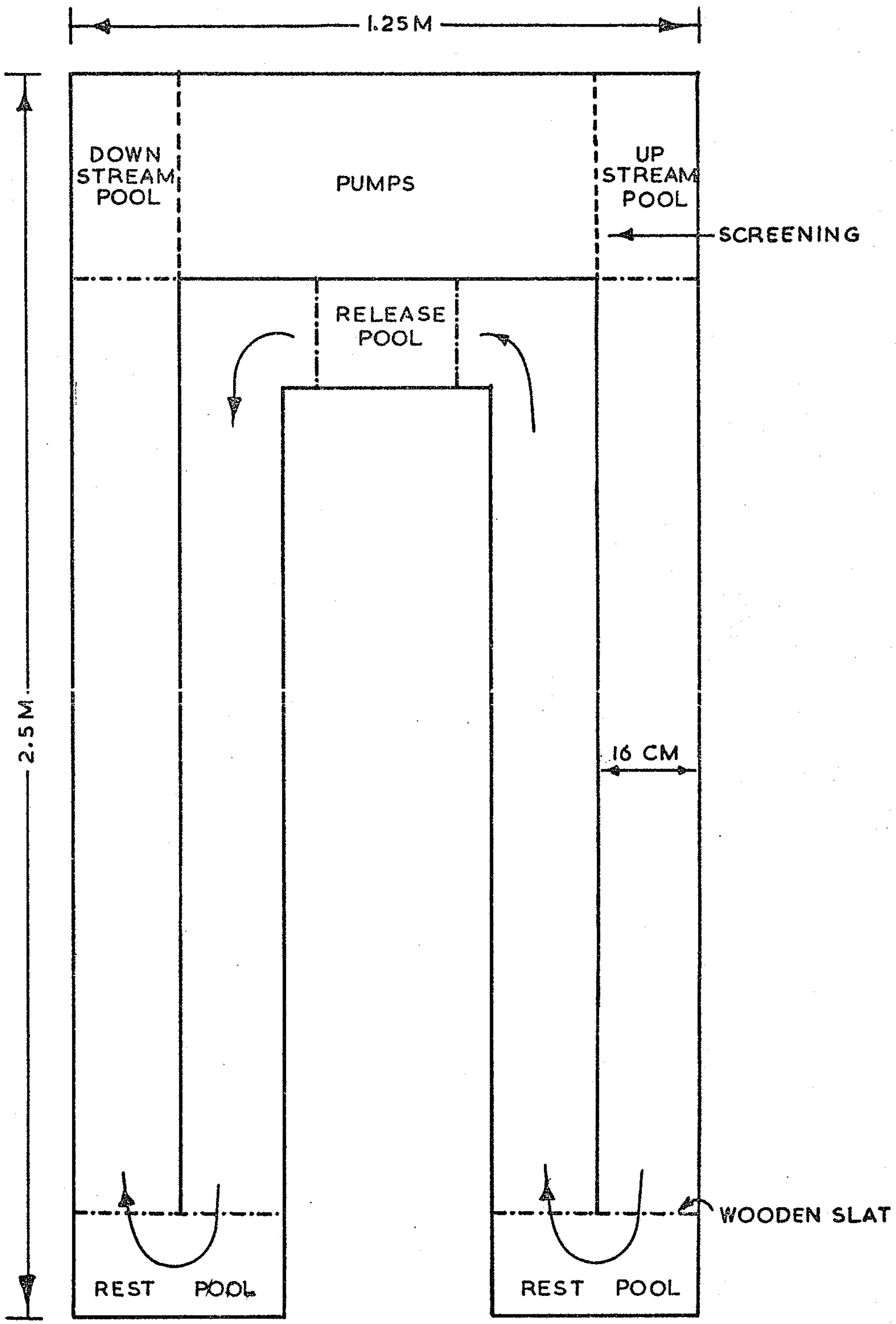
intensity and both velocity and temperature of water. The hypothesis tested in the laboratory was that light intensity and water temperature affect the movement of sticklebacks in current.

MATERIALS AND METHODS

An artificial stream channel, 9.15 m long, 16 cm wide and 30.5 cm deep was built (Figure 19), based on a design used by Raleigh (1967). The stream bed consisted of gravel (2 - 20 mm diameter), but five pools, without a gravel bottom, were spaced at equal intervals along the channel, to provide areas with a reduced velocity of water. There was an upstream and a downstream pool at opposite ends of the stream, a central release pool and two rest pools, midway between the central and end pools. Each pool could be sealed off with a wooden slat, so that the distribution of fish in each section could be determined at any time. Water flowed along the channel at a surface velocity of 8 cm/sec, maintained by two pumps, placed between the end pools. The pumps produced a gradient in the water depth along the channel. To ensure that both depth and velocity of water were even, there was a drop of 0.54 cm/m along the channel from the upstream end.

Twenty pre-reproductive adult brook sticklebacks were collected from the ditch at substation 56 on each of five consecutive days in early June, and 25 young were

Figure 19: Stream channel used to determine the responses of brook sticklebacks to current. Arrows represent the direction of water flow.


1.25 M
2.5 M
DOWN STREAM POOL
PUMPS
UP STREAM POOL
SCREENING
RELEASE POOL
16 CM
WOODEN SLAT
REST POOL
REST POOL

TOP VIEW

collected on each of four consecutive days in early July from the temporary pools at site 568. Each group was brought to the laboratory in a styrofoam cooler and placed in the release pool, which was sealed off from the rest of the stream channel. They were left in the release pool for six hours, to allow them to acclimate to water temperature in the stream, before the pumps were switched on and the group of sticklebacks was exposed to current for eight hours in complete darkness. The stream sections were then sealed off and the number of sticklebacks in each section was determined. The group was then replaced in the release pool and exposed to current for eight hours in light. Five lamps, evenly spaced around the trough, reflected light off a white ceiling and onto the stream.

