1.0

11

125

18607

NAME OF AUTHOR. Eugene Samuel Fritz.....................

TITLE OF THESIS. HYBRIDIZATION AND ISOLATING MECHANISMS
BETWEEN SYMPATRIC POPULATIONS OF
FUNDULUS HETEROCLITUS AND FUNDULUS DIAPHANUS
(PISCES : CYPRINODONTIDAE)

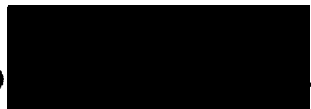UNIVERSITY. Dalhousie University.....................

DEGREE FOR WHICH THESIS WAS PRESENTED. Ph.D.............

YEAR THIS DEGREE GRANTED...1973.....................

(Signed)

PERMANENT ADDRESS:

106 Whitmore Dr.

Elkton, Maryland

U. S. A.

DATED....17 August.....19 73

NL-91 (10-68)

HYBRIDIZATION AND ISOLATING MECHANISMS

BETWEEN SYMPATRIC POPULATIONS OF

FUNDULUS HETEROCLITUS AND FUNDULUS DIAPHANUS

(PISCES: CYPRINODONTIDAE)

by

EUGENE SAMUEL FRITZ

Submitted in partial fulfilment

of the requirements for the

degree of Ph.D. at

Dalhousie University

Approved by   August 1973

DALHOUSIE UNIVERSITY

Date 17 August 1973

Author Eugene S. Fritz

Title HYBRIDIZATION AND ISOLATING MECHANISMS BETWEEN

SYMPATRIC POPULATIONS OF FUNDULUS HETEROCLITUS AND

FUNDULUS DIAPHANUS (PISCES: CYPRINODONTIDAE)

Department or School Biology

Degree Ph. D.   Convocation Fall   Year 1973

ABSTRACT

Field and laboratory study was made of hybridization and isolating mechanisms, with particular emphasis on premating mechanisms, between rarely hybridizing killifish, *Fundulus heteroclitus* mummichog, and *F. diaphanus* banded killifish, in Porters Lake, Nova Scotia, from 1971 to 1973. Phenotypic analyses of killifish in Porters Lake showed that there were two easily distinguishable species, mummichog and banded killifish, and an apparent hybrid which was phenotypically intermediate. Hybrid killifish having similar phenotypic intermediacy were also described from the St. Mary's River, Guysborough County, N.S. Hybrid index analysis and consistent electrophoretic patterns of muscle MDH and LDH suggest that hybrids are F_1, there being little or no backcrossing or production of F_2. Reciprocal interspecific fertility and viability was confirmed through laboratory crosses which displayed virtually identical hybrid indices and identical MDH electrophoretic patterns.

The major premating isolating mechanism appears to be salinity preference in which mummichog prefer highly brackish water and banded killifish prefer fresh water. Comparative age compositions, growth rates, length-weight relationships, and fecundity between sympatric and allopatric populations of both species indicated that mummichog are not affected by residence in slightly brackish water and banded killifish benefit from slightly brackish water. No seasonal or habitat isolation operates to isolate sympatric populations of mummichog and banded killifish reproductively. Both species mature and spawn during the same period

in the same areas and neither species has a substrate preference. Although interspecific spawning was observed in the laboratory, it could not be determined whether spawning was the result of crowding or underdeveloped ethological isolation.

Hybridization appears to result from an initial decline of salinity preferences so that both species come to inhabit an intergraded environment of fluctuating brackish water in which one species or the other but usually banded killifish predominates numerically. In Porters Lake suitable spawning sites also appear to be restricted. Thus, three criteria of the four which have been suggested in the literature as most instrumental in fish hybridization are encountered in Porters Lake.

The occurrence of these hybrids in the Atlantic provinces of Canada appears to be the result of recent localized sympatry along with a nominal cyprinodontid fauna which has limited the reinforcement of premating mechanisms. Apparent effective postmating mechanisms, however, suggest that evolution of isolating mechanisms between these species is still at an early stage, according to the hypothesis concerning evolution of isolating mechanisms proposed by Ernst Mayr.

ACKNOWLEDGEMENT

TABLE OF CONTENTS

Table of Contents continued

LIST OF FIGURES

List of Figures continued

List of Figures continued

(xi)

List of Figures continued

LIST OF TABLES

INTRODUCTION

Among the most important attributes of a species are the mechanisms that reproductively isolate the species from other closely related species. The importance of these isolating mechanisms becomes apparent when populations of two previously closely related allopatric species become sympatric. Absence of adequate isolating mechanisms can result in the introgressive fusion of the two forms, which, in most instances, is less fit for the existing environment and becomes a genetic loss to both species (Dobzhansky, 1951; Mayr, 1963). Acqusition of isolating mechanisms then can be equated with speciation itself (Mayr, 1963).

The term isolating mechanisms here defined, "biological properties of individuals that prevent the interbreeding of populations that are actually or potentially sympatric," was coined by Dobzhansky (1937). Many classifications of these mechanisms have been proposed. However, that proposed by Mechan (1961) and subsequently modified by Mayr (1963) has been adopted in this study.

"1. Mechanisms that prevent interspecific crosses
   (premating mechanisms)
   (a) Potential mates do not meet (seasonal
       and habitat isolation)
   (b) Potential mates do meet but do not mate
       (ethological isolation)
   (c) Copulation attempted but no transfer of sperm
       (mechanical isolation)

2. Mechanisms that reduce full success of interspecific
   crosses (postmating mechanisms)
   (a) Sperm transfer takes place but egg is not
       fertilized (gametic mortality)
   (b) Egg is fertilized but zygote dies
       (zygote mortality)

(c) Zygote produces F_1 hybrid of reduced viability
(hybrid mortality)
(d) F_1-hybrid zygote is fully viable but partially
or completely sterile or produces deficient F_2
(hybrid sterility)"

Detailed studies of isolating mechanisms between species of invertebrates, in particular insects, and of terrestrial vertebrates reviewed by Dobzhansky (1951), Mayr (1963), Sibley (1961), and Littlejohn (1969) have shown that, in general, premating mechanisms are evolutionarily more important. Littlejohn (1969) noted that premating mechanisms are gametically more economical than are postmating mechanisms and as such are more efficiently developed by natural selection.

Dobzhansky (1951) and Hubbs (1961) considered premating mechanisms to be most important among fish, especially seasonal and habitat isolation and ethological isolation. Evidence for the importance of premating mechanisms among fish was advanced by Hubbs and Strawn (1957), Hubbs and Drewry (1959), and Hubbs (1970), who after experimentation with interordinal, interfamilial, and intergeneric crosses showed that teleost hybrids are relatively easily produced in the laboratory, and in many instances easily reared, thus indicating a reduced role played by gametic, zygote and hybrid mortality among hybrid, fishes.

The consequence of a breakdown of any isolating mechanism that permits the crossing of genetically different individuals of taxonomically distinct populations is hybridization (Mayr, 1963). The first generation product (F_1) of animal hybridization is composed of

individuals that are usually phenotypically intermediate between the parental species (Mayr, 1963). This phenomenon has been demonstrated among fishes by Hubbs *et al.* (1943) and reviewed by Hubbs (1955). In his review and partial quantification of many of the known animal hybrids Mayr (1963) noted that hybridization is quite rare in most groups of animals, especially among the terrestrial vertebrates. Hubbs (1955, 1961), however, noted that natural hybridization in fishes is relatively common among northern freshwater species, a phenomenon which he attributed to the highly unstable environments in this relatively recently glaciated geographic area.

The investigations of natural hybridization in fish, partially reviewed by Hubbs (1955), together with investigations of laboratory crosses initiated by Moenkhaus (1910) and Newman (1908), have been so numerous that Schwartz (1972) was able to catalogue 1945 instances from all available sources dealing with fish hybrids, natural and induced. The task of further review will become greater still when it is noted that by the year 1975 more than 2200 reports are predicted which will concern fish hybrids (Schwartz, 1972).

Mayr (1961) stated "the study of hybridization ... is quite evidently an important area of the study of isolation mechanisms." In this statement it is clear that the study of hybridization itself is not the study of isolating mechanisms, but only a part of the study. However, most ichthyological investigations in this field have been limited to identifications and descriptions of hybrids along with a postulated explanation of causative environmental conditions. Despite

the availability of material few attempts have been made to identify
and describe the isolating mechanisms for a given pair of species. The
most noteworthy of the attempts have been those conducted by Nelson
(1968) on *Catastomus commersoni* and *C. macrocheilus* and by Hagen (1967)
who studied isolating mechanisms between two morphs of *Gasterosteus
aculeatus* which he believes are two species. Both studies were con-
ducted in areas where the hybridizing species or suspected species were
naturally sympatric.

My study was undertaken to investigate, by means of field and
experimental studies, hybridization and isolating mechanisms, in parti-
cular premating mechanisms, in *Fundulus heteroclitus* (Linneaus) 1776,
the mummichog, and *F. diaphanus* (Lesueur) 1817, the banded killifish,
two apparently rarely hybridizing species. Hybridization within the
genus is also considered rare (Hubbs *et al.*, 1943; Hubbs, 1955). How-
ever, other instances are known to have occurred between *F. kansae* and
F. sciadicus (Hubbs *et al.*, 1943) and between *F. notatus* and *F. olivaceus*
(Setzer, 1970).

Hybridization between mummichog and banded killifish was first
reported by Weed (1921), but this report was refuted by Hubbs *et al.*
(1943). However, Hubbs *et al.* (1943) described one female hybrid of
mummichog and banded killifish, collected in the Lake of Shining Waters,
Prince Edward Island. Griffith (1968 and 1972) reported hybrids of
these species for Mill River, Connecticut.

Because of the inferred scarcity of hybrids, hybridization experiments along with investigation of isolating mechanisms initially were to be conducted on fish gathered from allopatric populations. However, a population of suspected hybrids, a detailed description of which is included in this study, was discovered in Porters Lake, Halifax County, Nova Scotia, a lake in which mummichog and banded killifish are sympatric. Therefore the major portion of the study has been conducted with killifish from Porters Lake.

GENERAL MATERIALS AND METHODS

Most individuals of both species, mummichog and banded killifish, used in this study were collected in Porters Lake, Halifax County, N.S. (44°45'N, 63°18'W). Additional specimens were collected in Petpeswick Inlet, Halifax County (44°43'N, 63°10'W), Kejimkujik Lake, Annapolis and Queens County (44°23'N, 65°15'W), and St. Mary's River, Guysborough County (45°05'N, 61°46'W). Specimens in the Nova Scotia Museum were also used and are listed in Table 1. Mummichog and banded killifish were collected fortnightly in Porters Lake from May through November, 1971, and from May to September, 1972, except during the spawning season at which time collections were made weekly. Mummichog were collected in Petpeswick Inlet fortnightly from March to October, 1971, while the banded killifish of Kejimkujik Lake were sampled monthly from June to September, 1972.

All fish were captured by either a 1.8x3 metre or 1.8x6 metre nylon minnow seine. A minimum sample size was set at 30 fish irrespective of the species composition of the catches. Individuals comprising a sample were drawn without selective effort to favour particular individuals. Fish samples were fixed in the field in 10% formalin solution. After being taken to the laboratory, samples were washed in running fresh water within 24 hours of fixation, and preserved and stored in 40% isopropyl alcohol. Preserved fishes were used for structural comparisons, comparative age and growth analyses, comparative length-weight relationships, maturation and fecundity analyses, and food analyses.

TABLE 1

List of specimens of banded killifish, *Fundulus diaphanus*, and mummichog, *F. heteroclitus*, examined in the Nova Scotia Museum fish collection along with the locality in which each sample was taken. Those specimens above the broken line are from the Livingstone collection in the National Museum of Canada. S indicates that the sample is identified by date collected.

Species	Locality	County	Identification Number	Number of Specimens
F. *diaphanus*	Le Grand L.	Inverness	49-C-27-A	30
"	Goshen L.	Guysborough	S 3/8/48	15
"	Indian Hbr. R.	Guysborough	L#2	10
"	Loch Lomond	Cape Breton	S 26/9/48	3
"	Warren L.	Victoria	49-C-31-A	15
"	Wigmore L.	Cumberland	50-C-22-B	6
"	Big L.	Cumberland	50-C-23-B	15
"	Grand L.	Hants	49-C-39-K	15
"	Windsor Rd.	Hants	50-C-9-K	8
"	Minamkeak Br.	Lunenburg	50-C-2-B	15
"	Pretty Mary R.	Annapolis	49-C-4-B	8
"	Pearl L.	Yarmouth	S 15/9/48	15
"	Cameron L.	Hants	50-C-10-D	15
"	East R.	Halifax	50-C-11-B	15
"	Minard L.	Queens	49-C-5-A	5

Table 1 continued

F. diaphanus	Oxford	Cumberland	55-FRC-242	9
"	Maitland Br.	Annapolis	55-FRC-126	22
"	Medway R.	Queens	55-FRC-139	24
"	Fanoy L.	Yarmouth	55-FRC-169	11
"	Salmon L.	Yarmouth	55-FRC-83	12
"	Eden L.	Pictou	55-FRC-396	30
"	Lake George	Shelburne	55-FRC-151	10
"	Clyde R.	Shelburne	55-FRC-122	18
"	Carltown L.	Colchester	55-FRC-378	30
"	Digby	Digby	55-FRC-91	30
F. heteroclitus	Sandy Cove	Digby	55-FRC-48	31
"	Walton R.	Hants	55-FRC-205	16
"	Pubnic	Yarmouth	55-FRC-159	14
"	Wallace Bay	Cumberland	NMC59-291	4
"	Goose R.	Shelburne	55-FRC-160	30
"	Prospect Bay	Halifax	66-Z-5-3(18)	17

-8-

Mummichog and banded killifish used in laboratory experiments
were collected in Porters Lake in November 1971 and from June to August,
1972, and were transported from Porters Lake to the Dalhousie University
Biology Department aquarium facilities in 68-litre plastic containers.
There they were segregated by species and placed in either 0.9x0.6x0.6 metre
or 1.5x1.5x0.9 metre fiberglass aquaria serviced with compressed air,
filtered sea water, and dechlorinated fresh water. Unless being accli-
mated to specific salinities the fishes were kept in a constantly flow-
ing mixture of fresh water and sea water, the salinity of which fluc-
tuated between 5.8 and 16.2‰. Water temperature varied with season,
from 4 to 17 C. Laboratory experiments consisted of salinity preference,
rheotaxis, substrate preference, interspecific and intraspecific spawn-
ing behavior, interspecific crossing, and electrophoretic comparisons.

Water temperatures were measured with either a standard labora-
tory thermometer or a bucket thermometer. Salinities were measured
with a hydrometer registering densities of 1.000 to 1.070 specific
gravity. Specific gravities were converted to salinity by using the
sea water temperature and density reduction tables of Zerbe and Taylor
(1953). These hydrometer salinity estimates were checked by means of
analyzing water samples in a Buchler-Cotlove chloridometer and shown
to be accurate to 0.5 ‰ salinity. Turbidity was measured with a Hach
colorimeter and expressed in Jackson turbidity units. Measurements of
pH and dissolved oxygen were made in the field on an Orion 401 pH meter
and a Yellow Springs Instrument oxygen meter. Winkler titrimetry was
used on 12 samples as a check on oxygen meter readings.

More detailed procedures employed in the various portions of the study are described in the appropriate portions of the text.

DISTRIBUTION AND NATURAL HISTORY OF

FUNDULUS HETEROCLITUS AND *FUNDULUS DIAPHANUS*

Distribution

Of the 22 species of *Fundulus* that occur east of the continental divide of North America, eight occur in sea and brackish waters along the coasts of the Atlantic Ocean and the Gulf of Mexico (Brown, 1957; Griffith, 1972). *Fundulus diaphanus*, although considered a typically freshwater species is facultatively euryhaline (Garside and Jordan, 1968) and occurs occasionally in brackish water (Hubbs and Lagler, 1948; Brown, 1957; Leim and Scott, 1966; McAllister, 1970). The distribution of these brackish or occasionally brackish water species is such that south of New Hampshire it is theoretically possible for each of the nine species to occur sympatrically with at least two of the other species (Fig. 1). North of New Hampshire the maximum number of species that could occur sympatrically is two, *F. heteroclitus* and *F. diaphanus*, and these apparently do so only occasionally. Although the ranges of *F. heteroclitus* and *F. diaphanus* (Fig. 1) indicate that they are potentially sympatric as far south of New Hampshire as South Carolina it is only from the Atlantic provinces that hybridization between mummichog and banded killifish has been established (Hubbs *et al.*, 1943).

The Canadian distribution of mummichog is the Gulf of St. Lawrence, Quebec, Newfoundland, Anticosti Island, Magdalen Islands, along the coast of Prince Edward Island, Nova Scotia, including Sable Island and New Brunswick (Scott and Crossman, 1964; Leim and Scott, 1966; Garside, 1969;

FIGURE 1

Distribution of brackish water killifishes east of the
North American continental divide along with the distri-
bution of banded killifish. Distributions are based
upon published reports cited in the text. Different
lines are used to designate a given species' distribution,
letters are used to designate each species.

D	*F. diaphanus*
H	*F. heteroclitus*
M	*F. majalis*
G&S	*F. grandis & similis*
C	*F. confluentus*
P	*F. pluvereus*
Se	*F. seminolis*
L	*F. luciae*
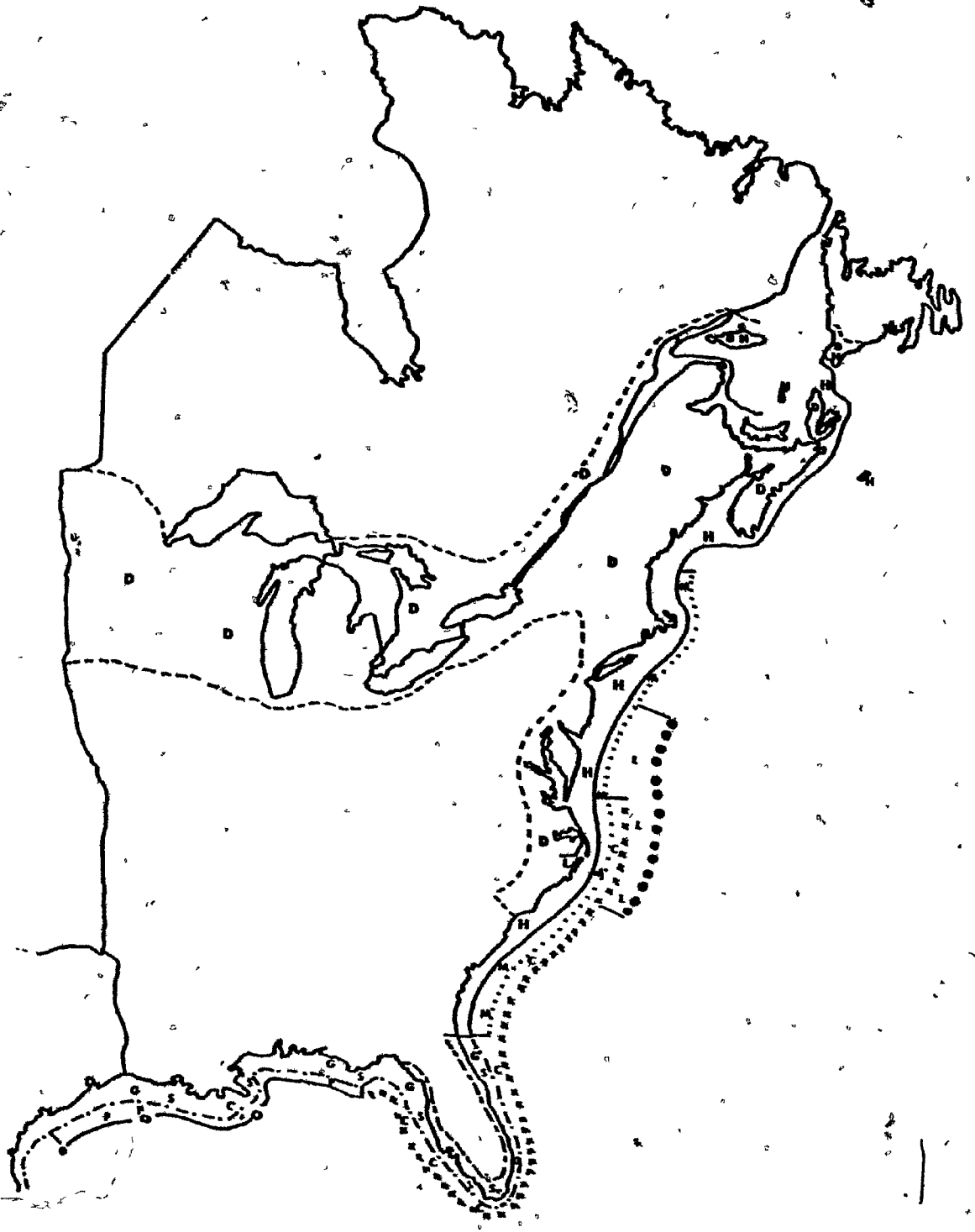
Garside *et al.*, 1972; McAllister, 1970). The Canadian distribution of banded killifish is from Newfoundland, the Magdalen Islands, and the Atlantic provinces, along the St. Lawrence River in southern Quebec, Lake Ontario, Lake Erie, Lake St. Clair, and Lake Huron north to the Spanish River (Scott, 1954; Scott and Crossman, 1964; Leim and Scott, 1966; Garside *et al.*, 1972).

Sympatric populations of mummichog and banded killifish have been reported by Garside *et al.* (1972) and Scott and Crossman (1964) from the Magdalen Islands and Newfoundland, respectively. However, little documented evidence can be found to indicate the occurrence of such populations in Nova Scotia. E.T. Garside (pers. comm.) has observed and collected sympatric mummichog and banded killifish in the Bras D'Or Lake and J. Gilhen (pers. comm.) has collected sympatric populations in the St. Mary's River, Guysborough County, N.S. The occurrence of these populations along with that described for Porters Lake indicates that sympatry may be more common than previously presumed. However, verification awaits further investigation.

Natural History

Fundulus as a genus has been a very popular source of experimental animals. However, little detailed information is known concerning the natural history of the species in the genus. Minckley and Klassen (1969) studied the life history of the plains killifish, *F. kansae*. Fritz (MS 1970) investigated the life history of the

California killifish, *F. kansas*. Fritz (MS 1970) investigated the life history of the California killifish, *F. parvipinnis*, while Foster (1967a) described and discussed the evolutionary significance of reproduction, behavior, and ecological differences of 12 species of *Fundulus*. Griffith (1972) has reviewed much of the physiological and systematic literature dealing with the genus.

Details of differing aspects of the biology of mummichog has been reviewed by Griffith (1972). However, with a few exceptions most of the studies described were conducted within the confines of the laboratory. The definitive life history of mummichog consists of a brief description by Chidester (1916) of a New Jersey populations in which it is noted that the fish inhabit salt marshes, coastal streams, and estuaries. Most mummichog movements are in response to the tide, moving up the gradient with the flowing tide and down the gradient into deeper water with the ebb. During the outward movement many fish either become trapped in or seek permanent pools, in which, during the spawning season, reproduction occurs. Young hatch in about 19 to 25 days depending on the water temperature. After the absorption of the yolk sac the young feed on minute plankton. Juveniles, however, feed on the same items as the adults. Stream-dwelling populations move into the marshes and into deep saline water during the autumn. Estuarine and salt-marsh populations also migrate into deeper water or deep holes during the autumn (Buttner and Brattstrom, 1960).

Mummichog feed throughout the water column, but the most commonly

occurring food items are benthic organisms. This has been confirmed
by Moore (1922) and Fritz (1972, in press). Until the present study
no attempt had been made to investigate such basic aspects of the
biology of mummichog as age, growth, maturation and fecundity.

With the exception of the information given in taxonomic studies
and systematic reviews (Griffith, 1972) very little has been reported
on the biology and natural history of the banded killifish. Richardson
(1939) and Foster (1967b) described spawning behavior and found it to
be quite similar to that of mummichog. Food habits were studied by
Forbes (1883), Forbes and Richardson (1908), Pears (1915), Smith (1947),
and Fritz (1972, in press). These studies indicate that, like mummi-
chog, banded killifish feed throughout the water column with the major
portion of the diet consisting of benthos. Age, growth, maturation,
and fecundity studies have, as in the case of mummichog, not been
reported prior to this study.

Comparative studies between mummichog and banded killifish
have to date consisted of Foster's (1967b) study of spawning behavior
and physiological studies conducted by Griffith (1972). Comparisons
by inspection and by Spearman rank correlation coefficients (Fritz,
1972, in press) of the diets of sympatric populations of mummichog and
banded killifish were made during this study. The results of these
comparisons (Table 2) show that both species consumed the same organ-
isms yet at significantly different frequencies (p < 0.01).

TABLE 2

Comparison of the diets of sympatric populations of banded
killifish and mummichog collected in Porters Lake from June
through August, 1971. Banded killifish ranged in size from
27 to 107 mm total length and mummichog ranged in size from
35 to 92 mm. (N_r = number of ranks, r_s = Spearman rank
correlation coefficient, %occ = percentage frequency.)

Food Items	F. diaphanus % occ.	rank	F. heteroclitus % occ.	rank
Nereis virens	4.8	16.5	0	1
Cladocera	3.4	12.5	7.7	20
Canthocampidae	2.0	7	4.6	13.5
Eurytemora affinis	15.2	26	1.5	5.5
Orthocyclops	11.8	24	13.1	27.5
Ostracoda	16.6	27	27.1	30
Gammarus fasciatus	11.1	22	11.6	24
Leptochela savignyi	43.7	31	3.8	11
Acarina	6.9	19	10.8	22
Entomobryidae	0.6	1.5	11.6	24
Odonata	3.4	12.5	6.6	17
Trichoptera	10.4	20	13.1	27.5
Veliidae	2.7	9.5	2.3	8
Corixidae	21.5	28	34.8	31
Chironomid Larva	26.3	29	18.6	29

F. diaphanus n-144 *F. heteroclitus* n=129; r_s=0.27073; N_r=31; t=1.5145;
p > 0.1; df = 29

Table 2 continued

Food Items	F. diaphanus		F. heteroclitus	
	% occ.	rank	% occ.	rank
Chronomid Pupa	2.7	9.5	1.5	5.5
Chironomid Adult	1.3	4	7.7	20
Ceratopogonid Larva	3.4	12.5	6.9	17
Ceratopogonid Pupa	4.1	15	3.8	11
Culicid Larva	13.1	25	11.6	24
Culicid Adult	2.0	7	3.8	11
Misc. Diptera	1.3	4	7.7	20
Unident. Diptera L.	5.5	18	0.7	2.5
Misc. Copeoptera	3.4	12.5	1.5	5.5
Misc. Hymenoptera	2.0	7	5.4	15
Insect Remains	4.8	16.5	6.9	17
Juv. Gastropoda	0.6	1.5	4.6	13.5
Hydrobia sp.	29.1	30	3.1	9
Pelecypoda	1.3	4	0.7	2.5
Fish Ova	11.1	22	12.4	26
Algae	11.1	22	1.5	5.5

F. diaphanus n=144; N_r=31; r_s=0.27073; t=1.5145; p > 0.1; df=29

F. heteroclitus n=129

HABITAT DESCRIPTION

Characteristics of Porters Lake

Porters Lake is located about 28 km northeast of Halifax, Nova Scotia. The lake is approximately 23 km long, 3 km wide at its widest, and 150 metres wide at its narrowest. The rocky shoreline is approximately 68 km long and the surface area of the lake is approximately 18.8 km^2. Like most lakes in Halifax County, Porters Lake appears to be relatively shallow, the greatest depth recorded during this study was 9.5 metres. However, the littoral profile is generally steep, such that within 2 metres horizontally from the shore the water depth increases from 0 to 1.5 metres.

The lake communicates directly to the sea via an opening at the south end (Fig. 2). During high tides sea water can be observed entering the lake through this opening. Interviews with local inhabitants suggested that the opening was formed some time between 1950 and 1955; however, no official records could be found to confirm either these dates or the manner in which the opening was formed.

Since no limnological information had been reported for Porters Lake, a short study was conducted to obtain some idea of the physical and chemical conditions of the lake. Twelve stations located throughout the lake were sampled over a two-day period from August 15 through August 16, 1972 (Fig. 2). Both surface and bottom waters were sampled

FIGURE 2

Locations of study areas and limnological stations on
Porters Lake, Halifax County, Nova Scotia. Study areas
are designated PO 1-6, stations are numbered from 1-12.

44°50'

44°45'

44°40'

7 HWY

N

PO1

PO2

PO3

PO4

PO5

ATLANTIC OCEAN

1000 m

63°20'
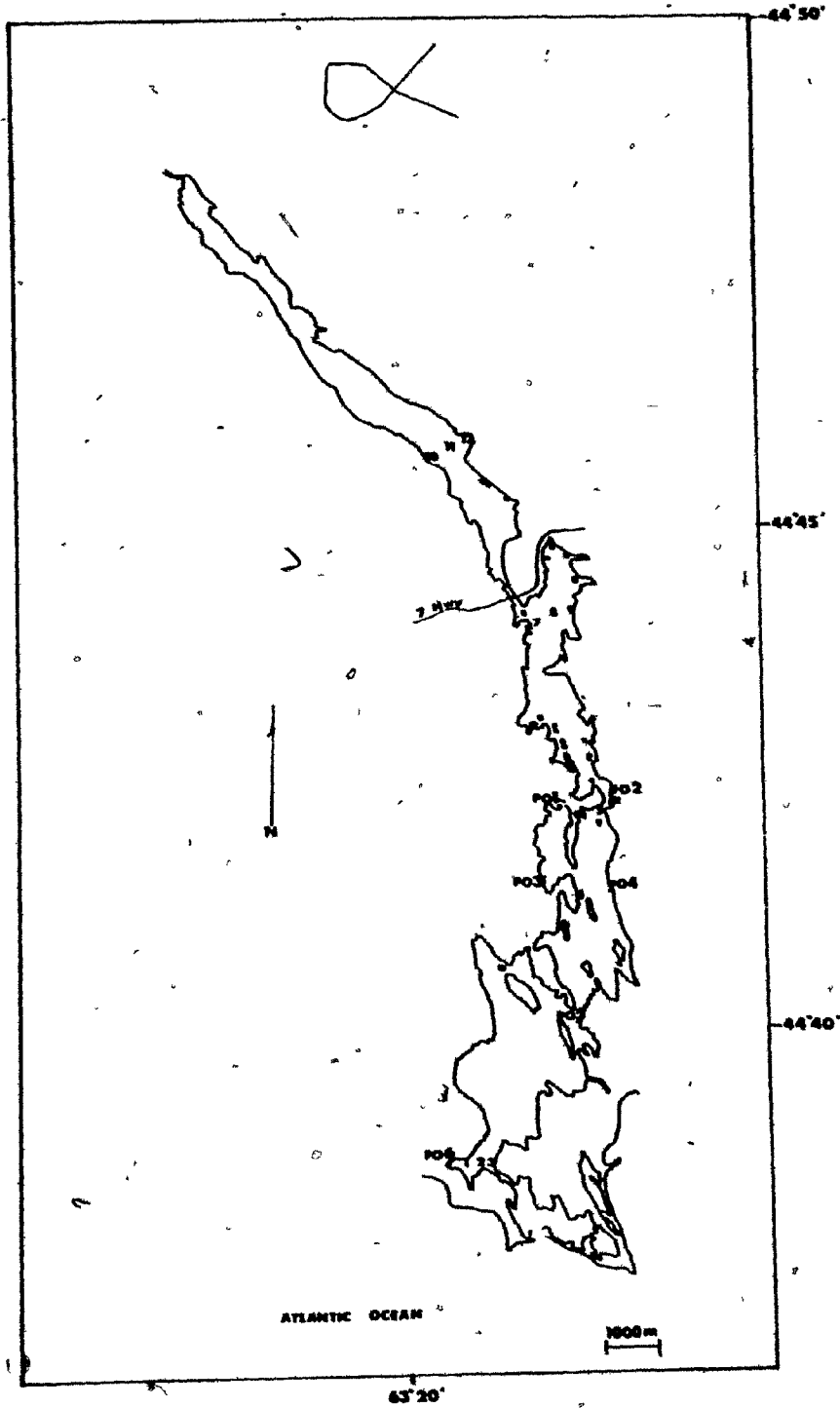
at all stations, while midwater samples were made at those stations at which the water depth exceeded 3 metres. The limnological characters measured were water temperature, salinity, pH, and dissolved oxygen (Table 3).

The results of the lake study (Table 3) indicate that Porters Lake is in fact two more or less distinct lakes. The upper lake north of Highway #7 (Fig. 2) appears to conform with the physical and chemical characteristics attributed to Nova Scotian oligotrophic lakes in igneous rock basins with relatively low total dissolved solids (Hayes and Anthony, 1958). The lower lake, south of Highway #7, is a brackish lake. The surface water appears to form an irregular horizontal salinity gradient towards the outlet to the sea. Midwater and bottom samples of water from stations 5, 7, and 8 show that the lake in these areas has both thermal stratification and a halocline. Station 8 is of particular interest since the samples of bottom water taken at this station were the most deficient in dissolved oxygen and released the distinct odor of hydrogen sulfide.

Study Areas

Because of the rocky and steep littoral zone the areas in which fish could be collected in Porters Lake were quite limited. Six areas, PO1 to PO6, were found which could be seined (Fig. 2). Of these areas three, PO1, PO2, and PO6, were accessible by road, while PO3, PO4, and PO5 were accessible only by boat. Features of the six study areas are shown in Table 4.

TABLE 3

Summary of the limnological survey made at Porters Lake August 15 and 16, 1972. All values are mean values for the two days. DO=dissolved oxygen. Position of each station can be located on Figure 1.

Station No.	1		2			3	
	surface	bottom	surface	middle	bottom	surface	bottom
Depth (m)	0.0	1.25	0.0	1.25	2.5	0.0	1.25
Temperature (C)	19.5	19.2	19.0	20.0	20.0	19.0	17.5
Salinity (%oo)	16.1	16.1	15.2	15.4	15.4	15.2	16.2
pH	7.1	7.1	7.2	7.1	6.9	7.2	7.2
DO (mg/l)	8.8	8.8	9.1	8.8	8.8	9.1	8.8

Station No.	4		5			6	
	surface	bottom	surface	middle	bottom	surface	bottom
Depth (m)	0.0	1.25	0.0	2.10	4.25	0.0	1.0
Temperature (C)	20.0	20.5	21.0	20.0	17.8	21.0	21.0
Salinity (%oo)	3.0	3.3	2.9	15.7	16.2	2.9	2.9
pH	7.2	7.2	7.6	7.5	7.2	7.4	7.4
DO (mg/l)	8.5	8.5	8.1	8.8	8.8	8.8	8.8

Table 3 continued

Station No.	7			8			9	
	surface	middle	bottom	surface	middle	bottom	surface	bottom
Depth (m)	0.0	2.25	4.5	0.0	4.5	9.5	0.0	1.0
Temperature (C)	21.0	19.0	17.0	21.0	17.0	11.5	20.0	20.0
Salinity (‰)	2.8	15.6	16.0	2.6	15.7	15.7	2.5	2.5
pH	7.1	7.5	7.3	7.1	7.5	7.4	7.3	7.3
DO (mg/1)	8.1	8.8	8.1	9.2	8.3	2.2	8.1	8.1

Station No.	10		11			12	
	surface	bottom	surface	middle	bottom	surface	bottom
Depth (m)	0.0	1.5	0.0	3.25	6.5	0.0	1.0
Temperature (C)	21.0	20.0	20.0	20.0	20.0	21.0	20.5
Salinity (‰)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
pH	6.6	6.6	6.5	6.5	6.5	6.6	6.5
DO (mg/1)	8.8	8.8	8.1	8.0	8.0	8.1	8.2

The area of the lake that includes PO1 and PO2 has been termed the 'hybrid' zone since this is the portion of the lake in which the greatest degree of sympatry between mummichog and banded killifish was encountered (Table 4) and in which the largest number of suspected hybrids was collected. Although both PO1 and PO2 occur approximately the same distance from the sea, the two areas are quite different (Table 4). Study area PO1 is quite shady and has a mixed mud and sand substrate with the green alga, *Chara* sp., and the spike rush, *Eleachoris palustris*, being the dominant aquatic macrophytes. Study area PO2, however, has little shade, substrate consisting of a mixture of mud, sand and wood chips, with creeping bent, *Agrostris stolonifer*, and broad-leaf cord grass, *Spartina pectinata*, being the dominant macrophytes.

