SOCIAL BEHAVIOR AND FEEDING ABILITY OF TWO
PHENOTYPES OF GASTEROSTEUS ACULEATUS IN RELATION
TO THEIR SPATIAL AND TROPHIC SEGREGATION IN A
TEMPERATE LAKE

by

GARY LEE LARSON

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Department of Zoology

The University of British Columbia
Vancouver 8, Canada

Date September 1, 1972.

ABSTRACT

Significant differences in morphology, vertical distribution, aggressiveness, and feeding habits were found between limnetic and benthic forms of the threespine stickleback (Gasterosteus aculeatus) in a small coastal British Columbian lake.

In deep laboratory aquaria, simulating lake pelagic and benthic regions, the benthic form was aggressive, solitary, and attracted to bottom cover, in which it remained. The limnetic form, in contrast, was gregarious, non-aggressive, attracted to surface cover, and seldom found near the bottom. In shallow aquaria, representing littoral lake regions, limnetic stickleback were aggressive and non-gregarious until cover was added, whereafter most individuals established residence in it and became significantly less aggressive. Benthic stickleback put into shallow aquaria huddled in small groups until cover was added, after which they spaced themselves out. These results were consistent with differences in the behavior of the two forms of stickleback in the field.

The limnetic form fed heavily on zooplankton in the lake, while the benthic form mostly ate macro-benthos, Gammarus being its principal prey. Laboratory studies compared the feeding rates of both phenotypes; that of the limnetic form was highest on plankton, while that of the benthic form

was highest on amphipods.

The extensive dissimilarities demonstrated in social behavior and feeding ability of the two forms of stickleback indicate that their spatial and trophic segregation in Paxton Lake are due mainly to innate behavioral differences, rather than interaction.

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INTRODUCTION

Many people have studied competition for food, space, or both among fishes in temperate waters (Larkin, 1956). Some authors agree that competition is real (e.g., Nilsson, 1967); others question whether interspecific competition exists to any measurable extent, and suggest natural selection favors species tolerant to the unstable environment (e.g., Larkin, 1956). Part of the disagreement likely develops from the inability to define the niche of each species, and also the supply and demand of the resources competed for. This information is necessary for an accurate interpretation of competitive situations in the field.

After an extensive literature review, and many years of field study of allopatric and sympatric populations of arctic char (Salvelinus alpinus) and brown trout (Salmo trutta) Nilsson (1955, '60, '63, '65, and '67) developed the hypothesis that closely related sympatric species of fish in temperate waters probably segregate through competitive interaction. Using the nomenclature of Brian (1956), Nilsson categorized the segregation as interactive, i.e., the result of interaction (competition) between two or more species for the same environmental resources. He suggested that overlap in ecological requirements emphasized differences between the species, resulting in a refined partitioning of the environmental resources available to each. Nilsson also refers to another

form of segregation in fishes, termed selective, i.e., the species select totally different habitats by instinctive behavior. Selective segregation may be the theoretical end-result of interactive segregation, and may best apply to sympatric fishes in tropical waters (Nilsson, 1967).

The discussion above only refers to those forms which, because they retain their identities through reproductive isolation, are classified as species. Relating the discussion to sympatric subspecies of the same species is difficult because they lack the necessary isolating mechanisms to prevent gene mixing (Mayr, 1965). The occurrence of sympatric subspecies is possible; however, their segregation is likely maintained primarily by interaction, i.e., intraspecific competition (Mettler, et al. 1969).

Two major problems often facing an analysis and interpretation of such competitive situations are: (1) lack of knowledge of whether the species or subspecies evolved in allopatry or sympatry; (2) identification of specific status between sympatric forms, i.e., species, subspecies, or races. Nevertheless, investigating the segregation of sympatric forms of the same species or genus may lead to a better understanding of the role of competition in evolution, because many of the differences seen from allopatric populations may have evolved through sympatric competition.

An opportunity arose to study the spatial and trophic segregation of two "forms" of stickleback inhabiting a lake. The specific status of these forms, be they species or subspecies, is uncertain, but is discussed later. Quantitative field observations and collections were made of feeding habits, distribution, and social behavior of both forms of stickleback. Abundance and distribution of food, and distribution of vegetative cover and other limnological characteristics of their habitats were also measured. Laboratory experiments were conducted to quantify the social behavior and feeding ability of the two forms held separately and together. With these laboratory data I hoped an accurate interpretation could be made of the competitive situation between the two stickleback forms in the lake. In addition, contemporaneous studies of the ecological genetics and habitat selection of these forms by Dr. J. D. McPhail and Mr. R. Jones , when completed, may contribute to an overall definition of the segregation mechanisms involved.

DESCRIPTION OF STUDY AREA

Paxton Lake lies at an elevation of 61 m on Texada Island, a small coastal island in Georgia Strait, British Columbia (Fig. 1). The lake is divided into two basins. Its outlet originally had access to the sea, but was dammed late in 1956, raising the water level of the lake approximately 1.5 m. Since 1957 water has been pumped from the system for industrial purposes resulting in marked annual fluctuations of the water

Fig. 1. Paxton Lake, British Columbia, showing location of the limnological stations ($\blacktriangledown$), bottom trap area, dock, pump, outlet dam and boundary between the basins (----). Depth contours in meters.

level (Fig. 2). In 1969 the fluctuation was nearly normal except for the draw-down in the fall. The water level was abnormally low in 1970, but high in 1971. The surface area of the lake at a level of 48 cm (Fig. 2) is 17 ha., 10 and 7 ha. for basins 1 and 2, respectively. The maximum depth is 16 m.

Trees and brush along the original shoreline to the present high water level are dead as a result of damming. Many dead trees have fallen into the lake, providing additional littoral cover.

Physical characteristics of the littoral areas vary with water level. At high water terrestrial plants dominate the vegetation, but at lower water levels the littoral regions may be covered by aquatic vegetation (predominately Chara), marl, or ooze. In 1969 Chara began to grow along the shoreline once the water level stabilized between September and October. In 1970 the water level was more stable and Chara grew to depths of over 3 m. In August, 1971, high water level prevented Chara growth, however, the previous years stand was still evident between 3 and 6 m.

Cutthroat trout (Salmo clarki) and stickleback apparently are indigenous to the lake. The former was almost completely exterminated when the lake was drawn-down to 11 m in 1957. In 1968, five-thousand coho (Oncorhynchus

Fig. 2. Water level fluctuations in Paxton Lake for 1969 (O), 1970 (Δ), and 1971 ($\blacktriangle$) compared with the mean and range of 1957 - 1968 (—•—). Based upon data provided by Texada Mines Ltd.

kisutch) fingerlings were planted in the lake.

GENERAL METHODS

Field

Observations of stickleback distribution and interaction were made with a viewing box from the dock, the shoreline, and a boat. The maximum viewing depth was about 3.5 m.

Sticklebacks were sampled by dipnet and with wire minnow traps (No. 12562, Canada Fishing Tackle and Sports Ltd.) every three hours for a 24 hour period at approximately monthly intervals. The traps were placed along the lake bottom and also suspended from floating cover (i.e., the dock and trees) to collect samples of benthic and limnetic forms of stickleback. Most bottom samples of fish were taken within a single sampling area (Fig. 1). The traps were placed on the lake bottom at 1 - m depth intervals from 0 - 9 m. Suspended traps were placed about 0.5 m below the lake surface. Only fish greater than 30 mm in length were captured by the traps.

Coho salmon and cutthroat trout were collected by angling and gill netting.

Plankton was sampled with a no. 10 Wisconsin type net (25 cm mouth diameter) in 1969 and with standard Clarke-Bumpus gear in 1970 (no. 10 net). Most samples were taken over the bottom trap area, but some were taken near the

dock and at the limnological stations (Fig. 1). Horizontal tows were made at the surface and vertical tows from 0 - 5 m in each area. Usually the samples were enumerated by triplicate subsamples and the results averaged. In some cases the entire sample was counted. Body measurements of crustacean zooplankters were made along the maximum carapace axis of 30 randomly selected individuals of each genus (Brooks and Dodson, 1965; Galbraith, 1967).

Bottom fauna was sampled with an Ekman dredge (175 cm²). Samples were taken along the bottom trap area at 1 - m depth intervals from 0 - 8 m between June and October, 1970. Most fine debris was removed at the lake by washing the samples in a bucket with a screen bottom (mesh size 1 mm). Each sample was later sorted three times for organisms. The organisms were preserved in 10 per cent formalin.

Growth rate of the amphipod Gammarus lacustris was estimated by measuring its "curl length". This species curled when attacked by stickleback and when preserved after collection. Curl length was measured by holding the cephalothorax parallel to the last posterior abdominal appendage and taking the distance between these structures.

Water temperature, oxygen concentration, and conductance were usually recorded at the limnological stations (Fig. 1), although some readings of temperature and oxygen were also

taken along the littoral areas close to the lake bottom. Water temperatures were recorded with a thermister (model 44TB - Y. S. I. Co. Inc.) or with a F. B. A. oxygen-temperature probe. Dissolved oxygen concentrations were determined by the winkler method in 1969 and with a F. B. A. oxygen-temperature probe in 1970. Conductance was determined with an Industrial Instrument Co. meter, model RB2 - 3341.

Laboratory

Individual fish were kept in 35 l glass aquaria (51 x 25 x 28 cm). Groups of fish were kept in 339 l wood aquaria (118 x 48 x 60 cm) with glass fronts. The aquaria were cleaned at monthly intervals. To reduce fish mortality 2 - 3 oz of rock salt and 2 - 3 drops of malachite green were added to each tank after water was renewed. Each aquarium had an inside filter and air was continually supplied. Water temperature was maintained at 15°C ($\pm 2^{\circ}$). A 12 hour light and dark photoperiod was controlled automatically.