Each group was tested in two light intensities at one of a series of water temperatures; either 7.2°, 10.0°, 15.6°, 21.1° and 23.9° C for adults and either 15.6°, 21.1°, 26.7° and 29.4° C for young sticklebacks. Water temperatures were regulated to within 0.3° C of the test temperature with two "Thermistemp" controllers, the heaters being placed between the upstream and downstream pools. Tests in darkness were conducted from 12 p.m. to 8 a.m., while tests in light were from 9 a.m. to 5 p.m. so that all tests on a group were completed within 24 hours.

The number of sticklebacks found upstream from the central release pool, the number found in the release pool, and the number found downstream were recorded for each test (Table XII).

To determine if water temperature affected the direction that sticklebacks moved in current, the independence of temperature and the ratio of the number of sticklebacks that moved upstream to the number that moved downstream was tested with 4 x 2 contingency tables. A significant Chi-square would indicate that the ratios differed between temperatures by more than chance and that the direction of movement was influenced by temperature.

To determine if light intensity affected movement in current, the homogeneity of the upstream to downstream ratios at each temperature in darkness and light was tested in a 2 x 2 contingency table. A significant Chi-square would indicate that the ratios differed by more than chance and that light intensity affected the direction of movement of sticklebacks in current.

RESULTS

(a) Movements of Adult Sticklebacks in Artificial Stream

Effect of Temperature

Temperature affected the direction that adults moved in the current of the experimental stream in darkness ($x^2 = 9.33$, $p \simeq 0.025$) (Table B-I of Appendix B). Net movement at all temperatures tested was downstream, but the percentage

Table XII: Results of experiments on movement of brook sticklebacks in current. Groups of 20 adults or 25 young were exposed to current in two light intensities (dark and light) at one of a series of water temperatures. Results are expressed as the number of sticklebacks found upstream, the number found in the central release pool, and the number found downstream after eight hours. The dashes indicate that no fish were tested.

Test Tempera- ture ° C	ADULTS						YOUNG					
	DARK			LIGHT			DARK			LIGHT		
	Upstream	Release Pool	Down- stream	Upstream	Release Pool	Down- stream	Upstream	Release Pool	Down- stream	Upstream	Release Pool	Down- stream
7.2	2	1	17	7	6	7	-	-	-	-	-	-
10.0	2	0	18	7	6	7	-	-	-	-	-	-
15.6	7	1	12	13	2	5	0	0	25	7	2	16
21.1	8	0	12	12	1	7	0	0	25	11	1	13
23.9	5	0	15	6	8	6	-	-	-	-	-	-
26.7	-	-	-	-	-	-	0	0	25	12	1	12
29.4	-	-	-	-	-	-	6	2	17	5	1	19
	24	2	74	45	23	32	6	2	92	35	5	60

of sticklebacks that moved upstream was greater at 15.6° and 21.1° C (35% and 40%, respectively) than at 7.2°, 10.0° and 23.9° C (10%, 10% and 25%, respectively). Thus upstream movement of adults in darkness appeared to increase within a temperature range that included 15.6° and 21.1° C, but did not include 10.0° and 23.9° C.

Temperature did not significantly affect the direction that adults moved in the current of the experimental stream in light ($x^2 = 3.41$, $p<0.50$), (Table B-II of Appendix B). Figure 20 A suggests that real differences may exist between temperatures in the number of adults moving upstream in light. The percentage that moved upstream appeared to be greater at 15.6° and 21.1° C (65% and 60%, respectively) than at 7.2°, 10.0° and 23.9° C (35%, 35% and 30%, respectively). Numbers moving upstream and downstream at 7.2°, 10.0° and 23.9° C were equal, but net movement at 15.6° and 21.1° C was upstream. If real differences exist between temperatures, sample size may have been too small to detect them.

