

A STUDY OF HYBRIDIZATION BETWEEN TWO SPECIES OF CYPRINID
FISHES, ACROCHEILUS ALUTACEUS AND PTYCHOCHEILUS OREGONENSIS

by

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B.Sc., Colorado State University, 1958

M.Sc., University of Miami, 1960

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THE REQUIREMENTS FOR THE DEGREE OF
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We accept this thesis as conforming to the
required standard

The University of British Columbia

June, 1966

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FACULTY OF GRADUATE STUDIES

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M.Sc., University of Miami, 1960

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ABSTRACT

Fish morphologically intermediate between Acrocheilus alutaceus and Ptychocheilus oregonensis have been collected infrequently from the Columbia River system. Morphological comparisons of wild Acrocheilus, Ptychocheilus, and intermediates, with artificial inter- and intra- species crosses indicated that wild intermediates were of hybrid origin. o

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Hybrids are largely sterile, but hybrid males produce fertile sperm infrequently. The parent species show no evidence of gene flow, but a few intermediates are apparently backcrosses. Gene flow is probably blocked by the rarity and partial sterility of hybrids and by selection against backcrosses.

The presence of wild backcrosses and partial fertility of hybrids suggest a large amount of genetic similarity between Acrocheilus and Ptychocheilus. This contention is strengthened by similarity in chromosome morphology and the presence of dominance effects in the inheritance of some parental characters in hybrids.

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PUBLICATIONS

Stewart, K.W., 1962. Observations on the morphology and optical properties of the adipose eyelid of fishes. J. Fish Res. Bd. Canada 19; 1161-1162.

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KENNETH WILLIAM STEWART. A STUDY OF HYBRIDIZATION BETWEEN TWO
SPECIES OF CYPRINID FISHES, ACROCHEILUS ALUTACEUS AND PTYCHOCHEILUSⁱ
OREGONENSIS

ABSTRACT

Supervisor: C. C. Lindsey.

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INTRODUCTION

Mayr defines a species as a population or group of populations which are reproductively isolated from all other populations. This is an objective but not entirely unambiguous definition. In nature, there is a continuum between freely interbreeding populations and those which occur together without hybridization.

Mechanisms affecting the occurrence and rate of gene flow between populations have received attention from many investigators, especially in the field of botany. Epling (1947) investigated the evidence for gene flow between plant species in California. Anderson (1949 and 1953) extensively reviewed introgressive hybridization in plants.

Mayr (1963) summarized knowledge regarding gene flow in animal populations, and many subsequent authors have modified or elaborated upon his views. Bigelow (1965) re-examines the concepts of reproductive isolation and gene flow in animal species.

Generally these authors conclude that if populations are closely related genetically and there is no selection against hybrids swamping will result, giving rise to a population which combines the adaptive characteristics of both parental types. Introgression occurs when one or a few genes or gene complexes introduced into the parental population by hybridization are

adaptively superior to the original genes or complexes. At the opposite extreme, only rare, sterile hybrids may be produced, or there may be no hybridization at all between related populations in zones of contact.

Prior to mating, behavioral, temporal and spatial isolation of breeding populations may limit or prevent hybridization. After mating, genetic factors causing zygote inviability or hybrid sterility can prevent hybrids from surviving or mating. If hybrid to hybrid matings or backcrossing do occur, many or all of the progeny will be selected against because recombination of the parental genotypes will leave them with unbalanced and poorly adapted genotypes.

Compared with other animals, fish hybridize rather frequently. Hubbs (1955) points out that hybrids are more common in freshwater than in marine fishes, in temperate and boreal areas than in the tropics, and in depauperate than in speciose fish faunas.

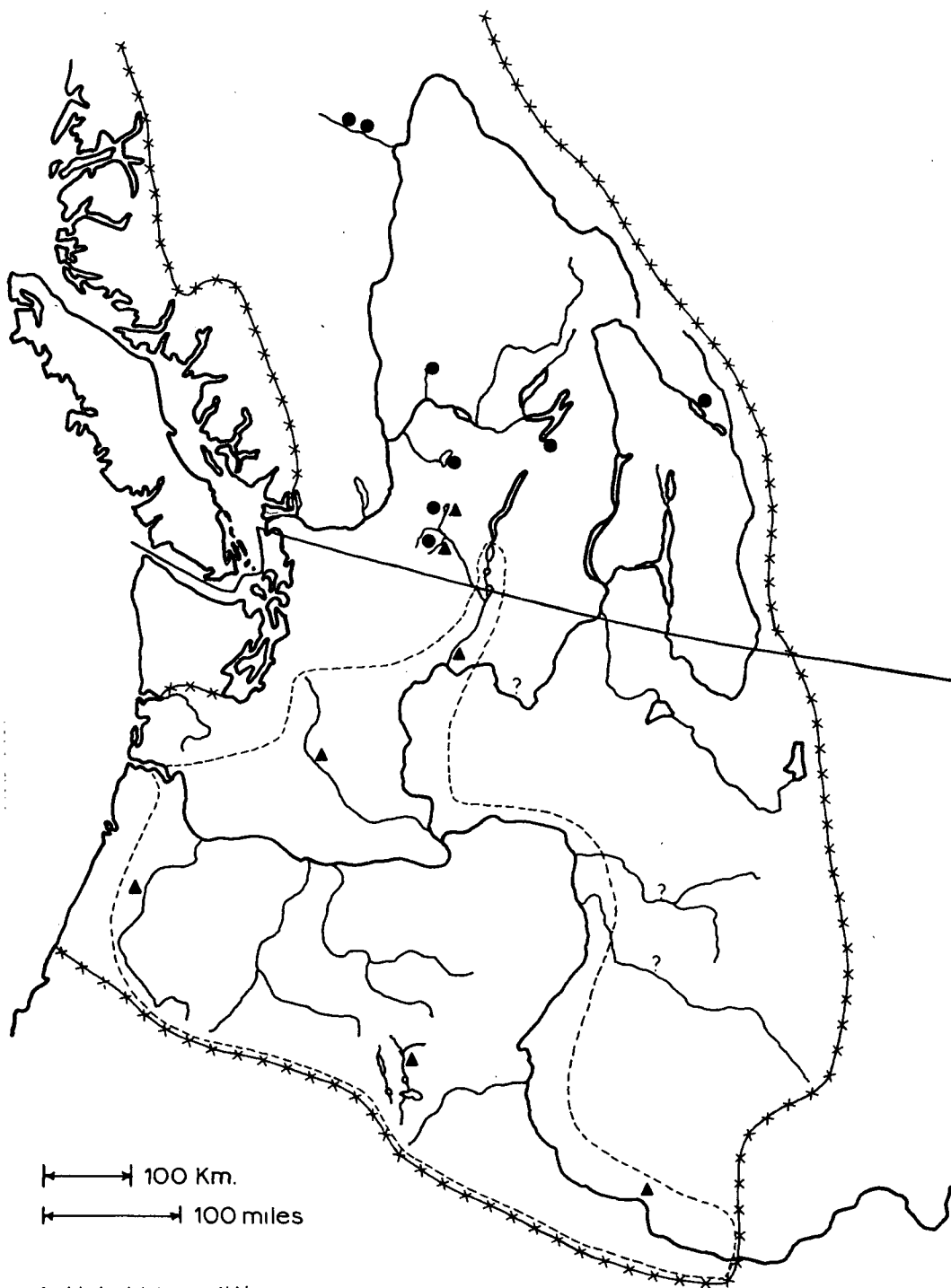
The North American cyprinids are among the more thoroughly studied groups of fishes with regard to natural hybridization. Hubbs, alone and with several co-workers has done much of this work. Gilbert (1961) investigated the relationship of Notropis cornutus and Notropis chrysocephalus chrysocephalus in several zones of contact of the two species of Michigan. In large part, the existing studies of cyprinid hybridization have been confined

to analysis of field collections of the parents and intermediates. Artificially produced F_1 hybrids in the etheostomatine fishes have been reared in the laboratory by Hubbs (1957, 1958 and 1959), Hubbs and Hubbs (1932a, 1932b and 1933) and have reared F_1 artificial crosses of Lepomis species and Mollienesis latipinna x M. mexicana.

Hybridization among the Pacific Northwestern cyprinid genera was first reported by Hubbs and Schultz (1931), for Acrocheilus alutaceus x Ptychocheilus oregonensis. Weisel (1954, 1955a and 1955b) reported combinations involving Richardsonius, Ptychocheilus, Mylocheilus and Rhinichthys. All of these studies were based solely upon morphological analysis of wild specimens.

The present study deals with hybridization between species of two nominal genera of cyprinid fishes, the northern squawfish, Ptychocheilus oregonensis (Richardson) and the chiselmouth, Acrocheilus alutaceus (Agassiz and Pickering). Ptychocheilus oregonensis and Acrocheilus alutaceus both are abundant in the Columbia River system. Ptychocheilus also occurs throughout the Fraser and Skeena River systems, and in all but a few coastal streams of Washington and British Columbia. Acrocheilus, however, is scattered in occurrence in British Columbia, with the exception of the Okanagan River (Fig. 1). Intermediates between these two species have been taken infrequently since 1905 in the Columbia River system in the United States. Snyder (1905

Fig. 1. . Distribution of Acrocheilus alutaceus and
Ptychocheilus oregonensis showing known hybrid
localities.



▲ Hybrid Localities

● Discontinuous Acrocheilus Localities

--- Boundary of Continuous Acrocheilus Distribution

- Boundary of Ptychocheilus oregonensis Distribution

and 1908) recorded an intermediate as being equivalent to the Leuciscus caurinus of Richardson (1836). Hubbs and Schultz (1931) on the basis of Snyder's specimen and some new material attributed this form to hybridization between Acrocheilus and Ptychocheilus. Schultz and Schaeffer (1936) and Schultz and De Lacey (1936) referred to a combination between Acrocheilus and Mylocheilus, citing Hubbs and Schultz (1931). This, in fact, is the Acrocheilus x Ptychocheilus combination. No subsequent references to this form appeared until Patten (1960) reported its occurrence in the Yakima River, Washington.

The present study has attempted first to verify that the wild intermediates are the result of hybridization. To this end, morphological comparisons were made for wild and reared fish of both parental species and the hybrids. The probable causes of hybridization were studied by means of field observations of the spawning habitat and behavior of the parental species. Also, gamete life was studied in order to determine the possibility of hybridization due to chance meeting of drifting gametes. Finally, the possibility of gene flow between the parental species was investigated. The parental species and hybrids were compared for a number of morphological characters, and for muscle electropherograms. Chromosome number and morphology in the parents and hybrids were studied, and hybrid fertility and the existence of naturally occurring backcrosses were

investigated. Comparisons of the relative abundances of hybrids to parental species were made in order to determine the possibility of a significant amount of gamete wastage due to hybridization.

METHODS

A. The Study Areas


Missezula and Wolfe Lakes and their inlet and outlet streams were the primary study areas. Both of these lakes drain into the Columbia River system via the Similkameen and Okanogan Rivers. The remaining localities, from which samples of juvenile and adult fish were collected, are the following.

1. Vidette Lake, which drains into the Fraser River system via the Thompson River.
2. Lorne and Izzits Lakes, which are respectively the uppermost and middle lakes in the Wolfe Creek drainage which also includes Wolfe Lake.
3. The Okanogan River, between the towns of Riverside and Brewster, Washington.

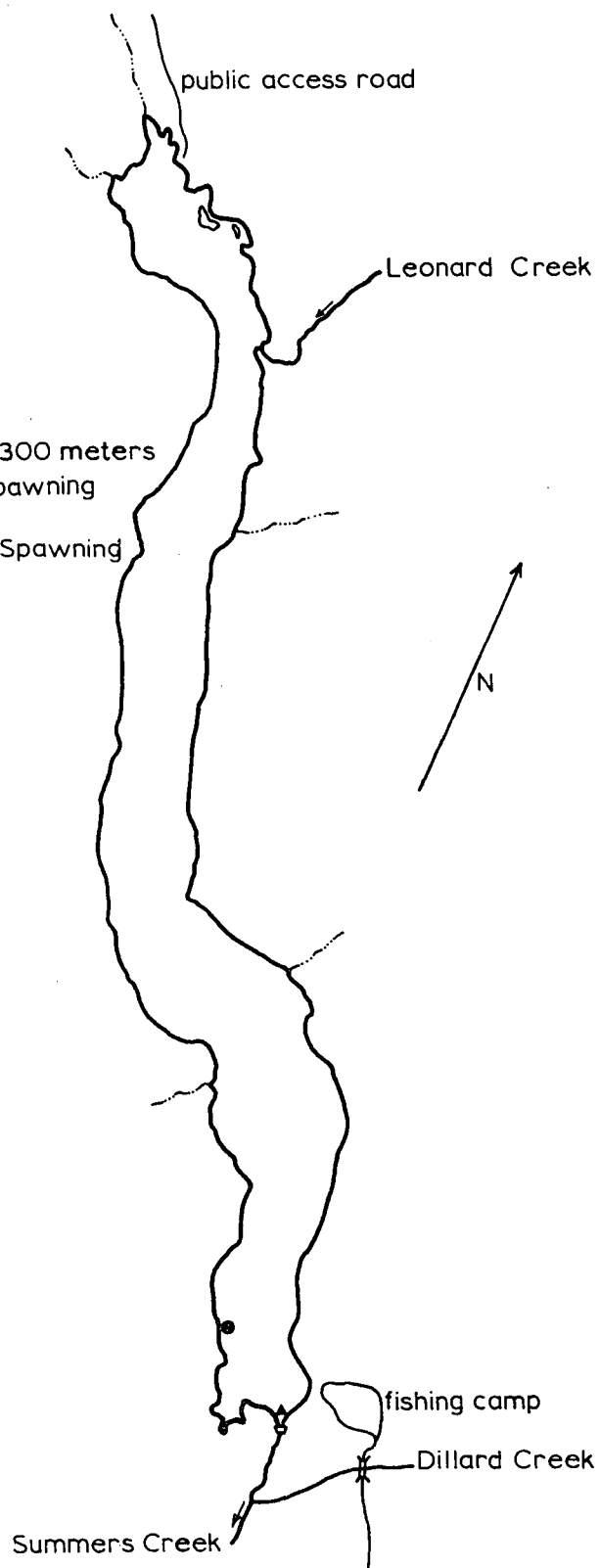
Missezula Lake is located 25 miles by road north of Princeton, British Columbia. It drains into the Similkameen River via Summers Creek and Allison Creek. Missezula is the larger of the two intensively studied lakes, being 7.3 Km. long, a maximum of 600 M. wide and a maximum depth of 65.3 M. (Fig. 2). There are several streams draining into Missezula Lake, only one of which, Leonard Creek, flows constantly. Leonard Creek is partly obstructed by beaver

Fig. 2. Missetula Lake, showing spawning localities for Acrocheilus and Ptychocheilus.

