THE UNIVERSITY OF MANITOBA

PHYSIOLOGICAL, ECOLOGICAL AND MORPHOLOGICAL DIFFERENCES

BETWEEN TWO FORMS OF NINESPINE STICKLEBACK

Pungitius pungitius, IN NORTH AMERICA

by TETSUYA NARITA

A THESIS

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i.

ABSTRACT

Field and laboratory studies were conducted at Churchill, Manitoba, where the two forms of *Pungitius pungitius*, which are allopatric in most of North America, come in contact. The coastal form spends the winter in the sea, and ascends creeks to spawn in early summer. The inland form remains in lakes and streams far inland throughout the year. An intermediate form, which apparently originated from the inland form, lives in the lower reaches of the Churchill River and tributary creeks.

The coastal form has higher average numbers of dorsal spines, lateral plates and caudal scutes, and a lower number of gill rakers, but the morphological differences are small and could not be used for clear-cut separation between the forms. However, distinct differences in salinity tolerance were found between the two. The coastal form tolerates higher salinity than the other forms, in both the adult and egg stages, and also shows a preference for higher salinity. Artificially-made hybrids were closer to the coastal form in morphological characters; the salinity tolerance of both reciprocal hybrid crosses resembled the coastal parent.

F_1 hybrids were viable, fertile, and no postmating isolating mechanisms were found. The only premating isolating mechanism seems to be spatial segregation which arises from hereditary differences in salinity tolerance. The inland and intermediate forms are apparently prevented from occupying any of the creeks directly entering Hudson Bay due to their low salinity tolerance thus preventing them from

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escaping to the sea when these creeks freeze solid.

The coastal and inland forms apparently are not different species, but their different geographic distribution and distinct physiological differences suggest that they may deserve subspecific recognition.

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INTRODUCTION

Geographic variation between populations of a species is essential for speciation. Spatial isolation, which leads populations to a gradual shift of gene frequency in response to selection by local environments, is the first step of speciation. Reproductive isolation may be established by the result of long term isolation in different environments. When two populations which have been isolated for a long time then come in contact again, the gene flow will be blocked if isolating mechanisms have been completed, but if they have not been completed, gene flow occurs between the two populations and introgression or swamping will result. The course of speciation has been discussed by many authors (DOBZHANSKY, 1937; MAYR, 1942; MOORE, 1949). MAYR (1963) especially discussed in detail the concept of species and of species development.

The ninespine stickleback, Pungitius pungitius (Linnaeus) is distributed widely in high latitudes of the northern hemisphere throughout Europe, Asia and North America. Many authors have described the variation of Pungitius. In Europe, WOLSKI (1927) reported the slight morphological differences between freshwater and marine populations. GASCHOTT (1929) also described the saline water form with bony armature and the freshwater form without armature. MUNZING (1969) recognized two subspecies, P. p. pungitius and P. p. laevis, depending on the presence or absence of keels on the caudal peduncle. In North America, VLADYKOV (1933) noticed that the numbers of dorsal spines, dorsal fin rays, and anal fin rays are higher in populations from the Hudson Bay region than in those from Lake Nipigon. WALTERS (1955)

examined the number of dorsal spines and suggested a cline of high
to low counts from north to south. He suggested a possible selective
adaptation of the spine count to local environments. McPHAIL (1963)
examined morphological variation of museum specimens in North America,
and distinguished two morphologically different types, a Bering form
and a Mississippi form. The former has a higher dorsal spine count, higher
lateral plate count and a lower gill raker count than the latter.

WALTERS suggested two glacial refugia for North American
Pungitius during Wisconsin glaciation (the Bering refugium and the
upper Mississippi Valley refugium), and the subsequent dispersion
throughout North America from these two refugia. McPHAIL also
interpreted the present distribution of these two forms in Canada in
terms of dispersion after deglaciation. According to McPHAIL, the
Bering form has dispersed to the south east through coastal waters
along the Arctic Ocean, Hudson Bay and the North Atlantic Ocean; the
Mississippi form dispersed northeast and northwest through fresh waters
of glacial lakes and their outlets during deglaciation.

There are many works on ecological and behavioural aspects of
Pungitius in Europe and North America, but North American studies
have been done almost entirely on the Mississippi form (or "inland form"
in the present study) because of its easy availability. Almost nothing
has been recorded concerning the life history, ecology and physiology
of the Bering form (or "coastal form" in the present study) in North
America, despite the fact that it may occupy different environments from
the Mississippi form.

The object of the present study is to find out the differences between two forms of <u>Pungitius pungitius</u> in North America and to determine their taxonomic status. Firstly, the distribution, migration, breeding and food of the Bering form in the Churchill area are described. The two forms then are compared to discover whether their morphological differences are accompanied by physiological or ecological differences which may be related to their long isolation during glaciation. The zone of possible contact between the Bering and Mississippi forms in the Churchill area is examined in detail. From these results the status of the two forms in North America is then discussed.

STUDY AREA

An intensive field study was carried out in the area of
Churchill, Manitoba, to find out the distribution and life history
of two forms on the west coast of Hudson Bay, from June to August
1967, May to August 1968 and June to July 1969. Field studies were
also conducted at Cranberry Portage, Manitoba, in the summer of
1969 (Fig. 1).

The creeks where the fish were collected in the Churchill area
may be divided into three categories: (i) creeks draining directly into
the saline water of the Hudson Bay, (ii) creeks draining into the
Churchill River and having tidal influence at their mouths, (iii)
creeks draining into the Churchill River but not having tidal influence
at their mouths.

The creeks in the first category, Paradox and Stygge Creek,
were of gravel or coarse sand bottom, and were approximately 3 to 10 m.
wide. The gravel bottomed rapids usually were shallow, but coarse sand
pools about 1 m. deep were found scattered among the rapids. At the
mouth of the creeks there were some patches of Fucus. Approximately
30 m. above the mouth of the creeks, both creeks had a curved pool
where some Fucus accumulated. The tidal water almost reached the pools
at high tide, but at low tide about 1.6 km. of intertidal flat with
numerous rocks was exposed.

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Fig.   1.   Locality of study area in Manitoba and location of collecting in the Churchill area.

HUDSON BAY

CHURCHILL

509 Cr. ISABELLE LAKE

PARADOX CR.

STYGGE CR.

STYGGE LAKE

FARNWORTH LAKE

MOSQUITO POINT

CHURCHILL RIVER

GOOSE CR.

Cr A1

Cr A2

Cr A3

Cr B

Cr C

Cr D

Cr E

Cr F

C N R

N

TWIN LAKE

0 1 2 3 4 Miles

WARKWORTH LAKE

Cr I

C N R

HUDSON BAY

CHURCHILL

CRANBERRY PORTAGE

MANITOBA

WINNIPEG
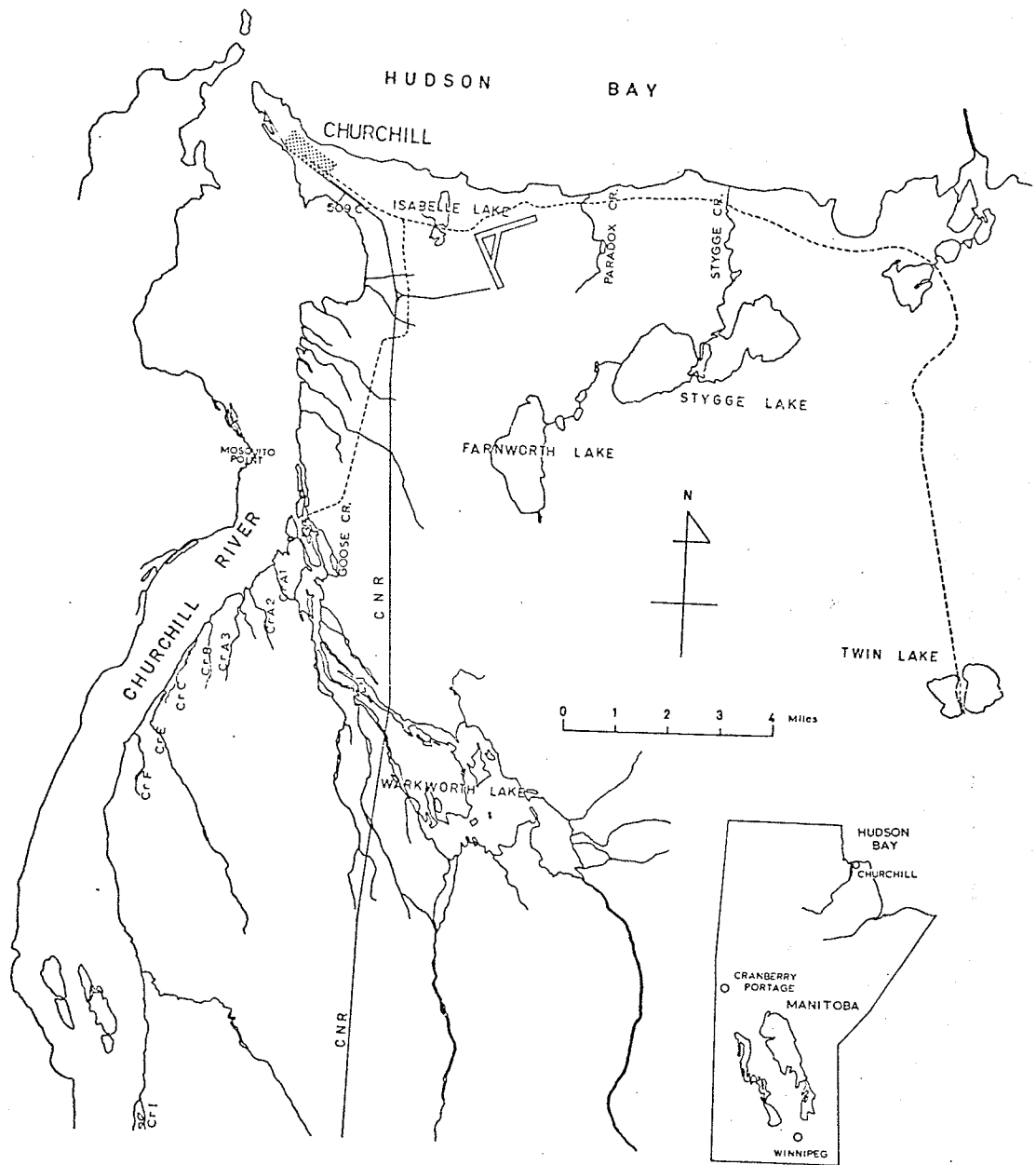
The creeks in the second category, from 509 Creek to Goose Creek, were narrow, approximately 1 to 5 m. wide, usually with a mud bottom but in many cases with a gravel bottom. There was tidal influence at high tide at the mouths of all these creeks except Goose Creek. The creeks in the third category, Creek A-1 to Creek I, had almost the same conditions as the creeks in the second category, but they had no tidal influence.

The ice broke up in late May in the creeks around Churchill, and the water levels were then high. In Stygge Creek, which is the outlet of Stygge Lake, the ice broke up rather late, at the end of May, and the water level was very high for about two weeks. In other creeks, which collect melt water from the tundra, the water level decreased gradually. In the middle of July, the water level was low in all creeks and in some creeks the water hardly flowed and was stagnant. Water temperature of the creeks varied from 0C at melting time to 20C in late July. The water colour was brown in all creeks around Churchill.

The salinity of the water of the creeks in (i) and (ii) categories was low, 0.15 o/oo in early summer when the water level was high, but it gradually increased to 0.5 o/oo*. The salinity of the creeks between Creek A-1 and Creek C was considerably higher, and showed 1.0 to 3.5 o/oo*. Generally speaking the salinity increased as the water level decreased, and in some isolated shallow pools it sometimes reached 6 o/oo in late summer. Also pH of the water increased from 7.5 to 8.5 in most of the creeks as the water level decreased.

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* The salinity of creeks was determined at least three times, at the beginning, in the middle and at the end of the summer. In most cases, the salinity was determined more than three times during summer.

Aquatic vegetation was not rich. Bulrush (Scirpus) and sedge (Dulichium) grew in the slow side current and the edges of the creeks, and bur reed (Sparganium) and water milfoil (Myriophyllum) grew in the middle of the creeks from midsummer to early autumn.

Culaea inconstans, the brook stickleback, was also collected in the Churchill area. Culaea was collected from Farnworth Lake, Twin Lake, Stygge Creek and Creek I, and was not collected from the same creeks or lakes where Pungitius was collected with the exception of Stygge Creek.

The only other species of fish in the creeks of the first and the second categories was burbot (Lota lota), but there were many species of fish, including northern pike (Esox lucius), grayling (Thymallus arcticus signifer), whitefish (Coregonus clupeaformis), and longnose sucker (Catostomus catostomus) in the creeks of the third category and Goose Creek.

In creeks of the first and the second categories, arctic tern (Sterna paradisaea) and some shore birds were abundant and the tern apparently fed on Pungitius in the creeks.

Around the Churchill area there are thousands of isolated shallow ponds with muddy beds. However, these ponds do not carry any fishes unless they are connected with creeks at flood time, apparently because they freeze right to the bottom in winter. During the winter the ice around Churchill is reputed to be about 6 feet; in the middle of June 1967, the ice in Farnworth Lake was still about 5 feet thick. Consequently, all the creeks and ponds less than 6 feet in depth probably freeze to the bottom in the Churchill area, thus making it impossible for any fish to survive there through winter.

MATERIALS AND METHODS

Specimens examined

The fish used in the study, except for museum specimens, were collected from the northern part of Manitoba during the study. The fish from creeks of the first and second categories were denoted as coastal form, and the fish from creeks of the third category were denoted as intermediate form in Churchill. Some fish were kept alive for experiments. For morphometric analysis, specimens were preserved in approximately 7 % formalin. Museum specimens were also examined from the National Museum of Natural Sciences, the Royal Ontario Museum, and the museum of the Institute of Animal Resource Ecology at the University of British Columbia.

The hybrids between the coastal and the inland forms were produced by introducing parent fish into natural freshwater ponds in Churchill. In early summer, coastal males and inland females were put in a pond (pH 8.0, salinity 300 ppm), and hybrids (hybrid FC) were collected at the end of the summer. The reciprocal hybrid (hybrid CF) was similarly produced in another pond (pH 9.0, salinity 150 ppm). Two ponds were similarly established at Cranberry Portage; of these only the coastal male and the inland female cross was successful, hence no hybrids from Cranberry Portage were used in this study.

Sampling methods

Seine   A pole seine net 3 m. wide was used for most collecting. On long sandy beaches of lakes a seine net 9 m. wide was employed. The mesh size of both seines ensured capture of the fish exceeding 2 cm. in length.

Trap   Small handmade traps were used to catch fish in small creeks and streams, especially at the time when they migrate up the creeks in early summer. The traps, 38 x 75 x 38 cm. in size, had a 2.5 x 5 cm. wooden frame, a V-shaped mouth and were screened with wire .

mosquito netting (Fig. 2). Wings of the same mosquito netting were
constructed to divert fish into the traps in the larger streams,
while rocks and woods were used to block the space between the trap
and creek edges in the smaller creeks. This method was effective in
small weedy and rocky creeks where the seine nets were not useful.
Also every size of fish could be caught by these traps.

Dip net   To collect fry and juveniles, a fine-meshed dip
net was used. Also a dip net of 5-mm. mesh was used for collecting
amongst dense weeds.

Morphometric methods

To determine whether morphological differences between the forms
exist, morphological measurements and counts were conducted. Procedures
for measurements   followed HUBBS and LAGLER (1958).

The following parameters were measured using dial calipers:
standard length (SL), body depth (BD), length of pectoral fin (Pec),
length of pelvic spine (Pel), depth of caudal peduncle (DCP), head
length (HL), predorsal length (the length from snout to the beginning
of dorsal fin, PL), preanal length (the length from snout to the anal
spine, PAF), prepelvic length (the length from snout to the pelvic
spine, PO).

For meristic studies, fish were stained with alizarin and
cleared by a modification of TAYLOR's method (1967). In this study
the trypsin enzyme was not used, because it makes the fish too soft for
later handling. The following meristic characters were counted: dorsal
spines, dorsal fin rays, anal fin rays (excluding anal spine), lateral
plates, scutes on the caudal peduncle (hereafter referred to as caudal

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Fig.   2.   Diagram of trap used at mouths of the creeks:
top view (above) and side view (below).

38 CM

75 CM

38 CM
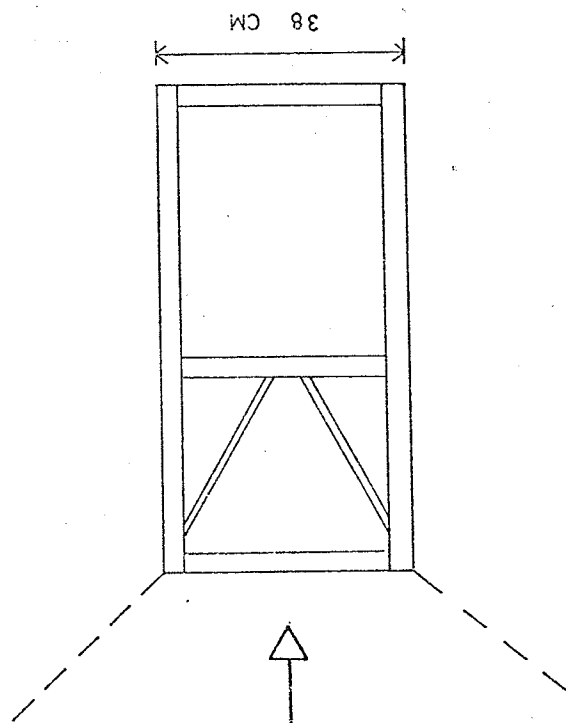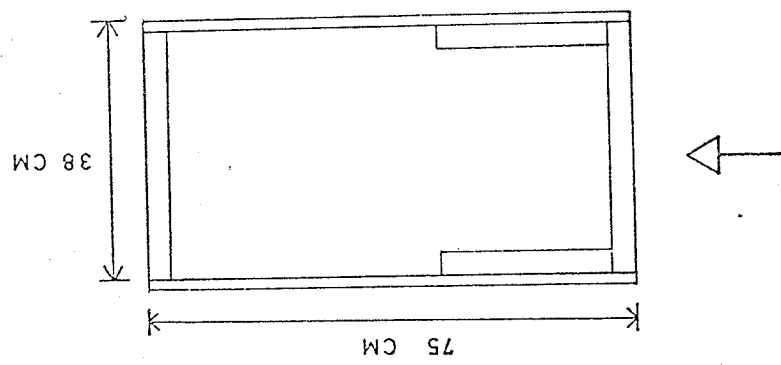
scutes), gill rakers and vertebrae. The counts were made in a Petri dish with glycerin under a dissecting miscroscope. Everymeristic element, even if rudimentary, was counted if it had been stained by alizarin.

The lateral plates are small round bones (less than 3 mm. in diameter) under the skin just above the lateral line. Sometimes the lateral plates had striations. In some cases the lateral plates were continuous with the caudal scutes which lay along the lateral line. The caudal scutes were rhombic and striated where they lay along the side of the caudal peduncle. Bony plates were considered to be caudal scutes when they lay on the lateral line.

Before staining and clearing, the viscera were removed. The sex of the fish was determined by the presence of testis or ovary. At the same time the stage of maturation of the eggs and the presence or absence of black nuptial appearance on thebelly of males was recorded. The maturation of eggs was assigned to one of the following stages.

Stage   I.   immature eggs, transparent or white colour, eggs
              not separated from each other.

Stage   II.   immature eggs, white and separated

Stage III.   mostly immature eggs, witha few yellow mature eggs.

Stage   IV.   more mature than immature eggs, mixed.

Stage   V.   eggs ripe, orange coloured and large: mature
              separated from immature eggs.

The stomach contents were examined in some fish, prior to staining and clearing.