Study areas PO3, PO4, and PO5 are essentially very similar. All are small coves with dense growths of ditch grass, *Ruppia* sp., and *Chara* sp., with large quantities of wood. Although closer to the sea than study areas PO1 and PO2, few mummichog were collected in these areas in several attempts, which indicates that mummichog distribution is rather disjunct in the lake.

Study area PO6 is quite distinct from the other study areas. The mud substrate, with an aerobic depth of about 40 mm covered to a great extent by mats of *Potamegeton* sp., pond weed, and *Enteromorpha* sp., is a habitat somewhat similar to those encountered in the salt pans of salt marshes.

TABLE 4

Synopsis of conditions of study areas in Porters Lake 1971. Physical and chemical data represent average conditions during the 1971 breeding season for both mummichog and banded killifish. Hybrids = the number of suspected hybrids collected at that study area; the upper number = 1971, the lower = 1972; JTU = Jackson turbidity units. Location of study areas are given in Figure 1.

Study Area	PO1	PO2	PO3
Water Temp. (C)	20.1 (16.0-24.4)	21.2 (16.0-27.0)	19.0
Salinity (‰)	7.3 (1.3-16.5)	6.6 (0.8-15.1)	5.2 (0.8-14.1)
Turbidity (JTU)	19 (10-40)	16 (10-20)	20
Substrate	Mud & sand, sunken wood	Wood chips, sand, mud, rocks	Mud, sand, wood chips
F. diaphanus	332 collected	517 collected	152 collected
F. heteroclitus	232 collected	128 collected	9 collected
Hybrids	30 collected (1971)	114 collected (1971)	0 collected (1971)
	0 collected (1972)	3 collected (1972)	0 collected (1972)
Vertebrates	*Roccus americanus* *Menidia menidia* *Anguilla rostrata* *Catostomus commersonii* *Apeltes quadracus* *Gasterosteus aculeatus* *Pungitius pungitius* *Alosa pseudoharengus*	*Roccus americanus* *Menidia menidia* *Anguilla rostrata* *Apeltes quadricus*	*Menidia menidia* *Apeltes quadracus* *Gasterosteus aculeatus*
Macrophytes	*Chara sp.* *Eleocharis palustris* *Agrostris stolonifera*	*Chara sp.* *Spartina pectinata* *Agrostris stolonifera* *Spirogira sp.*	*Ruppia sp.* *Chara sp.*

Table 4 continued

Study Area	PO4	PO5	PO6 (examined 1972 only)
Water Temp. (C)	26.7 (24.5-29.0)	25.6 (25.3-26.0)	24.1 (20.0-32.0)
Salinity (‰)	12.8 (12.0-13.6)	14.5	12.8 (7.5-15.3)
Turbidity (JTU)	12.5 (15-10)	15	16.6 (15-20)
Substrate	Mud, sand, wood chips	Mud, sand, wood chips	Mud, trash
F. diaphanus	131 collected	135 collected	48 collected
F. heteroclitus	28 collected	11 collected	250 collected
Hybrids	0 collected (1971)	0 collected (1971)	23
	0 collected (1972)	0 collected (1972)	
Vertebrates	Menidia menidia Apeltes quadracus	Menidia menidia	Roccus americanus Menidia menidia Anguilla rostrata Apeltes quadracus Gasterosteus aculeatus Pungitius pungitius Microgadus tomcod Pseudopleuronectes americanus
Macrophytes	Ruppia sp. Chara sp.	Ruppia sp. Chara sp.	Ruppia sp. Potamogeton sp.

All study areas show some degree of fluctuating salinity
(Table 4). These salinity fluctuations can, in part, be attributed
to the tides. As the tide rises sea water mixes with the fresh lake
water increasing the salinity and as the tide ebbs part of the mixed
water leaves the lake, thus reducing the salinity. The salinity is
further reduced during periods of rain when run-off increases the
fresh water input. Differences in temperature recorded at each station
(Table 3) may be attributed to the different times of the day at which
each temperature was taken. More reliable indicators of the surface
temperature of the littoral areas of the lake are the mean monthly
values calculated from the pooled temperatures taken at all stations
during each month (Fig. 3).

FIGURE 3

Mean monthly surface temperature of littoral areas
of Porters Lake, Halifax County, Nova Scotia. Values
were calculated by pooling temperatures taken at
each study area during each collecting trip.

PORTERS LAKE

1971
1972

MEAN TEMP. (C)

MONTH
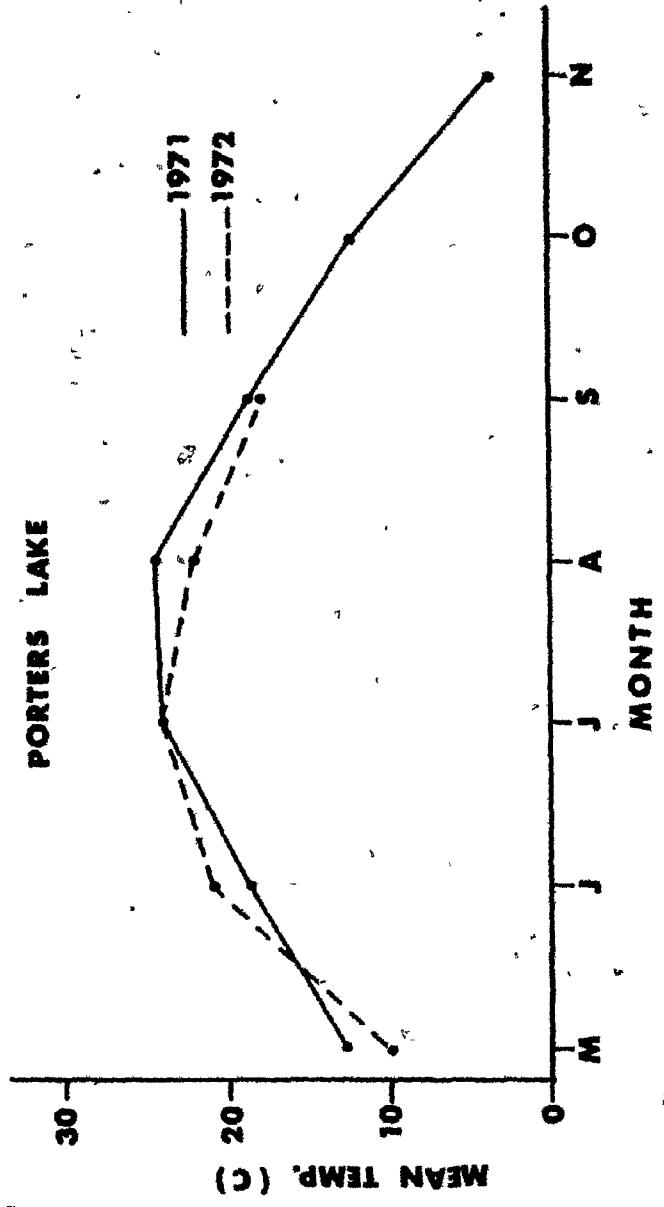
PHENOTYPIC ANALYSIS AND HYBRID IDENTIFICATION

Materials and Methods

Hubbs, Walker, and Johnson (1943) give a list of 32 characters by which they were able to distinguish mummichog, banded killifish, and their hybrid. Many of these characters, however, are relatively difficult to measure. My examination of the three forms revealed that eight characters, easy to measure, count, or calculate could be used to differentiate clearly these fishes. The characters are: distance from origin of the dorsal fin to the hypural plate (D-C), caudal peduncle depth (CP), ratio of D-C to CP, numbers of gill-rakers, caudal fin rays, and vertebrae, peritoneal color, and electrophoretic examination of muscle malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) patterns.

All external measurements and counts were made in accordance with the procedures of Hubbs and Lagler (1948). All measurements were made to 0.1 mm with vernier calipers. Any one of three methods were employed to count vertebrae, each being governed by size of specimen. Alizarin staining and stereoscopic magnification according to the methods of Taylor (1967) or conventional radiography were used for fish longer than 32 mm standard length, while 'soft' radiographic technique was used for smaller fish. Electrophoresis was performed according to the methods of Odense, Allen, and Leung (1966).

Hybrid determination is based on phenotypic intermediacy between

parental species (Hubbs *et al.*, 1943; Hubbs, 1955). Females of the three types of fishes were used in these comparisons because no male hybrids were identified from the 170 hybrids collected during 1971 and 1972. In order to determine any degree of character displacement that might have occurred within the Porters Lake mummichog and banded killifish populations, comparisons were made between these and representative allopatric populations from province-wide collections held in the Nova Scotia Museum.

Character Comparisons Between Sympatric Species

Origin of dorsal fin to hypural plate (D-C). Scott and Crossman (1964) showed, by means of the dorsal fin index (DFI) (that is, the difference between D-C and standard length), that insertion of the dorsal fin is located more anteriorly in banded killifish than in mummichog. The DFI, however, changes with growth to such an extent that in small individuals the DFI of each species tends to overlap each other somewhat (Scott and Crossman, 1964). To eliminate the problem of allometric growth incurred by using DFI, yet still utilizing this character, a relative-growth analysis (Martin, 1949) was performed on the D-C and body length (Fig. 4). Analysis of covariance of the resulting regressions indicated that while there is no significant difference between slopes ($P > 0.05$), there is a significant difference between adjusted means ($P < 0.001$). This indicates that more anterior location of the dorsal fin insertion in banded killifish is constant throughout the life span of the two species.

FIGURE 4

Relative growth regressions for (D-C) the distance from the
origin of the dorsal fin to the end of the hypural plate for
mummichog (H), banded killifish (D), and suspected hybrids (Q).
The upper two regressions were calculated from combined pro-
vincial data obtained from fishes held in the Nova Scotia
Museum fish collection. The lower three regressions were
calculated from killifish collected in Porters Lake. Results
of tests for statistical significance among the regressions
are given in the text or in Table 7.

D logD=-0.382+1.020 logL , r=0.978
H logD=-0.327+0.943 logL , r=0.961

D logD=-0.280+0.961 logL , r=0.961
Q logD=-0.438+1.039 logL , r=0.969
H logD=-0.446+1.023 logL , r=0.979

ORIGIN OF DORSAL FIN TO HYPURAL PLATE (mm)

STANDARD LENGTH (mm)
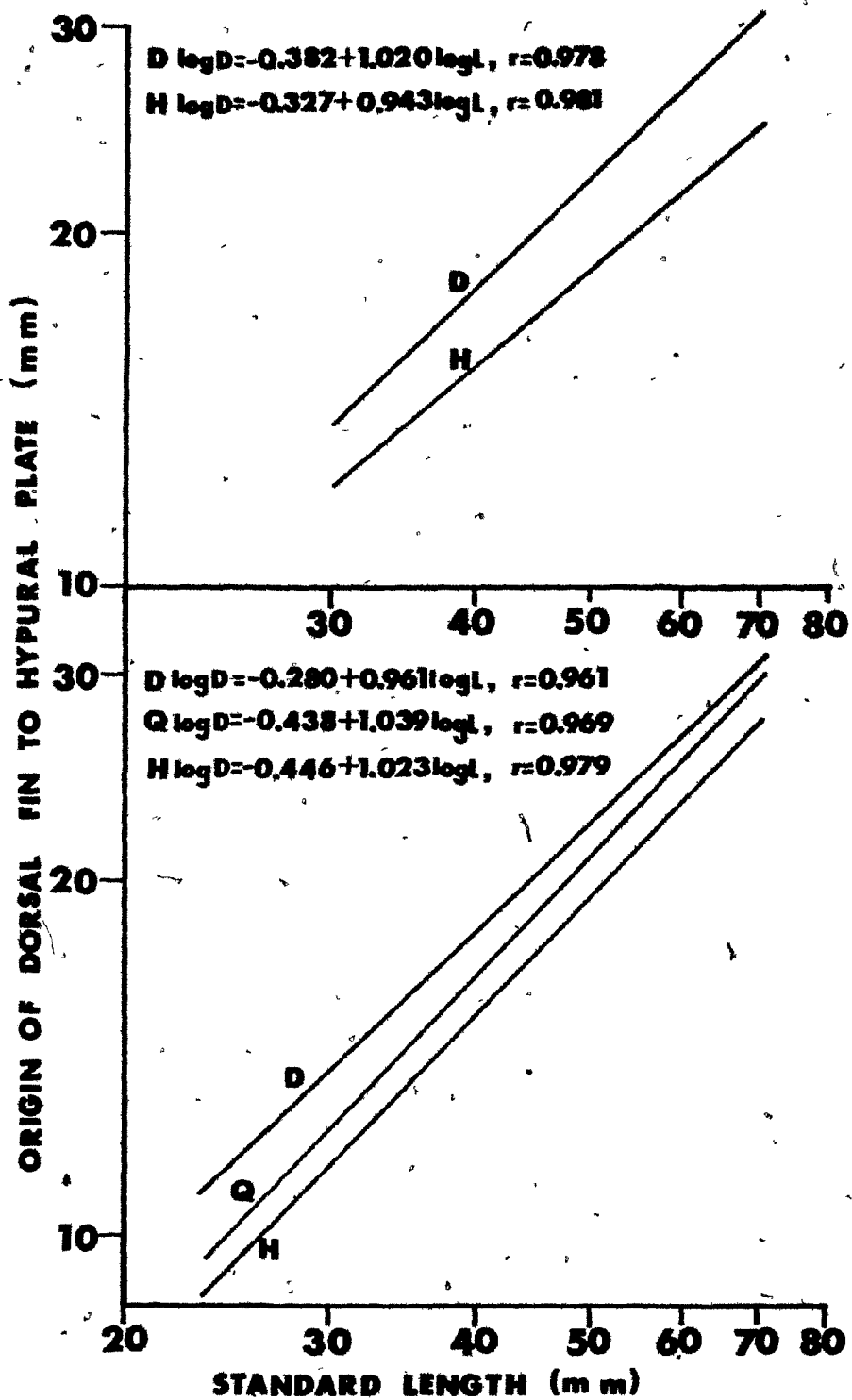
Depth of caudal peduncle (CP). Like D-C, this character is suscep-
tible to changes with growth (Martin, 1949). Therefore, it also was
analyzed by relative-growth methods (Fig. 5). Analysis of covariance
between the regression lines indicated that the slopes are not signi-
ficantly different (P > 0.05), but the adjusted means do differ signi-
ficantly (P < 0.001). This shows that the relative depth of the caudal
peduncle of the mummichog is greater throughout its life span that that
of banded killifish.

Ratio of D-C to CP. Brown (1957) used the ratio value of 3.3 or
more to separate *F. diaphanus*, *F. seminolis* and *F. waccamensis* from
F. heteroclitus, *F. grandis*, *F. pulvereus*, and *F. confluentus* which have
a ratio value of 3.2 or less. In Nova Scotia only one species is present
for each of these two series of species. Figure 6 shows that in Porters
Lake 13% of the mummichog have a value exceeding 3.2. A comparison by
't' test of the ratios of the two species shows a significant difference
(P < 0.001).

Gill-raker number. This character is of particular use in distin-
guishing the two species. Mummichog in Porters Lake have 8 to 10 gill-
rakers, while banded killifish have 5 or 6 gill-rakers (Fig. 7). No
specimens of either species have been collected in which the gill-raker
number of one overlapped that of individuals of the opposite species.

Caudal fin ray number. Figure 8 shows the frequency distributions
of caudal fin ray number for both species. Little overlap can be

FIGURE 5

Relative growth regressions for (CP) depth of caudal peduncle
for mummichog (H), banded killifish (D), and suspected hybrids
(Q). The upper two regressions were calculated from combined
provincial data obtained from fishes held in the Nova Scotia
Museum fish collection. The lower three regressions were
calculated from the three types of killifish collected in
Porters Lake. Results of tests for statistical significance
among the regressions are given in the text or in Table 7.

Upper plot:

H logD=-0.776+0.942logL, r=0.976
D logD=-1.090+1.056logL, r=0.966

Lower plot:

H logD=-0.681+0.885logL, r=0.964
Q logD=-1.279+1.204logL, r=0.980
D logD=-1.112+1.076logL, r=0.960

CAUDAL PEDUNCLE DEPTH (mm)

STANDARD LENGTH (mm)
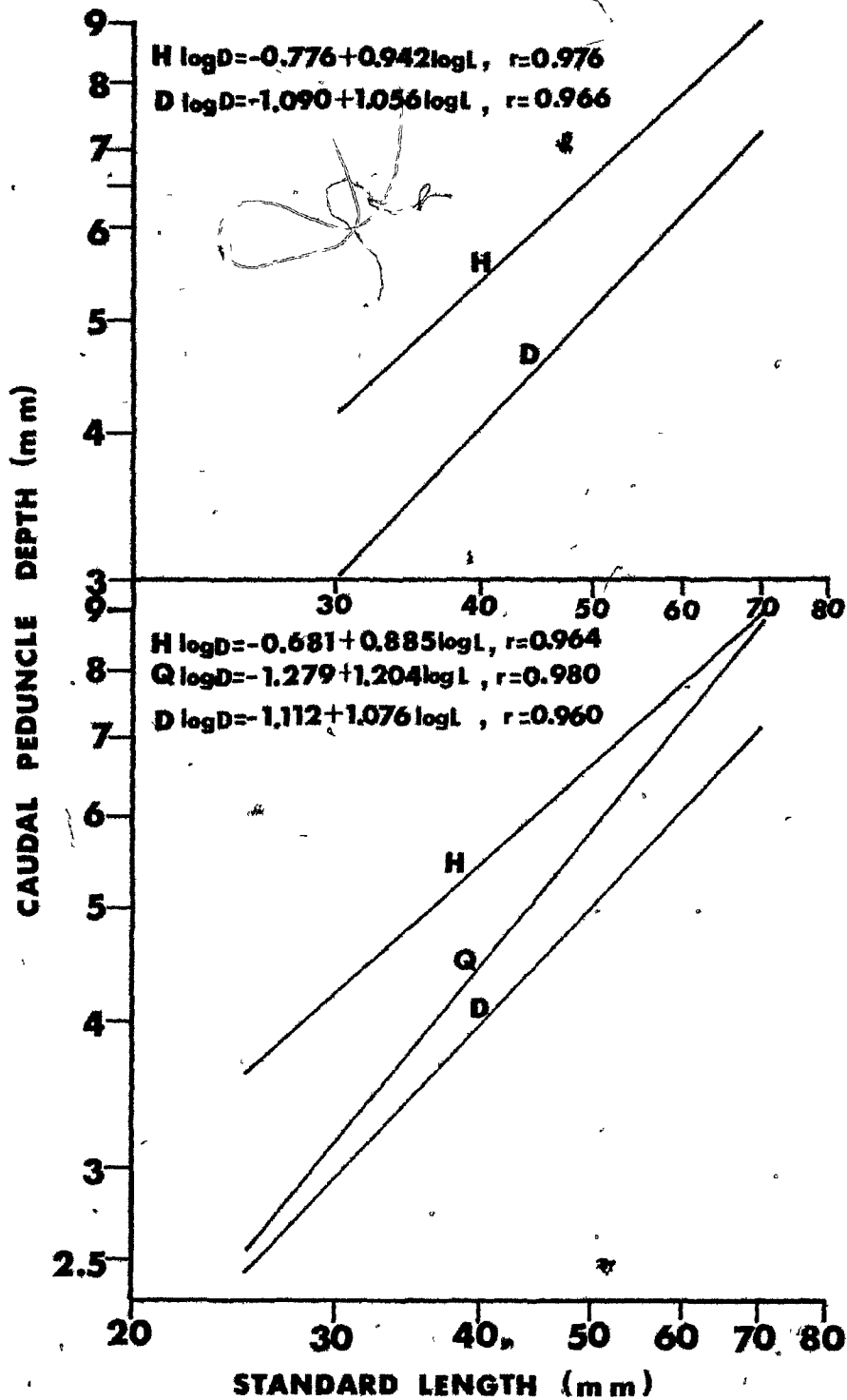
FIGURE 6

Frequency distributions for ratio of the distance from the
origin of the dorsal fin to the hypural plate divided by
depth of caudal peduncle (D-C/CP), for mummichog, banded
killifish, and suspected hybrids collected in Porters Lake
and banded killifish, and mummichog obtained from the Nova
Scotia Museum fish collection. Results of tests for
statistical significance among the members of these
populations are given in the text or in Table 7.

$\bar{x}$ = mean, n = sample size, s - standard deviation

F. diaphanus
(province)

$\bar{x} = 4.39$
$s = 0.32$
$n = 386$

F. heteroclitus
(province)

$\bar{x} = 2.78$
$s = 0.20$
$n = 95$

F. diaphanus

$\bar{x} = 4.29$
$s = 0.28$
$n = 101$

$\bar{x} = 3.67$
$s = 0.24$
$n = 110$

hybrid

F. heteroclitus

$\bar{x} = 2.85$
$s = 0.27$
$n = 101$

FREQUENCY

D-C/CP
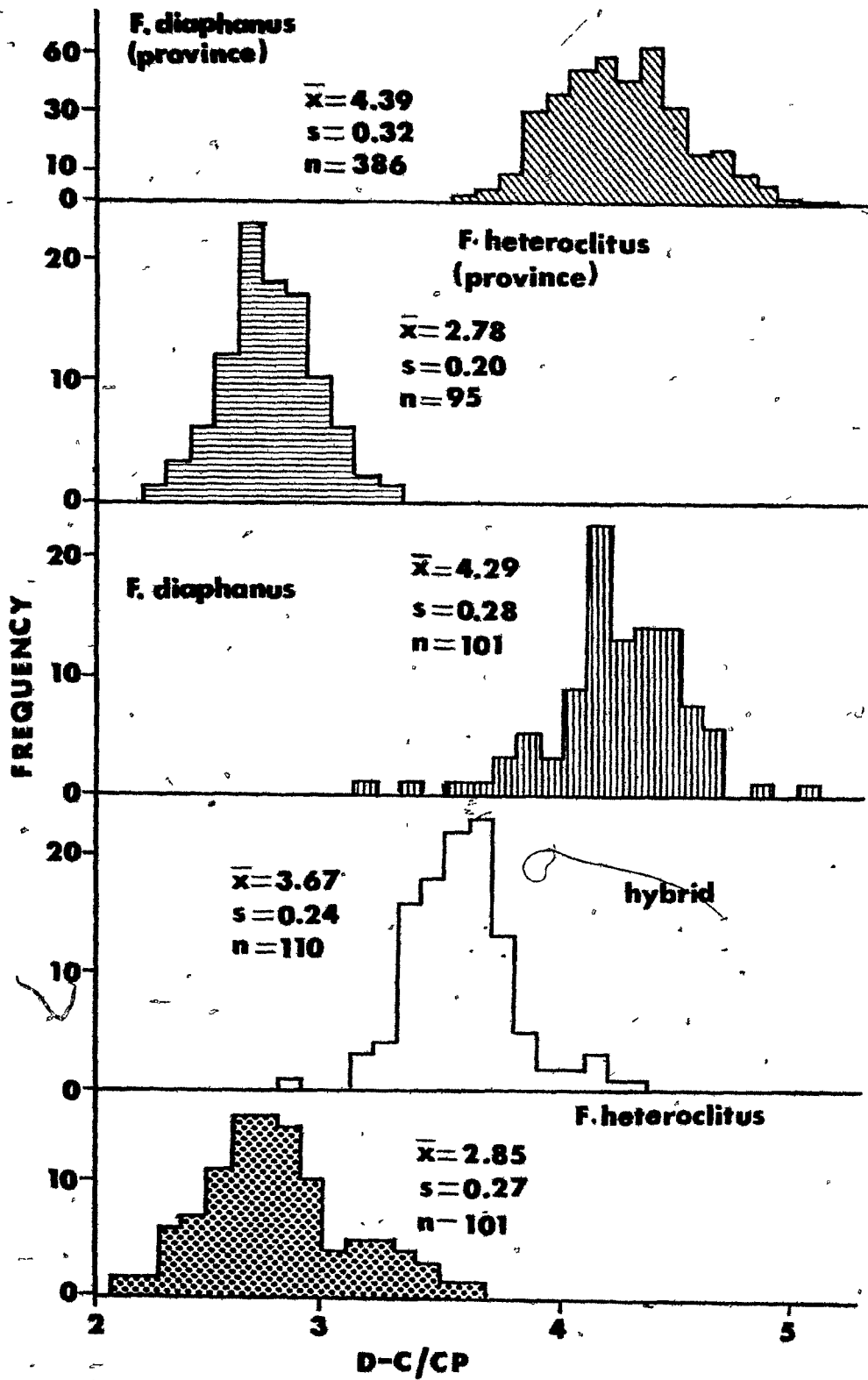
FIGURE 7

Frequency distributions for gill-raker number for mummichog,
banded killifish, and suspected hybrids collected in Porters
Lake and banded killifish and mummichog obtained from the
Nova Scotia Museum fish collection. Results for tests for
statistical significance among the members of these populations
are given in the text or in Table 7.

$\bar{x}$ = mean, n.= sample size, s - standard deviation

$\bar{x} = 5.47$
$s = 0.62$
$n = 386$

F. diaphanus
(province)

F. heteroclitus
(province)

$\bar{x} = 8.35$
$s = 0.70$
$n = 95$

$\bar{x} = 5.42$
$s = 0.52$
$n = 101$

F. diaphanus

hybrid

$\bar{x} = 7.10$
$s = 0.59$
$n = 110$

F. heteroclitus

$\bar{x} = 8.41$
$s = 0.71$
$n = 101$

FREQUENCY

GILL-RAKER NUMBER
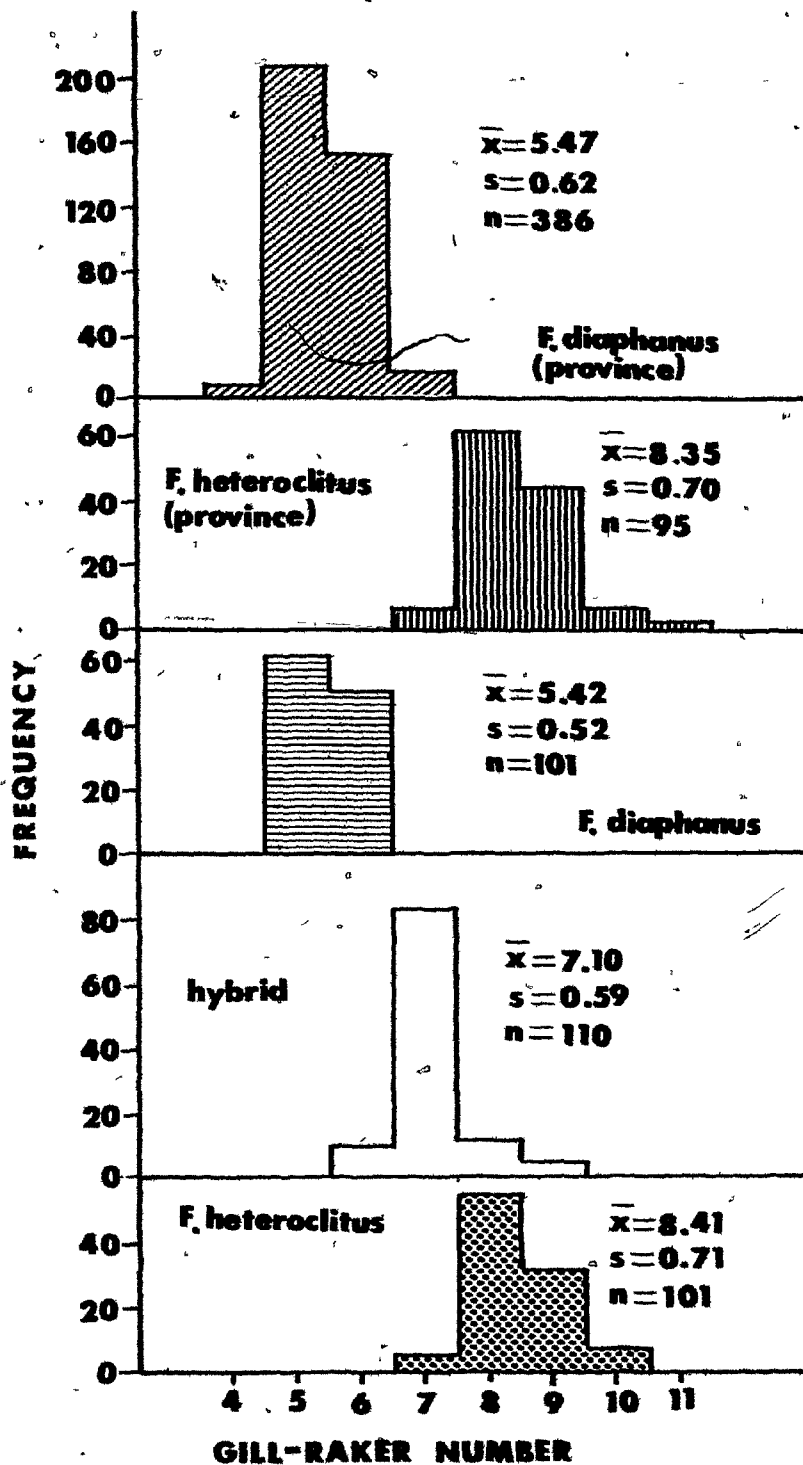
observed and a comparison between the two species by 't' test shows a highly significant difference (P < 0.001).

Vertebral number. This character, also, when compared by 't' test shows a significant difference (P < 0.001). The frequency distributions (Fig. 9), however, indicate that 6% of the mummichog have a vertebral number similar to banded killifish.

Peritoneal color. This character was analyzed qualitatively. Figure 10 shows examples of peritoneum of mummichog and banded killifish. These portions of peritoneum were photographed at different exposures and with different backgrounds. It can be noted that the peritoneum of mummichog is almost uniformly black, while that of banded killifish is dark on the dorsolateral surface and silvery on the ventrolateral surfaces (Fig. 10).

Electrophoretic patterns. The patterns of both MDH and LDH (Fig. 11) obtained from the Porters Lake mummichog and banded killifish indicate that both species are homozygous for both enzymes and that both species show distinctive electrophoretic mobilities of the two enzymes.

Other characters such as color pattern and gross morphologic appearance also show distinct differences (Fig. 12).

FIGURE 8

Frequency distributions for caudal fin ray number of mummichog, banded killifish, and suspected hybrids collected in Porters Lake. Results of tests for statistical significance among members of these populations are given in Table 7.

$\bar{x}$ = mean, n = sample size, s = standard deviation

$\bar{x}=15.87$
$s=0.42$
$n=101$

F. diaphanus

$\bar{x}=17.39$
$s=0.66$
$n=110$

hybrid

E. heteroclitus

$\bar{x}=19.51$
$s=1.44$
$n=101$

FREQUENCY

CAUDAL FIN RAY NUMBER
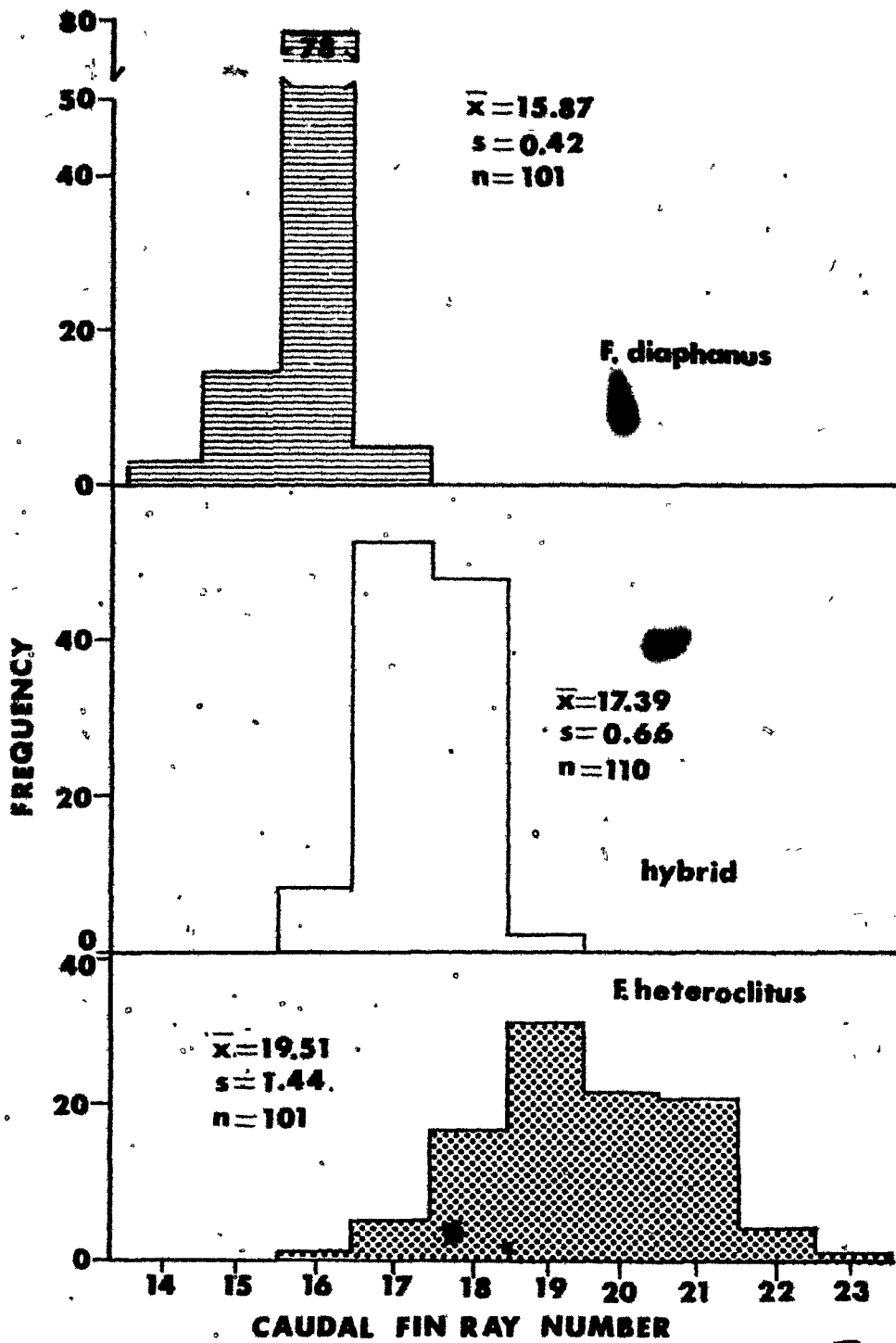
FIGURE 9

Frequency distributions for vertebral number of mummichog, banded killifish, and suspected hybrids collected in Porters Lake. Results of tests for statistical significance among members of these populations are given in Table 7. $\bar{x}$ = mean, n = sample size, s = standard deviation

$\bar{x}=34.71$
$s=0.58$
$n=62$

F.diaphanus

$\bar{x}=34.31$
$s=0.58$
$n=59$

hybrid

$\bar{x}=32.88$
$s=0.55$
$n=59$

F.heteroclitus

FREQUENCY

VERTEBRAL COUNTS
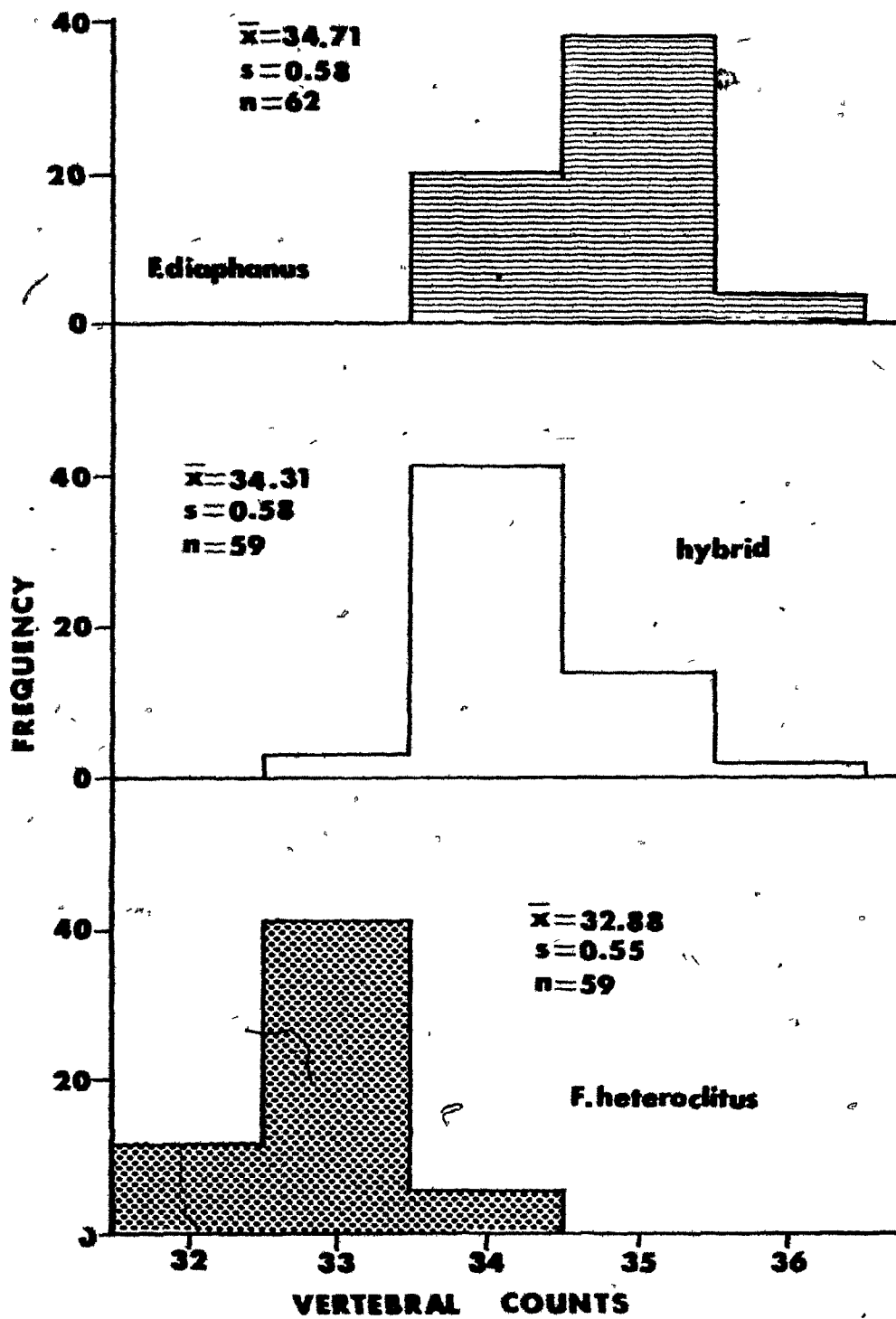
FIGURE 10

Peritoneum of banded killifish (D), suspected hybrids (Q),
and mummichog (H):   #1 was prepared on a white background,
#2 was prepared on a grey background, #3 was prepared on a
dark grey background.