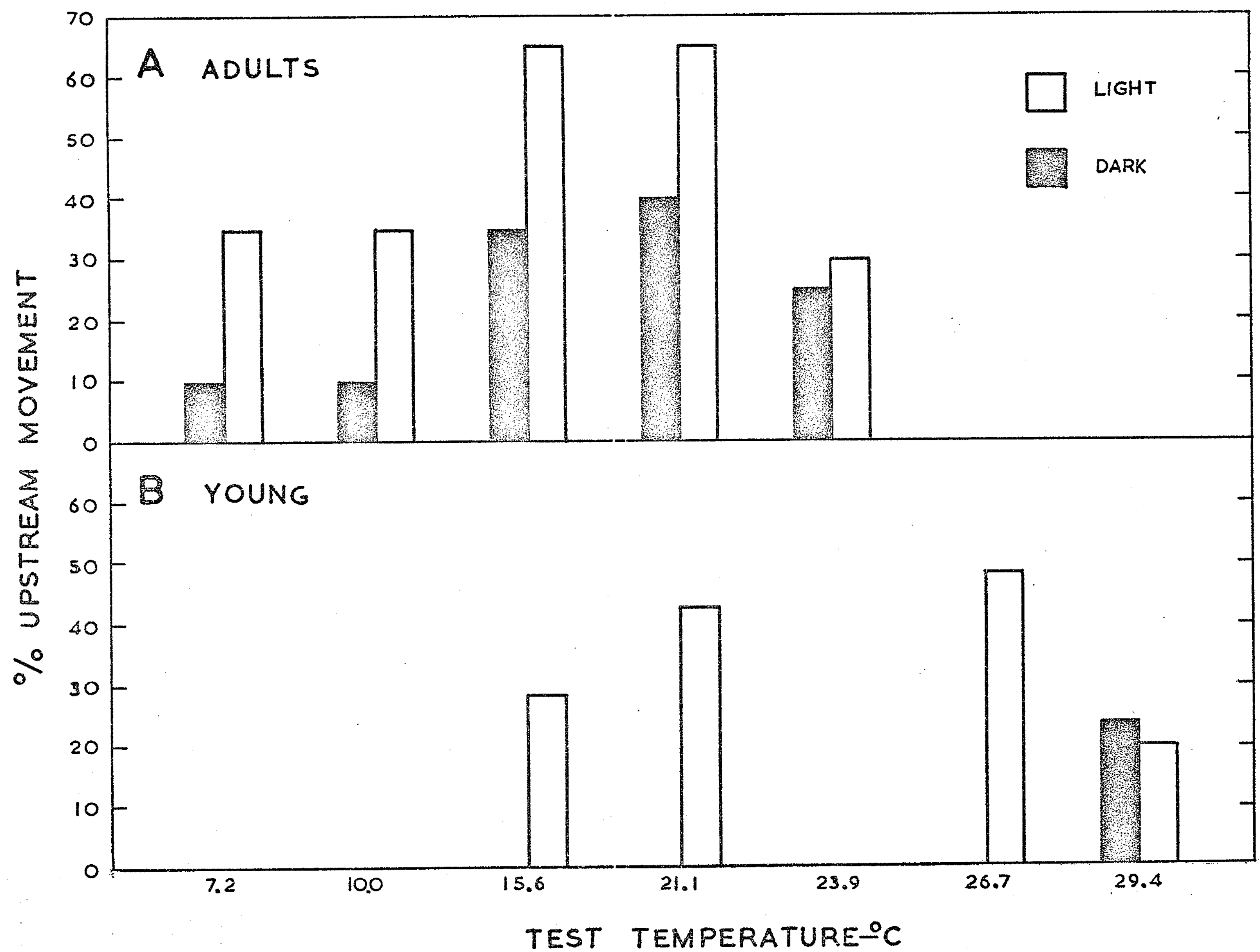
Temperature appeared to influence the number of adults in the release pool in light. Fewer adults appeared not to move at 15.6° and 21.1° C (10% and 5%, respectively) than at 7.2°, 10.0° and 23.9° C (30%, 30% and 40%, respectively).

These results suggest that water temperature affects the movement of adults in current. Upstream movement, in both darkness and light, appeared to be enhanced within a

Figure 20:

A Percentage of adult brook sticklebacks that moved upstream in current.

B Percentage of young brook sticklebacks that moved upstream in current. Groups of 20 adults or 20 young were exposed to current for eight hours at one of a series of test temperatures in two light intensities.


A ADULTS
B YOUNG
LIGHT
DARK
% UPSTREAM MOVEMENT
TEST TEMPERATURE–°C
7.2
10.0
15.6
21.1
23.9
26.7
29.4
0
10
20
30
40
50
60
70

temperature range that included 15.6° and 21.1° C, but did not include 10.0° and 23.9° C. The number of adults that moved downstream or remained in the release pool increased at temperatures outside this range.

Effect of Light Intensity

At all temperatures tested, the number of adults that moved upstream was greater in light than in darkness. When results from all temperatures were summed, 24% of all adults tested in darkness moved upstream, compared to 45% in light. Light intensity had a significant effect on the direction that adults moved at 7.2° - 10.0° C ($x^2 = 11.2$, $p<0.005$) (Table XIII). Results at these test temperatures were combined, to avoid the effect of small numbers on the Chi-square. Light intensity had no significant effect on the direction that adults moved at either 15.6°, 21.1° or 23.9° C.

Light intensity appeared to influence the number of adults in the release pool, as 23% of all adults tested in light were found in the pool, compared to 2% of those tested in darkness.

These results suggest that light intensity may influence the movement of adults in the current of the stream trough. The number of adults that moved upstream or remained in the release pool was greater in light, while downstream movement was greatest in darkness.

Table XIII: Summary of 2 x 2 contingency tests to test the homogeneity of movements of adults in current in darkness and light. Chi-square values were computed with Yate's correction. Number of adults moving up and downstream in current of experimental stream were tested.

Temperature °C	Darkness Up	Darkness Down	Light Up	Light Down	x^2	p
7.2-10	4	35	14	14	11.20	>0.005
15.6	7	12	13	5	3.34	>0.10
21.1	8	12	12	7	1.27	>0.50
23.9	5	15	6	6	1.12	>0.50

(b) Movements of Young Sticklebacks in Artificial Stream

Effect of Temperature

All young sticklebacks tested in darkness at 15.6°, 21.1° and 26.7° C moved downstream, while 24% of those tested at 29.4° C moved upstream. A contingency table could not be used to determine if temperature significantly affected the direction that young moved in the current of the experimental stream, because of the large number of zeros in the results. However, it appears to be improbable that significant differences would be found between temperatures.

Temperature did not significantly affect the direction that young moved in the current of the experimental stream in light ($x^2 = 5.41$, $p<0.25$) (Table B-III of Appendix B). Figure 20 B suggests that real differences may exist between temperatures in the number of young moving upstream in light. The percentage that moved upstream was greater at 21.1° and 26.7° C (44% and 48%, respectively) than at 15.6° and 29.4° C (28% and 20%, respectively). Thus, the upstream movement of young in light may be enhanced within a range of temperatures that included 21.1° C and 26.7° C, but did not include 15.6° and 29.4° C. If real differences exist between temperatures, sample size may have been too small to detect them. The range of temperatures within which upstream movement is enhanced appears to be higher for young than for adults.

These results suggest that temperature affects the movement of young in current in light, but not in darkness. In light, upstream movement increased within a range of temperatures that included 21.1 and 26.7 but not 15.6° and 29.4° C. At temperatures outside this range, downstream movement increased.