Missezula Lake

Scale:  = 300 meters

- ▲ Acrocheilus Spawning Area
- Ptychocheilus Spawning Area



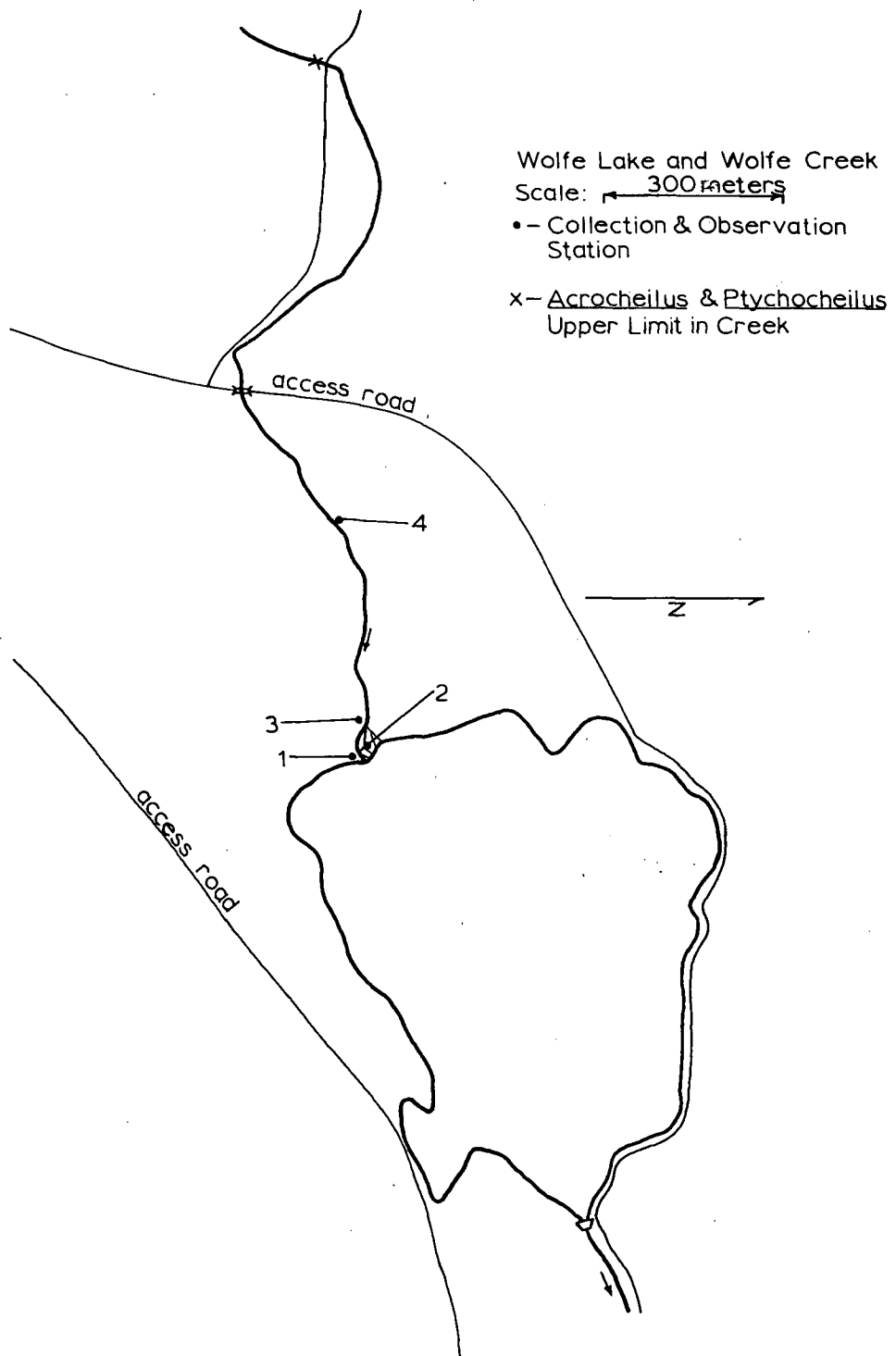
dams near its mouth and is quite cold. Its temperature was recorded in late June at 9.4°C. No cyprinids were ever seen spawning in the flowing part of Leonard Creek, although Richardsonius balteatus was observed in spawning condition in the shallow, weedy area of the lake around its mouth. Since none of the other creeks were flowing during the time in which cyprinids were in spawning condition (late May to early July) Leonard Creek offers the only opportunity for inlet spawning.

The outlet of Missezula Lake is blocked by a low earth dam which raises the level of the lake by a minimum of 1.2 M. The dam has also had the effect of altering the narrow area immediately above it from a shallow gradient stream bed to an area of standing water up to 1.8 M. in depth. Rounded stream worn stones could be found in the center of this area beneath approximately 60 cm. of accumulated silt. There is a directional current along the shores of the outlet area which attains up to 15 cm. per second velocity. The bottom near the shore consists of angular gravel which is kept free of silt, probably by the current. It is in this area that Acrocheilus and intermediates in spawning condition were taken. The dam does not constitute a barrier to fish movement, either into or out of the lake. Eastern brook trout were observed passing in both directions in overflow channels around the spillway. No cyprinids were either collected or observed in the stream

below the dam during the study however, so it may be assumed that all spawning fish are confined to the lake proper or the region immediately above the dam (Fig. 2).

Wolfe Lake is 10 miles by road east of Princeton. It lies near the mouth of a valley opening into the Similkameen canyon, and drains into the Similkameen River via a segment of Wolfe Creek about 1.6 Km. long. Wolfe Lake is roughly circular, about 600 M. in diameter, and the depth probably does not exceed 6.1 M. (Fig. 3). Wolfe Lake has a single inlet stream, Wolfe Creek, which continues below the lake as the outlet. No other surface drainage either enters or leaves the lake. The inlet reached a temperature of 19.4°C . in late June, only 1 degree cooler than the lake surface temperature and the outlet temperature. Around the mouth of the inlet there is an extensive marsh and the stream gives off a number of small channels, forming a delta. Above the marsh area the stream gradient is relatively shallow with pools separated by riffle sections. The banks of the inlet stream are covered with a heavy growth of brush over its entire length, and there are frequently piles of brush sunken in pools. Mr. E.E. Moodie observed a sharp decline in catch of adult cyprinids in the lake at the time spawning fish appeared in the stream, so it may be assumed that most of the cyprinids in the lake utilize the stream for spawning.

Fig. 3. Wolfe Lake and Wolfe Creek.



The outlet of Wolfe Lake, like Missezula Lake, has been blocked by an earth dam. The drop between the lake level and the level of the stream below is greater in Wolfe Lake, and, while the dam may not be a barrier to the movement of adult fish, it is probably an effective barrier to upstream movement of young cyprinids. In addition, the gradient of the outlet stream is steeper than that of the inlet, making it less probable that young cyprinids would be able to return to the lake.

B. Field Collections and Observations

Samples of adult fish were obtained by gillnetting, both from spawning and non-spawning fish. Seining and rotenone, as well as small-meshed gillnets, were used in collecting juvenile specimens.

When sampling standing water, stationary gillnet sets were used, left in most cases for a period of ten hours (overnight). In flowing water, the gillnets were allowed to drift through the desired area and the fish removed immediately afterward. Seining was of restricted use in most areas studied due to the presence of logs and boulders on the bottom. The most effective collections of juvenile fish were obtained either with rotenone or fine-mesh gillnets.

Gillnet collections were made daily during both the 1964 and 1965 field seasons from Missezula Lake. All

hybrids captured were saved for morphological analysis. Most of the Acrocheilus and Ptychocheilus used in the morphological studies were taken from Missezula Lake in 1964, although collections from all areas studied were compared with these.

Fish which were retained alive for later work were kept in styrofoam cooler chests for transport to the shore or laboratory, and fish which were to be retained alive for longer than a few days were treated with an intraperitoneal injection of 1,000 units of penicillin and 1,000 micrograms streptomycin (Difco Penicillin-Streptomycin T.C.) in order to prevent infection in net wounds. Fish to be preserved had their body cavities opened on the right side and were preserved in 10% Formalin immediately after collection. Samples of gonads for histological studies were preserved in Carnoy's fixative (3 parts ethanol :1 part glacial acetic acid) and refrigerated.

Groups of fish in spawning condition were observed both from above water and underwater. In Missezula Lake a small pier was built in the outlet adjacent to the area where fish were spawning. Observations were also made successfully by drifting along the shore of the lake in a boat, the water being clear enough for good vision to a depth of 4.6 - 6.1 M. Underwater observations were confined to Wolfe Creek and the outlet of Missezula Lake. In the nearly stationary water of the lake, it was necessary to

use an oxygen rebreather, since the noise produced by open circuit diving equipment or even by breathing through a snorkel tube frightened the fish. In Wolfe Creek, the flowing water produced sufficient background noise so that the fish were not disturbed by the breathing noises caused by a snorkel tube. Data from underwater observations were either recorded in pencil on a sheet of polyvinyl plastic while underwater or recorded with other field notes after the dive.

C. Rearing Experiments

Wild adult fish of both parental species as well as hybrids, when available, were used as parents for experimentally reared young. All but two of the parent fish were obtained from Missezula Lake. A total of 44 artificial crosses were made during the 1964 and 1965 seasons. The types of crosses and their fate are as follows.

During the 1964 season, 33 artificial crosses were made. Of these, 16 crosses hatched successfully and yielded young which could be used for morphological studies. They comprised 8 Acrocheilus x Acrocheilus crosses, 10 female Acrocheilus x male Ptychocheilus reciprocals and one backcross of female Acrocheilus x male presumed F₁ x Ptychocheilus parentage. Two female Ptychocheilus x

male Acrocheilus reciprocals died after 72 hours of development at 20°C, as did 6 Acrocheilus x Acrocheilus and 6 female Acrocheilus x male Ptychocheilus crosses.

During the 1965 season, a total of 11 crosses were made. Three of these produced young which were used for morphological analysis. These were 2 female Ptychocheilus x male Acrocheilus hybrid reciprocals and 1 female Acrocheilus x male Ptychocheilus reciprocal. In addition, young from 2 Ptychocheilus x Ptychocheilus crosses made by C.C. Lindsey were used. One of these originated from Lac la Hache parents, which are allopatric from Acrocheilus. Three wild presumed F₁ hybrids with free gametes were taken in 1965. Two of these were males, and each was crossed with two Acrocheilus females. None of the eggs survived beyond 72 hours. A single female presumed F₁ with free eggs was crossed with both a male Acrocheilus and a male Ptychocheilus. All but 200 of the resulting eggs were dead within 60 minutes of fertilization, and the remaining 200 all died after 24 hours of development at 18°C. A single cross of a female Richardsonius balteatus x male Acrocheilus yielded one offspring whose morphology was studied.

Parent fish were taken in overnight gillnet sets near areas where aggregations of fish in spawning condition or actual spawning had been observed. The fish were retained live in styrofoam cooler chests and brought to

shore. The eggs of a given female were stripped onto nylon crinoline screen in a dry stacking dish in lots of from one to three hundred per dish. In all cases, eggs from a single female were fertilized with sperm from at least one conspecific and one heterospecific male. Also a given male, where possible, was crossed with a female of both species. After fertilization, the eggs of each lot were spread into a single layer on the crinoline screen which was then carefully immersed in water in a second stacking dish. The eggs were always shielded from direct sunlight. During the spawning season, in 1964, the eggs were transported by air to Vancouver to be hatched in the laboratory. In 1965, most of the eggs were kept in screen enclosed boxes in the lake for hatching. The eggs were held in 7 x 9 x 10 cm. baskets made of nylon crinoline and lined with nylon chiffon. These were suspended in the water by means of a wooden frame inside the rearing box. After the eggs hatched, the young fish were retained in the crinoline baskets for about two months during the 1964 season, or transferred to 20 liter aquaria two weeks after hatching during the 1965 season.

Much of the pre-hatching mortality was apparently due to insufficient oxygen in the water in the crinoline baskets. Since fine mesh liners are required in order to retain the newly hatched fish, heavy aeration in the rearing box or aeration of each crinoline basket is necessary. The circulation within the screen enclosed rearing boxes held in

Misesezula Lake during the 1965 season was not sufficient to avoid mortality. In fact, the mortality to eggs held in these boxes was heavier than that of eggs hatched in the laboratory.

