

REPRODUCTIVE ISOLATION BETWEEN TWO SYMPATRIC SPECIES OF DACE,
RHINICHTHYS CATARACTAE AND RHINICHTHYS ATRATULUS, IN THE
MINK AND VALLEY RIVERS, MANITOBA.

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ABSTRACT

The Mink and Valley rivers, Manitoba, contain two sympatric species of dace (Cyprinidae: Rhinichthys). These fishes, although interfertile, have never been reported to hybridize with each other in nature.

Seasonal isolation is only partially developed, since overlap in time of spawning exists. During spawning, adults of the two species tend to occupy different habitats, the longnose dace, Rhinichthys cataractae, being most abundant in water velocities faster than 45 cm/sec and the blacknose dace, R. atratulus, in water velocities less than 45 cm/sec. But some overlap exists. However, the place of spawning of the two species is distinctly different. Nests of longnose dace were found in water velocities exceeding 45 cm/sec with large stones (upper limit > 5 cm) while nests of blacknose dace were found in slower water velocities with smaller stones. The patterns of spawning behaviour of these two cyprinids reveal marked differences and obvious incompatibilities. Males of both species actively defend territories prior to spawning but observations reveal distinct preferences for homospecific females over heterospecific females. Observations further indicate that females are receptive only to patterns of courtship behaviour shown by males of their own species.

The combination of different places of spawning and patterns of behaviour is considered to be a very powerful

barrier to interspecific hybridization. Sperm longevity of both species is of considerable duration (50% motility after 31-32 sec) and could, in the absence of other reinforcing mechanisms, permit accidental fertilization. However, the interplay of the pre-mating isolating mechanisms would appear to effectively maintain a high degree of reproductive isolation.

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INTRODUCTION

Preliminary ecological studies were conducted during the spring and summer of 1967, on the Mink and Valley rivers. These studies revealed the western blacknose dace, Rhinichthys atratulus meleagris Agassiz and the longnose dace, Rhinichthys cataractae (Valenciennes) to be well defined species which are sympatric, occur together in the same sections of the rivers, and spawn at similar times of the year. Although hybridization between these two species in the field has not been reported to date in the literature, interspecific hybrids produced in the laboratory show a survival rate comparable to that of homospecific matings (J. H. Gee, personal communication). Clayton and Gee (1969) have shown that both species and their laboratory-produced hybrids can readily be identified by electrophoresis of tissue extracts. However, of 19 dace taken from the Mink River and examined by this process, no hybrids could be identified.

Nelson (1966) has reviewed the literature on hybridization of Rhinichthys cataractae. The following species have been reported to hybridize with R. cataractae: Campostoma anomalum (Greeley, 1938), Hybopsis micropogon (Raney, 1940b), Rhinichthys osculus (Sigler and Miller, 1963), Richardsonius balteatus (Weisel, 1955) and Hybopsis plumbea (Nelson, 1966).

The object of this study was to determine how reproductive isolation is maintained so as to prevent interspecific hybridization between longnose and blacknose dace. The following isolating mechanisms are considered: time of spawning, place of spawning, spawning behaviour and longevity of sperm.

According to Breder and Rosen (1966), virtually nothing is known about the reproductive habits of Rhinichthys cataractae except that it spawns in early spring. Carl, Clemens and Lindsey (1959) wrote that spawning in Rhinichthys cataractae occurs in spring in shallow gravelly streams; no nest is constructed, but the males guard a territory over which they entice the females to spawn.

Raney (1940a) described the spawning act of Rhinichthys atratulus meleagris and stated that adult males establish and defend territories up to 2 feet in diameter for several days, spawning with many females. Raney (1940a) considered preferred spawning areas of this species to be over sand and fine gravel.

The present study investigates the breeding habits of both species. Spawning areas are described in detail and patterns of spawning behaviour are presented.

LITERATURE REVIEW

Dobzhansky (1937) introduced the term "isolating mechanisms" for all agents which prevent or hinder the interbreeding of species. Mayr (1963) defines isolating mechanisms as "biological properties of individuals that prevent the interbreeding of populations that are actually or potentially sympatric." There are a host of mechanisms that may isolate closely related species and several classifications have been proposed. The classification of Mayr (1963) was adopted in this investigation. This review will be restricted to isolating mechanisms in freshwater fishes as they pertain to the present study. The literature on the following isolating mechanisms will be reviewed: time of spawning, place of spawning, spawning behaviour, and longevity of sperm.

A. Time and Place of Spawning

According to Mayr (1963), the less often two potential mates in breeding condition come into contact with each other the less likely they are to interbreed. Many sympatric species are isolated only or in part by differences in time of breeding. Hagen (1967) has shown that seasonal isolation is partially developed between the sticklebacks, Gasterosteus leiurus and G. trachurus. One form breeds from early March through to July while the other breeds from late May to September. Hagen (1967) provides evidence to show that

ecological isolation is a very powerful barrier to hybridization between these sticklebacks. The two species show numerous adaptations to the distinctly different habitats that they frequent and each show a strong affinity for its own habitat. However, in localities with intermediate or contiguous habitats, co-existence and interbreeding occur.

Species which normally breed in different seasons may interbreed where conditions are such as to cause an overlap in the breeding seasons (Hubbs, 1955). In typical streams and lakes of western Montana containing temperature gradients, redside shiners, Richardsonius balteatus, ripen several weeks to a month earlier than the squawfish, Ptychocheilus oregonense. However, when both species occur in warm spring-fed sloughs, the warmth of the slough water, which remains constant at 17-18°C, causes the two species to ripen at approximately the same time. Weisel (1955) suggests that this factor is conducive to miscegenation between the squawfish and shiner.

Hubbs and Strawn (1957a) found seasonal isolation to be ineffective for the darters Etheostoma lepidum and E. spectabile which breed in response to different temperatures that overlap widely. Hubbs (1961a) states that selection of different areas for spawning often acts as a mechanism for reproductive segregation. The two darters Etheostoma lepidum and E. spectabile differ in substrate used for spawning (Hubbs, 1958). Etheostoma spectabile prefers gravel bottom

(Winn, 1957) and E. lepidum prefers filamentous algae (Hubbs and Strawn, 1957b). Winn (1957) states that in the subfamily Etheostomatinae (darters) the site where eggs are deposited appears to be relatively specific and constant for several species. The slight differences in gravel size utilized for the deposition of eggs by Etheostoma caeruleum and E. spectabile results in spatial isolation on a gravel riffle. Keenleyside (1967) has shown that where the sunfishes Lepomis megalotis and L. gibbosus are sympatric, the former usually breed in areas of faster water flow than the latter, although the two species may spawn within a few meters of each other. It has been observed that certain species of bitterling spawn and fertilize their eggs preferentially into distinct species of freshwater mussel. Duyvené de Wit (1962) notes that this predilection may thus act as an isolating mechanism. According to Miller (1964), Exoglossum sp. would not be able to maintain position and carry out spawning activities in the swift currents where many Hybopsis sp. and Semotilus corporalis nest. In this way somewhat different habitat preferences reinforce other isolating mechanisms (e.g. temporal) maintaining a fairly firm barrier against hybridization.

Nelson (1966) has stated that many closely related species that co-exist in undisturbed areas may hybridize if environmental changes occur. One of the most frequent causes of hybridization in freshwater fishes is due to such

changes, resulting mostly from human interference. Studies in which environmental changes are known to have been associated with the hybridization of fish species have been reviewed by Bailey and Lagler (1938), Hubbs and Miller (1943), Jurgens (1951), Aksiray (1952), Kosswig (1953), Hubbs, Kuehne and Ball (1953), Hubbs (1955), Weisel (1955), Hubbs and Strawn (1956), Gilbert (1961), Delco (1962) and Nelson (1966).

B. Spawning Behaviour

In most fish species the patterns of courtship behaviour are composed of a series of reciprocal or mutual signals involving both sexes. When individuals of two species meet, reproductive isolation may be maintained by the failure of one or both to give the appropriate signals and responses (Liley, 1966). Reproductive isolation through behaviour has been described for Catostomus commersonii and C. macrocheilus (Nelson, 1968), Gasterosteus wheatlandi and G. aculeatus (McInerney, 1969) and for Acheilognathus lanceolatus and A. limbatus (Duyvené de Wit, 1962). Miller (1964) states that the tilt display and spawning clasp of territorial male Notropis cornutus are not present in N. rubellus. Since these motor patterns associated with spawning differ markedly between the two species, Miller (1964) suggests that they are probably an important isolating mechanism.

Numerous studies have shown mate-recognition to be a major behavioural isolating mechanism. In his study on three species of sunfishes, Keenleyside (1967) found that males distinguished between females of their own and other species consistently, courting homospecific females in preference to the other two species. He concluded that this behaviour probably contributed to reproductive isolation between sympatric populations of these three species. Clark, Aronson and Gordon (1954) and Hubbs and Delco (1960, 1962) revealed similar behavioural isolation among sympatric species of xiphophorin and gambusiine fishes determined by means of such tests of preference. Picciolo (1964) found visual stimuli the most important of several possible mechanisms for discrimination in four species of anabantids.

However, Hubbs (1955) states that great scarcity of one species coupled with the abundance of another often leads to hybridization; individuals of the sparse species seem to have difficulty in finding their own mates. Weisel (1955) suggests that hybridization between Rhinichthys cataractae and Richardsonius balteatus may be enhanced by the fact that the dace is rare in some streams whereas the shiners are extremely abundant. Analogous cases were found by Hubbs, Hubbs and Johnson (1943) for instances of hybrids among suckers and by Hubbs, Walker and Johnson (1943) for hybrids among cyprinodonts.

C. Longevity of Sperm

According to Mayr (1963), ethological factors have only limited value as isolating mechanisms in aquatic animals with external fertilization. Eggs and sperm of many fishes are freely discharged into the water and if there is any intermingling of species, the eggs of one species might be fertilized by sperm of another. Laboratory experiments by Hubbs (1957, 1960, 1961b) have demonstrated that sperm longevity will permit accidental fertilization. However, Hubbs (1957, 1960, 1961b) and Hubbs and Drewry (1958) have found gametic incompatibility after a time-lapse which would reduce the chances of accidental interspecific fertilization. Since delayed fertilization may result in lower hatching survival, short temporal function of sperm may be a significant mechanism in restricted spawning areas (Nelson, 1968). Hubbs (1957) states that it is not surprising that in logperch, Percina caprodes, a fish with frequent natural interspecific hybrids, sperm longevity is greater than in the orangethroat darter, Etheostoma spectabile, a fish that seldom hybridizes. In many sympatric species of fishes whose temporal function of sperm is comparatively long, elements of chance linked with insufficient seasonal and spatial isolation have explained the occurrence of hybrids. Such cases have been noted by Hubbs and Brown (1929), Hubbs (1955), Gilbert (1961), Duyvené de Wit (1962), and Stewart (1966).

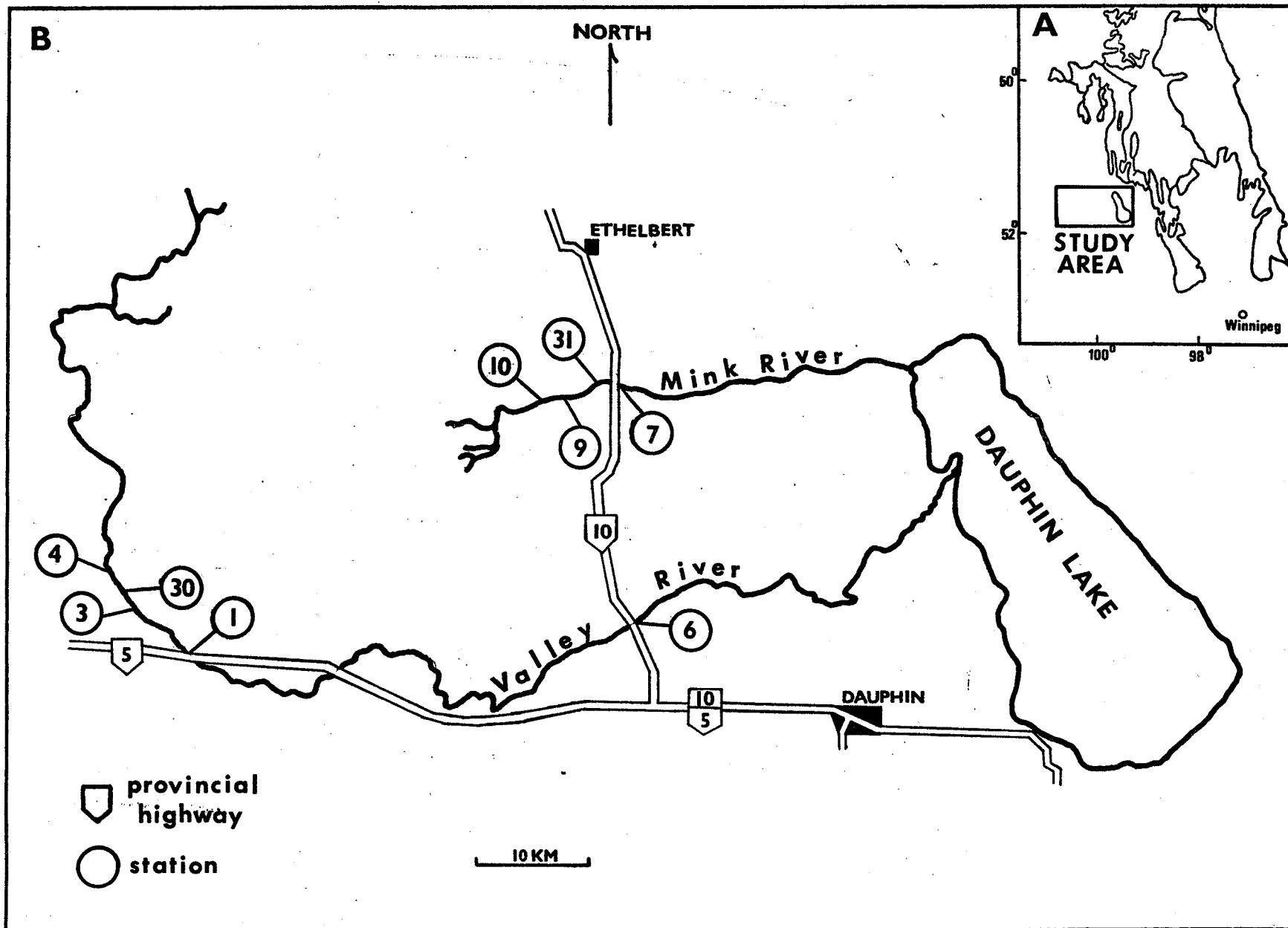
Most of the above studies on isolating mechanisms of freshwater fishes have been initiated by the recording of hybrids. The usual approach in these studies has been to determine which normally effective isolating mechanisms have broken down. The initiation for the present work, however, took a rather different form, since the two cyprinids concerned are interfertile yet have never been reported to hybridize with each other in nature. The present study attempts to determine the factors preventing interspecific hybridization and their significance in reproductive isolation, which must be considered as near-perfect.

DESCRIPTION OF THE STUDY AREA

The Mink and Valley rivers both originate in the Duck Mountains (Manitoba) and flow eastward, emptying into the northwestern shore of Dauphin Lake (Fig. 1). Several stations were established on each river system for the purpose of observing behaviour and collecting data on time and place of spawning. Both rivers are characterized by relatively high gradients. Although the Valley River is the larger, the two are similar, consisting of series of riffles and pools. They are deep and fast-flowing in early spring but generally become slower and more shallow later in summer and fall. All stations except Station 6 were situated on the upper reaches of the rivers where the width rarely exceeded 10 m. and the depth varied from less than 0.5 m. in riffles to between 1.0 and 1.5 m. in pools. Velocities throughout the study area ranged from less than 7.5 to 82.5 cm/sec.