Effect of Light Intensity

Upstream movement was greater in light than in darkness at 15.6°, 21.1° and 26.7° C, but not at 29.4° C. Light intensity had a significant effect on the direction that young moved in the experimental stream at 15.6°, 21.1° and 26.7° C, but had no effect at 29.4° C (Table XIV). Results in darkness were combined because temperature did not appear to affect the direction young moved in current. When results from all temperatures were summed, 6% of the young tested in darkness moved upstream, compared to 35% in light.

These results suggest that the movement of sticklebacks in current was influenced by light intensity and water temperature. Upstream movement increased in light and within a particular range of temperatures. This temperature range was higher for young than for adults. Downstream movement increased in darkness and at temperatures outside this range.

Table XIV: Summary of 2 x 2 contingency tables to test the homogeneity of movement of young sticklebacks in darkness and light.

Temperature °C	Darkness		Light		x^2	p
	Up	Down	Up	Down		
15.6	6	92	7	16	9.09	>0.005
21.1	6	92	11	13	22.15	>0.005
26.7	6	92	12	12	26.12	>0.005
29.4	6	92	5	19	3.45	>0.10

SUMMARY OF LABORATORY EXPERIMENTS

(1) SELECTED TEMPERATURES

Hypothesis: The seasonal distribution of brook sticklebacks observed in the field was related to their temperature preferences.

Results: (1) Pre-reproductive adults selected a mean of 17.3° C with a range from 14.9° to 20.2° C, while post-reproductive adults selected a mean of 16.3° C with a range from 8.9° to 25.6° C.

(2) Early in the summer, young selected a mean of 22.6° with a range from 21.1° to 25.2° C. At this time young selected a higher mean temperature than post-reproductive adults independent of acclimation temperature, although the degree of difference depended on the temperature of acclimation. Later in the summer there appeared to be little difference in the temperatures selected by young and by post-reproductive adults.

(3) Seasonal changes were found in the temperatures selected by brook sticklebacks, independent of the effects of acclimatization or acclimation temperature.

Sticlebacks appeared to select a higher temperature in spring and early fall than in winter. An exposure to high temperature appeared to reverse the hiemal decrease in selected temperature.

Conclusion: No evidence was found to refute the hypothesis.

(2) TEMPERATURE RESISTANCE

Hypothesis: Young sticklebacks have a greater resistance to sudden changes of water temperature than adults.

Results: Young sticklebacks tested in early summer had a greater resistance to lethal temperatures than adults, and there was a slight suggestion that the capacity of young to resist high temperatures decreases later in the summer.

Conclusion: The hypothesis is accepted.

(3) EXPERIMENTS IN ARTIFICIAL STREAM

Hypothesis: Light intensity and water temperature affect the movement of sticklebacks in current.

Results: (1) In the experimental stream, movement of sticklebacks in current was influenced by water temperature. Upstream movement of pre-reproductive adults was enhanced within a particular temperature range, that included 15.6° and 21.1° C, but not 10.0° and 23.9° C (Note: this range is similar to the range of temperatures selected by pre-reproductive adults in the experimental gradient). Tests also showed that upstream movement of young, in early summer, was enhanced over a range of temperatures that included 21.1° and 23.9° C, but not 15.6° and 26.7° C (Note: this range is similar to the range of temperatures selected by young in early summer, when tested in the experimental gradient). At temperatures outside these ranges, the number of sticklebacks remaining in the release pool or moving downstream increased.

(2) In the experimental stream, movement of adults was influenced by light intensity. Upstream movement increased in light, while downstream movement increased in darkness.

Conclusion: The hypothesis is accepted.

DISCUSSION

The distribution and movements of brook sticklebacks, observed in the Roseau River appear to be related to the temperatures they selected in a temperature gradient in the laboratory and to their responses to current as observed in an experimental stream. The effect of other factors on the observed distribution and movements must also be considered, as well as the mechanisms governing selected temperatures and current responses.

Distribution of Adult Brook Sticklebacks Prior to Reproduction

During spring in the Roseau River, adult brook sticklebacks moved from deep, cold water to shallow, warm water, where they reproduced. Reisman and Cade (1967) also described the movement, during spring, of pre-reproductive brook sticklebacks into shallow areas of a small pond. Several other authors have noted a similar change in distribution (Barker, 1918; Evermann and Clark, 1920; Winn, 1960; Breder, 1967).

In the present experiments, the temperatures selected by brook sticklebacks appeared to vary with season. Temperatures selected during winter were 6 - 10° C lower than those selected during the rest of the year, independent of the effects of either temperature acclimatization or acclimation.

Sullivan and Fisher (1953) recorded a sharp drop in the selected temperatures of brook trout, Salvelinus fontinalis, during fall and a corresponding rise during spring, independent of the effects of temperature acclimation. They suggested that the changes were controlled by photoperiod. Lustick (1963) found that the metabolic rates of brook sticklebacks decrease during winter and rose again during spring, and that the seasonal changes were controlled by photoperiod rather than water temperature.