After hatching, the young fish required about 5 days to absorb their remaining yolk (at 18-20°C.). They were then fed a mixture of brine shrimp (Artemia) nauplii and infusoria culture in the case of those reared in the Vancouver laboratory, or the infusoria culture and naturally occurring plankton (largely Diaptomus) for fish reared in the field. As the fish grew, the diet was supplemented with dry tropical fish food ("Tetra-Min Growth Food") in increasing proportions, and the fish were maintained entirely on this food after they had reached 2-2½ months of age.

All experimentally reared fish were kept alive until the fins were fully formed, and when possible, until the scales were formed.

D. Morphological Studies

Both wild and experimentally reared fish were used for morphological studies. Field identification of the parent species and hybrids is not difficult and notes regarding color, presence of spawning tubercles and general appearance could be made readily on uncaptured fish either while diving or observing from the surface.

In the case of small fish (20 mm. or smaller) it

was necessary to stain the specimens with alizarine red-s dye in order to count the scales and pharyngeal teeth. Measurements on these small fish were made using a stereomicroscope equipped with a graduated mechanical stage and a crosshair in the ocular. Larger fish were measured with dial calipers to the nearest 0.1 mm.

Sample sizes of fish for each morphological character are indicated on the figure or table relating to that character. A total of 2,622 adult cyprinids were collected during the 1964 and 1965 seasons. Only a portion of these were preserved and returned to Vancouver.

Most characters are defined in accordance with Hubbs and Lagler (1947). The exceptions are given below.

1. Lateral scale rows are the number of diagonal scale rows crossing the lateral line. The anteriormost scale row counted had the lateral line scale in contact with the pectoral girdle. The posteriormost row counted terminated with last lateral line scale on the base of the caudal fin. This count was found to be easier than was a count of pored lateral-line scales on Acrocheilus, on which the scales are imbedded, and on very small fish.
2. Gut length measurements are not detailed

in Hubbs and Lagler (1947). The gut was removed by severing at the transverse septum anteriorly and cutting the body wall around the anus posteriorly. Arbitrarily, the stomach was defined as the anterior longitudinal segment, and all portions from the first curvature to the anus were designated as intestine.

E. Electrophoretic Studies

Electrophoretic analysis of muscle proteins was carried out by Dr. Tsuyuki of the Fisheries Research Board of Canada Technological Station in Vancouver.

Wild fish of both parent species and the hybrids were collected in the field for this purpose. The fish were retained alive until the sample was taken. The sample consisted of a fillet cut from the longitudinal muscle mass which was labeled and frozen on CO₂ ice immediately after being removed from the fish. The samples remained frozen until analysis, at which time the skin and dark muscle was removed and discarded. The remaining white muscle was broken up in water by means of a blender, and the clear filtrate from this mixture was used to make the electrophoretic run. After developing, the resulting patterns were photographed and the photographs used in

making comparisons.

F. Cytological Studies

Chromosome number and morphology were determined for both parent species and for experimentally reared hybrids representing both reciprocal crosses.

Two techniques were used successfully to obtain chromosome preparations. The first of these was an adaptation of fish tissue culturing techniques as reported by Parker (1950), Wolfe and Dunbar (1956), Wolfe et al (1960), Townsley et al (1963) and Roberts (1964). Primary cultures of testis were obtained from both parent species by aseptically removing the testes, mincing them into pieces 1 mm. or less in diameter in sterile fish Ringer's solution, and explanting from five to fifteen pieces into a 30 ml. culture flask (Falcon Plastics sterile, disposable 30 ml tissue culture flask), 2 ml. of B.B.L. tissue culture medium 199, Hank's base, plus 10% human serum was added to the flask and the culture incubated at 18°C for seven days. After the incubation period, 2 ml of fresh medium and 0.01 ml. of 0.1% colchicine were added to the culture. After an additional incubation period of 36 hours, the tissues were treated for 10 minutes with a hypotonic solution consisting of 1 part medium to 6 parts distilled water. Fixing and staining were done simultaneously in 2% lactic-acetic

orcein (Natural orcein: 2 gm, Acetic acid: 45 ml, lactic acid 55 ml). Smears were prepared immediately by scraping a suspension of cells from the piece of tissue into a drop of stain on a cleaned slide. A coverslip (22 mm. square #0) was placed over the drop of stain, air bubbles excluded by light pressure with a dissecting needle, and the preparation squashed by the application of the author's full weight on a #4 rubber stopper placed on the coverslip. Examination and photography were done using a Zeiss photomicroscope equipped for phase contrast. All photos were made using the oil immersion objective.

Due to the extremely small size of the gonad in the reared hybrids, it was not possible to start a culture. For these specimens, a technique developed by J.D. McPhail (per. comm.) was employed. This involved the use of epithelial cells from the lamellae of the fourth gill arch. The fish (about 2 cm. in length) were treated with a subcutaneous injection of 0.05 ml of 0.01% colchicine in fish Ringer's and held alive in heavily aerated water for 1 hour. The fourth gill arch was then removed intact and placed in distilled water for 30 minutes and in lactic-acetic orcein for an additional 15 minutes. A cell suspension was then scraped from the terminal portions of the lamellae into a drop of stain on a slide and the smear made as described previously.

Chromosome morphology was compared between the

parent species and hybrids by means of photographs. A karyotype for each parent species, showing the probable pairing of the chromosomes, was constructed by arranging the chromosomes according to total length, centromere position, and the presence of constrictions. Comparisons were then made between the parent species, and hybrids. Diploid number and arm number could be determined with more certainty than the morphology of specific chromosomes with the restricted amount of material which was available. Some of the chromosomes were distinctive because of their size or morphology, however, and these could be seen in all the preparations.

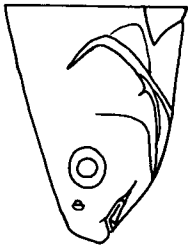
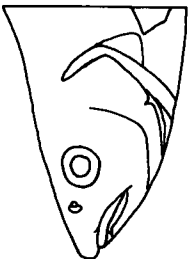
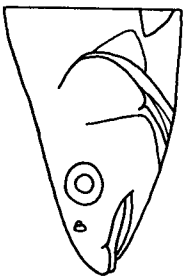
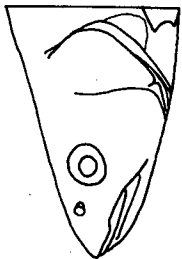
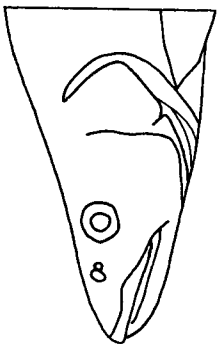
RESULTS

A. Morphology and Electrophoretic Studies

The morphological differences between Acrocheilus and Ptychocheilus may be summarized as follows:-

1. Scale counts: higher in Acrocheilus than Ptychocheilus, with little overlap (Fig. 4).
2. Pharyngeal teeth: Acrocheilus has a single row of teeth on each pharyngeal arch with 4 or 5 teeth (0,4-5,0). Ptychocheilus has 2 rows of teeth per arch, the most common count being 2,5-4,2, with rare variants showing 1,5-4,2; 2,5-4,3 or 3,5-4,2. (Table I).
3. Intestinal tract: complexly coiled in Acrocheilus (Fig. 5), a simple S-shaped tube in Ptychocheilus (Fig. 5).
4. Peritoneum color: black in Acrocheilus, silvery in Ptychocheilus.
5. Lower jaw: has a keratinized, chisel-like anterior margin in Acrocheilus, and is rounded, with no specialization in Ptychocheilus (Fig. 6).
6. Body proportions: Acrocheilus has a narrower caudal peduncle, shorter jaws and shorter snout than Ptychocheilus (Fig. 7).

- Fig. 4. Lateral and ventral profiles of Acrocheilus, Ptychocheilus and wild intermediates.
1. Ptychocheilus oregonensis, Missezula Lake.
 2. Presumed F₁ x Ptychocheilus, Missezula Lake.
 3. F₁ Hybrid, Missezula Lake.
 4. Presumed F₁ x Acrocheilus, Wolfe Lake.
 5. Acrocheilus alutaceus, Missezula Lake.



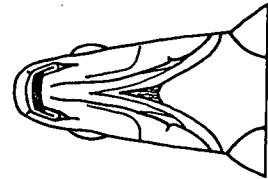
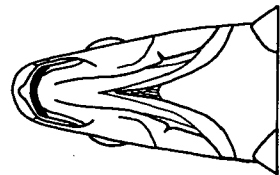
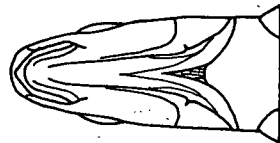
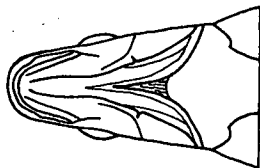
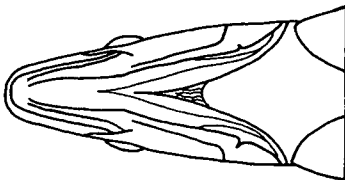
1

2

3

4

5



4 cm

Fig. 5. Numbers of diagonal scale rows in wild and reared Acrocheilus, Ptychocheilus and Intermediates.

Horizontal line = range; vertical line = mean;
solid rectangle = 2 standard deviations.

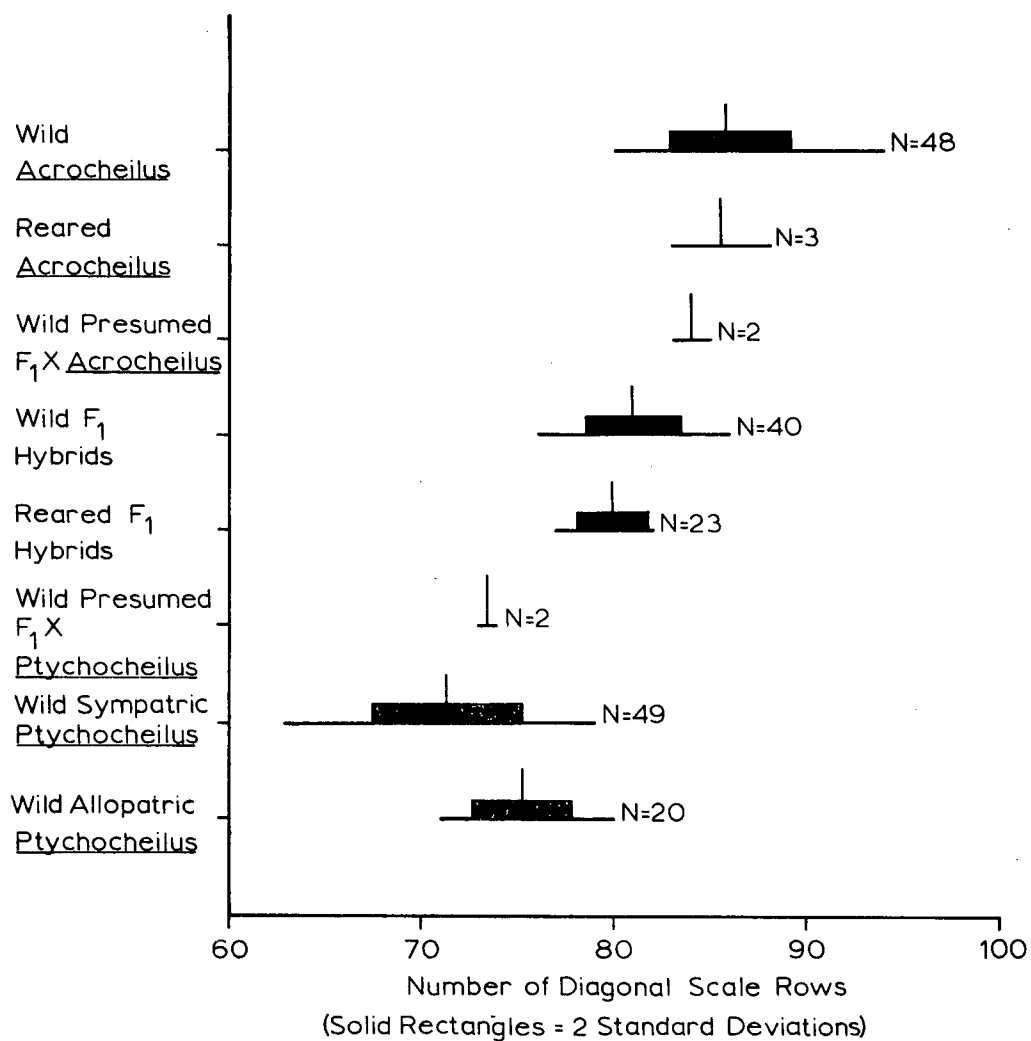
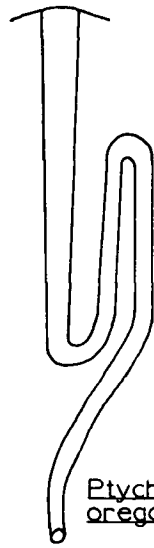


Fig. 6. Diagrams of gut coiling patterns for Acrocheilus, Ptychocheilus, wild intermediates, and juvenile Acrocheilus.



Ptychocheilus
oregonensis Adult



Acrocheilus
alutaceus Adult

Wild Adult Hybrids



F₁



F₁ X Acrocheilus



F₁ X Ptychocheilus

Acrocheilus Juveniles



39.0mm



58.0mm



76.7mm



80.7mm

Fig. 7. Relationships of various body proportions to standard length in wild Acrocheilus, Ptychocheilus and Intermediates.