Light intensities were measured at the surface of the water with a photometric cell (G. M. Submarine Photometer, model 15 - m -02/1).

Stomach analysis of the fish followed the per cent frequency of occurrence method outlined by Hynes (1950). Standard body length was taken for each fish.

FIELD RESULTS

Physical and Chemical Limnology

Seasonal changes of water temperature in 1969 and 1970

were similar (Fig. 3). In both years the maximal surface temperatures (about 23° C) occurred between June and July. The thermocline was usually between 2 to 5 m during summer. In winter water temperatures dropped to about 5° C.

Oxygen was present throughout the water column and along the littoral bottom regions during winter and early summer, but was much reduced or absent below 7 m from mid-summer to the fall turnover. H₂S was detected below 7 m in July and August, 1969.

The surface conductivity of Paxton Lake was high for a coastal lake (260 μ Mhos/ cm³ at 25° C on May 23, 1969; see Northcote and Larkin, 1956). The surface pH was slightly above neutrality (7.7 on November 16, 1970). Of the major cations (Na, K, Mg, and Ca) calcium was predominant (58.2 mg/l), the others being less than 2.5 mg/l when determined from surface water taken on November 16, 1970.

Zooplankton

The zooplankton was dominated numerically by cladocerans, especially Bosmina sp., Daphnia sp., and Polyphemus pediculus. Copepods and rotifers were also present but not identified. The population densities of all genera were high in 1969, Bosmina being the most abundant with a maximum density of about 105,000/ m³ in August (Fig. 4). However, all genera declined in abundance in 1970, except Daphnia.

Fig. 3. Isotherms ($^{\circ}\text{C}$) in basin 1 of Paxton Lake for 1969 and 1970.

Fig. 4. Mean number of zooplankton per m^3 for 0 - 5 m tows along the bottom trap area in Paxton Lake during 1969 and 1970. Rotifer data are in units of 10^2 , the other genera are 10^3 .

and Polyphemus. Daphnia was the most abundant form in 1970 reaching a maximum mean density of about 60,000/ m³ in July.

The mean body size of most zooplankters was similar in both years studied (Fig. 5). In 1970 individual Daphnia were four times larger than in 1969, reaching up to 1.3 mm in length.

Macro-benthos

The macro-benthos was dominated in number and probably biomass by a single species of amphipod, Gammarus lacustris (Table 1). Most amphipods were found in the Chara, which was distributed along the littoral areas (Table 2). The average size of Gammarus (Fig. 6) approximately doubled between June (2.6 mm) and October (4.9 mm).

Stickleback

Morphology

Many phenotypes of threespine stickleback occur in Paxton Lake. The predominant forms, "limnetics" and "benthics", occur in the pelagic and benthic regions of the lake respectively. The other forms are not abundant and appear to be intermediate between limnetics and benthics (J. D. McPhail, personal communication).

Limnetics and benthics differ in the number of body plates, number of gill rakers, and the presence or absence of a pelvic girdle and pelvic spines (Table 3). Benthics

Fig. 5. Mean body lengths of the crustacean zooplankton in Paxton Lake from May - September 1969, and July - October 1970. Symbols: copepods ($\square$), *Bosmina* ($\blacksquare$), *Polyphemus* ($\bullet$), and *Daphnia* ($\circ$).

Table 1. Total number of macro-benthic organisms/m²
collected from samples taken at 1-m depth
intervals from 0 - 8 m in Paxton Lake, 1970.

Month	<u>Gammarus</u> <u>lacustris</u>	Chironomid larvae	Baetid nymphs	Gastropods	Sphaeriidae
June	3168	88	44	220	178
July	624	88	0	44	44
August	3679	44	0	44	0
September	4011	0	0	220	0
October	1100	44	44	44	44

Table 2. Depth distribution¹ of Gammarus lacustris/ m² along the bottom trap area (0 - 8 m) in Paxton Lake from June to October, 1970. One sample was taken per month.

Depth	June	July	August	September	October	Mean	Per cent
m	Occurrence						
0	3124 ²	360	1012	3327	308	1626	80.4
1	0	0	0	0	528	106	5.2
2	0	44	44	44	44	35	1.7
3	44	264	220	88	88	141	7.0
4	0	0	88	352	132	114	5.7
5	0	0	0	0	0	0	0.0
6	0	0	0	0	0	0	0.0
7	0	0	0	0	-	0	0.0
8	0	0	0	0	-	0	0.0

¹ Depths not adjusted for fluctuations of water level.

² Vertical line represents the depth distribution of Chara.

Fig. 6. Mean curl length (95 per cent confidence limits) of Gammarus lacustris in Paxton Lake from June - October, 1970. Numbers refer to sample sizes.

Table 3. Some morphological differences between adult benthic and limnetic threespine stickleback in Paxton Lake.

Phenotype	Number of dorsal spines	Number of body plates	Pelvic girdle	Pelvic spines	Number of gill rakers ¹
Benthic	2 - 3	0	absent	absent	11 - 21
Limnetic	3	8 - 18	present	present	18 - 25

¹ Data provided by R. L. Jones.

had significantly ($p < 0.05$; analyses of covariance) heavier bodies, longer jaws, and wider mouths than limnetics of the same body length (Figs. 7, 8, and 9).

The perceptual fields of the two forms were not determined, but positioning of the eyes differed between the phenotypes. Limnetics probably could see well in frontal, caudal, dorsal, and ventral directions because of their narrow bodies and protrusive eyes, as could benthics less than about 55 mm in length. However, for larger benthic stickleback, vision was oriented more to dorsal and frontal directions. Their ventral vision (as indicated by the mm of eye visible from the ventral side of the head) was usually less than that for limnetics of the same length and was apparently negligible above lengths of about 85 mm (Fig. 10).

Body size

Benthic stickleback frequently attained body lengths of over 65 mm, while limnetics seldom grew larger than 60 mm (Fig. 11). In 1970 and 1971, mean body lengths of both phenotypes decreased from those observed in 1969 (Fig. 11). Young-of-the-year of both forms attained between 30 - 40 mm in length by October.

Distribution and substrate preferences

Both phenotypes bred near the shoreline between April and early July. Nests of benthic stickleback were located in cover,

Fig. 7. Relationship between the body length and body weight of benthic (Δ) and limnetic (X) threespine stickleback in Paxton Lake.

Fig. 8. Relationship between the body length and jaw length of benthic (—) and limnetic (-----) threespine stickleback in Paxton Lake. Data points are not presented but the lines (fitted by regression analysis) are based upon 95 benthic and 52 limnetic fish.

Fig. 9. Relationship between the body length and mouth width of benthic (●) and limnetic (○) threespine stickleback in Paxton Lake.

Fig. 10. Relationship between the body length and the ratio of the mm of the left eye visible from the ventral side of the fishes head (VE) to the body length (SBL) of the Paxton Lake benthic (Δ) and limnetic (X) stickleback.

Fig. 11. Frequency distribution of standard body lengths of Paxton Lake stickleback captured in 1969 - 1971.

and limnetic nests were found in open regions (J. D. McPhail, personal communication). After spawning, most limnetic stickleback in 1969 formed large aggregations near the lake surface, usually associated with surface cover such as trees or the dock (Fig. 12).

In 1969, benthic stickleback were usually taken at the lake bottom, in all but the deoxygenated regions below 7 m, where H_2S was present (Fig. 12). They were observed usually as solitary individuals amongst the Chara along the littoral zone, but small groups occurred near sunken logs. Benthics sometimes were found with aggregations of limnetic stickleback. Such individuals were generally less than 60 mm in length.

Both phenotypes were captured during winter, but only in the bottom traps. Most limnetics were captured in the deepest traps, and most benthics in the shallowest ones (Fig. 12).

The summer distribution of both phenotypes was altered in 1970 from that observed in 1969 (Fig. 12). Limnetics were much closer to the shoreline, rarely formed large aggregations in open water, and moved near the shore when travelling from one area of cover to another. Benthic stickleback were in shallower water than in 1969 and most were associated with cover along the lake bottom.

Information on stickleback depth and substrate

Fig. 12. Distribution of Paxton Lake limnetic (hatched bars) and benthic (solid bars) stickleback in 1969 and 1970 taken in surface and bottom traps. Data are presented as per cent frequency of occurrence. Numbers in parentheses give the sum of the number of fish captured/trap/depth zone.

preferences in summer were made by counting fish in standard portions of the shoreline representing different cover types and in five littoral areas near the dock. .

Data from the shore surveys demonstrated segregation in depth and substrate habitat between the phenotypes (Table 4). Limnetics were usually in shallower water than large benthics and associated with Chara or branch substrate. Small benthics were similar to limnetic stickleback in depth distribution and substrate preference except they often occupied open mud areas in greater densities. Large benthics were deeper than limnetics and near mud with cover areas. The extension of Chara from 0.5 m in July to about 3 m by September increased the amount of bottom cover, apparently permitting limnetics and small benthics to occupy deeper water (Table 4). In addition, increased cover appeared to aggregate limnetic stickleback and disperse benthic stickleback (Table 5).

Fish in five littoral areas near the dock (data not shown) were similar in substrate preference to those recorded in the shore surveys (Table 4). However, aggression from large benthics influenced the distribution of limnetic stickleback in the littoral areas. In July large benthics frequently chased limnetics, forcing them to seek refuge above Chara , where large benthics were less numerous (Fig. 13).