Figure 1:

- A Map of southwest Manitoba, showing location of the study area.
- B Map of study area, showing locations of stations on the Mink and Valley rivers during 1968-69. Numbers within circles refer to stations.



MATERIALS AND METHODS

A. Time of Spawning (Season)

On April 26, May 5, 16, 27, June 5 and 25, 1968, both species of dace were collected from a wide range of environments in the Mink and Valley rivers to obtain information on development of gonads. Collections were made with a two-man seine (5 meshes/cm). Dace collected were preserved in 10% formalin and later transferred to 40% isopropyl alcohol. Fish were dried lightly on paper toweling and weighed on a Sartorius electric single-pan balance (± 0.0025 gm). Then gonads were removed and weighed to the nearest .0025 gm. The weight of the gonad expressed as a percent of the body weight was used to determine the approximate interval of time over which the two species spawned. Only fish aged 2 years or older were used (determined by length-frequency).

B. Place of Spawning

a) Distribution of adult dace

Collections made on the above dates also provided evidence on distribution of adult dace in different environments prior to, during and after spawning. A data sheet was completed for each collection, recording the area seined, water velocity, substrate size and composition. Water velocity was measured on the surface by timing the movement of a floating object over a known distance. The area seined

in each collection was as homogeneous as possible with respect to each of the above environmental variables. Once these data were analysed, the number of adult dace per square meter taken in slow water velocities (< 45 cm/sec) and in fast water velocities (> 45 cm/sec) was calculated.

b) Distribution of redds

During the spring of 1968 and 1969, extensive searching throughout both rivers was carried out in order to locate nest-sites of the two species of dace. By finding recently deposited egg clutches or "redds", the environment utilized for egg deposition could be described for each species. This phase of the research was complicated by the presence of redds of common shiner, Notropis cornutus, northern creek chub, Semotilus atromaculatus and common sucker, Catostomus commersonii. Once collected, redds were returned to the laboratory, hatched and the fry reared to identification. Redds of these other species were not considered.

In searching fast-flowing rocky riffles, the bottom was examined by using a face-plate and snorkel. Since the depth in most riffles was generally shallow, less than 0.5 m, one could kneel on the bottom while lifting rocks and scanning the substrate for the presence of eggs. In the finer substrate areas eggs were found to be buried and were not visible. In these areas, the substrate was examined using a Surber sampler.

The search for redds in fast-flowing turbulent riffles was a completely random procedure. However, in slower flowing portions of the streams where the bottom was clearly visible, the search for redds was easier. In addition to random Surber sampling in these areas, tiny silt-free depressions often helped to indicate a recent site of spawning. Observations of territorial males also aided in locating several redds. When a redd was located in a rocky riffle, several of the rocks with adhering eggs were removed from the stream, placed in an enamel pan full of water and the eggs were nudged loose. A Surber sampler was then used to retrieve eggs from the underlying gravel. All eggs collected were then put into an egg-holding basket using a large-bore eye-dropper. To retard fungal growth, a methylene blue solution was added to the eggs. Egg-holding baskets were plastic containers (10 x 12.5 x 10 cm) with a nitex screen (15 meshes/cm) which covered openings on two of the opposing sides. This screen allowed water to circulate yet retained the eggs. After the basket was labelled and tightly closed it was weighted with a rock and placed in the stream. A data sheet was completed for each redd found, showing substrate size, surface velocity over the nest-site, depth and number of eggs collected. Diagrams and descriptions of the nest-site were often included. To reduce accidental drift of eggs from searching and to increase efficiency, searches for redds were always started at the downstream end

of a designated area to be sampled.

Surber samples taken from areas of finer substrate were emptied into an enamel pan full of water and examined for eggs. If the sample revealed a redd, it was dealt with in the same manner as previously mentioned.

Egg-baskets were returned to the laboratory within the next few days. Here each redd was incubated at room temperature (18-20°C) in egg-baskets suspended immediately below the surface of well aerated aquaria. Following hatching, alevins from each redd were transferred into an aquarium. As soon as the yolk sac was absorbed, fry were fed Tetramin paste and powdered Tetramin flake. Fry were reared to a size (greater than 9.5 mm) where they could easily be identified to species. Identification was made on pigmentation of lateral band and on snout length, defined as the distance from the tip of the snout to the anterior margin of the lower lip. Measurements were made using a dissecting microscope with a mounted vernier stage.

C. Spawning Behaviour

During the winter of 1968-69, pre-seasonal breeding was induced in the laboratory for purposes of studying spawning behaviour. Fish of both species were collected on October 5, 1968 from stations 7 and 31 on the Mink River. Fifty adults of each species were returned to the laboratory where they were held in a 100-gallon fiberglass tank located in a controlled temperature room. Photoperiod was regulated

by means of a time-clock connected to two lamps holding 60-w bulbs. Throughout the holding period the fish were fed Tetramine flake food, frozen brine shrimp, and white (enchytraeid) worms.

From October 8, 1968 to February 2, 1969, the conditions in the controlled environment room were regulated so as to give fish a winter regime of light and temperature (Table I). A cover over the holding tank was used to simulate ice cover during this artificial winter period. It was constructed from one-quarter inch plywood and contained many small holes (0.25 cm diam.) to allow a small amount of light to penetrate.

Following the above period of artificial winter, two stream tanks were used to compare the spawning behaviour of the two species. These stream tanks permitted observations that were difficult to make in the field because of the shallow, turbulent fast-flowing water. The design, construction and operation of the tanks is given in detail by Gee and Bartnik (1969). Water temperature in each tank was controlled by a 0.91-m length of chromalox tubular heating element (1000 watts). It was capable of raising the temperature 1.7°C/hr. above room temperature and was controlled by a thermostat and a time-clock. Thus the heater was operated over a particular period each day to result in a daily rise and fall in temperature similar to that in the streams. Day illumination was provided by two lights, each

Table I: Temperature and light conditions throughout
the artificial winter period.

Time Interval	Mean Temp. °C	Total Daily Hours of Light	Cover Present
Oct. 8 - Oct. 14/68	8.0	10.0	No
Oct. 15 - Oct. 22/68	5.0	9.0	No
Oct. 23/68 - Jan. 7/69	4.0	8.0	Yes
Jan. 8 - Jan. 14/69	4.0	9.0	No
Jan. 15 - Feb. 2/69	5.0	11.5	No

connected to a time-clock. A 60-watt bulb was held in a lamp above each aquarium and light was directed upwards to reflect off a white ceiling onto the stream tank. This source of illumination was provided over the complete length of day. A 150-watt spotlight held above each stream tank was used to provide bright midday illumination over part of the day.

The stream tanks were set up so as to produce an average velocity of 25 cm/sec in one and 50 cm/sec in the other. The bottom in the faster flowing stream tank was covered with various sized rocks (7.5-15 cm diam.) and coarse gravel (> 5 cm diam.) thus creating an irregular bottom. The bottom in the slower flowing stream tank consisted of a deep layer of uniformly fine to medium gravel (0.5-2.5 cm diam.) with some coarser gravel and rocks (5-10 cm diam.) at the upstream end to provide hiding places for the dace. All gravel and rocks used in the stream tanks were taken from areas of the Mink River where redds had been located the previous spring.

On February 3, 1969, ten longnose dace, five of each sex, were introduced into the fast-flowing stream tank and ten blacknose dace, five of each sex, were put into the slower flowing stream tank. A combination of small fin clips on different regions of the caudal fin were used so that each fish could be identified during observations. Once these dace were transferred to the stream tanks, the

controlled environment room was operated so as to duplicate the temperature regime and photoperiod as it had been recorded throughout the spring of 1968 (Table II). As a result of the short winter and early spring the reproductive cycle of the dace was advanced considerably. By investigating spawning behaviour during this induced pre-seasonal breeding period, the oncoming spring could be devoted entirely to field studies.

During the laboratory investigation, dace were transferred from one tank to the other for varying periods of time in order to make observations on interactions between species. Observations were made at different times of the day throughout the entire reproductive cycle.

An experiment was also conducted on sex discrimination by territorial longnose males using two-dimensional models. Models, shaped to resemble the ventral surface of a dace, were cut from waxed cardboard. Each of the six models used had a different colour and/or colour pattern (Fig. 2). A 20 cm piece of fine stiff wire was glued to the upper surface of each model. By attaching the free end of the wire to a thin, 60 cm, glass rod, the model could be introduced into the tank without casting shadows. Models were positioned approximately 8-10 cm overhead of a sedentary territorial longnose male, and his response recorded. They were presented to two different territorial males in a varied order. If no reaction was elicited from the territorial

Table II: Regime of temperature and illumination
received by the stream tanks.

Time Interval	Total Daily Hours of Light	Total Hours of Bright Midday Illumination	Mean Minimum Temperature °C	Mean Maximum Temperature °C
Feb. 3 - Feb. 12/69	14.5	3.5	4.0	9.0
Feb. 13 - Feb. 22/69	15.0	4.5	5.5	13.0
Feb. 23 - March 4/69	15.5	5.5	11.0	14.5
March 5 - March 14/69	16.0	6.5	12.0	18.0
March 15 - March 24/69	16.5	7.5	12.0	18.0
March 25 - April 13/69	16.5	7.5	12.0	21.0

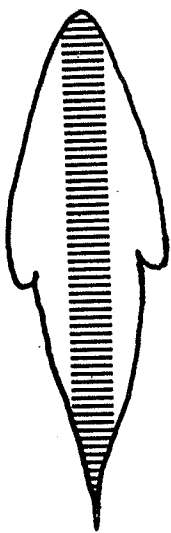
Figure 2: Two-dimensional models used to test the responses of territorial male R. cataractae to various colours and colour patterns.

- A Longitudinal red stripe
- B Red lips and lower fins
- C Green lips and lower fins
- D Uniformly green
- E Uniformly white
- F Uniformly red

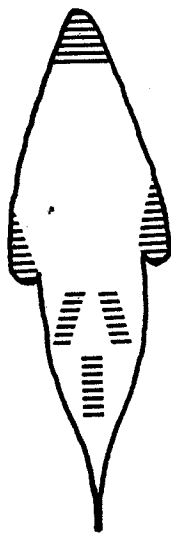
 red

 green

 white



A



B



C



D



E



F

male after a maximum of 10 seconds the model was removed. A 30 second pause between trials allowed normal encounters to take place. The models were removed from the tank just before the responding male could touch them, except in a few instances when the darting territorial male could not be avoided.

D. Longevity of Sperm

On May 30, 1969, five ripe males of each species were collected from the Mink River to determine duration of sperm motility. After thoroughly drying the ventral surface of each male by blotting on paper toweling, the abdomen was gently stroked so as to cause a small drop of sperm to be extruded. A thin smear of sperm was put on a glass slide and covered with a coverslip. The smear was then put under a Zeiss microscope and focussed. Sperm became motile only when placed into water. Water from the Mink River at a temperature of 14°C was used. By means of a stop watch, a record was kept of the time in seconds that it took for 50%, 75%, 95% and 100% of the spermatozoa to become non-motile. Average times for sperm longevity were determined for each species.

RESULTS

A. Time of Spawning (Season)

The most meaningful measure of temporal isolation is not timing of spawning, but overlap in time of ripe individuals in the spawning area (Nelson, 1968). In 1968 the percentage of body weight contributed by the gonads in female and male Rhinichthys cataractae reached a peak on May 16 (Fig. 3A). However, the percentage of body weight contributed by the gonads of male and female Rhinichthys atratulus reached a peak on May 27 (Fig. 3B). It is assumed that spawning commenced either during or just before the time when each species reached their maximum gonad development. The assumption is supported by the fact that the range and standard deviation of the relationships for the female dace show a noticeable increase once the peaks are reached (Fig. 3). This increase is interpreted as a direct result of the presence of both spawned and unspawned females in the samples of fish collected. Although Rhinichthys cataractae reached a peak of gonad development 11 days prior to R. atratulus, a definite overlap of ripe individuals still existed. Further evidence of overlap in ripe fish of both species was provided by field data. Dace examined in the field were stroked over the abdomen to check for the presence of clear ripe eggs or freely running milt. The results of these occasional field checks are shown in Table III.

Figure 3: Relationships between gonad weight and body weight of adult male and female dace in the Mink and Valley rivers, 1968. Vertical lines represent range while horizontal lines represent the mean. Vertical bars indicate two standard deviations, one above and one below the mean. Sample size for each sex and species was 25 except where otherwise indicated by the number in the brackets.

A Rhinichthys cataractae

B Rhinichthys atratulus

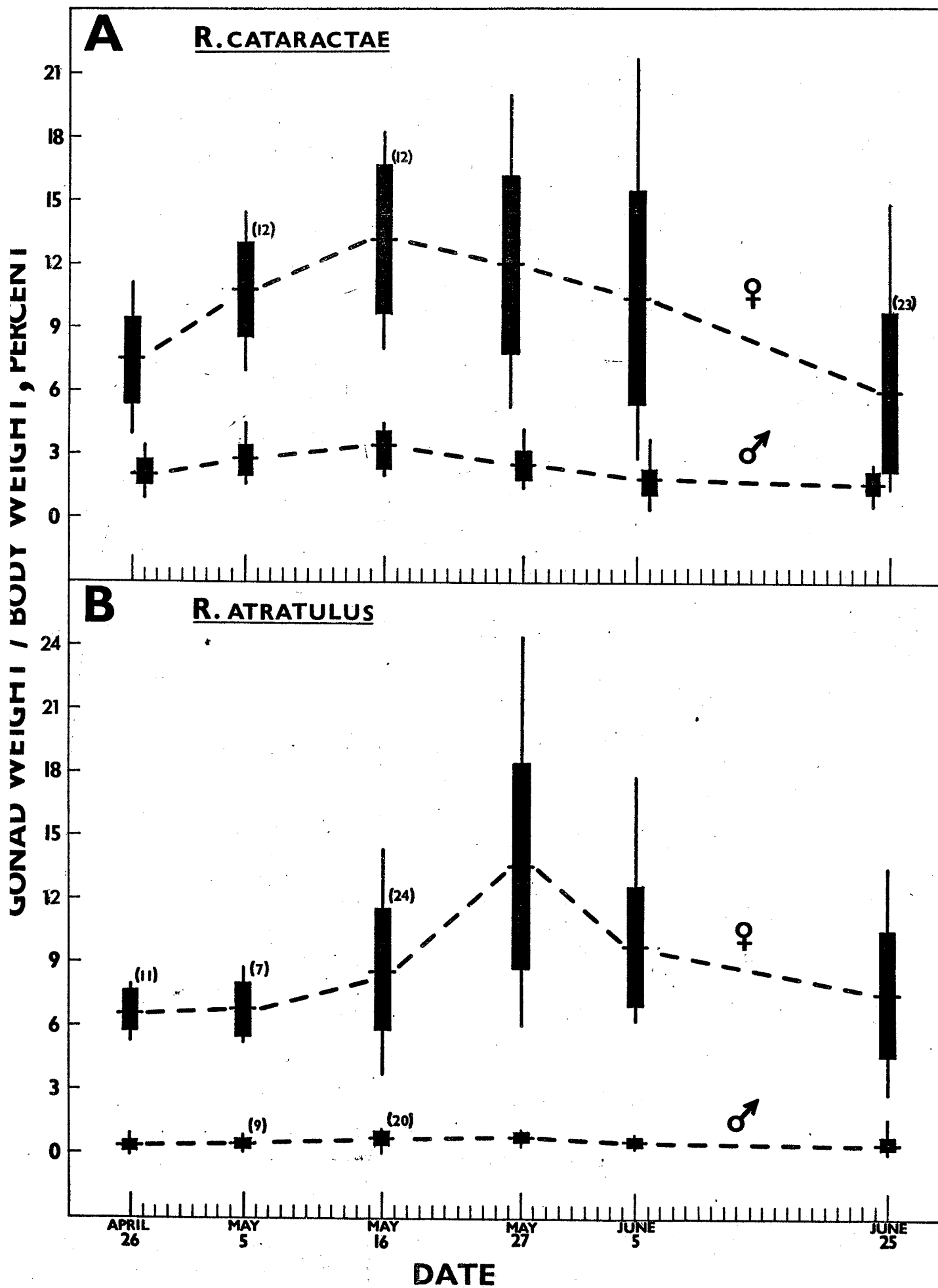


Table III. Numbers of ripe dace present on various
dates, 1968.