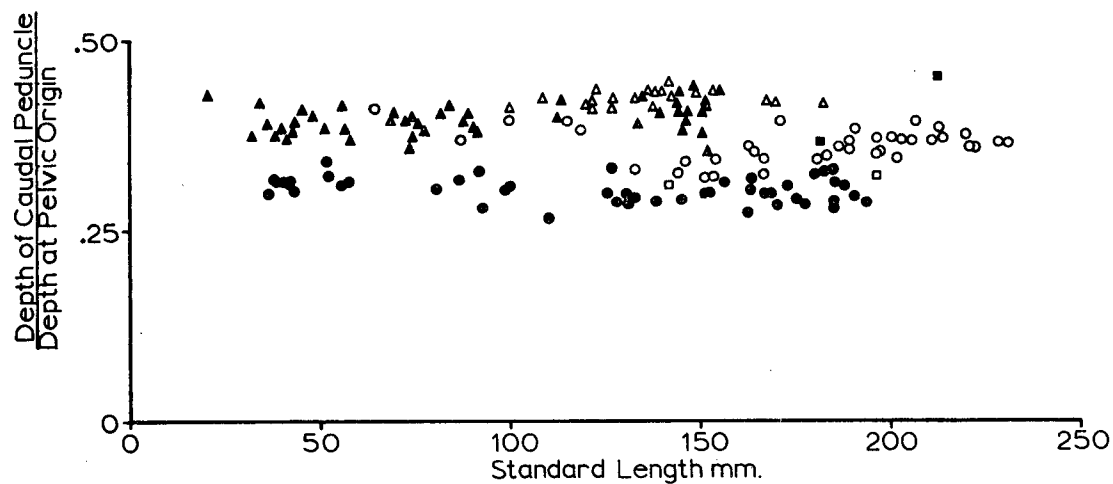
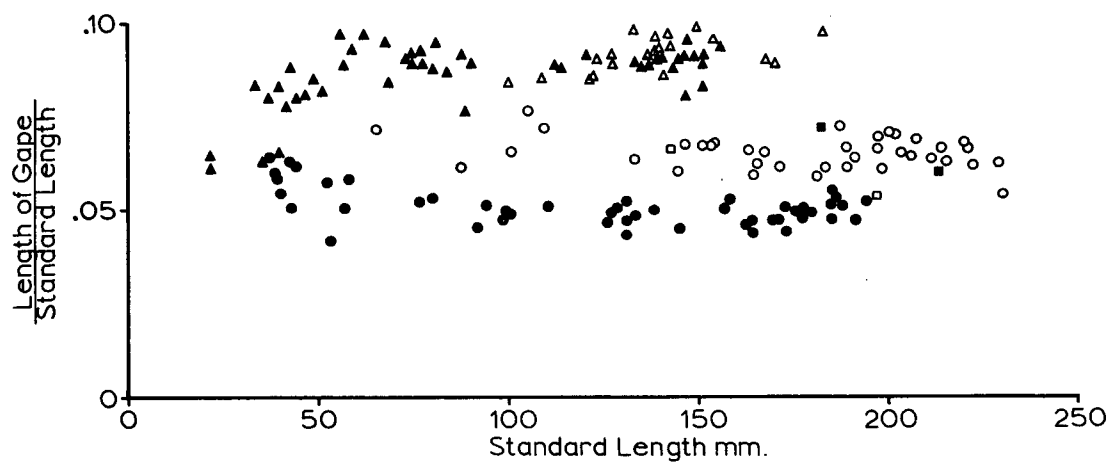
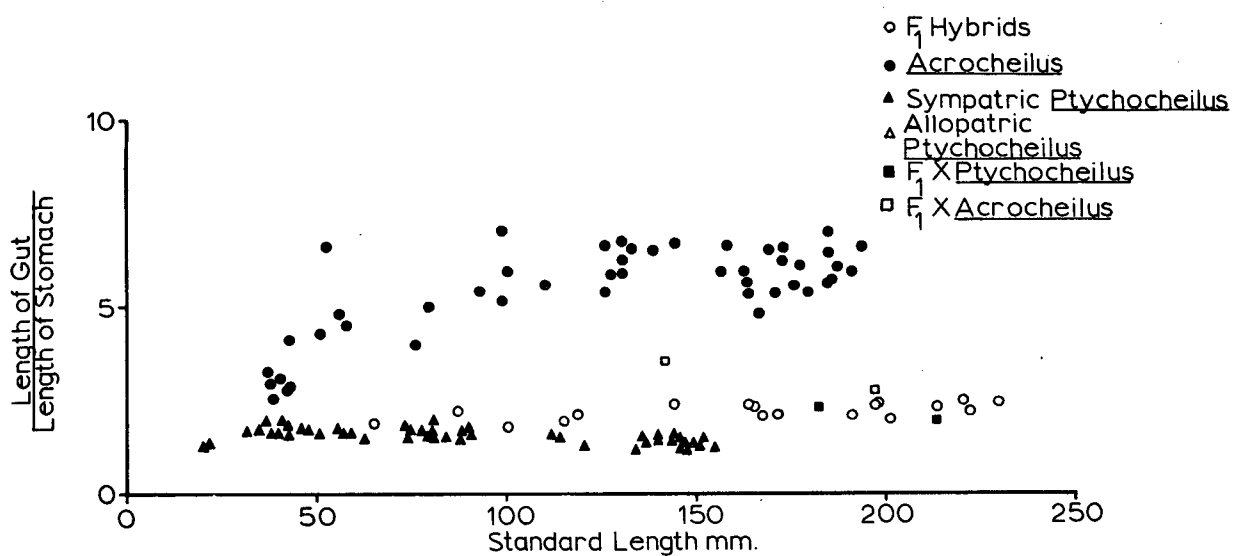


Table I Frequency of pharyngeal tooth counts in wild and reared parental species and hybrids.

		<u>Acrocheilus</u>		<u>F₁ H Y B R I D S</u>			<u>B A C K C R O S S E S</u>			<u>Ptychocheilus</u>		
Number of outer row pharyngeal teeth				Female <u>Acrocheilus</u> x male <u>Ptychocheilus</u> reared		Female <u>Ptychocheilus</u> x male <u>Acrocheilus</u> Reared	Wolfe Lake Presumed F ₁ x <u>Acrocheilus</u> Wild	Miszezula Lake Presumed F ₁ x <u>Ptychocheilus</u> Wild	Presumed male F ₁ x <u>Ptychocheilus</u> X female <u>Acrocheilus</u> Reared			
Left Arch	Right Arch	Wild	Reared		Wild					Sympatric Wild	Reared	Allopatric Wild
0 - 0		56	18						40			
0 - 1				1					3			
1 - 0				1					2			
1 - 1				14	4	1	2		15			
1 - 2				2	5			1				
2 - 1				4	7	1			1			
2 - 2				6	26	3		1	2	59	35	1
2 - 3										2		18
3 - 2										2	1	1
3 - 3											1	
Sample sizes		56	18	28	42	4	2	2	63	63	37	20

The body proportions of the parent species are similar in small fish and diverge with increasing size. The only reliable characters for distinguishing parent species and intermediates below one year in age are the pharyngeal tooth counts, the scale counts if scales are formed, and the peritoneal color. The chisel lip is not apparent in Acrocheilus until the end of the first summer at the earliest, and is rounded in outline in fish below 4-6 cm. Before the chisel has appeared, Acrocheilus tends to have a slightly subterminal mouth, which aids in distinguishing it from the squawfish, but this may be masked by deformation in preserved fish.

The intermediate forms collected in the wild, fall into three categories. By far the most common form agrees completely with the descriptions by Snyder (1906), Hubbs and Schultz (1931) and Patten (1960). The figures and tables referred to in the discussion of parent species morphology give the values of all three hybrid morphologies for each character.

One of the striking features of the morphology of the common intermediate type is the apparent dominance of some squawfish characters. No trace of a keratinized edge is to be found on the lower jaw, although in most other respects the mouth profile is intermediate (Fig. 6). The intestine shows none of the coiling to be seen in Acrocheilus, although the anterior loop of the intestine

is longer than in Ptychocheilus. In some cases, inflections are present anteriorly which are reminiscent of the gut morphology in young-of-the-year Acrocheilus. The pharyngeal teeth of wild intermediates are most commonly identical in count to Ptychocheilus, although a few individuals show a 1,5-4,1 count, which might arbitrarily be considered intermediate between 0,5-4,0 and 2,5-4,2.

Most other characters lie between mean values of the parent species. The peritoneum, (data not shown) varies from silvery with diffuse melanophores to almost black, with a silvery sheen. Most commonly, it is grey.

The other two intermediate types collected were represented by four specimens, two taken from Missezula Lake, and two from Wolfe Lake. The Missezula Lake specimens differed from typical intermediates in having lower scale counts, 73 and 74 diagonal rows, a much shorter anterior loop of the gut, and a straight rather than decurved mouth (Fig. 6). They tended therefore, to be more Ptychocheilus-like. The Wolfe Lake specimens were, on the other hand, more Acrocheilus-like. The scale counts were 83 and 85, the gut was strongly inflected, (Fig. 5), but not coiled, and the lower jaw had a rounded, keratinized margin (Fig. 6). The mouth was sharply deflected, as in Acrocheilus, rather than decurved, as in the typical intermediates.

The pattern observed in nature, then, consists of the two parent forms, and three readily distinguishable

intermediate forms, two of which are extremely rare. Since the body proportions are not distinctive in young-of-the-year fish, analysis of the morphology of reared specimens was limited for the most part to pharyngeal tooth counts and scale counts, both of which are distinctive in the natural populations.

Reared Acrocheilus all showed 0,5-4,0 pharyngeal tooth count, as do the wild fish (Table I). The mean scale count of the only three reared Acrocheilus which survived long enough for scales to be formed was 85.66 (range 83-86) as compared to a mean of 85.83, with a range of 80-94 for wild Acrocheilus (Fig. 4). In the three fish in which scales had formed (about 3 cm. body length) a rounded, keratinized margin was beginning to appear on the lower jaw. This was closely comparable to that observed in wild young-of-the-year Acrocheilus in Missezula Lake during the same month (October). The gut showed inflections much like those diagrammed for 38 mm. wild fish (Fig. 5).

The reared squawfish originated from Wolfe Lake and Lac la Hache parents, and were reared at three different constant temperatures. None was reared to a large enough size for scales to have formed, but pharyngeal tooth counts were readily made on all (Table I). The counts are the same as those observed in wild Wolfe Lake and Missezula Lake populations. The Lac la Hache specimens (shown separately in Table I.) are allopatric, so that there is no possibility

of introgression of chiselmouth genes into this population.

Table I shows the pharyngeal tooth counts of reared F_1 hybrids in comparison with wild intermediates and the parent species. The two reciprocal crosses are shown separately. The data for the female Acrocheilus reciprocal represent the pooled results of all crosses of this type which were made. Only five offspring of the female Ptychocheilus reciprocal, representing a single cross, survived. There is a difference in the modal pharyngeal tooth count of reared F_1 s of the female Acrocheilus reciprocal and the wild intermediates. The former are predominantly 1-1 and the latter 2-2. This may be due in part, to the small size of many of the reared F_1 s, which died at about 1.5 cm., before scales were formed. The five female Ptychocheilus reciprocals which were hatched successfully were reared to about 3 cm. and, of those, three showed a 2-2 count as is seen in the wild intermediates.

Scale counts of 18 female Acrocheilus reciprocals had a mean of 79.77, compared to a mean of 80.58 for the wild intermediates. This difference is not significant at the 95% confidence level (Fig. 4). The five female Ptychocheilus reciprocals had a mean scale count of 80.4, almost identical to the wild intermediates.

Reared specimens, then correspond closely in their morphology to wild specimens presumed to be of the same parentage.

In order to compare the muscle electropherograms of the parental species and hybrids, the bands present were designated with letters, beginning with A at the cathode side of the gel. The frequency of occurrence of each band present was then determined for the parental species and hybrids (Table II). Photographs of the electropherograms of all usable samples are given in Figs. 8, 9 and 10.

A number of the samples which originated from Lorne and Vidette Lakes and the Okanogan River were held in frozen storage for about three months before being run. The patterns obtained from these samples were anomalous, indicating that decomposition had occurred during storage.

No consistent differences could be found between Acrocheilus and Ptychocheilus, electropherograms. (Table II; Figs. 8, 9 and 10).

B. Relative Abundance of Hybrids

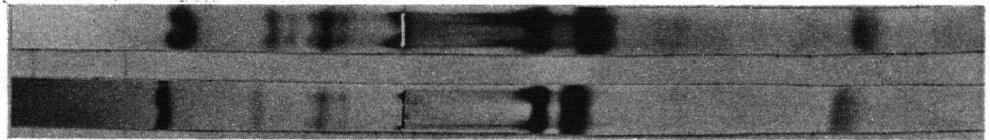
The quantitative composition of the catch by species was recorded for all samples of adult fish from each study area. Samples of juveniles were also analyzed for species composition, but are more restricted in the numbers of fish collected and in the area sampled. Also, it was mentioned previously that identification of hybrids is uncertain in young-of-the-year fish, so that estimates of hybrid abundance in juveniles is probably not as reliable

Fig. 8. Myogen electropherograms of Acrocheilus alutaceus.