Table. 4. Depth and substrate preferences of Paxton Lake stickleback in summer, 1970. Data presented as average number of fish observed per 10 m². About 1000 m² of lake bottom were surveyed each month.

Depth (m)	July			August			September		
	L ¹	B≤50 ²	B>50	L	B≤50	B>50	L	B≤50	B>50
shore - 1	7.8	4.1	0.7	3.0	0.8	0.3	4.0	2.2	0
1 - 2	0.1	12.5	0.6	2.9	0.3	0.4	19.1	3.2	1.4
2 - 3	0.3	92.5	2.3	0.5	0.1	0.6	7.0	1.3	2.2
<u>Substrate</u>									
<u>Above Chara</u>	9.2	1.1	0	2.7	0.3	0	30.8	3.8	0
<u>In Chara</u>	0	1.5	0.1	0	0.1	0	0	0	0
<u>Edge of Chara</u>	0	0.4	1.3	0	0	0.3	0	0	1.2
Open mud	0	91.1	0.2	1.0	0.6	0	0	0.9	0
Mud with cover	0	14.3	34.0	0	0.2	0.8	0	1.4	2.3
Branches	0	0	0	7.1	3.6	0.1	0	0	0

¹ Limnetic stickleback

² Benthic stickleback less than or equal to 50 mm in length

Table 5. Number of stickleback observed together in standard shore and cover type areas of Paxton Lake during summer, 1970.

Month	Stickleback Form	Number Together					
		1	2	3	4	5	5
July	Limnetic	1	1	1	0	0	1
	Benthic 50≤mm	20	12	6	0	2	5
	Benthic 50>mm	11	2	4	2	1	0
August	Limnetic	0	1	3	0	0	7
	Benthic 50≤mm	31	2	2	0	0	0
	Benthic 50>mm	35	0	0	0	0	0

Fig. 13. Relationship between the per cent vegetative cover (Chara) and the total number of limnetic and benthic sticklebacks /m² in the littoral observation areas at Paxton Lake in 1970.

Density of limnetic stickleback in the five littoral observation areas of the lake was negatively related ($p < 0.05$) to the number of large benthics there (Fig. 14). In August large benthics were rarely seen in the observation areas. The number of limnetics then decreased as the percent vegetative cover (Chara) in an area increased (Fig. 13). Thus, these data suggest that large benthics forced limnetic stickleback above the Chara in July.

In summer of 1971 both stickleback forms were only found in the vegetative region along the lake bottom from 3 - 6 m.

Summer activity

The frequency of fish capture in minnow traps was assumed a measure of fish activity, though trap capture efficiency was possibly reduced during darkness. In summer of 1969 benthic stickleback were most active in the evening and late morning (Fig. 15). Limnetics were most active from late morning to darkness. Both phenotypes were most active during the late afternoon in 1970.

Interactions

A series of aggressive encounters was recorded among the two stickleback forms in July and August, 1970. Benthic stickleback initiated most conflicts, which occurred in about equal frequency toward either phenotype. The encounters by both phenotypes are outlined below:

Fig. 14. Relationship between the total number of benthic and limnetic stickleback /m² in the littoral observation areas at Paxton Lake, July 17 - 18, 1970.

Fig. 15. Summer diel activity of Paxton Lake stickleback captured from bottom and suspended trap samples. Data are expressed as the number of fish captured per three hour intervals during 24 hour periods except for pooled data between 00 and 06 hours. The initial time of each interval is given.

Limnetic encounters

(1) Twenty limnetic fry (plus four adults) were distributed in an area of about 0.75 m^2 amongst tall vegetation in water 1 - m deep. The adults were about 20 cm apart and occupied the shadiest regions. The fry were about 5 to 10 cm from each other. Interactions were not observed between fry and adults, but several aggressive conflicts occurred within each group (average nips per five minutes for fry 1.1, adults 0.3). One adult limnetic stickleback attacked a 60 mm benthic swimming near the lake bottom beneath the vegetation. The limnetic nipped the benthic behind the head and then returned to the vegetation. The benthic quickly moved out of the area.

(2) A group of limnetics (58 fry and 10 adults) was distributed above Chara and on mud with cover regions in 1 - m water near the dock. Several aggressive conflicts occurred between individuals on the mud with cover regions; though no encounters were observed between individuals distributed above the Chara.

Benthic encounters

(1) A 65 mm benthic nipped a 40 mm benthic when the latter swam within 10 cm of the former. The conflict occurred above the shore vegetation in 0.5 m of water. The 65 mm benthic held its position and the smaller fish moved into the Chara.

(2) Three aggressive encounters occurred between two 40 mm benthics located above Chara in water 1 - m deep. Both fish retreated in opposite directions.

(3) An aggregation of 8 benthic stickleback (all greater than 50 mm in length) was distributed by a sunken log in 2- m of water. One fish was about 10 cm from the group and it remained in the shadiest region. By nipping the other fish it appeared to keep them out of its area.

(4) Two adult limnetics were moving close to the lake bottom on open mud in 1 - m of water. A 60 mm benthic attacked and nipped both limnetics three times; the latter retreated above the Chara and were not followed by the benthic.

(5) A group of 25 limnetic fry was close to the lake surface in 1 - m of water near the dock. A 65 mm benthic entered the area and quickly chased the limnetics into deeper water. No physical contact was made.

(6) A group of 58 limnetic fry and 10 adults occupied an area of about 2 m² in water 1 m deep near the dock. The fish were distributed above Chara and on mud with cover regions. A 70 mm benthic entered the area and chased the limnetics. No physical contact was made but the limnetics moved into an area of about 0.5 m² above the Chara.

(7) A group of 50 limnetic stickleback fry was attacked and nipped 5 times by a 65 mm benthic when the group passed near the latter's hiding place in 0.5 m of water.

The limnetics moved from the area and the benthic returned to its hiding place.

(8) A group of 30 limnetics was swimming along the edge of the Chara in about 2 -m of water. A 55 mm benthic attacked the group when they passed near the benthic's hiding place. No physical contact was made but the limnetics quickly moved from the area. The benthic returned to its hiding place.

Feeding habits

Benthic stickleback fed mainly upon Gammarus, chironomid larvae, and ostracods (Fig. 16). Some benthics (mostly fish < 55 mm in length) also fed upon plankton in 1969 and 1970 (Fig. 17).

In summer and fall of 1969 limnetic stickleback fed mostly upon plankton (predominantly cladocera). During winter and spring macro-benthos contributed about half of their diet. In summer and fall of 1970 limnetics still fed mostly upon plankton but more macro-benthos was consumed than during the same periods in 1969. The increased contribution of macro-benthos to limnetic diets in 1970 likely resulted from their greater association with the lake bottom (Fig. 12).

Phenotypes of similar lengths usually consumed zooplankton of approximately the same size (Table 6).

Salmon

In fall of 1968, 5000 coho salmon fingerlings (Oncorhynchus kisutch) were planted in Paxton Lake. The

Fig. 16. Feeding habits of Paxton Lake stickleback, 1969 and 1970. Data are expressed as per cent frequency of occurrence and numbers without parentheses indicate the number of stomachs containing each prey type. Numbers in parentheses refer to the number of fish examined. The abbreviations are defined as: zooplankton (hatched bars) - copepods (Z), *Daphnia* and *Bosmina* (D), *Polyphemus pediculus* (P); macro-benthos (solid bars) - *Gammarus lacustris* (A), ostracods (O), chironomid larvae (C), baetid nymphs (B), Sphaeriidae (S), gastropods (G), and miscellaneous (open bar; M).

Fig. 17. Relationship between the body length of Paxton Lake benthic stickleback and the average number of zooplankton consumed per fish size class (bars). Data points are also given.

Table 6. Mean¹ size (mm) of plankters consumed by
Paxton Lake stickleback

Date	Phenotype	Fish Size (mm)	<u>Daphnia</u>	<u>Bosmina</u>	Copepod
July 1969	Limnetic	30 - 40	0.40	0.32	-
		41 - 55	0.36	0.32	0.71
	Benthic	41 - 55	-	0.35	0.57
		55 - 75	-	0.35	0.64
August 1969	Limnetic	41 - 55	0.42	0.36	0.91
		55 - 65	0.40	0.34	0.62
	Benthic	41 - 55	0.40	0.38	-
October 1970	Limnetic	41 - 55	1.15	-	-
	Benthic	41 - 55	1.14	-	-

¹
Based upon body measurements of at least 20 individuals of
each predator type.

fish were 12 - 15 cm in length next summer and attained lengths up to 34 cm by August 1971.

Salmon consumed mostly insects in July and August, 1969, and May, 1970 (Fig. 18). In June, 1970, they fed mainly upon insects and Gammarus. Chaoborus, Gammarus, and stickleback were the predominant prey in July. Daphnia and Gammarus were the predominant prey in October. In August, 1971, stickleback were the predominant prey.

The salmon attacked limnetic stickleback near the dock during daylight hours in July, 1970, frequently causing the latter to move onto the littoral region. Observations were made for 30 minutes once per 3 hours from 0600 - 2100. The mean rate of attack was 2 per 30 minutes.

LABORATORY RESULTS

Social Behavior

Vertical distribution and aggression

Adults

An experiment was conducted to determine if adult benthic and limnetic stickleback differed in vertical distribution and aggressive behavior in standard 339 l aquaria, simulating pelagic and benthic habitats in the lake.