Date	Species	Females		Males	
		# examined	# ripe	#examined	# ripe
May 16	<u>R. cataractae</u>	5	3	60	59
May 19	<u>R. cataractae</u>	-	-	3	3
	<u>R. atratulus</u>	7	0	1	0
May 21	<u>R. cataractae</u>	10	0	-	-
	<u>R. atratulus</u>	12	0	-	-
May 22	<u>R. atratulus</u>	10	0	-	-
May 23	<u>R. cataractae</u>	70	5	-	-
May 26	<u>R. cataractae</u>	6	3	10	10
	<u>R. atratulus</u>	12	2	10	2
May 27	<u>R. cataractae</u>	16	0	-	-
	<u>R. atratulus</u>	28	1	-	-
May 31	<u>R. cataractae</u>	15	5	-	-
	<u>R. atratulus</u>	6	5	-	-
June 1	<u>R. cataractae</u>	1	1	-	-
June 3	<u>R. atratulus</u>	1	1	-	-

Temperature fluctuations, recorded in the Mink and Valley rivers using a maximum-minimum thermometer, were similar during the spring of 1968 (Fig. 4B). These temperature data reveal that longnose dace reached a peak of gonad development and began spawning at lower temperatures than did blacknose dace (Fig. 4A and Fig. 4B).

Pre-seasonal breeding induced in the laboratory during the winter of 1969 revealed this same pattern of reproductive development in each species. The first longnose spawning was recorded 18 days prior to the first blacknose spawning, and a total of six longnose spawning acts had occurred before the first blacknose spawning.

B. Place of Spawning

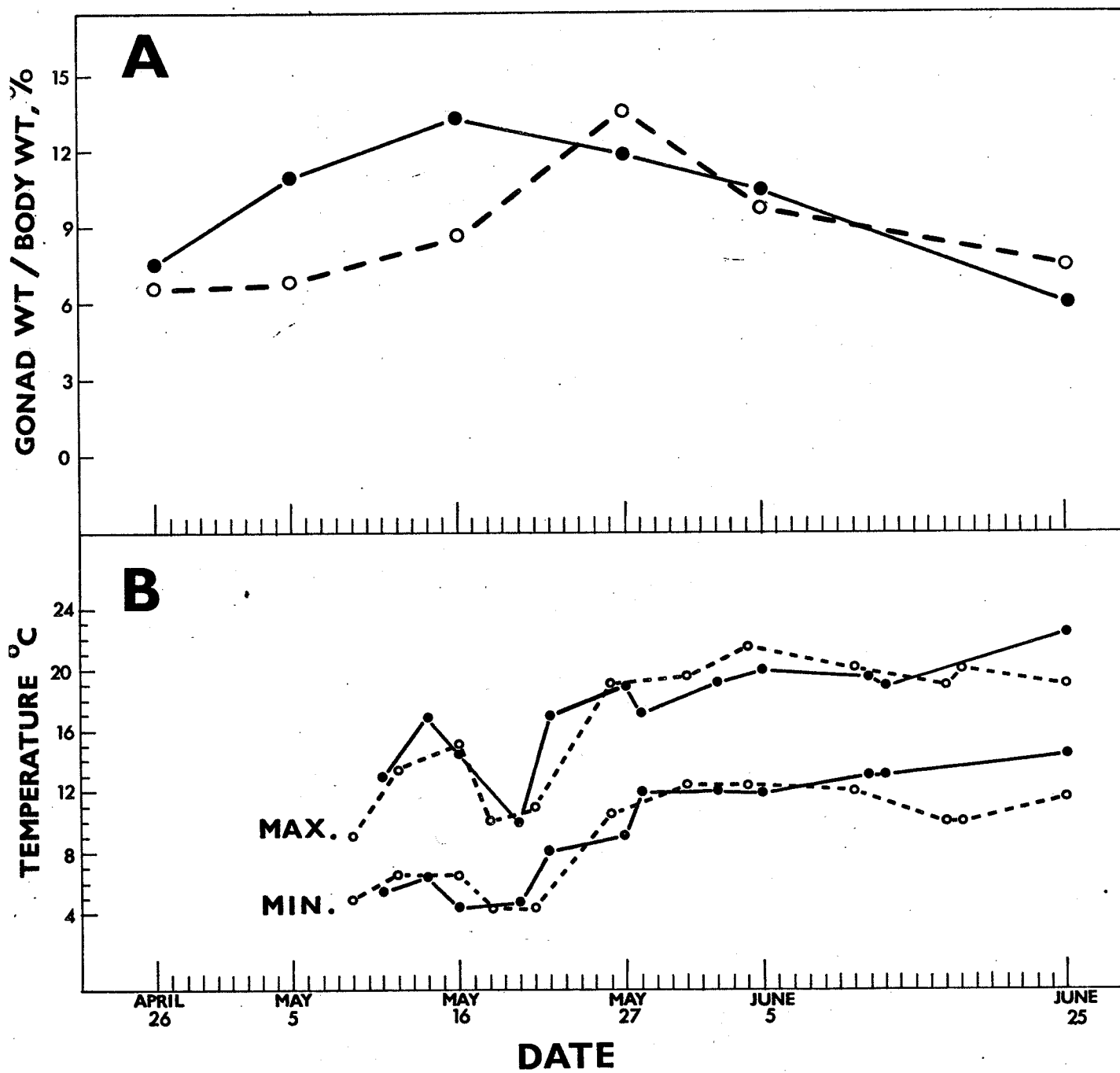
a) Distribution of adult dace

The purpose of this section is to examine the distribution of the two species in different environments in the Mink and Valley rivers, and to determine if there is any spatial isolation prior to or during spawning. Although several environmental variables were measured, results of the field study presented here will concentrate on density of adult dace (age 2 years or older) in relation to water velocity. Other variables may affect distribution, but will not be described.

Division of water velocities into slow (< 45 cm/sec) and fast (> 45 cm/sec) categories produced the best separation of the two species. Densities in Fig. 5 are calculated from

Figure 4A: Mean percent gonad weight of body weight for adult female dace in the Mink and Valley rivers, 1968. Solid lines and closed circles represent R. cataractae females, broken lines and open circles represent R. atratulus females.

4B: Maximum and minimum temperatures in the Mink and Valley rivers, during the spring of 1968. Solid lines and closed circles represent the Valley River, broken lines and open circles represent the Mink River.



total area sampled and total number of fish caught on these dates. The assumption is made that both species were caught in each environment with the same degree of efficiency.

Throughout the spring of 1968, adult longnose dace were most abundant in the fast water velocities (>45 cm/sec), and blacknose dace were most abundant in the slow water velocities. Although this distribution was maintained during spawning, considerable overlap still existed (Fig. 5).

b) Distribution of Redds

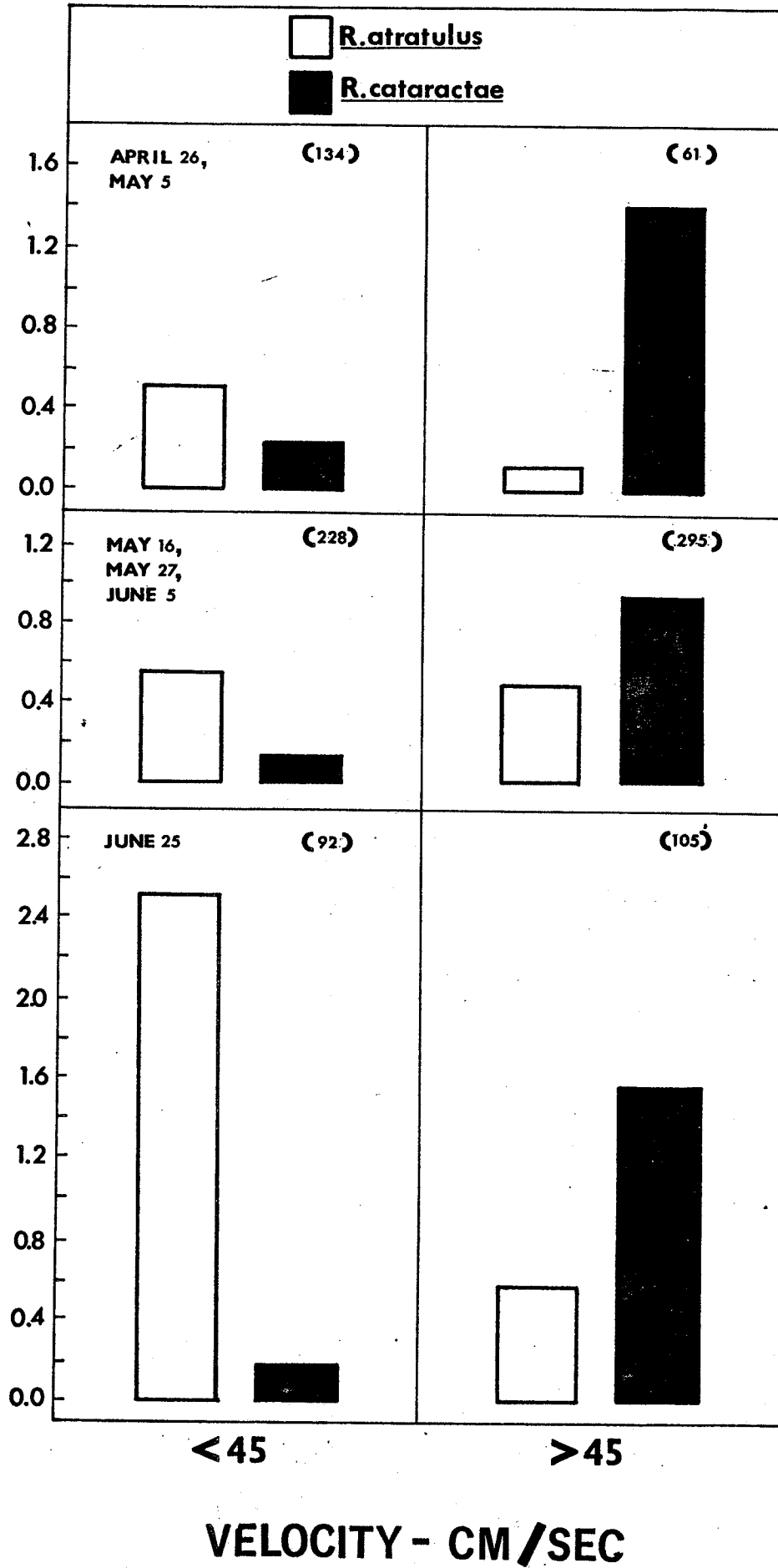
Recently deposited egg clutches or redds were collected from the Mink and Valley rivers, hatched and the fry were reared. By identification of fry from each redd to species, places of spawning could be described.

i) Identification of fry

High mortalities of eggs and fry were incurred in 1968 and 1969. In 1968, only 19% of the eggs collected survived to the fry stage. The greatest percentage of this mortality occurred prior to hatching. Mortality may have been due to insufficient oxygen in the water in the egg baskets or to mechanical damage while collecting eggs. The addition of one more screened opening to the egg baskets and the holding of fewer eggs per basket (50-70 from each redd) during 1969 increased survival to the fry stage to 25% of all eggs collected.

Figure 5: Distribution of adult dace in slow and fast water velocities prior to spawning (April 26 and May 5), during spawning (May 16, May 27 and June 5) and after spawning (June 25), 1968. Numbers in brackets refer to number of square meters sampled.

NUMBER OF FISH/M²



Fry of the two species can easily be identified on the basis of pigmentation alone. Blacknose dace fry have a broad band of black chromatophores along the body extending from the tip of the snout and terminating in a black spot at the base of the caudal fin and extending onto the caudal fin. Longnose dace fry are characterized by a thin lateral band forming a small spot at the base of the caudal which is much fainter than in R. atratulus. The striking difference in the lateral bands of the two species is a direct result of the number of chromatophores forming the band (Fig. 6).

Although fry of both species can be identified by pigmentation alone, snout length also can be used to identify the two species. Fry of blacknose dace are easily distinguished from those of longnose dace because in the former the snout scarcely projects beyond the slightly oblique mouth (Fig. 7A). In longnose dace the protruding snout is evident even in the smaller specimens. Although snout length of laboratory-produced hybrids is intermediate between that of the two species, there is overlap with either species over a narrow zone (Fig. 7A).

Fry reared from eggs collected from the Mink and Valley rivers were first identified to species by the width of the lateral band. Then snout length, relative to fork length was measured confirming the former method of identification. Both methods revealed two well separated groups, one of each species (Fig. 7B), which approximated those of

Figure 6: Width of lateral band expressed as the number of chromatophores touching a vertical line perpendicular to the band at the level of the origin of the dorsal fin.