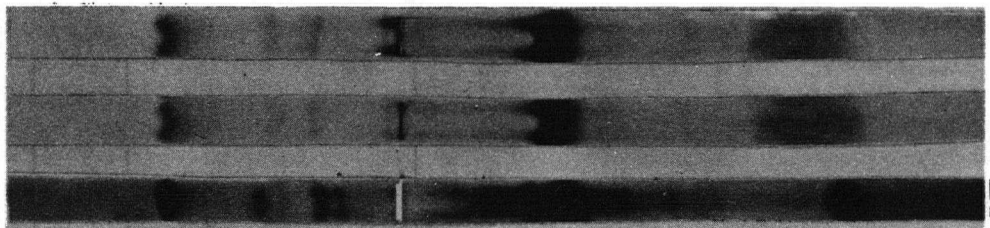
Acrocheilus alutaceus

Missezula Lake

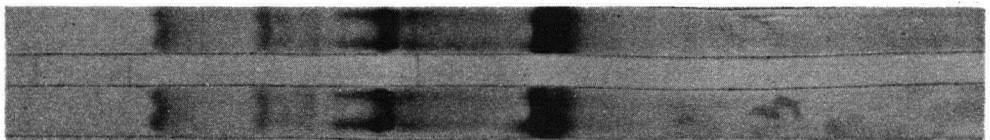
15cm.



Wolfe Lake



Okanogan River



Vidette Lake

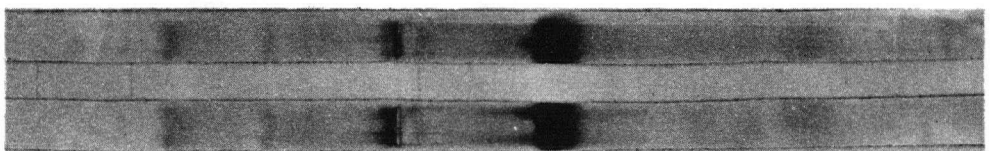
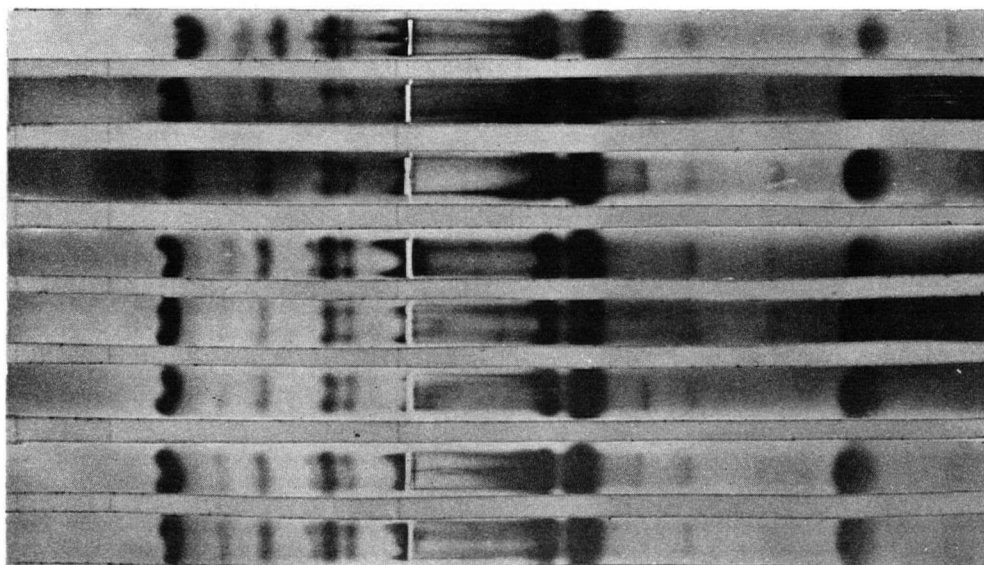


Fig. 9. Myogen electropherograms of Ptychocheilus oregonensis.

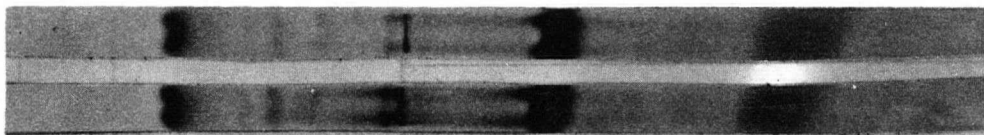
Ptychocheilus oregonensis

Missezula Lake

15cm.



Lorne Lake



Vidette Lake

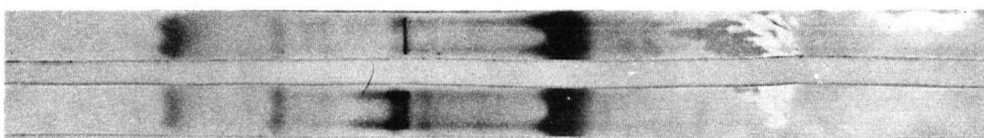


Fig. 10. Myogen electropherograms of wild intermediates.

Wild Intermediates

F_1

Missezula Lake

15cm.



Wolfe Lake



Presumed F_1 X Acrocheilus

Wolfe Lake



Table II Frequency of occurrence of bands in myogen electropherograms of wild parental species and hybrids.

	<u>L E F T</u>							origin	<u>R I G H T</u>			
	A	B	C	D	E	F	G		H	I	J	K
Missezula Lake												
<u>Acrocheilus</u>	2	2	2	2	2	2			2	2		2
<u>Ptychocheilus</u>	8	6	8	5	8	8			8	8		8
<u>F₁ Hybrids</u>	1		1		1	1			1	1		1
Wolfe Lake												
<u>Acrocheilus</u>	2	1	1		2	1			2	2		2
<u>Ptychocheilus</u>	none	sampled	-	-	-	-	-	-	-	-	-	-
<u>F₁ Hybrids</u>	1		1		1	1			1	1		1
<u>F₁ x <u>Acrocheilus</u></u>	1	1	1	1	1	1			1	1		1
Lorne Lake												
<u>Ptychocheilus</u>	2	2	2	2	2		2		2	2		2
Vidette Lake												
<u>Acrocheilus</u>	2	2	2		2	2	2		2	2		2
<u>Ptychocheilus</u>	2		2	2	2		2		2	2		
Okanogan River												
<u>Acrocheilus</u>	2	2	2		2	2	2		2	2		
Fraser River												
<u>Ptychocheilus</u>	1		1		1	1			1	1	1	1
Pooled Results												
<u>Acrocheilus</u>	8	6	8	6	8	7	6		8	8		6
<u>Ptychocheilus</u>	13	9	13	9	12	9	3		13	13	1	11
<u>F₁ Hybrids</u>	2		1		2	2			2	2		2
<u>F₁ x <u>Acrocheilus</u></u>	1	1	1		1				1	1		1

as are the estimates for adult fish.

Missezula Lake had the highest relative abundance for adult hybrids, 14.58 per cent relative to Acrocheilus. In Wolfe Lake, hybrids constituted 1.31 per cent relative to the Acrocheilus. The samples on which these figures are based include a total of 110 Acrocheilus and hybrids collected in daily samples over a three month period in Missezula Lake and 232 Acrocheilus and hybrids collected in daily samples over a one month period in Wolfe Lake (Table III).

A single sample of juveniles from the Okanogan River, near Brewster, Washington contained 13.2 percent hybrids relative to Acrocheilus juveniles. No adult hybrids were taken from the Okanogan River, although 70 Acrocheilus were collected. Patten (1960) reports the collection of 11 hybrids and 4,260 Acrocheilus from the Yakima River. Although he says that this is a high incidence of hybridization, the relative abundance of 0.3 percent is the lowest figure which was obtained for any hybrid locality.

Two rotenone collections made in Wolfe Lake in 1965 contained one year old cyprinids. At this stage positive identification of the hybrids was possible, and pooled abundance of hybrids for the two samples was 3.45 per cent relative to Acrocheilus. The decrease in abundance of hybrids between this sample and the adult fish does not necessarily suggest selection against hybrids. It was mentioned previously that the samples of juveniles were

Table III Species composition of pooled catch from Missezula Lake and Wolfe Lake

	Missezula Lake, 1965 Adult Fish		Wolfe Lake, 1965 Adult Fish (E.E.Moodie, per. comm.)		Wolfe Lake, 1965 Juveniles	
	Number	Percent	Number	Percent	Number	Percent
<u>Ptychocheilus</u>	246	19.63	occurs but not counted	-	57	9.27
<u>Acrocheilus</u>	96	7.66	229	98.69	58	9.43
<u>Ptychocheilus</u> x <u>Acrocheilus</u> Hybrids	14	1.12	3	1.31	2	0.33
<u>Mylocheilus</u>	893	71.27	occurs but not counted	-	373	60.65
<u>Mylocheilus</u> x <u>Ptychocheilus</u> Hybrids	4	0.32	may not occur	-	may not occur	-
<u>Richardsonius</u>	occurs but not counted	-	occurs but not counted	-	125	20.33
Total	1,253	100.00	232	100.00	615	100.02
<u>Ptychocheilus</u> x <u>Acrocheilus</u> <u>Acrocheilus</u>		14.58		1.31		3.45
<u>Ptychocheilus</u> x <u>Ptychocheilus</u> <u>Acrocheilus</u>		5.69		-		3.51

more restricted both in time period and area covered. Also, the juvenile and adult samples represent different year classes, and the relative abundance would not be comparable unless it could be shown that hybrids are produced at the same rate every year, and that environmental factors causing mortality had operated at the same intensity over the span of years separating the adults sampled from the juveniles.

C. Fertility and Viability

During the spawning season in 1964, all eggs collected were returned to Vancouver for hatching. Eggs were taken in lots of about 300 each, and survival to hatching was highly variable. In general, survival was more dependent upon the individual female which was the source of eggs than it was on the cross which was made. One female chiselmouth in particular yielded 90 per cent hatching success in crosses both with male chiselmouth and male squawfish.

Several lots of eggs from both chiselmouth and squawfish, representing both intra-species crosses and hybrid crosses, were observed during the first four days of development. In all cases, rearing temperature was 20°C. At this temperature, development appeared normal for all crosses. By the beginning of the third day, 15

somites were visible, and by 84 hours ($3\frac{1}{2}$ days) the blastopore had closed. In other lots which were reared through to hatching, eye pigmentation was apparent by the tenth day, and hatching was complete by 14 - 17 days.

Hybrids with mature gametes were infrequent. During the 1964 season, a single male was collected. This individual differed in some respects from a typical hybrid morphology, and was the male parent of the backcross offspring discussed previously. Three typical hybrids, with free gametes, two males and one female, were collected during the 1965 season. Each of the two males was used to fertilize both chiselmouth and squawfish eggs. Qualitatively, the milt from these males was translucent, rather than the opaque white typical for parent species males, having a "watery" appearance. In both cases, about 40% (range 30-70%) of the eggs showed malformed blastodiscs after 6 hours incubation at 18°C. After 24 hours incubation, 50% - 70% of the eggs fertilized by the hybrid males were dead. The remaining live embryos appeared normal, but all died within four days of fertilization. It should be noted, however, that at this time nearly all the eggs of intra-species crosses being reared also died. While the mortality of the first 24 hours cannot be explained by environmental causes, the later mortality at 3-4 days was probably due to insufficient oxygen in the water, and affected all the eggs being reared at this time.

The female hybrid yielded eight lots of about 200 apparently normal eggs each. Four of these lots were fertilized with chiselmouth sperm and four with squawfish sperm. All but one of these lots began to swell and turn clear on immersion in water after fertilization, and were obviously dead after 10 minutes. The single lot which did not behave in this way had about 50% obviously dead eggs after 6 hours and 100% mortality in 24 hours. Although swelling did not follow immersion as rapidly in this lot, the dead eggs appeared to have suffered the same fate over a longer period of time, all appearing swollen and clear when dead rather than opaque white which was typical of dead eggs of the parent species.

During the 1964 season, the duration of sperm motility was examined for both parent species. Sperm became motile when placed into water. At 18°C., motility was lost at the same rate in both species. From 15-20 seconds after immersion, there appeared to be no reduction in motility. By 30 seconds, 50% of the sperm was no longer moving, and by 50-60 seconds, there was no remaining motility.

A gamete life of 30 seconds would allow viable sperm to drift from 18.3 - 54.9 M. in the water velocities observed in Wolfe Creek. This equals or exceeds the dimensions of all the pools and runs in which both species were observed so that accidental fertilization by drifting

sperm is a distinct possibility in producing hybrids in Wolfe Creek.

D. Cytology

The diploid number most frequently recorded for Acrocheilus is 46, and for Ptychocheilus, 52. (Table IV). In both cases, these were also the highest counts observed. J.D. McPhail (per. comm.) observed counts of both 50 and 52 for Chehalis and Columbia River Ptychocheilus. On the basis of these counts, one would expect F_1 hybrids to have a diploid number equal to the sum of the haploid numbers of the two parents, or 49. In fact, counts of 49 and 50 were most commonly observed, and appeared to depend upon which hybrid reciprocal was involved, the female Ptychocheilus reciprocal showing counts of 50 and the female Acrocheilus reciprocal giving counts of 49.

In constructing karyograms of the best nuclei, there was close agreement between different nuclei from the same species, such that, when chromosomes were ranked in descending order at total length, the morphology of the chromosome at any given position in the rank was like the one from the same position in other nuclei of the same species. A total of six Ptychocheilus and four Acrocheilus nuclei were sufficiently free of chromosome overlaps to permit construction of karyograms (Figs. 11,

Fig. 11. Colchicine treated mitotic metaphase from cultured testis of Acrocheilus alutaceus. Probable pairing of chromosomes is shown below photograph of nucleus. $2N = 46$.