The fish were fed Tubifex once each day. All fish were held under standard laboratory conditions for 2 - 3 months before testing. Three groups of each phenotype

Fig. 18. Feeding habits of coho salmon in Paxton Lake, 1969 to 1971. Data are expressed as per cent frequency of occurrence and numbers without parentheses indicate the number of stomachs containing each prey type. Number in parentheses refer to the number of fish examined. The abbreviations are defined as: zooplankton (hatched bars) - Polyphemus pediculus (P), Daphnia (D); macro-benthos (solid bars) - Gammarus lacustris (A), gastropods (G), chironomid larvae (C), Chaoborus (L); insects (I), and stickleback (S).

(20 fish/ group) were tested separately.

A horizontal line on the glass front divided the water column into upper and lower halves. Vertical distributions of the fish were recorded as the number of individuals in the upper and lower halves of the aquaria. Counts were made from behind a partition once per hour for 6 hours on each of three days. Light intensity was 10.4 lux at the water surface.

Limnetic stickleback were in the upper half of the aquaria more than benthics were (Table 7). They usually remained in a group and showed little aggressive behavior. Benthic stickleback usually remained in the lower half of the aquaria in scattered groups of up to 10 individuals. All fish were aggressive, especially solitary individuals.

After termination of some experiments water temperatures in the aquaria dropped below 8°C. In such cases most individuals of both phenotypes were observed on the aquaria bottoms where they remained inactive.

Fry

An experiment similar to the above was conducted with benthic and limnetic stickleback young-of-the-year (fry). Benthic fry were hatched and reared in the laboratory. No pregnant limnetics were available, so lake reared fry were used. Limnetics were heavier than benthics (mean weights 0.21 and 0.14 gm, respectively). Both forms seemed in good condition.

Table 7. Mean number of Paxton Lake stickleback in the upper and lower halves of the standard 339 l aquaria.

Phenotype	Group	Number in upper half	Number in lower half
Limnetic	1	56	64
	2	48	72
	3	40	80
Benthic	1	5	115
	2	10	110
	3	9	111

Standard 35 l aquaria were used for rearing and testing. Four groups of twenty fish of each phenotype were used; two of each phenotype were fed chopped Tubifex, the rest were fed Artemia salina nauplii.

Vertical distributions were recorded as for adults. Counts were made once per hour for 5 hours each day. Light intensity at the water surface ranged between 13 - 15 lux. No food was present during observation periods.

Limnetics were in the upper half of the water column more than benthic stickleback ($p < 0.05$; Fig. 19). Food types did not influence this relationship between the phenotypes (Fig. 19). Most limnetics remained together, generally in the central tank region, and rarely hovered close to the tank bottom. Benthics generally hovered in small groups close to the tank bottom, usually in corners. Aggression was rarely observed within either phenotype.

Individuals and pairs with groups

An experiment was conducted to determine if individual adult benthic and limnetic stickleback were equally attracted and aggressive to groups of fish and if equal social status was established between paired fishes of the same and different phenotype.

A standard 339 l aquarium was divided in half with a vertical wooden partition. Single clear glass one gallon jars (16 cm diameter) were suspended near the water surface

Fig. 19. Total daily number of Paxton Lake benthic and limnetic stickleback fry in the upper halves of 35 l aquaria.

in each half of the tank. A line was drawn around the middle of the jar parallel to the tank bottom, dividing it into upper and lower sections. Light intensity was 10.4 lux at the water surface.

Five fish of the same phenotype were placed in each jar, and individual fish ("rovers") added to each half of the tank. Rover behavior was recorded for four 5 minute periods, each 20 minutes apart. The time rovers spent along the upper and lower sections of the jar (within 15 cm of it) and the number of nips to fish in the jars were recorded. An additional rover was then added to the aquarium and the positions of each at the jar as well as aggression between them were recorded continuously for 15 minutes immediately after introduction and then for three additional 5 minute periods, each 20 minutes apart. Rover pairs of the same and different phenotypes were tested. Body lengths of the fish tested ranged between 45 - 75 mm for benthics and 45 - 61 mm for limnetics.

Social status in paired rover tests were recorded as dominance when one fish apparently controlled the jar position of the other, and no dominance when both fish had free access to jar positions.

A two way analysis of variance tested single rover data for time at the jars and aggression toward the fish in the jars. Analysis of jar positions compared the subordinate

fishes frequency at upper and lower jar sections while solitary and when paired with another rover. Analysis of aggression in paired tests compare: (1) aggression by dominant fish to subordinate of each phenotype, and (2) aggression by subordinate to dominants of each phenotype.

A pilot series was conducted with no fish in the jars. Limnetic rovers never came to the jar; benthic rovers occasionally visited the jar for short periods.

The analysis of solitary rover behavior (Tables 8 and 9) indicated : (1) individual rover benthics spent more time with the jar groups of either phenotype than did limnetic rovers; (2) benthic rovers spent more time with groups of benthics, while limnetic rovers spent equal time with groups of either phenotype; (3) for each rover phenotype aggression was equal toward individual jar groups of benthics and limnetics; and (4) benthic rovers were more aggressive than limnetics to the groups.

Dominance was observed in all paired rover tests. In the tests with mixed phenotypes, benthics dominated limnetics significantly more frequently (sign test, $p = 0.01$, $N = 19$, $X = 4$).

Subordinate fish moved to the upper jar section when dominated by benthics ($p < 0.05$), but not when dominated by limnetics ($p > 0.05$; Fig. 20); however, subordinate were positioned a few centimeters from the dominant limnetics

Table 8. Statistical analysis of the time solitary benthic and limnetic stickleback (rovers) spent with fish of either phenotype in the jars

Source of Variation	df	SS	MS	F	Significance (p = 0.05)
Treatments	3	12681.9	4227.3	16.3	S
L ¹ x B ¹ vs Rovers	1	10848.0	10848.0	41.9	S
L vs B with B ² Rovers	1	1826.0	1826.0	7.1	S
L vs B with L ³ Rovers	1	7.9	7.9	0.03	NS
Within	49	12685.0	258.9	-	
Total	52				

- 1
L = Limnetic stickleback in the jars
B = benthic stickleback in the jars
- 2
Benthic rovers
- 3
Limnetic rovers

Table 9. Statistical analysis of aggressive activity of solitary benthic and limnetic stickleback (rovers) toward fish of either phenotype in the jars.

Source of Variation	df	SS	MS	F	Significance (p = 0.05)
Treatments	3	52375.5	17458.5	8.85	S
L ¹ and B ¹ vs rovers	1	47581.0	47581.0	24.12	S
L vs B with B ² rovers	1	4793.0	4793.0	2.43	NS
L vs B with L ³ rovers	1	1.5	1.5	< 0.00	NS
Within	54	106504.0	1972.3	-	
Total	57				

¹
L = Limnetic stickleback in jars
B = Benthic stickleback in jars

²
Benthic rover

³
Limnetic rover

Fig. 20. Average time subordinate benthic and limnetic stickleback spent at jar groups without and with dominant fishes present in aquaria.
 Symbols: Dom - dominant, Sub - subordinate,
 B - benthic, L - limnetic

at the bottom of the jar.

Dominant benthics were more aggressive toward subordinates than were dominant limnetics ($p < 0.05$; Fig. 21). However, dominants of either phenotype were most aggressive toward subordinate benthics ($p < 0.05$). Subordinate fish were equally aggressive to dominants of either phenotype ($p < 0.05$).

The above data indicated that benthic rovers were more aggressive toward individuals and groups of either phenotype than were limnetic rovers. Most benthics attacked the fish. Solitary limnetic rovers showed little aggressive behavior and usually positioned themselves near the jar groups. Defence of the jar bottom by dominant limnetics from other rovers in paired tests was probably similar to their territorial behavior in tall vegetation.

A significant relationship ($p < 0.05$) was observed between the total number of aggressive acts (attempted nips) to fish in the jars by each benthic rover in solitary tests, and the number of nips they made to other rovers in paired tests (Fig. 22). The number of nips to fish in the jars was used as a test of the aggressiveness of solitary shore benthics and individuals from groups deeper in Paxton Lake. Shore benthics were more aggressive (Table 10).

Groups of limnetics with single benthics

An experiment was conducted to determine if individual

Fig. 21. Average number of aggressive acts (nips) between subordinate and dominant Paxton Lake stickleback in the paired rover tests. Data compares benthic vs benthic, limnetic vs limnetic, and benthic vs limnetic rovers. Symbols: Dom - dominant, Sub - subordinate, B - benthic, and L - limnetic.

Fig. 22. Relationship between the total number of nips to fish in the jars when benthic rovers were solitary and the number of nips they made to other rovers in paired tests.

Table 10. Statistical analysis of the aggressiveness of benthic stickleback captured from shallow and deep areas in Paxton Lake, 1970.

Depth Captured	Number of Fish	Mean Nips ($\pm$ 95% C.L.) ¹	t-Test unpaired	Significance p = 0.05
1 m	5	17.2 $\pm$ 7.3	calc. = 3.12	p < 0.05
3 m	4	4.4 $\pm$ 1.8	df = 34	

¹
95 % confidence limits

benthic stickleback were aggressive to groups of limnetics, and if they altered the latter's vertical distribution.

A standard 339 l aquarium was used in all tests. A horizontal line across the tank front divided the water column into upper and lower sections. Fish positions were recorded photographically every 15 minutes from 1100 to 1745 hours and scored as the number of fish in each section. Statistical analysis compared limnetic vertical distributions before and during contact with each benthic. Aggression was recorded by counting the number of nips between the phenotypes for five minutes once per hour.