An exposure to high temperature may also be capable of inducing the increase in selected temperatures of brook sticklebacks during spring. Adults acclimated to 21.1° C in August and November selected similar mean temperatures, while those acclimated to 4.4° C and 12.8° C selected much lower means in August than in November. In addition, many of the adults acclimated to 21.1° C in November developed secondary sexual characteristics. Baggerman (1960) stated that sudden rises of temperature during spring may cause the onset of mass migrations of Gasterosteus aculeatus, the three-spined stickleback. Reisman and Cade (1967) suggest that the initiation of the breeding season of brook sticklebacks may be dependent on water temperature. Controls of the seasonal changes in preferred temperature appear to be similar to controls of salinity preference of G. aculeatus (Baggerman, 1957; 1960). Light controls the change in salinity preference which occurs prior to migration from salt to fresh water where reproduction occurs, but a sudden increase in water temperature can also induce the same change. Baggerman (1962) suggests that "migration only occurs when the animals are in the proper physiological condition (migration--disposition), and at the same time under the influence of appropriate external stimuli, which may act as 'releasers'", and considered that the change in salinity preference was part of migration--disposition. Thus, the increase in selected temperatures may be part of the migration disposition of brook sticklebacks, and may be necessary prior to movement into shallow water.

The relationship between the density of sticklebacks and water temperature, observed in the Roseau River during spring, suggested that the movement of brook sticklebacks into shallow water prior to reproduction may be orientated by water temperature. Orientation is used here in the sense of "any reaction which guides the animal into its normal habitat, or into other situations of importance to it" (Fraenkel and Gunn, 1940). During spring, pre-reproductive brook sticklebacks appeared to select the warmest water available to them, but avoided water temperatures above 19 - 22° C. Thus they moved from the cold river into the warmest areas of the meltwater ponds and ditches. Densities of sticklebacks in both the ponds and the runoffs, connecting the ponds to the river, thus appeared to be correlated with water temperature.

In laboratory experiments, acclimatized pre-reproductive adult brook sticklebacks selected a mean of 17.3° C and a range from 14.9° to 20.2° C. Brook sticklebacks have definite thermal requirements for successful reproduction. Optimal reproductive activity occurs between 15° and 19° C, and outside this range nest-building and courtship are seriously impaired (Winn, 1960; Reisman and Cade, 1967). Nest-building of brook sticklebacks has been observed in water temperatures as low as 8° C, although successful nests are rarely built below 15° C (Jacobs, 1948). Above 20° C, nest-building ceases completely. Lamsa (1963) related a sudden downstream movement of brook sticklebacks in water temperatures above 19° C to the temperatures required for successful reproduction. McPhail (personal communication) has suggested that optimal temperatures for reproductive activity may be highly correlated with optimal temperatures for survival of eggs to hatching. Temperature requirements for successful reproduction and optimal survival of eggs to young may, therefore, influence the distribution and movements of adult brook sticklebacks prior to reproduction.

Water current also appeared to influence the distribution of pre-reproductive adults during spring. In both field and laboratory, pre-reproductive adults moved upstream in current, suggesting that a positive rheotactic response may be involved in the orientation of brook sticklebacks during movement into areas suitable for reproduction.

Brook sticklebacks were observed to face upstream in current at all times of the year. In the laboratory, upstream movement of sticklebacks was greatest in light, while downstream movement was greatest in darkness, suggesting that visual cues may be used to determine position in current. Harden Jones (1963) showed that G. aculeatus used visual cues to determine their direction of movement in current, and that, during summer, adults appeared to maintain a fixed position in current. Several authors (van Iersel, 1953; Hoar, 1962; van den Assem, 1968) have described the horizontal swimming movements of maturing G. aculeatus, referred to as "fluttering". Fluttering occurs prior to reproduction in spring, and is considered a true migratory behaviour (van Iersel, 1953). Sticklebacks swim back and forth, near either the sides of a glass aquarium or fixed objects on the bottom (Morris, 1958). Reisman and Cade (1967) observed similar movements with maturing brook sticklebacks, but considered the behaviour as exploratory. Van den Assem (1968) showed that the fluttering phase, characterized by long horizontal movements, was followed by a settling phase, in which the movements became gradually shorter and concentrated within one area. This area was eventually defended as a territory. During the period of migration or movement, sticklebacks may move so that visual stimuli pass in a anterio-posterior direction over the retina (Lyon, 1909; Harden Jones, 1968). When combined with an upstream orientation to current, sticklebacks would tend to move upstream in current.

The ability of brook sticklebacks to move upstream appeared to be affected by light intensity, water velocity, and water temperature. Upstream movement appeared to increase in light, while downstream movement appeared to increase in darkness. Light intensity affects the ability of fish to perceive visual cu es (Harden Jones, 1968). Thus, sticklebacks would have a greater chance of losing their sense of direction, and of being swept downstream, in darkness. In the present experiments, upstream movement of pre-reproductive adults appeared to increase within a range of temperatures that included 15.6° and 21.1° C. This range appears similar to that selected by pre-reproductive adults in a gradient (14.9° to 20.2° C). Outside this range, downstream movement appears to increase. To move upstream in the current, of the experimental stream, sticklebacks must swim at a speed slightly faster than the water velocity (8 cm/sec). The swimming speed of fish is optimal within a particular range of temperatures (Fry and Hart, 1948; Brett, Hollands and Alderdice, 1958). Fry and Hart (1948) suggested that fish may select temperatures associated with the maximum cruising speed. Thus, within a range of water temperatures that includes 15.6 and 21.1° C, but not 10.0° and 23.9° C, there appears to be sufficient scope for activity for sticklebacks to move upstream in the trough, and this same range was selected in a temperature gradient. The critical water velocity, at which fish must lose ground, will be just above its cruising speed (Harden Jones, 1968). The maximum visual gains would be made in the slowest velocity consistent with receiving an adequate visual stimulus, which, during spring, would be near the stream banks (Morisawa, 1968). In the field, sticklebacks were found in the lowest velocity of water, and appeared to move upstream near the banks of runoffs and ditches. During early spring, brook sticklebacks would move to the banks of the river, where they would come into contact with the warm water from the runoffs. The positive rheotactic response to current may be orientated by water temperature, so that brook sticklebacks would move upstream from the cold river into the warm meltwater ponds and ditches.