For museum specimens the following characters were measured
and counted without staining or clearing: standard length, body
depth, depth of caudal peduncle, length of pectoral fin, length of
pelvic spine, dorsal spine number, and caudal scute number. Out of
these characters, dorsal spine, caudal scutes and BD/SL ratio were
used for scoring. The score for each character was as follows:

Dorsal spine		Caudal scutes		BD/SL ratio	
Count	Score	Count	Score	Ratio	Score
7	0	6- 7	0	-0.129	0
8	4	8- 9	2.5	0.130-0.139	2
9	8	10-11	5	0.140-0.149	4
10	12	12-13	7.5	0.150-0.159	6
11	16	14-15	10	0.160-0.169	8
12	20	16-17	12.5	0.170-0.179	10
		18-19	15	0.180-0.189	12
		20-21	17.5	0.190-0.199	14
		22-	20	0.200-0.209	16
				0.210-0.219	18
				0.220-0.229	20

The score for each fish was the sum of the individual scores
for each of its three characters. For example, a typical coastal form
fish which has 10 dorsal spines, 13 caudal scutes and 0.180 BD/SL
ratio, had 31.5 points as its score; an inland form fish having nine
caudal scutes and 0.160 BD/SL ratio had 18.5 points as its score.

Physiology experiments

Experiments on salinity tolerance and salinity preference were carried out in laboratories at Fort Churchill, Cranberry Portage and Winnipeg to determine if there were differences between the three forms.

After capture, the fish were brought back to the laboratory and were kept in freshwater tanks. In Winnipeg in winter the fish were acclimated in a cold room at 4C with 8L16D (8 hours light and 16 hours dark) day length, and fed frozen brine shrimp once a day. In the Churchill laboratory in summer, the day length using artificial lights simulated in nature: the temperature of the holding tank was not necessarily controlled, although it was kept below 20C most of the time. The fish were fed various kinds of food:mosquito larvae, zooplankton, gammarids, frozen brine shrimp and "Tetramin" dried food.

"Instant Ocean" synthetic sea salt was used for experiments of salinity tolerance and salinity preference. The salt was dissolved to 50 o/oo in distilled water in summer, or dechlorinated tap water in winter. This original solution was diluted with lake water or dechlorinated tap water to each different concentration for the experiments as a salt solution.

MOORE's titration method was used to determine the salinity in the experiments. In field, "HACH" chemicals was used to determine the salinity of water in creeks.

For salinity tolerance experiments one-liter plastic containers were employed. Five hundred ml. of solution was put into the container, covered with a lid and aerated by an airstone. These containers were hung in a 48 x 95 x 40 cm. wooden tank in which water temperature was controlled at 4, 10, 15 or 20 C, within + 0.4 C°. In the course of experiments, the day length was controlled at 8L16D in the cold room

during winter time, but it followed natural day length in the field during the summer.

Five to eleven fish were put in each container, depending on the size of the fish. They were not fed during the experiments. The solution of the experiments was changed on the second day and then on alternate days. The criterion for death was cessation of opercular movement.

For experiments on salinity tolerance of eggs, mature males with nuptial black bellies and some ripe females were kept in 10-gal. glass aquaria containing sand and some aquatic plants. The eggs were obtained from a fully ripe female by pressing and squeezing her abdomen. The testes were removed from a fully mature male which had made a nest in the aquarium. The eggs and the testes were placed in a Petri dish with a small amount of lake water, and the testes were chopped into small pieces to fertilize the eggs. The eggs were left for about 30 minutes to one hour; then, after they had hardened and formed a perivitelline space, they were separated into several groups to be put into different concentrations of salt solution (10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30o/oo). Each group of eggs was placed in a plastic Petri dish, which was screened by 0.5 mm. plastic netting, and immersed in a plastic container with aeration.

The developmental stages of the eggs, numbered according to ICHIKAWA and YAMAMOTO (1956), were recorded approximately every 24 hours until the hatched fry completed yolk absorption. Dead eggs and embryos were picked out at the same time and their numbers were recorded. The hatching rate of the eggs in each concentration was determined by the number of survivors up to the time of yolk absorption.

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Methods for the salinity preference experiments were modified after McINERNEY (1964). Three 10-gal. glass aquaria(26 x 50 x 30cm.) were each divided into two equal compartments by a glass partition 12.5 cm. high. Each compartment was equipped with an air stone, and a glass tube was used to fill each compartment with water. An approximately 3 cm. layer of sand was placed on the bottom of each compartment. The conditions in the two compartments were arranged to be as similar as possible. These three experimental aquaria were placed in a wooden frame which was lined with black tar paper and black cloth. Observations were made through slits in the black cloth; illumination and mirrors were so arranged that the fish could not see the observer.

The experiment was conducted at 4C under short day length (8L16D) in the cold room, where the fish had been acclimated. To begin the experiment, salt solution of 5, 15 or 25 o/oo was filled to the level of the partition in one side, and the dechlorinated tap water (freshwater) was filled to the same level on the other side of the compartment, and then the solution were left over night to settle the sand particles to the bottom. One fish was put into the freshwater compartment in each aquarium and left to adjust quietly to the new environment for at least 30 minutes, and meanwhile the air flow was started in both compartments. After the transfer into the experimental aquaria, the fish usually remained inactive in the bottom corner for a while, and then started to move about slowly. After confirming that the fish was swimming freely in the compartment, the air flow was stopped. The level of the freshwater was then raised slowly until a freshwater bridge of 1 to 2 cm. was created, and a stratification of salt water and freshwater was formed in the salt water compartment. Observations

were started five minutes after the formation of the bridge, and each compartment was checked every minute for 100 minutes. The side of the salt water compartment was alternated in some experiments in order to eliminate bias.

Electrophoresis

Electrophoretic analysis of Lactate Dehydrogenase (LDH) of skeletal muscle, and of hemoglobin of blood, were made to determine whether these were biochemical differences between forms by Mr. W. G. FRANZIN, Department of Zoology, University of Manitoba.

The fish used for the analysis were kept in a freshwater tank until the examinations were made. The fish were 0+ year old for the LDH analysis, and 0+ and 1+ year old for the hemoglobin analysis. The largest available fish were used in order to obtain large enough samples of muscle and blood. To obtain the blood, the caudal peduncle of a fish was cut off at 5 to 10 mm. from the urostyle and the blood was collected in a fine glass capillary tube.

The method of CLAYTON and GEE (1969) was employed for the analysis of LDH, and the method by TSUYUKI et al (1965) was used for the analysis of blood hemoglobin. For hemoglobin analysis, a buffer of 0.3 M Boric Acid (pH 8.5 NaOH) was used.

Mating behaviour

Observations of mating behaviour were made by use of 10-gal. aquaria with some aquatic plants and sand. For the observations of a mating choice, a nuptial male of the inland form was put into a

test aquarium, and after he made a nest a nuptial male of the coastal form was then introduced to the same test aquarium. After the coastal male made a nest, a ripe female of either form was introduced into the aquarium. The aquaria were placed under artificial illuminations, and no special enclosures were prepared for the observations.

LIFE HISTORY OF POPULATIONS IN CHURCHILL AREA

In the Churchill area there were found to be two forms of
Pungitius pungitius, a coastal form and an intermediate form, which
differ morphologically and physiologically. The coastal form lives in
brackish water in the winter and ascends creeks to spawn in summer.
The intermediate form, whose origin will be discussed in a later
section, is distributed in creeks which enter the lower reaches of
the Churchill River, and stays away from sea water throughout its life.

Distribution in summer

Coastal form The coastal form was collected on the Hudson
Bay shore, in creeks of the first and second categories, and in
Isabelle Lake (Fig. 3). In creeks of the first category, Paradox
and Stygge Creek, fish were present only downstream from road culverts
which are about 400 m. from the mouth in Paradox Creek and 1.2 km.
in Stygge Creek. Due to fast current (greater than 0.5 m/sec.) the
fish could not pass through the culverts, except in late summer when
water current was slow (0.16 m/sec.) a few fish ascended past the
culvert in Paradox Creek. In creeks of the second category fish were
collected as much as three miles from the mouth. In Goose Creek, fish
were very rarely collected in early summer, but in late summer many
yearling fish gather in slow currents of pools near the creek mouth.
In Creek A-1, belonging to the third category, only two fish of the
coastal form were caught by a trap at the end of June in 1969.

Few coastal fish came up far from tidal influence in early summer when the current was still fast, but in late summer the current in the creeks and in the Churchill River was slow and immature yearling fish then ascended to the creeks tributary to the Churchill River. However, even in late summer the coastal fish stayed within 5 km. - range of the tidal influence.

The coastal form was collected in Isabelle Lake, which does not have a direct outlet to the Hudson Bay. The fish seemed to come up to the lake through a chain of shallow pools during flooding in early summer.

Intermediate form   The intermediate form was collected in creeks of the third category, from Creek A-2 to Creek F (Fig. 3). The fish stayed mostly in shallow pools connected to the creeks, and usually stayed within a 3 km. range from the creek mouth.

Distribution in winter

Pungitius did not seem to stay in the lakes or pools throughout the winter, although Culaea apparently survives through winter in lakes. The coastal form spent the winter in Hudson Bay or in the mouth of Churchill River, and came into the creeks in early summer, where they were caught by traps at the mouth of the creeks. A 1 cm. mesh gill net was set in the sea near the mouth of Stygge Creek from June 3 to 12, but no fish were caught. However, 29 fish were caught by seining on the shore of the Hudson Bay in brackish water near the mouth of the Paradox Creek on June 3rd, 1968. The salinity was 2.2 o/oo and temperature was 3.8°C.

Fig. 3.   Distribution of the coastal form (black circles) and
            the intermediate form (white circles) near Churchill.

HUDSON   BAY

ISABELLE LAKE

509 c

PARADOX CR.

STYGGE CR.

STYGGE LAKE

FARNWORTH LAKE

MOSQUITO
POINT

CHURCHILL RIVER

N

GOOSE CR.

CrA 2
CrA 3
CrB-0
CrC-0
CrD
CrE
CrF

C N R

TWIN LAKE

0   1   2   3   4   Miles

WARKWORTH LAKE

Cr1
509

C N R

● Coastal form
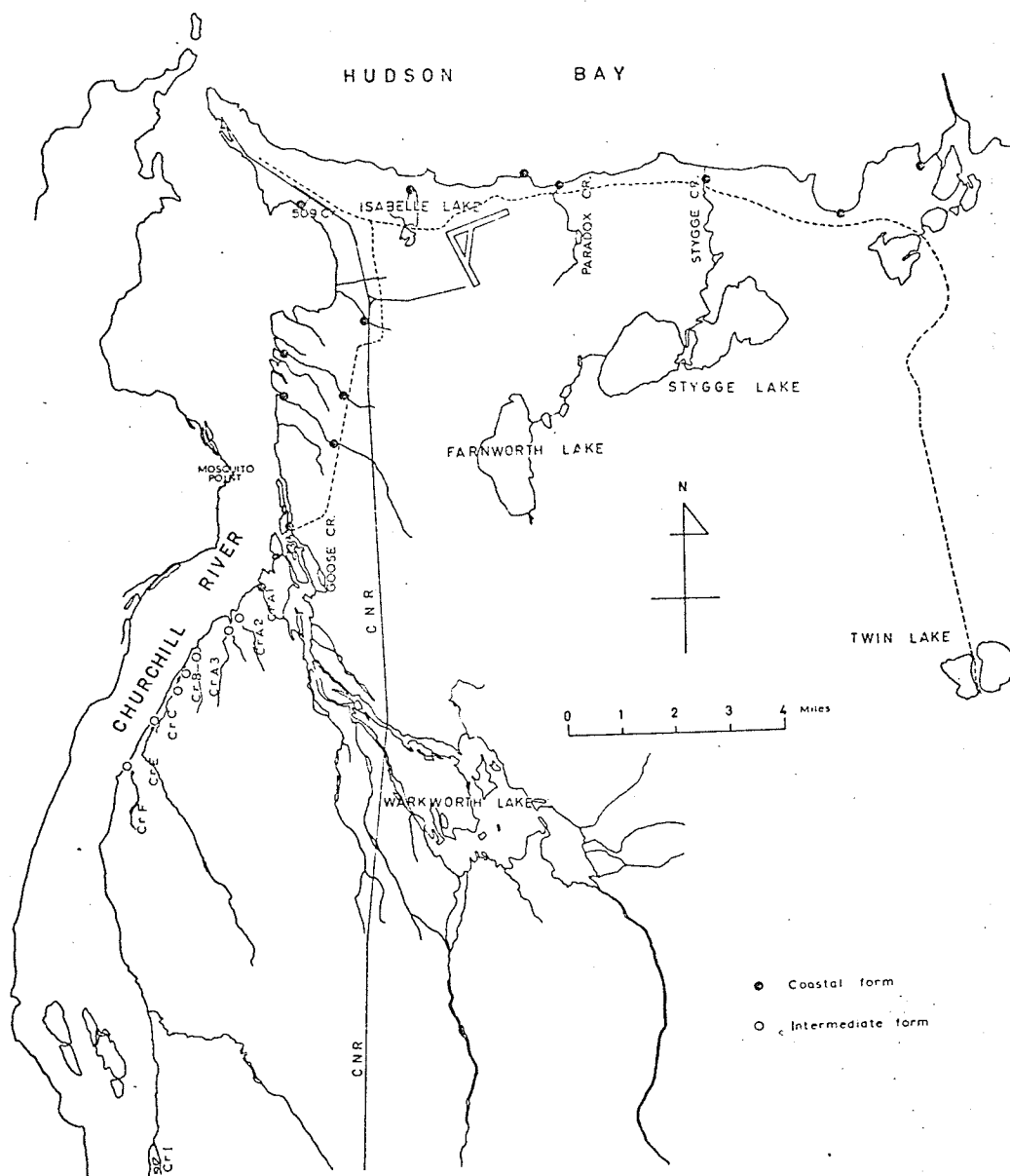
○ Intermediate form

The intermediate form probably spent the winter in a deeper part of the Churchill River, because they could not tolerate more than 20 o/oo salt water.

Upstream migration

Coastal form   Anadromous migration of the coastal form occurs from the end of May to the middle of July. Catches by traps at the mouths of 509 Creek, Paradox Creek and Stygge Creek in 1968 are shown in Fig. 4.

The wide difference in timing of upstream migration in the three creeks seems to be correlated with water level rather than with temperature. In 509 Creek, the fish came up at the end of May just after the ice on the creek had thawed. The water temperature rose to about 5°C in mid day, but dropped to almost 0°C at night. Fish did not come up Paradox Creek then because at the end of May the water level was very high, and the current probably too fast. The ice in Stygge Creek thawed on May 28, and the water level was very high for two weeks making it impossible for the fish to ascend during that period.

Figure 5 indicates the number of the fish caught by the trap at the mouth of 509 Creek on four successive days. It shows that many fish were caught just after high tide, and particularly just after high tide at night.

Similar results were found in Paradox Creek (Fig. 6). On June 12, the migration of fish was observed at the mouth of Paradox Creek. Between 2100 hour and 2130 hour five schools of the fish, each with about 100 to 500 individuals, came up from the Bay to the creek. They

Fig. 4. Number of the coastal form fish caught in traps at the
mouths of 509 Creek, Paradox Creek, and Stygge Creek
in 1968. Black circles show the daily maximum temperature.
White arrows show days with high tide at midnight.
Cross indicates no fish caught.

22a

TEMPERATURE

16
12
8
4
0 C

20
16
12
8
4
0 C

16
12
8
4 C

509 Creek

Paradox Creek

Stygge Creek

837

1956

water level high

water level high

THAW

x x x x

400
300
200
100

300
200
100

200
100

NUMBER OF FISH

30   1   5   10   15   20   25   30   1   5   10   15

23
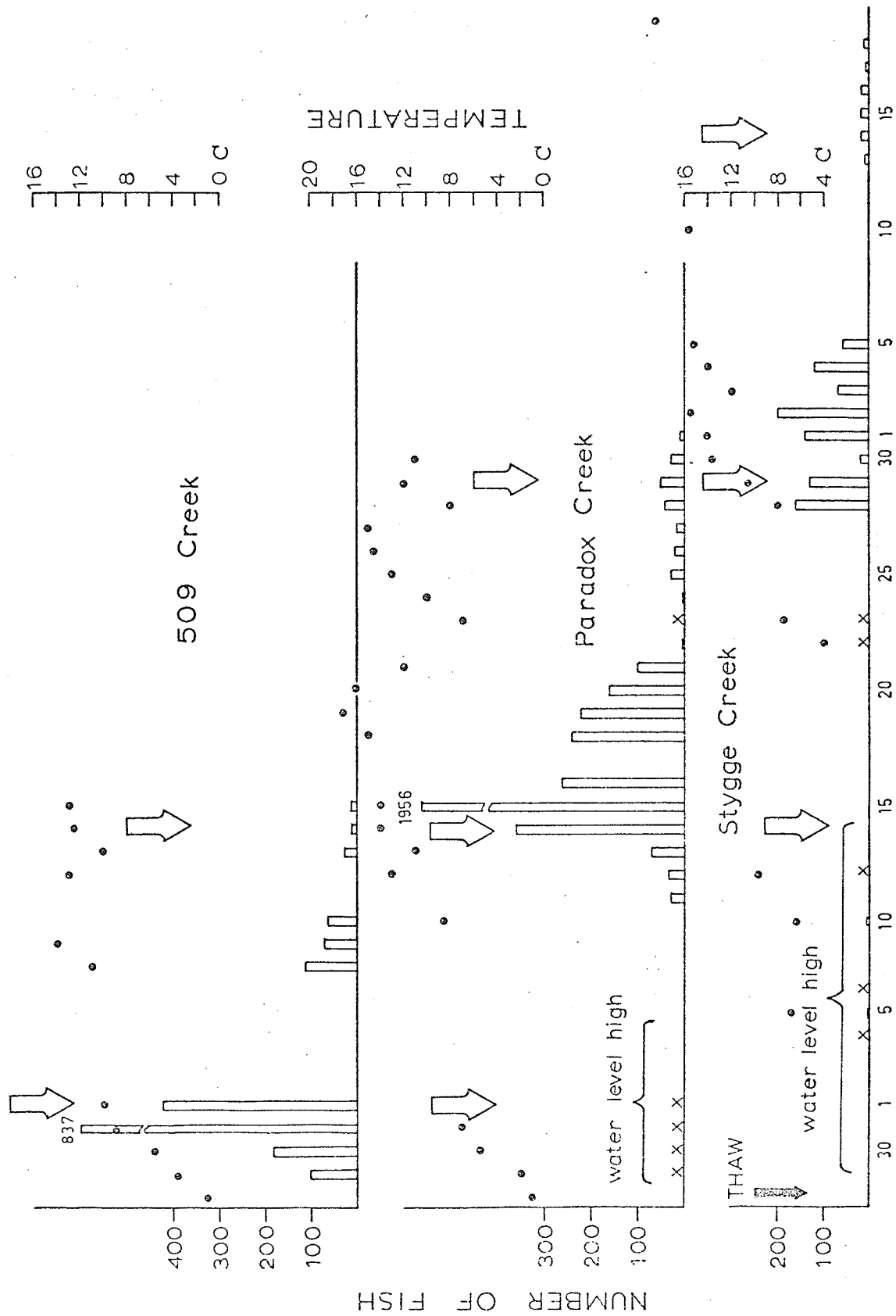
Fig. 5. Catch of coastal fish entering the trap at the mouth of 509 Creek. Stippled bars indicate darkness.