Each benthic was tested separately; the smallest benthics were used first in each test, followed by increasingly large fish. The number of days of recording per fish, body size of each fish, order of benthics used, and the conditioning period before testing are presented in Table 11. Light intensity was 15 lux at the water surface. The fish were fed at 1800 hours each day.

All benthic stickleback usually remained near the tank bottom. Vertical distribution of limnetic stickleback shifted downward after contact with the very aggressive 53 mm benthic in test 1, and during contact with the 55 mm benthic in test 2 ($p < 0.05$); thereafter the limnetics remained down in the aquaria with or without larger benthics in each test (Fig. 23). The 45 mm benthic in test 2 did not

Table 11. Number of days per test, body length of the fish, order of benthics used, and the conditioning period before each test

Test	Days Each Benthic Examined	Mean Length Limnetics	Benthic Lengths and Order Used	Conditioning Period
1	5	55.7	53, 65, and 75 mm	5 months
2	2	50.9	45, 55, 65, and 75 mm	1 month

Fig. 23. Total number of limnetic stickleback in the upper half of a standard 339 l aquarium per session without and with individual benthics of different body lengths in test 1 and test 2.

change the vertical distribution of limnetics ($p > 0.05$).

Benthic aggression generally increased after 1400 hours, probably in response to the limnetics' increased occupation of the upper water column during that time in "anticipation" of food. The relationship between the mean number of limnetics in the upper half of the water column each day and the mean number of benthic nips to limnetics each day was significant ($p < 0.05$), indicating that when limnetic stickleback remained in the lower half of the water column benthic aggression was reduced (Fig. 24).

Intermediate sized benthics were more aggressive than were the smaller or larger ones used (Fig. 25). Limnetic stickleback showed little aggression.

The data indicated that benthic stickleback are aggressive to groups of limnetics and can change the latter's vertical distribution. The downward shift of the limnetics distribution was likely caused by their confinement to a small aquarium, since they showed no such behavior in the field.

Cover effects

An experiment was conducted to determine if the limnetics association with floating cover and the benthics association with bottom cover in Paxton Lake were normal behavior patterns.

A standard 339 l aquarium was used for all tests. Vertical distributions were determined by counting the number

Fig. 24. Relationship between the mean number of limnetic stickleback in the upper half of a 339 l aquarium and the mean number of benthic nips to limnetics each day. Symbols: test 1 (O), test 2(●).

Fig. 25. Aggression between single benthics of different lengths and groups of 20 limnetics. Benthic data presented as the mean number of nips to limnetic stickleback each day; limnetic data as the mean nips to benthic stickleback each day (test 1(●), test 2(○)).

of fish in the upper and lower halves of the water column at hourly intervals (1100 to 1800 for limnetics, and 1200 to 1600 for benthics). One group (15 fish) of each phenotype was tested without and with cover (a dead pine branch) in the tank. Cover was positioned in upper and lower halves of the water column, occupying about half the volume of each half. One aggressive 65 mm benthic was used in the second test with limnetic stickleback.

When cover was absent most benthics remained in the lower portion of the water column (Fig. 26). No distributional changes occurred when cover was in the lower half of the water column ($p > 0.05$); however, 1 - 4 benthics defended portions of the cover from other individuals. The number of benthics in the upper half of the water column increased when floating cover was present ($p < 0.05$), but two individuals accounted for most of the increase.

The number of limnetic stickleback in the upper portion of the water column increased when cover was in either portion of the water column ($p < 0.05$; Fig. 27). However, most individuals moved into the upper half when floating cover was present (Fig. 27).

The aggressive benthic did not alter the limnetics vertical distribution when floating cover was present ($p > 0.05$; Fig. 27). However, it increased the number of limnetics in the upper half when cover was in the lower half

Fig. 26. Total number of benthic stickleback in the upper half of the standard 339 l aquarium each day without and with cover in the lower and upper halves of the water column. Maximum possible total count in the upper half each day would be 75.

Fig. 27. The effects of cover and benthic aggression on the total number of limnetic stickleback in the upper half of standard 339 l aquaria each day. Maximum possible total count in the upper half each day would be 120.

of the water column ($p < 0.05$). When cover was removed the number of limnetics in the lower half of the water column increased from that observed prior to the addition of cover or the benthic ($p < 0.05$).

The selection of different cover position by the two stickleback forms in the lake was also found in the laboratory experiments. The data strongly suggest the summer distribution of limnetic stickleback near floating cover and benthic stickleback near submerged cover to be normal features of their behavior and not the result of interference between the two forms.

Distribution and aggression in shallow water

An experiment was conducted to examine aggression and aggregation behavior of each phenotype in aquaria representing littoral regions of Paxton Lake.

Two groups of limnetic and one group of benthic stickleback were used. A group contained 14 fish and was randomly divided into lots and placed into deep or shallow aquaria. Later the lots were switched to opposite aquaria and retested.

The deep aquarium (a standard 339 l tank) was divided into three vertical sections by lines on the glass front. The shallow tank measured 116 x 91 x 20 cm, held 211 l of water, and was marked into 6 sections (each 38.5 x 45.5 cm) by lines on its bottom.

Distributions were recorded as the sum of individuals

in groups of four or more per tank section from observations made once per half hour for 7 hours each day. In limnetic tests aggression was recorded for five minute periods once per hour for 7 hours each day. Aggression was not recorded in benthic tests.

Limnetic and benthic stickleback were tested without and with cover (a dead pine branch) in the shallow tank. The cover filled one tank section. Light intensity was held at 0.7, 5.0, or 8.6 lux in the limnetic tests when cover was absent, and was 8.6 lux in others. One aggressive 65 mm benthic was used in the second tests with limnetic stickleback.

When cover was absent limnetic stickleback were less gregarious in the shallow tank than in the deep tank at all light intensities (Fig. 28). However, in the shallow tank the number of fish together increased as did aggression with increasing light intensity (Figs. 28 and 29). The relationship between the number of fish together and the number of aggressive acts each day was significant ($p < 0.05$). Aggression was low in the deep tank and apparently not influenced by light intensity.

When cover was added to the shallow tank most limnetics moved into it and the total number of fish together each day increased significantly ($p < 0.05$; Fig. 30). The addition of the benthic further increased the number of limnetics occupying the cover in run 1 ($p < 0.05$), but not in run 2

Fig. 28. Relationship between light intensity (lux) at the water surface and the total number of limnetics together in groups of 4 or more each day in shallow (X) and deep (Δ) aquaria. Maximum possible total number of fish together each session would be 98.

Fig. 29. Relationship between light intensity (lux) at the water surface and the total number of nips by limnetics each day in the shallow aquarium (test 1 (X), test 2 (Δ)).

Fig. 30. The effects of cover and an individual benthic on the aggression (nips) and aggregation behaviors of limnetic stickleback in shallow aquaria. Symbols as total each day for: number together in groups of 4 or more (●—●), number in tank section where cover was placed (○—○), limnetic to limnetic aggression (Δ—Δ), and benthic to limnetic aggression (■—■).

($p > 0.05$). In run 2 benthic aggression increased and forced some limnetics from the cover.

Limnetic - limnetic aggression decreased when cover was added to the shallow tank ($p < 0.05$; Table 12) and further decreased when the benthic was present (Fig. 30).

Most benthic stickleback hovered in small groups in the corners of both aquaria when cover was absent. When cover was added to the shallow tank they dispersed ($p < 0.05$; Fig. 31) and 1 - 3 individuals defended portions of the cover from other fishes.

The laboratory results on the aggression and aggregation behavior of the two forms of Paxton Lake stickleback on simulated lake regions of open mud and mud with tall vegetation were consistent with field observations, strongly suggesting that these activities represent normal features of their behavior.

Feeding Ability

A series of experiments was conducted to compare the feeding ability of the two stickleback forms on types of prey they utilized in Paxton Lake. All tests were conducted in standard 35-l aquaria illuminated with single 40 and 10 watt bulbs (surface light intensity of 14.9 lux). Observations were made from behind one-way plastic mirrors. All data were recorded on a Rustrak 4 channel tape recorder. Tubifex sp., Artemia salina nauplii, Daphnia sp., and the amphipod Hyalella azteca were used for prey.

Table 12. Statistical analysis of the effects of cover on the aggressive behavior between limnetic stickleback in the shallow aquarium.

Tank Condition	Number of Observations	Mean nips per day	t- Test ¹
No cover	6	41.8	calc. = 6.9 p < 0.05
Cover	6	23.5	

¹ Level of significance = 0.05

Fig. 31. The effects of cover on benthic stickleback aggregation behavior in the shallow aquarium. Symbols as total each day for: number together in groups of 4 or more (●—●), and number in tank section where cover was placed (○—○). Maximum possible together each session would be 98.

The effect of starvation on hunger

Benthic stickleback weigh more than limnetics of the same body length (Fig. 7), and therefore may have higher rates of food consumption. Consequently the weight of food consumed by each phenotype after being starved for various periods of time were determined in order to standardize stomach fullness at the start of each feeding experiment.

The amount of Tubifex eaten was determined as the difference between the ration provided and that remaining after 30 minute feeding sessions. Prey was filtered on Watman paper (No. 1) for two minutes with constant suction to remove excess moisture before weighing. The basic methodology of Holling (1966) was followed to determine the amount of food eaten. Fish were starved 12, 24, 48, and in two cases, 72 hours between feeding. It was assumed that no food would be consumed with zero hours of starvation. For statistical analysis mean rations for each fish (five replicates per fish per deprivation level) were proportioned to the body weight of the heaviest benthic (4.5 gm; Appendix 1).