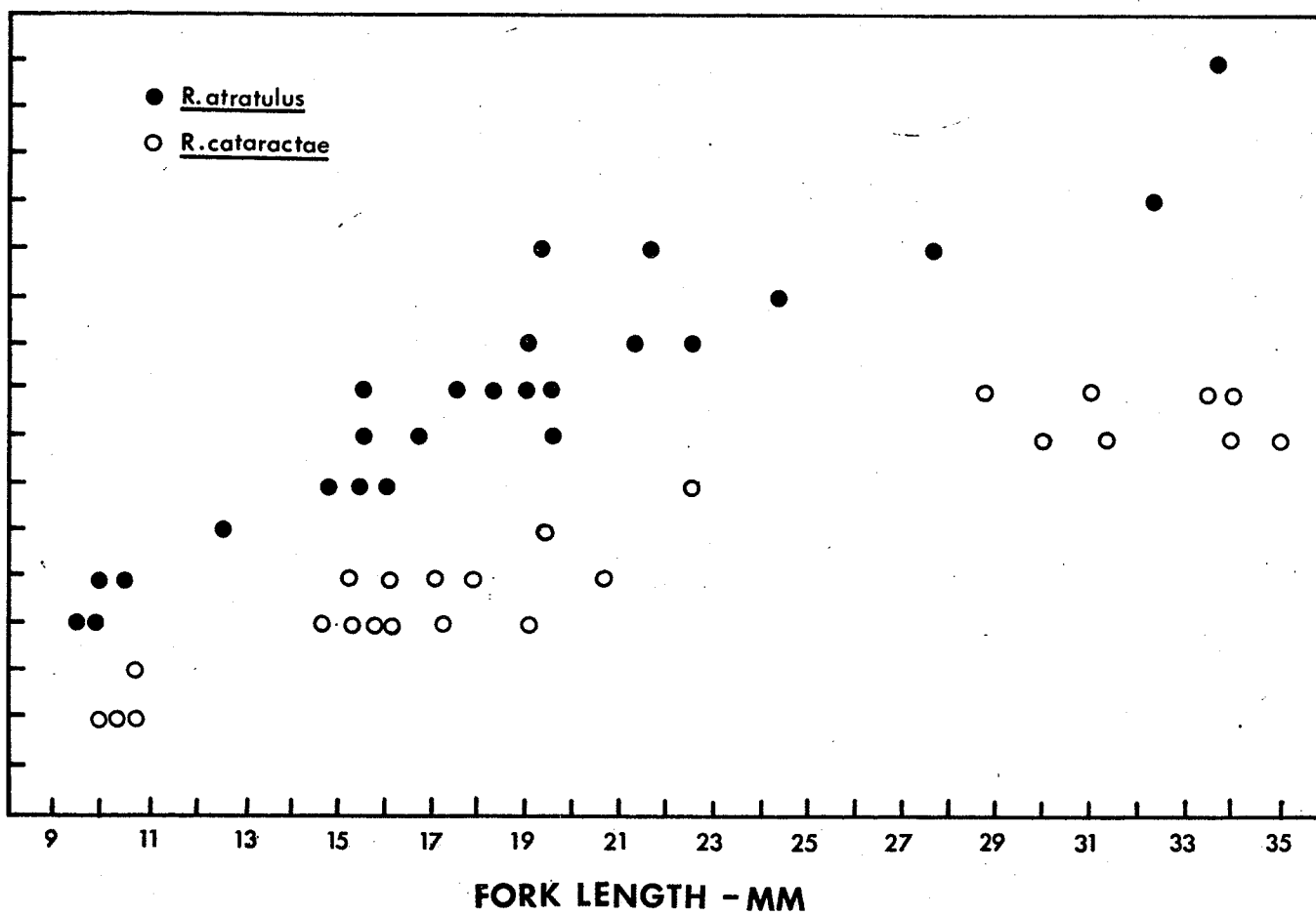
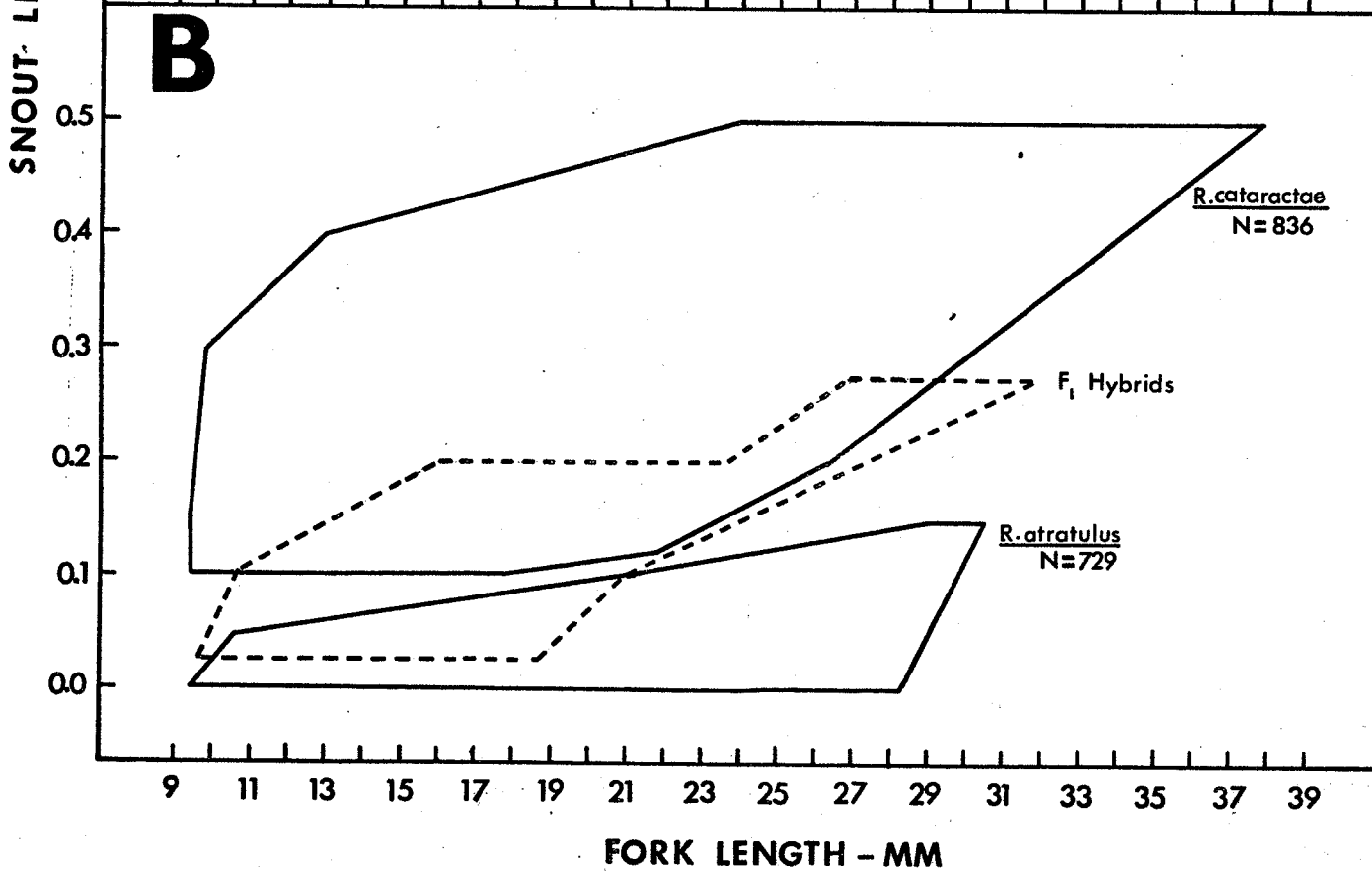
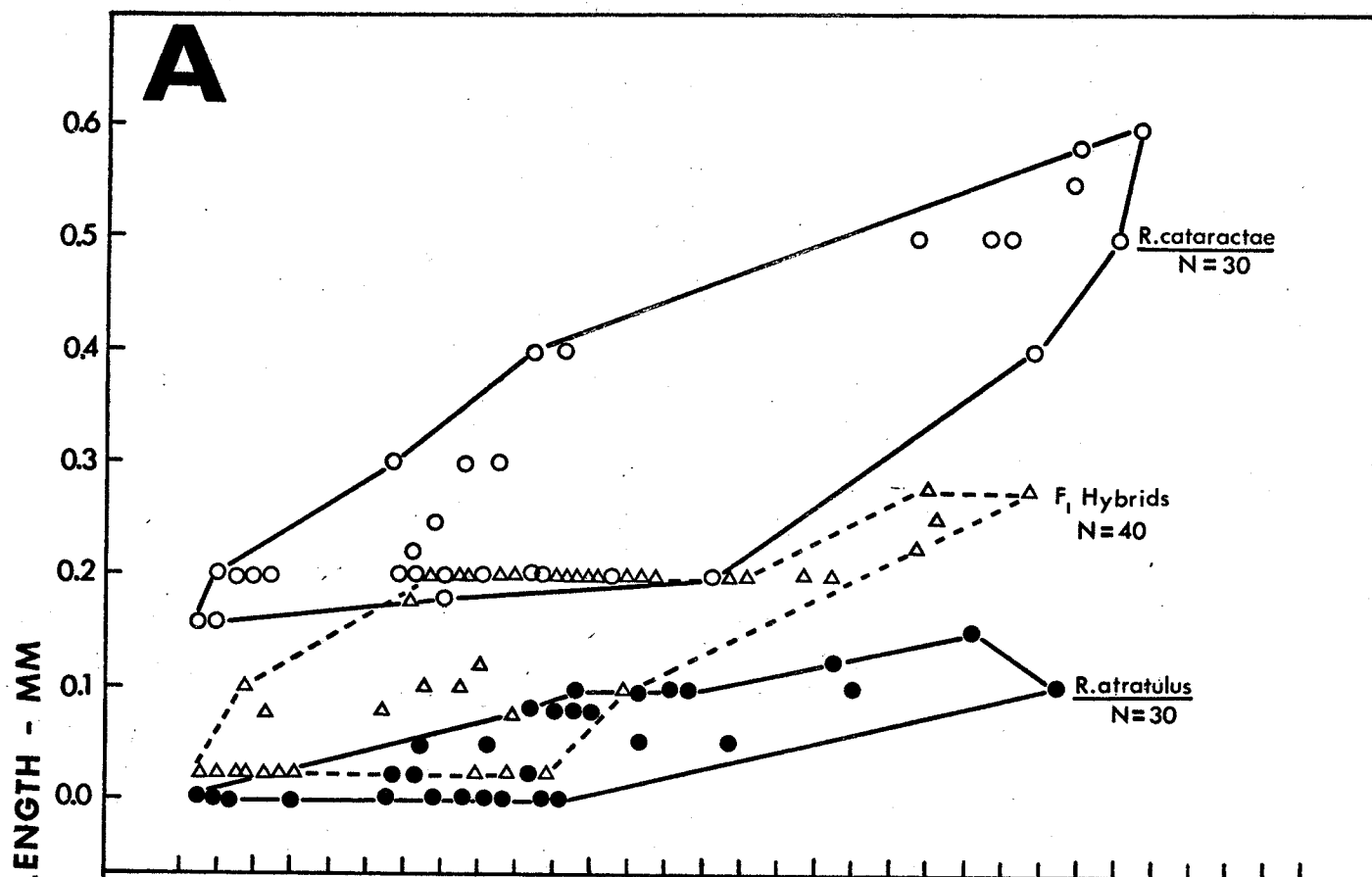


Figure 7: Outer ranges of snout length - fork length relationships for fry of the two species and F_1 hybrids. Boundaries are drawn by joining up the extreme points.

A Comparison of snout length of longnose and blacknose fry of known parents with F_1 hybrids produced in the lab.

B Comparison of snout length of fry of two species reared from collected eggs with F_1 hybrids produced in the lab.



the two species from known parents (compare Fig. 7A and Fig. 7B).

Laboratory-produced F_1 hybrids, on the basis of snout length relative to fork length, may be confused with (1) fry of either species of known parents (Fig. 7A) or (2) fry reared from eggs collected in the field and identified to species on the basis of both snout length and width of lateral band (Fig. 7B). Thus it is possible that a few of the fry reared from eggs and identified as either longnose or blacknose actually may have been hybrids.

Six or more fry survived in 89 redds that were reared in the laboratory. Of these, 80 contained fry of only one species (Table IV). The remaining nine contained fry of both species. In each of these nine redds it was found that the fry of one species was rare (Table V). This suggests either the drifting of eggs from one nest into that of another or accidental fertilization of some eggs had occurred as a result of drifting heterospecific sperm. The fry accounting for the rare species in eight of these nine mixed redds were found to have a snout length - fork length relationship which fell well outside the zone where the hybrids overlapped that particular species. This would suggest that these eggs of the rare species were from a homospecific mating but had drifted into a heterospecific nest. However, in one of the nine redds collected, 15 blacknose fry and one longnose fry survived and the latter

Table IV: Size and number of redds in which fry of only one species were found.

Table V: Number of fry of each species reared from nine redds found to contain both species.

* fry which did not fall into the hybrid zone

** fry which did fall into the hybrid zone

Number of fry in redd	Number of Redds	
	all <u>R. atratulus</u>	all <u>R. cataractae</u>
6	7	3
7	7	2
8	2	6
9	2	3
10	2	2
11-25	15	18
26-50	4	5
53	0	1
123	0	1

Number of each species	
<u>R. atratulus</u>	<u>R. cataractae</u>
2*	6
7	1*
1*	7
22	2*
12	2*
15	1**
12	2*
18	1*
15	3*

fell within the hybrid zone of overlap (Table V). This fry may have been a hybrid although the pigmentation of the lateral band was that of a longnose dace.

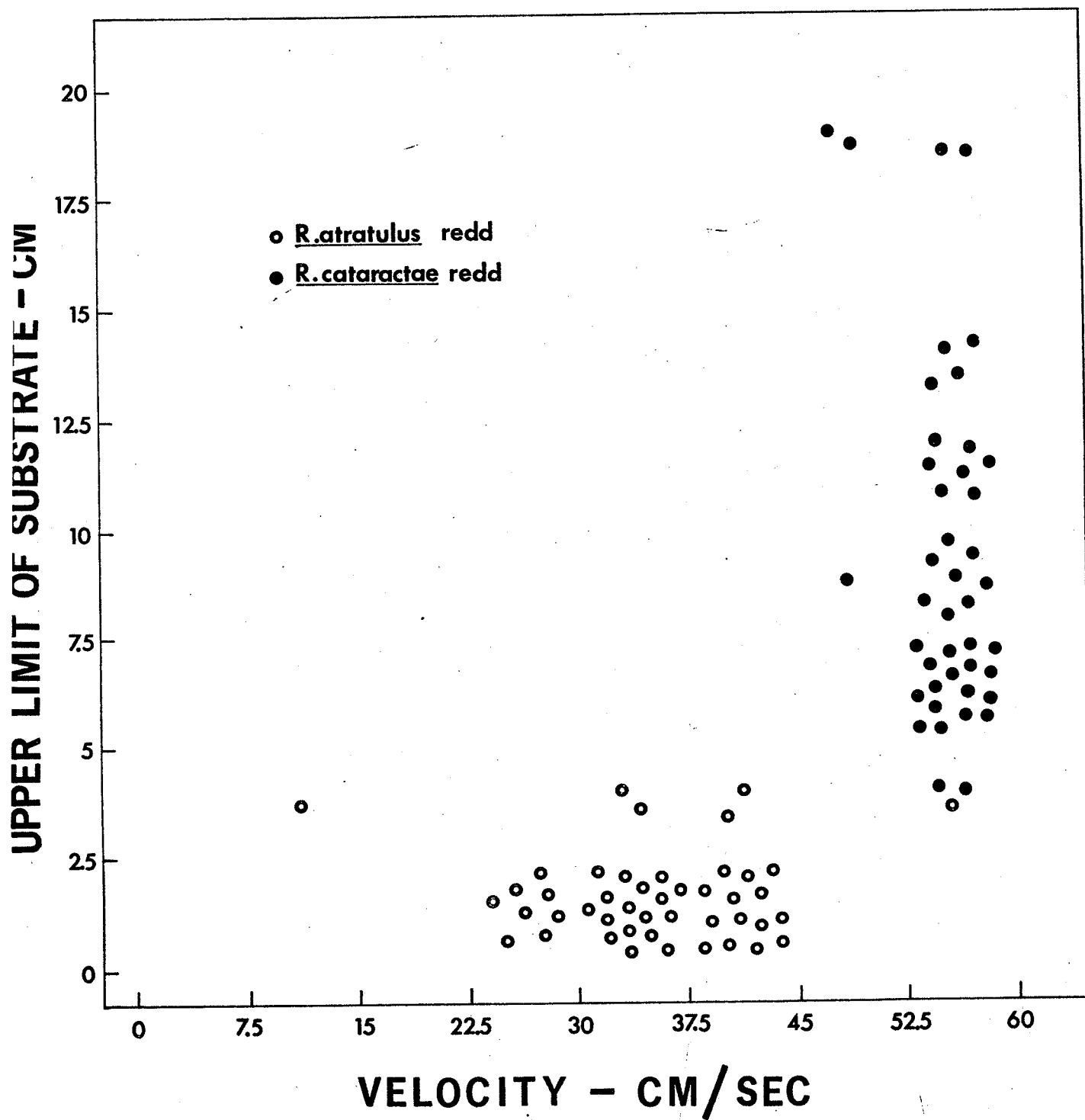
ii) Distribution of redds

Although several environmental variables were measured, only the number of redds in relation to water velocity and size of substrate forming the nest will be described. Other variables may affect distribution of redds, but they are not described here as they revealed no obvious differences between the two species.

The combined data on distribution of redds for 1968 and 1969 indicate that the blacknose and longnose dace display strong segregation in spawning habitat. Figure 8 shows that the majority of redds of blacknose dace were located in water velocities ranging from 7.5 to 45 cm/sec. In fact, only one of the 46 redds of this species collected was located in a water velocity exceeding 45 cm/sec. All these blacknose redds were situated in nest substrates with an upper limit of 5 cm or less. "Upper limit," as used herein, refers to the diameter of the largest piece of gravel or rock forming part of the nest substrate. The majority of blacknose redds were found in nest substrates with an upper limit of 2.5 cm or less (Fig. 8).