H   Q   D

1

H   Q   D

2

(H)   Q   D

3
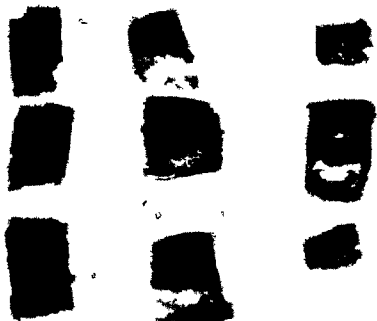
FIGURE 11

Electrophoretic patterns of muscle malate dehydrogenase (MDH)
and lactate dehydrogenase (LDH) for 5 mummichog (H), 5 suspected
hybrids (Q), and 5 banded killifish (D). X indicates a suspected
hybrid that was inadvertently placed among the mummichog. C'C
is the hybrid band in MDH, the undesignated arrow indicates the
hybrid band in LDH.

AA

B

BB
CC

C'C

C'C'

H ——— Q ——— D

MDH

D ——— Q ——— D

x

LDH

FIGURE 12

Killifishes collected in Porters Lake, Halifax County, Nova
Scotia. F.H. mummichog, F.q. suspected hybrids, F.d. banded
killifish.

Fd

Fq

Fh

FISHER SCIENTIFIC
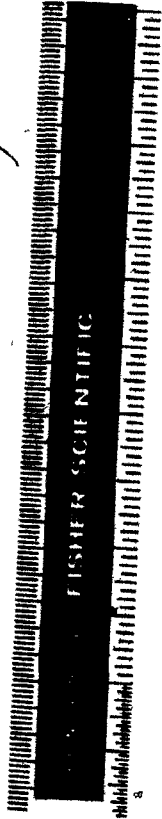
Character Comparisons Among Allopatric Populations

When stable populations of closely related species are sympatric over a period of time that encompasses a large number of generations, the phenomenon of character displacement can take place (Brown and Wilson, 1956). As was mentioned previously, Porters Lake has been open to the sea for about 15 years. If mummichog apparently began to enter the lake at that time it is possible that some character displacement could have occurred within the banded killifish and mummichog populations.

Three characters were initially used as potential indicators of character displacement, D-C/CP, gill-raker number, and DFI. Figure 13 shows the mean values for these characters for both species for the county in which the fishes were collected. Analysis of variance for D-C/CP and gill-raker number of allopatric provincial populations of each species showed that these characters, in both species, differ significantly (Tables 5 and 7). These differences, however, do not appear to be geographically clinal in nature (Fig. 13).

The D-C/CP and gill-raker number of Porters Lake mummichog and banded killifish populations were compared by 't' tests with the combined provincial values of these characters for each species (Fig. 6 and 7). Results of the comparisons showed that there were no significant differences (P > 0.05) between the Porters Lake populations and the combined provincial values. No attempt was made to

FIGURE 13

Mean values of D-C/CP, gill-raker number, and dorsal fin index
(in vertically descending order) of samples of mummichog (H) and
banded killifish (D) collected throughout the provnice and held
in the Nova Scotia Museum.
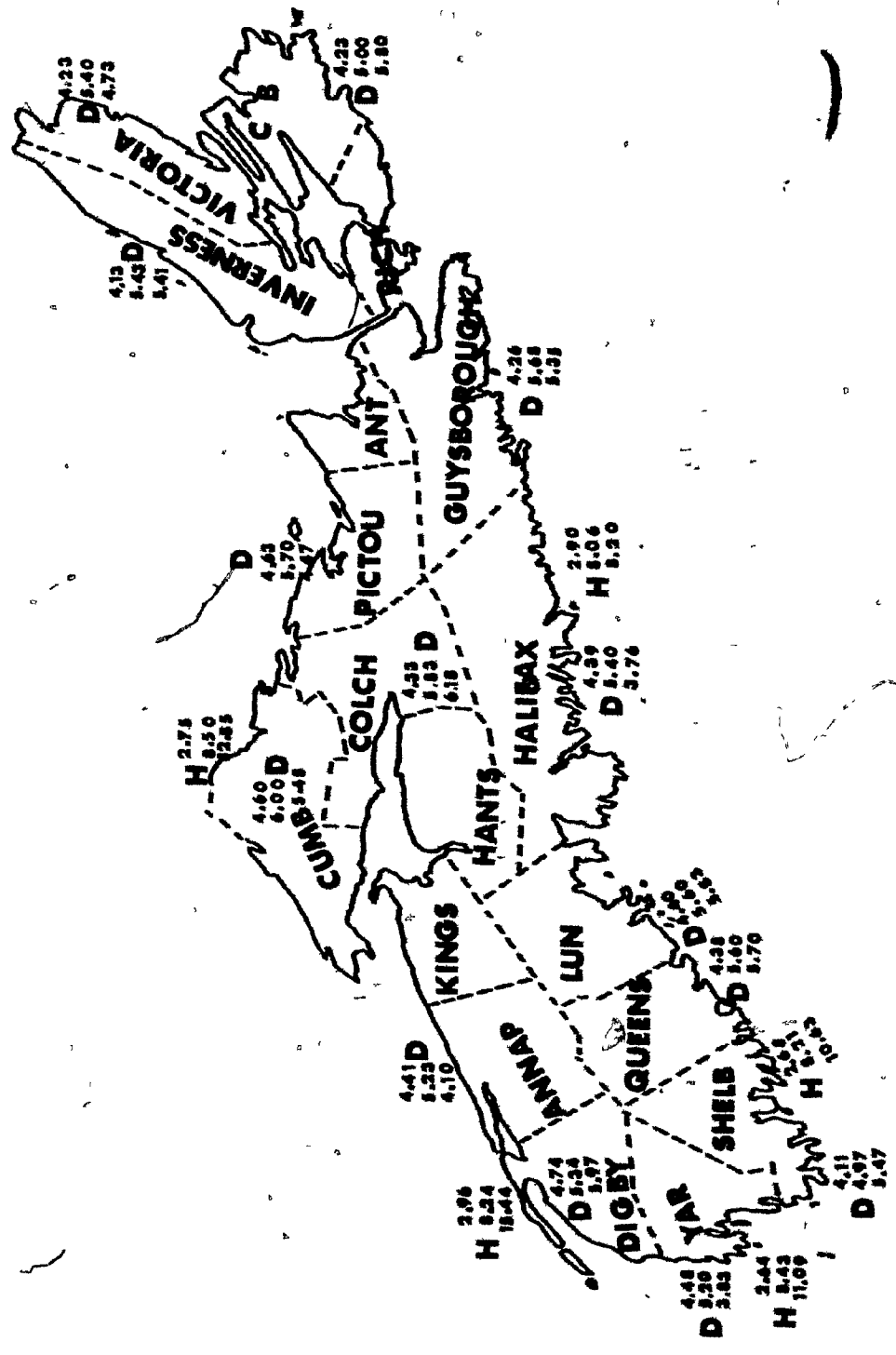
TABLE 5

Analysis of variance for one-way design for the ratio of D-C:CP of province-wide samples of mummichog and banded killifish obtained from the Nova Scotia Museum fish collection. Localities from which samples were taken are given in Table 1.

Species: *Fundulus diaphanus* (banded killifish)

Source	df	Sum of Squares	Mean Square	F Ratio	Probability
Between Groups	13	12.754	0.981	14.171	> 0.05
Within Groups	369	25.548	0.069		
Total	382	38.302			

Species: *Fundulus heteroclitus* (mummichog)

Source	df	Sum of Squares	Mean Square	F Ratio	Probability
Between Groups	5	1.366	0.273	10.949	> 0.05
Within Groups	89	2.220	0.029		
Total	94	3.586			

TABLE 6

Analysis of variance for one-way design for gill-raker number of province-wide samples of mummichog and banded killifish obtained from the Nova Scotia Museum fish collection. Localities from which samples were taken are given in Table 1.

Species: *Fundulus diaphanus* (banded killifish)

Source	df	Sum of Squares	Mean Square	F Ratio	Probability
Between Groups	13	28.356	2.1481	6.752	< 0.05
Within Groups	369	119.206	0.323		
Total	382	147.562			

Species: *Fundulus heteroclitus* (mummichog)

Source	df	Sum of Squares	Mean Square	F Ratio	Probability
Between Groups	5	7.461	1.492	3.488	< 0.05
Within Groups	89	38.076	0.428		
Total	94	45.537			

compare the dorsal fin indices of the allopatric provincial populations, since the size ranges of fish in each sample varied too greatly. To reduce this variation D-C and CP relative-growth rates were calculated from the pooled data for each species (Fig. 4 and 5), and these were compared with those of the Porters Lake mummichog and banded killifish populations. Results of the analyses covariance indicate no significant differences in either slopes or adjusted means for both characters. The results of these four comparisons indicate that no detectable character divergence has occurred within the Porters Lake mummichog and banded killifish populations.

Hybrid Character Analysis

Since neither the Porters Lake mummichog or banded killifish appear to differ structurally and morphometrically from others of the respective species collected throughout the province and since any hybrid between these species should be intermediate between parental populations only the Porters Lake populations of both species were utilized in the analysis of suspected hybrids.

Inspection of the morphometric relationships, meristic frequency distributions and electrophoretic patterns of the two species and suspected hybrid (Fig. 4 to 11) indicates that the suspected hybrid is distinctly intermediate to mummichog and banded killifish in all characters analyzed. The frequency distributions of caudal fin ray number and gill-raker number (Fig. 8 and 7) are

slightly skewed toward mummichog, while vertebral number appears to be skewed slightly toward banded killifish (Fig. 9). The frequency distribution of the D-C/CP ratios shows little skewness toward either species (Fig. 6). Although some skewness is noticeable in three characters, the degrees of skewness do not appear to indicate any degree of back-crossing. This hypothesis is supported by peritoneal color and by MDH and LDH electrophoresis (Fig. 10 and 11). The hybrid bands that are noticeable in both MDH and LDH appear to have an electrophoretic mobility as consistent as any of the bands observed in each species. Peritoneal color, also, has a consistently similar intermediate color pattern (Fig. 10).

Table 7 summarizes the results of statistical analysis of the six morphometric and meristic characters used to distinguish the suspected hybrid from the two established species. In all instances the suspected hybrid, as may be expected from the graphic representations (Fig. 4 to 12), differ significantly from either of the established species. No quantitative analyses were performed on peritoneal color or electrophoretic patterns. However, inspection of these characters show that they are qualitatively quite distinct.

Further structural and morphometric evidence for hybridization between mummichog and banded killifish was obtained from collections from the St. Mary's River. Frequency distributions of D-C/CP, gill-raker number, and caudal fin ray number along with analysis of relative-growth rate of D-C and CP (Fig. 14 to 18) show relationships and

TABLE 7

Tests for statistical significance for characters used to distinguish among the three types of killifish caught in Porters Lake. F.d. = *Fundulus diaphanus*, F.h. = *F. heteroclitus*, F.q. - suspected hybrids. At mean of F.q. = the values taken from relative growth regressions at the mean length of the suspected hybrids (47 mm) standard length. Reg. coeff. = regression coefficient for the preceding character. D-C, CP, and D-C/CP are defined in the text. SE = standard error.

Morphometric Character	At Mean of F.q.			Probability of Significance		
	F.d.	F.q.	F.h.	F.d.-F.q.	F.h.-F.q.	F.d.-F.h.
D-C	21.3	20.0	18.5	< 0.001	< 0.001	< 0.001
Reg. coeff.	0.96	1.04	1.02	> 0.05	> 0.05	> 0.05
CP	4.7	5.4	6.3	< 0.001	< 0.001	< 0.001
Reg. coeff.	1.07	1.20	0.88	< 0.05	< 0.05	> 0.05
Meristic Characters or Ratios	**Mean ± SE**					
D-C/CP	4.24 ±0.03	3.67 ±0.02	2.85 ±0.03	< 0.001	< 0.001	< 0.001
Gill-Rakers	5.42 ±0.05	7.10 ±0.06	8.41 ±0.07	< 0.001	< 0.001	< 0.001
Caudal Fin Rays	15.87 ±0.04	17.39 ±0.06	19.51 ±0.14	< 0.001	< 0.001	< 0.001
Vertebrae	34.71 ±0.07	34.32 ±0.08	32.91 ±0.07	< 0.001	< 0.001	< 0.001

FIGURE 14

Relative growth regressions of distance from origin of the
dorsal fin to hypural plate for banded killifish (A),
suspected hybrids (B), and mummichog (H) collected in the
St. Mary's River, Guysborough County, Nova Scotia. Results
of tests for statistical significance among the three types
of fishes are given in Table 8.

A logD=-0.303+0.971logL , r=0.999
B logD=-0.277+0.925logL, r=0.965
C logD=-0.250+0.8921 gl, r=0.976
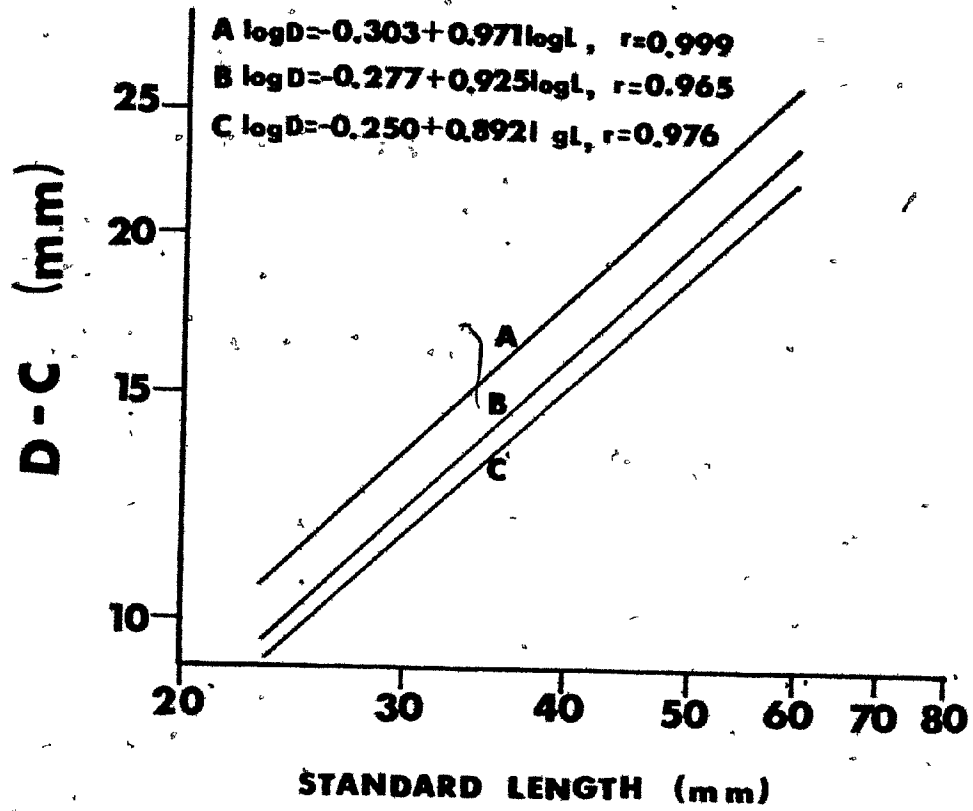
FIGURE 15

Relative growth regressions for depth of caudal peduncle
of mummichog (A), suspected hybrids (B), and banded killi-
fish (C) collected in the St. Mary's River, Guysborough
County, Nova Scotia. Results of tests for statistical
significance among the three types of killifish are given
in Table 8.

A $\log D = -1.182 + 1.185 \log L$, $r = 0.975$
B $\log D = -1.033 + 1.034 \log L$, $r = 0.984$
C $\log D = -1.195 + 1.113 \log L$, $r = 0.995$
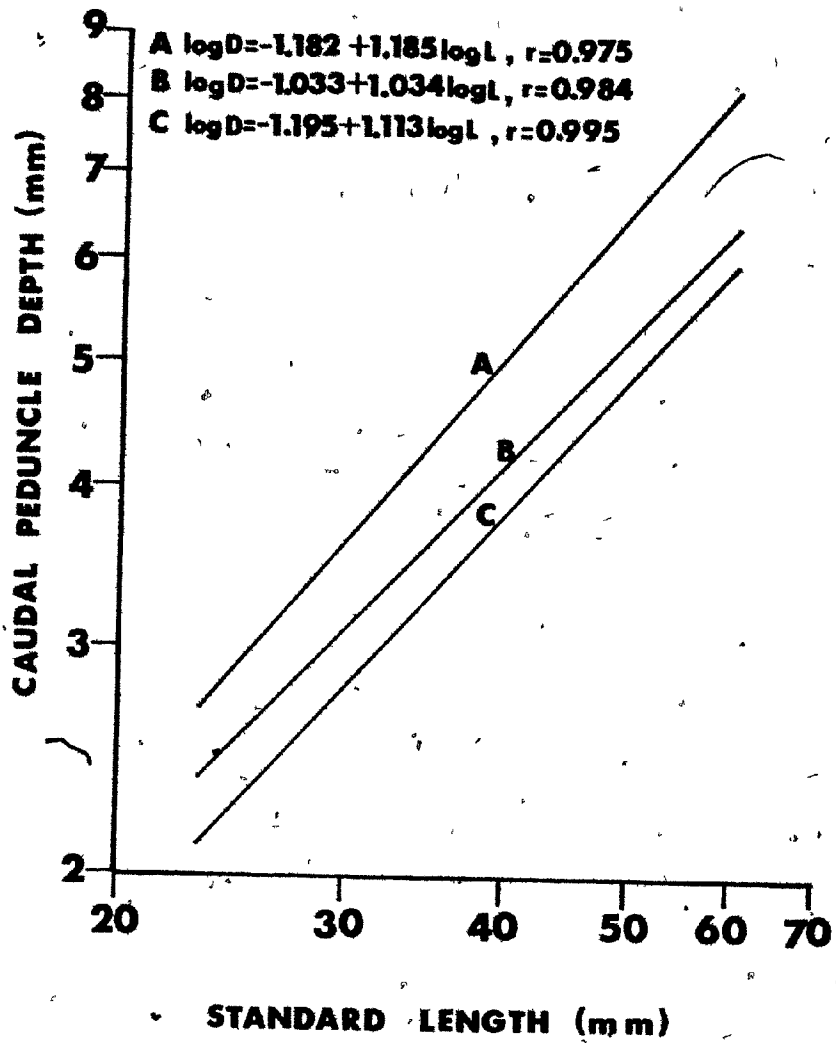
FIGURE 16

Frequency distributions for ratio of the distance from
origin of dorsal fin to hypural plate divided by depth
of caudal peduncle for mummichog, banded killifish, and
suspected hybrids collected in the St. Mary's River,
Guysborough County, Nova Scotia. Results of tests for
statistical significance among the three types of
killifish are given in Table 8.

$\bar{x}$ = mean, n = sample size, s = standard deviation

F. diaphanus

$\bar{x} = 4.69$
$s = 0.34$
$n = 24$

hybrid

$\bar{x} = 3.86$
$s = 0.26$
$n = 19$

F. heteroclitus

$\bar{x} = 3.06$
$s = 0.27$
$n = 15$

FREQUENCY

D—C/CP
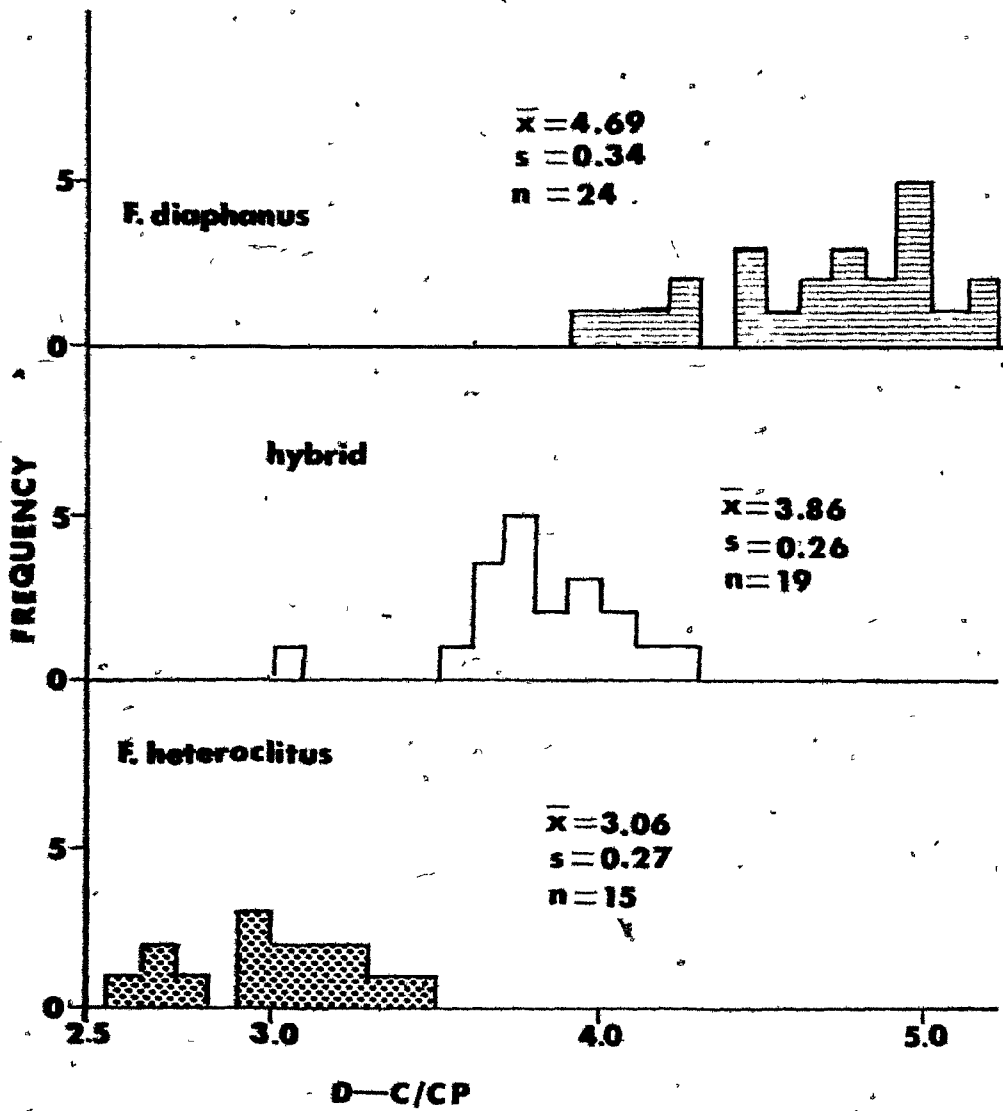
FIGURE 17

Frequency distributions for gill-raker number for mummi-
chog, banded killifish, and suspected hybrids collected
in the St. Mary's River, Guysborough County, Nova Scotia.
Results of tests for statistical significance among the
three types of killifish are given in Table 8.

$\bar{x}$ = mean,   n = sample size,   s = standard deviation

F. diaphanus

$\bar{x} = 5.38$
$s = 0.65$
$n = 24$

$\bar{x} = 7.16$
$s = 0.37$
$n = 19$

hybrid

F. heteroclitus

$\bar{x} = 7.80$
$s = 0.41$
$n = 15$

FREQENCY

GILL - RAKER NUMBER
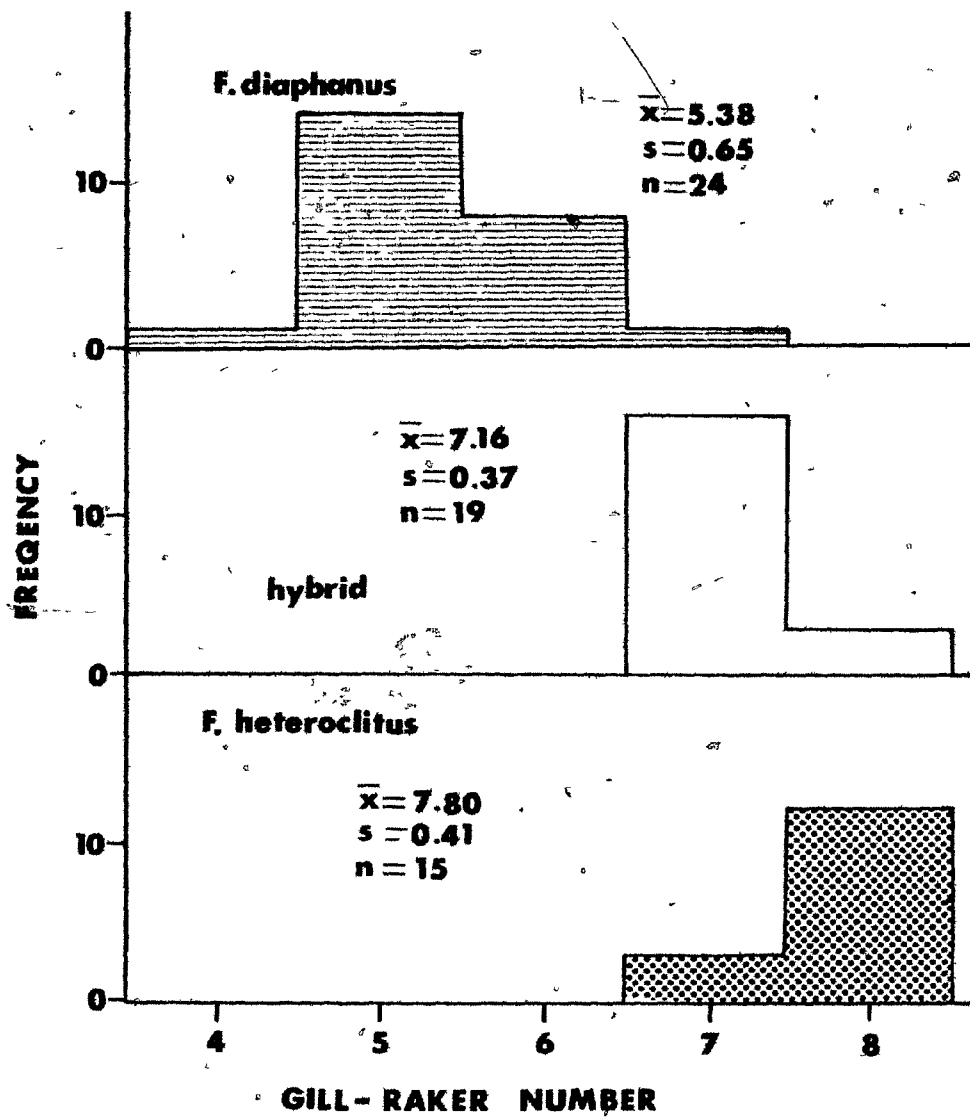
FIGURE 18

Frequency distributions for caudal fin ray number for mummichog, banded killifish, and suspected hybrids collected in the St. Mary's River, Guysborough County, Nova Scotia. Results of tests for statistical significance among the three types of killifish are given in Table 8.

$\bar{x}$ = mean, n = sample, s = standard deviation

$\bar{x}=15.79$
$s=0.65$
$n=24$

F. diaphanus

$\bar{x}=17.11$
$s=0.57$
$n=19$

hybrid

$\bar{x}=19.20$
$s=0.41$
$n=15$

F. heteroclitus

FREQUENCY

14 15 16 17 18 19 20 21

CAUDAL FIN RAY NUMBER
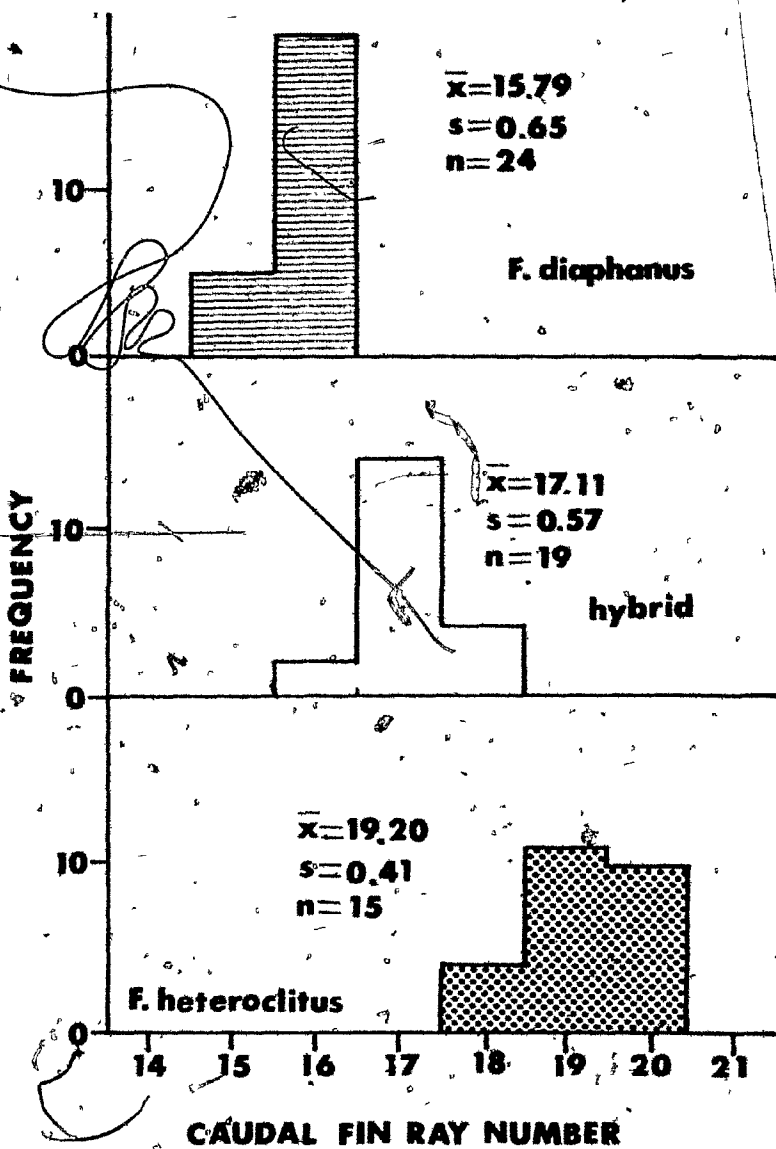
differences similar to those observed among the killifishes of Porters Lake. Statistical analyses (Table 8) show that the suspected hybrids differ significantly from local mummichog and banded killifish by approximately the same degree as that observed among the Porters Lake hybrids. Comparisons between the hybrids of Porters Lake and the hybrids of St. Mary's River show that the two differ significantly in all characters except gill-raker number (Tables 7 and 8). These analyses indicate the presence of hybrids, rather than a morphotype of one or the other species.

Hybrid Index

Hybrid indices are used to determine the extent of intermediacy possessed by a hybrid (Hubbs, Walker and Johnson, 1943), as a means of estimating the degree of back-crossing (Hagen, 1967; Greenfield and Greenfield, 1972) and as a means of identifying hyorids (Nelson, 1968).

Four characters were combined to calculate hybrid indices (Fig. 19) according to the methods of Greenfield and Greenfield (1972). The characters used were the ratio D-C/CP, gill-raker number, caudal fin ray number, and vertebral number. The total provincial numerical range of values for each character was transformed to an arbitrary scale of 10 units in which the lowest value for banded killifish was assigned the value of 1.0 index units and the highest value for

TABLE 8

Tests for statistical significance of characters used to distinguish among the three types of killifish caught in St. Mary's River. F.d. = *Fundulus diaphanus*, F.h. = *F. heteroclitus*, F.q. = suspected hybrids. At mean of F.q. = the values taken from relative growth regressions at the mean length of the suspected hybrids (32 mm standard length). Reg. coeff. = regression coefficients. D-C, CP, and D-C/CP are defined in the text. SE = standard error.

Morphometric Character	At Mean of F.q.			Probability of Significance		
	F.d.	F.q.	F.h.	F.d.-F.q.	F.h.-F.q.	F.d.-F.h.
D-C	14.8	13.5	12.5	< 0.001	< 0.001	< 0.001
Reg. coeff.	0.97	0.92	0.89	> 0.05	> 0.05	> 0.05
CP	3.2	3.5	4.1	< 0.001	< 0.001	< 0.001
Reg. coeff.	1.11	1.03	1.18	< 0.05	< 0.05	> 0.05
Meristic Number or Ratios	Mean ± SE					
D-C/CP	4.69 ±0.07	3.86 ±0.06	3.06 ±0.07	< 0.001	< 0.001	< 0.001
Gill-Rakers	5.38 ±0.13	7.16 ±0.09	7.80 ±0.11	< 0.001	< 0.001	< 0.001
Caudal Fin Rays	15.79 ±0.09	17.11 ±0.13	19.20 ±0.11	< 0.001	< 0.001	< 0.001

FIGURE 19

Frequency distributions of individual hybrid indices based on D-C/CP, gill-raker number, caudal fin ray number, and vertebral number of mummichog, banded killifish, and suspected hybrids collected in Porters Lake, Halifax County, Nova Scotia.