The amount of food eaten is a measure of hunger (H) if it represents the quantity of food passing from the gut during the times of deprivation. Thus,

$$dH/dTF = AD (HK - H) \quad (1)$$

where TF is the time of food deprivation, AD is a constant,

the rate of food disappearance, and HK is the maximum amount of food the gut can hold. This equation integrates to

$$H = HK(1 - e^{-AD(TF)}), \quad (2)$$

which in turn can be transformed into the linear form of

$$(HK - H)/HK = e^{-AD(TF)} \quad (3)$$

or

$$\ln \left(\frac{HK}{HK - H} \right) = AD(TF) \quad (4)$$

The last equation (4) provides a way to test the descriptive powers of the original assumption in equation 1, for if equation 2 does describe the relationship adequately, then a plot of $\ln \left(\frac{HK}{HK - H} \right)$ vs TF should yield a straight line passing through the origin, with slope AD (Holling, 1966).

Benthic and limnetic stickleback consumed equal amounts of food in proportion to their body weights. Per cent body weight of food consumed at all levels of deprivation ranged from 2.47 - 7.41 and 2.18 - 8.48 for benthics and limnetics, respectively (Appendix 2).

HK was 0.31 at 72 hours of deprivation for 69 mm benthics (Fig. 32). Hunger did not differ between the phenotypes up to 24 hours of deprivation ($p > 0.05$; Fig. 33). However, after 24 hours of deprivation hunger decreased for all limnetics and those benthics ≤ 59 mm in length (benthics between 60 and 68 mm were not examined). Such a decrease may reflect an incapacity to consume as much food

Fig. 32. Relationship between hours of deprivation and weight (gm) of Tubifex eaten by three 69 mm benthic stickleback. All data are proportioned to the heaviest individual (4.5 gm).

Fig. 33. Relationship between hours of deprivation and the proportion of the maximum food ration eaten for all limnetic and benthic stickleback up to 24 hours ($p < 0.05$; ----), and individual benthics (69 mm (●), ≤ 59 mm (■)) and limnetics (X) at 48 hours.

after 48 hours of starvation, than an actual decrease in the rate of food passage from the gut. The two 69 mm benthics tested showed no such decrease in food intake up to 72 hours of deprivation (Fig. 33).

All fish were starved 24 hours before subsequent experiments.

Feeding

On Tubifex

An experiment was conducted to examine effects of mouth size on feeding rates of benthic and limnetic stickleback.

The mean daily ration of Tubifex for each fish was placed into a 10 x 5 x 1 cm aluminium cup on the tank bottom. Prey animals were allowed to bunch together before each fish was tested. The number of grasps at the food by each fish tested as well as its stomach fullness were recorded after 30 minute feeding periods. The amount of food eaten was determined as outlined in the previous experiment. The index of stomach fullness was determined from the following relationship:

$$H = \frac{\text{Obs}}{\text{Max}}$$

where H is an index of stomach fullness, Obs the amount of food consumed, and Max the mean daily ration of each fish.

Limnetic stickleback of 45, 55, and 60 mm and benthics of 55, 60, and 69 mm in length were used.

Benthic stickleback usually consumed more prey per grasp than did limnetics (Fig. 34); they frequently took 90 per cent or more of their total ration in 10 or fewer grasps. Most benthics grasped loose worms after consuming much of their ration and while this activity greatly increased the number of grasps, additional prey added little to the final H values.

On Artemia salina nauplii

This experiment was conducted to compare feeding ability of the two stickleback forms on a small prey, Artemia salina nauplii.

Brine shrimp nauplii were hatched and separated from unhatched eggs and empty egg cases following the methodology of Larson (1970). Test fish were fed nauplii for at least two weeks prior to experiments. Large benthics (> 55 mm) fed poorly on nauplii during the conditioning period, and their diets were supplemented periodically with Tubifex.

Nauplii usually sank to the tank bottom, and most were captured there by each phenotype. Nauplii used in the experiment were 0.6 - 0.7 mm in length, presented in densities of 1750 - 2300/ tank.

Data on feeding ability were obtained by recording

Fig. 34. Relationship between number of grasps per feeding session and index of stomach fullness at the end of the feeding session for benthic (●) and limnetic (○) stickleback.

the interval between grasps at individual nauplii, the manipulation (swallowing) time, and the search + fixation + approach time (collectively called search time). Search time was equal to the interval between grasps minus manipulation time. Data represent means of at least 40 consumptions per fish. Most fish were tested 2 - 3 times.

Limnetics ≤ 50 mm in length had the shortest interval between grasps, manipulated the fastest and searched the least (Fig. 35). Small benthics (≤ 45 mm) performed as well as limnetics over 50 mm in length. Larger benthics fed at a low rate and those longer than 64 mm ate no nauplii at all.

Manipulation time contributed most to the interval between grasps for limnetics, while search time contributed most for benthics, especially the larger individuals.

On Daphnia

To examine feeding ability of limnetic and benthic forms of stickleback on prey swimming actively in the water column a series of experiments was conducted using Daphnia.

The prey was similar in size (average 1.09 ± 0.14 mm) to larger Daphnia in Paxton Lake (Fig. 5). Prey density was fixed at 2.9 per l at the start of one set of experiments (Fig. 36) and tested over a range of 0.1 to 2.9 per l in another (Fig. 37). Data were collected from 7 - 25 consumptions per fish in experiments with variable

Fig. 35. Relationship between fish length and interval between grasps, manipulation time, and search time for benthic (●) and limnetic (○) stickleback feeding on Artemia salina nauplii.

prey densities and from 25 consumptions per fish in the other tests. All fish were fed Daphnia for two weeks before the experiment started. Only fish of each phenotype with the highest feeding rate on Daphnia were used in the experiment with variable prey densities.

Limnetic stickleback searched less, manipulated the prey faster, and consumed them at a higher rate than did most benthics (Fig. 36). Large limnetics had the highest feeding and manipulation rates; however, search time was about the same for limnetics of all sizes. Small (≤ 51 mm) benthic stickleback fed at higher rates, manipulated prey faster, and searched less than large benthics.

Search time contributed most to the low feeding rates of benthic stickleback, especially for large individuals; manipulation time made the largest contribution to the high feeding rates of limnetic stickleback.

The 60 mm limnetic fed at a higher rate than the 51 mm benthic at all prey densities (Fig. 37). Feeding rate did not increase with prey densities above 1.1 per liter for either phenotype.

On Hyaella

A final block of experiments examined various aspects of benthic and limnetic stickleback feeding upon amphipods, an important food item for the benthic form in Paxton Lake.

Fig. 36. Relationship between fish length and interval between grasps, manipulation time, and search time for benthic (●) and limnetic (○) stickleback feeding on Daphnia.

Fig. 37. Relationship between prey density and interval between grasps for the 60 mm limnetic (O) and the 51 mm benthic (●) stickleback feeding on Daphnia. Ninety-five per cent confidence limits are included.

Maximum prey size

Fish were fed Hyaletella for one week before the experiments started. During tests four or five amphipods of known size were placed into experimental aquaria and exposed to predation by individual fish for 45 minutes. Consumed prey were recorded. Each fish was tested at least four times.

Over the size range examined, benthic stickleback consistently consumed larger amphipods than did limnetics (Fig. 38). The 60-mm limnetic and 45-mm benthic consumed prey of similar size. Benthics larger than 51-mm were not tested because such individuals could consume the largest amphipods in Paxton Lake.

Manipulation time

Up to 6 amphipods were fed individually to each fish tested and manipulation (swallowing) times recorded. Manipulation time did not equal handling time because prey were often rejected several times before being consumed.

Manipulation times increased linearly for each predator as prey size increased (Fig. 39) and all slopes (from regression analysis) were significantly different from zero except for the 54-mm limnetic (Appendix 2). Slopes decreased as predator size increased and were smaller for benthics than limnetics of similar body length (Fig. 40).

Fig. 38. Relationship between fish length and maximum size of Hyalella consumed by limnetic (Δ) and benthic (●) stickleback. Projecting lines indicate the next available prey size which was not eaten.

Fig. 39. A comparison between Hyalella length and manipulation time for benthic and limnetic stickleback of similar size. Data for a large benthic are also presented. Symbols: benthics- 48 mm ($\circ$), 51 mm (∇), 54 mm (∇), 59 mm ($\bullet$), and 69 mm ($\circ$); limnetics - 54 mm ($\blacktriangledown$), 58 mm ($\blacktriangle$), and 60 mm ($\blacktriangleleft$).

However, for limnetics and benthics with about the same mouth width, the former manipulated prey faster (Fig. 40).

Time between successful grasps

Each stickleback was offered a range of prey sizes. Fifteen amphipods were used in each test. Variances around mean prey sizes were small (Appendix 3). The intervals between successful grasps (that grasp before a prey was consumed) of individual prey were recorded.

For benthic and limnetic stickleback of comparable length, the former had shorter intervals between successful grasps on prey of the same size (Fig. 41). Grasping rates decreased as prey size increased for all predators; however, the 69 -mm benthic presumably had an optimal prey size between 3 and 4 mm before its feeding rate decreased. Furthermore, benthics consumed larger amphipods than did limnetics of comparable length feeding at most successful grasping rates, e.g., 10 seconds (Fig. 42; data from Fig. 41).

Search time

Limnetics (58 and 60 mm) searched less than did benthics of comparable and longer body size on prey less than 2 mm in length (Fig. 43). With larger prey, limnetics searched more than did benthics; limnetics seemed "reluctant" to consume any prey not immediately swallowable. However, small benthics (48 and 51 mm) searched less than

Fig. 40. Relationship between mouth width and slopes of manipulation time vs amphipod size (data from Fig. 39) for benthic (▲) and limnetic (X) stickleback.