TEMPERATURE

°C

12 10 8 6 4 2 0

557

JUNE 1   MAY 31   MAY 30   MAY 29

12 8 4 0 20 16 12 8 4 0 20 16 12 8 4 0 20 16 12 8 4 0 16 12

TIDE(feet)

12 8 4 0

NUMBER OF FISH

200 150 100 50
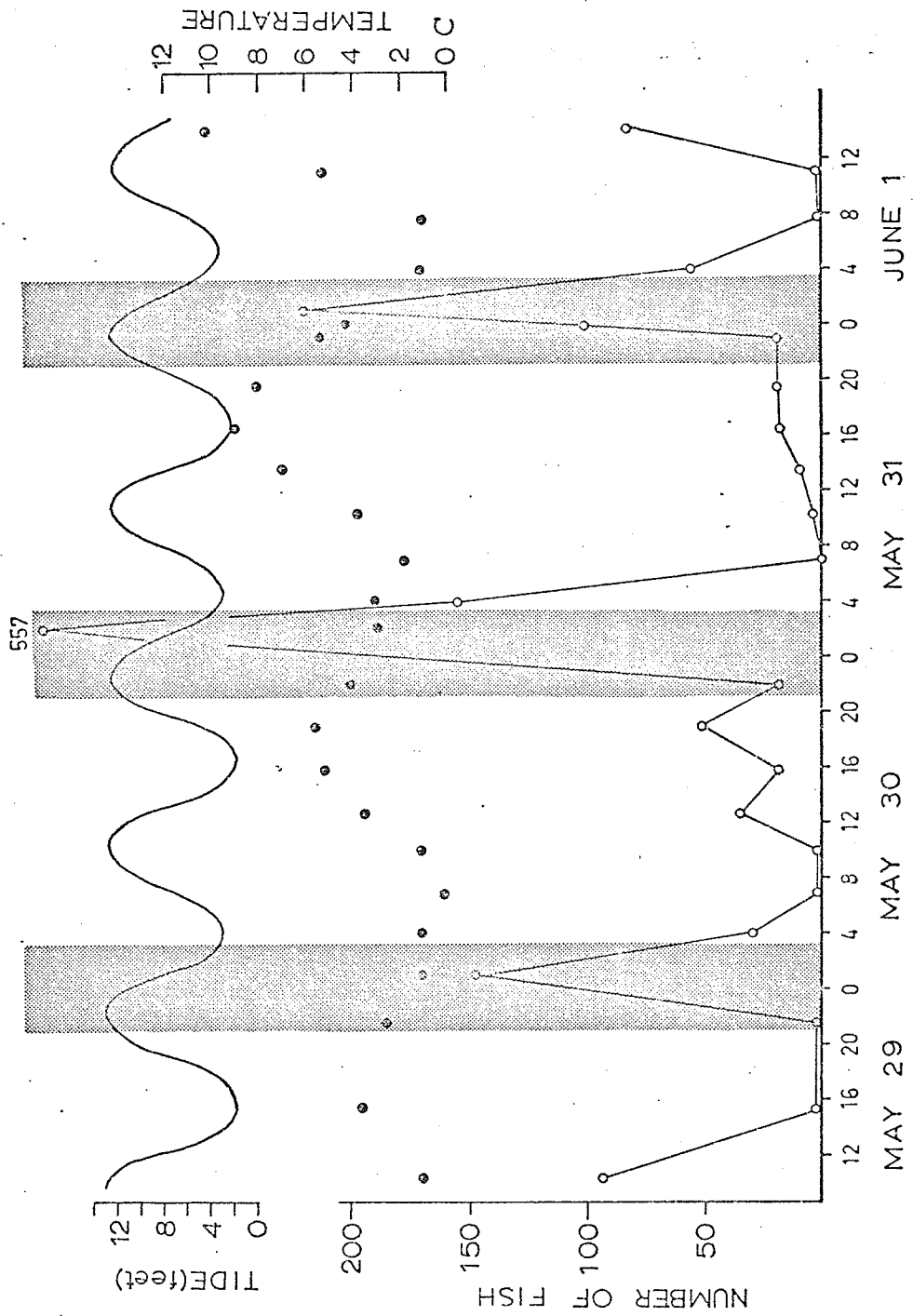
Fig.　6.　Catch of coastal fish by traps at the mouth of Paradox Creek.

TEMPERATURE

°C

14 12 10 8 6 4 2

925
864

traps
submerged

TIDE(feet)

12 8 4 0

200 150 100 50 0

NUMBER OF FISH

12 16 20 0 4 8 12 16 20 0 4 8 12 16 20 0 4 8 12 16

JUNE 12   JUNE 13   JUNE 14   JUNE 15
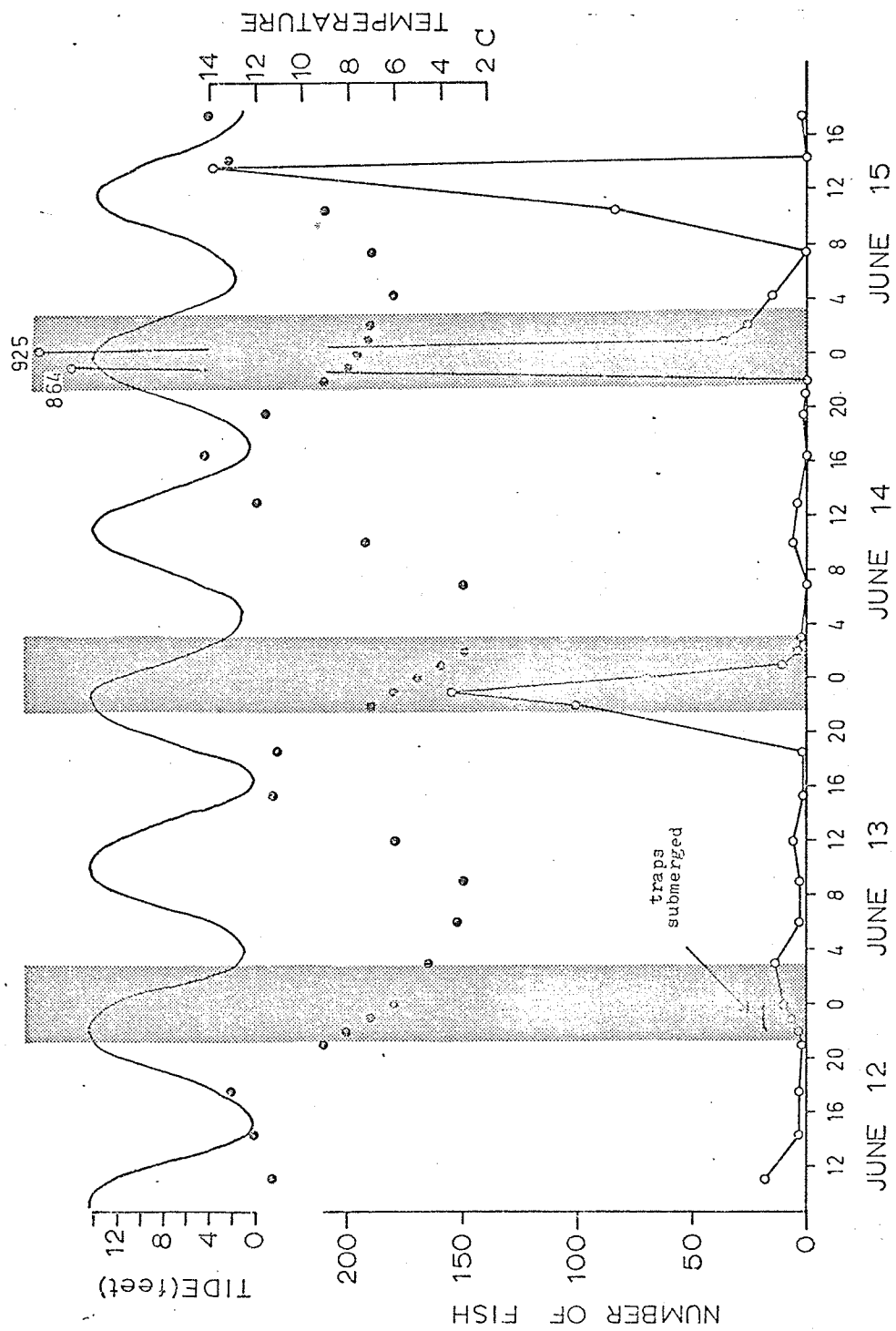
swam very slowly, and zigzagged. The tide was very high from 2100 hour to 2230 hour on that day. However, the catch by the traps at that time of the night (2100 to 2400) was not large (Fig. 6), because the traps and the wings were submerged with the high tide so that fish passed through. In subsequent days the traps and the wings were modified, and large numbers of fish were then caught.

The peak of the migration in Paradox Creek on June 15 was two weeks after that of the 509 Creek on May 31 (Fig. 4), and the second (small) peak of Paradox Creek on July 29 was also two weeks after the first peak, even though the second peak was considerably smaller comparing to the first. This two-week cycle of migration correlates with the two-week cycle of high tide at night (white arrows in Fig. 4), and hence the anadromous migration of the fish is mostly at night, at high tides in daytime small peaks of catch were also seen (Fig 5, June 1; Fig. 6, June 15).

The peak of the migration was not clear in Stygge Creek, probably because it was not possible to completely block the creek by the wings of the trap, so that fish could by-pass the traps. However, very small catch of fish at the middle of July even at high tide indicated the termination of fish migration then.

Intermediate form   The catch of the intermediate form by upstream traps at the mouths of Creek B and Creek C in 1969 is shown in Fig. 7. The migration was late, probably because breakup of ice was two weeks later in 1969 than 1968. At the beginning of July the

water flow greatly decreased, the water became stagnant, and water temperatures in most of the creeks exceeded 20·C (Fig. 7). The migration of the intermediate form had probably finished by that time.

Downstream migration

Coastal form   The coastal form started to go down the creeks to Hudson Bay or to the Churchill River in the middle of summer. On the shore of the Hudson Bay at the mouth of Stygge Creek some fry and juveniles were caught by seine net on July 10, but at this time most of the fish were around the mouth of the creek. At the end of August rain increased the water level and dropped the water temperature in 509 Creek and Stygge Creek, the juvenile fish went down the creeks, and after then very few fish were caught in the creeks.

Intermediate form   On September 14, 1969, downstream traps were set to catch the migrating fish in Creek A-1 to Creek F. The catches of the traps were large, mostly 100 to 300 fish, and more than 1000 fish in Creek A-2 (but only 15 fish in Creek A-1). Almost all were 0+ year fish.

Breeding time and place

Coastal form   The age of the spawning population of the coastal form was determined from the otoliths. One-year-old fish (i.e. had completed one winter) comprised 82.5% of the population and two-year-old fish comprised 17.5% (Table 1). The two year old fish migrated upstream early in the migration period.

The breeding time of the coastal form was estimated from the presence of the black nuptial belly of males, the maturation stages of eggs of the females, the presence of nesting and guarding males in the

Fig.   7.   Numbers of intermediate form fish caught by traps at
the mouths of Creek B and Creek C. Cross indicates
no fish caught.

Creek B

Creek C

TEMPERATURE (C)

22 20 18 16 14 12 10

NUMBER OF FISH

60 40 20 0

25 26 27 28 29 30 1 2 3 4 5 6 7

JUNE   JULY
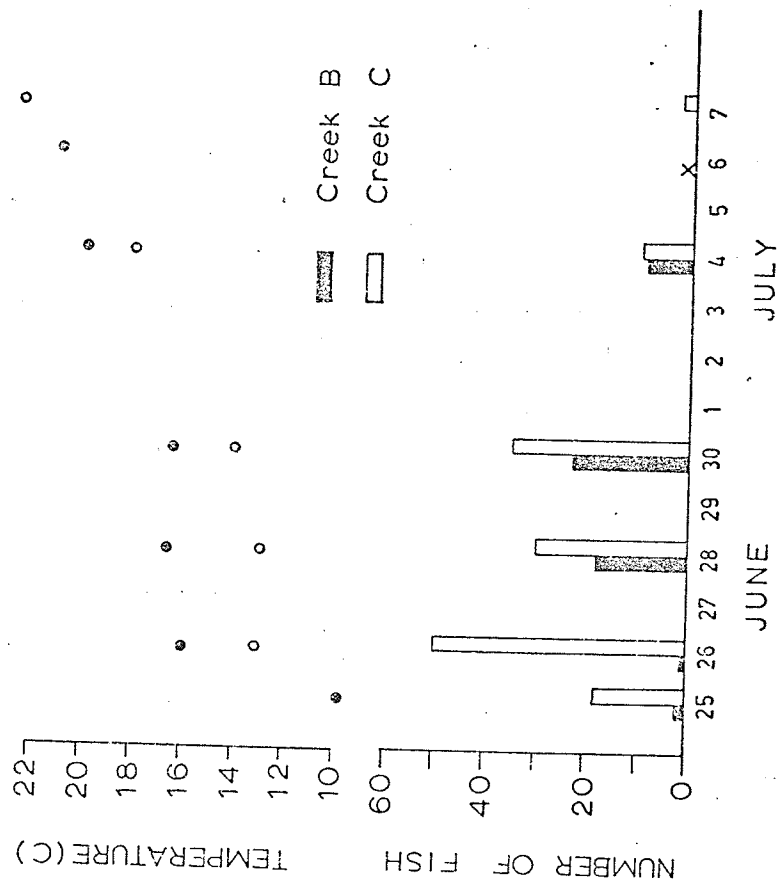
Table   I.   Age composition of ascending samples of the coastal form.

509 Creek		1 year	2 year
27	May	17	8
1	June	29	0
9	June	72	15
		118	23
Paradox Creek			
29	May	14	13
11	June	55	5
13	June	34	5
14	June	18	7
25	June	15	0
		136	30
Stygge Creek			
10	June	3	9
12	June	6	8
22	June	37	0
5	July	30	0
		76	17
Total		330	70
Per cent		82.5	17.5

creeks, and the emergence of newly hatched fry. Mature males of
Pungitius became black on the underside of the head and chest and belly,
and also milky white pelvic spines. In the peak of maturation, when
a male was building and guarding a nest, the black pigment sometimes
spread to the whole head and lower part of the body.

Some black nuptial males were present from the end of May to
the middle of July. In 509 Creek the ratio of nuptial to non-nuptial
males was high in the middle of June (Table II).

Females with mature eggs (stages IV and V) were collected from
the middle of June to the middle of July (Table II).

The eggs hatched in 5 to 15 days depending on the water temperature.
Newly-hatched fry were collected with a net in Stygge Creek on July 9
Fig. 8). On the same day two males guarding a nest and two males
building a nest were observed in Stygge Creek. Also a nest with some
hatched fry but without a guarding male was seen. Nine more nests were
seen in Stygge Creek until July 20. Out of these nine nests, the eggs
hatched in five and the hatched fry were seen, but the other nests
seemed to be abandoned. In conclusion, the breeding time of the coastal
form was from the middle of June to the end of July.

The nesting sites of the coastal form were varied: on the
bottom among grasses, under rocks, in the concave bank of the creeks,
or even on open bottom with coarse sand. However, all nests were built in
places with very slow current (less than 5 cm/sec.) or no current, and
without the influence of sea water. The materials used for the nests
were dead or dying plant leaves and stems and rootlets. The males built

Fig.   8.   Length frequency distribution of the coastal fish in
Stygge Creek, 1967. Fish over one year old indicated
by shading.

AUG 24

AUG 5

JULY 26

JULY 9

JUNE 22

10

10

5

10

10

NUMBER

49
46
43
40
37
34
31
28
25
22
19
16
13
10
7
4

STANDARD LENGTH (mm)

Table   II.   Number of nuptial males and maturation stage of *eggs* of females of coastal form.

		Male			Female (egg stages)					
		non-nuptial	nuptial	N	I	II	III	IV	V	N
509 Creek	27 May	14	1	15	0	6	4	0	0	10
	1 June	61	8	69	0	38	6	0	0	44
1968	9 June	17	29	46	1	15	15	4	4	39
Paradox Creek	22 June	14	4	18	12	1	2	3	0	18
	9 July	9	2	11	16	0	0	1	1	18
1967	26 July	6	0	6	3	0	0	0	0	3
	5 August	16	0	16	14	0	0	0	0	14

nests with live aquatic plants in aquaria, but no nest was seen in nature built with living aquatic plants, probably because of the scarcity of live plants in the creeks.

All nests observed in nature and even in aquaria were built on the bottom, and no nest was found hung on plants, even in an aquarium which had many living aquatic plants.

Intermediate form   Judging from the fish collected by traps at the creek mouths, the breeding migration lasted from the end of June to the beginning of July. Nuptial males were most abundant at the end of June, and females carrying mature eggs were also present from the end of June to the beginning of July (Table III). Three nests were observed in Creek B on July 6. The actual breeding time seems to be rather later than that estimated from the migrating fish at the mouth of the Creeks, and probably lasts for an additional two weeks.

Judging from three nests found in nature, the intermediate form constructs a nest on the bottom of open water with a muddy substrate, using dead plants as nesting material. In an aquarium, they also used some living aquatic plants as nesting material and some nests were found hung on aquatic plants.

Food

Stomach contents of the coastal and intermediate forms were examined in fish collected from various places: the creeks, a bog in Isabelle Lake, and on the shore of Hudson Bay for the coastal form, and Creek A-2 to Creek F for the intermediate form. The food

Table   III.   Number of nuptial males and maturation stage of eggs of females of intermediate form, 1969.

	Male			Female (egg stage)					
	non-nuptial	nuptial	N	I	II	III	IV	V	N
June 24	4	0	4	0	3	1	0	0	4
25	0	2	2	0	4	2	1	0	7
26	4	0	4	2	6	6	2	0	16
28	14	1	15	6	8	7	4	0	25
30	14	12	26	3	12	16	10	3	44
July 2	0	2	2	0	0	2	0	0	2
4	2	0	2	0	0	1	0	0	1
7	0	0	0	0	0	2	2	0	4

items in the stomachs were identified and subsequently grouped into three categories according to their habitat: (i) pelagic or surface, (ii) benthos or benthic, and (iii) uncertain items (Table IV). The dominant food items in the stomachs were recorded in the manner used by HYNES (1950) for English populations of _Pungitius_. Both the coastal and intermediate form used various food items (Table IV), but their dominant food consisted of benthic organisms (74% for coastal form and 80% for intermediate form). There was no significant difference in proportions of the three categories of food between the two forms (Chi-square = 1.79, $0.50 < P < 0.25$).

Some coastal fish appeared to be undernourished when they first entered the creeks, but they fed vigorously and grew fast in the creeks, and most spawned in the same summer. Juveniles also grew fast in the creeks in summer. The size of the fish at the beginning of summer when they came into creeks was not much greater than the size of the fish at the end of the previous summer (Fig. 8). Apparently almost all growth occurred during the three-month summer period in the creeks, and little during the nine-month period in the sea.

Table   IV.   Number of stomachs classified according to dominant food item.

Dominant item	Coastal*	Intermediate**	Inland***
Pelagic or surface			
Anostraca	15		
Cladocera	1		6
Copepoda	8	20	26
Terrestrial insects	2	1	1
per cent	18.2	15.6	40.2
Uncertain			
Ostracoda	3	6	
Amphipoda	8		
per cent	7.7	4.4	0
Benthos or benthic			
Harpacticoid	14		
Chironomid	34	80	43
Ephemeraida	48		
Plecoptera	2		
Hemiptera		4	
Odonata		18	
Trichoptera	2	1	
Unidentified insects			5
Fish eggs		5	1
Oligochaeta	4		
Gastropoda	2		
per cent	74.1	80.0	59.8

*   Churchill area, early June to early August

**   Churchill area, end of June and middle of September

***   Heming Lake (47 fish, 11 August) and Athapapuskow Lake (35 fish, 27 May)

LIFE HISTORY OF INLAND FORM

Literature

WHITAKER (1968) found that Pungitius in lakes in Saskatchewan was most common in open beaches with sandy substrate. He also suggested that Pungitius moved from shallow to deeper parts in late summer in Heming Lake. NELSON (1968) found Pungitius in Crooked Lake, Indiana at the depths between 5 to 30 m., with smaller fish in shallower water. He found fully ripe females from April to the end of August. McKENZIE and KEENLEYSIDE (1970) described that Pungitius of South Bay, Lake Huron, made a nest mostly under or between rocks on the bottom, about 25 to 150 cm. from shore and 25 to 80 cm. deep, using rootlets and plant fragments as nest material.

Observations

The inland form in Athapapuskow Lake at Cranberry Portage spawned on the lake shore from the beginning of June to the end of July. The fish did not seem to ascend creeks, because two traps set in two creeks which drained into the lake caught no Pungitius. However, just below a dam in an outlet of the lake, many fish were seen swimming in a school against the current. In Heming Lake, the fish ascended a creek at the end of April and were caught in traps (personal communication, Mr. J. MARTIN). On May 23 about 20 fish were caught at the creek by seine net, but all of them were mature females and neither mature males nor nests were found in the creeks.

The inland form ate benthic organisms more than pelagic organisms, but the proportion of pelagic organisms was significantly larger than for the coastal and the intermediate forms (Table IV).

MORPHOLOGY

Manitoba fish

Morphological divergence between the coastal and the inland form was small, and none of the morphological characters could be used for clear cut separation, although mean values were quite different in some characters.

The development of lateral plates and caudal scutes was completed by 3 cm. standard length (Figs. 9 and 10), and the body proportions of the fish larger than 3 cm. did not vary with standard length, other than the depth of caudal peduncle (Fig. 15). Therefore only fish larger than 3 cm. were used for morphological analysis. Gravid females which had mature eggs (Stages III, IV and V), and some inland form fish which were badly infected with a parasite, Schistocephalus, were omitted for measuring the body depth because the swollen abdomen contributed errors.