Other components of the environment and biological phenomena may influence the distribution and movements of adult brook sticklebacks prior to reproduction. The schooling of adults, observed during movement, could have several functions. Sticklebacks are more exposed to predation during movement, and schooling may reduce the rate of predation (Fortunatov,1959; Breder, 1967). Schooling would also ensure that groups of sticklebacks, including both sexes, moved into the same area prior to reproduction. Both sexes appeared to move into breeding areas at the same time, although sexual segregation may occur during the formation of territories and nest-building (Reisman and Cade, 1967). Morris (1958) reported that males and females of Pungitius pungitius, the nine-spined stickleback, migrated together. This was related to the fact that nesting occurs in vegetation, where females would have difficulty in locating males if sexual segregation did occur during migration. Sexual segregation during migration occurs with G. aculeatus (van Iersel, 1953) and Apeltes quadracus, the four-spined stickleback (Seal, 1932). Both species reproduce on the bottom in open areas. The presence of other males and females in the same area would stimulate reproductive activity (van den Assem, 1968; Reisman, 1968). Schooling may also tend to increase upstream movement, since sticklebacks in a school will follow each other (Keenleyside, 1955; Harden Jones, 1968).

Food may also have an effect on the distribution of pre-reproductive adults. The meltwater ponds would be more productive than the river during spring, and large amounts of drift food may pass through the runoffs. Sticklebacks may accumulate at the mouth of the runoff during early spring, to feed on the drift. However, sticklebacks appeared to be found in the runoff only when there is a temperature gradient between the runoff and the river, suggesting that they are orientated by temperature rather than food.

To explain the movement of adult brook sticklebacks into shallow, warm water during spring, a temperature--current hypothesis is proposed. The hypothesis is that a positive rheotactic response is orientated by a preference for water temperatures between 15° and 19° C. An alternate hypothesis is that homing may occur. Sticklebacks may return to their parental runoff and pond, but this would be of limited selective value in the Roseau, where the same breeding areas are not present from year to year. Sticklebacks may be attracted by a characteristic component of the water discharged by the runoffs. Such a component might be concentrated in upstream areas, and may act as a cue, guiding sticklebacks into the meltwater ponds. Either the olfactory, gustatory or the common chemical receptors might be used in detecting the chemical component. Several authors have described the relative reduction of both the olfactory and gustatory apparatus in _G. aculeatus_ (Nieuwenhuys, 1959; Segaar, 1961; Freihofer, 1963; Bannister, 1965). In comparison, the olfactory apparatus of salmon is well developed (Hasler, 1966 Harden Jones, 1968). The common chemical sense is the least discriminating of the chemical senses and is only stimulated at high concentrations of acids, alkalis and various irritating compounds (Hoar, 1966). Thus, although it may have an effect, the role of any chemical component, as a cue to stickleback movement, would appear to be of secondary importance.

Another alternate hypothesis is that current may have little effect on the movement of pre-reproductive adults, and that sticklebacks may move along a gradient of water temperatures. However, although temperature stimuli may vary in intensity, they do not have directional properties (Harden Jones, 1968).

Distribution of Adult Brook Sticklebacks After Reproduction

Following reproduction, male adults remained at the nest while females moved into deeper, colder water. After one week to ten days, males also moved into colder areas. Evermann and Clark (1920) observed that adult brook sticklebacks were found in shallow water during spring and in deep water during summer. Adults of both P. pungitius and G. aculeatus also appear to move from shallow to deep water soon after reproduction (Craig-Bennett, 1931; Baggerman, 1957; Morris, 1958; Mullem and Vlught, 1964; Nelson, 1968a; b).

Post-reproductive adults selected a mean of 16.3° C with a range from 8.9° to 25.6° C. The range of temperatures selected by post-reproductive adults appeared to be considerably wider than that selected by pre-reproductive adults. The Coefficient of Variability was also greater for post-reproductive adults, so that their selection response appears to be less precise. Brett (1956) suggested that migrating fishes may be able to detect much smaller temperature changes than at other times. Reproduction, and in particular both the fertilization and early survival of eggs, requires a narrower range of temperatures than other functions (Brett, 1958; McCauley, 1963).

The lack of precision of the reaction of post-reproductive adults to temperature may be important to the survival of both eggs and newly hatched sticklebacks. The parental behaviour of males, including fanning and protection from predators, is necessary to ensure a high rate of survival of the small number of eggs produced by sticklebacks (van Iersel, 1953; Morris, 1958; Sevenster, 1961). Pre-reproductive brook sticklebacks appeared to avoid water temperatures above 19 - 22° C, but post-reproductive adults were found in water temperatures above this level. Thus males will stay at the nest, despite rising water temperatures, for the two weeks to ten days during which parental care is necessary.

Distribution of Young Brook Sticklebacks

During the summer, newly hatched brook sticklebacks in the Roseau River appeared to remain in shallower and warmer water than adults. Faber (1967) noted that young brook sticklebacks have a littoral distribution during summer. Nelson (1968a; b) showed that young *P. pungitius* occupied shallower and warmer areas of a lake than adults during summer. Baggerman (1957) and Craig-Bennett (1931) noted that young *G. aculeatus* remained in nesting areas during summer, while adults moved into deeper water in late spring.

In June, young brook sticklebacks selected a mean of 22.6° C, much higher than that selected by post-reproductive adults. The selected temperatures of young fishes are higher than those selected by adults, especially during any littoral phase in the life history (Ferguson, 1958). Upstream movement of young in light appeared to increase within a range of water temperatures that included 21.1° and 26.7° C, but not 15.6 and 29.4° C. This range appears to be related to the range of temperatures selected by young in a temperature gradient in June (21.1° to 25.2° C). The range of temperatures within which upstream movement appears to be increased is also higher for young than adults. If the temperature range within which upstream movement was enhanced can be related to scope for activity, as was suggested in the discussion of adult movements in current, then the maximum scope for activity appears to occur at a higher temperature for young than for adults. Young selected a range, in the experimental gradient, that was similar to that within which upstream movement was enhanced. Young also have a greater resistance to upward fluctuations of water temperature, and are thus able to survive in warm, shallow environments better than adults.