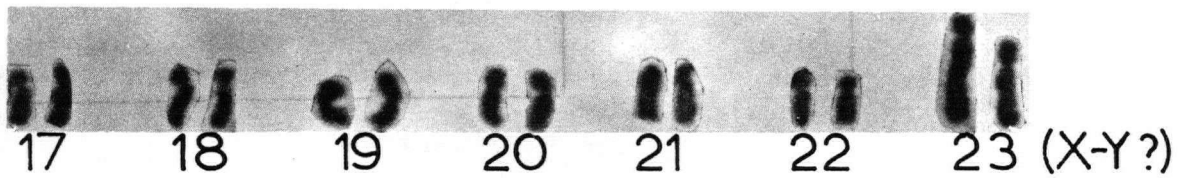
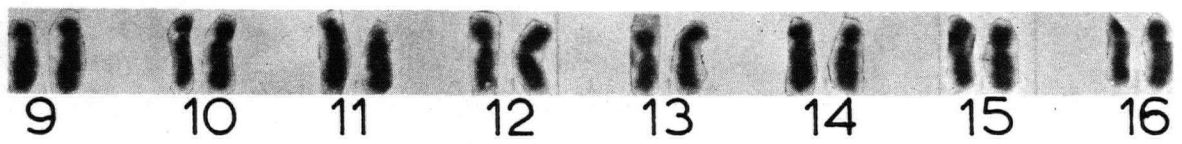
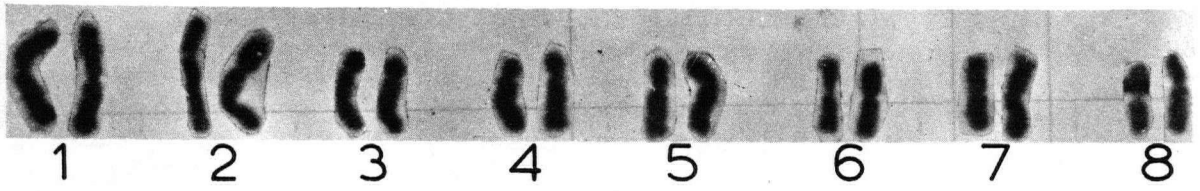
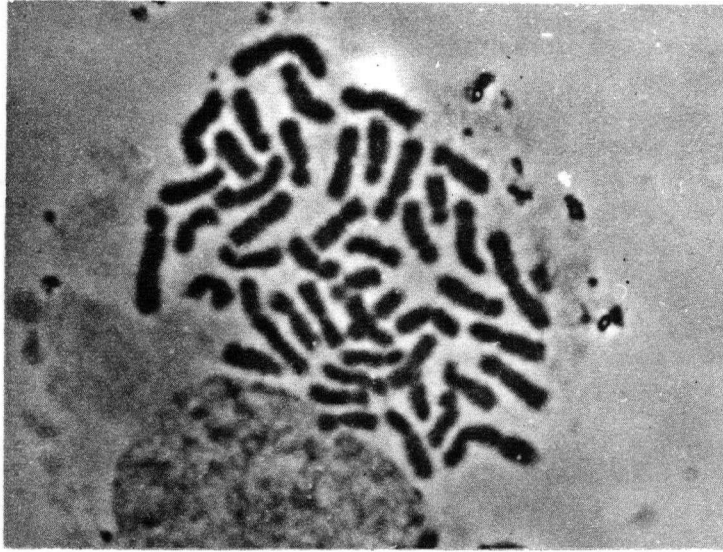


Table IV Frequency of diploid chromosome numbers for wild parental species and reared F₁ Hybrids

		Observed Chromosome Numbers										
Species		41	42	43	44	45	46	47	48	49	50	51 . 52
<u>Acrocheilus alutaceus</u>		1	1	2	0	1	9					
Reared F ₁ Hybrids	Female <u>Acrocheilus</u> <u>reciprocal</u>									6	2	
	Female <u>Ptychocheilus</u> <u>reciprocal</u>									3	8	
	<u>Ptychocheilus</u> <u>oregonensis</u>									2	3	1 10

12 and 13).

If the observed number of chromosomes was grossly above or below the actual number, one would expect to find cases of triplets or unpaired chromosomes, but none occurred.

All the material from the parent species was obtained from males. The asymmetrical pair of chromosomes (See Figs. 11 and 12) possibly represent sex chromosomes. Again, McPhail has observed the same asymmetrical pair in Chehalis River Ptychocheilus. There is no evidence, however, that this pair is heterochromatic, and data on their meiotic behavior are lacking.

Among the larger chromosomes, in which the morphology is distinctive, close agreement can be seen between Acrocheilus and Ptychocheilus. One notable difference is in the smaller of the two possible sex chromosomes, which is a very small metacentric in Ptychocheilus and a larger submetacentric in Acrocheilus. Arm numbers calculated from the karyograms are given in Table V. From this it cannot be shown that any of the Acrocheilus chromosomes represent centric fusions of two Ptychocheilus chromosomes. Also, there is no higher proportion of nearly symmetrical chromosomes in Acrocheilus than in Ptychocheilus, which could indicate translocations. It can only be concluded that Ptychocheilus has more chromatin.

Fig. 12. Colchicine treated mitotic metaphase from Ptychocheilus oregonensis cultured testis. Probable pairing of chromosomes is shown below photograph of nucleus. $2N = 52$.

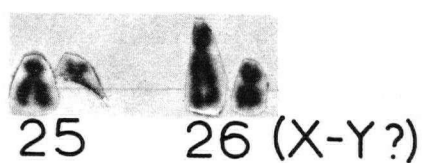
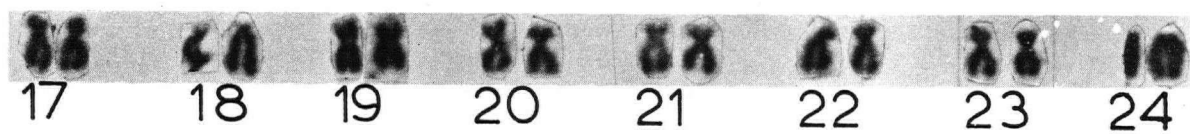
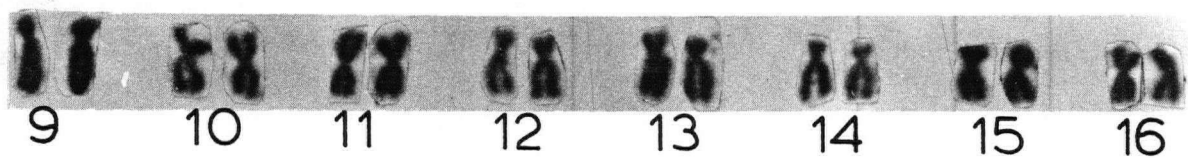
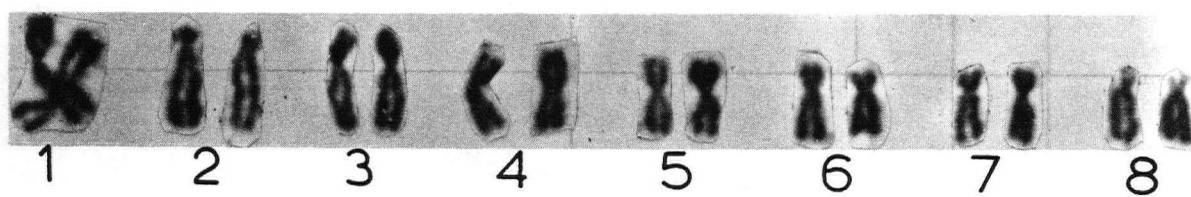
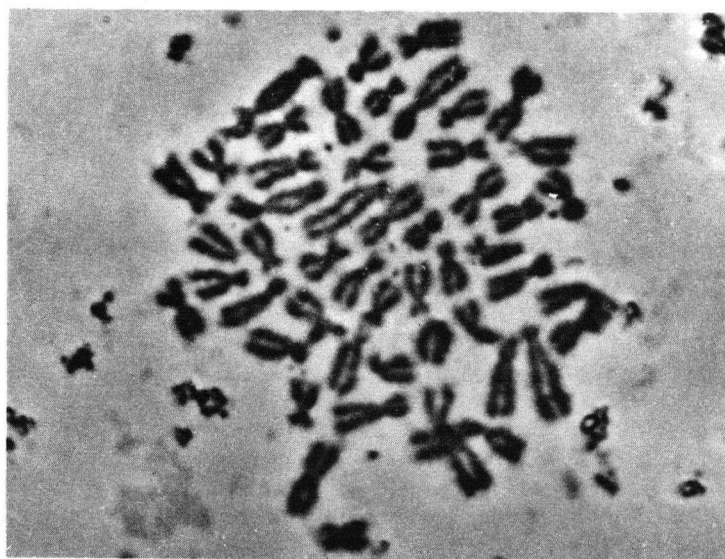


Fig. 13. Colchicine treated mitotic metaphases from
branchial epithelium of reared F₁ hybrids.
Above: female Acrocheilus reciprocal: $2N = 50$.
Below: female Ptychocheilus reciprocal:
 $2N = 49$.

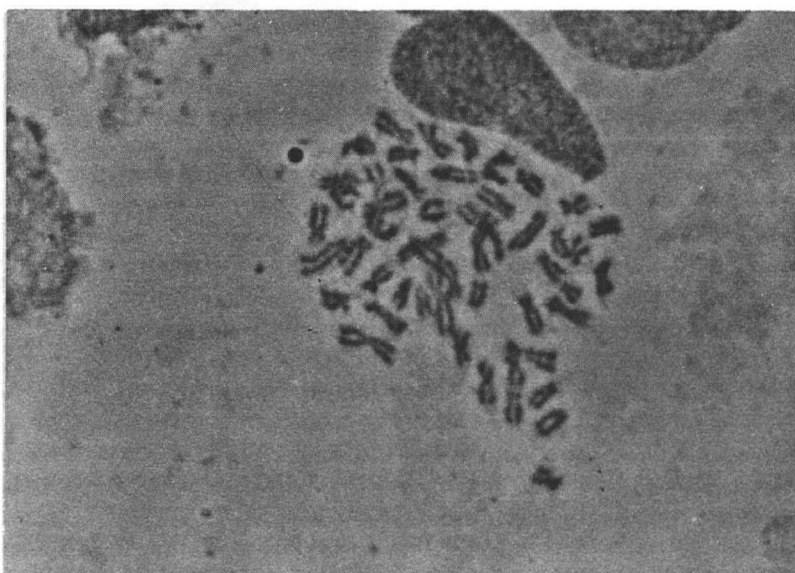
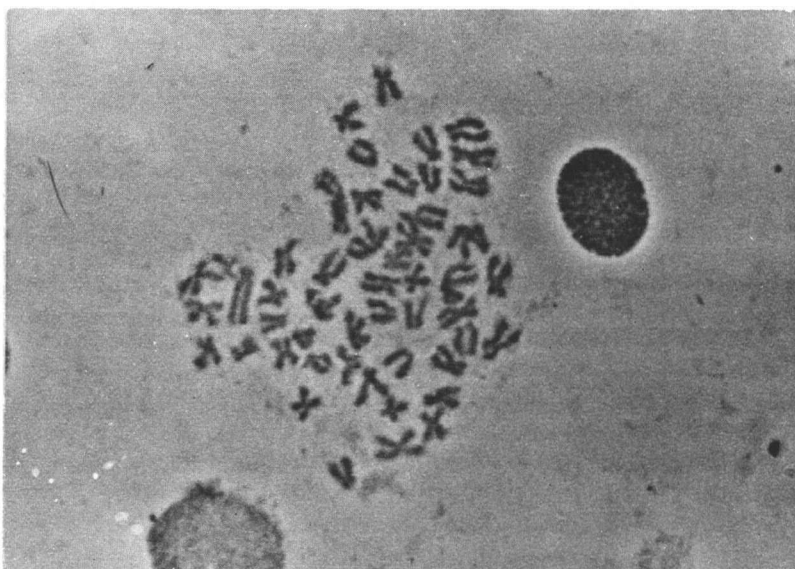


Table V Chromosome arm numbers observed for wild parental species from Wolfe Lake and the Chehalis River

Species	Observed Arm Number	Number of Nuclei
<u>Acrocheilus</u> <u>alutaceus</u> (Wolfe Lake)	88	4
<u>Ptychocheilus</u> <u>oregonensis</u> (Wolfe Lake)	98	6
<u>Ptychocheilus</u> <u>oregonensis</u> (Chehalis River, McPhail, per. comm.)	96	1

In the absence of data on meiotic behavior of the hybrid chromosome the amount of homology between the two species can only be inferred. Because of the generally close resemblance in chromosome morphology, it is possible that there is a high degree of homology, with the three extra Ptychocheilus chromosomes acting as univalents. Further evidence for a high degree of homology is the presence of probable backcrosses in the population. If there were a large number of non homologous chromosomes, then pairing of the chromosomes during the prophase I of meiosis in F_1 hybrids would be completely blocked, and the hybrids would be completely sterile. Since some F_1 hybrids were observed to have at least partial fertility, and since presumed backcrosses were collected, a high degree of chromosomal homology must be assumed.

E. Spawning Habitat and Behavior

All the data on natural spawning areas originate from Missezula and Wolfe Lakes. The localities where fish in spawning condition were observed or taken are shown in Figures 2 and 3.

Acrocheilus with free gametes were first taken in gillnet samples on June 9 during the 1965 season. During the 1964 season, they were first taken on June 12, but may have been there earlier. Surface water temperature in all

regions of the lake was 18°C. when spawning fish of both species were first observed.

During clear weather, mature Acrocheilus appeared to move into the outlet region each night, and remain there, sheltering under brush and logs during the day. Cloudy weather appeared to stop the movement into the outlet and cause those fish which were there to move back into the lake.