Among the seven meristic characters counted, the mean numbers of dorsal spines, lateral plates, caudal scutes and gill rakers were each significantly different between allopatric coastal and inland forms (Fig. 12). Sixty-seven per cent of the coastal form had ten or more dorsal spines, but 88% of the inland form and 73% of the intermediate form had nine or fewer; 82% of the coastal form had three or more lateral plates, but 73% of the inland form and 88% of the intermediate form had two or fewer; 84% of the coastal form had 12 or more caudal scutes but 85% of the inland form and 68% of the intermediate form had 11 or fewer; 54% of the coastal form and 56%

38

Fig. 9. Development of lateral plates with length. All fish are
from Manitoba localities.

39
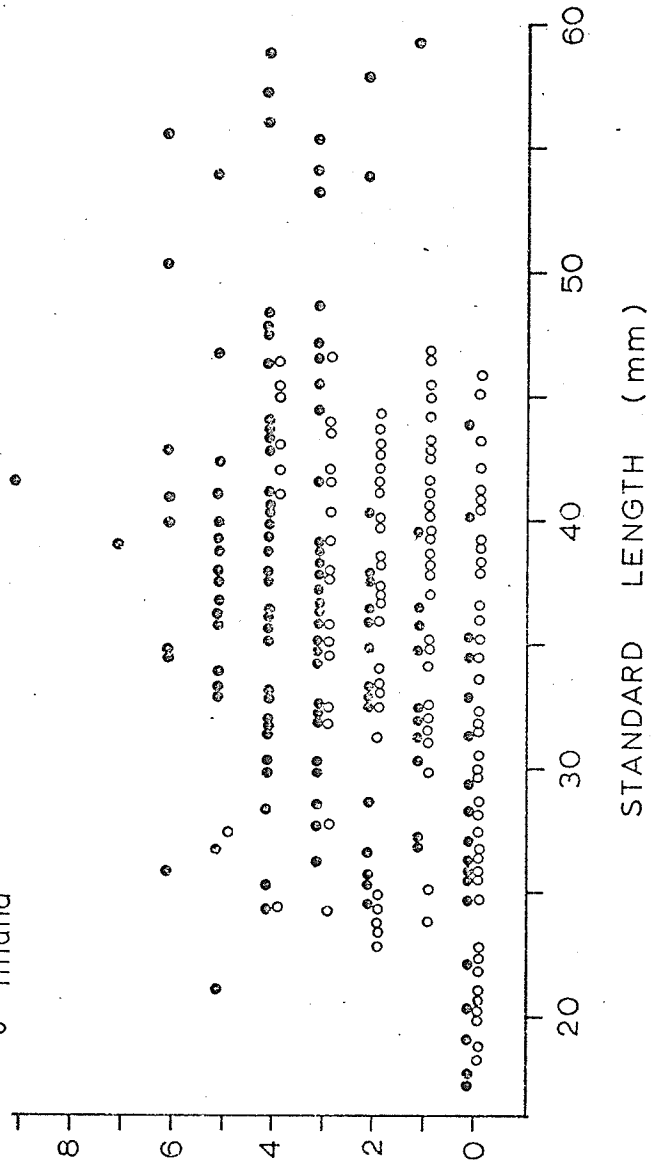
Fig. 10. Development of caudal scutes with length. All fish are from Manitoba localities.

NUMBER OF CAUDAL SCUTES

STANDARD LENGTH (mm)

• coastal

○ inland

20

15

10

5

0

20

30

40

50

60
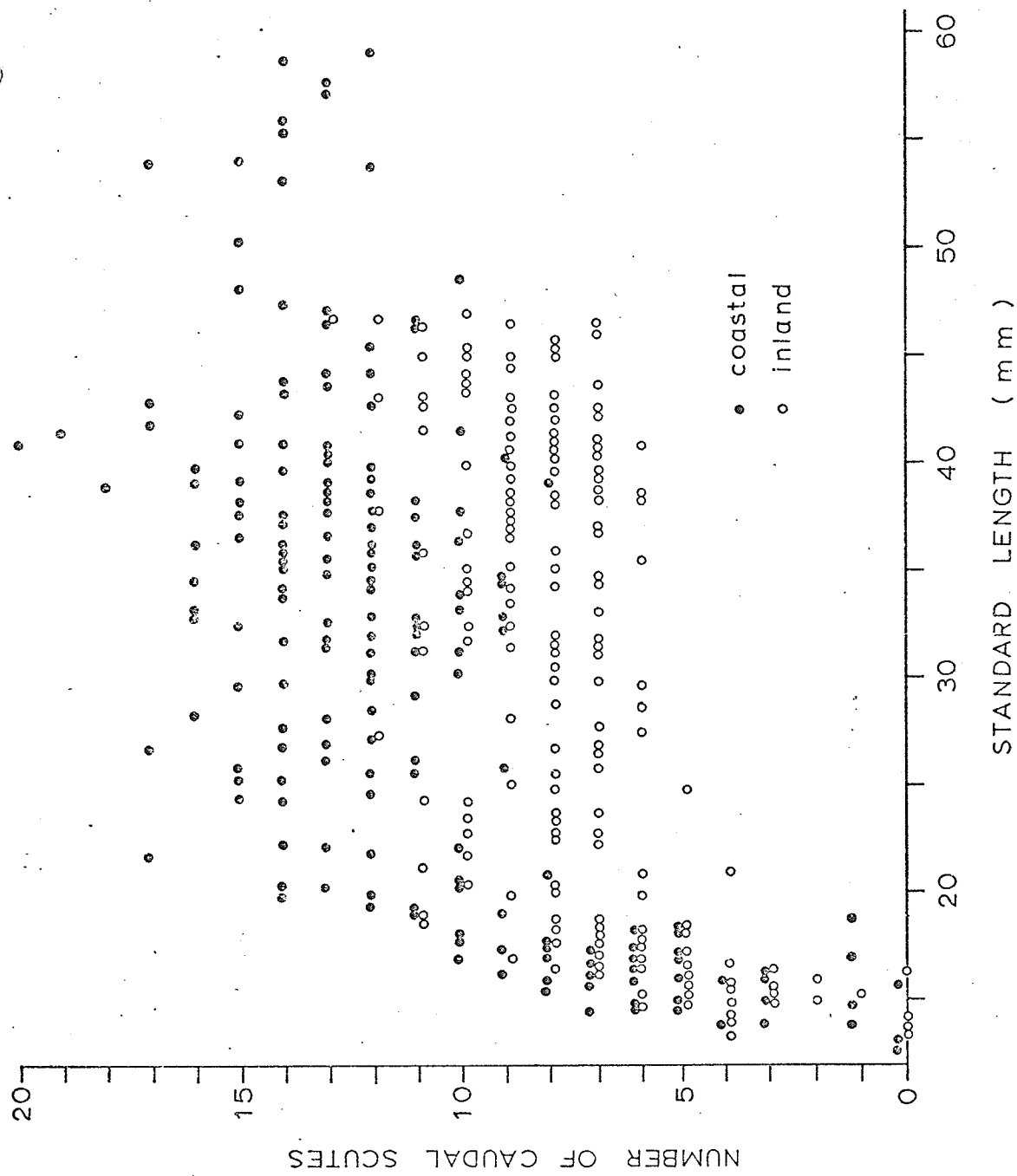
of the intermediate form had 12 or fewer gill rakers, but 77% of the inland form had 13 or more (Table V).

The mean values of the preceeding four characters for both reciprocal hybrids lay between the two parental forms, and all except gill raker number were significantly different ($P < 0.05$) from both parental forms. In these characters the hybrids were closer in mean to the maternal than to the paternal form (Fig. 11). No mean numbers of any meristic characters were significantly different between the two reciprocal hybrids.

In the other three characters, dorsal fin rays, anal fin rays and vertebrae, allopatric coastal and inland form were not significantly different, and the hybrids did not lie between the two parental forms (Fig. 11).

The intermediate form was significantly different in mean from both coastal and inland forms in all meristic characters except gill rakers, which were not different from the coastal form. Only the dorsal spines and caudal scutes of the intermediate form fell between coastal and inland forms. The intermediate form had one more count in dorsal fin rays, anal fin rays and vertebrae than both the coastal and inland forms (Fig. 12).

Body proportions were expressed as a ratio of standard length except the pectoral fin length which was expressed as a ratio of the pelvic spine length. The mean values of the allopatric coastal and the inland forms were significantly different in all measured body proportions (Fig. 13).

Fig. 11.   Frequency distribution of meristic characters. Sample
             sizes: coastal 367, inland 201 (Heming Lake,
             Athapapuskow Lake, and Manitoba Lake), intermediate
             155, hybrid FC 95, and hybrid CF 45.

42

Fig. 12.   Range, two standard deviations and mean of meristic
characters, and result of multiple range test between
means. Means are not significantly different (5 %)
within the same underline.
C: coastal, F: inland, I: intermediate,
CF: hybrid CF, FC: hybrid FC. Localities and sample
sizes as in Fig. 11.

LATERAL PLATES   ANAL FIN RAYS   DORSAL FIN RAYS   DORSAL SPINES
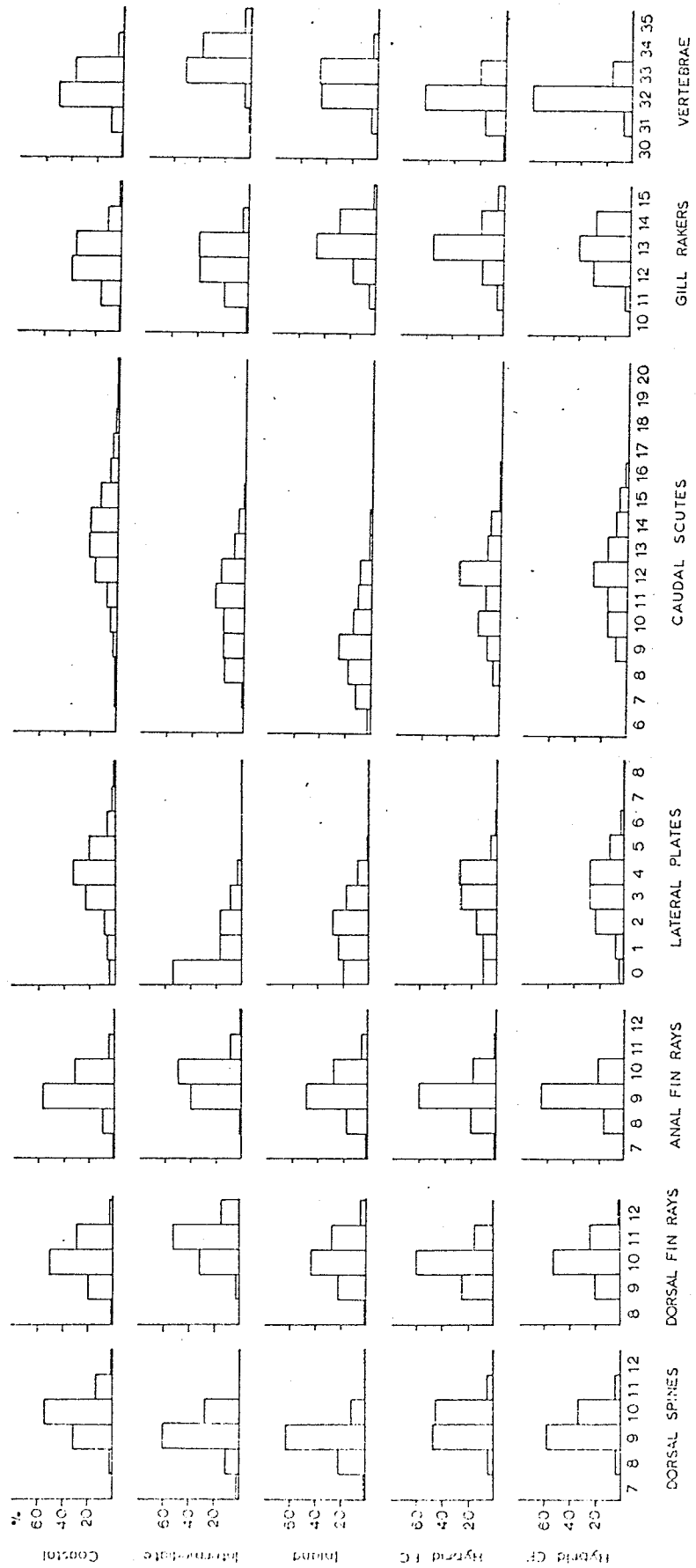
VERTEBRAE   GILL RAKERS   CAUDAL SCUTES

43
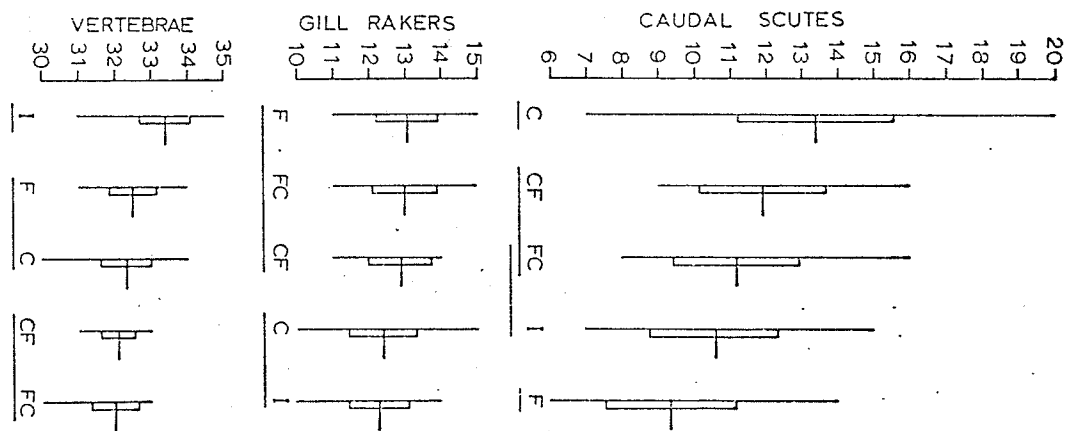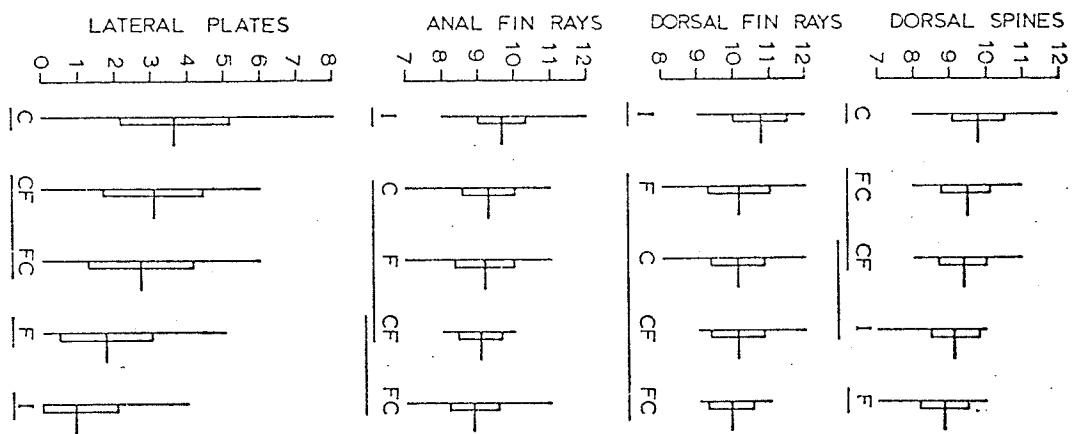
Fig. 13.   Range, two standard deviations and mean of the ratio of morphometric characters, and result of multiple range test between means. Designations are same as Fig. 12.

PL/SL 0.69 0.67 0.65 0.63 0.61 0.59 0.57 0.55 F I C FC CF

PO/SL 0.49 0.47 0.45 0.43 0.41 0.39 0.37 0.35 C I F FC CF

HL/SL 0.35 0.33 0.31 0.29 0.27 0.25 C I FC F CF

BD/SL 0.225 0.215 0.205 0.195 0.185 0.175 0.165 0.155 0.145 0.135 0.125 F I FC CF C

Pec/Pel 2.50 2.30 2.10 1.90 1.70 1.50 1.30 1.10 0.90 CF F FC I C

PAF/SL 0.69 0.67 0.65 0.63 0.61 0.59 0.57 I F C FC CF

44
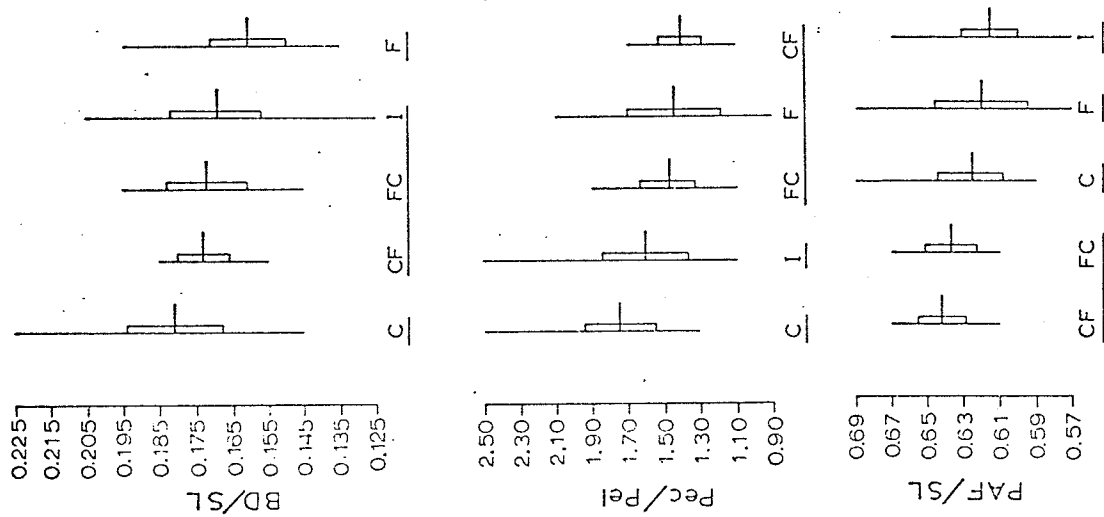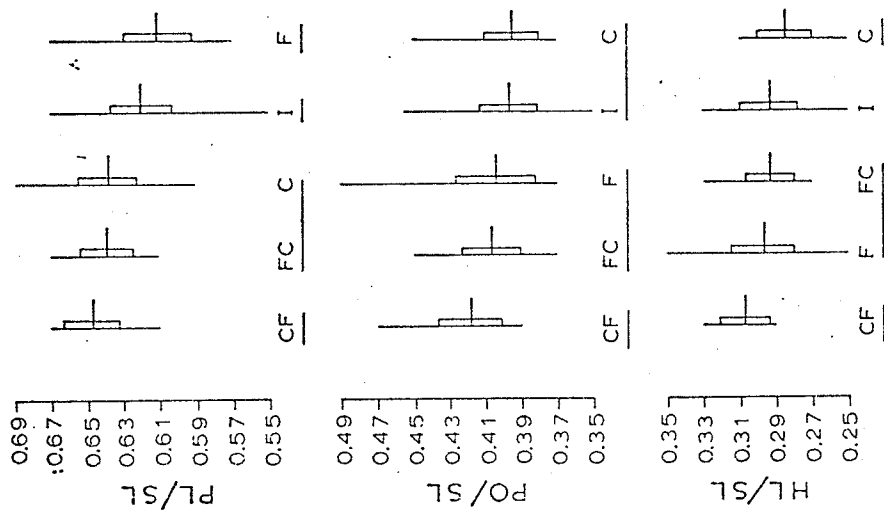
Table   V.   Comparison of numbers of dorsal spine, lateral plate, caudal scute and gill raker between coastal, intermediate and inland forms.

	Dorsal spines		Lateral plates		Caudal scutes		Gill rakers	
	≤9	≥10	0-2	≥3	≤11	≥12	≤12	≥13
Coastal	33 %	67 %	18 %	82 %	16 %	84 %	54 %	46 %
Intermediate	73	27	88	12	68	32	56	44
Inland	88	12	73	27	85	15	23	77

The standard lengths of mature fish (nuptial coloured males and gravid females) were also different. The coastal form had a larger mature size than the inland and intermediate forms, and females grew larger than males (Fig. 14).