On the other hand, all 43 redds of longnose dace were found in water velocities ranging from 45 to 60 cm/sec. All these redds except three, were found in water with a

Figure 8: Numbers of blacknose and longnose redds found in various combinations of substrate size and water velocity. Velocities were measured in 7.5 cm/sec ($\frac{1}{4}$ ft/sec) intervals. Substrate sizes were grouped into 2.5 cm (one inch) intervals.



velocity of 52.5 to 60 cm/sec. These longnose redds were distributed in substrates with an upper limit ranging from 2.5 to 20 cm. The majority were in substrates with an upper limit of 5 to 20 cm (Fig. 8).

Plates 1 and 2 illustrate the distinctly different spawning habitats of the two species of dace.

iii) Spatial distribution of redds

Field data indicate that redds of longnose dace are often found in close proximity to one another (Fig. 9A). Redds of this species have been found as close as .25 m from each other. The redds of the blacknose dace, however, show greater spatial separation (Fig. 9B). Redds of this species are usually found at least .5 m apart.

Although redds of each species were located in very different habitats, the nature of the streams is such that the segregation is often slight in terms of distance. The blacknose dace generally spawn either upstream or downstream of the riffles while longnose dace restrict spawning within riffles. However, redds of both species, at various stages of development, have been collected from the same station over a number of days (Table VI). Plate 3 illustrates the proximity of the spawning areas of the two species of dace in a typical section of the Mink River.

Plate 1: Nest-site of a longnose dace located in the
Valley River.

Plate 2: Nest-site of a blacknose dace located in the
Mink River.



PLATE 1



PLATE 2

Figure 9: Spatial distribution of redds and territorial males. Arrows indicate direction of flow.

- A Distribution of longnose dace redds over a fast-flowing (52.5-60 cm/sec) rocky riffle, in the Mink River at Station 7, May 16, 1969. Closed circles represent redds of longnose dace. Open circles represent redds of longnose dace with an attending territorial longnose male.
- B Distribution of territorial blacknose males over a moderately flowing (30-37.5 cm/sec) gravel riffle, in the Mink River at Station 7, June 7, 1968. Broken lines represent territorial boundaries as determined by encounters with rival males. Closed squares represent males defending redds; open squares, males without redds. Hatching represents islands or large stones.

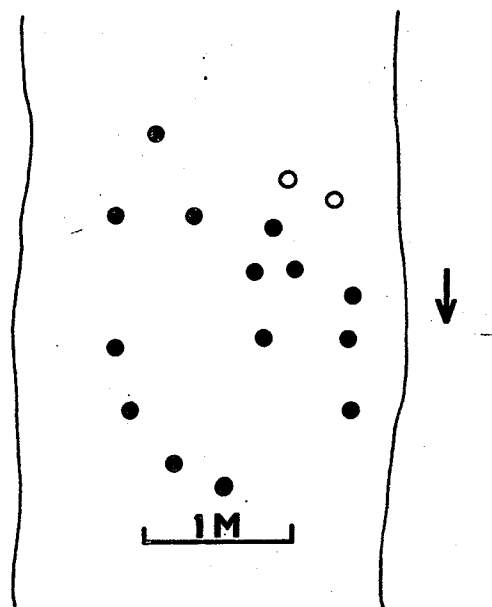
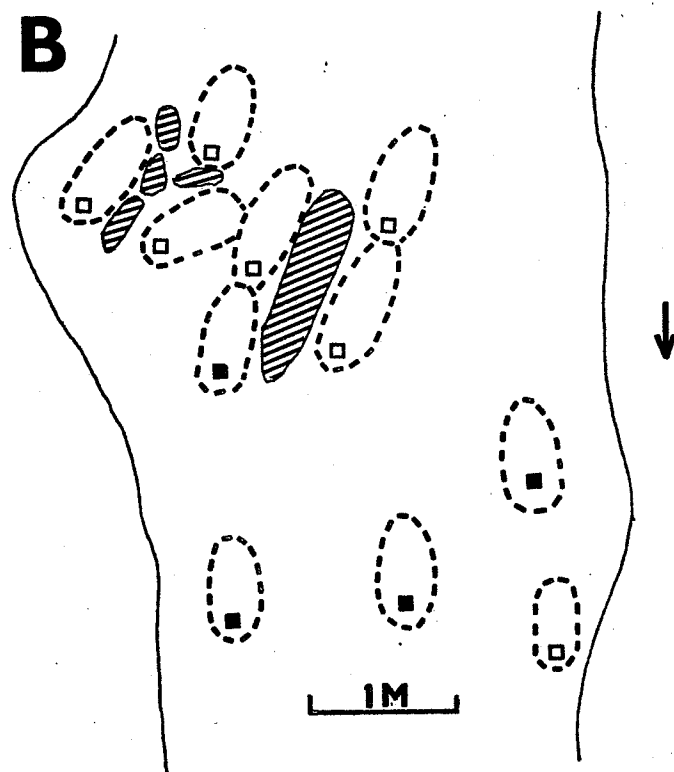
A**B**

Table VI: Number of redds of either species of dace found within 15 m of each other, over a given number of days.

Time Interval	River	Station	Number of redds of either species found within 15 m. of each other		Minimum distance between a longnose and a blacknose redd
			<u>R. cataractae</u>	<u>R. atratulus</u>	
May 28-30, 1968	Valley	4	4	3	4.0 m
June 7-8, 1968	Mink	7	1	2	2.5 m
June 17-18, 1968	Mink	10	1	5	10.0 m
May 14-20, 1969	Valley	6	5	7	5.0 m

Plate 3: Proximity of the spawning areas of the two species in the Mink River. Solid arrows indicate nest-sites of longnose dace. The broken arrow indicates a blacknose nest-site.



PLATE 3

C. Spawning Behaviour

Except where otherwise stated all information on spawning behaviour was obtained from laboratory observations.

a) Longnose dace behaviour patterns

The following behaviour patterns are observed in both sexes but are much more common in males, except where otherwise specified.

i) Aggressive behaviour patterns

Dart: The fish darts at an intruder very quickly. The dart is short-lived, usually because the intruder either retreats or the aggressor moves too far away from its territory.

Butt: The fish thrusts with the snout or side of the head, striking the opponent's body without attempting to bite.

Chase: If the intruder flees from a dart or a butt, it may be pursued, the defending fish usually following as closely as it can and biting whenever the fleeing fish slows or stops.

Bite: The dart and chase frequently lead to biting. Biting occurs when the aggressor quickly closes its mouth over some part of the intruder's body, usually in the region of the caudal fin. No apparent damage is caused by this action.

Fight: Two males parallel to one another, with their heads together, go through a series of quick sweeping lateral head-butts, the majority of which are glancing blows causing the fish to slide over or under the opponent's head. When this crossing over takes place the two combatants have merely reversed positions and they immediately butt each other again.

ii) Courtship behaviour patterns

Follow: One fish swims behind the other without making contact, stopping when it stops.

Nudge: In lateral-nudging, one fish takes up a position parallel to a second and pushes its side against that of the other in a swaying motion. In nose-nudging, the fish pushes the snout and anterior portion of the dorsal surface of the head against the posterior region of the abdomen of the second.

Nibble: One fish gently opens and closes its mouth on the dorsal fin, caudal fin or nape of the neck of the second.

Tremble: Trembling is restricted to territorial males.

When a female passes overhead, the male remaining on the bottom in its station goes through a series of high frequency vibrations in which the entire body trembles. These trembles last $\frac{1}{2}$ to 2 seconds and are repeated every $\frac{1}{4}$ to 1 second, as long as the female remains overhead.

Quiver: The quiver is distinctly different from the tremble and is performed only by males. While parallel to a female the male's body goes through a series of forceful muscular contractions. These last approximately one to two seconds and cause lateral undulations to pass down the length of the body. The male usually goes through several quivers (5-6) in succession with very short pauses ($\frac{1}{4}$ - $\frac{1}{2}$ sec) between each.

b) Blacknose dace behaviour patterns

The following behaviour patterns are observed in both sexes but are more common in males, except where otherwise specified.

i) Aggressive behaviour patterns

The aggressive behaviour patterns of darting, butting, chasing and biting in blacknose dace are similar to those of longnose dace.

Fight: Two rival males swim parallel to one another, laterally butting each other with their heads, the point of contact being the tuberculated opercula. This action usually leads to the males chasing one another in a roundabout circular manner. The males do not contact one another during this roundabout chasing.

ii) Courtship behaviour patterns

The courtship or enticement behaviour patterns of following, nudging, nibbling and quivering in blacknose dace are similar to those of longnose dace.

Circling: A male and female circle around one another several times and then resume mutual nibbling and nudging. Although circling is very similar to the roundabout fighting between rival males, it is a noticeably slower non-aggressive action.

Dance: The male swims some 3 to 5 cms in front of the female and at right angles to her. He swims approximately 15 cms one way, turns around and swims back the same distance. The male repeats this back and forth motion several times, quivering his body as he does so, giving the swimming motion a wobbly appearance.

The reproductive cycle of these two fishes has been divided into four phases: pre-territorial, territorial, sexual, and parental. While there are qualitative and quantitative behavioural differences in longnose and blacknose dace, the phases are basically the same in the two species.

c) Longnose dace spawning behaviour

i) Pre-territorial phase

Prior to the onset of reproductive activities, longnose dace display a very sedentary behaviour, usually

remaining secluded in spaces between or under rocks. Both males and females in such places have been observed to show a mild form of aggression towards a trespasser, in the way of a dart or butt. However, for the most part, fish take no interest in one another despite frequent encounters.

When temperature and light conditions in the stream tank simulated those of early May 1968, the fish became more active. Sexual dimorphism developed with angles of the lips, cheeks, bases of the paired fins and the anal fin of males taking on a bright crimson colour. Females, however, showed only a light pink colouration in these areas. In the male, the snout, dorsal edges of the opercula, pectoral fins and entire dorsal surface became tuberculated. Females were non-tuberculate. Correlated with this sexual dimorphism was the appearance of male territoriality.

ii) Territorial phase

A territory refers to any defended area (Noble, 1939). The term "station" used frequently in the study is defined by Kalleberg (1958) as the place in which the occupant of the territory spends most of its time and where it also displays the maximum aggressiveness.

In longnose dace, only males become territorial. Although the territory guarded is only 10 to 20 cms in diameter, the male spends most of its time at a small central station which usually becomes the nest-site.

The first sign of territorial behaviour occurred 36 days prior to the first spawning. At this time two of the five males present, temporarily held territories. However, seven days prior to the first spawning all five males held territories and were aggressive. Correlated with the increase in aggressive behaviour was an increase in the intensity of spawning colouration.

At the onset of territorial behaviour, males showed aggression towards both sexes in the tank. Later, males began to entice females onto their stations. These early enticements were in the form of frequent nudgings. At this early stage in the reproductive cycle, females either ignored this pattern of behaviour or retreated to another part of the tank. Although territorial males showed only aggressive behaviour towards other males, they often showed both enticement and aggression towards females. As territorial males approached spawning condition, they showed stronger interest in females by beginning to follow, nibble and nudge them more frequently. The comparatively inactive females, however, still remained non-receptive and spent most of their time concealed under rocks.

Territorial males were soon observed to quiver beside females. If the female failed to respond, the male aggressively forced her from the territory. In the presence of a female a territorial longnose male often pushed his snout in a quivering motion between the rocks below him, the

long axis of his body making an angle of approximately 45 to 90° with the bottom. The station substrate, composed of large pieces of gravel and rocks (> 5 cm in diam.), was not moved by this action.

As a result of the irregular bottom two territorial males were often quite close to one another yet remained out of each other's field of vision. As more males established territories, the aggression in the tank became more intense. Instead of a single dart, butt or bite to an intruder, the guarding males frequently engaged in savage fights. Since territories were close together males sometimes shared a common boundary. When two such neighbouring males encountered one another both were reluctant to flee from an attack and intense fighting resulted. This fighting was carried on for several seconds, sometimes lasting for up to a minute. When one male delivered a direct butt to another it usually tipped the other male over onto one side, momentarily ending the fight. Fighting was so intense and prolonged at times that visible exhaustion followed with the combatants remaining motionless on the bottom gasping heavily.

A day or two prior to spawning, territorial males are easily excited by the presence of females. With the approach of a female the male immediately begins to entice her onto his station. When a female passes above a territorial male, he quickly moves directly over the station in his territory, and goes through a series of trembles while the female remains overhead.

Males have been observed to guard territories throughout the daily period of illumination. During this interval, males have been observed to display both enticement and aggressive behaviour patterns.

Territories are sometimes exchanged. A territory held by one male may be held by another the following day. Generally the same male will hold a given territory over 3 to 4 days. Even though territories are sometimes exchanged, the boundaries taken up by the new territorial male are usually the same and the exact positioning of the station is retained. Therefore, it would appear that the location of a station is not random but rather precise, in that it obviously must meet certain requirements. The station, usually destined to become the nest-site, is directly over some natural depression in the substrate which will allow eggs to be deposited such that they are not washed downstream by the fast-flowing water but filter down between the rocks and adhere to the underlying stones. The territorial male usually establishes its territory in a place where strong current is diverted by surrounding rocks. This observation is strongly supported by descriptions and diagrams made of spawning sites located in the field.

iii) Sexual phase

As females ripen they no longer are as retiring but spend more time fully exposed, resting on the bottom, often near a male's territory. In the presence of a female with a

distended abdomen, territorial males frequently push their snouts between rocks in the station. The function of this substrate-probing is not clear but it would appear to be a form of preparation for egg deposition and/or a form of courtship behaviour.

Females no longer avoid or flee from males but enter their territories to rest on the bottom, often vigorously pushing their snouts between rocks in the station, in the same manner as described for territorial males. The female leaves the territory immediately after this substrate-probing but returns shortly to once again thrust her snout between the rocks. When the female leaves the territory, the male probes this same substrate with his snout. While the female pushes her snout between the rocks in the station the male often quivers parallel to her as she does so. As the male goes through these quiverings beside the female, both fish, their snouts thrust between pieces of substrate, are angled toward the bottom. The two fish then assume a position on the bottom over the depression they probed. The pair, touching sides, go through a spawning act in which both quiver for 1 to 2 seconds. The eggs are squirted into the depression and fall between the rocks and adhere to the surfaces of the stones. After the spawning act, the fish remain motionless on the bottom gasping heavily. However, the male recovers quickly and forces the female from his territory with a lateral sway.

The same female was observed to return to the same nest-site several times, first pushing her snout between the rocks then spawning with the male. In each of the observed spawning acts no detectable spawning clasp was observed. Each successful spawning showed a noticeable reduction in the size of the abdomen of the female.

Figure 10 illustrates some of these behaviour patterns.

iv) Parental phase

Once spawning is complete the male becomes most aggressive. He spends almost all his time remaining directly over the nest-site, defending the area vigorously against all fish with exception of gravid females with which he may spawn. There is no apparent fanning of eggs by the parental male but he does on occasion push his snout between the rocks beneath him. The parental male, however, was never observed to eat the eggs he was guarding.

Of the 43 longnose nest-sites located in the field, a guarding male was present with 11. These guarding males were not easily frightened off and were never seen to eat the eggs they defended. However, when a site was disturbed during the collection of eggs other longnose males were often observed to enter the nest-site where they began to devour eggs.