Fig. 41. Relationship between Hyalella length and time between successful grasps for benthic and limnetic stickleback of similar and different sizes. Symbols: benthics - 48 mm (+), 51 mm (x), 59 mm (●), 64 mm (□), and 69 mm (○); limnetics - 54 mm (▽), 58 mm (▲) and 60 mm (△).

Fig. 42. Relationship between fish body length and amphipod length consumed at a feeding rate of 10 seconds for benthic (●) and limnetic (○) stickleback.

did limnetics for prey of any size (Fig. 43). For each large benthic (59, 64, and 69 mm) search time was minimal within a specific range of prey sizes; however, with smaller or larger prey the search time increased. The optimal range of prey sizes shifted to larger prey as benthics increased in size.

Search time amongst cover

Loose leaf litter five cm in depth was placed on the bottom of each tank. Approximately twenty Hyaella of various lengths were present in the cover for each test. Search time amongst cover was recorded over three separate 10 minute periods for each fish.

Benthic stickleback spent significantly more time searching the leaf litter than did limnetics (unpaired t-test, calc. $t = 2.479$, d.f. = 20; level of significance = 0.05). The 51 mm benthic often spent periods of 2 - 3 minutes amongst the cover (Fig. 44). Limnetics usually searched the water column or the surface of the cover.

DISCUSSION

In the present study I attempted to evaluate the importance of social behavior and feeding ability as mechanisms maintaining the segregation of two forms of threespine stickleback (Gasterosteus aculeatus) in a small coastal lake.

Fig. 43. Relationship between search time and *Hyaletella* length for benthic and limnetic stickleback of similar and different lengths. Symbols: benthics - 48 mm (+), 51 mm (X), 59 mm (●), 64 mm (□), and 69 mm (○); limnetics - 58 mm (▲) and 60 mm (Δ).

Fig. 44. Mean search time amongst leaf litter for benthic (●) and limnetic (○) stickleback between 30 and 75 mm long.

A distinct spatial segregation occurred between the two forms of stickleback in the lake, during summer 1969. The limnetic form (limnetics) was usually found in large aggregations associated with floating cover, while the benthic form (benthics) was usually solitary and associated with bottom cover. Both forms were found along the lake bottom during winter, but limnetics were deeper than benthics. Segregation of limnetic and benthic stickleback was altered in summers of 1970 and 1971, presumably owing to severe predation from salmonids.

Large benthics (> 50 mm) along the shoreline of Paxton Lake were solitary, and from laboratory tests seemed more aggressive than those deeper in the lake. Limnetics which moved into shoreline areas in 1970 apparently contended with this aggressiveness by occupying regions uninhabited by large benthics, i.e., tall vegetation and open water above Chara. Limnetics were aggressive and held small territories when in tall vegetation, but were gregarious and non-aggressive when above Chara. In the absence of large benthics, limnetics did occupy shore areas with open mud or mud with cover substrate, and became slightly territorial and aggressive toward each other.

Trophic differences between the two stickleback forms were greatest during summer. Limnetics consumed mostly

plankton, and benthics fed heavily on macro-benthos.

Limnetic stickleback ate more macro-benthos during winter than in summer.

Laboratory studies demonstrated significant differences in social behavior between limnetic and benthic stickleback, which seemed relevant to their behavior in the field. In deep aquaria (representing pelagic and benthic regions of Paxton Lake) benthics were: (1) near the bottom of the water column; (2) aggressive; (3) territorial ; and (4) attracted to bottom cover. In contrast limnetics were: (1) high in the water column; (2) only slightly aggressive; (3) gregarious; and (4) attracted to surface (floating) cover. In shallow aquaria (representing littoral regions of Paxton Lake) limnetics were aggressive and not gregarious if cover was absent. When cover was present most individuals went into it and became less aggressive. Behavior of benthic stickleback in shallow aquaria differed from that of the limnetics. They aggregated in small groups when cover was absent, and became territorial when cover was present, 1 - 3 individuals defending the cover from other fish.

In the laboratory experiments limnetics fed on plankton at a higher rate than did benthics. Their prey manipulation and search abilities probably contributed most to this success. Benthics fed on amphipods at a higher rate than did limnetics. A wide mouth probably contributed much to

the benthics' feeding advantage on this prey type.

The phenotypic differences between the two stickleback forms have a genetic basis, and artificial "hybridization" does not produce discontinuous phenotypes (J. D. McPhail, personal communication). However, behavior of "hybrids" may be intermediate between that of the parental phenotypes (R. L. Jones, personal communication). Thus, it is unlikely that polymorphism contributed to the behavior and morphology of the Paxton Lake stickleback.

The morphological and social behavioral differences between lake-reared adult stickleback were also found between limnetic and benthic young-of-the-year that were hatched and reared under standard laboratory conditions (R. L. Jones, personal communication). It appears that non-genetic differences make, at best, a minor contribution to the behavior and morphology of the two stickleback forms.

Benthic and limnetic stickleback appear to differ genetically, but their specific status is in question. Males of benthic and limnetic stickleback usually build nests in different shore regions of Paxton Lake; the former seeking covered areas, while the latter nests in the open. Nothing is known about mate preferences, but limnetic males were observed to successfully court several benthic females in 1969. Some benthic females appeared, however, too large to

enter the nests. Perhaps this could be a form of pre-mating isolation mechanism preventing, to some extent benthic females from depositing eggs in limnetic nests.

The extent of interbreeding between the two stickleback forms is unknown, but it does occur (J. D. McPhail, personal communication). In the laboratory, offspring from artificial crosses between limnetics and benthics appear viable (F₁ and backcross) and possibly occupy intermediate habitats (R. L. Jones, personal communication). In the field they have intermediate feeding habits.

The above information could suggest that the two stickleback forms are species, but until further data are available we must consider them possible subspecies (races).

If the two stickleback forms represent different species, their segregation should be of the interactive type as suggested by the hypothesis of Nilsson (1967). However, it is not clear that the mechanisms proposed by Nilsson adequately cover this as a case of interactive segregation, as may be illustrated by considering the proposed mechanisms in relation to the results of this study:

- 1) Exploitation - This is defined as the ability of one species to use vacant resources faster, or get there first, than another species (Brian, 1956). Food seems the main concern in the Paxton Lake case. The two stickleback ate

similar foods during winter, but the fish were less active at the lower temperatures. Exploitation then seemed unlikely or unimportant. In summer, 1970, little trophic overlap occurred between the two stickleback forms. Limnetics moved near the shoreline in 1970 and along the lake bottom between 3 and 6 m in 1971, probably in response to increased salmonid predation. Both stickleback forms ate more of the same foods than in summer 1969, and food exploitation may have occurred.

2) Territoriality - This could be a possible mechanism for maintaining spatial segregation between the stickleback forms. Benthic stickleback were shown to be territorial and aggressive, attacking aggregations of limnetic stickleback moving along littoral regions in 1970. Breakdown of spatial segregation after 1969 probably increased the frequency of benthic - limnetic interaction.

In the laboratory, benthic stickleback were shown to interact with both forms of stickleback, especially their own form, causing changes in vertical distribution of subordinate fishes. Thus, benthic-limnetic interaction in the field likely represents a normal feature of the benthics' behavior. Vertical distribution of individual groups of limnetic and benthic stickleback in aquaria were dissimilar, but consistent with field observations.

Futhermore, differences between the stickleback in morphology, social behavior, and feeding ability were consistent with that of other fish species inhabiting pelagic or benthic environments (see page 105). Therefore, the interaction between the two stickleback forms likely played only a minor role maintaining their segregation in 1969.

3) Food-fighting - This was never observed in the lake or laboratory. In the laboratory benthics grasped food and moved to an unoccupied tank section to consume it. Limnetic stickleback usually remained together while feeding.

4) Predation - A few large benthics preyed upon both forms of stickleback in 1969, but the level of cannibalism was low. Thus, predation probably was unimportant as a factor maintaining segregation of the two forms.

5) Other interferences - Nilsson (1967) suggested that avoidance experiments of Ivlev (1961) and Breder (1929) indicated that stimuli released by strange shapes, movements, sounds, or odours could be important to segregation in fishes. Nilsson (1967) mentions that alteration of the environment by introduced species may also be important, citing Cahn (1929), Threinen and Helm (1954) as examples. The impact of salmon upon decreased spatial and trophic differences between the stickleback forms in 1970 and 1971 may be most relevant to interferences of this type.

However, little is known of such interferences between stickleback, or between salmonids and stickleback prior to 1970.

The discussion above suggested that in summer 1969, interspecific competition was of reduced importance in maintaining the segregation of the two stickleback forms. This is not unexpected because evolution is a continuous process and can lead to reduced interference between sympatric species. Reduction of interspecific interference through character displacement (Kohn and Orians, 1962; Mayr, 1965) is dependent on many processes which likely vary in importance with specific situations, but always an important one is environmental stability.

Interference between closely related species, or unrelated species with similar ecological demands may be greatly reduced if an environment is sufficiently stable to permit adaptation by species to specific portions of the environment. Adaptive radiation may be extensive, but as Fryer (1959) has shown it may be restricted with many related species or genera frequently co-existing in the same habitats and consuming the same foods. Fryer emphasized that for the cichlid fishes in Lake Nyasa specialization has been enhanced by the lakes' large size, long stability, and sparse number of habitats as well as by predation and mouth breeding characteristics of its fishes. Competition is

probably not absent between the cichlid fishes, but no doubt these mechanisms have greatly reduced interference between the species.