F. diaphanus hybrid F. heteroclitus

$\bar{x}=5.22$
$s=0.34$
$n=81$

FREQUENCY

HYBRID INDEX
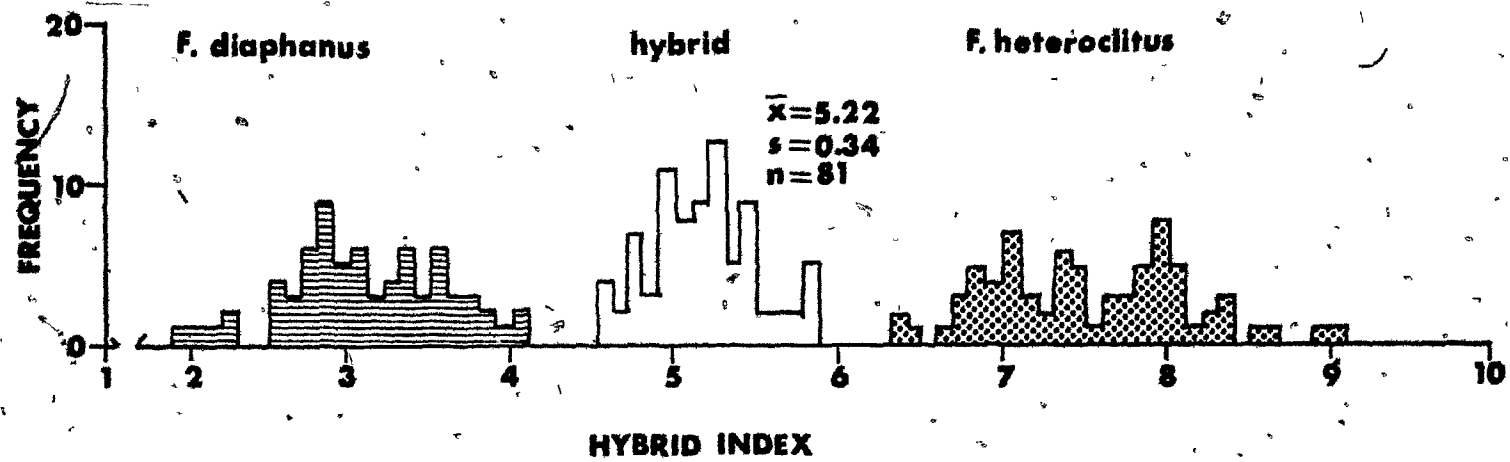
mummichog was assigned the value of 10.0 index units and all other
original character values interpolated accordingly. Thus, the 32
values for the ratio D-C/CP for all samples were converted to trans-
formed values of 0.31 index units (10.0/32 = 0.31). Gill-raker
counts contained 8 values and were assigned index units of 1.3. In
the same way the number of caudal fin rays were assigned 1.0 index
units, and 2.5 index units were given for vertebral number. After
the index values were assigned to each character, a mean character
index value was calculated for each specimen, this value being the
hybrid index for that individual. According to this method an
organism showing perfect intermediacy should have a hybrid index of
5.0. Neither D-C nor CP were utilized separately in the calculations
of hybrid indices because these characters constantly change with
growth, thus making the assigning of index units extremely difficult.
The subjectiveness inherent in distinguishing gradations in color is
too great to warrant the inclusion of peritoneal color into the hybrid
index. Subjectively, however, examination of differences in peritoneal
color and the relative-growth rates of D-C and CP show similar degrees
of separation among the three types of fish as those observed in the
characters utilized in calculating the hybrid index.

The resulting hybrid index (Fig. 19) shows clearly that the
suspected hybrid is quite intermediate between the two parental
species. The mean value and standard deviation of the combined indi-
vidual hybrid indices is 5.22 ±0.34. This value compares quite

favorably with the 50% (5.0) combined hybrid index value obtained by
Hubbs *et al.* (1943). The distinct structural separation of the three
populations of fish also implies that the hybrids are of the F_1 genera-
tion (Hubbs, 1955). This inference is substantiated somewhat by the
consistently disjunct nature of electrophoretic patterns.

Breeding Experiments

Successful laboratory crossing of two species does not, in
itself, substantiate natural hybridization (Hubbs, 1955). Many suc-
cessful laboratory crosses have been made between non-hybridizing
cyprinodont species. (Hubbs (1970) reviewed the literature on recent
laboratory crossings among *Fundulus* and other cyprinodont species,
and showed that most allopatric species easily produce hybrids and
that those produced from morphologically similar parents are easily
reared to adult size. However, a laboratory produced hybrid which
possesses characteristics that are similar to those of a suspected
natural hybrid does provide some substantial evidence for natural
hybridization (Nelson, 1968; Simon and Noble, 1968).

Parents used in laboratory crossing experiments were collected
in Porters Lake at the end of June 1972, the time shown elsewhere to
be the peak spawning period for both species. The two species were
segregated and held in the laboratory. Fishes were starved for 24
to 36 hours prior to crossing. This was done because experience
showed that ova could be stripped more easily after a delay than when

fish were initially brought into the laboratory.

Induced fertilization was accomplished by expressing ova of one species into a moistened finger bowl into which had been placed the minced testes of the other species. This mixture was agitated slightly in filtered sea water for five minutes and allowed to stand for ten minutes. Ova were then placed into 0.58x0.33x0.07 metre polyethylene trays, clumps were separated, and incubated in brackish water (salinity 10 ±2‰) which was supplied from a constant-flow head tank (Fig. 20). Temperature was maintained at 20 ±1 C by thermostat controlled submersible heaters. The fertilized ova were maintained at a 16-hour day, 8-hour night photoperiod. Following initial difficulties with fungus infections, 4 ml of Squibb Mycostatin suspension was added to each incubation tray at four-day intervals. Although infection was not eliminated it was reduced appreciably. Reciprocal crosses were made between the sexes of the two species.

When embryos reached developmental stage 34 (lower jaw well developed and mouth open) as described for mummichog by Armstrong and Child (1965) incubating trays were placed into aquaria in which hatching occurred. These aquaria were supplied with the same type of water that had been supplied to the incubation trays. Following absorption of the yolk-sac, fry were maintained for 12 weeks on a diet of freshly collected plankton. From week 10 on until the completion of the study fry were fed ground Tetramin. Twenty weeks after hatching 50 fishes of each cross were unselectively/drawn and preserved for morphometric examinations, while an additional 50 of each cross were frozen for

FIGURE 20

Constant flow egg incubator used in laboratory crossing
experiments between mummichog and banded killifish

    A Reservoir with submersible heater

    B Polyethylene trays with stand pipes

    C Water inputs
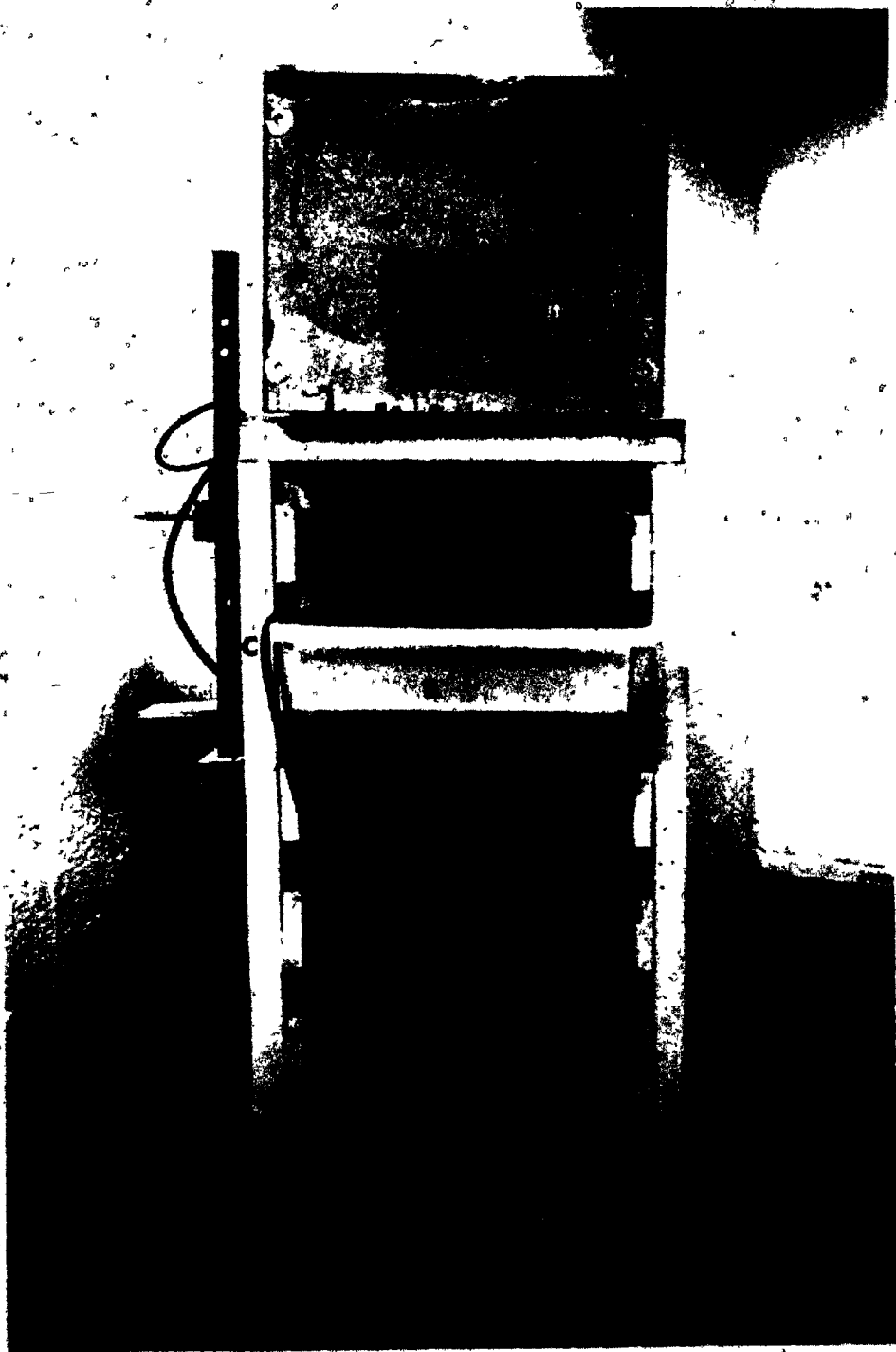
electrophorétic examination.

Because of difficulties resulting from repeated fungus infections no attempt was made to analyze quantitatively mortality rates. However, examinations showed that the cross of *F. diaphanus* (F.d.) ♂ x *F. heteroclitus* (F.h.) ♀ had high hatching success followed by rapidly decreasing survival. The opposite cross had a low hatching success with higher survival. Possible crowding in the F.d. ♂ x F.h. ♀ cross was the only appreciable environmental condition that differed between the two crosses. However, subsequent elimination of this crowding did not enhance survival detectably.

Four characters of the laboratory-produced hybrids were used in comparisons with suspected natural hybrids, D-C/CP, gill-raker number, vertebral number, and MDH electrophoresis. Neither cross produced a significant regression (P < 0.001) in the relative-growth rate analysis of D-C and CP. This may be, in part, due to the small size range of both types of fishes. Caudal fin ray number was not included in the analysis becasue fin ray number did not appear to be fixed at the time the fish were sampled.

D-C/CP. Figure 21 shows the distribution of this character among the three hybrid types. When compared by 't' tests there was no significant difference in D-C/CP among the hybrid types (P > 0.05).

Gill-raker number. This character also has similar frequency distributions among the three lots of hybrids (Fig. 22), and no

FIGURE 21

Frequency distributions for D-C/CP for suspected natural
hybrid and the two laboratory-produced hybrid crosses.
$\bar{x}$ = mean, n = sample size, s = standard deviation. Results
for tests for statistical significance, among the three types
of hybrids are given in the text.

F. d.♂ X F. h.♀

$\bar{x}=3.56$
$s=0.25$
$n=43$

hybrid

$\bar{x}=3.52$
$s=0.25$
$n=110$

F. h.♂ X F. d.♀

$\bar{x}=3.49$
$s=0.37$
$n=50$

FREQUENCY

2.5   3.0   3.5   4.0

D—C/CP
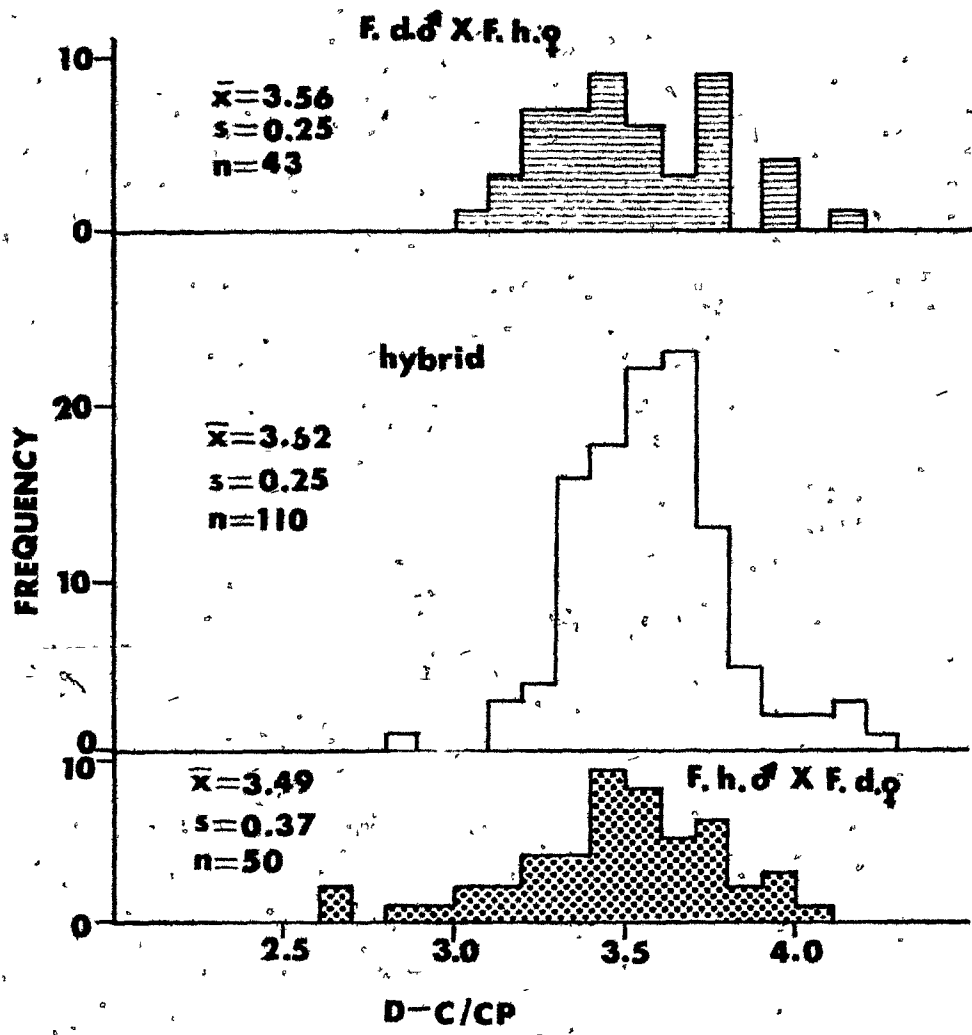
FIGURE 22

Frequency distributions for gill-raker number for suspected
natural hybrids and the two laboratory produced hybrid
crosses. $\bar{x}$ = mean, n = sample size, s = standard deviation.
Results for tests for statistical significance among the
three types of hybrids are given in the text.

7

2 OF/DE 3

1.0

125

ARC-374

$\bar{x}=7.04$
$s=0.60$
$n=50$

F.d.♂ X F.h.♀

84

hybrid

$\bar{x}=7.10$
$s=0.59$
$n=110$

F.h.♂ X F.d.♀

$\bar{x}=7.04$
$s=0.53$
$n=50$

FREQUENCY

GILL-RAKER NUMBER
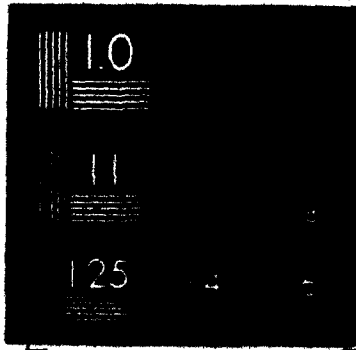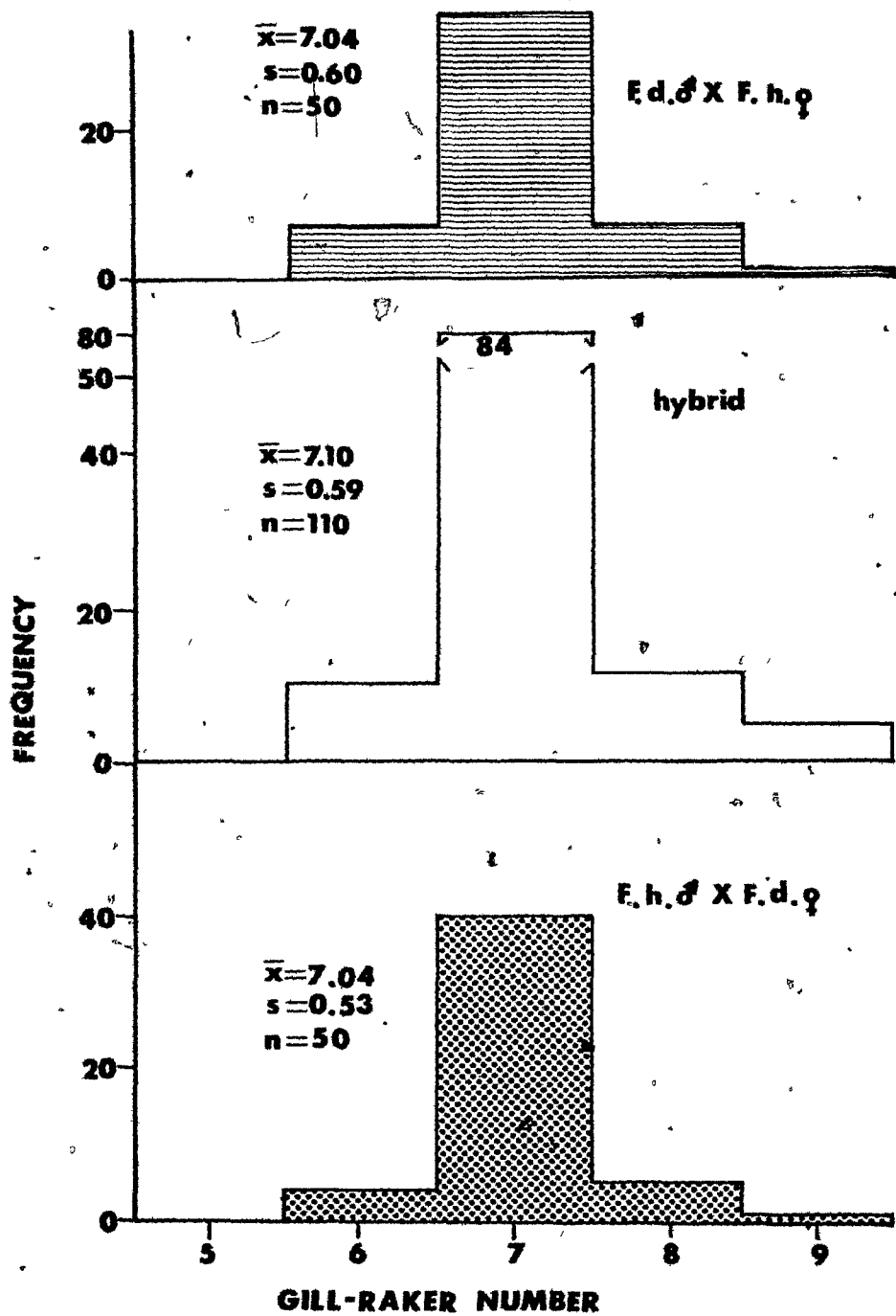
significant differences are indicated when they are compared by 't' test (P > 0.05 in both comparisons).

Vertebral number. Comparisons of the frequency distributions of the three hybrids for this character (Fig. 23) shows that the F.h. σ x F.d. φ cross corresponds quite well to that observed for the suspected natural hybrid. This is verified by a 't' test which indicates no significant difference (P > 0.05). The reciprocal cross, however, is significantly different from the suspected natural hybrid. Although lack of intermediacy of characters in the F_1 hybrid generation have been reported (literature review Simon and Noble, 1968) the opposite results observed in the two crosses raise questions that cannot be answered at this time.

Hybrid indices based upon D-C/CP, gill-raker number, and vertebral number were calculated for each individual of both crosses (Fig. 24). Comparison by 't' test indicates that there is no significant difference between the two crosses ($\bar{x} \pm SD = 5.2 \pm 0.62$, F.h. σ x F.d. φ and $\bar{x} \pm SD = 4.94 \pm 0.72$ F.d. σ x F.h. φ) (P > 0.05). The frequency distribution of hybrid indices for the combined laboratory crosses shows a strong similarity to those calculated for the same three characters of the suspected natural hybrid. Statistical comparisons support the observation in that no significant difference could be demonstrated (P > 0.05).

While gross morphometric and meristic characters have been shown to be plastic and greatly affected by environmental conditions

such as temperature and salinity, little evidence has been put forth
to show similar effects upon enzyme systems. The pattern of banding
produced by MDH in both laboratory crosses are indistinguishable from
that produced in the suspected natural hybrids (Fig. 25). Thus,
the results of comparison of electrophoretic patterns of MDH (Fig. 12
and 25) provide the most conclusive evidence for natural hybridization
between mummichog and banded killifish in Porters Lake, and by infer-
ence, in the St. Mary's River, Nova Scotia.

FIGURE 23

Frequency distributions for vertebral number for suspected

hybrids and the two laboratory-produced hybrids. $\bar{x}$ = mean,

n = sample size, s = standard deviation. Results for tests

for statistical significance among the three types of hybrids

are given in the text.

F.d.♂ X F.h.♀

$\bar{x}=34.70$
$s=0.67$
$n=48$

hybrid

$\bar{x}=34.31$
$s=0.59$
$n=59$

F.h.♂ X F.d.♀

$\bar{x}=34.11$
$s=0.63$
$n=43$

FREQUENCY

VERTEBRAL COUNTS
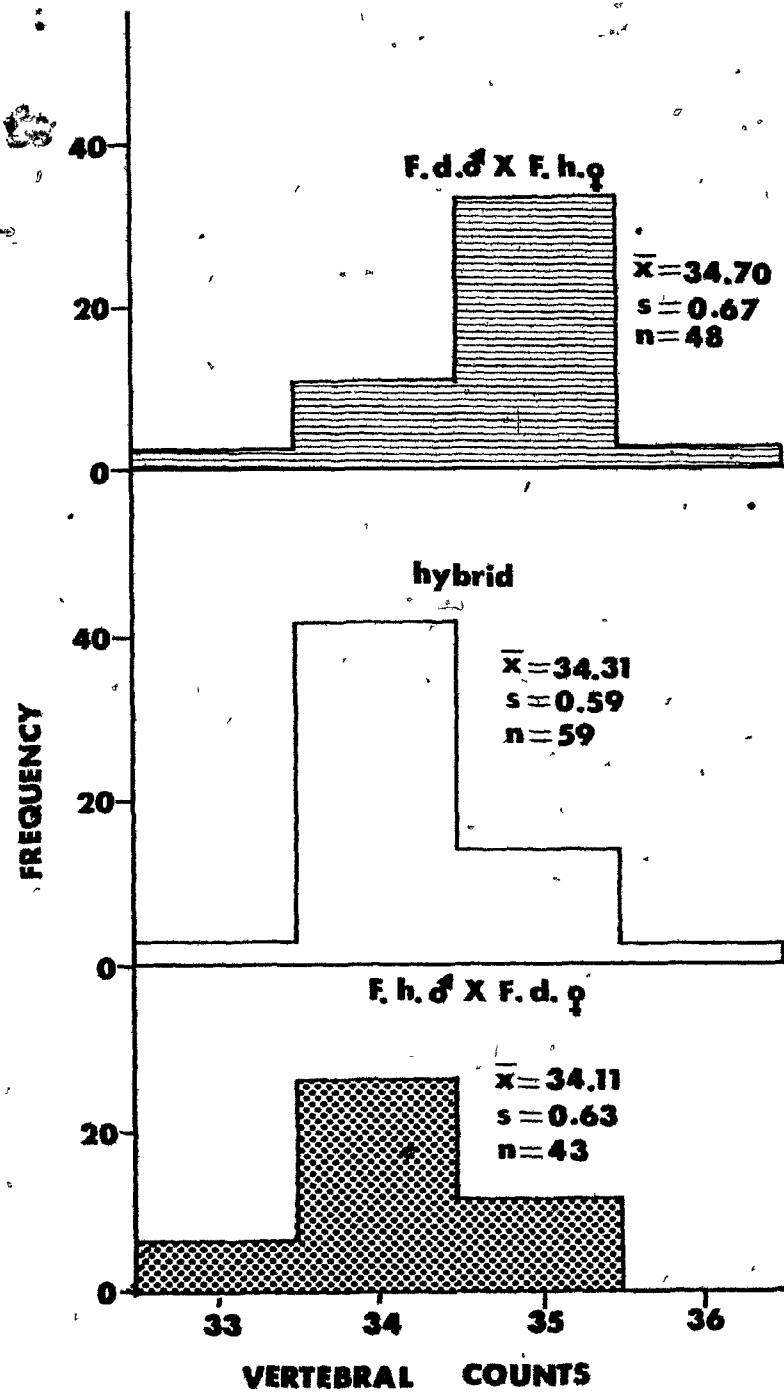
FIGURE 24

Frequency distributions of individual hybrid indices based on D-C/CP, gill-raker, and vertebra number of suspected natural hybrids and the two laboratory-produced hybrids. $\bar{x}$ = mean, n = sample size,

s = standard deviation.

FREQUENCY

HYBRID INDEX

X̄=5,16
s=0,48
n=81

hybrid

X̄=5.05
s=0.62
n=100

crosses
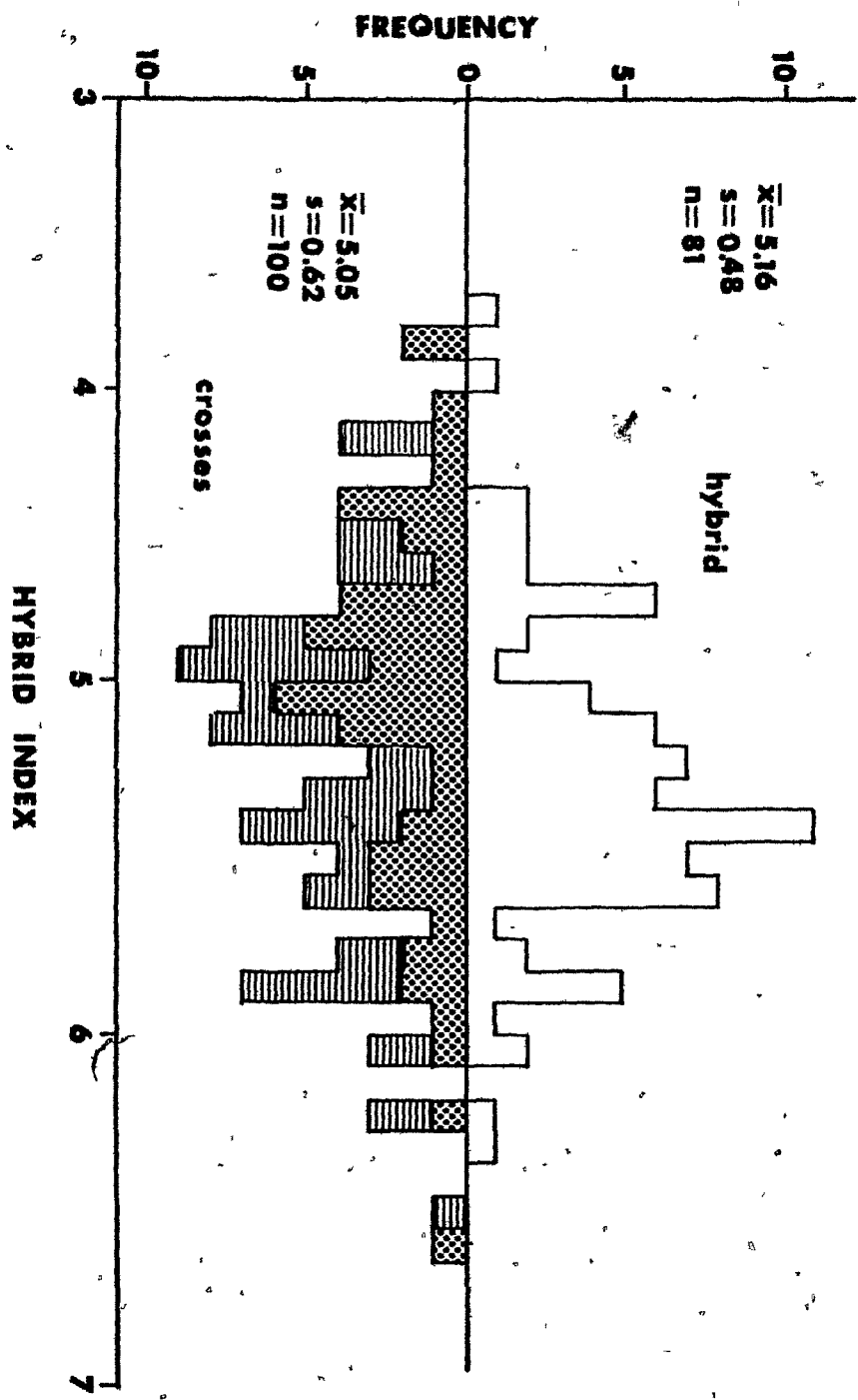
FIGURE 25

Electrophoretic patterns of muscle maltate dehydrogenase (MDH) for
three mummichog (H), three banded killifish (D), three F.h. $\vec{O}$ x F.d. φ
(h), and three F.d. $\vec{O}$ x F.h. φ (d). C'C is the hybrid band. Compare
this to the C'C band of the suspected hybrid.

AA

AB

BB
CC

c'c

c'c'

BEHAVIORAL ISOLATING MECHANISMS

Salinity Preference

Salinity is probably the most distinctive environmental factor, to influence the geographic distributions of mummichog and banded killifish. Although the two species are considered physiologically euryhaline, thus being able to tolerate great changes in salinity, the distribution of the two species in Nova Scotia is such that, as mentioned previously, mummichog are considered mostly marine organisms, while banded killifish are considered fresh water organisms. These salinity related distributional differences suggest that differing salinity preferences may act as an effective isolating mechanism.

Materials and Methods. The experiment used in determining salinity preference in the two species was designed not only to determine whether each species has a salinity preference but also whether salinity acclimation would affect the potential for salinity preference. The salinity habituations tested were $0.0\%_{oo}$, $14.0\%_{oo}$, and $31.0\%_{oo}$. Habituation of mummichog was accomplished by diluting sea water in the holding tank with fresh water at a rate of $2\%_{oo}S$ per day until the desired salinity was reached. Banded killifish habituation was attained similarly except that the salinity of the holding tank was increased by adding sea water to the fresh water of the holding tank so as to produce a change in salinity of $2\%_{oo}S$ per day. Fish were held at the required

salinity for a minimum of one week prior to commencement of testing. With the exception of the banded killifish used in 0.0‰S acclimation test, the same group of fish was used in all three acclimation tests.

Preference tests were conducted in a vertical salinity gradient and results checked in a horizontal salinity gradient. The vertical salinity gradient apparatus used (Fig. 26) was essentially that used by Hurley and Woodall (1968). The dimensions of the apparatus were 1.3x0.65x0.55 metres. A vertical salinity gradient was produced by successively pumping water of six different salinities into tapered 165x55x140 mm diffusers the upper edge of which delimited a salinity layer and were spaced 200 mm apart. Water to be used in the formation of a salinity gradient was mixed in 68-litre containers and aerated vigorously until used, while water temperature was kept at 19 C, by maintaining room temperature at 19 C throughout the experimental period.

The basic procedure used in this experiment consisted of two parts, a control and an experimental test. A control test consisted of placing one of five groups of five fish into the gradient apparatus which contained only water of the salinity in which the fish had been habituated. Fish were allowed to become accustomed to the tank for a minimum of one hour, and positions were recorded only after the fish were observed swimming from top to bottom at least twice. The positions of the five fish were recorded at one-minute intervals for the first ten minutes then at five-minute intervals for the next 50 minutes. Preliminary

FIGURE 26

Vertical salinity gradient apparatus adapted from Hurley
and Woodall (1968). The dimensions are 1.3x0.65x0.55 metres.
The sides are constructed from 3/4 inch plywood. The front
is 5/8 inch plate glass. The entire apparatus is supported
by a steel Dexion frame. The diffusers (A) are tapered
165x55x140 mm, 1/4 inch plywood boxes, the upper edges
of each are spaced 200 mm apart and delimit a compartment (B).
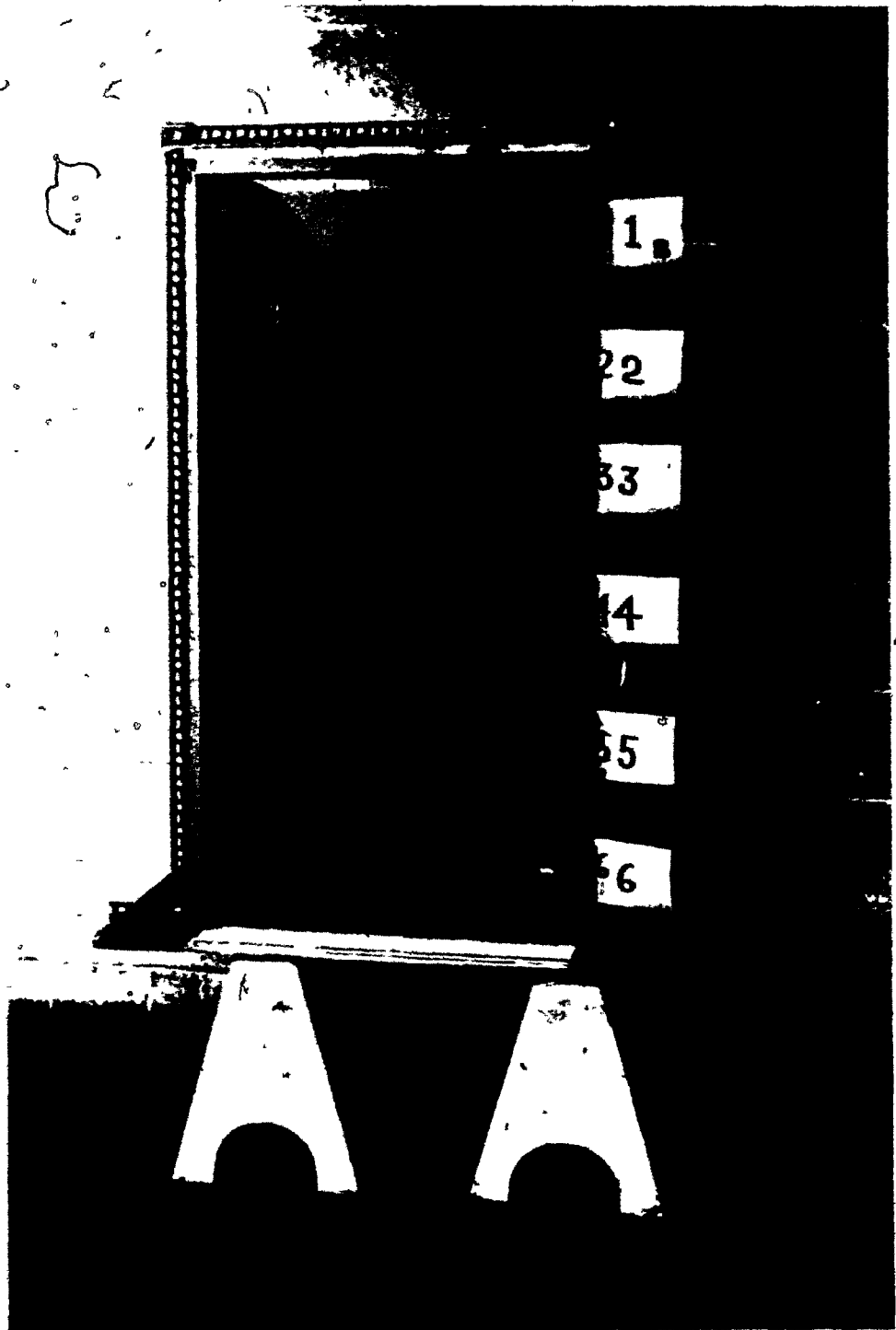
testing showed that the results obtained from this sequence of obser-
vations did not differ from those made at one-minute intervals for
60 minutes. Upon completion of the control test the fish were returned
to a segregated portion of the acclimation holding tank. The gradient
tank was then emptied, rinsed with sea water and a salinity gradient
established consisting of water layers from bottom to top of 31, 26,
20, 14, 8, and 0‰S. Water samples were then taken from each layer
and the salinity recorded. The five fish previously tested in the
control test were then placed into the gradient and observed in the
same manner described for the control test. Upon completion of the
test the salinity in each layer was again determined to determine
gradient stability and the fish returned to a segregated portion of
the acclimation tank.