The parental male displays a low tolerance to trespassing fish. In fact, a dart will often be made towards

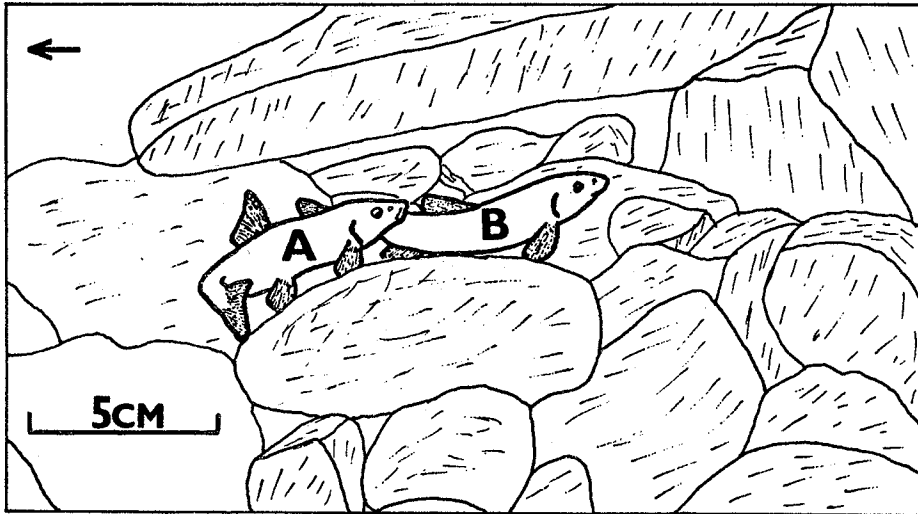
Figure 10: Outline tracings of photographs taken in the fast-flowing stream tank. Arrows indicate direction of flow.

A Territorial longnose male A, with erected dorsal fin, aggressively removing intruding male B from its territory with a bite to the caudal peduncle.

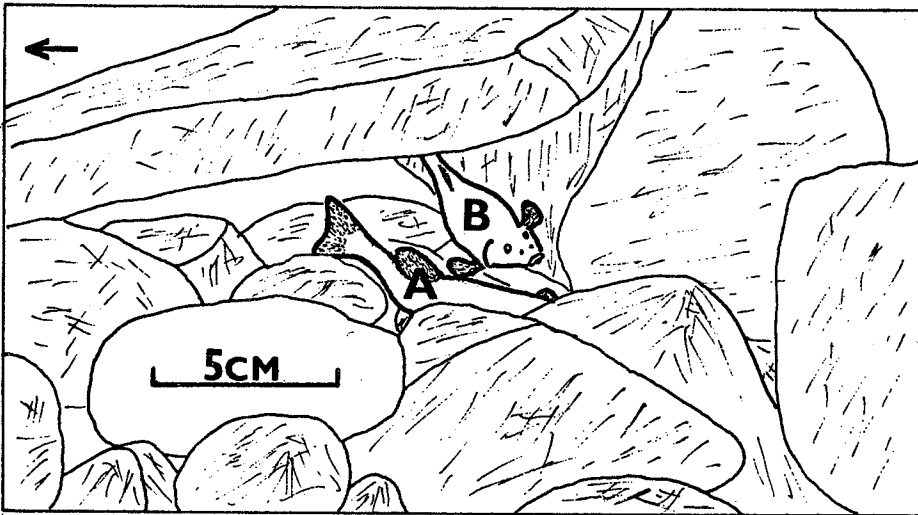
B Gravid longnose female B, approaching territorial male A, resting directly over the station in the territory.

C Territorial longnose male A hovering above the station substrate which later became a nest-site.

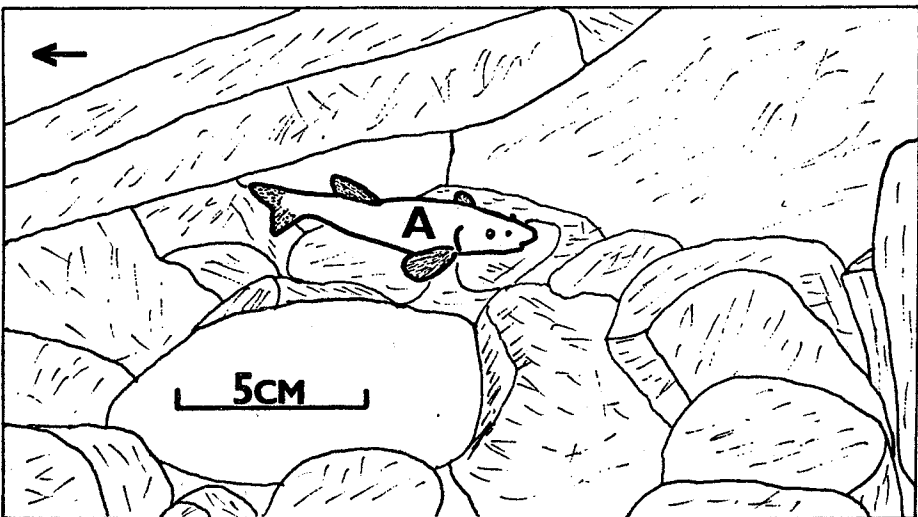
A



B



C



an approaching fish 10 to 15 cms from the nest-site. Immediately after spawning, parental males were never observed to leave the nest-site for the purpose of following females or fighting rival males.

However, as time passes, the aggressive behaviour of parental males recedes. The parental male begins to leave the nest-site for longer periods of time and shows a higher tolerance to trespassing fish. Observations in the stream tank indicate that the length of time a parental male actively guards its nest-site appears to vary from several hours to three days.

Longnose males spawn with several females or the same female a number of times. In the stream tank a longnose male spawned with two different females over a several day period. One of these females had also spawned with another male previously. Since redds of longnose dace located in the Mink and Valley rivers ranged in size from approximately 100 to 900 eggs, it would appear that the number of spawnings by territorial males varies considerably.

v) Sex discrimination by territorial longnose males

Although prior to spawning longnose males immediately attack an intruding male, the approach of a female does not evoke this same aggressive response. Instead, the male courts her.

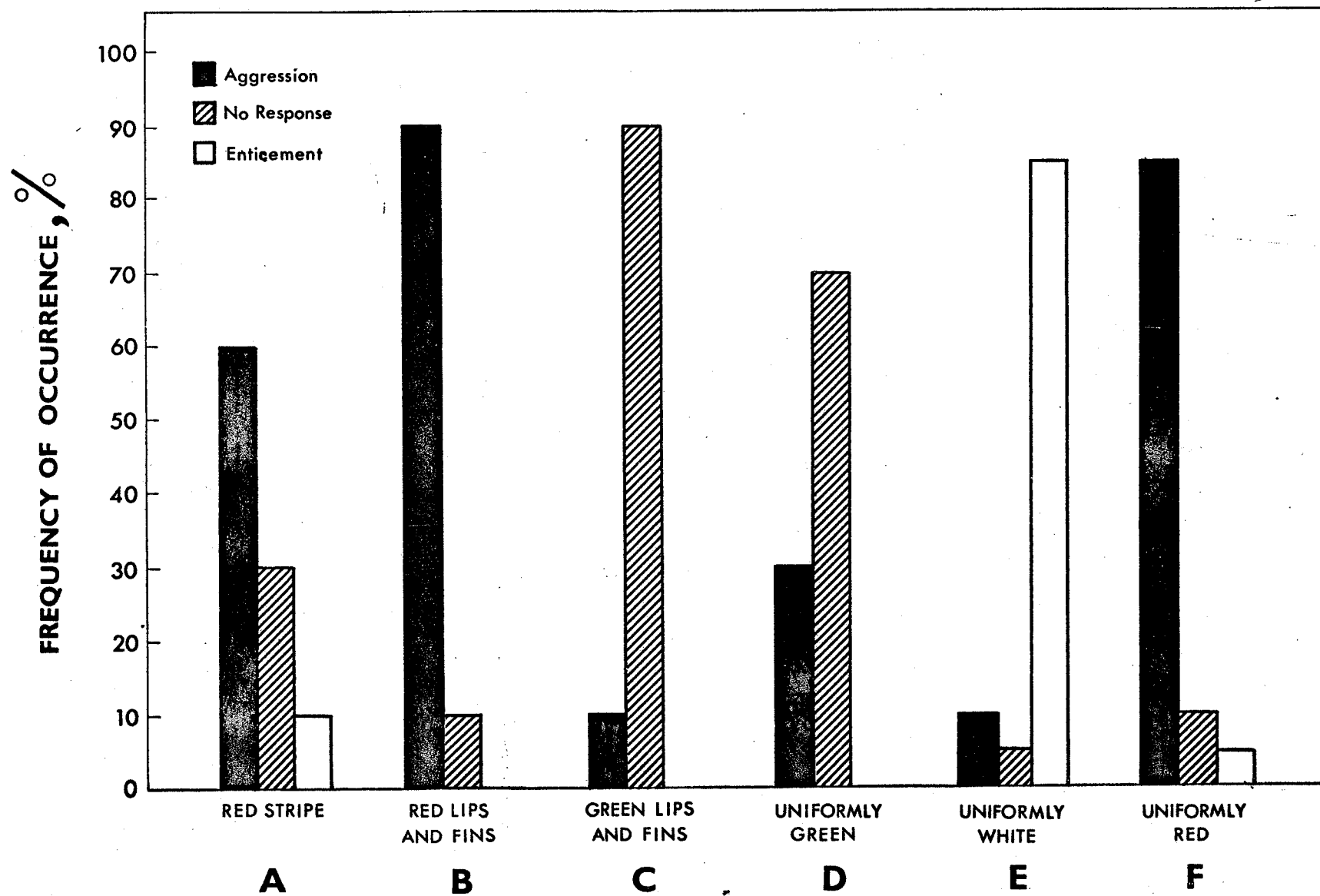
Since sexual dimorphism in the form of marked colour differences exists, it seems most probable that colour and/or

colour pattern plays an important role in sex discrimination. To test this hypothesis, a series of tests were conducted using two-dimensional models (Fig. 2). Aggressive responses were always in the form of a dart, the test male swimming directly to the model. Enticement responses, however, were noticeably different. The test male slowly lifted from the station substrate, hovered below the model, and then attempted to nudge it with its nose and dorsal part of the head.

Males were able to discriminate visually between models (Fig. 11). A high percentage of aggressive responses were elicited by models with red colouration, regardless of pattern. On the other hand, test males displayed a high percentage of enticement patterns to the uniformly white model. The uniformly white model apparently resembled a female. The models with green colouration, however, failed to elicit a high percentage of either enticement or aggressive behaviour patterns. The test males usually showed no response to these models.

The results strongly suggest that colour, rather than colour pattern, functions as a visual cue for sex discrimination in male longnose dace.

Figure 11: Frequency of occurrence of different responses by territorial longnose males to six different two-dimensional models with different colours and/or colour patterns. Results for models A, B, C and D are based on 10 trials. Results for models E and F are based on 20 trials.



d) Blacknose dace spawning behaviour

1) Pre-territorial phase

Prior to the onset of reproductive activities the blacknose dace are also quite inactive, the majority of their time being spent on the bottom hidden from view. Occasional encounters between fish take place at mid-water with mild forms of aggression (head-butts) being shown. Aggressive females dart, bite and occasionally chase both males and females. Females are also observed to nibble and nudge males. However, in these early encounters the male avoids the female and retreats to some other part of the tank.

Marked sexual dimorphism is exhibited by the blacknose dace. Males in breeding condition possess nuptial tubercles on the head, opercula, anal fin, pelvic fins, scales and the ventral surface of the caudal peduncle. Nuptial pads, light orange in colour, are present on the pectoral fins. Females are non-tuberculate. The tuberculate males have a brick red lateral band of chromatophores, while females have a dark black lateral band. When males exhibit aggressive behaviour it is manifested by an intensification of colouration. The colouration in the lateral stripe disperses, covering the greater part of the male's sides, and the dark black spots on the sides become more pronounced. Males not engaged in any form of interaction do not display this flushing of colour. Females engaged in encounters also

exhibit a flushing of colour. The normally dark black lateral stripe takes on a silvery appearance and the dark spots on the lateral side become darker, giving the female a blotched appearance.

ii) Territorial phase

In blacknose dace also it is only the male that becomes territorial. The territories, which are roughly circular, vary from 30 to 60 cm in diameter. Field observations on 24 territorial males revealed that all established their territories over areas of fine to medium textured gravel with an upper limit of 2 to 3 cms. The territorial male spends most of its time at a station measuring 10 to 12 cm in diameter. The stations, which usually become nest-sites, are generally located at the downstream end of the territory. Since the bottom over the territories is comparatively uniform, the male has a wide field of vision. The surface velocity over all 24 observed holdings in the Mink River varied from 30 to 45 cm/sec. The depth was relatively shallow, being from 10 to 20 cms.

The first behavioural evidence of reproductive activity is aggressiveness in males. Early in the territorial phase, males show only aggressive behaviour towards females. Males defend territories aggressively against other territorial males and show marked flushing of colouration. Initially, territorial behaviour is desultory but as males approach spawning condition they display stronger

territorial behaviour and begin to court females. These early patterns of courtship are in the form of following, nudging and nibbling. Females, however, continue to be more retiring and are for the most part not in view.

Males were observed to defend territories most aggressively during the bright midday illumination hours. This correlates with field observations, where territorial behaviour was observed from 11:00 am. to 5:00 p.m., being most intense during midday and early afternoon. A male defends a territory for several days. Field observations on the 24 territorial males showed that 15 males defended a territory for one day, seven for a 3 day period and two males for a two day interval.

The defending male directs his attacks to fish as far as 45 cm away from the small station he rests at. For the most part, the male hovers over the station, occasionally rubbing the lower paired fins on the bottom as he moves about. As other males establish territories, there is an increase in the amount of aggression in the tank. The aggression between two rival males is often in the form of fighting during which the fish make as many as ten rotations, often drifting as much as one meter downstream while engaged in this combat. The fight often ends with a short chase, the intruder fleeing.

iii) Sexual phase

As females reach spawning condition they become more active and show interest towards territorial males, often engaging in mutual nudging and following. Territorial males show particular interest in females that settle on the substrate in their territory, quickly moving over them and nibbling on the dorsal fin and the nape of the neck. Males, in addition to nibbling and nudging, also now go through quiverings while lying parallel to a female. Although territorial males occasionally show aggression towards non-receptive females they are generally preoccupied with receptive females. The territorial males respond quickly to the approach of gravid females into their territory. A male attempting to maneuver a female back to his station, swims slightly ahead and below the female, constantly quivering his body. If the courted female leaves the territory the male quickly returns to hover over the station substrate.

Territorial blacknose males were repeatedly observed to respond to approaching females from as far as 60 cm. Territorial males gave chase to intruding males while they were as much as 45 cm away. These observations indicated that blacknose males possess good visual sex discrimination.

Receptive females circle, nibble and nudge with males, and allow themselves to be maneuvered to the station. Once a receptive female is over the station the male may

begin to display or dance before her. Once the receptive female is settled in the station, she pushes her nose into the substrate, moving small pieces of gravel. The female then moves over this substrate and the male quickly assumes a position parallel to her. The female arches her body, thrusting her anal fin into the gravel. The male throws his caudal peduncle over hers, curling his body so as to hold the female tightly on the inside curl. Both then go through a violent quivering motion lasting about 2 seconds during which bits of gravel are displaced, leaving a small depression in the substrate. Immediately after spawning the male releases the female from his clasp and both fish remain motionless on the bottom, gasping heavily. The male recovers quickly and forces the female out of the territory with a dart and butt.

Within a few minutes the now parental male began to show sexual behaviour, courting females once more. Successive spawnings in the male's nest-site appeared to be at random, since no care was taken to avoid substrate already containing eggs. Numerous observed spawnings by one blacknose male were all, except for one, in the immediate vicinity of the station. This one spawning outside the station was still, however, within the territorial boundaries. In one observation after spawning, the female retired to the upstream end of the tank, but returned shortly afterwards to spawn again. Males spawned with several females and/or the

same female a number of times.