Species adaptations are more generalized in less stable environments. The overall stability of a temperate lake, for example, is low due to seasonal fluctuations in temperature, vegetative cover, food type, food density, etc. Therefore selection favors retention of generalized features of species. However, stability still exists but on a more coarse level in that species adapt to regions of lakes such as pelagic or benthic rather than the more specific micro-habitats of the type noted by Fryer (1959). Several species might inhabit pelagic or benthic regions of a lake, but each may possess variations in behavior, mouth size, mouth position, or body shape providing an advantage for exploiting certain environmental resources better than the others. However, these adaptations, while reducing interspecific interaction, are not complete, maintaining within each species a plasticity to environmental changes. Two examples of supporting evidence are provided from the study by Keast and Webb (1966) on 14 cohabiting fish species in Lake Opinicon and an experimental study of feeding behavior and interaction of coastal cutthroat trout and dolly varden char by Schutz (MS 1969). The extensive adaptations of fishes in tropical lakes (e.g., Lake Nyasa)

as compared with those in temperate waters may in part be due to the greater age of the former.

If the two stickleback forms represent a single species, their extensive adaptations likely represent an attempt to optimize the potential resources of the pelagic and benthic regions of Paxton Lake. Their failure to perfect isolating mechanisms to attain specific status may have resulted from insufficient time, or a response (possibly introgression) to the less stable conditions since 1957, as a means of maintaining genetic variation that increases the species chances of survival. Nonetheless, the level of intraspecific competition appeared to be low in summer, 1969.

The evolution of the Paxton Lake stickleback is not clear. Allopatric processes are possible: (1) each stickleback form could have evolved in one of the two lake basins; (2) double invasion of the freshwater (*leiurus*) and marine (*trachurus*) forms of Gasterosteus.

Sympatric dimorphic selection may also have been operative. Intraspecific competition for food, space, reproduction sites, etc., could have provided a separating force for selection of phenotypic extremes in the population (i.e., disruptive selection) subjected to selective pressures in different lake regions (i.e., pelagic and benthic). Such divergences would require stabilized selection (i.e., favoring average individuals and eliminating extreme variants) after

the phenotypes were separated (Mayr, 1965). The extensive adaptations and genetic differences between the two stickleback forms may suggest the establishment of stabilized selection in this case.

Whether allopatric or sympatric processes are at work is thus in question, but perhaps the following mechanisms may provide additional insight into the evolution of the two stickleback forms:

1) Parasitic influence - Gasterosteus is frequently infested with Schistocephalis. The parasite is acquired from eating copepods. Infested individuals have a higher metabolic rate than do those uninfested (Lester, 1971). Lester suggested this metabolic increase as a reason for the segregation of infested from uninfested individuals, the former seeking the high oxygenated water near creek mouths or at lake surfaces. Such a situation might be applicable to the Paxton Lake stickleback. A high percentage of limnetics were infested with the parasite in 1969. If the trait for plankton feeding had a genetic basis and if infested individuals lived long enough to spawn, with time directional selection might favor offspring with increased fitness to living a pelagic existence. This process could result in the evolution of a limnetic phenotype from a common gene pool, or refine an interaction between two gene pools.

2) Stickleback predation - A few large benthic stickleback consumed stickleback as prey in 1969. This occurred at a low level, but the suggestion that more intense predation occurred in the past is obvious. It is possible that benthic predation on limnetics and possibly other benthics, generated sufficient directional force to either refine or develop the present ecological relationships between the two stickleback forms.

3) Salmonid predation - Salmonid predation may have acted as a selective force favoring those phenotypes occupying the pelagic and benthic regions of the lake (see Fryer, 1959, for a discussion of the role of predation in fish evolution). Laboratory studies suggested that the two forms of stickleback became well adapted to different resources of Paxton Lake. Limnetic stickleback's "attraction" to surface cover and shore behavior, and the benthic stickleback's territorial behavior and occupation of bottom cover may have been mechanisms reducing salmonid predation before the cut-throat trout population decreased in 1957. This interpretation seems reasonable, since limnetics were usually associated with surface cover in 1968 (before salmon were planted in Paxton Lake; J. D. McPhail, personal communication), and in 1969 when salmon predation was low; but they moved near and into littoral areas when predation levels

increased in 1970. High water in 1971 apparently permitted salmon to explore shallow shore areas where no suitable cover was present for the stickleback. Both forms of stickleback then sought refuge in the Chara between 3 and 6 m along the lake bottom.

Hoogland et al. (1958) presented experimental evidence that stickleback pelvic spines are important defense mechanisms. Despined individuals were eaten more often than were spined individuals. Application of these results to Paxton Lake stickleback, however, presents many difficulties. Since limnetic stickleback likely has a higher risk of predation to salmonids than benthic stickleback, their spines could serve as a defence mechanism. But what about benthic stickleback which had neither pelvic spines nor body plates? Perhaps the loss of armorment permitted them to move quickly into bottom cover as a means of avoiding predation.

Benthic and limnetic fishes differ in distribution, as well as social and feeding behavior (e.g., benthic species- Gadus morhua, Brawn, 1969; Hypsypops rubicunda, Clarke, 1970; Cottus asper and C. rhotheus, Northcote, 1954: limnetic species - Clupea harengus, Blaxter, 1965, and Blaxter and Holliday, 1963; and Engraulis mordax, Leong and O'Connell, 1968). In general, benthic species are aggressive and territorial; they remain near bottom cover, possess a large

mouth (relative to body size), and consume mostly benthic foods. Limnetic fishes are gregarious and non-aggressive; they inhabit open waters, possess a small mouth, and consume mostly plankton. These characteristics also pertain to benthic and limnetic stickleback in Paxton Lake. Furthermore, experimental demonstration of these characteristics suggested that the spatial and trophic segregation of the stickleback was not the result of intense interference in 1969.

In conclusion, this study indicated that the two stickleback forms, whether species or subspecies, had evolved means for co-existence with much reduced interference prior to salmonid introduction. If they represented two species, I believe the mechanisms of their segregation do not fully support the hypothesis of Nilsson that closely related species, or unrelated species with similar ecological demands, segregate through competitive interaction. Little evidence was gathered to support this hypothesis. In fact, I suggest this segregation to be, in Nilsson's terms, closer to the selective, than the interactive type. If the two stickleback forms are subspecies of Gasterosteus aculeatus, a lack of intense intraspecific competition was strongly suggested.

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APPENDIX 1. Mean rations of Tubifex and per cent body weight of food eaten at 12, 24, 48, and 72 hours of deprivation by benthic and limnetic stickleback.

Phenotype	Tank	Size mm	Weight gm	Weight of food eaten at deprivation levels of : (hours)			
				12	24	48	72
Benthic	A	69	4.48	.172 (3.82)	.253 (5.62)	.288 (6.40)	.283 (6.29) ¹
Benthic	B	69	4.50	.183 (4.09)	.267 (6.00)	.286 (6.40)	.332 (7.41)
Benthic	C	69	4.39	.128 (2.92)	.201 (4.58)	.277 (6.31)	-
Limnetic	D	60	2.11	.046 (2.18)	.074 (3.51)	.062 (2.94)	-
Benthic	E	47	1.50	.069 (4.60)	.037 (2.47)	.075 (5.00)	-
Limnetic	F	54	1.62	.083 (5.12)	.099 (6.11)	.077 (4.75)	-
Benthic	G	58	2.75	.097 (3.42)	.140 (5.09)	.112 (4.07)	-
Benthic	H	54	2.25	.097 (4.31)	.128 (5.69)	.104 (4.62)	-
Limnetic	I	47	1.12	.036 (3.21)	.074 (6.61)	.050 (4.45)	-
Limnetic	J	54	1.71	.051 (2.98)	.099 (5.79)	.145 (8.48)	-
Limnetic	K	45	0.94	.046 (4.90)	.088 (9.36)	.036 (3.83)	-

¹
() = per cent body weight

APPENDIX 2. Regression analysis of manipulation times
on amphipods by Paxton Lake stickleback

Phenotype	Size mm	Regression	n ¹	Prob. b = 0	Sign. p = .05
Benthic	69	$y = -.14 + 1.05X$	5	.038	S
	59	$y = -.15 + 1.48X$	5	.033	S
	56	$y = -.22 + 2.21X$	5	.000	S
	51	$y = -.19 + 3.39X$	7	.000	S
	45	$y = -.58 + 7.19X$	7	.000	S
Limnetic	60	$y = -.37 + 3.92X$	5	.000	S
	58	$y = -.60 + 5.83X$	7	.000	S
	54	$y = -.83 + 9.32X$	4	.104	NS

¹

Number of amphipods presented to each predator.

APPENDIX 3. Selected examples of variation around mean size of Hyaella in the time between grasps experiment

Date	Phenotype	Fish size mm	Mean <u>Hyaella</u> length $\pm$ 95% C. L. mm
May 6	Benthic	69	4.11 (3.95 - 4.27)
	Benthic	51	3.30 (3.15 - 3.44)
	Benthic	59	3.19 (3.06 - 3.33)
	Limnetic	60	3.20 (3.00 - 3.40)
May 9	Benthic	69	2.01 (1.84 - 2.18)
	Benthic	48	2.31 (2.16 - 2.46)
	Benthic	59	2.43 (2.34 - 2.53)
	Limnetic	60	2.43 (2.33 - 2.53)
	Limnetic	58	2.37 (2.28 - 2.47)