An acclimation test consisted of repeating both control and
experimental tests, for each group of five fish, at a given
acclimation.

Extraction of possible geotactic effects in a vertical gradient
was accomplished by two procedures. One was the comparison of pooled
observations of the distributional patterns in both control and experi-
mental conditions and the other was a test of any vertically determined
preferences in a horizontal gradient. The groups of fish used in the
vertical gradient experiments were also used in the horizontal gradient
experiments. The apparatus used for establishing a horizontal salinity
gradient was similar to the divided chamber with a water bridge used

by Houston (1957). The gradient (Fig. 27) was established by filling
one of the 10-litre compartments with the apparently preferred salinity
and the other compartment with the acclimation salinity. If a prefer-
ence for more than one salinity was displayed, these salinities were
also tested in the horizontal gradient. The 50 mm water bridge join-
ing the two salinity compartments was always made up of the least
dense of the two test waters. During the observation periods a fish
was not considered to be in a compartment unless it was below the
level of the water bridge. Once the gradient was established with
well aerated water five fish were placed into one compartment and
allowed to become quiescent in the tank for at least one hour. This
adjustment was considered complete when the fish were observed swimming
across the water bridge twice. Observations of positions were made at
one-minute intervals for 60 minutes. Four replicates were conducted
for each apparently preferred salinity for both species. Both the
positions of the salinities and the compartment in which the fish were
placed were shifted after each replicate run. Prior to and immediately
after the test the salinity of each compartment was checked.

Results. The salinity in each layer of the vertical gradient or
compartment of the horizontal system was not observed to change in
either salinity preference apparatus during the course of any test
(Fig. 28).

Factorial analysis of variance was used to detect the effects
of salinity, habituation and the interaction of the two on the

FIGURE 27

Horizontal salinity gradient apparatus adapted from
Houston (1957). The apparatus is constructed of ½ inch
plywood with a front of ¼ inch plate glass. The
dimension of each compartment is 0.3x0.3x0.11 metres
and a 50-mm water bridge (B) is used. Water input-
outlet tubes (A) are constructed from ¼-inch plexiglass
tubes 0.13 metres long into which small holes have been
drilled.
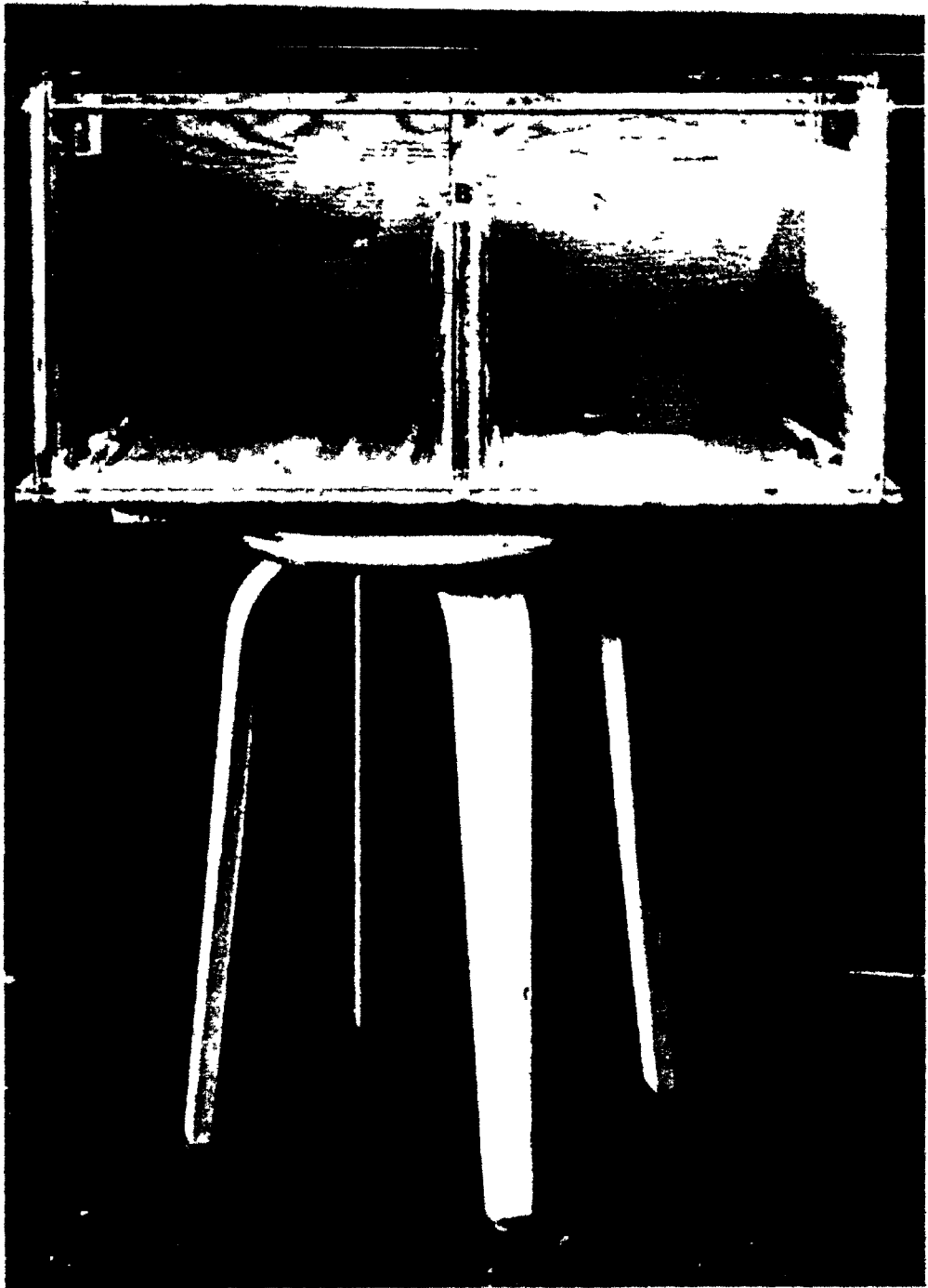
FIGURE 28

Frequency distributions for mummichog and banded killifish in the
vertical salinity gradient apparatus under control and experimental
conditions. Data represent pooled observations at each acclimation
salinity. Salinity values given alongside experimental test
frequencies are the mean salinities of water in the associated
compartment before the tests began (upper) and at the end of the
tests (lower). Shaded areas indicate experimental test, blank areas
indicate control test.

ACCLIMATION SALINITY

31.0‰   14.0‰   0.0‰

DEPTH & SALINITY

F. heteroclitus

F. diaphanus

FREQUENCY
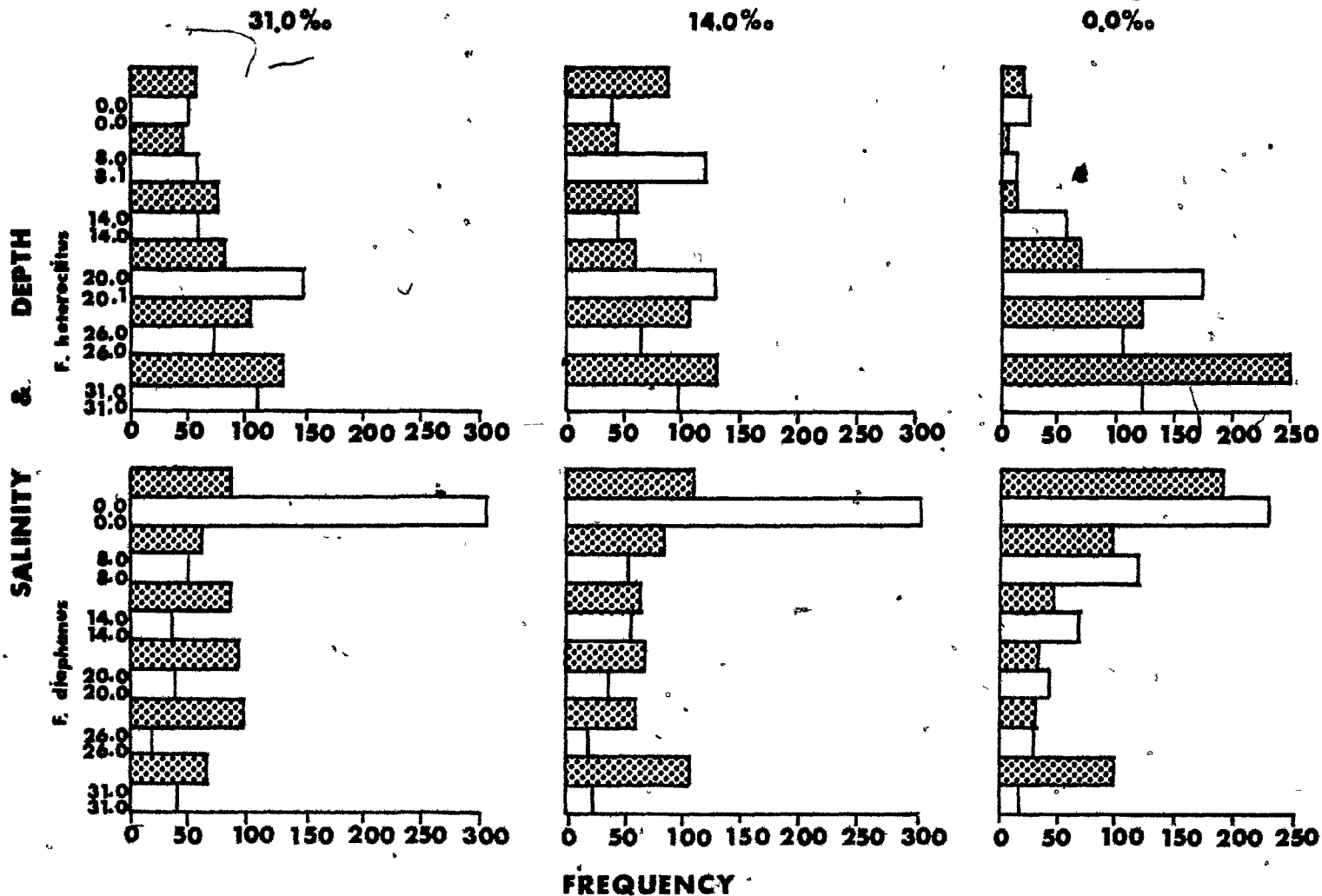
distribution of mummichog and banded killifish in the vertical salinity gradient (Tables 9 and 10). Neither habituation, nor interaction of habituation and differing salinities, appear to affect the distribution of either mummichog or banded killifish within a vertical salinity gradient (P > 0.05). However, the salinity gradient does appear to affect the distribution of each species (P < 0.05).

Although the distribution of mummichog in the salinity gradient seems to indicate a salinity preference (Fig. 28), analysis of variance of the control tests (Table 9) shows that depth also has a significant effect on distribution of this species throughout the trials. The conclusion that salinity preference determines the distribution pattern in the experimental runs cannot be accepted. A χ^2 analysis (Fig. 29) of the pooled results of the control experimental tests indicates that the distributions observed in the two types of tests were significantly different (P < 0.001). In using the χ^2 analysis one assumption had to be made, that being that the distribution represented by the pooled results of the control tests were assumed to indicate the distributional pattern that could be attributed to a factor other than differing salinities and therefore be the expected value of the χ^2 statistic.

Comparison of pooled control with the pooled experimental data of the mummichog salinity preference experiment indicates that this species shows slight preference for a salinity of 20‰ (Fig. 29). Tests in the horizontal gradient showed that 20‰ S was the preferred salinity no matter the acclimation (Table 11). Analysis by χ^2 test

TABLE 9

Factorial analysis of variance for salinity preference trials of mummichog
and banded killifish in relation to salinity acclimation and salinity choice.
Results of five replicates with three acclimations (see text).

Species: *Fundulus heteroclitus* (mummichog).

Source	df	Sum of Squares	Mean Squared	F Ratio	Probability
Acclimation	2	5.36	2.68	0.037	> 0.05
Salinity	5	5000.00	999.99	13.861	< 0.001
Acclimation x Salinity	10	1561.40	156.14	2.164	> 0.05
Error	72	5194.40	72.14		

Species: *Fundulus diaphanus* (banded killifish)

Source	df	Sum of Squares	Mean Squared	F Ratio	Probability
Acclimation	2	1.76×10^{-23}	8.80×10^{-24}	-	> 0.05
Salinity	5	29212.00	5842.4	83.56	< 0.001
Acclimation x Salinity	10	1491.90	149.19	2.13	> 0.05

TABLE 10

Factorial analysis of variance for depth preference trials of mummichog and banded killifish in relation to salinity acclimation and depth choice. Results of five replicates with three acclimations (see text).

Species: *Fundulus heteroclitus* (mummichog)

Source	df	Sum of Squares	Mean Squared	F Ratio	Probability
Acclimation	2	5.21×10^{-24}	2.6×10^{-24}	–	> 0.05
Depth	5	8609.10	1721.80	24.87	< 0.001
Acclimation x Depth	10	3793.30	379.33	5.478	< 0.05
Error	72	4985.60	69.24		

Species: *Fundulus diaphanus* (banded killifish)

Source	df	Sum of Squares	Mean Squared	F Ratio	Probability
Acclimation	2	1.67×10^{-23}	5.83×10^{-24}	–	> 0.05
Depth	10	1960.10	392.03	3.85	> 0.05
Acclimation x Depth	15	2532.30	253.23	2.49	> 0.05
Error	72	7333.60	101.86		

FIGURE 29

Pooled frequency distributions for (A) mummichog and (B) banded killifish in the vertical salinity gradient apparatus under control and experimental conditions. Salinity values given alongside the experimental test frequencies are mean salinities of water in the associated compartment before the tests began (upper) and at the end of the tests (lower). Chi square for control = expected and experimental = observed for mummichog is 481.87 @ df = 5 P < 0.001 and for banded killifish is 867.72 @ df = 5 P < 0.001. Shaded areas indicate experimental tests, blank area indicates control tests.

A

B

SALINITY & DEPTH

0.0
0.0

8.0
8.1

14.0
14.0

20.0
20.1

26.0
26.0

31.0
31.0

8.0
8.0

14.0
14.0

20.0
20.0

26.0
26.0

31.0
31.0

0 100 200 300 400 500 600

0 100 200 300 400 500 600 700 800 900

FREQUENCY
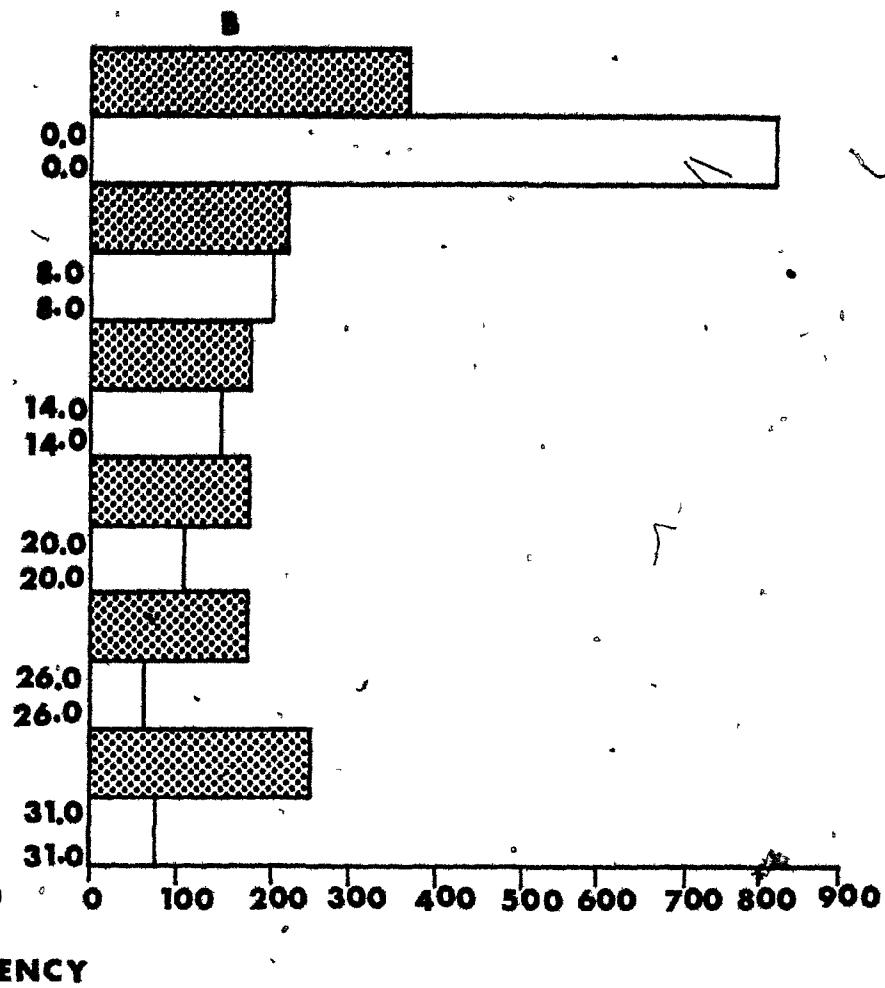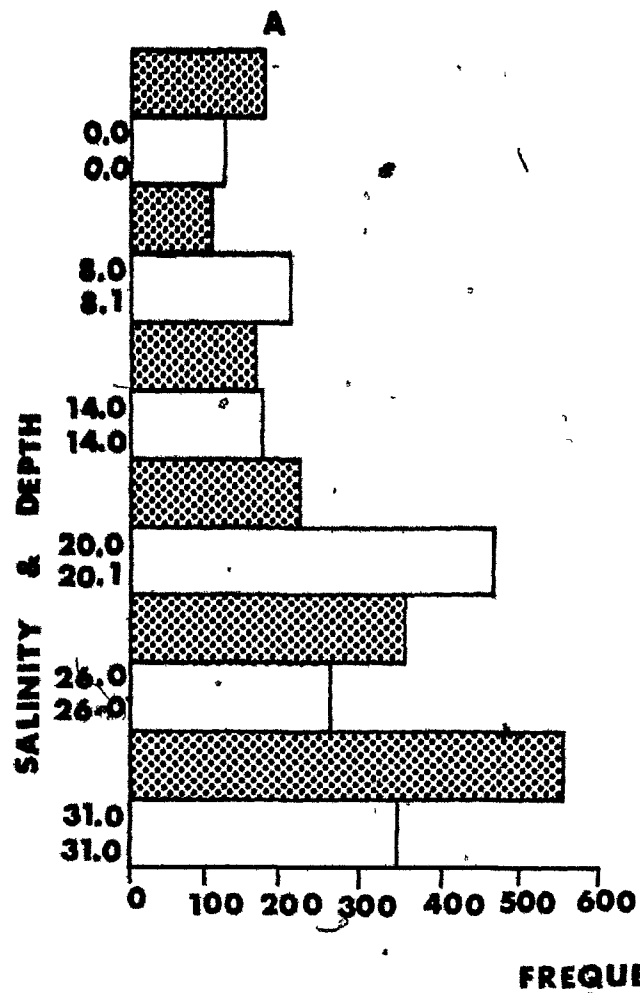
of the pooled and unpooled observations of the replicates showed that in each instance there was a highly significant difference in the salinity selected, and in all instances 20%₀ was the preferred salinity when available.

Results of the analysis of variance for the control tests of banded killifish (Table 10) indicates that depth does not have a significant effect on the distribution of this species within the vertical salinity apparatus. Thus, the factor that appears to affect the distribution of banded killifish within a vertical salinity gradient is salinity. A comparison between pooled control and experimental tests by χ^2 shows a highly significant difference (P < 0.001) in distribution (Fig. 29). The comparison also indicates that banded killifish have a distinct preference for fresh water (Fig. 28 and 29). This preference is corroborated by horizontal tests (Table 11).

Discussion. The results obtained in these salinity preference experiments do not completely agree with those obtained by Griffith (1972) who also tested mummichog and banded killifish for salinity preferences. Griffith noted that banded killifish showed a fresh water preference, while mummichog showed no salinity preference. A comparison of the experimental distribution patterns of the two species indicates that banded killifish have more sharply defined salinity preferences than do mummichog (Fig. 28 and 29 and Tables 9 and 10). The apparently less defined salinity preference displayed by mummichog may, in part, account for the differences between these experiments and those conducted by

TABLE 11

Anaylses of χ^2 for horizontal salinity gradient salinity preference experiments for mummichog and banded killifish. Yates' correction $\left(\Sigma[(o-e)-\frac{1}{2}]^2/e\right)$ was used in all calculations. χ_r^2 = chi square for each replicate, χ_p^2 = chi square for pooled replicates.

Species	Acclimation %o	Replicate	Side	Test Salinity %o	No. of Individuals	χ_r^2	P_r	χ_p^2	P_p
mummichog	31.0	1	right	31	171				
			left	14	129	5.88	< 0.05		
		2	right	14	120				
			left	31	180	12.00	< 0.001		
		3	right	31	184				
			left	14	116	15.41	< 0.001		
		4	right	14	125				
			left	31	175	8.33	< 0.001	40.33	< 0.001
mummichog	31.0	1	right	31	117				
			left	20	183	14.52	< 0.001		
		2	right	20	204				
			left	31	96	38.88	< 0.001		
		3	right	31	121				
			left	20	179	11.21	< 0.001		
		4	right	20	184				
			left	31	116	15.41	< 0.001	75.0	< 0.001

Table 11 continued

Species	Acclimation ‰	Replicate	Side	Test Salinity ‰	No. of Individuals	X_r^2	P_r	X_p^2	P_p
mummichog	14	1	right	31	115				
			left	20	185	16.33	0.001		
		2	right	20	184				
			left	31	116	15.41	0.001		
		3	right	31	117				
			left	20	183	14.52	< 0.001		
		4	right	20	187				
			left	31	113	18.25	< 0.001	64.4	< 0.001
mummichog	0.0	1	right	0	33				
			left	20	117	47.04	< 0.001		
		2	right	20	118				
			left	0	32	49.30	< 0.001		
		3	right	0	15				
			left	20	135	96.0	< 0.001		
		4	right	20	117				
			left	0	33	47.04	< 0.001	233.12	< 0.001
mummichog	0.0	1	right	31	86				
			left	20	114	3.87	< 0.05		
		2	right	20	183				
			left	31	17	137.78	< 0.001		

Table 11 continued

Species	Acclimation %o	Replicate	Side	Test Salinity %oo	No. of Individuals	X_r^2	P_r	X_p^2	P_p
mummichog	0.0	3	right	31	49				
			left	20	151	52.02	< 0.001		
		4	right	20	125				
			left	31	75	12.50	< 0.001	147.92	< 0.001
banded killifish		1	right	14	38				
			left	0	112	36.5	< 0.001		
		2	right	0	108				
			left	14	42	29.04	< 0.001		
		3	right	14	40				
			left	0	110	32.66	< 0.001		
		4	right	0	106				
			left	14	44	25.62	< 0.001	123.0	< 0.001
banded killifish	14	1	right	14	39				
			left	0	111	34.56	< 0.001		
		2	right	0	109				
			left	14	41	30.82	< 0.001		
		3	right	14	40				
			left	0	110	32.66	< 0.001		

Table 11 continued

Species	Acclimation %oo	Replicate	Side	Test Salinity %oo	No. of Individuals	χ_r^2	P_r	χ_p^2	P_p
banded killifish	14	4	right	0	108				
			left	14	42	29.04	< 0.000	126.96	< 0.001
banded killifish	31	1	right	0	111				
			left	31	39	34.56	< 0.001		
		2	right	31	42				
			left	0	108	29.04	< 0.001		
		3	right	0	126				
			left	31	24	69.36	< 0.001		
		r	right	31	47				
			left	0	103	20.90	< 0.001	146.02	< 0.001

Griffith. However, the results of two separate experiments tend to indicate that mummichog do show a preference for 20‰S. K. L. Tay (1973, pers. comm.) has shown that the hatching success of mummichog is maximum when ova are incubated in a salinity of 20‰, thus indicating that a preference for 20‰S may have some survival benefits for the species.

The salinity preferences observed in both species can, in part, explain the salinity related distributional differences of mummichog and banded killifish in Nova Scotia. However, perhaps even of more significance is that salinity preference can act as an effective isolating mechanism.

Rheotaxis

Water currents are an environmental factor that must be encountered and overcome by both mummichog and banded killifish at some time if either species is to enter the habitat of the other. An experiment was designed to determine the behavior of both species in respect to water currents, and to determine whether or not the behavior patterns could act as isolating mechanisms.

Materials and Methods. An apparatus was designed to produce water currents of any desired flow rate (Fig. 30). The apparatus consisted of an oval channel 140 mm wide, 65 mm high, and 1.8 metres centre channel circumference. The channel was constructed from two

2.3 metres long, 32 mm o.d. rubber tubes taped together one on top
of the other. The tubes were filled with water, the ends joined
together by means of a 30 mm plexiglass adapter, and the oval placed
into a 0.9x0.6x0.6 metre aquarium provided with a 60 mm standpipe.
A smaller 65 mm high, 1.3 metre in circumference oval was placed
within the larger oval, thus producing the 140 mm width of the cur-
rent channel. Two water input tubes, one on either side of the chan-
nel, were placed between the two rubber hoses of the outer oval in
such a way that they not only projected into the channel, but also
were directed along the side of the outer oval. Each water input
hose was regulated by means of a screw clamp while constant pressure
was maintained by using a submersible pump in a reservoir that was
constantly being supplied with water. Once the channel was filled
a current could be produced by adding water from one or the other
water input tubes. Current directions could be reversed by shutting
down one input tube and turning on the other input tube. Flow rates
in the channel could be adjusted by controlling the input flow rate.

The flow rate of the currents produced were estimated by
timing, with a stopwatch, one complete uninterrupted circumvention
of the channel by a cork, weighted to neutral buoyancy. Although
the apparatus produced eddies and side currents which caused the cork
to speed up or slow down in different portions of the channel, the
total time to circumvent provided a value from which the average
flow rate could be calculated.

FIGURE 30

Overhead view of rheotaxis apparatus constructed from
two 2.3 metres long, 32 mm o.d. rubber tubes and two
1.3 metres long, 32.0 mm o.d. rubber tubes arranged in
a 0.9x0.6x0.6 metre aquarium to produce a 140 mm wide
oval channel. Water input is from a submersible pump
(A) kept in a reservoir. Current is produced and
controlled by adjusting water flow from the input tubes
(B). Fish in this figure display positive rheotaxis.
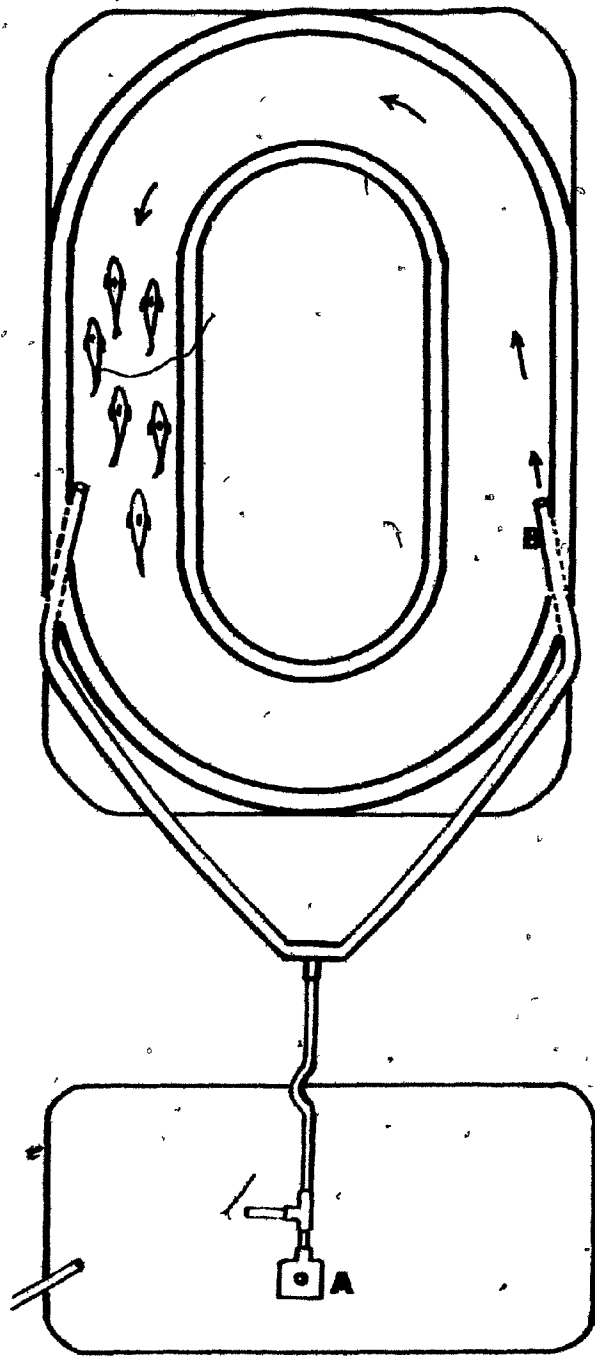
Once the apparatus was set up six fish were placed into the channel and permitted to become habituated for one hour. After the habituation period a current was produced, its flow rate estimated, and the responses of the fish recorded for two minutes. The flow rate was then increased and responses observed. This procedure was repeated for 11 different flow rates. After the completion of the 11th the current was reversed and the response to the 11 flow rates recorded again. Four replicates were made for both species. Three types of responses were recorded, positive (+) one in which the fish oriented themselves into the current, negative (-), one in which the fish oriented themselves away from the direction of the current, and no response (0), when apparently random swimming was observed.

Results. With the exception of slightly mixed responses displayed at flow rates of 0.06 m/sec and 0.08 m/sec, mummichog responded either positively or not at all (Table 12). No rheotactic responses were recorded at flow rates below 0.04 m/sec. Both no response and positive responses were observed at 0.04 m/sec indicating that this flow rate is close to the rheotactic threshold of this species. At flow rates greater than 0.13 m/sec mummichog did not appear to be able to make upstream headway. However, at flow rates below 0.13 m/sec these fish had little difficulty in making headway upstream.

Banded killifish respond to currents differently at differing flow rates (Table 12). No rheotaxis was observed in this species at

TABLE 12

Rheotactic responses of 4 replicates of 6 fish for both mummichog and banded killifish. + = fish face into the current, - fish face away from the current, o = apparent random movement. I = the initial direction of current, R = reversal of current direction.

Species: *Fundulus heteroclitus* (mummichog)								
	Replicates							
Flow Rate m/sec	1		2		3		4	
	Response		Response		Response		Response	
	I	R	I	R	I	R	I	R
0.04	o	o	o	o	o	o	o	o
0.05	o	o	+	+	+	+	o	o
0.06	±	±	+	+	+	+	+	+
0.07	+	+	+	+	+	+	+	+
0.08	+	+	+	+	+	+	+	+
0.09	+	+	+	+	+	+	+	+
0.11	+	+	+	+	+	+	+	+
0.13	+	+	+	+	+	+	+	+
0.14	+	+	+	+	+	+	+	+
0.16	+	+	+	+	+	+	+	+
0.23	+	+	+	+	+	+	+	+

Species: *Fundulus diaphanus* (banded killifish)								
	Replicates							
Flow Rate m/sec	1		2		3		4	
	Response		Response		Response		Response	
	I	R	I	R	I	R	I	R
0.04	o	o	o	o	o	o	o	o
0.05	-	-	-	-	-	-	-	-
0.06	-	-	-	-	-	-	-	-
0.07	±	±	±	±	-	-	-	-
0.08	±	±	±	±	±	±	-	-
0.09	+	+	+	+	+	+	+	+

Table 12 continued

Flow Rate m/sec	Replicates							
	1 Response		2 Response		3 Response		4 Response	
	I	R	I	R	I	R	I	R
0.11	+	+	+	+	+	+	+	+
0.13	+	+	+	+	+	+	+	+
0.14	+	+	+	+	+	+	+	+
0.16	+	+	+	+	+	+	+	+
0.23	+	+	+	+	+	+	+	+

flow rates of 0.04 m/sec and below, while negative rheotaxis was observed at flow rates between 0.04 and 0.06 m/sec. Mixed rheotactic responses are observed between flow rates of 0.06 and 0.08 m/sec and positive rheotaxis was displayed at flow rates greater than 0.08 m/sec. The mixed responses shown at flow rates between 0.06 m/sec and 0.08 m/sec indicate that the threshold for positive and negative responses occurs between these flow rates. Banded killifish could make headway in the current of flow rates of 0.14 m/sec and below while at rates greater than 0.14 m/sec the fish could only maintain their position.

Discussion. The positive rheotactic responses made by mummichog concur with the observations of Chidester (1916), who noted that this species migrates into the streams of New Jersey. The mixed responses noted at 0.06 and 0.08 m/sec may be an indication that these flow rates act as cues which cause the fish to seek some body of water either upstream or down. Similar water seeking behavior has been reported for mummichog by Chidester (1916). Current initiated water seeking behavior could also account for the tidally induced movements of mummichog described by Moore (1922) and Buttner and Brattsom (1960).

Livingstone (1951) reported that the banded killifish of Nova Scotia inhabit rapidly moving streams as well as slow moving rivers and lakes. This ability to inhabit such diverse water systems is reflected by banded killifish rheotactic response. The extent of

negative rheotaxis observed indicates that banded killifish could be induced to move downstream, thus providing a mechanism for species dispersal and range extension. Such movements also indicate that banded killifish could be induced by slow currents to move seaward.

Although rheotaxis experiments indicate that swift currents could act as an effective isolating mechanism, the positive response made by mummichog to moderate currents, rates below 0.13 m/sec, indicate that currents would be an ineffective isolating mechanism. Thus, moderate currents could attract mummichog into areas inhabited by banded killifish, while the negative rheotactic responses of banded killifish could provide a mechanism by which these fish would be attracted to areas inhabited by mummichog.

COMPARISON OF INDICES OF PHYSIOLOGIC CONDITION

Although collections of mummichog have been made in moderately brackish water in Nova Scotia (Table 1) mummichog most commonly inhabit highly saline estuaries and salt marshes. Livingstone (1951) noted that with the exception of one specimen he did not find mummichog in fresh water or in the tidal reaches of rivers. Banded killifish, however, have only rarely been found inhabiting brackish or marine waters in Nova Scotia. The water of middle and lower Porters Lake, as mentioned previously, ranges from slightly to moderately brackish water (0.6 to 15.5‰S) a condition which is approximately intermediate between typical environments of mummichog and banded killifish.

In order to determine the possible effects of habitation in an environment intermediate to the norm, comparisons were made of such indicators of population condition as age structure, growth rates, length-weight relationships, and fecundity. The comparisons were made between an estuarine population of mummichog collected at Petpeswick Inlet, Nova Scotia, and the population in Porters Lake, and between a fresh water population of banded killifish collected in Kejimkujik Lake, Nova Scotia, and the population in Porters Lake.

Age and Growth

Age-classes present in the four populations were determined according to the graphic method of polymodal separation of Cassie

(1963) (Fig. 31 to 34). Tests for statistical significance of aging of all populations indicate no significant departures between observed and expected normal distribution. These results were verified through aging by counts of annual growth rings of otolith (Fig. 35). Overlaps that may have occurred between young of the year and members of age-class I were eliminated by analyzing samples from each population collected during peak spawning periods. Thus, the youngest and smallest fish in any collection had to be, at least, a member of age-class I.