Figure 12 illustrates some of these behaviour patterns.

iv) Parental phase

Blacknose males maintain a territory from one to three days. They spawn several times over this period without any visible change in behaviour following spawning.

e) Species interactions

i) Longnose male vs. heterospecific females

Although longnose males generally either ignore heterospecific females or show aggression towards them, they have also been observed to show interest in them as spawning partners. Longnose males have been observed to court blacknose females but blacknose females have never been observed to respond. Although blacknose females have entered the territories of longnose males, they were never observed to push their snouts between the rocks in the manner that the longnose females do prior to spawning.

During the laboratory study, a territorial longnose male encountered both a blacknose and a longnose female simultaneously on 12 occasions. Each time the male was observed to show some form of enticement to the female of his own species but to either completely ignore the heterospecific female or to show aggression to her.

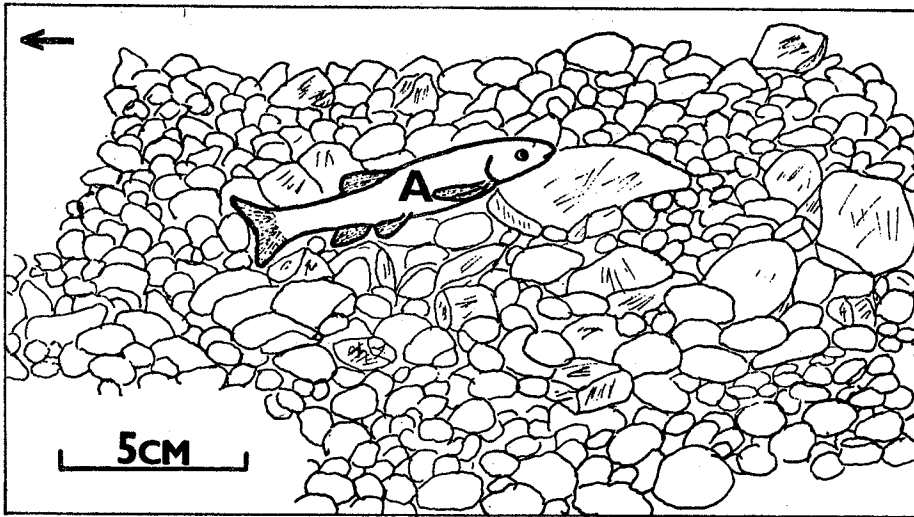
Figure 12: Outline tracings of photographs taken in the slower flowing stream tank. Arrows indicate direction of flow.

- A Territorial blacknose male A hovering over the station substrate in its territory.

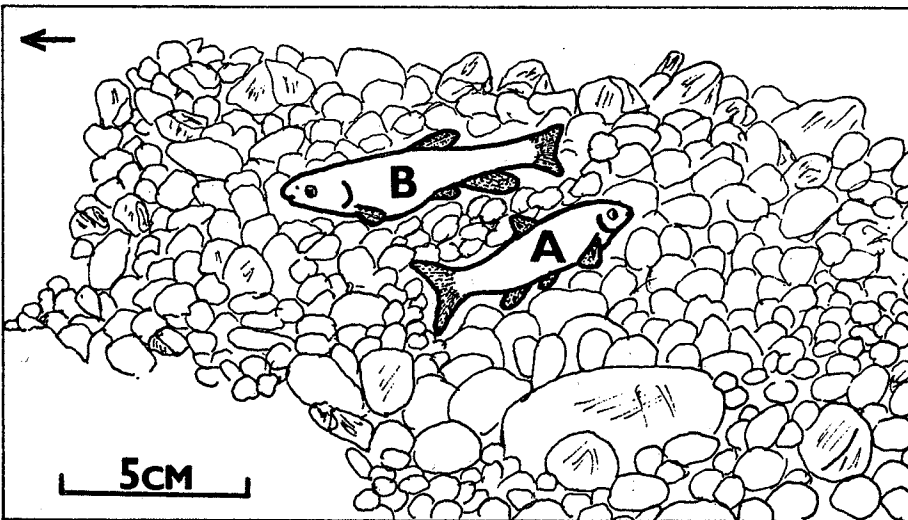
- B Territorial blacknose male A maneuvering blacknose female B over the station substrate.

- C Territorial blacknose male A dancing before settled blacknose female B.

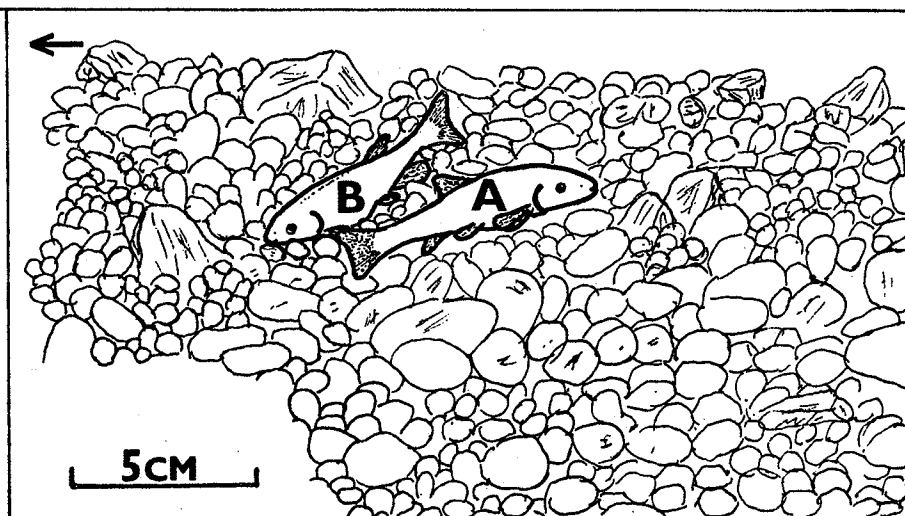
A



B



C



ii) Blacknose male vs. heterospecific females

Blacknose males are generally either aggressive towards or uninterested in heterospecific females. However, some observations were made of territorial blacknose males showing courtship behaviour towards heterospecific females by following, nudging and quivering. Almost all such instances were terminated by the retreat of the heterospecific female. Although longnose females generally flee from territorial blacknose males, in one case a gravid longnose female displayed strong interest in a blacknose territorial male. The female nibbled and nudged the blacknose male but the male did not show mutual interest toward her despite the fact that she had settled in his station. Instead, the blacknose male became aggressive and removed her from his territory. The gravid longnose female did not push her snout into the substrate in the station nor did she arch her body in the manner that blacknose females do when a blacknose male is beside them. Approximately one hour after this observation was made, this same longnose female was observed to spawn with a lone longnose male in another part of this same stream tank. Blacknose males were never observed to dance before heterospecific females or attempt to clasp them.

On 22 occasions in the stream tank a territorial blacknose male encountered simultaneously both a blacknose and a longnose female. On each occasion the male showed

enticement to the homospecific female but either ignored the heterospecific female or showed aggression to her.

iii) Longnose and blacknose males vs. heterospecific males

Both territorial longnose and blacknose males generally either ignore heterospecific males or aggressively remove them from their territories.

f) Comparison of spawning behaviour patterns in longnose and blacknose dace

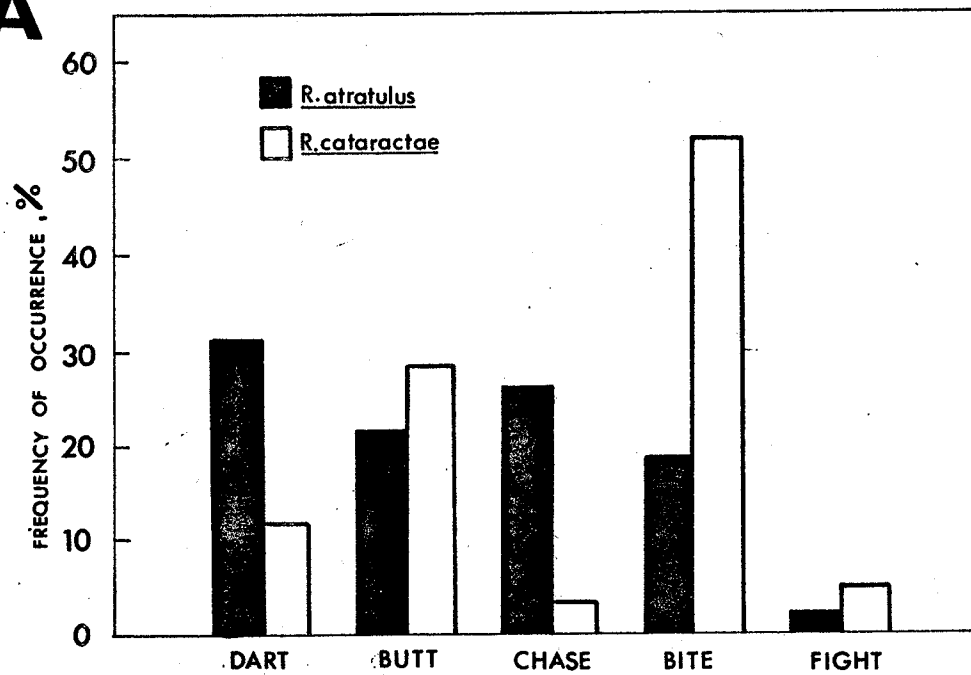
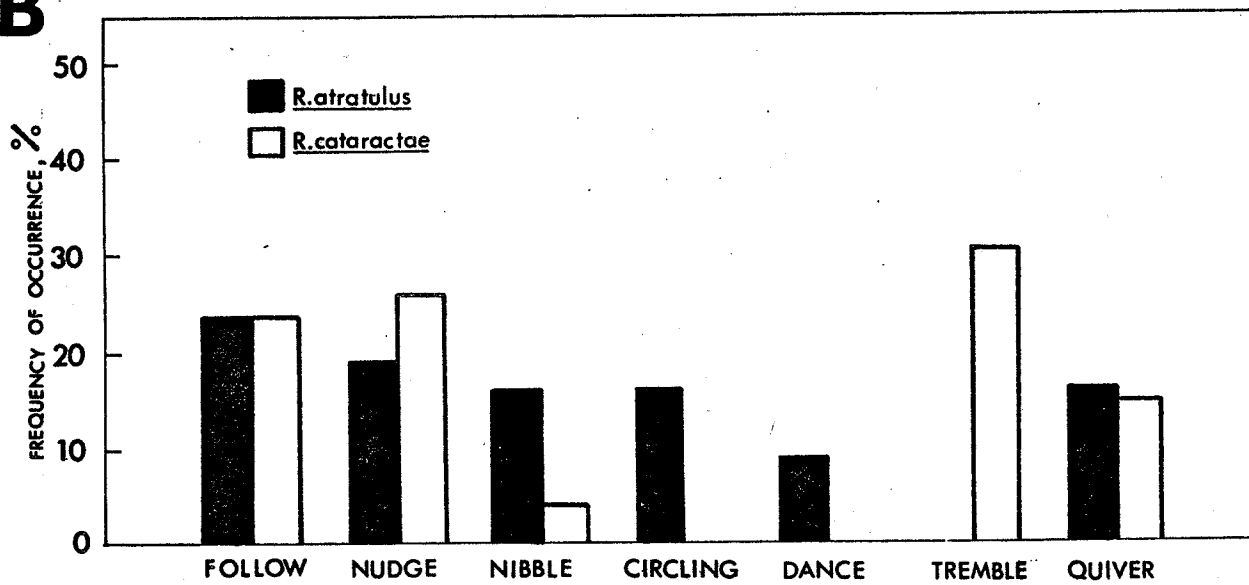
Although the patterns of aggressive behaviour of the longnose and blacknose males are basically the same, marked quantitative differences exist (Fig. 13A). These quantitative differences between the two species are probably a reflection of the size and location of the territory defended.

Longnose territories are of comparatively small size and aggression takes place over a short distance. Thus the patterns of aggressive behaviour shown by territorial longnose males are primarily those of butting and biting. On the other hand, the territory defended by a blacknose male is considerably larger. The uniform bottom of the blacknose territory affords a wide field of vision and unlike the longnose male, the patterns of aggressive behaviour are predominantly those of darting and chasing.

Figure 13: Frequency of occurrence of various patterns of behaviour observed in longnose and blacknose dace. Frequency data are compiled from quantitative observations made throughout the entire reproductive cycle.

A Aggressive behaviour patterns

B Courtship behaviour patterns

A**B**

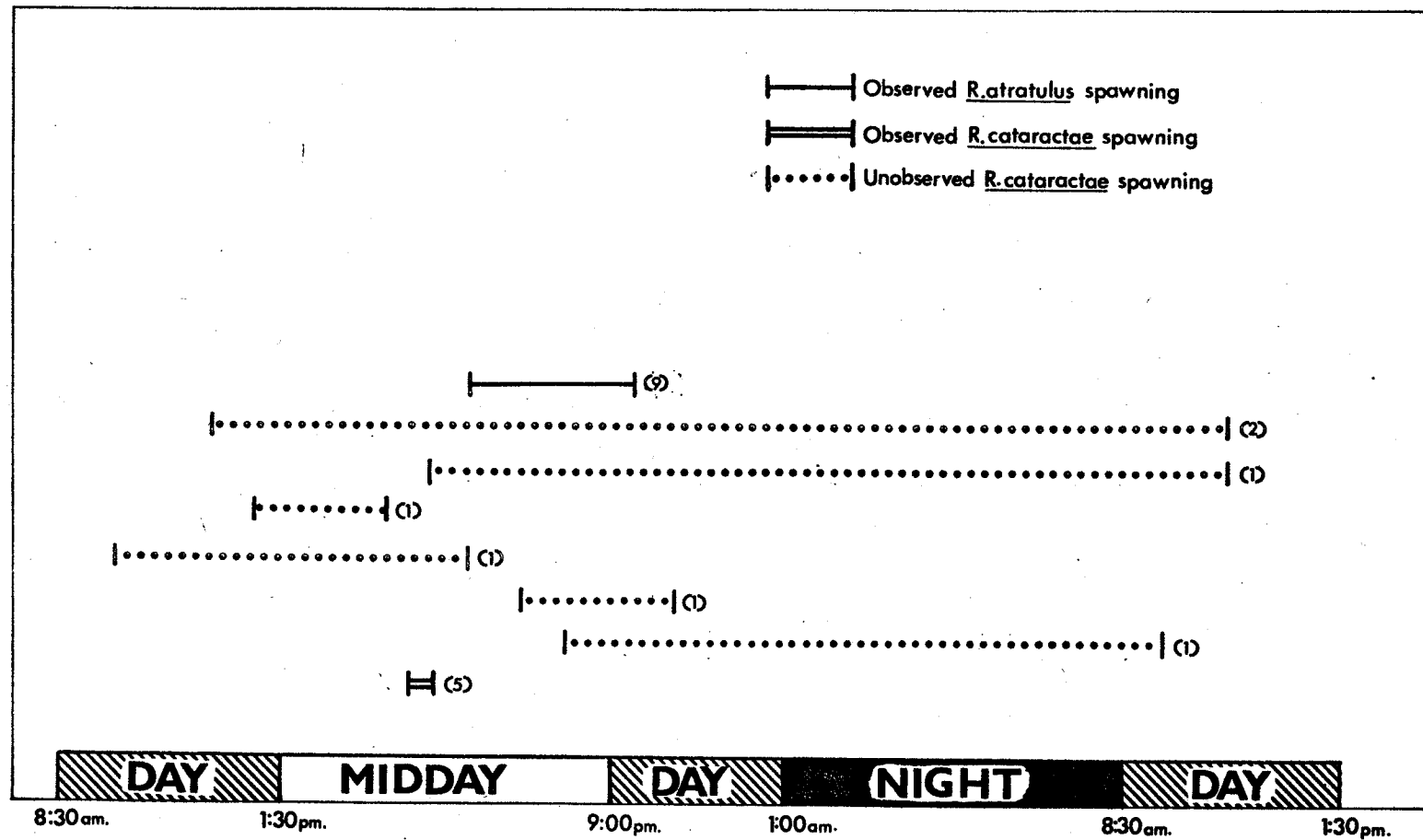
When courtship behaviour patterns of the two species are compared a number of qualitative differences are revealed (Fig. 13B). The blacknose male demonstrates considerable mobility in its courtship behaviour patterns. In contrast, longnose males display rather sedentary types of courtship behaviour, rarely straying far from the station. During the trembling pattern of courtship behaviour, for example, the male remains on the bottom of the station. The blacknose male, however, is much more mobile over the uniform bottom of its territory. The trembling behaviour pattern is absent in this species but circling and dancing occur.

g) Time of spawning (day)

Although several spawnings by both species were observed, a number occurred in the absence of the observer. Since females show a noticeable reduction in abdomen size immediately after spawning, the interval of time during which unobserved spawnings occurred could be determined. The finding of eggs in the tank verified unobserved spawnings.