There are several possible functions of the ecological separation of young and adult sticklebacks:

(1) to reduce cannibalism. Van Iersel (1953) observed that males replace young, which stray from the nest, during the parental phase, but young were eaten once parental behaviour had ceased.

(2) to prevent competition for food between age groups. Abdel' Malek (1968) showed that young and adults of _G. aculeatus_ eat similar food, although large food items were of more importance to adults.

(3) to reduce predation of young sticklebacks by adults of other species. Few predators are found in the shallow environment of young sticklebacks, although _Umbra limi_ was collected in such areas in late spring.

The temperatures selected by young brook sticklebacks in late summer (a mean of 16.9° C with a range from 14.5 to 19.8° C), appeared to be similar to those selected by adults during spring. Young of _G. aculeatus_ move into deeper water during late summer and early fall (Craig-Bennett,1931;

Baggerman, 1957; Mullem and Vlught, 1964). Mullem and Vlught (1964) suggested that a factor associated with length, displaced young into deeper water at this time, as longer individuals appear to move before shorter ones. A relationship may occur between the length of the individual and the temperature it selects.

Temperature and current appear to play an important role in the seasonal distribution and movements of brook sticklebacks. Brook sticklebacks appear to have evolved a pattern of responses to both temperature and current which guides them to areas in the environment where their chances for survival and reproduction are greatest.

SUMMARY AND CONCLUSIONS

The purpose of this study was to study the effect of the environment on the distribution and movements of brook sticklebacks, *Culaea inconstans*. Distribution and movements were studied in the Roseau River during 1967 and 1968. During early spring, large numbers of adults were found in association with runoffs draining meltwater ponds and ditches into either the river or a permanent pond. Later, adults moved from the runoffs into the meltwater ponds, where they reproduced. Following reproduction, adults appeared to move into deep water, while young remained in shallow water during the summer.

Water temperature appeared to influence the distribution and movements of brook sticklebacks. Pre-reproductive adults were found in the runoffs only when water temperatures in the runoff were higher than those of the river. Density of sticklebacks appeared to be correlated with water temperature, although current velocity and the schooling behaviour of adults influenced this relationship. Prior to reproduction, adults appeared to prefer the warmest water available, but they appear to avoid temperatures above 19 - 22° C, by moving downstream. Following reproduction, adults were found in water temperatures above 22° C, but young appeared to remain in warmer water than adults during the summer.

Current appeared to influence the distribution and movements of pre-reproductive adults. They appeared to move upstream in the currents of the runoffs and ditches, suggesting that they may be positively rheotactic. Movement in current appeared to be affected by light intensity and by both the temperature and velocity of water.

The population of brook sticklebacks in the spring, prior to reproduction, appeared to consist mainly of one year olds and a few with an age of two plus. Size may influence the movement of pre-reproductive adults from the runoffs into the meltwater ponds, as larger individuals appeared to be the first to move. Both sexes appear to move into breeding areas at the same time, but sexual segregation may occur during the territorial and parental phases of reproduction.

A temperature gradient was produced in a copper trough in the laboratory to test the hypothesis that the distribution of brook sticklebacks, observed in the field, was influenced by temperature preferences. Pre-reproductive adults appeared to select a range of temperatures associated with the optimal range for reproductive activities. The reaction of post-reproductive adults to temperature appeared to be less precise than that of pre-reproductive adults. In early summer, young appeared to select a higher mean temperature than adults, but the amount of difference depended on the temperature of acclimation. There appeared to be little difference between the mean temperatures selected by adults and young during late summer. Temperatures selected during winter were 6 - 10° C lower than those selected during the rest of the year. By exposing groups of both young and adult brook sticklebacks to a sudden increase in water temperature, young were found to have a greater resistance to such fluctuations, but the capacity of young to resist may decrease during the summer.

An artificial stream trough was used to test the hypothesis that water temperature and light intensity affected the movement of brook sticklebacks in current. Upstream movement of pre-reproductive adults increased within a range of temperatures that included 15.6° and 21.1° C, but not 10.0° C and 23.9° C while upstream movement of young appeared to increase within a range that included 21.1° and 26.7° C, but not 15.6° and 29.4° C. Downstream movement increased at temperatures outside these ranges. A relationship may exist between the ranges within which upstream movement increased and those selected in a temperature gradient. Upstream movement increased in light and downstream movement increased in darkness for both age groups, suggesting that visual cues may be used to determine position in current. Thus both temperature and light intensity appear to influence the movement of sticklebacks in current.

Water temperature and current appear to act as cues, guiding brook sticklebacks to areas in the environment where the chances to survive and reproduce are greatest.

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APPENDIX A: Summary of collection records for station 1, 3, and substation 52 during 1967 and substation 56 during 1968.

TABLE A-1: Summary of collection records from station 1 during Spring 1967, relating the density of sticklebacks to water temperatures.