BD/SL   81% of the coastal form had a ratio of 0.17 or more, but 84% of the inland form and 50% of the intermediate form had 0.169 or less (Fig. 16).

Pec/Pel   82% of the coastal form had 1.6 or more, but 69% of the inland form and 50% of the intermediate form had 1.59 or less. However, this ratio varied considerably between different lakes in the inland form.

PAF/SL   63% of the coastal form had 0.62 or more, but 62% of the intermediate form and 60% of the inland form had 0.619 or less.

PL/SL   86% of the coastal form had 0.62 or more, but 70% of the inland form and 50% of the intermediate form had 0.619 or less. The length from snout to the dorsal fin ray correlated with the number of dorsal spines, so this ratio should be high in the coastal form which had more dorsal spines than other two forms.

PO/SL   61% of the coastal form and 55% of the intermediate form had 0.399 or less, but 53% of the inland form had 0.40 or more.

HL/SL   87% of the coastal form and 63% of the intermediate form had 0.299 or less, but 47% of the inland form had 0.30 or more.

Fig. 14. Length of mature males and females, their means, and the multiple range test between means.

Coastal ♀ Inland ♂ Intermediate ♂

♂ ♀ ♂ ♀

60
56
52
48
44
40
36
32
28
24

STANDARD LENGTH (MM)

mean 49.1 43.5 42.7 41.9 38.4 34.3
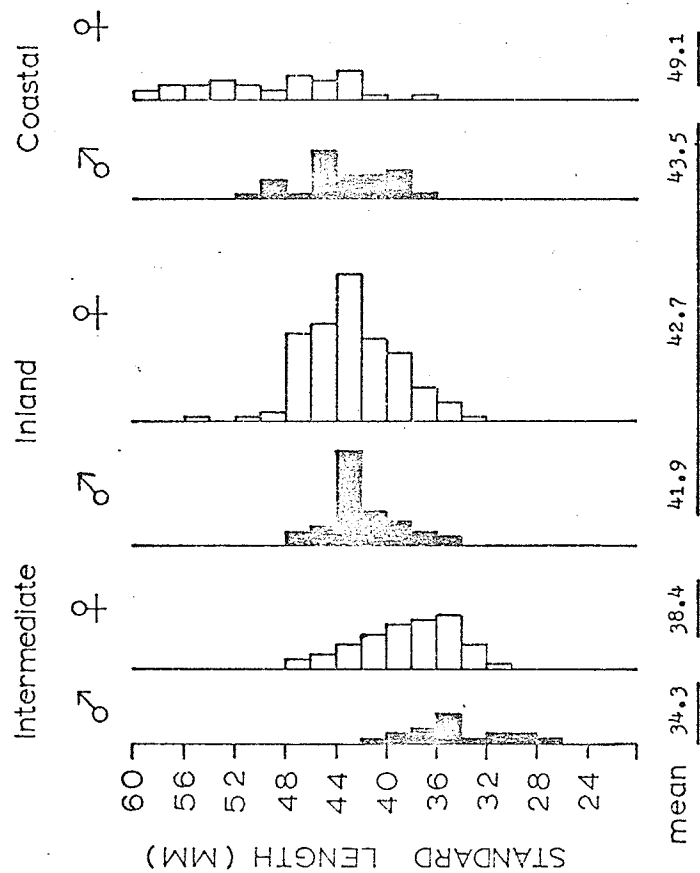
Among these morphometric characters, BD/SL ratio is the most reliable for distinguishing the coastal and inland forms. Although the DCP/SL ratio varied with the size of the fish, the ratio was quite different between the coastal and inland forms (Fig. 15). As a whole, the coastal form had deeper body, deeper caudal peduncle and chunky thicker body; the inland form had a generally slim body shape.

The BD/SL ratio of both reciprocal hybrids lay between the parental forms and closer to their maternal form. The ratio of the intermediate form also lay between the coastal and inland forms, and was significantly different from either (P<0.05).

In summary, dorsal spine, lateral plates and caudal scutes were the most reliable characters to distinguish the forms.

North American forms from other areas

To confirm the morphological differences between the forms found in Manitoba fish, morphometric analysis of museum specimens was made. McPHAIL (1963) has previously demonstrated differences in the numbers of dorsal spines, lateral plates, and gill rakers, and in the Pec/Pel ratio. Although, as shown above in the present study, none of the morphological characters was clear-cut for distinguishing the forms, dorsal spines, lateral plates, caudal scutes and BD/SL ratio were the most reliable to distinguish the forms in Manitoba. However, the counting of lateral plates without staining was not reliable, so lateral plate was not counted on museum specimens. The number of lateral plates is correlated with the number of the scutes (Fig. 17), so caudal scute number reflected the lateral plate number to some extent.

Fig. 15.   Change of the DCP/SL (depth of caudal peduncle into
            standard length) ratio with standard length. Line
            of separation drawn by eye.

Coastal form

Inland form

STANDARD LENGTH (MM)

DCF/SL
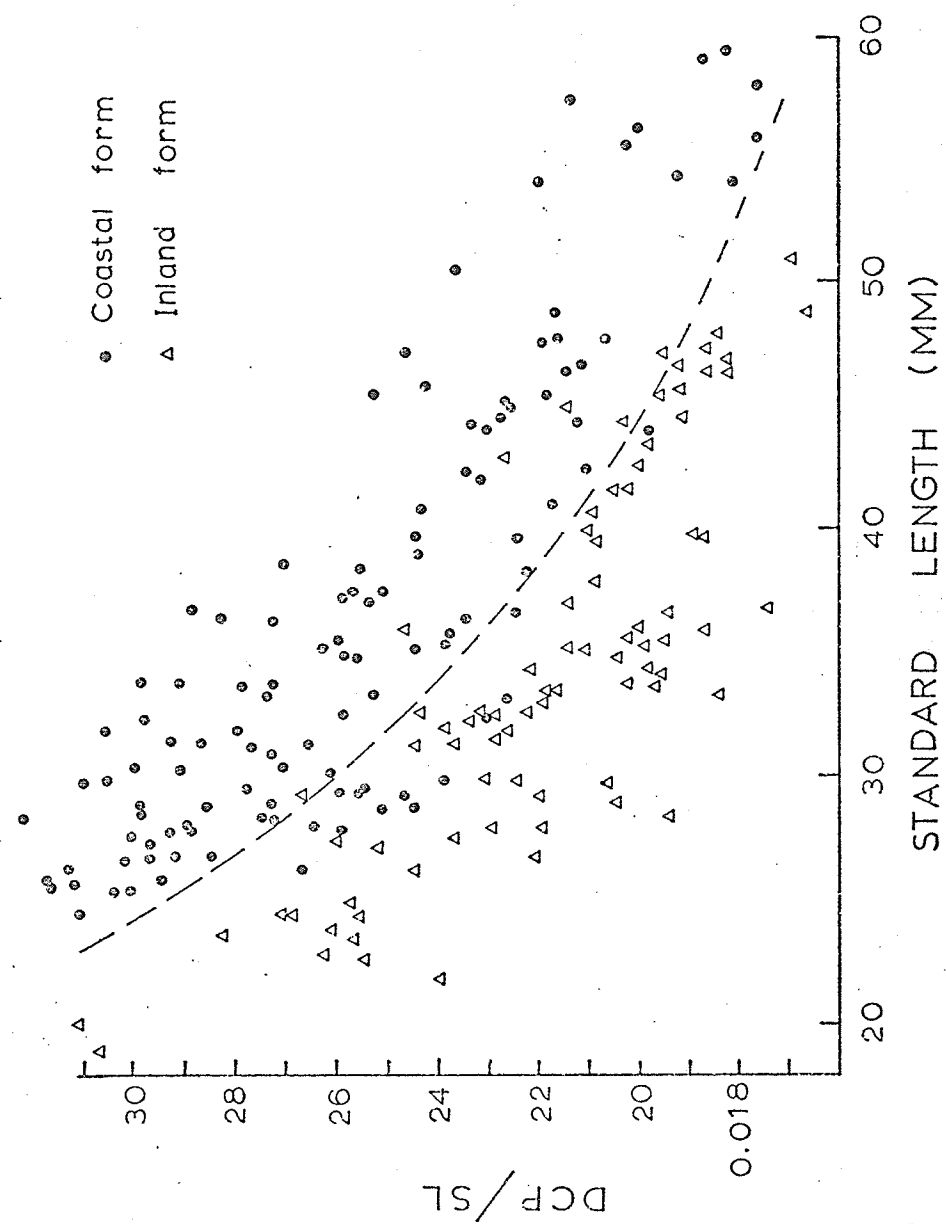
Fig.   16.   Frequency distribution of BD/SL ratio.

Coastal

Intermediate

Inland

Hybrid FC

Hybrid CF

%
60
40
20

60
40
20

60
40
20

60
40
20

60
40
20

0.130 0.140 0.150 0.160 0.170 0.180 0.190 0.200 0.210 0.220 0.230
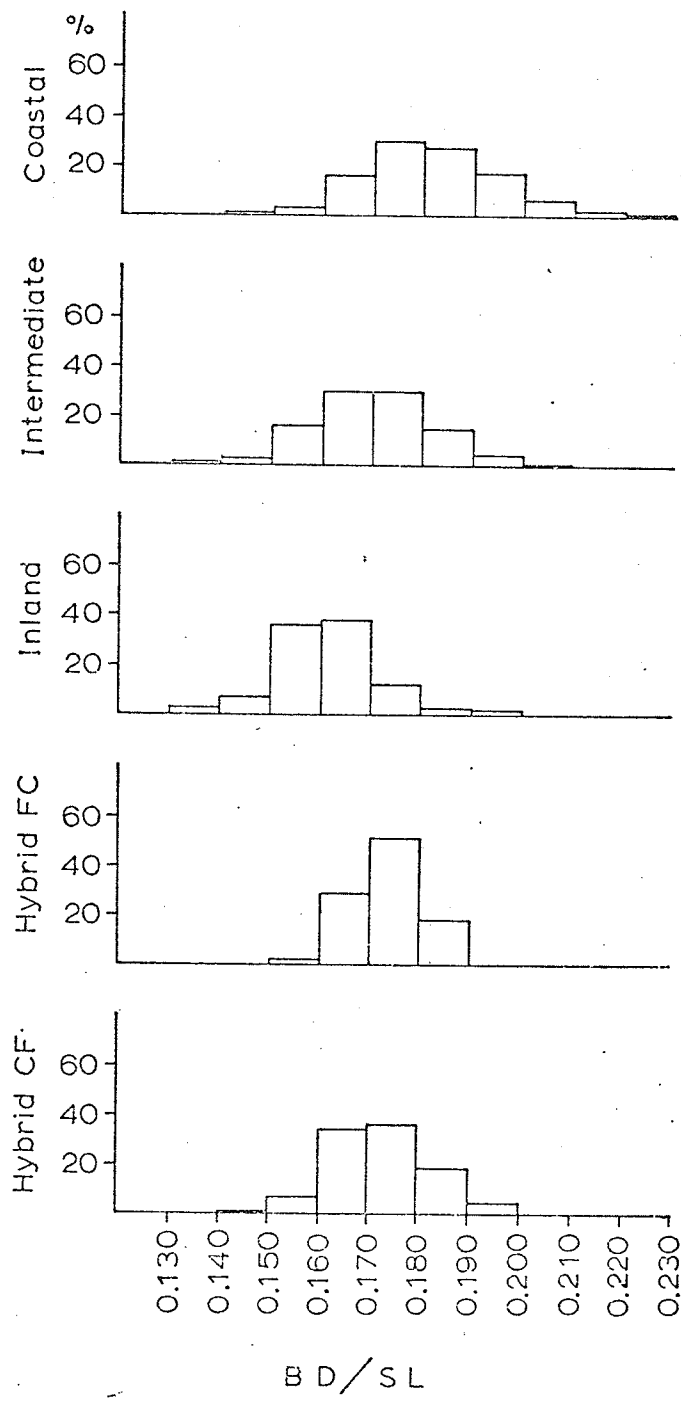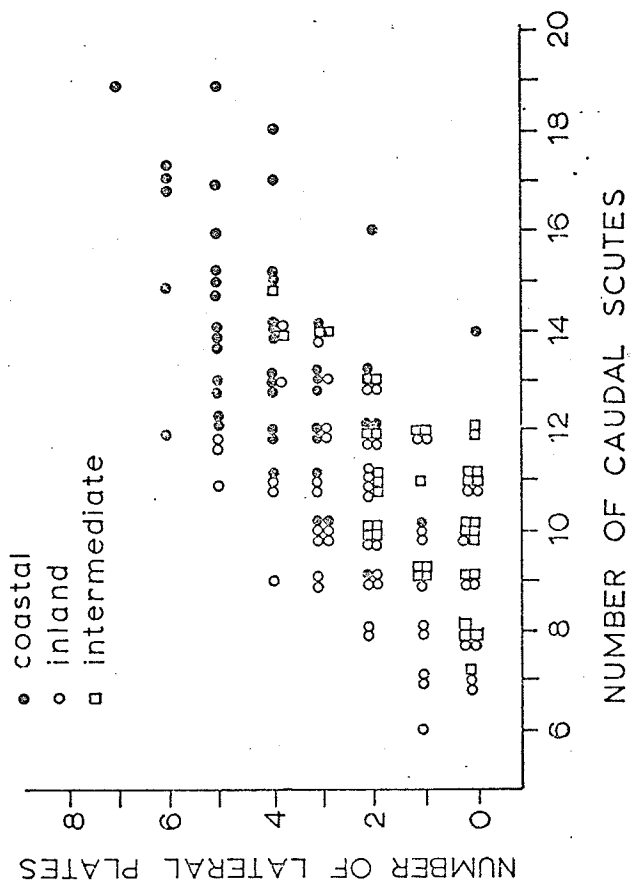
B D / S L

Fig.   17.   Relation between the numbers of caudal scutes and
             of lateral plates.

The areas from which specimens were examined was mainly restricted to regions where the two forms may come in contact. Hence collections from the coastal areas of the Arctic Ocean, Hudson Bay and Atlantic Ocean predominantly, and not many collections from far inland were studied.

The dorsal spine number was higher for fish in coastal waters than in inland waters (Fig. 18) as McPHAIL showed. The number of caudal scutes was also higher for coastal fish, except on the Arctic coast, where the caudal scute number was low (Fig. 19). The BD/SL ratio, as shown previously in Manitoba fish, was also generally higher in the coastal area fish than the inland fish, but the difference was not distinct (Fig. 20).

By the use of the BD/SL ratio plus dorsal spine and caudal scutes, the score of each fish was calculated as described under Methods, and the mean value of each population was estimated. The same scoring was used for the Manitoba fish, and mean values were 29 to 32 for the coastal form, 14 to 22 for the inland form, 22 for the intermediate form and the 26 for the artificially made hybrids (Fig. 22). As shown in Fig. 21, the mean scores for the coastal water fish were generally higher than for inland water fish, even though there were some exceptions.

52

Fig. 18. Geographic variation of dorsal spine number.

DORSAL SPINE

	10 or more
	9.7 9.9
	9.4 9.6
	9.1 9.3
	9.0 or less

☐ tidal water

○ inland water
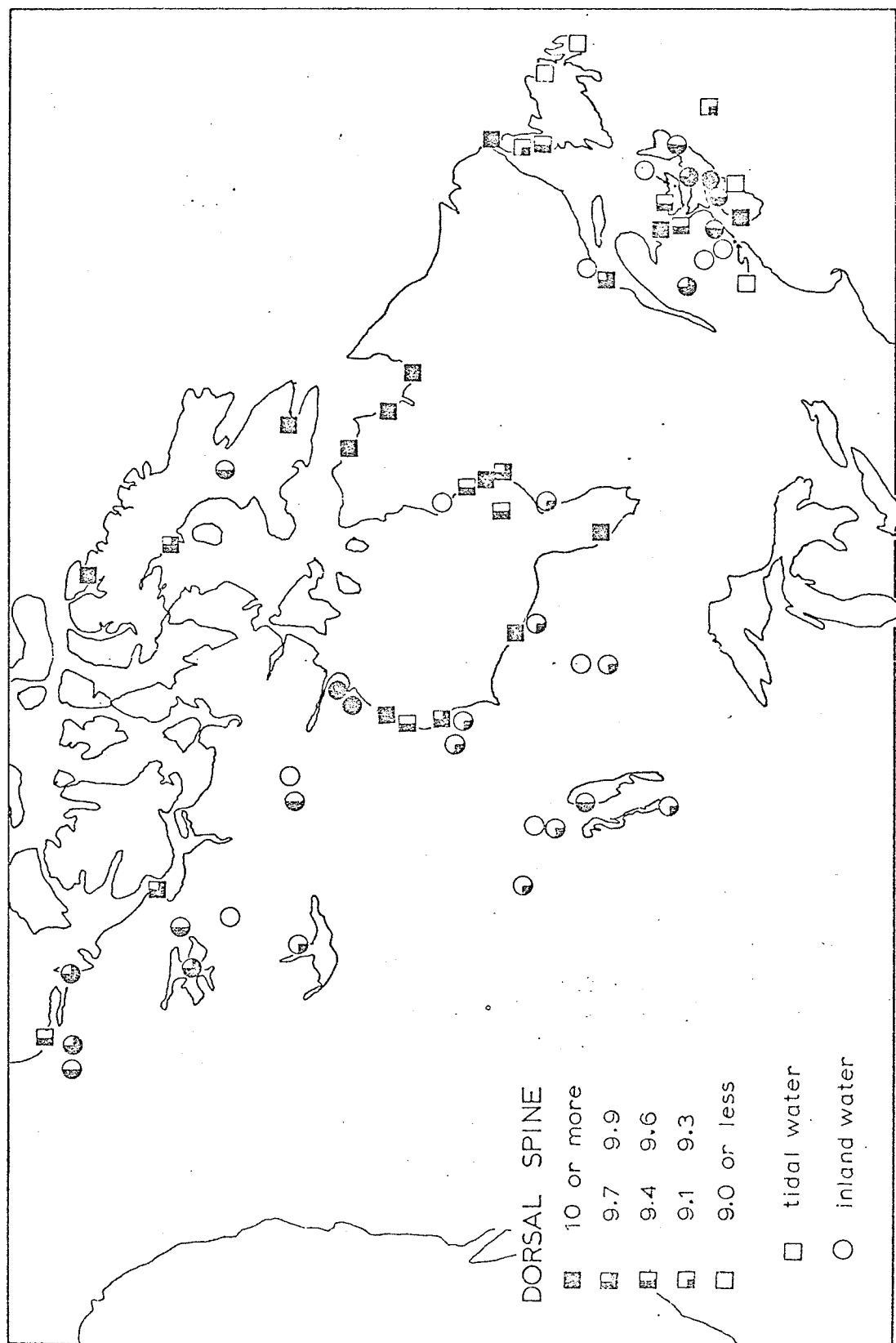
Fig. 19. Geographic variation of caudal scute number.