All spawnings, except four, were observed or noted and verified to have occurred during the daylight illumination period (Fig. 14). Since neither species restricts spawning to any particular time of the day, overlap in time of spawning apparently exists.

Figure 14: Time intervals over which dace spawnings were observed or noted and verified. Numbers in brackets indicate the number of spawnings recorded over that interval of time.



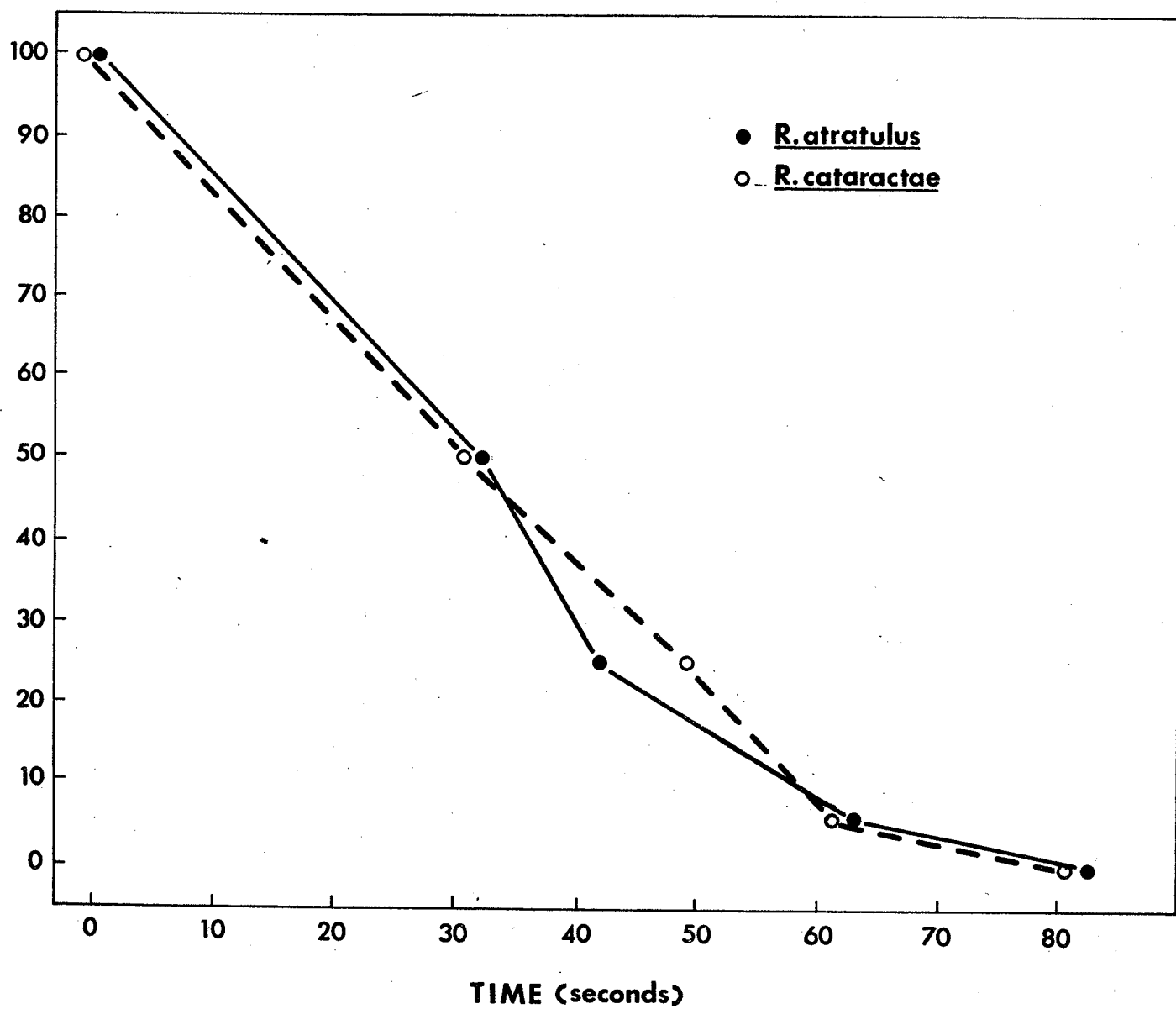
ILLUMINATION PATTERN

D. Longevity of sperm

Since the two species concerned show gametic compatibility, it seems important to examine the longevity of sperm in order to determine the possibility of hybridization due to chance meetings of drifting gametes. The purpose of this section therefore is to examine the duration of sperm motility in order to determine if it may act as a post-mating isolating mechanism.

The means of five trials for each species revealed that at 14°C, sperm motility was lost at a similar rate in both species. By 31 to 32 seconds, 50% of the spermatozoa were no longer moving and by 81 to 83 seconds no remaining motility was visible (Fig. 15). A gamete life of 32 seconds would allow viable sperm to drift from 2.4 to 19.2 meters in the water velocities observed in spawning areas. Since this distance equals or exceeds the distance between pools and riffles where redds of both species were collected, accidental fertilization by drifting sperm is a distinct possibility.

Figure 15: Mean time in seconds for loss in motility
of dace sperm in 14°C stream water.
Sample size was five for each species.



DISCUSSION

The reproductive isolation of the sympatrically occurring longnose and blacknose dace is based on the interaction of a number of mechanisms. The factors I have been able to ascertain which serve as isolating mechanisms include: time of spawning (season), place of spawning, and spawning behaviour.

A. Time of spawning (season and day)

Although the present study reveals that peaks of breeding activity for the two species are not identical, their time of spawning does overlap. However, many longnose dace spawn before blacknose dace and thus do so at lower temperatures. This fact may perhaps explain the more northerly distribution of longnose dace in North America as reported by Keleher (1956).

Time of day for spawning is not considered to act as an isolating mechanism between the two species of dace, since no differences in time of spawning were revealed. Mayr (1963) states that no case is known of two species that are isolated by restriction of their courtship period to different parts of the day.

B. Place of spawning

a) Distribution of adults

Becker (1962) generally found R. cataractae to frequent fast shallow waters with gravel bottoms while

R. atratulus meleagris was usually found between fast and slow water or in eddies behind rocks.

The present study also indicates that longnose and blacknose dace tend to occupy different habitats, with longnose preferring swifter waters and blacknose the calmer waters. Evidence from morphology and behaviour reveals that the two species are adapted to these different habitats. The terete shape and negative buoyancy of the longnose dace (Gee, 1968) are adaptations to life in swift-flowing waters. The large testes of longnose males would also appear to be an adaptation to spawning in fast velocities of water where perhaps more sperm are required to fertilize eggs. Although there are differences in adult distribution of the two species in water currents during spawning, considerable overlap exists. Such differences result in a reduction of meetings of heterospecific individuals but this is considered to be only a partially effective isolating mechanism.

b) Distribution of redds

Greeley and Bishop (1933) reported breeding R. cataractae over fine gravel in strong current. Dobie et al. (1956) reported longnose dace to breed over sand or gravel bottom in clear swift currents. Raney (1940a) considered the downstream end of pools over sand and fine gravel to be preferred spawning areas for R. atratulus meleagris. Noble (1965) stated that mature female and breeding male

R. atratulus meleagris were most often taken from swift water 3 to 6 inches deep over gravel.

The present work shows that breeding individuals of both species of dace differ markedly in substrate and water velocity preference and that breeding site selection serves as an important device to prevent interspecific mating. The spawning areas of longnose dace are characterized by a rocky bottom (nest substrate upper limit > 5 cm) with swift-flowing currents (> 45 cm/sec). Blacknose dace spawning areas are characterized by a gravel bottom (nest substrate upper limit < 5 cm) with slow to moderately flowing currents (< 45 cm/sec). Close correlation with respect to water velocity is shown between distribution of adults of each species and their redds.

In light of the laboratory observations on spawning behaviour the abrupt break in distribution of redds of blacknose and longnose dace appears to stem from the fact that one species moves substrate during spawning while the other does not. Burying its eggs, blacknose restrict spawning to areas where the substrate is small and moveable (upper limit < 5 cm). Longnose dace, whose eggs are found adhering to rocks and underlying gravel (upper limit > 5 cm), restrict spawning to places where the substrate is coarse enough to provide natural depressions between pieces of substrate for egg deposition.

C. Spawning Behaviour

Mayr (1963) states that males of every species have specific courtships or displays to which on the whole only females of the same species are receptive. The spawning behaviour of the longnose dace R. cataractae has remained obscure up to the present study. The only record available on the breeding behaviour of R. cataractae comes from Greeley and Bishop (1933) who reported spawning in Tributary 2 of Clear Pond, New York. Observations were made on July 16, 1932,:

" . . . A school of at least 25 fishes was watched at close range, about 3 feet, in this small stream where they were swimming over an area of fine gravel in strong current. The water was only 2 to 4 inches deep and the fishes could be seen plainly. Evidently there were several females present and those which could be identified as females were larger than the attending males, which were several times more numerous than females. A single spawning act was seen. The female stopped on the bottom and a dense group of at least six males, individuals which had been following her closely, hid the female from view for a brief second or two as they crowded against her sides and dorsal area. Following this, several males were seen to thrust their noses downward in an evident search for eggs. A handful of the gravel was picked up and found to be well supplied with eggs distributed between the pebbles. . . ."

Raney (1940a) made detailed observations on breeding in R. atratulus meleagris. He stated that blacknose males remain at one spot and spawn with many females for several days until a small depression is worn near the center of the 2 foot diameter territory. Raney (1940a) observed that the male clasped the female during spawning by throwing his

caudal peduncle over hers. The actual contact of the adults against the bottom during the breeding act tossed some sand and pebbles aside. Traver (1929) described for the eastern blacknose dace, R. atratulus atratulus a type of activity preliminary to spawning where a male swims back and forth beneath, in front or over a female. This activity is similar to that referred to as the dance of male R. atratulus meleagris in the present study, yet Raney (1940a) observed no such action in this species.

Laboratory observations have shown that several of the aggressive behaviour patterns of the blacknose and longnose dace are similar, including darting, butting, chasing, and biting. Blacknose males, however, dart and chase with greater frequency than longnose males which predominately butt and bite. Although the courtship behaviour patterns of following, nudging, nibbling and quivering are similar for the two species, a number of courtship patterns occur in one species and not the other. Blacknose males circle with females, lead them, dance before them, and clasp them during spawning. Longnose males display a trembling courtship behaviour pattern but have no noticeable spawning clasp.

The different spawning behaviour patterns of the two species of dace would appear to be a reflection of the size and location of the territory defended. Breeding longnose dace display rather sedentary behaviour patterns as

a result of the irregular bottom and high velocities of water which restrict both vision and movement. The spawning behaviour patterns of the blacknose dace, however, exhibit greater mobility, the uniform bottom and moderate water velocities characteristic of its spawning areas affording a wide field of vision and more freedom of movement.

Laboratory observations made in the present investigation indicate recognition of potential mates by males of both species of dace. The inquiry into sex discrimination further suggests that visual cues may play an important role in mate recognition by longnose males. Observations have shown that males show a distinct preference for homospecific females over heterospecific females and that females respond only to patterns of courtship behaviour shown by males of their own species.

D. Longevity of Sperm

The inquiry into sperm motility of longnose and blacknose dace revealed that longevity of sperm does not act as a post-mating isolating mechanism. Accidental fertilization of eggs of one species by drifting sperm of the other is shown to be a distinct possibility in the absence of other reinforcing mechanisms.

SUMMARY AND CONCLUSIONS

The results of the present study are summarized using Mayr's (1963) classification of isolating mechanisms.

I. MECHANISMS THAT PREVENT INTERSPECIFIC CROSSES (PRE-MATING MECHANISMS)

a. POTENTIAL MATES DO NOT MEET (SEASONAL AND HABITAT ISOLATION)

Potential mates do meet. Overlap in time of spawning (season) exists, although many longnose dace spawn before blacknose. Differences in distribution of adults in waters of differing velocities are revealed during the spawning period, but considerable overlap occurs. These differences are considered to be partially effective in reducing encounters.

Places of spawning of the two species differ markedly. Longnose spawning areas are characterized by rocky bottoms and high velocities of water (> 45 cm/sec). In strong contrast blacknose spawning areas have a uniform gravel bottom and slow to moderate water velocities (< 45 cm/sec). Since these areas differ greatly, place of spawning is considered to be an important isolating mechanism.

b. POTENTIAL MATES MEET BUT DO NOT MATE (ETHOLOGICAL ISOLATION)

Breeding males of either species become territorial and spend the majority of time over a small station within the territory which eventually becomes the nest-site. Unlike the larger blacknose territories (30-60 cm in diam.) longnose territories are quite small (10-20 cm in diam.).

Potential mates do meet but males of both species show distinct preferences for homospecific females over heterospecific females. Longnose males entice females over the station by nose-nudging and trembling. Blacknose males, however, lead females to the station and dance before them. Laboratory observations show that females respond only to patterns of courtship behaviour shown by males of their own species. Longnose females respond by thrusting their snout into the station substrate while blacknose females respond by settling on the station substrate and arching their body. Once the longnose female has responded to the enticements of the male, both fish thrust their snouts into the substrate and then assume a side by side position on the substrate while spawning. Blacknose males mount the arched females and clasp them during the spawning act which follows. These behavioural incompatibilities as outlined above are considered to effectively prevent hybridization.

- c. COPULATION ATTEMPTED BUT NO TRANSFER OF SPERM
TAKES PLACE (MECHANICAL ISOLATION)

Not applicable here because of external fertilization.

II. MECHANISMS THAT REDUCE FULL SUCCESS OF INTERSPECIFIC CROSSES (POST-MATING MECHANISMS)

- a. SPERM TRANSFER TAKES PLACE BUT EGG IS NOT
FERTILIZED (GAMETIC MORTALITY)

Previous work (J. H. Gee, personal communication) shows that fertilization occurs in interspecific crosses. The present work reveals that longevity of sperm in both species is of sufficient duration to allow accidental fertilization by drifting sperm.

- b. EGG IS FERTILIZED BUT ZYGOTE DIES (ZYGOTE
MORTALITY)

Zygotes from interspecific fertilizations show a hatching success comparable to that of either parental species (J. H. Gee, personal communication).

- c. ZYGOTE PRODUCES AN F_1 HYBRID OF REDUCED VIABILITY
(HYBRID INVIABILITY)

In the laboratory, F_1 hybrids show a survival rate comparable to that of either parental species (J. H. Gee, personal communication).

- d. F_1 HYBRID ZYGOTE IS FULLY VIABLE BUT PARTIALLY OR COMPLETELY STERILE OR PRODUCE DEFICIENT F_2 (HYBRID STERILITY)

Unknown as yet.

The interaction of the isolating mechanisms of temporal, spatial, and ethological isolation are considered to act as a very powerful barrier to hybridization between longnose and blacknose dace in the Mink and Valley rivers. Although post-mating isolating mechanisms are lacking, these pre-mating isolating mechanisms are considered to effectively maintain a high degree of reproductive isolation.

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