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-existance 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.

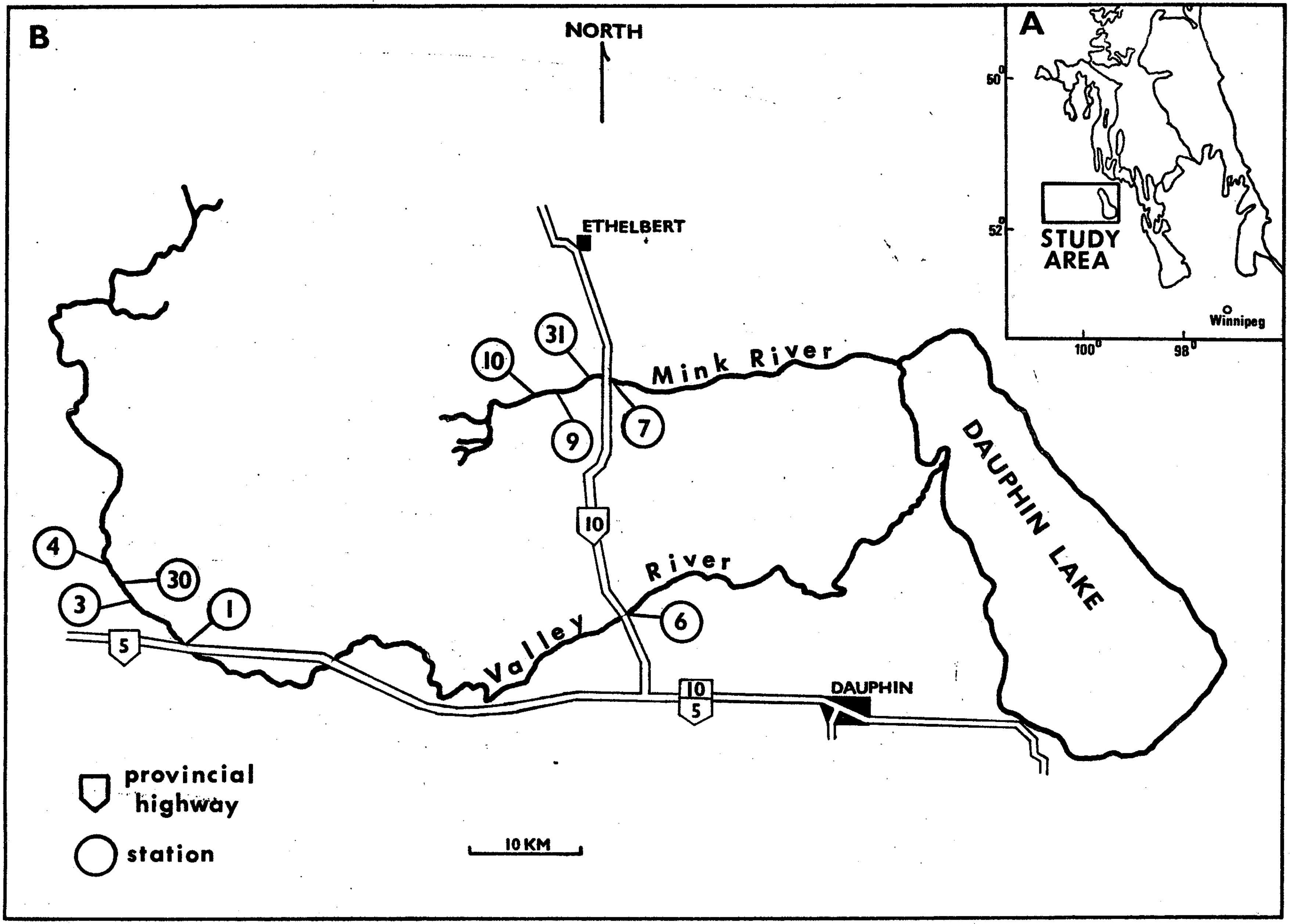
DESCRIPTION OF THE STUDY AREA

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.


B
NORTH
A
50°
52°
STUDY AREA
Winnipeg
100°
98°
ETHELBERT
31
10
9
7
Mink River
DAUPHIN LAKE
10
River
Valley
4
30
3
1
6
5
10
5
DAUPHIN
provincial highway
station
10 KM

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 (±.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