NUMBER OF SCUTES
OF CAUDAL KEEL

	12 or more
	11
	10
	9
	8 or less

☐ tidal water
○ inland water

54
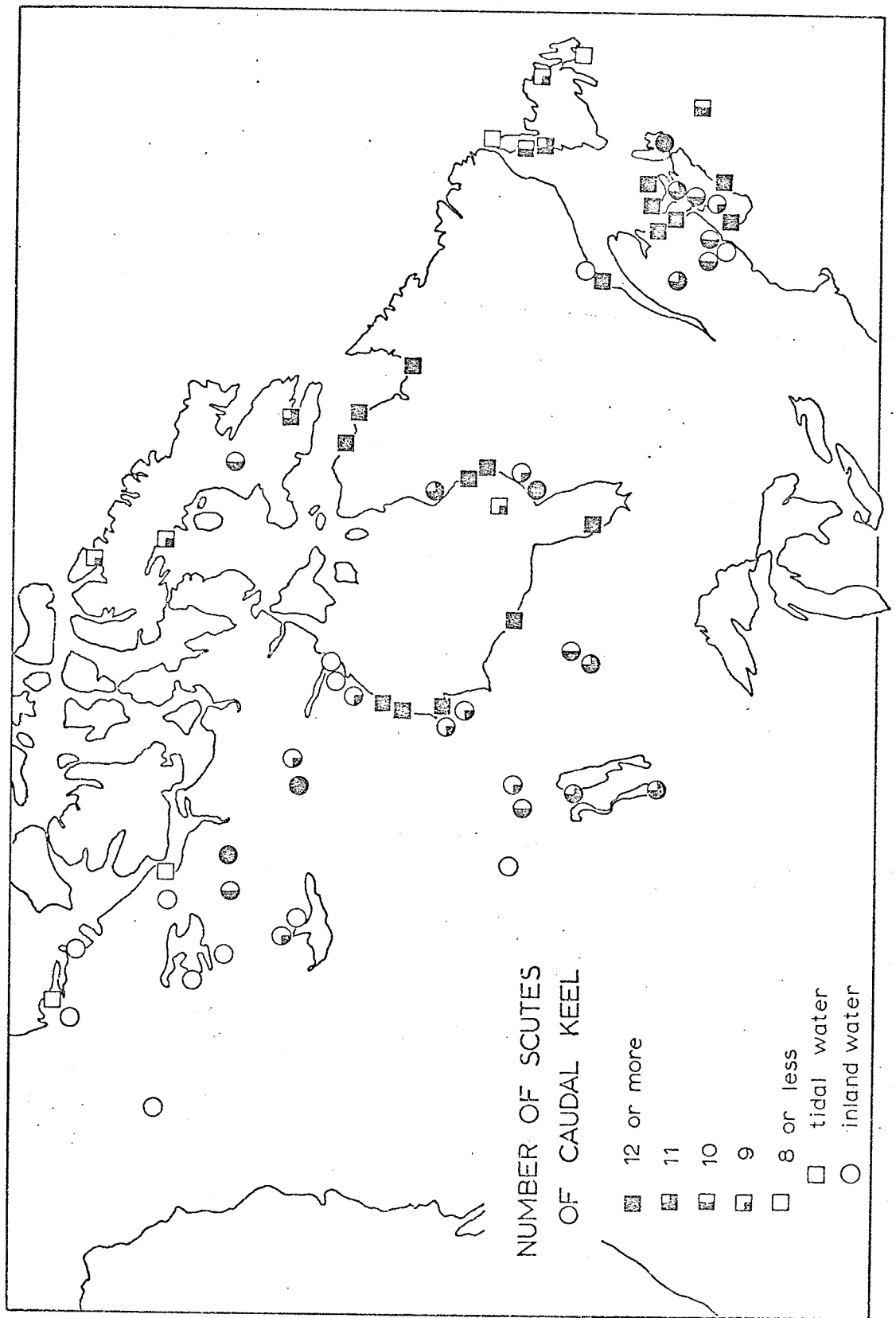
Fig. 20. Geographic variation of BD/SL ratio.

BODY DEPTH / STANDARD LENGTH

0.19 or more
0.18
0.17
0.16
0.15 or less

☐ tidal water
○ inland water
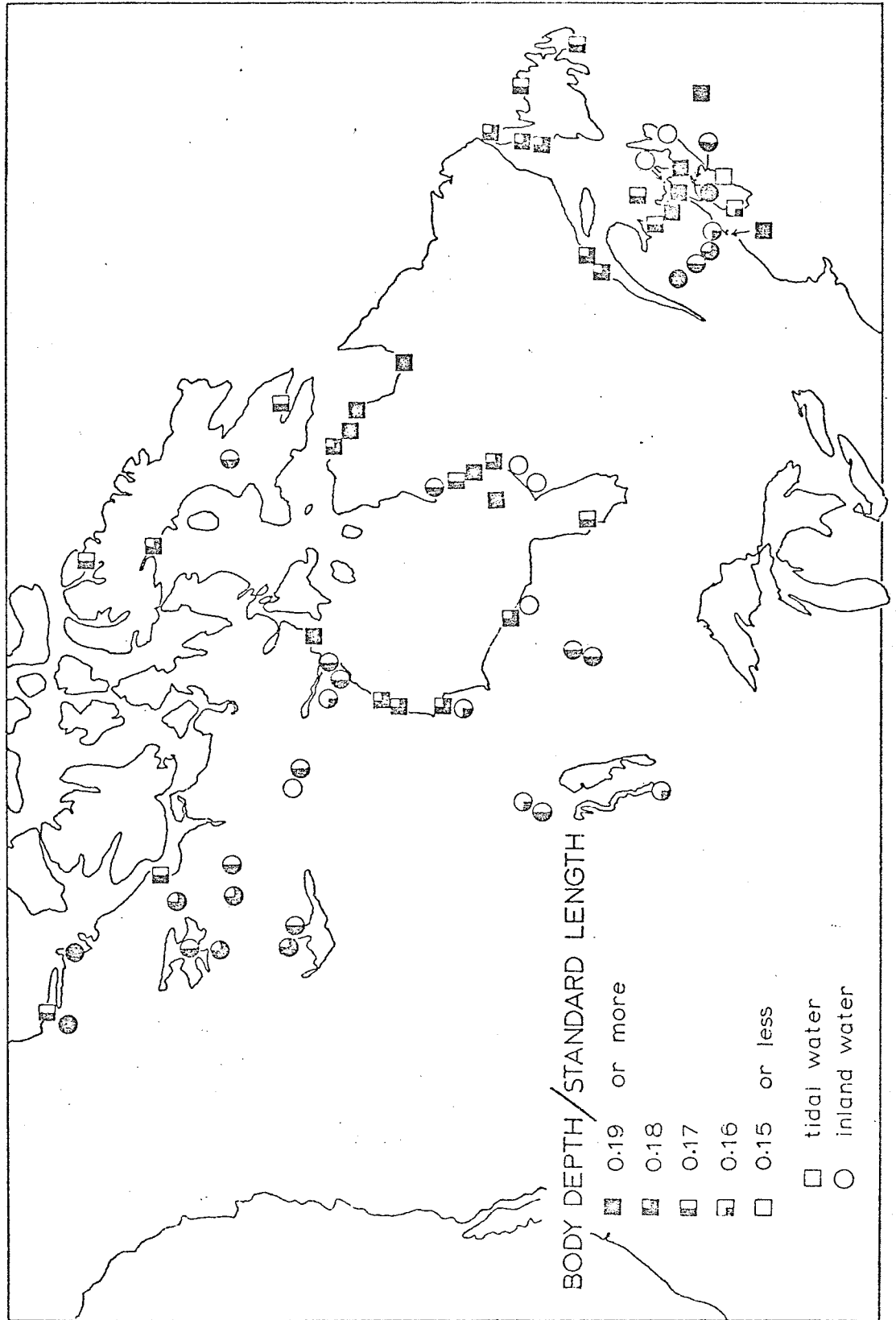
Fig.   21.   Geographic variation of score based on dorsal
spine, caudal scute, and BD/SL ratio.

SCORE OF THREE
CHARACTERS

29 or more

27 28

25 26

23 24

22 or less

tidal water

inland water
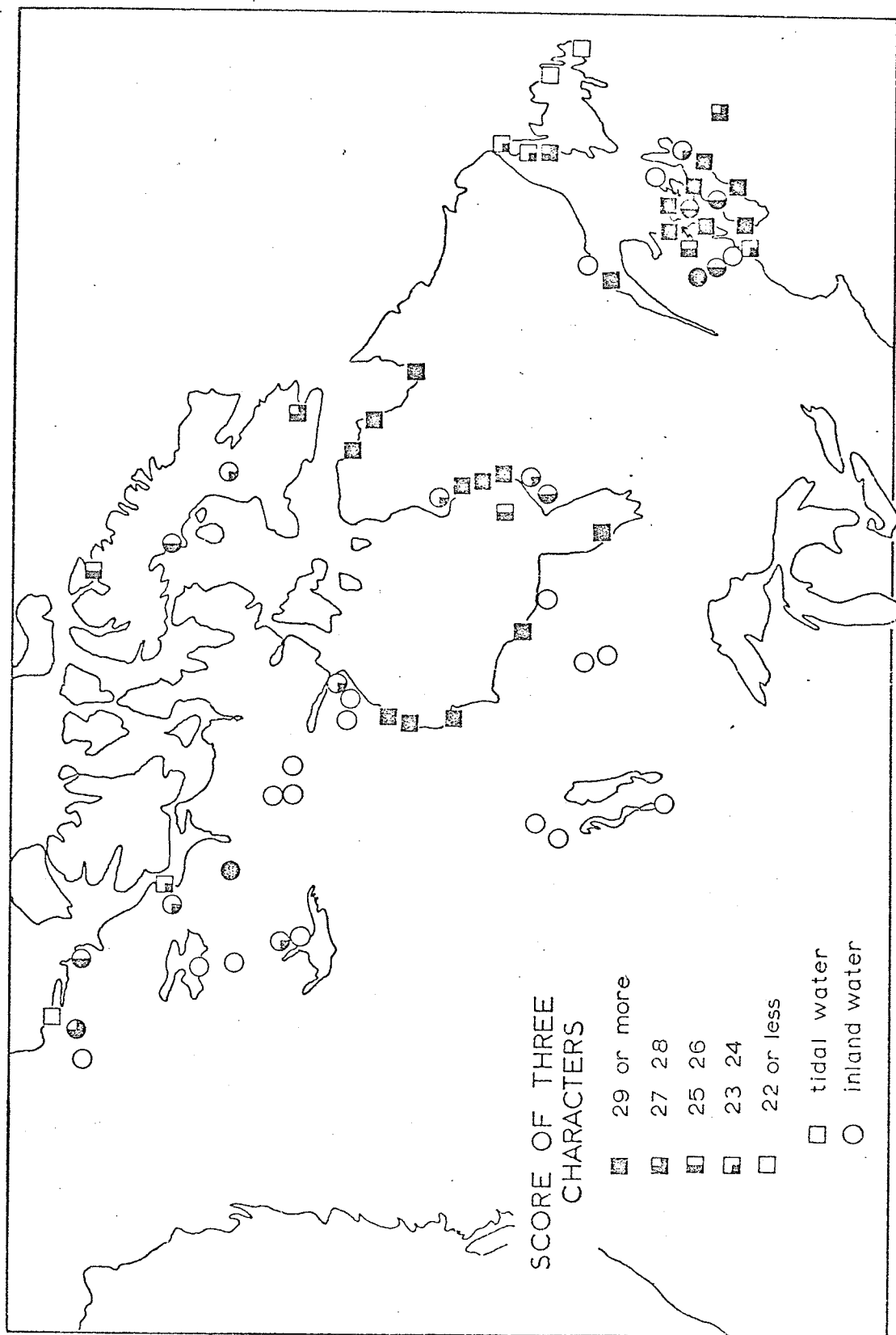
Fig. 22.   Morphological scores of Manitoba fish based on dorsal spine, caudal scute and BD/SL ratio. Inland form (Heming Lake 30, Athapapuskow Lake 38, Manitoba Lake 14); coastal form (Goose Creek 10, 509 Creek 100, Isabelle Lake 29, Paradox Creek 65, Stygge Creek 54, and Hudson Bay shore 27); intermediate form (Creek C 70, and Creek F 40).

PERCENTAGE

20   20   20   20   20

SCORE

41-42
39-40
37-38
35-36
33-34
31-32
29-30
27-28
25-26
23-24
21-22
19-20
17-18
15-16
13-14
11-12
-10

	inland	intermediate	hybrid FC	hybrid CF	coastal
number	82	110	95	45	283
mean	18.9	21.8	25.5	26.0	30.8
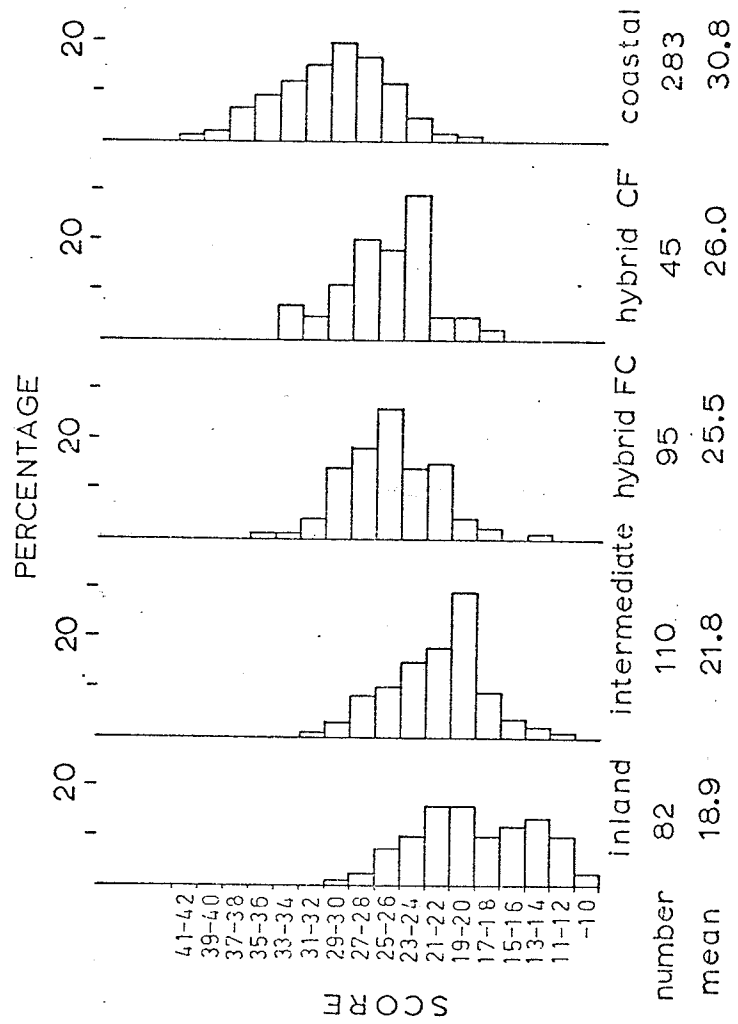
PHYSIOLOGY

Salinity tolerance

Differences in salinity tolerance between the coastal
and the inland forms might be expected from differences in their
life history. As described before, salinity tolerance tests were
conducted for the coastal, intermediate, and inland forms, and for
both reciprocal hybrids. Tests were conducted at several temperatures,
and at different times of the year. The life span of the fish was two
years and most of the fish died at age one, so the fish older than one
year were treated as adult, and no attempt was made to find differences
between ages one and two. As WHITAKER (1968) described, the tested
fish which could not tolerate salt solution died usually after five
days of exposure, and they turned dark brown or black before they died.

A distinct difference in salinity tolerance was found to
exist between the coastal form and the inland form. No adult inland
fish could tolerate 25 o/oo or greater, but 92% of the examined adult
coastal fish could survive in 27.5 o/oo (Fig. 23). The salt concentration
for 50% mortality (LD 50) was 19.6 o/oo for the adult inland form
but 34 o/oo for the adult coastal form (Fig. 24). The salinity tolerance
of adults depended on the test temperature; adult of both forms were
more tolerant at 10C and 15C than at 4C, so the experiments were conducted
at 10C.

Fig.   23.   Salinity tolerance of adult <u>Pungitius</u> in early summer, tested at 10 C. Each point based on one experiment using 7 fish.
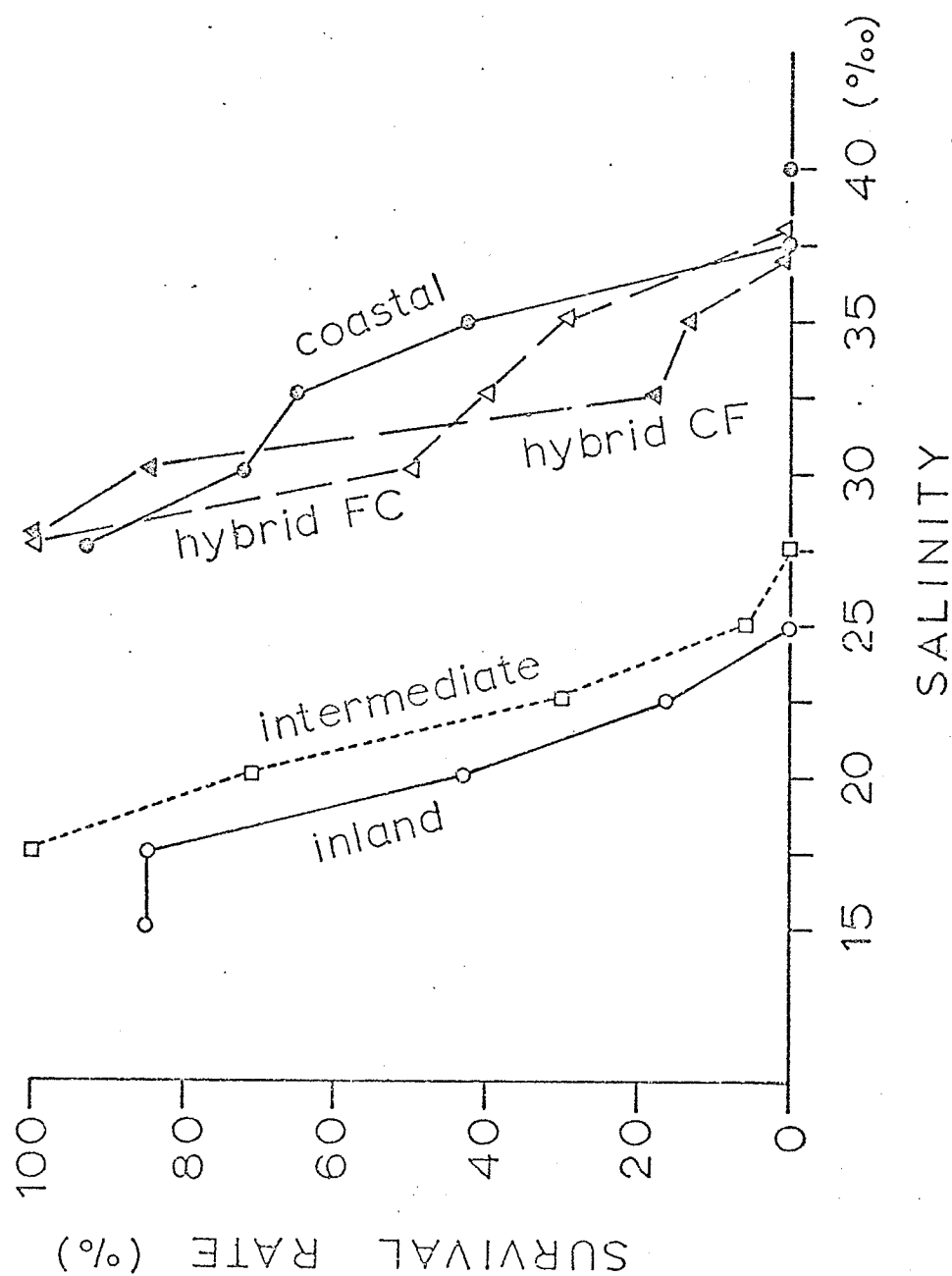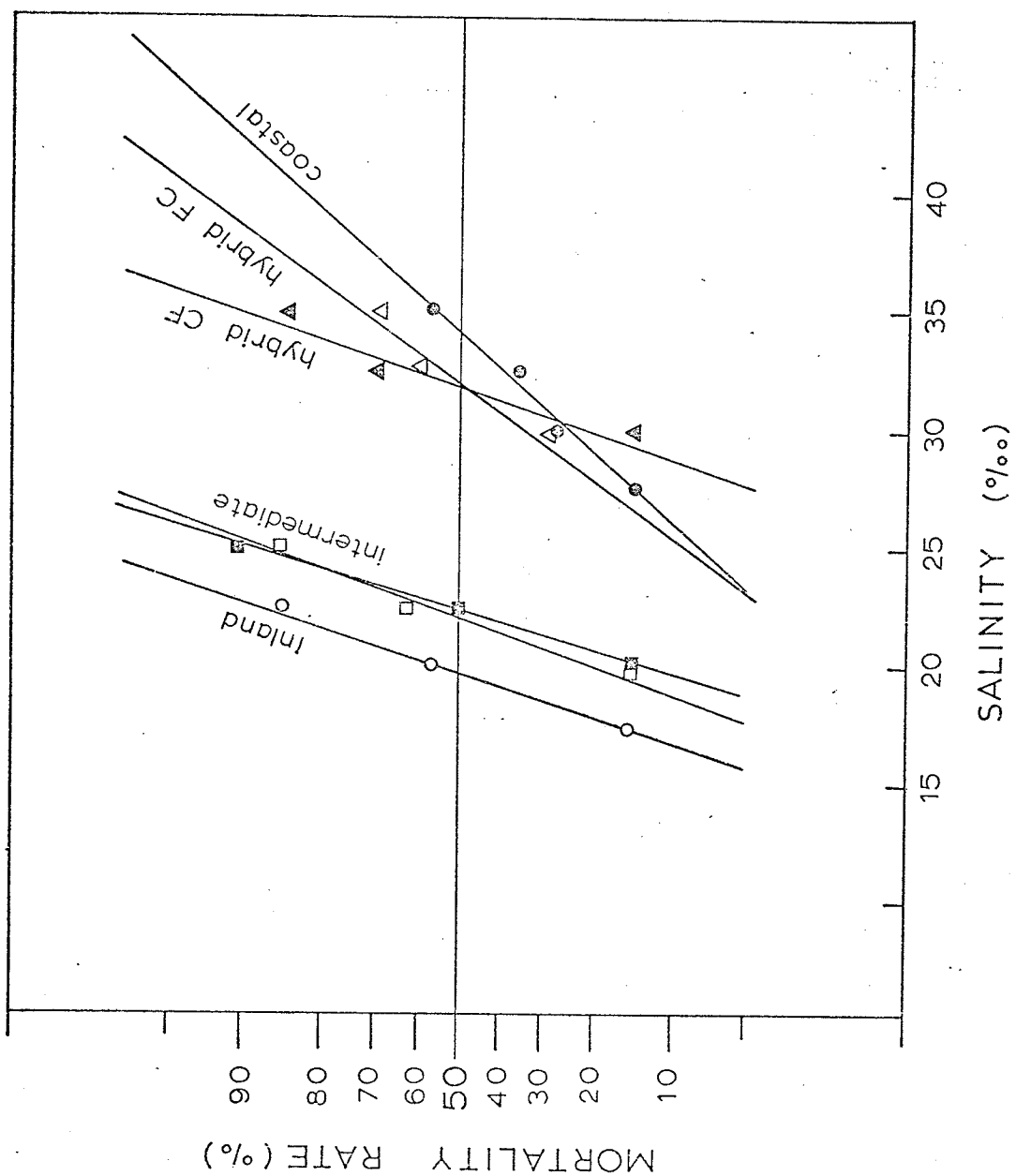
Fig.   24.   Salinity tolerance (LD 50) in logistic transformation based on same data as Fig. 23.