Site	April 7			April 23			May 5			May 15			May 23		
	Area Seined (m^2)	Temperature C	Stickleback Density Number/m^2	Area Seined (m^2)	Temperature C	Stickleback Density Number/m^2	Area Seined (m^2)	Temperature C	Stickleback Density Number/m^2	Area Seined (m^2)	Temperature C	Stickleback Density Number/m^2	Area Seined (m^2)	Temperature C	Stickleback Density Number/m^2
11	0	-	-	0	-	-	0	-	-	3.5	26.7-23.9	40.8	0	-	-
12	0	-	-	0	-	-	0	-	-	5.5	23.8-21.1	6.2	0	-	-
13	0	-	-	0	-	-	0	-	-	13.7	21.0-18.3	1.1	0	-	-
14	0	-	-	0	-	-	0	-	-	9.3	18.2-15.6	0.1	0	-	-
15	0.42	7.2	119.4	0.84	6.1	32.1	0.56	14.4	37.6	5.2	18.2	2.7	0.56	22.8	12.9
16	0.74	7.2-0	250.7	4.18	6.1-0	0.2	3.71	14.4-5.6	1.2	2.4	17.8	0	0.93	22.8-13.3	16.1
17	6.13	0	0.6	1.86	1.7	0	9.21	5.6	0	2.8	17.0	0	3.35	13.3	0
18	5.96	0	0.2	0	1.7	-	0	5.6	-	0	17.0	-	0	13.3	-

TABLE A-II: Summary of collection records from substation 52 during Spring 1967, relating the density of sticklebacks to water temperature.

Site	May 11			May 23			May 26			June 5		
	Area Seined (m^2)	Temperature ° C	Stickleback Density Number/m^2	Area Seined (m^2)	Temperature ° C	Stickleback Density Number/m^2	Area Seined (m^2)	Temperature ° C	Stickleback Density Number/m^2	Area Seined (m^2)	Temperature ° C	Stickleback Density Number/m^2
521	2.2	8.3	0	0.37	16.7	344.3	0.37	21.1	449.2	D	R	Y
522	13.15	8.3-6.1	0.7	0.56	16.7-15.0	0.7	2.32	21.1-16.7	36.1	2.97	18.9	0
523	2.79	6.1	0	1.23	15.0	0	6.04	16.7	0	18.96	18.9	0.4

TABLE A-III: Summary of collection records from station 3 on April 7, 1967, relating the density of sticklebacks to water temperature and current.

Sampling Site	Area Seined (m^2)	Temperature °C	Current (cm/sec)	Stickleback Density (Number/m^2)
31	10.59	6.1	0	0
32	0.84	6.1	122	30.1
33	13.2	6.1	30	61.1
34	0.84	3.3	92	1.2
35	0.19	3.3	0	5207.8
36	3.44	2.2	30	7.2

TABLE A-IV: Summary of collection records from substation 56 during Spring 1968, relating the density of sticklebacks to water temperatures.

Date		Site									
		560	561	562	563	564	565	566	567	568	569
April 18	Temperature ° C	10.6	12.8	12.8	12.8	12.8	16.1	16.4	16.1	16.4	12.8
	Density (Number/m^2)	0	0	0.6	3.2	35.5	0.6	5.3	3.3	7.6	0
April 25	Temperature ° C	5.6	6.7	7.8	7.8	10.6	12.2	12.8	12.2	12.8	10.0
	Density (Number/m^2)	0	0	0.1	1.7	1.7	0.5	5.3	0.2	0.6	0.1

APPENDIX B: R x 2 contingency tables to test the independence of water temperature and the ratio of the number of brook sticklebacks moving upstream to the number moving downstream.

TABLE B-I: Contingency table to determine the interaction between temperature and direction of movement of adult brook sticklebacks in current in darkness.

Temperature °C	Number Upstream No	Number Downstream No	Total Number N	Fraction Upstream $\hat{p}$	$\hat{p}$ No
7.2+10.0	4	35	39	0.10	0.40
15.6	7	12	19	0.37	2.59
21.1	8	12	20	0.40	3.20
23.9	5	15	20	0.25	1.25
Σ	24	74	98		7.44

$p = \Sigma No/\Sigma N = 24/98 = 0.24$ $\qquad p\Sigma No = 5.76$

$$x^2 = \frac{\Sigma\hat{p}No - p\Sigma No}{p(1 - p)} = \frac{7.44 - 5.76}{0.24 \times 0.76} = 9.33,\ 3df,\ p \simeq 0.025$$

TABLE B-II: Contingency table to determine the interaction between temperature and direction of movement of adult brook sticklebacks in current in light.

Temperature °C	Number Upstream No	Number Downstream No	Total Number N	Fraction Upstream $\hat{p}$	$\hat{p}$ No
7.2	7	7	14	0.50	3.50
10.0	7	7	14	0.50	3.50
15.6	13	5	18	0.72	9.36
21.1	12	7	19	0.63	7.56
23.9	6	6	12	0.50	3.00
Σ	45	32	77		26.92

$p = \Sigma No/\Sigma N = 45/77 = 0.58$ $\qquad p\Sigma No = 26.10$

$$x^2 = \frac{\Sigma\hat{p}No - p\Sigma No}{p(1 - p)} = \frac{26.92 - 26.10}{0.58 \times 0.42}$$

$$= 3.41,\ 4df,\ p<0.50$$

TABLE B-III: Contingency table to determine the interaction between temperature and the direction of movement of young brook sticklebacks in current in light.

Temperature °C	Number Upstream No	Number Downstream No	Total Number N	Fraction Upstream $\hat{p}$	$\hat{p}$ No
15.6	7	16	23	0.30	2.10
21.1	11	13	24	0.46	5.06
26.7	12	12	24	0.50	6.00
29.4	5	19	24	0.21	1.05
Σ	35	60	95		14.21

$p = \Sigma No/\Sigma N = 35/95 = 0.37$ $\qquad p\Sigma No = 12.95$

$$x^2 = \frac{\Sigma \hat{p}No - p\Sigma No}{p(1-p)} = \frac{19.21 - 12.95}{0.37 \times 0.63}$$

$$= 5.41,\ 3df,\ p<0.25$$