Development of annuli is similar among the mummichog populations. The first annulus is found among fish ranging in size from 35 to 50 mm total length (TL), the second in fish ranging in size from 55 to 74 mm TL, the third in fish ranging in size from 66 to 83 mm TL, the fourth in fish ranging in size from 78 to 95 mm TL. The first annulus in the banded killifish occurs in fish ranging between 24 to 48 mm TL, the second in fish 49 to 68 mm TL, the third in fish 67 to 80 mm TL, and the fourth 75 to 110 mm TL. These size ranges in the four populations fall within the size ranges of the four year classes as determined by the method of Cassie (Fig. 31 to 34).

Growth rate of each population was calculated by plotting the mean size at each age, as derived from the Cassie cumulative frequency analysis, against age-class (Fig. 36 and 37). Growth rate estimates were then calculated from semilogarithmic relationships

FIGURE 31

Cumulative frequency analysis for age-class determination from
length distribution of 210 mummichog collected in Petpeswick
Inlet during June 1971. Test for significance for this analysis:
$\chi^2 = 20.5$ @ $df = 20$, $P > 0.05$; circles = total cumulative
frequency distributions, crosses = cumulative frequency distribution
of age-class, numbers = age-classes 1,2,3,4.
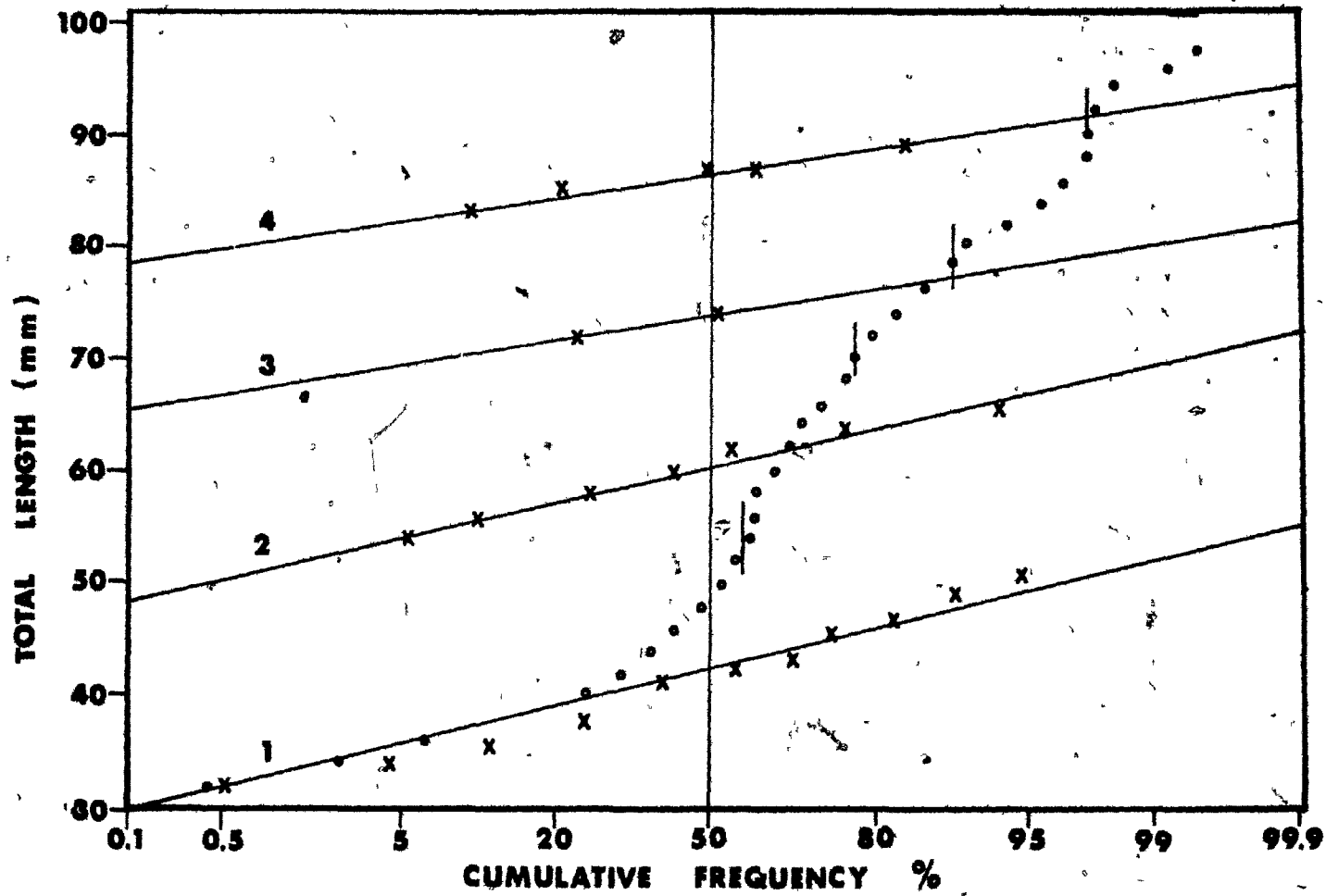
FIGURE 32

Cumulative frequency analysis for age-class determination from
length distribution of 202 mummichog collected in Porters Lake
during June 1971. Test for significance for this analysis:
$\chi^2 = 14.91$ @ df = 21, P > 0.05; circles = total cumulative
frequency distribution, crosses = cumulative frequency
distributions of age-class; numbers = age-classes 1,2,3,4.
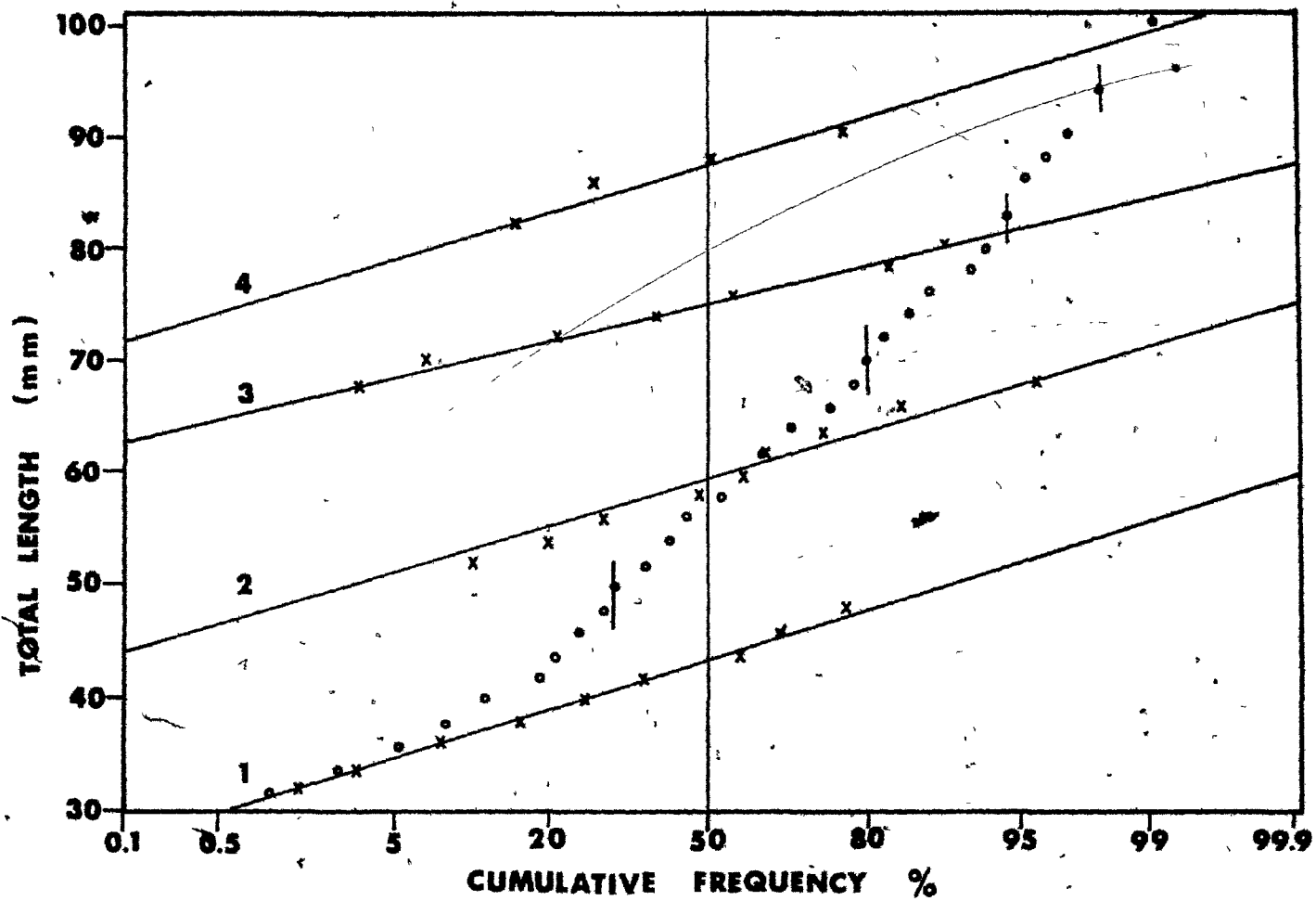
FIGURE 33

Cumulative frequency analysis for age-class determination from length distribution of 225 banded killifish collected in Porters Lake in June 1971. Test for significance for this analysis: $\chi^2 = 31.2$ @ $df = 29$, $P > 0.05$; circles = total cumulative frequency distribution, crosses = cumulative frequency distributions of age-class, numbers = age-classes 1,2,3,4.
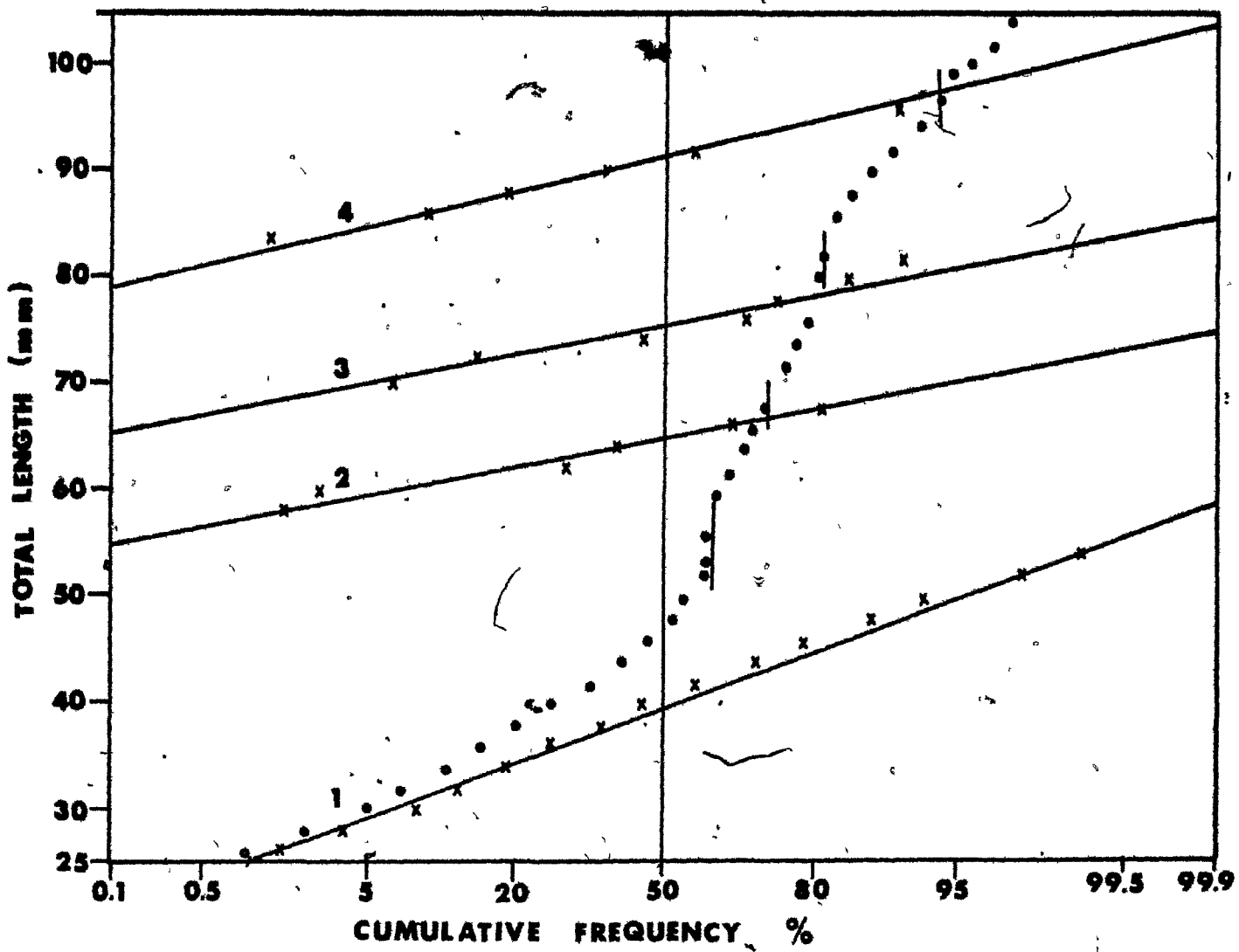
FIGURE 34

Cumulative frequency analysis for age-class determination from
length distribution of 200 banded killifish collected in
Kejimkujik Lake during June 1972. Test for significance for
this analysis: $x^2 = 21.6$ @ df = 22, P > 0.05; circles = total
cumulative frequency distribution, crosses = cumulative
frequency distributions of age-class, numbers = age-classes 1,2,3,4.
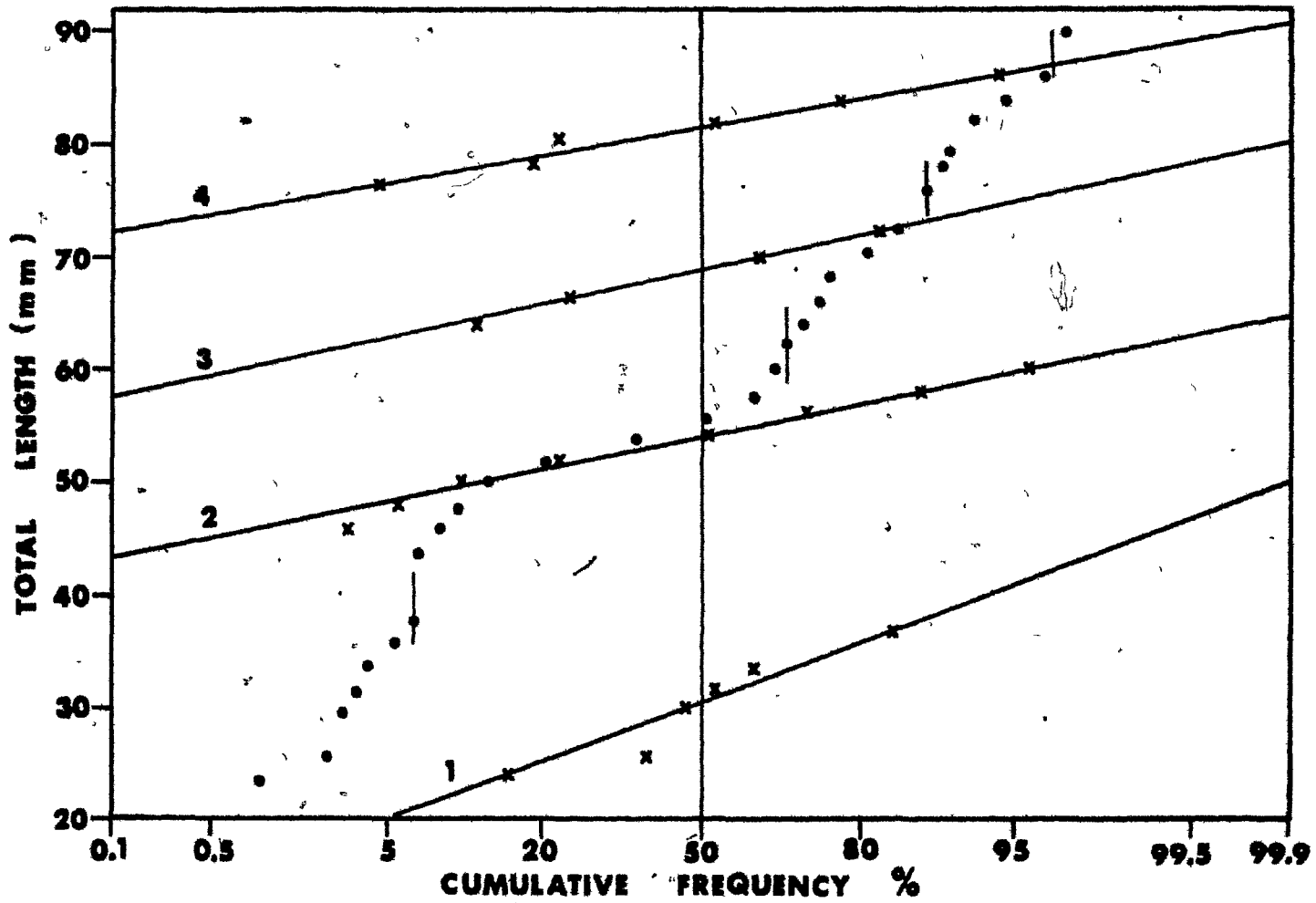
FIGURE 35

Otoliths showing annual growth rings taken from mummichog
(A-D) and banded killifish (E-H) collected in Porters Lake
during June 1971.

A   85 mm ♂ age-class IV   E   88 mm ♀   age-class IV

B   73 mm ♂ age-class III   F   77 mm ♀   age-class III

C   59 mm ♂ age-class II   G   66 mm ♂   age-class II

D   46 mm ♂ age-class I   H   46 mm ♂   age-class I
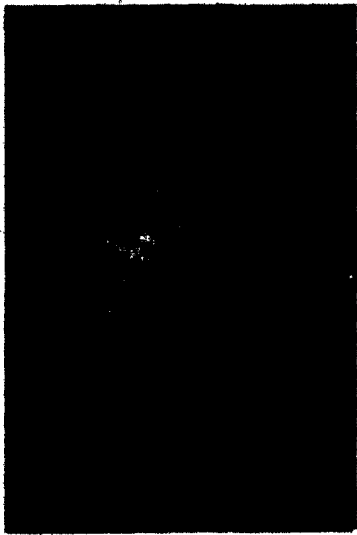
E   F
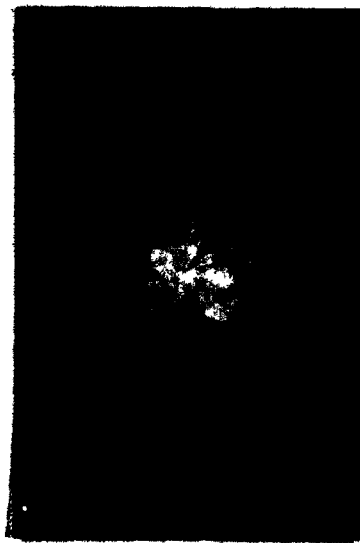
G   H
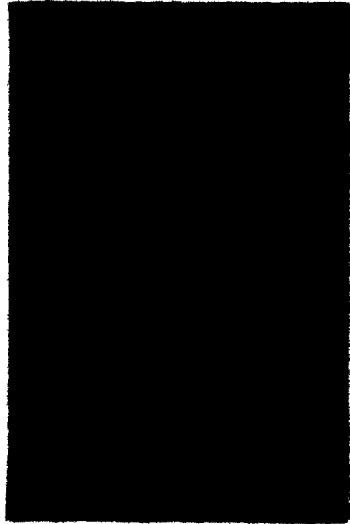
A   B
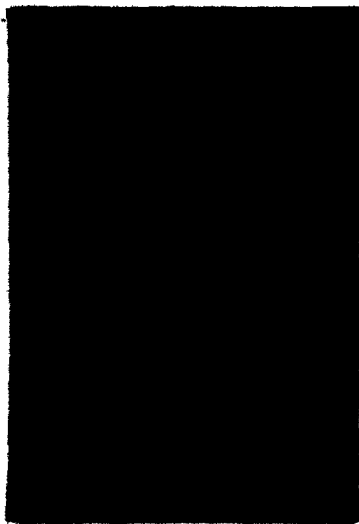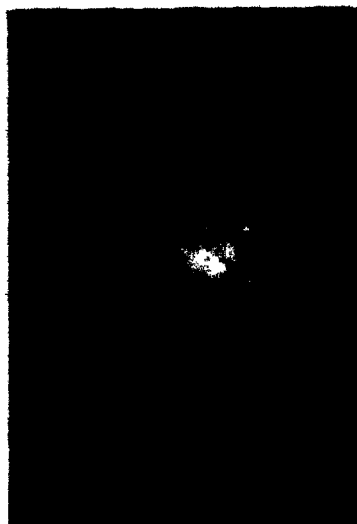
C   D

FIGURE 36

Growth rates of mummichog collected in Porters Lake (A)
and Petpeswick Inlet (B) expressed as semilogarithmic
regressions of mean length at each age to respective
age-class. Mean length at each age is taken from age-
class analysis.

Figure showing MEAN TOTAL LENGTH (mm) versus AGE CLASS.

A ln L = 3.65 + 0.21 T

B ln L = 3.56 + 0.23 T
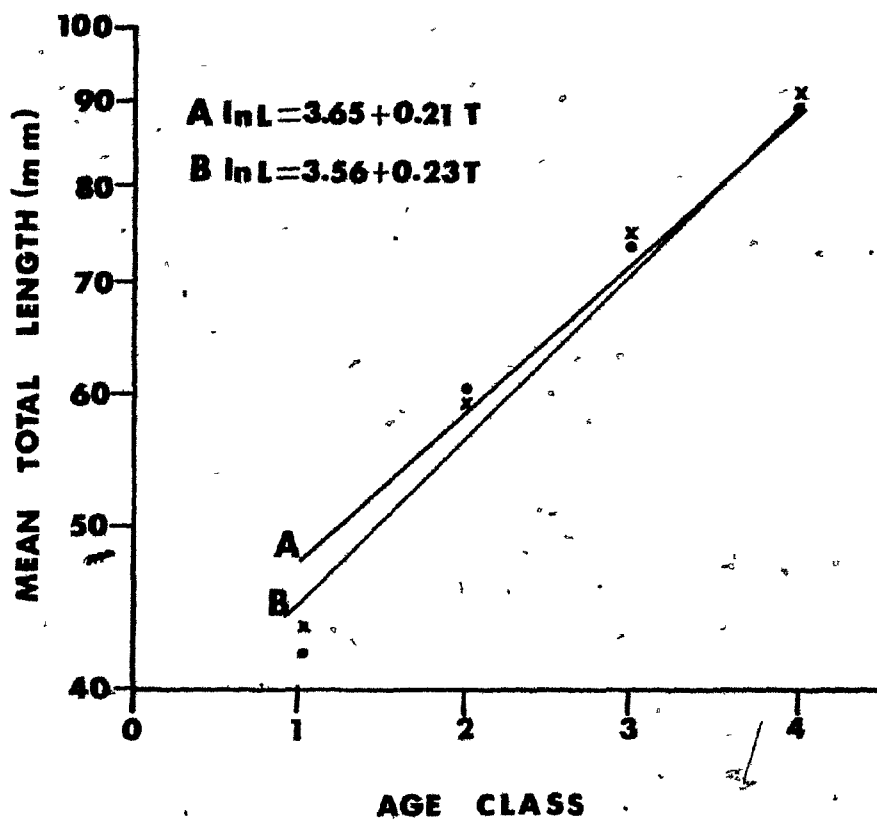
FIGURE 37

Growth rates of banded killifish collected in Porters
Lake (A) and Kejimkujik Lake (B) expressed as a semi-
logarithmic regression of mean length at each age to
respective age-class. Mean length at each age is taken
from age-class analysis.

A ln L = 3.587 + 0.244 T
B ln L = 3.231 + 0.313 T
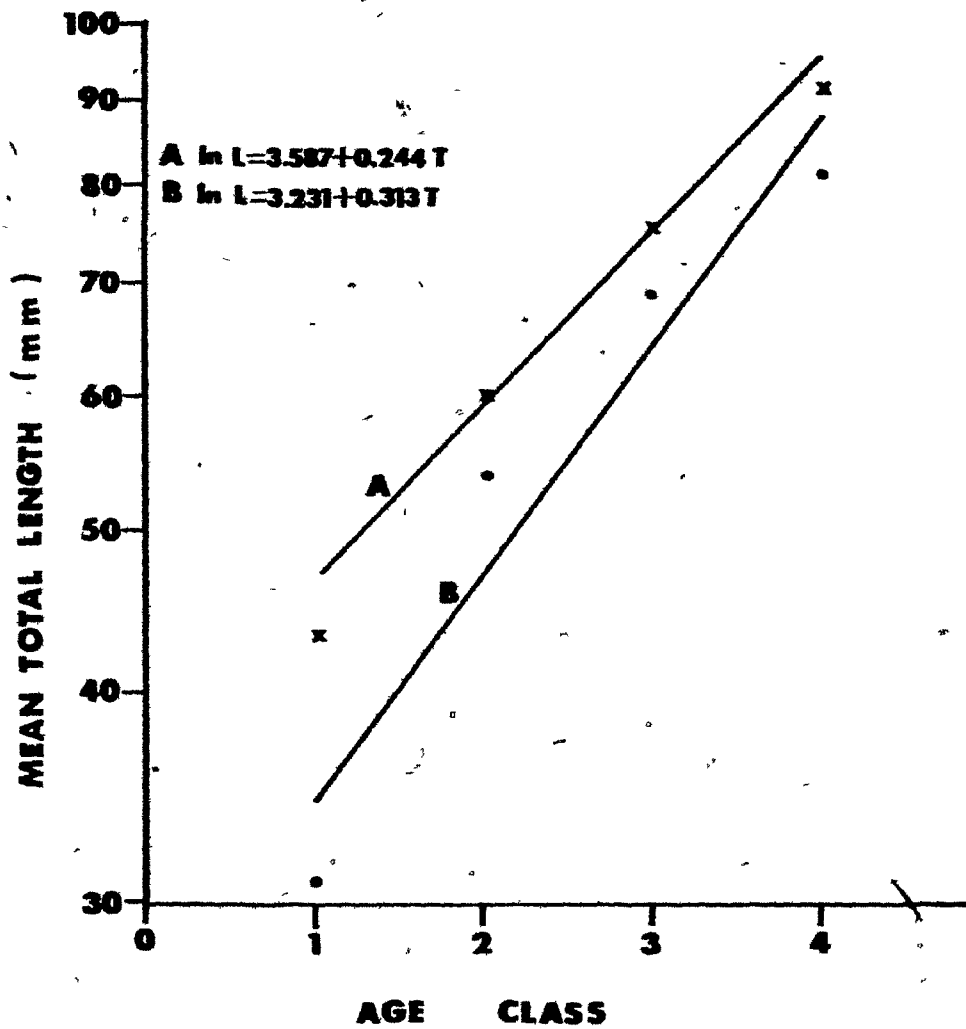
(Fig. 36 and 37). Growth rates of the mummichog of Porters Lake and Petpeswick Inlet respectively are described by the equations,

$$\ln L = 3.655 + 0.210T$$

and

$$\ln L = 3.563 + 0.233T$$

where
L = total length

T = age-class

the regression coefficients 0.210 and 0.233 being the instantaneous growth rates of each population. Comparison of the two growth rates by inspection shows that there is no difference between members of the two populations. A slight difference appears to exist between the growth rate of members of the two banded killifish populations (Fig. 37). The Kejimkujik Lake population grows slightly faster than the Porters Lake population. Growth in these populations is described by the equations,

$$\ln L = 3.587 + 0.244T$$

$$\ln L = 3.231 + 0.313T$$

for the Porters Lake and Kejimkujik Lake fishes respectively. There is, also, a conspicuous difference between mean sizes at each age of these two populations. This difference indicates that those fish in Porters Lake grow faster than those in Kejimkujik Lake during the first year, and that the difference incurred is not

compensated throughout the life span of the fishes of both populations.

Length-Weight Relationships

Length-weight relationships for the four populations were determined according to the methods of Ricker (1959). Data gathered throughout the collecting period (March to October 1971 and 1972), were used to calculate the relationships of the members of the mummichog populations, while data collected from June to September were used to calculate the relationships for the members of the two banded killifish populations. This was done to reduce the influence of seasonal variations that might have occurred within the two populations, since Kejimkujik Lake fish were only sampled from June to September, 1972.

Both mummichog populations show isometric growth (Fig. 38) described by the equations,

$$\log W = -5.287 + 3.22 \log L$$

and

$$\log W = -5.232 + 3.172 \log L$$

where W = weight in grams

L = length in mm

for Porters Lake and Petpeswick Inlet fish respectively. The correlation coefficients (r) for these regressions are 0.974 for

Porters Lake and 0.972 for Petpeswick Inlet. Analysis of covariance between the two regressions indicates there is no significant difference (P > 0.05) between the slopes and adjusted means.

Banded killifish also display isometric growth (Fig. 39). With the Porters Lake fish described by the regression equation

$$\log W = 5.090 + 3.041 \log L$$

and the Kejimkujik Lake fishes described by the equation

$$\log W = 4.830 + 2.880 \log L$$

the (r) values for these regressions are 0.986 and 0.987. Analysis of covariance between these regressions indicates that there is no significant difference between both the slopes and the adjusted means (P > 0.05).

Fecundity

Ovaries of fishes collected in Porters Lake and Petpeswick Inlet were examined after each sampling period. Fish bearing ripe ovaries were weighed, measured, ovaries removed, weighed and total number of ova counted. When ripe banded killifish appeared in the Porters Lake samples during 1972 a collection of banded killifish was made at Kejimkujik Lake. Regressions were calculated for total number of ova against total length for each of the four populations. The resultant scatter diagrams indicate that the relationships

FIGURE 38

Length-weight regressions for 1068 mummichog collected
in Petpeswick Inlet between March 1971 and September
1971 (A) and 325 mummichog collected in Porters Lake
between May and October, 1971 and 1972 (B).

A logW=-5.232+3.172 logL

B logW=-5.287+3.222 logL
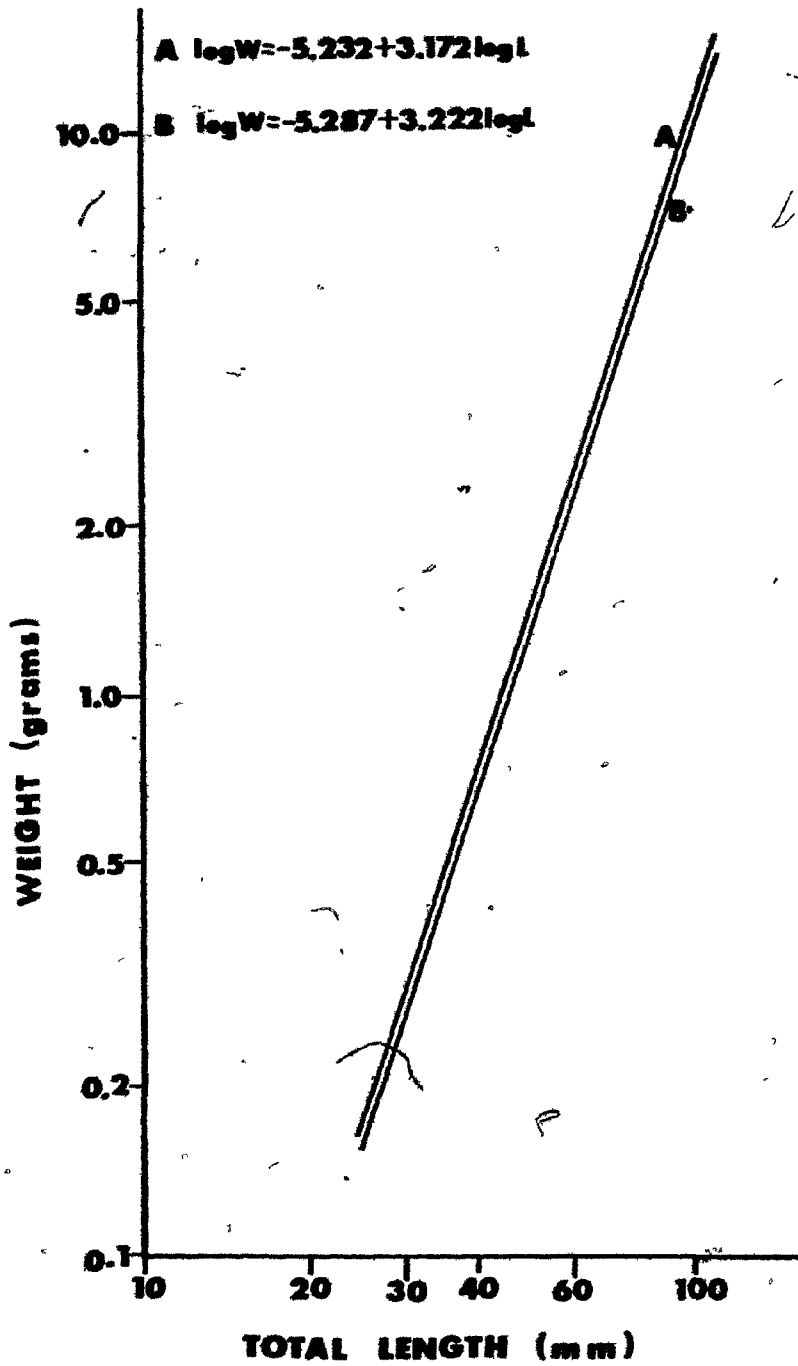
FIGURE 39

Length-weight regressions for 423 banded killifish
collected in Porters Lake between June and September
1971 (A) and 203 banded killifish collected in
Kejimkujik Lake between June 1972 and September 1972 (B).

A $\log W = -5.090 + 3.041 \log L$
B $\log W = -4.830 + 2.880 \log L$

WEIGHT (grams)

TOTAL LENGTH (mm)
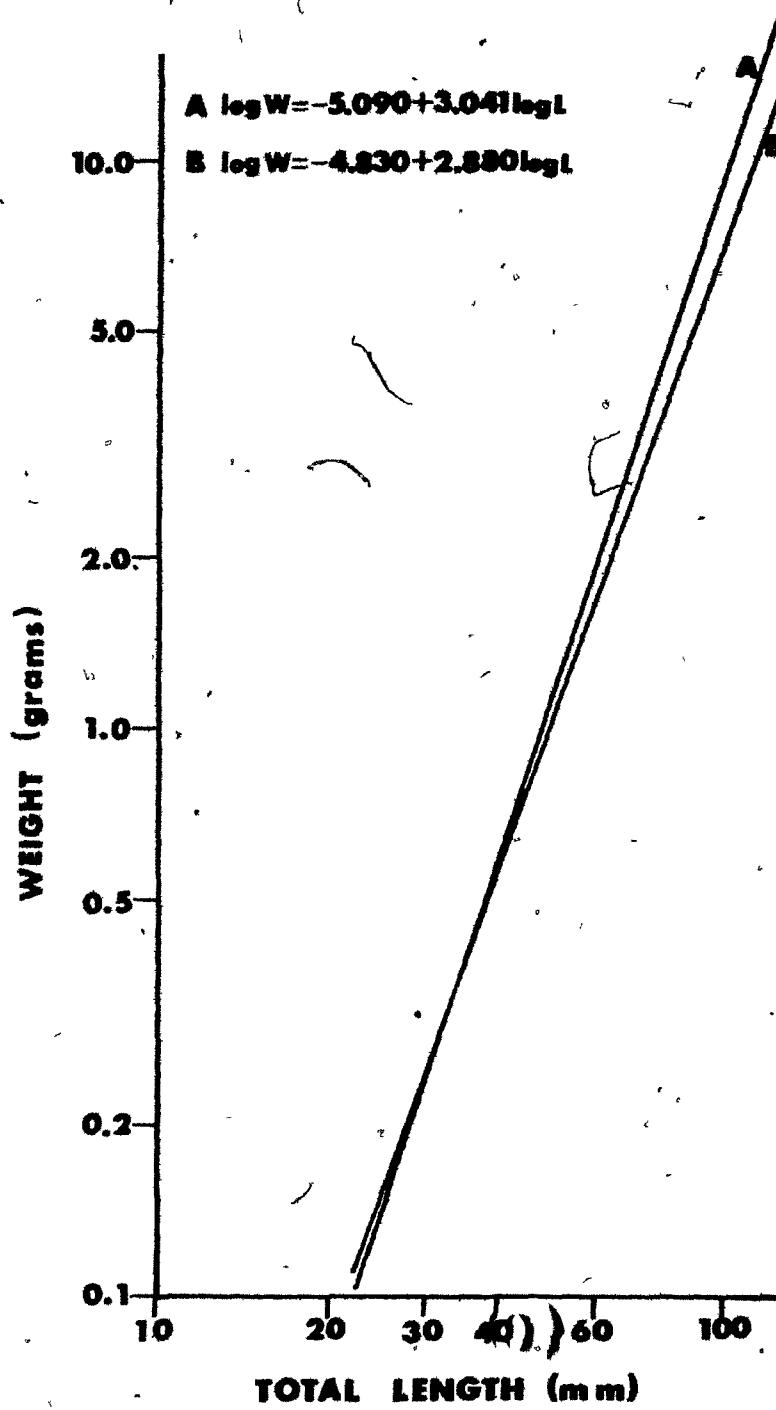
obtained are similar to that described by Bagnel (1966), that is,
$F = aL^b$. Logarithmic transformation of these data (Fig. 40 and 41)
were used to compare fecundity between populations of the same
species.