MORTALITY RATE (%)

90 80 70 60 50 40 30 20 10

SALINITY (‰)

15 20 25 30 35 40

coastal

hybrid FC

hybrid CF

intermediate

Inland

None of the adult intermediate form could tolerate more than 27.5 o/oo salt water (Fig. 23). The LD 50 was 22.5 o/oo, slightly higher than for the inland form (Fig. 24).

Both reciprocal hybrids could survive in 27.5 o/oo. Their salinity tolerance was close to the coastal form, with an LD 50 of 32 o/oo, only slightly lower than the coastal form (Fig. 23 and 24).

The salinity tolerance of adults may be subject to change by acclimation before the tests. On the other hand, the tests of salinity tolerance of eggs, by hatching them directly in different salt concentrations, probably eliminate the effects of the acclimation; any difference in response of the eggs would be due to a genetic difference between the forms.

At the highest salinities, some eggs died by shrinking, probably due to osmotic water loss. Even if they did not die, they developed as abnormal embryos (lacking one or both eyes, or with a deformed body), and they eventually died by the time of yolk absorption.

The salt concentrations at which 50% of the eggs hatched (LD 50) were 15 o/oo for the inland form and 22.5 o/oo for the coastal form, and were 20 o/oo for the inland form eggs fertilized by a coastal male, and more than 22.5 o/oo for the coastal form eggs fertilized by an inland male; in this case 73% of the eggs hatched in 22.5 o/oo. The salt concentrations for 100% mortality of eggs were 17.5 o/oo for the inland form and 30 o/oo for the coastal form (Fig. 25).

Fig.   25.   Percentage of fish surviving to yolk absorption
at 20 C.   Inland and hybrid points are each based
on one experiment using 30 eggs each, coastal
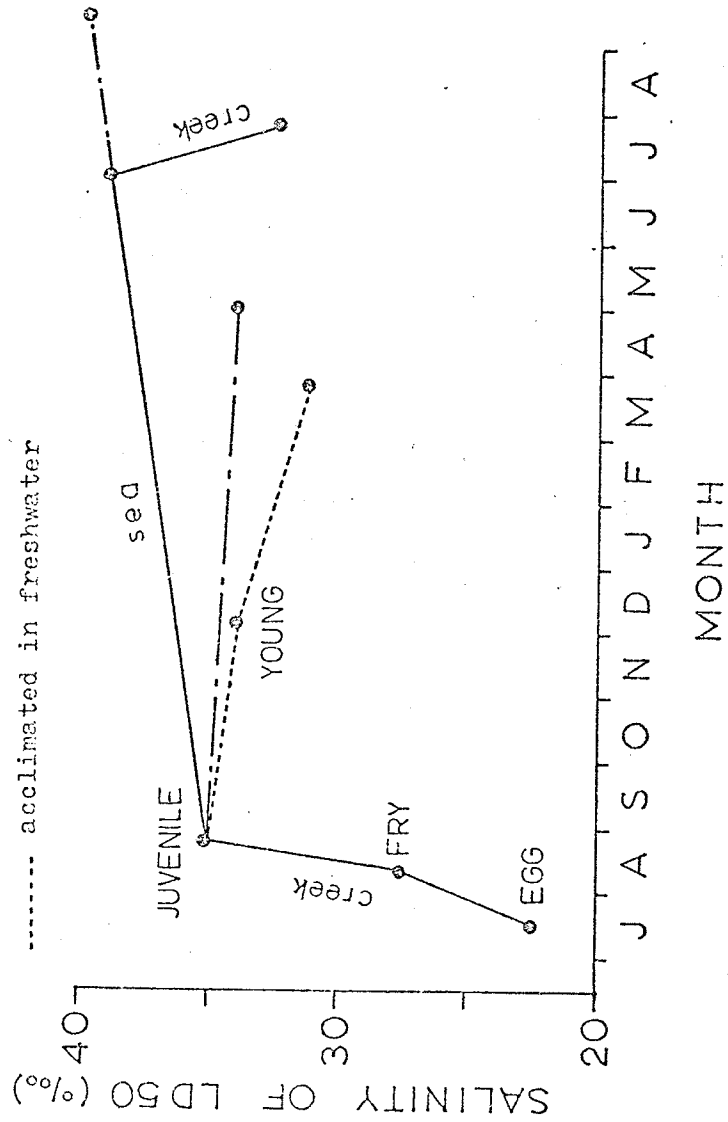form based on 3 experiments for each point.
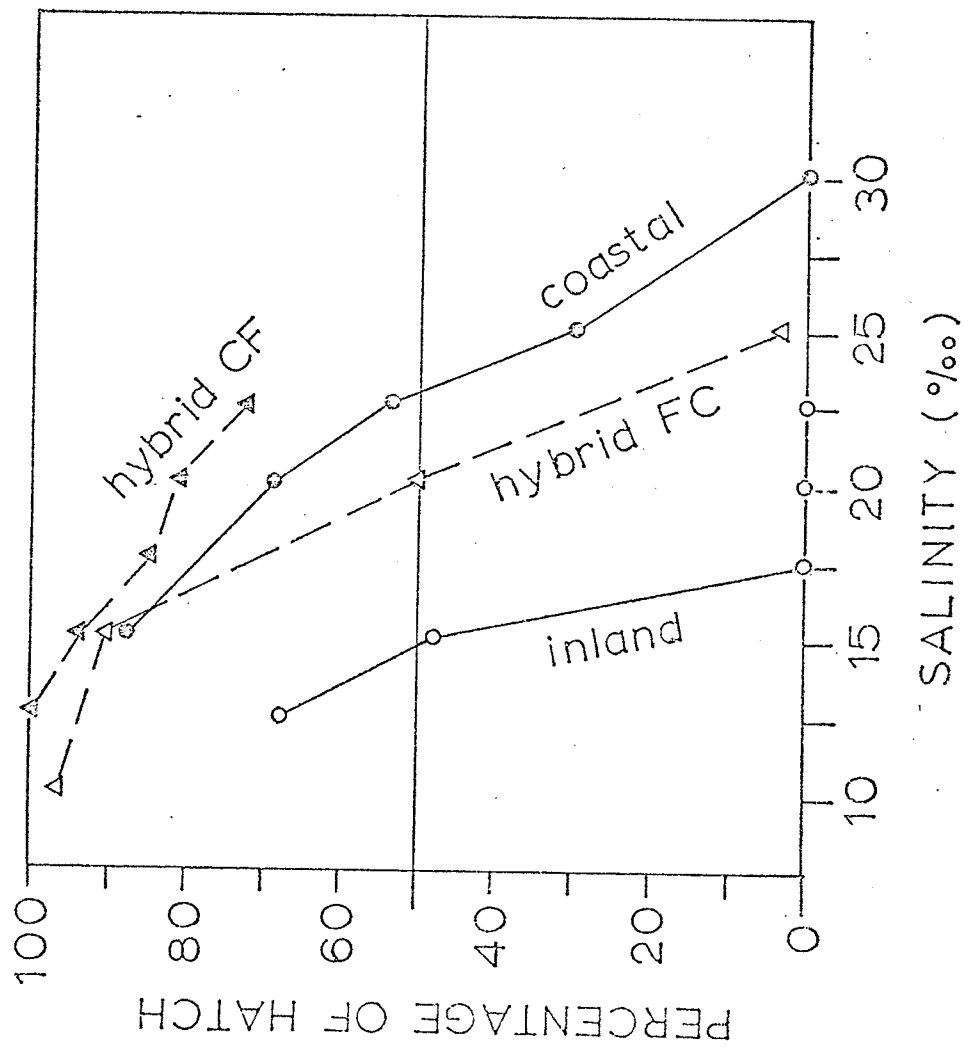
In summary, the salinity tolerances of both adult and egg were quite different between the coastal and the inland form, and the salinity tolerance of both reciprocal hybrids was closer to the coastal form than to the inland form, for both adults and eggs.

The salinity tolerance of the coastal form changed with the size of the fish, the season and the acclimation. After hatching, the fish developed increased resistance to salinity, and in autumn their LD 50 was 35 o/oo. In the natural environment they then went down the creeks to the Hudson Bay. They presumably maintained the tolerance to high salinity throughout the winter, because at the beginning of the next summer when the fish came into the creeks, their LD 50 was 37.5 o/oo at 15C. However, if coastal fish were acclimated in a freshwater tank since autumn, their LD 50 decreased to 33 o/oo by next spring (Fig. 26). In the freshwater tanks the coastal fish had an exceptionally high mortality rate in the autumn compared to the inland fish. However, unlike the marine form of Gasterosteus aculeatus (trachurus form) which cannot survive in freshwater through winter without an injection of prolactin (LAM and HOAR 1967), the coastal form of Pungitius survived in freshwater through winter without special treatment, once the autumn period of mortality had passed.

After the coastal fish entered a creek in the summer, the salinity tolerance decreased to 32 o/oo in the middle of the summer, probably because they were acclimated in freshwater after entering the creeks. After entering the creek in early summer some fish were captured and when acclimated in 20 o/oo salt water, were able to tolerate 37.5 o/oo in salinity at the end of the summer (Fig. 26).

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Fig.   26.   Changes of salinity tolerance (LD 50) of the coastal
form with season and by acclimation.

in nature

acclimated in 20 o/oo salt water

acclimated in freshwater

creek

sea

YOUNG

JUVENILE

creek

FRY

EGG

J A S O N D J F M A M J J A

MONTH

SALINITY OF LD 50 (°/oo)

40

30

20

No seasonal change of salinity tolerance was observed in the inland form. At the end of August the inland juveniles tolerated 20 o/oo, and there was no change found between August and next Spring.

Relation between salinity tolerance and morphology

The coastal fish which had been acclimated in freshwater throughout the winter were exposed to 32.5 o/oo salt solution at 15C. After four days, 21 fish had died and 17 survived. The morphological analysis of meristic characters of these fish is shown in Table VI. The survivors had a higher mean number of dorsal spines, lateral plates and caudal scutes, and had fewer gill rakers than the dead fish. The means of lateral plate counts between the survivors and the dead were significantly different. Hence it may be said that within this sample of coastal fish the individuals which were more tolerant to high salinity had a higher number of dorsal spines, lateral plates and caudal scutes and a lower number of gill rakers, all of which are features of the coastal as compared to the inland form.

The inland form fish, which had been collected a week before the test and acclimated at 4C in a laboratory, were also exposed to 20 o/oo at 4 and 15C. At 15C no fish died but at 4C all the fish died. There was no apparent correlation between time to death and any of the measured morphological characters.

Salinity preference

Laboratory tests of salinity preference were done in spring and autumn, using freshwater acclimated fish of both coastal form and inland form. The coastal and inland fish responded differently to

Table VI.   Relation between salinity tolerance and meristic characters.

Dorsal spines	N	Mean	Variance	
Dead fish	21	9.8	0.462	t=0.952
Live fish	17	10.0	0.376	(0.4 > P > 0.3)
Lateral plates				
Dead fish	21	2.62	1.55	t=2.448 *
Live fish	17	3.53	1.64	(0.025 > P > 0.01)
Caudal scutes				
Dead fish	21	12.57	2.957	t=0.765
Live fish	16	13.00	2.800	(0.5 > P > 0.4)
Gill rakers				
Dead fish	21	12.67	1.033	t=0.336
Live fish	17	12.58	0.382	(P > 0.5)

Mean of standard length

Dead fish	44.0	mm
Live fish	46.6	mm

the saltwater solution, and each responded differently in different seasons, as shown in Table VII.

In a series of choices between fresh water and a given salinity, the coastal fish had in autumn, a slight preference for 5 o/oo, no preference for or against 15 o/oo, and avoided 25 o/oo. The inland fish, on the other hand strongly avoided 15 o/oo.

Biochemical structure

In order to determine if there were biochemical differences, electrophoretic study of Lactate Dehydrogenase (LDH) of the skeletal muscle, and of hemoglobin of the blood was conducted for the coastal, inland and intermediate forms. The samples were from fish which had been acclimated in freshwater in the laboratory for more than three months.

Lactate Dehydrogenase   There was just one anodally migrating band and no difference between the forms (Fig. 27). KUSA (1966) also demonstrated one band of LDH in Pungitius pungitius in Japan, but the band was cathodally migrating, probably because he used a different pH of buffer (pH, 8.6).

Hemoglobin   There were three to eight bands on the anode side of the gel. Although the number of the bands seems to be variable within a form (Table VIII), the coastal form seemed to have rather more bands than the inland form (Fig. 28). No inland or intermediate forms had the bands I, VII, or VIII, but out of 20 coastal fishes 5, 7 and 6 fishes had bands I, VII, and VIII respectively (Table VIII).

Table   VII.   The time spent in salt solution compartment
during 100-minute test period.   Number in
bracket is repetitive.

Spring

	5 o/oo	15 o/oo
Coastal	60 % (15)	50 % (18)
Inland	45 % (15)	25 % (18)

Autumn

	5 o/oo	15 o/oo	25 o/oo
Coastal	57 % (17)	48 % (18)	40 % (18)
Inland	58 % (18)	35 % (17)	

Table VIII. Frequency of occurrence of the bands in hemoglobin electropherogram of three forms of P. pungitius.

	N	I	II	III	IV	V	VI	VII	VIII
				Band	Number				
Coastal	20	5	19	20	20	20	20	7	6
Intermediate	8	0	7	8	8	8	5	0	0
Inland	20	0	18	20	20	18	15	0	0

Fig.　27.　　Pattern of Lactate Dehydrogenase in body muscle of
coastal, inland and intermediate forms of Pungitius
pungitius. C: coastal,　F:.inland,　I: intermediate.

Fig.　28.　　Electropherogram of hemoglobin of three forms of
Pungitius pungitius. Roman numerals indicate
the band number. C: coastal, F: inland,
I: intermediate.

61a

(-)
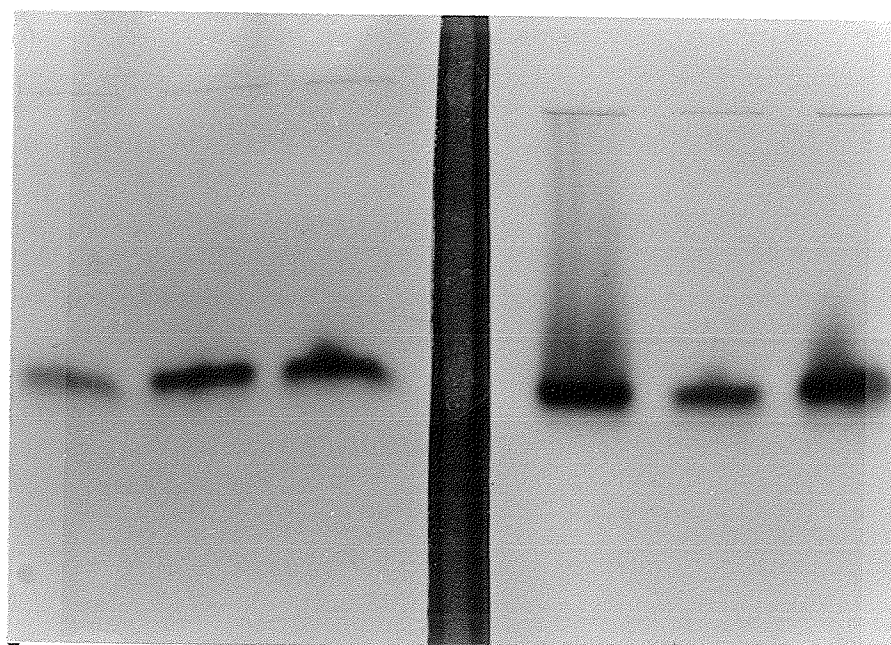
——origin

C   F   I   C   F   I   (+)

(-)

——origin
——I
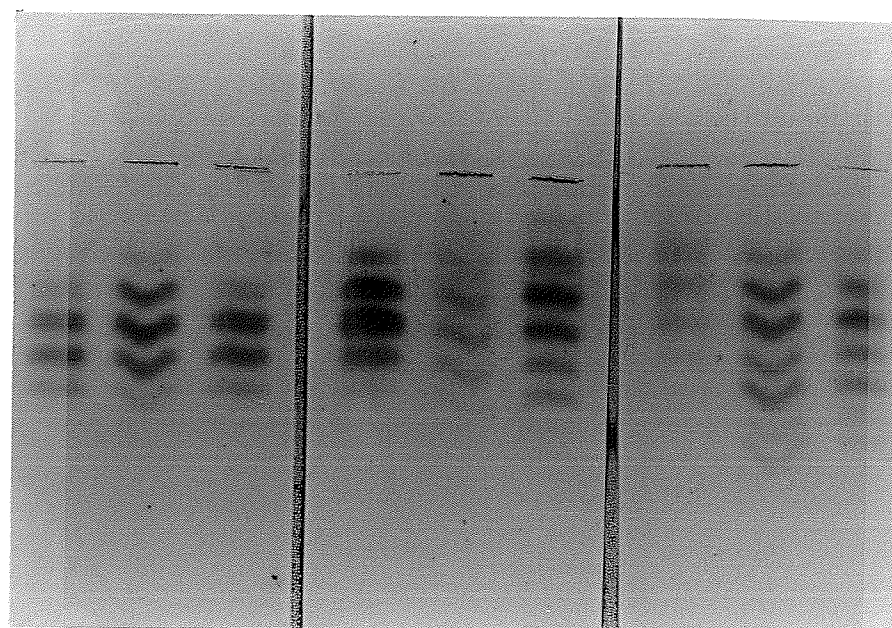——II
——III
——IV
——V
——VI
——VII
——VIII

F F I   I I C   C C C (+)

There are two possible explanations for the variation of the number of bands between and within the forms. The first is that it is due to the technique. Although the fish used were fully adult (4 to 5 cm. standard length), the volume of the blood obtained may not have been enough in some samples. The inland forms were generally smaller than the coastal form, and thus the smaller volume of the blood might have caused the fewer bands (or certain faintly stained bands could not be detected). However, even among the strongly stained specimens and those having enough volume of blood, the coastal form had rather more bands than had the inland form.