Observations by diving during the 1964 season showed that a school of Acrocheilus estimated to contain from 20 to 50 fish was present in the outlet on several clear days in succession. A few Ptychocheilus tentatively identified as males because of their small size and the presence of well-developed tubercles on the head and pectoral fins, were included in the school. The best visual estimate of Ptychocheilus abundance was a count of six individuals in a school estimated to contain about 50 Acrocheilus and one hybrid.

Male Ptychocheilus were also taken in the gillnet samples from the outlet region at the rate of one to about ten Acrocheilus. During both seasons, only three sexually mature female Ptychocheilus were taken from the outlet.

Ptychocheilus in Missezula Lake were observed to spawn in an area along the west shore of the lake, about 0.8 Km. north of the outlet region (Fig. 2). Sexually mature male Ptychocheilus were also found in the outlet of Missezula Lake at the same time that Acrocheilus began to

appear, although spawning in Ptychocheilus was not observed until June 21 during the 1965 season. This, however, was probably due to difficulty in finding Ptychocheilus spawning localities, since a mature female was taken in the outlet on June 12 during the 1964 season and on June 15 during the 1965 season.

Spawning was observed on four days during the 1965 season, each time at dusk, from 7.30 p.m. to 10 p.m. A dense mass of fish, apparently males, judging from their size, occupied the area immediately offshore. Some of the fish closest to shore were mostly out of the water. The dimensions of the aggregation were about 3.1 M. from inshore to offshore extremity and about 4.6 M. parallel to the shore. In the deeper water, offshore from the aggregation of males, larger fish, almost certainly females, were swimming around the periphery of the aggregation. Females apparently entered the aggregation near the shore, and spawning took place in water too shallow to cover the fish, accompanied by much splashing by the males. In a few cases, the female was sufficiently visible to see her body vibrating, presumably during emission of the eggs. On one occasion the aggregation dispersed due to disturbance but re-formed in about 30 minutes. At nightfall, the school dispersed, and no further activity was heard or seen when observations were extended to one hour after nightfall. No spawning activity was observed during the early morning, just after dawn.

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Examination of the spawning locality revealed large numbers of eggs, all very close to shore, in less than 15 cm. of water. The area of egg deposition extended for about 3.6 M. along the shore.

Gillnet collections from this locality yielded large numbers of ripe male Ptychocheilus and smaller numbers of females in nearly mature, ripe, or recently spent condition. Only one Acrocheilus, a spent male, was collected in this area near the end of the spawning season (July 12).

Ptychocheilus probably spawns in other areas of the lake, but these were not located during the course of the study. It cannot be shown conclusively that Acrocheilus spawns only in the outlet region. It is not a common fish in the lake, however, and its high rate of occurrence in the outlet region during the spawning season would seem to be sufficient to account for the spawning of the entire lake population.

In Wolfe Lake, both Acrocheilus and Ptychocheilus utilized the inlet stream, Wolfe Creek, for spawning. Underwater observations showed Acrocheilus and Ptychocheilus to be abundant in the deeper pools and runs in the lower half of the stream's length. A large portion of the remaining distance upstream to Izzit's Lake consisted of straight runs with little cover and almost no fish. For about 91.4 meters below Izzit's Lake, pools were present,

occupied again by Acrocheilus and Ptychocheilus. These fish may, however, have originated from Izzit's Lake rather than Wolfe Lake.

Unlike Missezula Lake, there was no spatial segregation of Acrocheilus and Ptychocheilus in Wolfe Creek. Both were abundant wherever conditions were suitable. Also, the Acrocheilus appeared to form groups which moved together, independent of the Ptychocheilus. Non-sexual interactions, primarily nudging, chasing, and grouping together, appeared to be confined to within each species and no interspecific acts were observed. This is in contrast to observations in the outlet of Missezula Lake, where the Ptychocheilus males actively oriented themselves with, and moved about with Acrocheilus.

Spawning was not observed in Wolfe Creek, but eggs collected from two of the runs where both parent species were present were reared in the laboratory. The fry produced were mostly Ptychocheilus, but a number of Acrocheilus were also present.

DISCUSSION

A. Origin of the Wild Intermediates

Four lines of evidence are available which indicate that the wild intermediates are the result of hybridization between Ptychocheilus and Acrocheilus, and that most of them are F_1 hybrids. First, the comparative morphology of the wild intermediates and the parental species shows that the intermediates, for the most part, lie between the values for the parents in the characters examined. There would appear to be some dominance of squawfish features, however, since the intermediates lack the keratinized margin of the lower jaw, typically show two teeth on the outer pharyngeal tooth row, and lack the coiling typical of the chiselmouth gut.

Second, with the exception of the disagreement in outer row pharyngeal tooth counts in the female Acrocheilus reciprocals, the reared F_1 hybrids are similar in all respects to the wild intermediates.

Third, the offspring of the backcross experiment, in which the aberrant male from Missezula Lake was crossed to a female Acrocheilus, show a pharyngeal tooth count segregation that can only be explained by assuming that the male had chiselmouth genes. This male shared with typical intermediates the dark peritoneum and the long anterior fold of the intestine. It differed in having a

straight, rather than decurved mouth and in a lower scale count. It differed from presumed Mylocheilus x Ptychocheilus hybrids from Missezula Lake in that the latter have a silvery peritoneum, a short anterior fold of the intestine and, like Mylocheilus, the ventral two pharyngeal teeth of the inner row are short and blunt rather than pointed as in Acrocheilus, Ptychocheilus, and in the Missezula Lake aberrant male. This male also differed from the reared F_1 hybrids between Ptychocheilus and Richardsonius in that the latter show a silvery peritoneum with diffuse melanophores, a high anal ray count (11, as opposed to 7, 8 or 9 for Ptychocheilus and Acrocheilus in Missezula Lake) and in the more oblique mouth in Ptychocheilus x Richardsonius hybrids. This leaves open only two possibilities; either the aberrant male was an unusual Ptychocheilus, or was of hybrid origin. It has been shown that reared F_1 hybrids and wild intermediates always have at least one outer row pharyngeal tooth. If the male in question were an aberrant Ptychocheilus, then the same result would be expected upon crossing him to a female Acrocheilus. The fact that 62 per cent of the offspring showed a 0-0 count excludes the possibility that he was an aberrant Ptychocheilus, leaving only hybrid origin as an explanation.

Fourth, the wild intermediates are partially sterile. Of those which develop sex products, the females appear to produce inviable eggs and about 40 per cent of

parental eggs fertilized with sperm from intermediate males show abnormal cleavage and die. These observations exclude the possibility that the wild intermediates might be a rare and hitherto unrecognized species which reproduces itself.

The probability that most of the wild hybrids are F_1 s is indicated by their agreement in morphology with reared F_1 s, and the observed high degree of sterility in wild hybrids. Because of their low abundance and high degree of sterility, the chances of hybrid to hybrid matings is extremely low (Hubbs, 1955) if not absent altogether in view of the apparent total inviability of hybrid eggs. If other than F_1 hybrids are to occur, they must almost certainly be the result of backcrossing to one or the other parent species. In fact, the four wild intermediates of aberrant morphology seem to suggest this type of origin. The two from Missezula Lake might be interpreted as F_1 x Ptychocheilus backcrosses, while the two Wolfe Lake examples might be F_1 x Acrocheilus backcrosses. It seems certain that the Missezula Lake individuals are of hybrid origin, from the argument outlined above. The differences between these individuals and typical hybrids are in the more Ptychocheilus-like values for some characters. Because of this, F_1 to Ptychocheilus backcrosses would seem to be the most logical explanation of their origin.

The Wolfe Lake aberrants show a morphology

intermediate between an F_1 and Acrocheilus, which would suggest backcrossing with Acrocheilus as their origin. The evidence in this case is not as direct, however. Acrocheilus is known to hybridize only with Ptychocheilus, but Mylocheilus and Richardsonius are also in the same area during the spawning period. An Acrocheilus male x Richardsonius female cross was made at Wolfe lake using 10 Richardsonius eggs. One individual survived to a size at which scales were formed, and its morphology is different from the Wolfe Lake aberrants. The possible morphology of Acrocheilus x Mylocheilus is a speculative matter. In the two Acrocheilus hybrid crosses which were made artificially however, the keratinized edge on the lower jaw was totally suppressed, as was the gut coiling. If an Acrocheilus x Mylocheilus cross could be expected to behave similarly, then the Wolfe Lake aberrants would differ since they have a keratinized margin on the lower jaw and an inflected gut.

B. Causes of Hybridization

In all known hybrid localities except Missezula Lake, the hybrids are relatively rare. Wolfe Lake, with a 1.3 per cent relative abundance of hybrids is the highest figure recorded except for Missezula Lake, where the abundance of hybrids is 13.2 per cent relative to Acrocheilus. The large gap between Missezula Lake and all the other

hybrid localities in relative abundance of hybrids suggests that two different mechanisms may be operating to cause hybridization.

In Missezula Lake, both species spawn in the lake. Acrocheilus uses the outlet narrows above the dam, apparently to the exclusion of all other areas, and Ptychocheilus spawns in the main body of the lake. Some sexually mature Ptychocheilus are found in the outlet region, away from their observed spawning area. Because of this, the school in the outlet is mixed, containing mostly sexually mature Acrocheilus and a few, mostly mature male Ptychocheilus.

Ptychocheilus was observed to be a group spawner, with a number of males spawning with one or a few females at the same time. Schultz (1935) observed a similar spawning habit in Mylocheilus, and in Wolfe Creek, Richardsonius was observed to spawn in large groups with no apparent pair formation. Acrocheilus was not seen spawning, but the pattern is presumably similar to that observed for the other three related genera.

If group spawning is assumed for Acrocheilus, then hybridization may arise in one or both of the following ways. First, since there are few male Ptychocheilus schooling with the Acrocheilus in the outlet, these might participate in fertilization of the eggs of an Acrocheilus female. Second, since female Ptychocheilus with free eggs occur occasionally in the outlet, they may also spawn there.

In the case of the Missezula Lake outlet, the only male Ptychocheilus available are schooling with the Acrocheilus, and it would seem, on the basis of relative numbers of the two species present in the outlet, that a spawning female Ptychocheilus would more likely hybridize than spawn with a male of her own species. If the difference in pharyngeal tooth counts between reared female Acrocheilus and female Ptychocheilus reciprocal crosses is consistent it indicates that the bulk of wild hybrids are from Ptychocheilus eggs. Possibly, hybridization via female Ptychocheilus is the best explanation for the high relative abundance of hybrids in Missezula Lake. If hybridization was occurring only through mixed males fertilizing female Acrocheilus, the numerically less abundant Ptychocheilus males could fertilize only a small proportion of the eggs of any female, and adult hybrids would be less abundant than observed. On the other hand, a female Ptychocheilus in the same school of fish would lose most of her eggs to hybridization because of the greater abundance of male Acrocheilus.

Hubbs, Walker and Johnson (1943), and Hubbs, Hubbs and Johnson (1943) report cases where interspecific hybridization of atherinids and catostomids respectively are probably due to a disproportionate relative abundance of the parental species. Mayr (1963) also cites cases in birds where zones of contact established on the periphery

of the range of one parental form can lead to hybridization. This also applies to rare strays in normally segregated reproductive groups within a small area. The strays, being within the mating area of the other species, will be unable to find conspecific mates and will hybridize (Mayr, 1963).

In Wolfe Creek, the circumstances are quite different. Here there is no observable segregation of sexually mature Acrocheilus and Ptychocheilus. Both species are concentrated in the deeper pools and runs, using whatever top cover is available. Spawning was not observed in Wolfe Creek, but both species must spawn in the same areas where the aggregations were seen, since eggs taken from those areas and reared proved to belong to both species.

Since the relative abundance of adult hybrids is much lower in Wolfe Lake than in Missezula Lake, species recognition must take place, since there is no spatial segregation. The relative abundance of Acrocheilus and Ptychocheilus as estimated from visual counts of sexually mature fish in Wolfe Creek, is nearly equal. In contrast, in the outlet of Missezula Lake, Ptychocheilus is rare relative to Acrocheilus, and schools with Acrocheilus.

The only observations made in Wolfe Creek which are applicable to the problem of mate selection in the two species are that, in the aggregations, the Acrocheilus seem to cluster together, rather than being scattered uniformly among the Ptychocheilus. Also, the only interactions

which were observed were intraspecific, suggesting that species recognition is operating, at least in non-sexual interactions.

If, in fact, species recognition and segregation are operative when relative abundance is more nearly equal, then chance encounters of drifting gametes may account for the level of hybridization observed. From experiments on sperm of Missetzula Lake Acrocheilus and Ptychocheilus it would appear that the sperm can be in contact with water for 15 to 20 seconds before motility begins to decline. At the water velocities measured in Wolfe Creek in areas where sexually mature fish were observed, sperm could drift from 4.6 to 36.3 meters, depending on the water velocity before their motility began to decline. Since the two species were observed to be closer together than this, and since eggs of both were taken from the same area, chance fertilization probably does occur. This could be a general phenomenon, since the spawning habitat used by the two species is similar in most respects.

Hubbs (1957, 1960 and 1961) has studied duration of sperm function and gamete compatibility as related to interspecific hybrid formation. The delay times he observed before fertilization percentages began to decline are comparable to the delay times observed for Acrocheilus and Ptychocheilus before motility declined sharply.

C. Consequences of Hybridization

Swamping of either parental form has not occurred, even where the rate of hybridization is as high as in Missezula Lake. If introgression has taken place, it is not revealed in the morphology of either parent species. The wild populations appear to consist of distinct and unaltered parent species, with intermediates, mostly F_1 hybrids appearing in scattered localities throughout the sympatric range of the parent species.