The regression equations for mummichog of Porters Lake and
Petpeswick Inlet are

$$\log F = -2.244 + 2.499 \log L$$

and

$$\log F = -1.278 + 2.038 \log L$$

where F = total number of ova

              L = total length in mm

The correlation coefficients for these data are respectively 0.925
and 0.867.

Comparison of these two regressions by analysis of covariance
indicates that there is a significant difference in fecundity
($P < 0.001$). Although fecundity overlaps somewhat among the largest
individuals, those from Petpeswick Inlet are more fecund than those
of Porters Lake (Fig. 40). The average length of spawning mummichog
in Porters Lake is 60 mm TL, with an average of 161 ova. The average
length of spawning fish in Petpeswick Inlet is 65 mm TL, but these
produce an average of 243 ova.

Fecundity of banded killifish is described by the equations,

$$\log F = -0.7864 + 1.566 \log L$$
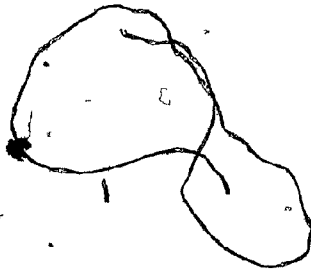
FIGURE 40

Fecundity regressions for 100 mummichog collected in
Petpeswick Inlet (A) and 73 mummichog collected in
Porters Lake (B) between 8 June and 21 July 1971 and 1972.

OVA NUMBER / TOTAL LENGTH (mm)

A log F = −1.278 + 2.038 log L

B log F = −2.244 + 2.499 log L
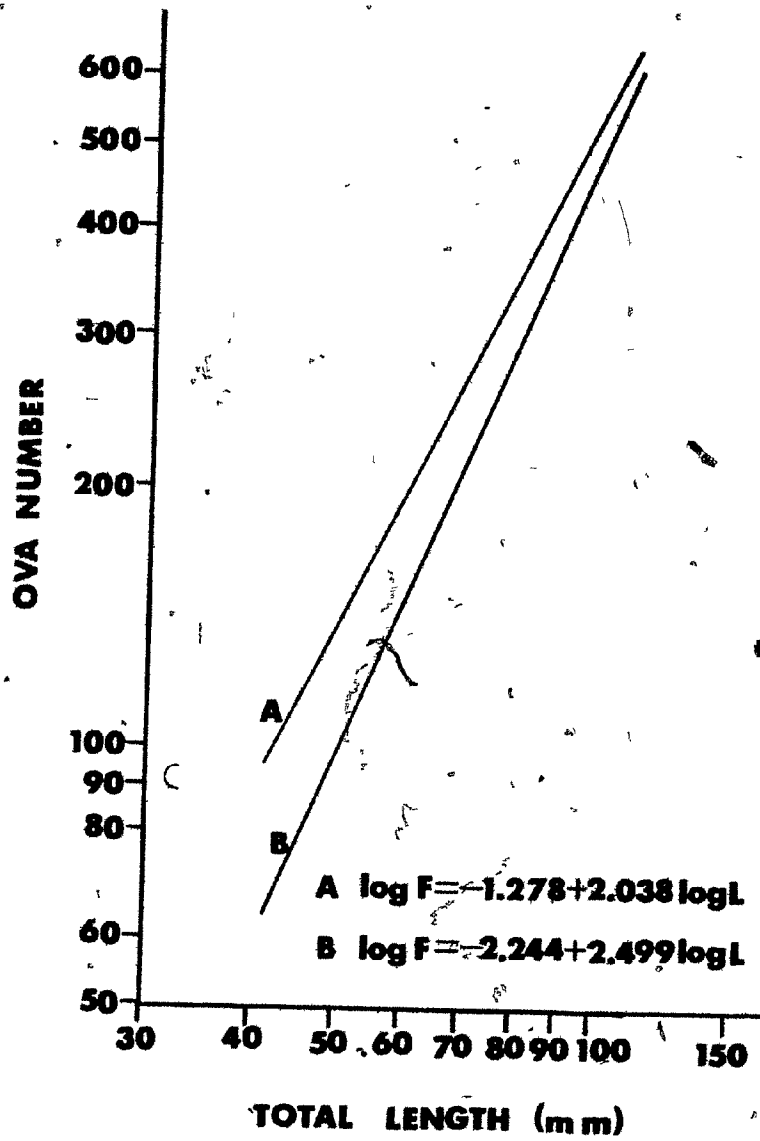
FIGURE 41

.Fecundity regression for 100 banded killifish collected
in Porters Lake (A) from 8 June to 21 July 1971 and
1972, and 35 banded killifish collected in Kejimkujik
Lake (B) from 8 June to 21 July 1972.

Graph showing OVA NUMBER (y-axis, log scale from 25 to 250) versus TOTAL LENGTH (mm) (x-axis, log scale from 40 to 150), with two lines labeled A and B:

A $\log F = -0.786 + 1.566 \log L$
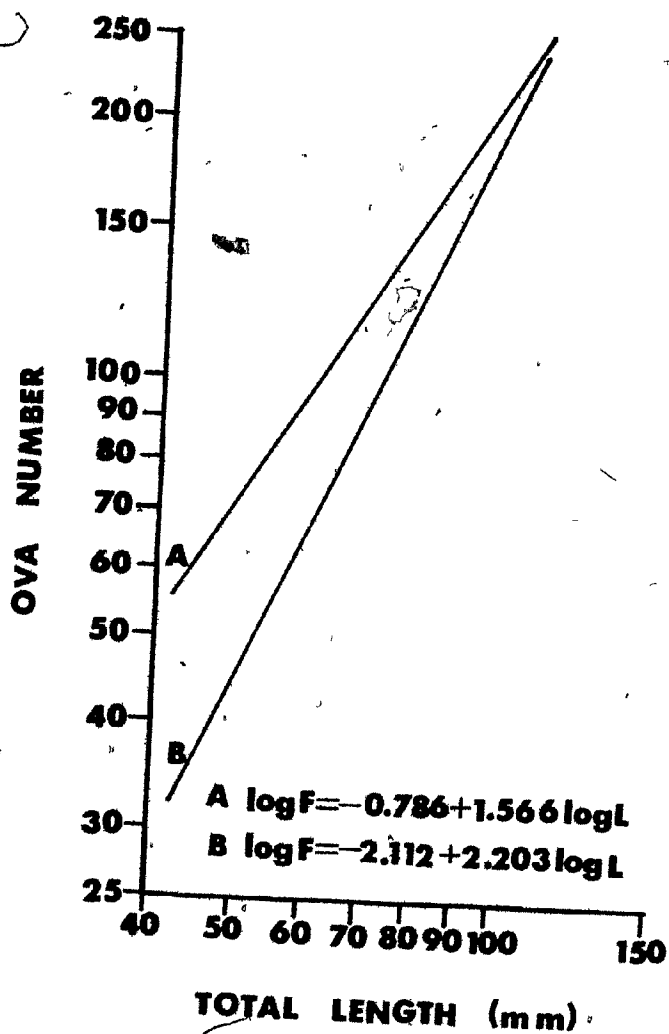
B $\log F = -2.112 + 2.203 \log L$

and

$$\log F = -2.112 + 2.203 \log L$$

for the populations of Porters Lake and Kejimkujik Lake respectively. The (r) values for these regressions are 0.719 and 0.904. Analysis of covariance between fecundity regressions of the members of the two banded killifish populations indicate a significant difference (P < 0.001) both in slope and adjusted mean. There is a slight overlap among the largest fish of both populations yet Porters Lake banded killifish are the more fecund of the two populations (Fig. 41). The average length of spawning females in Porters Lake is 73 mm TL, these produce an average of 128 ova, while the mean spawning length of females in Kejimkujik Lake is 69 mm TL, with a mean production of 88 ova.

Discussion

The results of the comparisons of growth rate and length-weight relationship show that no significant differences exist between the two mummichog populations. If growth, both in length and weight, is considered an activity determined in considerable measure by the environment (Warren and Davis, 1967) then the similar growth rates observed may indicate that the two populations are responding to the two different environments in a very similar manner.

Differences in growth observed between the two banded

killifish populations show that the Porters Lake fish grow larger
than the Kejimkujik Lake fish. Ivlev (1961) and Warren and Davis
(1967) showed that growth in fishes increased with increasing food
density. Kerekes (MS, 1973) stated on the basis of oxygen deficits,
Kejimkujik Lake can be considered very oligotrophic and low in pro-
duction. Although no indices of production were estimated for
Porters Lake, it can be considered more productive than Lake Kejimkujik.
The basis for this consideration is a comparison of production indices
obtained by Geen and Hargrave (1966) in Bras d'Or Lake, an environment
similar to middle and lower Porters Lake, with the results obtained
for Kejimkujik Lake which indicate that production is generally higher
in brackish lakes than in oligotrophic lakes in Nova Scotia. There-
fore, it may be concluded that food density is higher in Porters Lake
than in Kejimkujik Lake. This suspected increase in food density
may account for the increase in growth observed in the banded
killifish of Porters Lake.

Further evidence of Porters Lake being a more favorable
environment for banded killifish than the typical environment exem-
plified by Kejimkujik Lake may be derived from comparison of fecundity.
As can be seen in Figure 41 banded killifish of Porters Lake are more
fecund than those of Kejimkujik Lake. Such increases in fecundity
have been attributed to environmental fertility and/or food density
by Bagenal (1966), McFadden *et al.* (1965), and Wydowski and Cooper
(1966).

The lower fecundity observed in the mummichog of Porters Lake population (Fig. 40) may indicate that the food density or environmental fertility is lower in Porters Lake than in the typical environment of mummichog exemplified by the estuarine littoral environment of Petpeswick Inlet. This conclusion does not conflict with that derived from the growth comparisons. Fecundity among mummichog populations could be acting as a density-dependent population regulator similar to that proposed by Bagenal (1966) for populations of plaice, in that the total energy provided by the Porters Lake environment may be insufficient to maintain both the normal growth, as observed, and a normally higher fecundity as observed in the estuarine population. Comparison of the fecundity of the mummichog and banded killifish of Porters Lake (Fig. 42), however, indicates that mummichog still maintain a very high degree of reproductive potential, a potential high enough to permit the assumption that the Porters Lake environment is not particularly deleterious to this species.

FIGURE 42

Fecundity regressions for both mummichog (H) and
banded killifish (D) collected in Porters Lake.
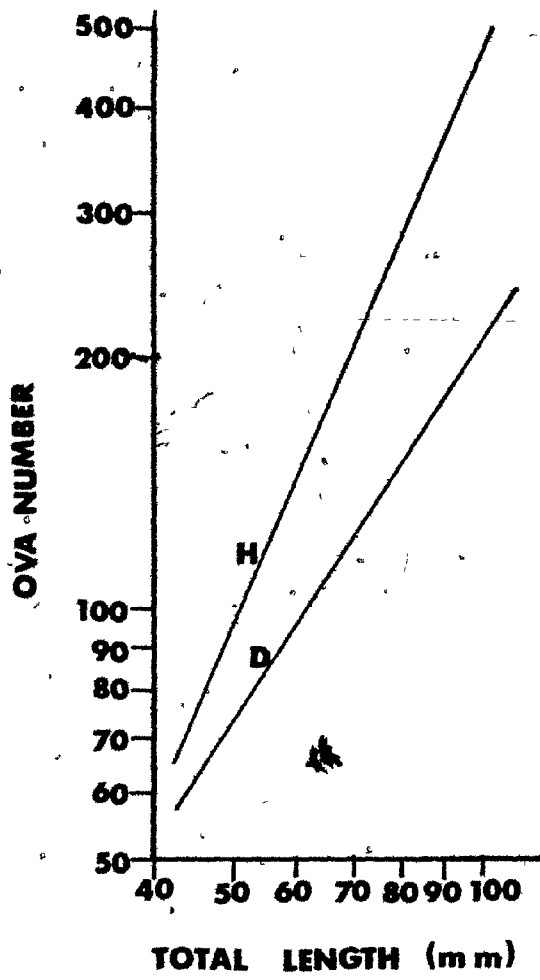
SEASONAL AND HABITAT ISOLATION

Maturation and Breeding Period

Methods. Data were gathered to determine whether there is a difference in maturation and breeding times and the contribution that such a difference would make toward reproductive isolation. The information used in the study was derived from the samples collected in the hybrid zone of Middle Porters Lake during 1971 and verified by samples collected in 1972. Mummichog collected in Petpeswick Inlet and banded killifish sampled in Kejimkujik Lake were used as indicators of maturation and breeding time among allopatric populations of each species.

The time of maturation and breeding was determined by two methods. First, the calculation, for both sexes, of mean monthly gonosomatic indices (gonad weight divided by total body weight) and second, the identification of mature ovaries. Both methods were used on samples collected in Porters Lake and Petpeswick Inlet. However, only the second method was employed on banded killifish sampled from Kejimkujik Lake.

Results and conclusions. Both mummichog populations and the Porters Lake banded killifish population mature at the same rate during the same period of time (Fig. 43 and 44). Although no data, prior to May, were available for the fishes of Porters Lake and

FIGURE 43

Mean calendar monthly gonosomatic indices of male mummichog collected
both in Porters Lake (----) and Petpeswick Inlet (——) (A), and
male mummichog (—·—·—) and banded killifish (——) both collected in
Porters Lake (B).

♂ MEAN MONTHLY GONOSOMATIC INDEX × 1000
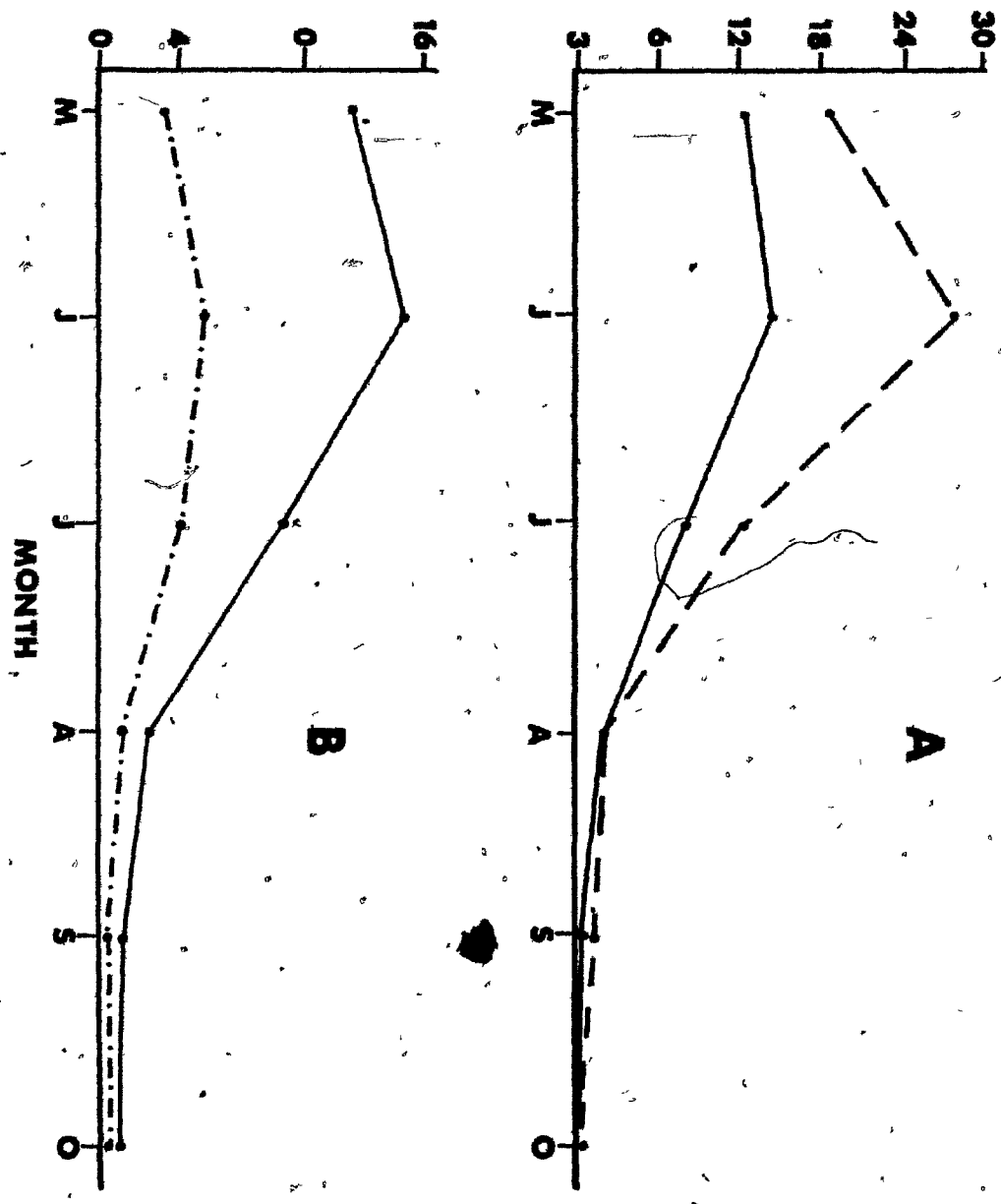
FIGURE 44

Mean calendar monthly gonosomatic indices of female mummichog
collected in both Porters Lake (-----) and Petpeswick Inlet
(———) (A), and female mummichog (—·—·—) and banded
killifish (———) both collected in Porters Lake (B).

♀ MEAN MONTHLY GONOSOMATIC INDEX × 100

MONTH

A

B
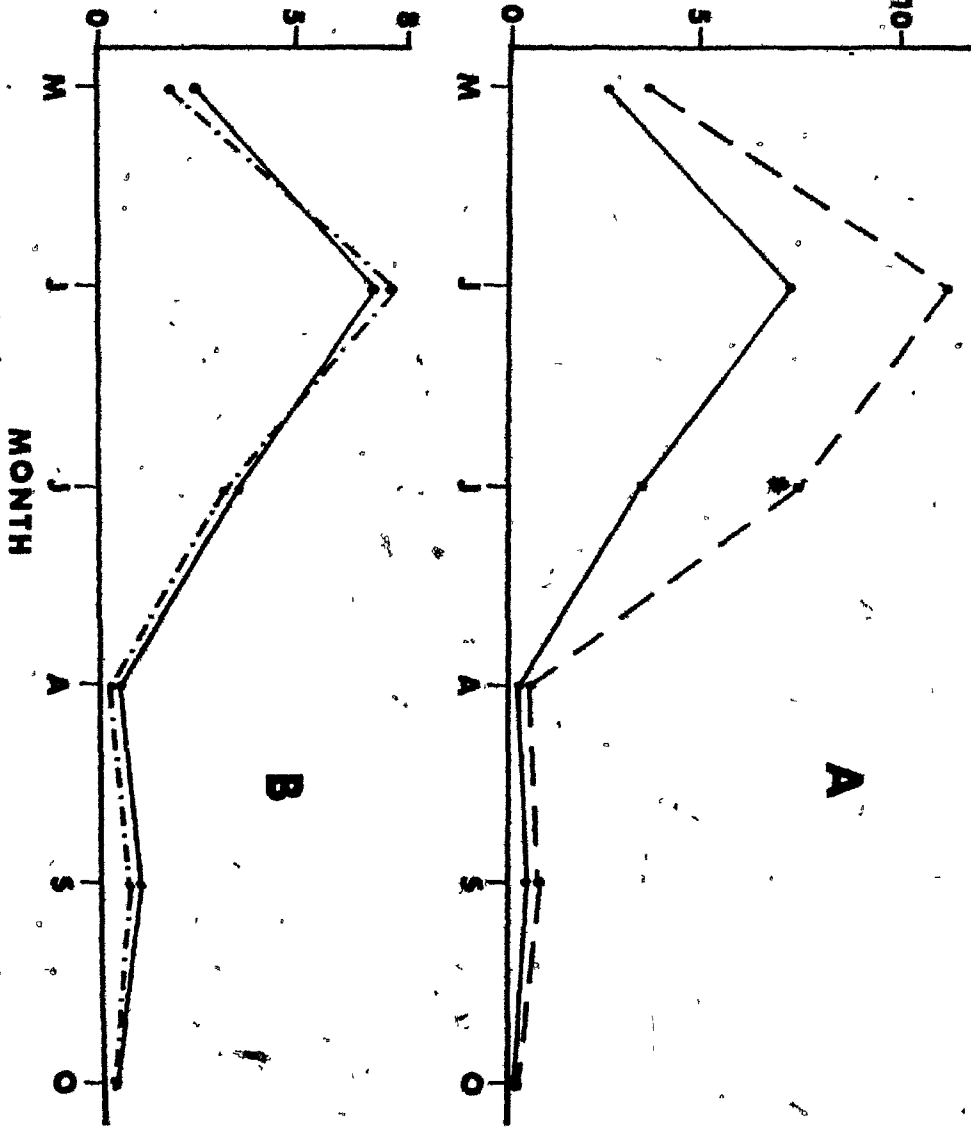
Kejimkujik Lake, the similarities in changes in gonosomatic indices
of all populations throughout the overlapping collecting periods
indicate that the changes observed in Petpeswick Inlet prior to May
(Fig. 43 and 44) also occurred in the two other populations. Thus,
it may be assumed that maturation in both sexes commences in April
and peaks in June. This June peak coincides with the initial occur-
rence of ripe females in all populations. In 1971, ripe females of
the two mummichog populations and the Porters Lake banded killifish
population appeared in samples collected from 8 June to 21 July.
From 6 June to 18 July, 1972, ripe females occurred in all four
populations thereby indicating that the peak spawning period for
both species extends from mid-June to mid-July. Also, suspected
hybrids were ripe during this same one-month period. Of note also
is that the mean recorded water temperatures during these calendar
months were approximately 20 C at all collecting sites (Fig. 3 and
Table 4). The optimum temperature for maximum hatching success for
mummichog is 20 C (K.L. Tay, pers. comm.).

Selection of Spawning Habitat

Differences in selection of spawning areas can act as a
premating isolating mechanism, especially when the spawning periods
of two sympatric species overlap (Hubbs, 1955 and 1961). A compari-
son of the species composition of catches made in the study area in
which hybrids occur (Table 4) show that during the spawning period

banded killifish are more plentiful than mummichog in study areas
PO1 and especially PO2, while in study area PO6 mummichog exceed in
number banded killifish. Such differences in the catch make-up
indicated that the two species have different habitat preference.
This hypothesis was tested by means of habitat preference experiments.

Materials and Methods. Mummichog and banded killifish used in the
experiments were collected during the height of the spawning period.
The fish were segregated by species and held in the laboratory facil-
ities and under the conditions described previously. Substrate
preferences of both species were evaluated for eight weeks from the
middle of July (just prior to the end of peak spawning period) to the
beginning of September so that any changes in preference that might
occur could probably be detected.

Two types of preference tests were conducted in succession,
both mummichog and banded killifish together, and mummichog and
banded killifish separately. Five replicates were performed for the
individual species, while four replicates were performed for the two
species combined.

The apparatus used was a 1.2x0.6x0.6 metre wooden aquarium,
waterproofed by vinyl paint, partitioned down the centre by a 50 mm
high piece of wood and provided with a 0.4 metre stand pipe (Fig. 45).
Water was continuously supplied to the aquarium at a constant rate.

FIGURE 45

Substrate preference apparatus the dimensions of which are 1.2x0.6x0.6 metres
is partitioned down the centre by a 50 mm high piece of wood, and provided
with a 0.4 metre stand pipe. The apparatus was constructed entirely from
3/4 inch plywood, waterproofed by vinyl paint. Water was continuously supplied
from a reservoir (A) into the selection chambers (B) by means of inlet tubes (C).
Substrate of either type, PO6 or PO2 (see text for components), was placed
into one selection chamber and the other type into the remaining chamber.
Initially three fish were placed into each selection chamber All observations
were made from above at random intervals.
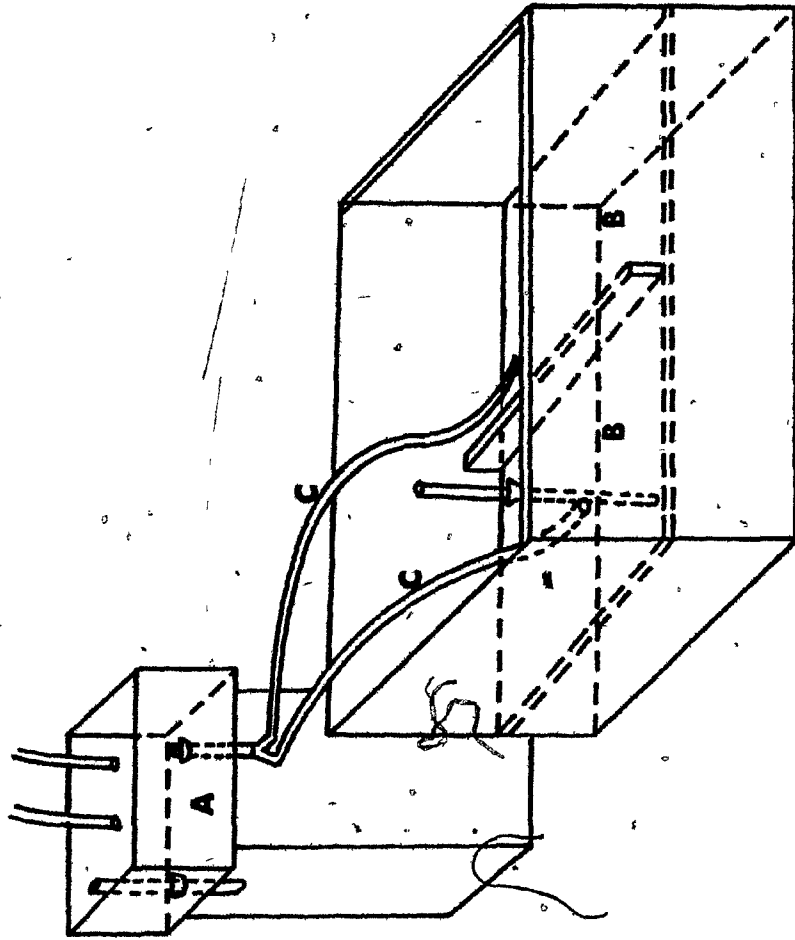
The salinity of the water was regulated variously from 4.2 to 11.3‰S to duplicate, as closely as possible, changes recorded in the hybrid zone in Middle Porters Lake. Two substrate types were collected from Porters Lake. The station PO6 type (Table 4) consisted of dense adhesive mud with associated vegetation (an area in which mummichog was the predominant fundulid). The station PO2 type (an area of the lake in which banded killifish were the predominant killifish) consisted of a combination of sand, clay and wood chips along with associated flora (Table 4).

Fish to be tested were allowed to habituate in the aquarium for 24 hours prior to the beginning of observations. A test consisted of a total of ten observations made over a three-day period of the locations of six fish. Intervals between observations were determined by using random numbers tables. Ten random observations were decided upon to reduce the influence of fright reaction displayed by the fish after each observation period.

Results and Conclusions. Contingency χ^2 tests calculated for the replicates of the three tests indicated that there were no significant differences among replicates (Table 13) so that pooling of the replicate data is permissible. Adjusted χ^2 tests calculated from the pooled data (Table 13) indicate that these species neither individually nor together have a substrate preference.

TABLE 13

Contingency Chi-square and pooled Chi-square tests for bottom habitat preference experiments for mummichog and banded killifish both separately and combined. Yates' correction $([(o-e)-\frac{1}{2}]^2/e)$ was applied to both tests. χ_c^2 = contingency, χ^2 = pooled, P = probability. PO6 and PO2 are the substrate types found in these study areas.

Species	Substrate Type	Replicates						Statistical Significance					
		1	2	3	4	5	Totals	χ_c^2	df	P	χ_p^2	df	P
F. heteroclitus	PO6	25	28	36	35	37	161						
	PO2	35	32	24	25	23	139				1.47	1	> 0.20
	Total	60	60	60	60	60	300	7.695	4	> 0.05			
F. diaphanus	PO6	32	26	26	24	32	141						
	PO2	28	34	34	25	28	159				0.96	1	> 0.3
	Total	60	60	60	60	60	300	3.265	4	> 0.50			
F. heteroclitus	PO6	17	20	17	18		72						
F. diaphanus		17	14	15	15		61						
F. heteroclitus	PO2	13	10	13	12		48						
F. diaphanus		13	16	15	15		59				3.941	3	> 0.20
	Total	60	60	60	60		240	2.167	6	> 0.90			

Since the experiment was conducted during parts of both the spawning and post spawning periods and the contingency χ^2 tests indicate homogeneity among replicates, it may be concluded that neither species has substrate preference either during or after the spawning period. Therefore, the apparent predominance of one species or the other over one substrate or the other in Porters Lake can be considered to be regulated by other conditions than that of the quality of the substrate.

ETHOLOGIC ISOLATION

Courtship and displays inherent in the spawning behavior of closely related species and genera are well known as effective isolating mechanisms. However, Mayr (1963) notes that among many species such ethologic isolating mechanisms readily become dissociated. The spawning behavior of mummichog has been described in some detail by Newman (1907) and this description has been corroborated and embellished to some extent by Chidester (1916). Spawning behavior of banded killifish has been described by Richardson (1939) and Foster (1967b). Observations of spawning made during this study agree with those reported. The descriptions along with observations of spawning behaviors of the two species indicated that the spawning activities of mummichog differ only slightly from those of banded killifish. Although time and cost did not permit a detailed study of the courtship behavior of the two species during this study, observations that were made showed that mummichog males tended to display their medial fins and flanks more vigorously than do banded killifish males, yet mummichog defend their territories against other of their own species less rigorously than do banded killifish.

Due to the general similarity of courtship and spawning activity noted in both species an investigation was made to determine the role that spawning behavior plays as an isolating mechanism.

Materials and Methods

Fish used in the investigation were collected in the hybrid zone of Porters Lake during the peak spawning period (the beginning of July). Four 40-litre glass aquaria were used in the study (Fig. 46). The bottoms of two aquaria were covered with substrate gathered from Porters Lake in order to provide a habitat somewhat similar to the natural environment. All aquaria were gravity fed at a constant flow rate with fluctuating brackish water (3.2 to 11.3‰ S). A 16-hour light, 8-hour dark photoperiod was maintained throughout the study.

Observations of spawning activity were first made between members of the same species. This was followed by observing spawning activities between members of the two species. Two males of one species and two females of the other were placed into two aquaria. The reciprocal complement was placed into the remaining two aquaria. Observations were made during all daylight hours for 14 days and all activities recorded.

Results

One observation was made of interspecific spawning between a male banded killifish and a female mummichog. Ovulation was observed, but the ova were eaten by the male before they could be recovered and examined for fertilization.

Female mummichog were observed extruding ova while vibrating their bodies against the aquarium stand pipe, sides of the aquarium and the vegetation in the aquarium. Similar observations have also been reported by Chidester (1916).

Both the observations of spawning and of self-induced ovulation were made between 1000 and 1200 Atlantic Standard Time. A general increase in activity was observed in all aquaria by all the fish during this same time period. Daily spawning periodicity has been reported for *Fundulus olivaceus* and *F. notatus* (Thomerson, 1966).

Conclusions

Although no spawning activity was observed between mummichog males and banded killifish females, such a mating could occur. For Chidester (1916) and Newmann (1907) noted male mummichog will actively mate with any female that enters its territory. The activity of females of both species in response to males does not appear to differ in any way. The only difference noted was the degree of ripeness of the females used in the experiment, mummichog being the riper and therefore possibly more receptive than banded killifish.

FIGURE 46

Spawning behavior observation apparatus consisting of four
40-litre glass aquaria that were gravity fed from a reservoir
(A) and drained by means of rubber tubing (B) connected to
stand pipes. Four fish (two males and two females) were placed
into each aquarium. During interspecific spawning experiments
reciprocal pairs were placed into each set of aquaria. The
upper two aquaria had PO2 type substrate. The lower two had
PO6 type substrate.

GENERAL DISCUSSION

Hybrids

Hybridization between mummichog and banded killifish has been reported as being a rare phenomenon (Hubbs, 1955; Griffith, 1972). Prior to this study only one acceptable report of hybridization between these fishes had been made and that for a single individual collected in the Lake of Shining Waters, Prince Edward Island (Hubbs *et al.*, 1943). A possible hybrid has been reported by Griffith (1972) from the Mill River, Connecticut. However, no morphological data have been published to verify the identification and serological data that have been reported are insufficient, in themselves, to permit unqualified acceptance of the suspected hybrid at this time. Two populations of hybrids of mummichog and banded killifish are reported in this study, one in Porters Lake, Nova Scotia, the other collected in the tidal reaches of the St. Mary's River, Nova Scotia. In each instance the hybrids have the anticipated phenotypic intermediacy to the parental species. Also, they have similar degrees of variability. These characteristics have been attributed to most F_1 hybrids (Hubbs *et al.*, 1943, 1955). Although male hybrids have been produced in the laboratory, no male hybrids were observed in any field samples made during this study. The hybrid described by Hubbs *et al.* (1943) was also a female. The occurrence of only females may be an expression of Haldane's rule, "when in first generation hybrids between two species, one sex is absent, rare or sterile, that sex is always the heterogametic sex," (Haldane,

1922). A review of this subject in fish and other lower vertebrates has been conducted by Ohno (1967), who arrived at the same general conclusion.

The occurrence of hybrids from three and possibly four geographically separated areas along with differences noted among the populations of both species and among hybrid populations indicates that the procedure of comparing suspected hybrids with allopatric populations of parental species could be misleading on theoretical bases, since an F_1 hybrid reflects the characters of its parental populations. And populations are rarely phenotypically identical in localized areas of a species range. This is particularly true among populations both of mummichog and banded killifish (Scott and Crossman, 1964; Tay and Garside, 1972). The mummichog and banded killifish hybrids of Porters Lake are phenotypically intermediate to the Porters Lake mummichog and banded killifish populations. The mummichog and banded killifish hybrids of the St. Mary's River are intermediate to St. Mary's River mummichog and banded killifish populations. The only measured characters that are similar among all hybrid populations are caudal fin ray number, and gill raker number, characters that are also constant among all parental populations. Initial comparison between sympatric and allopatric populations should indicate the extent that these phenotypically confusing phenomena are present. However, utilization of allopatric populations could aid in some instances in eliminating potential problems created by introgression or character displacement.