The second possibility is that of genetic polymorphism, as seen in the myogen of muscle between the two forms of Gasterosteus aculeatus (HAGEN 1967) or in the hemoglobin of whitefish, Coregonus clupeaformis (LINDSEY et al, 1970). But there was no clear-cut difference in the uniform presence or absence of a band between the coastal and inland form, such as had been found in Gasterosteus.

Hence, there is evidently no detectable difference in structure of LDH between the forms; there may possibly be a difference in proportions of different hemoglobin polymorphisms, but the evidence is inconclusive.

EXPERIMENTAL OBSERVATIONS ON ISOLATING MECHANISMS

Mating behaviour

The role of mating behaviour as an isolating mechanism
between closely related species (or forms) has been demonstrated
for many fish species. The complex reproductive behaviour of stickle-
backs has been studied by many authors, especially MORRIS (1958) who
described in detail the reproductive behaviour of Pungitius pungitius
in freshwater in Britain. McKENZIE and KEENLEYSIDE (1970) described
the reproductive behaviour of the inland form of Pungitius pungitius
in South Bay in Lake Huron in Canada.

The observations of mating behaviour in this study were
made in 10-gal. aquaria, in which sand and some aquatic plants were
put. As shown in Fig. 14, the size of sexual maturity was different
between the forms. The intermediate and inland form had smaller body
size, and the intermediate and inland form males generally made smaller
nests than did the coastal form. The coastal female had the largest
body size amongst the fish.

The mature male in all forms had a black nuptial belly and
milky white pelvic spines. But the coastal male had a jet black,
while on the contrary the inland and intermediate male had rather
grayish black or weak black as nuptial colour (even though the weak
black turned to deep black after they were killed). In females, the
coastal form had a very silvery shiny abdomen and dark green head and
back, and the fully mature coastal females had weakly developed milky

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white on the pelvic spines. The inland and intermediate females had brown or dark brown head and back, and silvery abdomen, which was not as shiny as the coastal female; the inland and intermediate female did not have the white pelvic spines.

As described in detail on European Pungitius by MORRIS (1958), the sequence of the mating behaviour was as follows:

male	female
approach to female	turning towards the male tail-down posture
dancing off towards the nest	follow the male to the nest
lead the female to the nest entrance	follow to the entrance
ritual fanning	enter the nest
shivering on her tail	lay eggs, leave the nest
enter the nest, fertilize the eggs.	

McKENZIE and KEENLEYSIDE (1970) reported a difference in the leading phase of male courtship between the European Pungitius and the freshwater Pungitius at South Bay. They write "The European males perform dance jumps while leading the female to the nest; South Bay males did not. A South Bay male courted by swimming straight at a ripe female, stopping near her, briefly twisting his body into a slight S-bend, erecting both ventral spines, then turning and swimming straight back to the nest. ..... The male usually responded to such a hesitant female by quickly approaching her and dancing, in which he tilted head-down and jumped around the female in a series of short jerky

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movements, turning slightly laterally between jumps. After a bout of dancing he swam directly to the nest again."

In agreement with McKENZIE and KEENLEYSIDE, in the present observations the inland form did not frquently show a "zigzag dance". The inland male approached a female and tried to lead her to the nest. Sometimes he showed a weak "zigzag dance" around the female, but the dancing was not distinct. On the contrary, the coastal male showed a distinct "zigzag dance" towards a female, around her, and away from the female towards the nest with head-down posture, and showed the same sexual behaviour as MORRIS described except that the "zigzag dance" was not a real zigzag, but rather the male made a jerky circle around or in front of the female. To such a distinct dance of the coastal male, both the coastal and inland females responded very easily and were led to the entrance of the nest, though in most cases this courting was not followed by the sequence of entering and laying eggs. On the other hand, both the coastal and the inland females hardly responded to the inland male; especially, very few coastal females responded to an inland male. The male of the intermediate form showed almost the same courting behaviour as the inland male.

In aquaria in the laboratory, four coastal females layed eggs in the nests of two intermediate males and four inland females out of eight layed eggs in a nest of a coastal male, but no coastal female layed eggs in an inland male nest.

Two ponds at Churchill were established, one with coastal males and inland females, the other with inland males and coastal females. Both crosses produced offspring in Churchill.

As seen in the successful crossings in the hybrid ponds, the barrier in the mating behaviour between the forms was not rigid, but females of both forms seemed less responsive to the intermediate or inland males than to the coastal male. The reason is not clear, but the body size, the nuptial colour, and the courting behaviour seem all to be more pronounced in the coastal male.

Hybrid fertility and viability

As the postmating isolating mechanisms, MAYR (1963) lists (a) gamete mortality, (b) zygote mortality, (c) hybrid inviability and (d) hybrid sterility.

Crosses between the forms showed no evidence of gamete mortality. The mortality of zygotes before hatching was variable, from 4 to 32% among the crosses of the coastal male and the coastal female. The single cross between an inland female and an inland male showed 32% mortality. The mortality of the eggs between a coastal female and an inland male, and an inland female and a coastal male, was 7.4% and 7.5% respectively. Thus there seemed to be no differences in zygote mortality between the intra-form and inter-form crosses.

With respect to hybrid inviability, both reciprocal hybrids produced in the hybrid ponds in Churchill were collected in autumn and brought back to the laboratory and kept in freshwater tanks. The hybrid CF (the hybrid of the coastal female and the inland male) was very small at capture, 1 to 2 cm. in total length, and had a quite large mortality, probably because of a shortage of proper foods for small fish. But after they attained 2.5 cm. in length, the survivors had low mortality and became mature the next spring. The hybrid FC

fish (the hybrid of the inland female and the coastal male) were more than 2.5 cm. long at capture, had a small mortality in the tank, and also matured in the next spring.

With respect to hybrid fertility, the following phases of the reproductive behaviour of the fish may be separated:

(i)   whether hybrid males make a nest?

(ii)   whether hybrid males show black nuptial colour, and which type of colour they show?

(iii)   whether hybrid males show normal mating behaviour?

(iv)   whether hybrid females respond to males and deposit eggs?

(v)   whether hybrid females produce fertile eggs?

(vi)   whether hybrid males successfully fertilize eggs?

(vii)   whether the eggs of hybrid female fertilized by the hybrid male hatch?

(viii) whether F_2 fry survive to adult?

Most of these questions can be answered from the present experiments. The F_1 male made a normal nest and showed black nuptial colour. The black nuptial colour varied individually and seemed no different between CF and FC hybrids. The hybrid males also showed normal mating behaviour and frequently showed a "zigzag dance". The hybrid females deposited eggs in the nests. Eight F_1 females deposited eggs in 8 nests of F_1 males, and four nests had hatched fry in aquaria. Four hybrid crosses were produced experimentally, and hatching rates were 0, 23, 67, and 70%. All hatched F_2 fry died within four weeks, but the hatched fry of the parental forms also usually died in early

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stages, probably because of lack of proper food for these small fry.
Thus it is not clear whether hybrid break-down may have existed at
this point.

The above questions from (i) to (vi) were positive and
F_1 hybrids were fully viable. Both F_1 males and females were fertile,
but one could not say whether they are "fully fertile" or not, because
the hatching rate of the F_2 was very variable.

DISCUSSION

Morphology and physiology

Differences in morphological characters are small between

the two forms of *Pungitius pungitius* in North America. Amongst

meristic characters the mean numbers of dorsal spines, lateral

plates, caudal scutes and gill rakers, and amongst morphometric

characters the body thickness (expressed by BD/SL ratio) are all

useful to distinguish between populations of the coastal and inland

forms. It is rather difficult to identify the forms individually

by the morphological characters, but the foregoing characters can

be combined into a score which allows identification of roughly

45% of all individual wild fish as belonging to one or other form.

As described in many other fish species (HUBBS, 1955;

HAGEN, 1967), the hybrids showed intermediacy in morphology. The

distinction between the hybrids and the coastal and inland forms is

impossible individually and also was very difficult even as populations,

although the means of some characters are significantly different.

Therefore in this study it was not possible to positively identify as

hybrids any fish other than those which had been artificially created.

The salinity tolerances of the forms of *Pungitius* are

distinctly different. None of the adult inland form survived in

25 o/oo but all of the adult coastal form survived in 25 o/oo.

Thus the salinity tolerance can be used to distinguish the two forms

individually. Physiological characters, although certainly less

convenient, are in this case more precise than morphological characters

as taxonomic indicators.

The salinity tolerances of both reciprocal hybrids are closer to the coastal form than to the inland form. HEUTS (1947) demonstrated that the salinity tolerance of hybrid eggs produced by crossing the two forms of Gasterosteus aculeatus was not intermediate between the parental forms, but showed a maternal influence. On the other hand MINAMORI (1957) showed that the temperature tolerance of hybrid eggs from three forms of the loach, Cobitis taenea striata, was intermediate between the parental forms. Both reciprocal hybrids in this study showed the characteristic of the coastal form in salinity tolerance, no matter which form produced eggs. Therefore this physiological character in Pungitius seems to be genetically controlled and the coastal form character is dominant.

HEUTS (1943) showed that two adult specimens of Pungitius pungitius collected from freshwater in Belgium tolerated approximately 33 o/oo salinity, but fry could not tolerate more than 22 o/oo. RAWSON and MOORE (1944) reported that Pungitius survived up to 20 o/oo in saline lakes in Saskatchewan and North Dakota, while WHITAKER (1968) reported that the LD 50 of Pungitius of Saskatchewan was 29 o/oo T D S (Total Dissolved Solids). The data of the inland form in the present study agree more with RAWSON and MOORE than with WHITAKER, whose results were rather lower than those in the present study (29 o/oo T D S equals to 14 o/oo salinity in Little Manitou Lake in Saskatchewan). However, temperatures, acclimation and test conditions (18-20 C, 12L12D, acclimated to 400 ppm T D S) were different.

HEUTS (1943) also demonstrated that the chloride of the
blood (salinity of blood) in fry of _Pungitius_ washomeosmotic at
5 o/oo when exposed to 10 o/oo salinity in the external medium.
But at greater than 10 o/oo in the external salinity, the salinity
of the blood increased as did that of the external salinity, that
is, the fry became poikilosmotic. In the salinity preference tests
of this study, both the coastal and the inland forms prefered 5 o/oo
salt water solution rather than freshwater in autumn. If the salinity
of blood of both the forms is the same as that in the Belgian
Pungitius, that is 5 o/oo, the preference for 5 o/oo is not surprising.

Origin of the intermediate form

Morphological analysis and the salinity tolerance tests lead
to the conclusion that the intermediate form in creeks tributary of
the lower Churchill River originated from the inland form. The
intermediate form is morphologically closer to the inland form in
5 out of 7 numerical characters, and in BD/SL ratio. Also the salinity
tolerance of the intermediate form is very close to the inland form.
Moreover, both reciprocal hybrids between the coastal and inland forms
were much closer to the coastal form in salinity tolerance, so the
intermediate form is apparently not the hybrid between the coastal
and inland forms. Instead, the intermediate form seems to be a
locally established population from the inland form, which has
become modified slightly in morphological and physiological characters
by the local environment conditions. The dorsal fin ray, anal fin
ray and vertebral numbers are higher than in the two other forms or
the hybrids. HEUTS (1947) demonstrated that the numbers of dorsal
and anal fin rays were changeable by differences of temperature and

salinity in the course of rearing Gasterosteus aculeatus. LINDSEY
(1962) found no correlation between dorsal or anal fin ray counts and
rearing temperature in English Pungitius, although there was a
correlation between the dorsal or anal fin basals and rearing temperature.
LINDSEY also demonstrated a change in the vertebral number by the
rearing temperature. There is no evidence that the creeks where the
Churchill intermediate form lived had higher temperature than other
creeks and lakes, but salinity in the creeks where intermediates bred
was much higher than in the other creeks.

The LD 50 of salinity tolerance was 22.5 o/oo for the intermediate
form and 19.6 o/oo for the inland form. The LD 50 of the intermediate
was much closer to the inland form than to the coastal form and to
both hybrids, but slightly higher than the inland form. The linear
regression by logistic transformation of salinity tolerance showed
that the inland form and intermediate forms are significantly different.
Two possible reasons could be considered. The first is that gene flow
from the coastal form to the inland form has produced introgression.
The second is modification by the environmental conditions. The chance of
gene flow seems to be small. As seen in Fig. 23, the intermediate forms
were not F_1 hybrids (from morphological and physiological evidence),
although the possibility exists that a few F_1 hybrids mingled among the
intermediate form and that some gene flow might occur by back crossing
between the intermediate form and F_1 hybrids.

The second alternative seems to be more probable. The salinity
of the lakes where the inland form was collected was less than 0.05 o/oo,
but the salinity of the creeks with the intermediate form was 1.5 to
6 o/oo, usually 2 to 3 o/oo. This difference in salinity where the

fish have been living might well influence to a slight degree of their salinity tolerance (LD 50).

Isolating mechanisms and hybridization

In the creeks of the lower reaches of the Churchill River the coastal form and the intermediate form live side by side, but spatial segregation due to their physiological characters was well established. The coastal form was caught upstream as far as Goose Creek and its tributary Creek A-1, while in the early summer the intermediate form was caught in Creek A-2 and farther upstream. The fish caught in Creek A-2 at the end of the summer had bimodal distributions in the numbers of lateral plates and caudal scutes, with modes corresponding to those of the coastal and the intermediate forms. As mentioned, the hybrids have almost the same morphological characters as the coastal form, so some Creek A-2 fish could have been hybrids.

Both the coastal and the intermediate forms spawned in June and July, so that temporal isolation of breeding times was not evident.

The ethological isolation is partially effective. The coastal female seemed to be restricted in her response to the intermediate male, but the intermediate female responded readily to the coastal male. Although unbalance of the sex ratio can induce the mating with other species (HUBBS 1955), there was no evidence of unbalanced sex ratio in either form in this study. The chance that eggs and sperms of different forms might meet to make a hybrid accidently is very unlikely, because of their complex breeding processes and nest building.

In summary, the isolating mechanisms in the premating phase are small except for spatial segregation.

The postmating isolation mechanisms were not developed. Neither gamete mortality, zygote mortality, hybrid inviability, nor hybrid sterility were evident in the F_1 hybrids. As a whole, no effective isolating mechanism between the coastal and the intermediate form was evident. However, the presence side by side of the intermediate form and the coastal form which differ morphologically and physiologically suggests that gene flow must somehow be restricted to prevent mass hybridization.

Spatial segregation between the coastal and the intermediate forms is almost distinct, due to their physiological characters. Spread of intermediate form farther downstream is not possible, because the short coastal creeks freeze in winter and the intermediate form cannot enter the sea. But the factor preventing the coastal form from moving far inland (here and elsewhere in North America) is not known .

Evolutionary significance of difference

The physiological characters of the forms are well adapted to their habitat. The coastal form, which migrates between sea and freshwater had the ability to tolerate highly saline water, while on the contrary the inland form, which stays in freshwater throughout its life, cannot tolerate high salinity, and, moreover, the coastal form displays a preference for more saline waters. The number of lateral plates is high in the fish which are tolerant to high salinity. HEUTS (1947) showed that Gasterosteus aculeatus hatched in saline water had more lateral plate numbers than the fish in freshwater. Lateral plate number seems to be associated with salinity tolerance not only

in Gasterosteus, but also in Pungitius; the present experiments showed
such a correlation not only between the two forms but also between
individual fish of the coastal form. The selective advantage of higher
plate numbers in coastal fish is conjectural, but the selective
advantage of greater salinity tolerance and preference seems clear.

McPHAIL (1963) suggested that the Bering form had a refugium
where contact with the sea was maintained, while the Mississippi form
had an inland refugium in the Mississippi Valley during the Wisconsin
glaciation. The Bering form, which is equivalent to the coastal form
in this study, developed (or maintained) their salinity tolerance
during the glaciation, and hence were able subsequently to disperse
through coastal waters to Hudson Bay and perhaps as far east as New
England. The Mississippi form, which had no contact with the sea
during the glaciation did not develop the ability to tolerate salinity,
and hence cannot occupy coastal streams which freeze during winter.

HAGEN (1967) demonstrated that Gasterosteus aculeatus trachurus
(the marine form) has a terete shape as an adaptation to its pelagic
habits, while leiurus (the freshwater form) has a deeper shape as an
adaptation to a more benthic life. In this study, on the contrary,
the coastal form of Pungitius had a deeper and heavier body shape, and
the inland and the intermediate forms had a slim body shape. The slim
body shape of the inland form seems to be adaptive to a pelagic life
in lakes. The number of gill rakers is also higher in the inland form
than in the coastal and intermediate forms. The inland form utilized
more pelagic food than did the coastal or intermediate forms which fed
prominently on bottom organisms. Similarly in Gasterosteus HAGEN

demonstrated that <u>trachurus</u> with many and longer gill rakers feeds on planktonic or suspended organisms while <u>leiurus</u> with few and short gill rakers feeds on bottom dwelling organisms (although again the pelagic adaptation is in the marine form in <u>Gasterosteus</u> but in the inland form in <u>Pungitius</u>). Although coastal form of <u>Pungitius</u> spend many months in the sea each year, it has been shown that the greater part of their growth occurs during summer when they are in creeks and are feeding mostly on benthic organisms.

The inland form was more retiring in captivity than was the coastal form, possibly because the inland form is liable to predation by several species of piscivorous fish, none of which occurred in the creeks in Churchill occupied in summer by the coastal form.

Taxonomic status of Pungitius pungitius in North America

BERG (1949) recognized four species in <u>Pungitius</u> in Europe and in Asia. Recently MUNZING (1969) recognized two species, <u>P. pungitius</u> and <u>P. platygaster</u>, and four subspecies in <u>P. pungitius</u>. He claimed that North American <u>Pungitius</u> should all be included in the subspecies, <u>P. p. pungitius</u>. McPHAIL (1963) suggested not recognizing as subspecies the two forms of North American <u>Pungitius pungitius</u> through morphometric analysis, even though he noticed the constant morphological differences of two forms throughout North America.

MAYR (1963) defined the subspecies as "an aggregate of local populations of a species, inhabiting a geographic subdivision of the range of the species, and differing taxonomically from other populations of the species". HUBBS (1943) defined the subspecies as "any genetic form which shows reasonable geographical or ecological consistency, and which can usually be distinguished on its totality of characters".

The morphometric analysis of the forms of North American *Pungitius* revealed the differences as being very small, and were distinguishable by "the average characters that need statistical treatment" (which is the definition of a race, according to HUBBS). However, the differences in physiological characters are distinct and genetically based, and 92% of the individual fish can be distinguished thereby. Thus, the two forms meet the subspecies requirement. They do not, however, warrant recognition as separate species, since hybridization and other experiments suggest that isolating mechanisms are only weakly developed, and the two forms have never been taken sympatrically. If a study of other areas of contact between the two forms of *Pungitius pungitius* in North America confirms the findings in the Churchill area, then the two should be recognized formally as distinct subspecies.

SUMMARY

1.   Three forms of _Pungitius pungitius_, coastal, intermediate, and
      inland forms, were collected in Manitoba.

2.   The coastal and the intermediate forms are distributed side
      by side in the Churchill area.

3.   The morphological divergence was small and none of the
      morphological characters can be used for clear-cut separation.
      The coastal form had higher average numbers of dorsal
      spines, lateral plates, and caudal scutes, and fewer number
      of gill rakers than the others, and heavily-made body.

4.   Distinct differences in salinity tolerance were found
      between the forms in both adult and egg stages. The
      coastal form tolerated higher salinity than the others.
      The coastal form showed a preference to higher salinity
      than the inland form.

5.   The intermediate form probably originated from the inland
      form, but has been modified by environmental conditions.

6.   Both reciprocal hybrids between the coastal and inland forms
      were closer to the coastal parent in salinity tolerance.
      In morphology, the hybrids were intermediate between the
      parental forms except in lateral plate number, which was
      closer to the coastal parent.

7. The F_1 hybrids were viable and fertile, and no postmating isolating mechanisms were evident.

8. Spatial segregation due to the physiological characters was the only evident mechanism preventing mass hybridization.

9. Salinity tolerance was adaptive to the habitat of each form, and seems to have been produced by long term selection.

10. The coastal form and the inland form fulfill the requirement of subspecific recognition, and they may deserve to be recognized as subspecies if study in other areas confirm findings in the Churchill area.

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