McAllister (1970) in an investigation of the pisci-fauna of
Sable Island, suggested that certain characters observed in insular
mummichog could, because of their seemingly intermediate nature,
have resulted from previous hybridization and introgression of mummi-
chog and banded killifish. However, comparisons of the characters
used for the differentiation between each parental species and
hybrids (Fig. 4 to 12) indicate that either backcrossing has been
rare or more probably, lacking. The apparent absence of intermediate
males, which are aggressive partners in spawning in the mummichog
and banded killifish, certainly would greatly reduce the potential
for backcrossing. Overlaps in numerical values of morphological
and structural characters that occur between these species and their
hybrids appear to be the result of individual variation or of environ-
mental effects on the development of these characters. This is par-
tially verified through comparisons between diagnostic characters of
laboratory hybrids and natural hybrids (Fig. 21 to 23); since the
value of each character of the natural hybrid falls within the range
of variation of the laboratory produced hybrid. Further evidence
for the lack of backcrossing can be derived from hybrid indices.
Greenfield and Greenfield (1972), and Hagen (1967), showed that
backcrossing is indicated when a high frequency of individual hybrid
indices overlap the indices of either one or both of the parental
species. Hybrid indices calculated for the Porters Lake populations
(Fig. 13) show that there is no overlap between the hybrid and the
parental species, thus indicating little or no detectable backcrossing.
This is partially verified by comparison of the indices of natural

hybrids with the indices of laboratory produced hybrids (Fig. 24), which shows that the frequency and range of the hybrid indices of both types of hybrids are very similar.

When it is possible for an observer to segregate visually from a mixed sample of killifish specimens, suspected hybrids which consistently display electrophoretic patterns for MDH and LDH that are identical to those in artificially produced F_1 hybrids adds still more evidence to support the notion that backcrossing does not occur. This apparent inability to backcross prevents introgression with the parental species, thus ensuring the integrity of each species.

Causes of Hybridization

Hubbs et al. (1943) and Hubbs (1955) noted that hybridization is conditioned by environmental factors, the most common of which are: disproportionate numbers of individuals of two species in sympatry, restricted spawning areas, disturbance or intergradation of the environment, and species introduction. Evidence obtained in this study suggests that three factors operating with one another are instrumental in the initiation of hybridization between mummichog and banded killifish, intergradation of the environment, disproportionate numbers, and restricted spawning areas.

Although, as has been previously noted, apparently non-hybridizing sympatric populations of mummichog and banded killifish

in brackish habitats have been reported, the establishment of sym-
patry in slightly but fluctuating brackish water appears to be a
prior condition for hybridization. Based on the natural distribu-
tion of the two species (Brown, 1957), the slightly to moderately
brackish water, in which hybrids occur, can be considered as a form
of environmental intergradataion, especially for banded killifish.
In salinity preference experiments neither species showed a preference
for the salinities recorded in the areas of hybridization. Contrarily,
each species demonstrated preferences for salinities that corresponded
to natural distributional patterns, that is, fresh water for banded
killifish and strongly brackish water for mummichog. However, popu-
lations of both species have in some way overcome salinity preferences
and established themselves in areas of environmental intergradation.

A possible explanation for the apparent breakdown of salinity
preferences may have some basis in the physiologic condition of the
two species. The mummichog population of Porters Lake does not
show any significant differences in growth, from a highly brackish
allopatric population of mummichog. Fecundity does differ between
these populations, and as previously mentioned, the differences may
be attributed to a density-dependent population regulator rather than
a deleterious effect caused by the environment. Condition of banded
killifish indicates that the slightly brackish environment has a posi-
tive effect on the over-all physiologic condition of the species.
Comparisons of the Porters Lake banded killifish populations with

fresh water populations showed that the Porters Lake population contained the largest fish collected, both in this study and in museum collections.

Evidence for the beneficial qualities of brackish water habitation of the mummichog and banded killifish can be derived from the physiological work of Garside and Jordan (1968). In that study it was shown that the upper lethal temperature of both species was higher in an isosmotic medium (14‰ S) than in water of more typical salinities. The inference which follows is that as salinity approaches isosmoticy, osmoregulatory stress is reduced and the fish becomes physiologically more capable of withstanding other metabolic stresses.

The breakdown of the salinity preference as an isolating mechanism does not, in itself, always result in hybridization. Among those sympatric populations in which hybridization between mummichog and banded killifish has been reported one species is usually more abundant than the other. This situation, which could result in the inability of an individual to encounter a conspecific mate, has been noted by Hubbs et al. (1943), Sibley (1961), Hubbs and Laritz (1961) and others as being quite instrumental in causing hybridization between species. Hubbs et al. (1943) attributed the observed hybridization between mummichog and banded killifish in the Lake of Shining Waters to this phenomenon. Inspection of the catch composition in Porters Lake during the spawning season (Table 3) shows that in all

areas in which hybrids occurred, one or the other species excessively predominates. With the exception of the catches made at study area PO6 (Fig. 2) banded killifish was the predominant species. The predominance of banded killifish was also noted in the Lake of Shining Water collection (Hubbs *et al.*, 1943). Although mummichog predominated in study area PO6, hybridization does appear to be occurring in this area.

Cross-mating experiments, in which the reciprocal crosses produced viable hybrids, indicate that, genetically, hybridization between these species is not restricted to any particular set of parents. However, in all instances in which hybridization is suspected between mummichog and banded killifish, mummichog appear to be the invading species. Areas in which mummichog is the predominant of the two species are close to apparent invasion routes, such as tidal reaches of rivers, a tidally effected outlet of a lake, and barrachois close to the mouths of small streams. Experiments on comparative rheotaxis suggest that mummichog are attracted by currents. This behavioral pattern could provide a mechanism by which mummichog would be induced to enter banded killifish habitats. Negative rheotaxis may also account in part for the movement of banded killifish into saline water since it has been shown that banded killifish are negatively rheotactic at current speeds of 0.04 to 0.06 m/sec.

The effect of disproportionate numbers as an instrument of hybridization can be further enhanced when spawning areas are

relatively restricted. Crowding, a possible natural result of
restricted spawning areas, has been suggested as the cause for hybridi-
zation between normally non-hybridizing closely related sympatric
species kept in captivity (Hubbs, 1955; Thomerson, 1966).

The extent and number of possible spawning sites are not
known in areas of hybridization; however, the littoral profile of
Porters Lake indicates that in this area of hybridization spawning
sites are quite restricted. Both mummichog and banded killifish spawn
in very shallow water (Scott, 1954; Chidester, 1916; Newman, 1907),
a condition infrequently encountered in the zone of hybridization of
Porters Lake. Although two types of habitat could be distinguished
and identified by a preponderance of one or the other species of
Fundulus, benthic preference experiments indicated that neither species
displayed a preference for either type of bottom both during and after
the spawning period. This lack of preference may indicate that the
habitat associated with each species may be the product of spatial
distribution rather than habitat selection and that this distribution
pattern may be the result of shallow depth selection, especially
during the spawning period. Indirect evidence for this is an increase
in the number of specimens of both species in catches made in shallow
water of PO1 and PO2 during the spawning season (Appendix I).

Hybridization potential represented by restricted spawning
areas is, itself, increased by overlapping spawning season. Hagen
(1967) showed that temporal isolation played a role in reducing

3 3

OF/DE

10

125
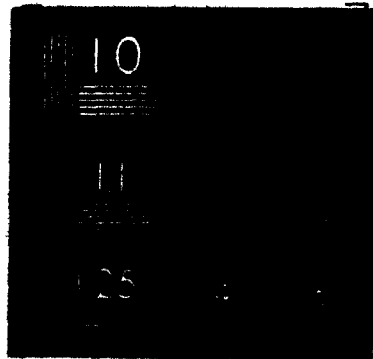
hybridization between euryhaline morphs of the *Gasterosteus aculeatus*
complex. Maturation time and spawning seasons of both sympatric and
allopatric populations of mummichog and banded killifish are completely
overlapping (Fig. 43 and 44). This phenomenon along with observations
of self-induced ovulation in mummichog made during this study and
noted by Chidester (1916) and demonstrated longevity of sperm (Hubbs,
1957, 1960, and 1961) indicate that even accidental fertilization is
quite possible between these two species.

Further evidence of restricted spawning sites as a means of
causing hybridization between mummichog and banded killifish may be
derived from interspecific spawning experiments. Foster (1967a) noted
that among sympatric species of killifish species ethologic isolation
was the most effective of the premating isolating mechanisms. Similar
conclusions were also arrived at in studies of other animals
(Dobzhansky, 1951; Speith, 1958; Sibley, 1961; Mayr, 1963; Littlejohn,
1967; and others). Within the confines of a small aquarium, however,
a mummichog female was observed spawning with a banded killifish male.
Although this observation does not by itself prove natural interspeci-
fic spawning (Hubbs, 1955; Thomerson, 1966), it does indicate that
under certain conditions this type of spawning can occur. Natural
crowding, simulated by the aquarium, could act as a behavioral releaser
that would permit interspecific spawning.

Although Hubbs (1955 and 1961) suggests that the occurrence
of one of the described environmental factors could result in the
hybridization of two fish species, the occurrence of apparently non-

hybridizing sympatric populations of mummichog and banded killifish in Newfoundland, Magdalen Islands, Quebec, and Cape Breton, Nova Scotia, indicates that more than one factor is involved as causative agents in hybridization between these two species. Evidence gained in this study suggests that at least two factors are always involved in mummichog and banded killifish hybridization, environmental intergradation and disproportionate numbers. In Porters Lake, however, restricted spawning sites also appear to be instrumental in hybridization. The occurrence of three factors in Porters Lake may account for the large number of hybrids collected (144) during the first year of the study. Since the number of hybrids declined so drastically to three during the second year of the study, hybridization probably does not occur regularly. Collecting intensities did not differ greatly between the two years nor did it appear that spawning areas had been affected by seining operations. Discontinuities in the frequency of hybridization could possibly account for the apparent rarity of the cross. If conditions necessary for hybridization occur infrequently then the results of chance hybridization would be lost due to natural mortality within four years. Age determination of the two parental species indicates that hybrids should have a four-year life span. Also, unless present in quantity, hybrids could be possibly overlooked in a mixed collection.

Evolution of Isolating Mechanisms

To date, three hypotheses on the evolution of isolating mech-
anisms between two species have been proposed. According to one,
natural selection is responsible for the establishment and re-enforce-
ment of isolating barriers (Dobzhansky, 1951). This hypothesis argues
that when species are in sympatry, individuals with poorly developed
isolating mechanisms will hybridize and produce offspring of reduced
fitness. These offspring will be diminished by natural selection,
thus resulting in the loss or reduction of the genotypes of hybridi-
zing individuals. Evidence in support of this was shown by Dobzhansky
(1951) in work done on *Drosophila*, by Mechan (1961) in studies of
anuran amphibians, and by Hubbs and Delco (1960) for cyprinodontoid
fish *Gambusia* spp. The main flaw in this hypothesis is introgressive
hybridization. As Mayr (1963) suggests, backcrossing should result
in a weakening of isolating mechanisms due to the diluting effect of
hybridization.

An alternative proposed by Muller (1940) suggests that isola-
ting mechanisms arise as an incidental byproduct of genetic divergence.
Evidence in support of this is that isolating mechanisms that have an
ecologic basis are overcome only when the two hybridizing species have
similar niches and come into contact after the breakdown of geographi-
ical barriers, a phenomenon inferred to occur among fishes by Hubbs
(1955, 1961). Further support for this is given by the necessity for
genetic reconstitution of the polygenic characters that must be involved

in the evolution of an isolating mechanism (Littlejohn, 1961).

Differences between the two hypotheses have been overcome
somewhat by Mayr (1963) who suggested that large category "isolation
mechanisms" be subdivided into primary and secondary mechanisms. The
initial evolutionary step is the acquisition of at least one primary
isolating mechanism through geographic isolation. This mechanism
usually, but not necessarily, is a form of cross-sterility or a post-
mating mechanism. The second step is selection for additional isola-
ting mechanisms, usually premating mechanisms, which as mentioned
previously, prevents the wastage of gametes.

The hypothesis proposed by Mayr (1963) appears to be applic-
able in explaining the current state of the evolution of isolating
mechanisms between mummichog and banded killifish. These two species
are considered to be quite taxonomically distinct being placed into
separate subgenera, mummichog in the subgenus *Fundulus* (Brown, 1957;
Griffith, 1972) and banded killifish in the subgenus *Fontinus* (Brown,
1957) or *Planterus* (Griffith, 1972). According to Griffith (1972)
the two species have been phylogenetically separated for a long period.
Distributional patterns of sympatric populations observed during this
study indicate that the two species are ecologically quite similar.
Comparative analysis of diet of sympatric populations of mummichog
and banded killifish partially substantiate the idea of ecologic
similarity, since both species feed on the same food items and at
similar sites in the water column. Thus when the salinity preference

barriers which in themselves could be an effective primary isolating mechanism are overcome, two ecologically similar yet phylogenetically well separated species come into contact. The hybridization that does occur results in the production of sterile F_1 hybrids which could indicate the occurrence of a second primary isolating mechanism.

The natural distribution of both species indicates that sympatry between mummichog and banded killifish occurs in relatively few isolated areas. From indirect evidence gained in this study it appears that in most instances sympatry between these species is a recent occurrence. It is, therefore, possible that insufficient time has passed for reinforcement of existing but poorly developed secondary isolating mechanisms such as those ecologic and ethologic mechanisms described for other sympatric species of *Fundulus* (Foster, 1967a).

Although Griffith (1968, 1972) reported hybrids of mummichog and banded killifish from Connecticut, hybridization between these fishes appears, from this study and that conducted by Hubbs *et al.* (1943), to be more common in the Maritime provinces of Canada than in other areas of proximal allopatry. A possible explanation for this may be lack of reinforcement of premating isolating mechanisms. Both species are potentially sympatric with at least one other cyprinodontid species throughout their respective overlapping ranges, with the single exception of Atlantic Canada. In areas beyond Atlantic Canada the other cyprinodontid species could potentially aid in

reinforcing premating isolating mechanisms. Such reinforcement has been postulated to occur among other sympatric killifish species (Foster, 1967). In Atlantic Canada no such reinforcement is possible until the two species under consideration come into sympatry. This combined with reduced genetic variability common to peripheral popu- lations (Mayr, 1963; Dobzhansky, 1951) make Atlantic Canada an area well suited for hybridization between the two species of *Fundulus*.

SUMMARY

1. Mummichog and banded killifish appear to hybridize rarely and then only in specific localized areas.

2. All hybrids produced appear to be F_1 hybrids and there is no indication of further stages of hybridization or of backcrossing or that introgression occurs.

3. Hybridization takes place in slightly but variably brackish water in which banded killifish are indigenous and in which mummichog have invaded more recently.

4. In Porters Lake three environmental factors appear to be instrumental in causing or allowing hybridization between mummichog and banded killifish:

   a. Environmental intergradation may produce conditions in which salinity preferences that normally separate the species are overcome.

   b. Disproportionate numbers have possibly brought about conditions in which individuals of either of the species do not find a conspecific mate at a critical moment of the spawning period.

   c. Restricted spawning sites have possibly caused crowding of the two species which in turn could have led to the overcoming of ethologic mechanisms.

5. Disproportionate numbers and restricted spawning sites are further enhanced as factors contributing to hybridization

by complete overlap in maturation time and an apparent lack of habitat preference.

6. Interspecific spawning, as well as accidental fertilization, could result in hybrids.

7. Hybridization is noted only in the northeastern limit of the ranges of both species. However, both species are known to occur sympatrically throughout their common Atlantic coast range.

8. Maritime hybridization may be the result of recent localized occurrences of sympatry. The time of sympatry may not have been sufficient to reinforce possible primary isolating mechanisms. However, effective postmating mechanisms appear to be sufficient to maintain species integrity.

LITERATURE CITED.

Armstrong, P.B. and J.S. Child. 1965. Stages in the normal develop-
ment of *Fundulus heteroclitus*. Biol. Bull. 128 (2): 143-168.

Bagenal, T.B. 1966. The ecological and geographical aspects of
the fecundity of the plaice. J. Mar. Biol. Ass. U.K. 46:
161-186.

Brown, J.L. 1957. A key to the species and subspecies of the
cyprinodont genus *Fundulus* in the United States and Canada
east of the continental divide. J. Wash. Acad. Sci. 47
(3): 69-77.

Brown, W.L. and E.O. Wilson. 1956. Character displacement.
Syst. Zool. 3 (2): 49-63.

Butner, A. and B.H. Brattstrom. 1960. Local movement *Menidia*
and *Fundulus*. Copeia 1960: 139-141.

Cassie, R.M. 1963. Tests of significance for probability paper
analysis. N.Z.J. Sci. 6 (4): 474-482.

Chidester, F.E. 1916. A biological study of the more important
of the fish enemies of the salt marsh mosquito. N.J. Agr.
Exper. Sta. Bull. 300. 16 pp.

Dobzhansky, T. 1937. Genetics and the origin of species. 1st ed.
Columbia University Press, New York.

Dobzhansky, T. 1951. Genetics and the origin of the species. 2nd ed. Columbia University Press, New York. 364 pp.

Forbes, S.A. 1883. The food of the smaller freshwater fishes. Bull. Illinois State Lab. Nat. Hist. 1 (6): 61-86.

Forbes, S.A. and R.E. Richardson. 1908. The fishes of Illinois. Illinois State Lab. Nat. Hist. 3: 357 pp.

Foster, N.R. 1967a. Trends in the evolution of the reproductive behavior in killifish. In Proceedings of the International Conference on Tropical Oceanography. U. of Miami Inst. of Mar. Sci.: 549-566.

Foster, N.R. 1967b. Comparative studies on the biology of killifishes (Pisces, Cyprinodontidae) Ph.D. thesis. Cornell University, Ithica, New York. 369 pp.

Fritz, E.S. 1970. Life history of the California killifish *Fundulus parvipinnis*, Girard, in Anaheim Bay, California. M.A. thesis. California State University at Long Beach, Calif. 97 pp.

Fritz, E.S. 1972. Total diet comparison in fishes by Spearman rank correlation coefficients. Copeia, in press.

Garside, E.T. 1969. Distribution of insular fishes of Sable Island, Nova Scotia. J. Fish. Res. Bd. Canada 26 (5): 1390-1392.

Garside, E.T. and C.M. Jordan. 1968. Upper lethal temperatures at various levels of salinity in the euryhaline Cyprinodontids *Fundulus heteroclitus* and *F. diaphanus* after isosmotic acclimation. J. Fish. Res. Bd. Canada 25 (12): 2717-2720.

Garside, E.T., J.M. Snair, and D.L. Waugh. 1972. Insular fishes of Iles de la Madeleine, Gulf of St. Lawrence, Canada. Can. Field Nat. 86: 275-278.

Geen, G.H. and B.T. Hargrave. 1966. Primary and secondary production in Bras d'Or Lake, Nova Scotia, Canada. Verh. Internat. Verein. Limnol. 16: 333-340.

Greenfield, D.W. and T. Greenfield. 1972. Introgressive hybridization between *Gila orcutti* and *Hesperoleucus symmetricus* (Pisces: Cyprinidae) in the Cuyama River basin, California: I meristics, morphometrics, and breeding. Copeia 1972 (4): 849-859.

Griffith, R.W. 1968. Analysis of serum protein in the teleost genus *Fundulus*. Amer. Zool. 8 (4): 806.

Griffith, R.W. 1972. Studies on the physiology and evolution of killifishes of the genus *Fundulus*. Ph.D. thesis. Yale University, New Haven, Conn. 470 pp.

Hagen, D.W. 1967. Isolating mechanisms in three spine sticklebacks *(Gasterosteus)*. J. Fish. Res. Bd. Canada 24 (8): 1637-1692.

Haldane, J.B.S. 1922. Sex ratio and unisexual sterility in hybrid animals. J. Genet. 12: 101-109.

Hayes, F.R. and E.H. Anthony. 1958. Lake water and sediments. I characteristics and water chemistry of some Canadian east coast lakes. Limnol. Oceanog. 3 (3): 299-307.

Houston, A.H. 1957. Responses of juvenile chum, pink, and coho salmon to sharp sea water gradients. Can. J. Zool. 35: 371-383.

Hubbs, C.L. 1955. Hybridization between fish species in nature. Syst. Zool. 4: 1-20.

Hubbs, C.L. 1961. Isolating mechanisms in the speciation of fishes. *In* W.F. Blair (ed) Vertebrate speciation. U. of Texas Press, Austin, Texas: 5-23.

Hubbs, C.L., B.W. Walker, and R.E. Johnson. 1943. Hybridization in nature between species of American Cyprinodont fishes. Contr. Lab. Vertebr. Biol. Univ. Mich. 23: 1-21.

Hubbs, C.L. and K.F. Lagler. 1948. Fishes of the Great Lakes region. Cranbrook Inst. Sci. Bull. 26: 186 pp.

Hubbs, C. 1957. Duration of sperm viability as a factor in frequency of fish hybridization. Tex. J. Sci. 9 (4): 472-474.

Hubbs, C. 1960. Duration of sperm function in the percid fishes *Etheostoma lepidum* and *E. spectabile* associated with sympatry of the parental populations. Copeia 1960 (1): 1-8.

Hubbs, C.   1961.   Differences between the gametic compatibility of
Etheostoma lepidum sperm and eggs of related percid fishes
from allopatric and sympatric populations. Tex. J. Sci.
13 (4): 427-433.

Hubbs, C. · 1970. Teleost hybridization studies. Proc. Cal. Acad.
Sci. 38 (15): 289-298.

Hubbs, C. and K. Strawn. 1957. Survival of F_1 hybrids between fishes
of the subfamily Etheostominae. J. Exptl. Zool. 134 (13):
33-62.

Hubbs, C. and G.E. Drewry. 1959. Survival of F_1 hybrids between
cyprinodont fishes, with a discussion of the correlation
between hybridization and phylogenetic relationships.
Inst. Mar. Sci. 4: 81-91.

Hubbs, C. and E.A. Delco. 1960. Mate preferences in males of four
species of Gambusiine fishes. Evolution, 14: 145-152.

Hubbs, C. and C.M. Laritz. 1961. Natural hybridization between
Hadropterus scierus and Percina caprodes. S.W. Nat. 6:
188-192.

Hurley, D.A. and W.L. Woodal. 1968. Responses of young pink salmon
to vertical temperature and salinity gradients. Int. Pac.
Sal. Fish. Comm. Prog. Rept. (19): 80 pp.

Iylev, V.S. 1961. On the utilization of food by plankton eating
fishes. Trudy Sevastapol Biol. Sta, 14: 188-201.
(Fish. Res. Bd. Canada Transl. Ser. 447.)

Kerekes, J. MS 1973. Aquatic resource inventory, Kejimkujik
National Park, N.S. Limnological survey of 29 lakes.
Canadian Wildlife Serv. MS reports.

Liem, A.H. and W.B. Scott. 1966. Fishes of the Atlantic coast of
Canada. Fish. Res. Bd. Canada Bull. 155: 485 pp.

Littlejohn, M.J. 1969. The systematic significance of isolating
mechanisms. In Syst. Biol. Publ. 1692. Nat. Acad. Sci.:
459-482.

Livingstone, D.A. 1951. The fresh water fishes of Nova Scotia.
Proc. Nova Scotia Inst. Sci. 23 (1): 90 pp.

McAllister, D.W. 1970. Fauna of Sable Island and its zoogeographic
affinities. Nat. Mus. Nat. Sci. Publ. in Zool. 4: 38-43.

McFadden, J.J., E.L. Cooper, and J.K. Andersen. 1965. Some effects
of environment on egg production in brown trout *(Salmo tratta)*.
Limnol. Oceanog. 10: 88-95.

Martin, W.R. 1949. The mechanics of environmental control of the
body form in fishes. Publ. Ontario Fish. Res. Lab. 58: 76 pp.

Mayr, E. 1961. Introduction. In W.F. Blair (ed) Vertebrate
speciation. U. of Texas Press, Austin, Texas: 1-4.

Mayr, E. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge, Mass. 797 pp.

Mecham, J.S. 1961. Isolating mechanisms in Anuran amphibians. *In* W.F. Blair (ed) Vertebrate speciation. U. of Texas Press, Austin, Texas: 24-61.

Minckley, C.O. and H.E. Klassen. 1969. Life history of the plains killifish, *Fundulus kansae* (Garman) in the Smoky Hill River, Kansas. Trans. Am. Fish. Soc. 98 (63): 460-465.

Moenkaus, J. 1910. Cross fertilization among fishes. Proc. Indiana Acad. Sci. 1910: 353-393.

Moore, P.J. 1922. Use of fishes for control of mosquitoes in northern freshwaters of the United States. U.S. Com. Fish. Append. IV: 1-60.

Muller, H.J. 1940. Bearings of the *Drosophila* work on systematics. *In* J.S. Huxley (ed) The new systematics. Clarendon Press, Oxford: 185-268.

Nelson, J.S. 1968. Hybridization and isolating mechanisms between *Catostomus commersonii* and *C. macrocheilus* (Pisces: Catostomidae). J. Fish. Res. Bd. Canada 25 (1): 101-150.

Newmann, H.H. 1907. Spawning behavior and sexual dimorphism in *Fundulus heteroclitus* and allied fish. Biol. Bull. 12 (5): 314-349.

Newman, H.H. 1908. The process of heredity as exhibited by the development of *Fundulus* hybrids. J. Exp. Zool. 5: 503-561.

Odense, P.H., T.C. Leung, and C. Annard. 1972. Comparative biochemical genetics of Atlantic herring populations. Unpublished manuscript.

Ohno, S. 1967. Sex chromosomes and sex-linked genes. Springer-Velag. New York. 192 pp.

Pearse, A.S. 1915. The food of the shore fishes of certain Wisconsin Lakes. Bull. U.S. Bur. Fish. 35: 245-292.

Richardson, L.R. 1939. The spawning behavior of *Fundulus diaphanus* (Le Sueur). Copeia 1939: 165-167.

Ricker, W.E. 1958. Handbook of computations for biological statistics of fish populations. Bull. Fish. Res. Bd., Canada 119, Ottawa. 300 pp.

Schwartz, F.J. 1972. World literature to fish hybrids with an analysis by family, species, and hybrid. Publ. Gulf Coast Res. Lab. Mus. 3: 328 pp.

Scott, W.B. 1954. Freshwater fishes of eastern Canada. Univ. Toronto Press. 128 pp.

Scott, W.B. and E.J. Crossman. 1964. Fishes occurring in the freshwaters of insular Newfoundland. Can. Dept. Fish. Queen's Printer, Ottawa, Canada.

Setzer, P.Y. 1970. An analysis of a natural hybrid swarm by means of chromosome morphology. Trans. Am. Fish. Soc. 99 (1): 139-146.

Sibley, C.G. 1961. Hybridization and isolating mechanisms. *In*
W.F. Blair (ed) Vertebrate speciation. U. of Texas Press,
Austin, Texas: 69-88.

Simon, R.C. and R.E. Noble. 1968. Hybridization in *Oncorhynchus*
(Salmonidae). I. variability and inheritance in artificial
crosses of chum and pink salmon. Trans. Am. Fish. Soc.
97 (2): 109-118.

Smith, M.W. 1947. Food of killifish and white perch in relation
to supply. J. Fish. Res. Bd. Canada 7: 22-34.

Speith, H.T. 1958. Behavior and isolating mechanisms. *In* A. Roe
and G.G. Simpson (eds) Behavior and evolution. Yale Univ.
Press, New Haven, Conn: 363-389.

Tay, K.L. and E.T. Garside. 1972. Meristic comparisons of popula-
tions of mummichog *Fundulus heteroclitus* (L.) from Sable
Island and mainland Nova Scotia. Can. J. Zool. 5 (1): 13-17.

Taylor, W.R. 1967. An enzyme method of clearing and staining small
vertebrates. Proc. U.S. Nat. Mus. 122 (3596): 1-15.

Thomerson, J.F. 1966. A comparative biosystematic study of *Fundulus
notatus* and *Fundulus olivaceus* (Pisces: Cyprinodontidae).
Tulane Stud. Zool. 3 (1): 29-47.

Warren, C.E. and G.E. Davis. 1967. Laboratory studies on the feed-
ing, bioenergetics, and growth of fish. *In* S.D. Gerking (ed)
Freshwater fish production. Blackwell Sci. Publ. Oxford.
175-214.

Weed, A.C. 1921. Fishes from southern Wisconsin. Copeia 1921. 69-72.

Wydoski, R.S. and E.L. Cooper. 1966. Maturation and fecundity of brook trout from infertile streams. J. Fish. Res. Bd. Canada. 23: 623-641.

Zerbe, W.B. and C.B. Taylor. 1953. Sea water temperature and density reductive tables. U.S. Dept. Comm. C. & G.S. special Publ. (298). 21 pp.

APPENDIX I

Catch composition and water characteristics for each

collecting day at each study area in Porters Lake

during 1971

Date	Study Area	Number F.d.	Number F.h.	Water Temp.	Salinity	Turbidity
10 May	PO1	71	22	13.0	0.4	30
10 May	PO2	60	50	12.0	0.4	30
8 June	PO1	11	10	16.0	1.3	40
8 June	PO2	61	10	16.0	0.8	20
21 June	PO1	104	49	20.0	2.4	10
21 June	PO2	160	26	20.5	0.9	10
29 June	PO1	139	65	20.0	5.0	10
29 June	PO2	101	17	21.0	3.5	15
6 July	PO3	60	6	19.0	0.0	20
12 July	PO1	18	44	24.0	16.5	20
12 July	PO2	80	27	27.0	13.0	20
12 July	PO4	59	18	24.5	12.0	15
12 July	PO5	85	0	26.0	14.5	15
21 July	PO4	32	10	29.0	13.6	10
21 July	PO5	50	18	26.0	14.5	15
21 July	PO1	17	23	27.0	13.0	20
21 July	PO2	82	18	29.0	13.4	20
27 July	PO1	17	30	20.5	11.6	15
27 July	PO2	38	20	21.5	15.1	15
10 August	PO1	46	10	28.0	13.6	20
10 August	PO2	53	10	28.0	13.9	20
10 August	PO3	62	3	26.0	16.5	20
30 August	PO1	21	19	21.0	8.1	20
30 August	PO2	33	22	21.0	8.6	18
15 Sept.	PO1	24	28	19.0	8.2	23
4 Oct.	PO1	35	39	12.5	12.4	22

APPENDIX II

Characteristics of relative growth regressions of morphometric data used to distinguish among three types of killifish collected or encountered in Nova Scotia. (n = sample size; s = standard deviation)

Locale	Species	Character	n	Mean X	Mean Y	Slope ± s	Intercept ± s
P. L.	Hybrid	D-C	110	47.0	20.0	1.038 ±0.03	0.365 ±0.05
P. L.	F. diaphanus	D-C	101	51.5	22.8	0.961 ±0.028	0.525 ±0.05
P. L.	F. heteroclitus	D-C	101	46.7	17.7	1.023 ±0.026	0.358 ±0.04
P. L.	Hybrid	DCP	110	47.0	5.4	1.204 ±0.048	0.053 ±0.05
P. L.	F. diaphanus	DCP	101	51.5	5.1	1.077 ±0.038	0.073 ±0.07
P. L.	F. heteroclitus	DCP	101	26.7	6.0	0.885 ±0.150	0.208 ±0.04
Museum	F. diaphanus	D-C	386	48.3	21.6	1.020 ±0.011	0.4151 ±0.02
Museum	F. heteroclitus	D-C	95	41.3	15.7	0.942 ±0.019	0.471 ±0.03
Museum	F. diaphanus	DCP	386	48.3	4.8	1.056 ±0.014	0.082 ±0.02
Museum	F. heteroclitus	DCP	95	41.3	5.4	0.942 ±0.022	0.167 ±0.04
S.M.R.	Hybrid	D-C	19	33.4	13.6	0.925 ±0.061	0.528 ±0.09
S.M.R.	F. diaphanus	D-C	24	34.4	14.4	0.971 ±0.011	0.498 ±0.04
S.M.R.	F. heteroclitus	D-C	14	31.3	12.1	0.895 ±0.055	0.562 ±0.11
S.M.R.	Hybrid	DCP	19	33.4	3.5	1.033 ±0.045	0.093 ±0.07
S.M.R.	F. diaphanus	DCP	24	34.4	3.2	1.113 ±0.025	0.064 ±0.04
S.M.R.	F. heteroclitus	DCP	14	31.3	3.9	1.185 ±0.074	0.068 ±0.11

APPENDIX III

Observations made of mummichog and banded killifish in each replicate in the vertical salinity gradient apparatus under control and experimental conditions for each acclimation. (C = control; E = experimental)

Species	Acclimation Salinity	Compartment Salinity	C^1E		C^2E		C^3E		C^4E		C^5E	
F.h.	0.0	0.0	0	6	4	0	14	9	0	10	0	0
		8.0	0	5	0	2	7	8	0	2	0	0
		14.0	1	19	7	12	6	7	0	11	2	7
		20.0	17	32	9	42	6	32	12	33	23	32
		26.0	23	17	21	32	20	17	22	15	35	27
		31.0	59	20	59	12	47	27	66	29	40	34
F.h.	14.0	0.0	24	15	8	3	49	2	0	2	8	18
		8.0	2	18	14	26	22	22	5	25	2	30
		14.0	9	3	9	13	7	14	24	11	14	8
		20.0	16	22	10	26	9	27	12	31	12	21
		26.0	19	11	33	12	7	13	34	19	20	10
		31.0	30	21	26	21	6	22	25	12	44	13
F.h.	31.0	0.0	20	3	7	0	6	41	6	5	17	2
		8.0	11	24	15	0	10	16	4	7	9	12
		14.0	16	18	13	12	13	14	19	10	15	4
		20.0	14	37	11	37	15	25	26	23	16	26
		26.0	16	15	27	21	17	1	23	25	22	12
		31.0	23	3	27	30	39	3	22	30	21	44
F.h.	0.0	0.0	31	33	45	67	25	48	63	45	30	37
		8.0	55	23	13	14	1	22	5	35	22	24
		14.0	7	16	13	3	9	7	4	14	13	27
		20.0	3	16	10	7	7	7	3	5	9	5
		26.0	1	9	7	5	13	12	5	1	7	1
		31.0	3	3	12	4	45	4	20	0	19	6

Appendix III continued

Species	Acclimation Salinity	Compartment Salinity	Replicate									
			C^1E		C^2E		C^3E		C^4E		C^5E	
F.d.	14.0	0.0	15	63	17	78	29	42	22	50	27	73
		8.0	5	18	23	6	24	6	12	15	21	10
		14.0	5	11	10	3	13	15	14	19	25	9
		20.0	17	3	19	5	13	16	9	9	11	4
		26.0	20	1	16	2	6	12	12	4	9	2
		31.0	38	4	16	6	15	9	30	3	7	2
F.d.	31.0	0.0	22	42	38	79	9	46	9	60	11	80
		8.0	7	11	19	7	5	4	23	21	9	9
		14.0	12	11	14	7	15	9	30	10	15	2
		20.0	19	22	10	1	24	10	14	5	28	2
		26.0	16	3	9	3	36	11	15	2	24	1
		31.0	24	11	10	3	11	23	7	2	13	3

APPENDIX IV

Characteristics of length-weight and fecundity regressions used in comparing mummichog and banded killifish collected in Porters Lake with those collected in more typical environments. (n = sample size; s = standard deviation; P.L. = Porters Lake; P.I. = Petpeswick Inlet; Kej = Kejimkujik Lake)

Locale	Species	Character	n	Mean X	Mean Y	Slope ± s	Intercept ± s
P.I.	*F. heteroclitus*	Lt-Wt	1068	49.3	1.37	3.171 ±0.023	58×10^{-5} ±0.039
P.L.	*F. heteroclitus*	Lt-Wt	296	49.8	1.53	3.222 ±0.044	52×10^{-5} ±0.074
Kej	*F. diaphanus*	Lt-Wt	204	58.1	1.78	2.880 ±0.033	15×10^{-4} ±0.059
P.L.	*F. diaphanus*	Lt-Wt	425	58.1	1.88	3.041 ±0.025	81×10^{-5} ±0.044
P.I.	*F. heteroclitus*	Fecundity	100	64.7	243	2.024 ±0.119	0.053 ±0.217
P.L.	*F. heteroclitus*	Fecundity	75	60.4	161	2.499 ±0.218	0.006 ±0.389
Kej	*F. diaphanus*	Fecundity	35	69.2	88	2.203 ±0.096	0.008 ±0.176
P.L.	*F. diaphanus*	Fecundity	100	70.7	129	1.566 ±0.153	0.164 ±0.283