Since the F_1 hybrids are partially fertile, and rare backcrosses are known, barriers other than sterility must prevent gene flow between the parent species. The low abundance and partial sterility of the hybrids, combined with the strong morphological and ecological divergence of the parent species, could operate to limit the rate at which backcrossing takes place, and also, to restrict severely the survival of backcross offspring because of their unbalanced and non-adaptive genotypes.

Swamping or introgression can take place only if the hybrids themselves are at a selective advantage, or if certain gene complexes of one parent are at a selective advantage to their alternatives in the other parent (Anderson, 1953, Mayr, 1963, Bigelow, 1965). In order for F_1 hybrids to survive at all, there must be a suitable environment open to them. For hybrid generations beyond

the F_1 , or for backcrosses, recombination will cause much greater variability than in F_1 hybrids, with the result that many recombinants will be inviable or poorly adapted. In organisms which are widely divergent, the number of possible adaptive recombinants would be only a small fraction of the total number of possible recombinants. This would severely restrict the survival of backcross offspring, or of hybrid generations beyond the F_1 , though survival of F_1 s may be good.

Since the F_1 hybrids are numerically rare, they will probably backcross to one of the parent species, rather than mating with one another (Hubbs, 1955). It has been shown that Ptychocheilus is a mass-spawner, and that Acrocheilus probably is. Also, the evidence indicates in female F_1 hybrids, any eggs which may be produced are inviable. The only channel open for gene flow, then, would be the male F_1 s, of which only 3 out of 20 examined had free gametes. In order for a male F_1 to fertilize any parental eggs, he would have to compete with a relatively large number of Acrocheilus or Ptychocheilus males which were spawning with the same female simultaneously. At best, then, a male hybrid could fertilize only a small portion of the eggs of a given female. From the evidence given, about 40 per cent of these eggs would die before cleavage was complete. If all the remainder hatched, there would still be heavy mortality in the offspring for

the reasons outlined above.

Only one backcross, the second Missezula Lake male, had free gametes. The first Missezula Lake backcross was collected on 26th July, or about two weeks after spawning in both parent species was finished in the two seasons of observation, and appeared to be unspawned. Neither of the Wolfe Lake backcrosses had free eggs, although the ovaries of both appeared grossly normal and well developed.

The second Missezula Lake backcross male produced sperm with high fertility, but this does not mean that all backcrosses would necessarily be fertile. While the lack of free eggs in Wolfe Lake backcross females might mean only that they had not yet completed maturation, the unspawned condition of the first Missezula Lake male backcross is positive evidence that he was somehow barred from reproduction. Post-spawning samples of both parent species all had spent gonads. The presence of the well-developed gonads in this backcross is exceptional.

Apparently introgression or swamping are completely blocked in both directions. The parent species maintain themselves as distinct and separate populations with no gene exchange, and the gametes which enter into hybridization are lost.

None of the available evidence indicates whether the rate of hybridization is static, or varying in some manner. If the gamete wastage were significant, isolating

mechanisms might be expected to have developed which would prevent hybridization, but this does not seem to be the case. In all the areas studied, Ptychocheilus population levels are high enough that wastage due to hybridization is probably not significant. In the case of Missezula Lake, Acrocheilus is sufficiently rare for the observed level of hybridization to affect its abundance. This does not imply, however, that the rarity of Acrocheilus is due to hybridization. Missezula Lake affords only marginal habitat for a bottom-feeding cyprinid such as Acrocheilus because of the restricted littoral area. The relatively recent damming of the lake may also have affected adversely the survival of eggs and young, since no other stream is available for spawning. Hybridization alone, then, cannot account for the low abundance of Acrocheilus in Missezula Lake but further drops in the population level, if they do occur, may be due to gamete wastage from hybridization. In the other areas where hybrids have been collected, the abundance of Acrocheilus is much greater, and the incidence of hybridization much lower.

Since hybrids have been taken in widely scattered localities, and were known before the Columbia River System had been modified by man, it can be concluded that hybridization at a low level has taken place between Acrocheilus and Ptychocheilus since the two species came into contact.

D. Relationship of Ptychocheilus and Acrocheilus

Acrocheilus and Ptychocheilus are strongly divergent in their morphology and ecology, but much of the evidence collected during the present study indicates that they are rather closely related genetically. Several morphological characters, especially the keratinized margin of the lower jaw, the gut coiling, and the pharyngeal tooth counts, show partial or complete dominance effects in F_1 hybrids and backcrosses. Also, the myogen electropherograms failed to show any consistent differences between the two. The best single evidence of genetic similarity, however, is the indication of partial hybrid fertility and the presence of wild backcrosses.

It is possible to produce viable F_1 hybrids artificially from parents with strongly divergent genotypes. Such hybrids can also be found in the wild. In order for a hybrid to be fertile, however, there must be sufficient homology between the parental chromosome complements to allow proper pairing of the chromosomes during prophase I of meiosis. Even relatively small differences can lead to partial infertility, since failure of chromosomes to pair normally will cause them to behave abnormally during the metaphase and anaphase portions of the first meiotic division (Sinott, Dunn and Dobzhansky, 1958; Darlington, 1958). This results in the secondary gametocytes

receiving imperfect sets of chromosomes, and thus renders them inviable. Because of the mechanics of chromosome pairing involved in successful meiosis, the fact that Acrocheilus x Ptychocheilus hybrids are capable of occasionally producing viable gametes indicates a high degree of chromosomal homology.

Further evidence of genetic similarity is provided by the morphology of the chromosomes. It has been noted earlier that in readily identifiable pairs of chromosomes, there is close comparison in the morphology of these pairs between Acrocheilus and Ptychocheilus. J.D. McPhail (per. comm.) has found that this similarity can also be seen in Richardsonius and Mylocheilus chromosomes.

The available evidence, then, suggests a close genetic similarity, not only between Acrocheilus and Ptychocheilus, but also these two and Richardsonius and Mylocheilus. All permutations of hybrids between these four genera except Acrocheilus x Mylocheilus and Acrocheilus x Richardsonius are known to occur naturally.

The question of the taxonomic position of Acrocheilus and Ptychocheilus is, however, a subjective matter. Obviously, they meet all the criteria of good species, and this has never been questioned. Whether they should be regarded as distinct genera is an arbitrary decision. If total hybrid sterility were the criterion,

they should be placed in the same genus. Many congeneric species, however, show total hybrid sterility, and far less morphological and ecological divergence than do Acrocheilus and Ptychocheilus. Should these be placed in separate genera? If the criterion for taxonomic rank were to be recentness of divergence from a common ancestor, there is no direct evidence which is relevant to the problem at hand. In fact, genetic divergence, or most commonly, morphological divergence, is used as an indicator of the time of divergence from a common ancestor. The morphological and genetic evidence, however, are often in conflict for the same species pair. A further problem in reassessment of the generic status of Acrocheilus and Ptychocheilus is that there are not sufficient data available on their degree of relationship to the many other genera of western cyprinids not considered here.

If Acrocheilus and Ptychocheilus were placed in the same genus, Richardsonius and Mylocheilus should probably also be placed with them. However, since the relationship of these four to other genera is unknown, the best position to adopt seems to be to retain the present classification until the required data are available.

SUMMARY

1. The wild intermediates between Acrocheilus and Ptychocheilus are of hybrid origin, and most of them are F_1 hybrids.
2. When relative abundance of the two parental species is disproportionate, as in Missezula Lake, active interspecific mating probably takes place.
3. The occurrence of rare F_1 hybrids from many other localities suggests that habitat modification is not prerequisite to hybridization. All known hybrid localities other than Missezula Lake are in streams, and the evidence collected in Wolfe Creek suggests that hybridization under unaltered conditions may be caused by drifting gametes meeting by chance.
4. None of the morphological evidence suggests that swamping or introgression has taken place, although rare, aberrant individuals are almost certainly backcrosses.
5. The mechanisms which have prevented gene flow between the parental species are a high degree of sterility in the F_1 hybrids, the numerical rarity of hybrids, which lowers the probability of hybrid to hybrid mating or of backcrossing, and probable severe selection against recombinants in any

backcrosses which are produced.

6. Only in Missezula Lake is the rate of hybridization high enough, relative to the abundance of either parental species, to suggest that gamete loss through hybridization may affect the population size of a parental species. In the other known hybrid localities, both parental species have high levels of abundance, and hybrids constitute 1.3 per cent or less of the parental populations.
7. The presence of dominance effects in the inheritance of some parental characters, combined with the observed partial fertility of the hybrids, the similarity in chromosome morphology, and the presence of backcrosses in the wild suggest that Acrocheilus and Ptychocheilus are genetically very similar.

LITERATURE CITED

- Anderson, E. 1949. Introgressive hybridization. J. Wiley, New York: ix + 109.
1953. Introgressive hybridization. Biol. Rev. 28: 280-307.
- Bigelow, R.S. 1965. Hybrid zones and reproductive isolation. Evolution, Lancaster, Pa. 19: 449-458.
- Darlington, C.D. 1958. Evolution of genetic systems. Oliver & Boyd, Edinburgh: xii + 265.
- Epling, Carl 1947. Genetic aspects of natural populations. Actual and potential gene flow in natural populations. Am. Nat. 81: 104-113.
- Gilbert, Carter R. 1961. Hybridization versus intergradation: an inquiry into the relationship of two cyprinid fishes. Copeia, 1961: 181-192.
- Hubbs, Carl L. 1955. Hybridization between fish species in nature. Syst. Zool. 4: 1-20.
- Hubbs, Carl L. and L.C. Hubbs, 1932a. Apparent parthenogenesis in nature in a form of fish of hybrid origin. Science, N.Y. 76: 628-630.
- 1932b. Experimental verification of natural hybridization between distinct genera of sunfishes. Pap. Mich. Acad. Sci. 15: 427-437.
1933. The increased growth, predominant maleness, and apparent infertility of hybrid sunfishes. Pap. Mich. Acad. Sci. 17: 613-641.
- Hubbs, Carl L., L.C. Hubbs and Raymond E. Johnson, 1943. Hybridization in nature between species of suckers. Contr. Lab. Vertebr. Biol. Univ. Mich. 22: 1-76.
- Hubbs, Carl L. and K.F. Lagler, 1947. Fishes of the Great Lakes Region. Bull. Cranb. Inst. Sci. 26: 1-186.
- Hubbs, Carl L. and L.P. Schultz, 1931. The scientific name of the Columbia River chub. Occ. Pap. Mus. Zool. Univ. Mich. 232: 1-7.

- Hubbs, Carl L., Boyd W. Walker and Raymond E. Johnson, 1943. Hybridization in nature between species of American cyprinodont fishes. Contr. Lab. Vertebr. Biol. Univ. Mich. 23: 1-21.
- Hubbs, Clark, 1957. Duration of sperm viability as a factor in frequency of fish hybridization. Tex. J. Sci. 9: 472-474.
1958. Fertility of F₁ hybrids between the percid fishes, Etheostoma spectabile and E. lepidum. Copeia, 1958: 57-59.
1959. Laboratory hybrid combinations among etheostomatine fishes. Tex. J. Sci. 11: 49-56.
1960. Duration of sperm function in the percid fishes Etheostoma lepidum and E. spectabile, associated with sympatry of the parental populations. Copeia, 1960: 1-8.
1961. Differences between the gamete compatibility of Etheostoma lepidum sperm and eggs of related percid fishes from allopatric and sympatric populations. Tex. J. Sci. 13: 427-433.
- Mayr, Ernst, 1963. Animal species and evolution. Belknap Press, Harvard University Press, Cambridge: xiv + 797.
- Parker, Raymond C., 1950. Methods of tissue culture. Paul B. Hoeber, New York: xv + 294.
- Patten, Benjamin G., 1960. A high incidence of the hybrid Acrocheilus alutaceum x Ptychocheilus oregonense. Copeia, 1960: 71-73.
- Richardson, John, 1836. Fauna Boreali - Americana (Part 3, the Fish). Richard Bently, London: 1-327.
- Roberts, Franklin L., 1964. Chromosome studies of twenty species of centrarchidae. J. Morph. 115: 401-407.
- Schultz, L.P., 1935. The spawning habits of the chub, Mylocheilus caurinus - a forage fish of some value. Trans. Am. Fish. Soc. 65: 143-147.
- Schultz, L.P. and Allan C. DeLacey, 1935. Fishes of the American Northwest. A catalogue of the fishes of Washington and Oregon, with distributional records and a bibliography. J. Pan-Pacif. Res. Instn. 10: 366-380.

1936. Addenda to Part I. J. Pan-Pacif. Res. Instn. 11: 211-290.
- Schultz, L.P. and Milner B. Schaefer, 1936. Descriptions of new intergeneric hybrids between certain cyprinid fishes of the northwestern United States. Proc. Biol. Soc. Wash. 49: 1-10.
- Sinnott, Edmund W., L.C. Dunn and T. Dobzhansky, 1958. Principles of genetics. McGraw-Hill, New York: xiv + 459.
- Snyder, John Otterbein, 1905. Critical notes on Mylocheilus lateralis and Leuciscus caurinus. Rept. U.S. Commnr. Fish. 1904: 341-342.
1908. Fishes of the coastal streams of Oregon and Northern California. Bull. Bur. Fish. Wash. 27, 1907: 153-159.
- Townsley, P.M., H.G. Wright and M.A. Scott, 1963. Marine fish tissue culture. J. Fish. Res. Bd. Can. 20: 679-684.
- Weisel, George F., 1954. A rediscovered cyprinid hybrid from Western Montana, Mylocheilus caurinus x Richardsonius balteatus balteatus. Copeia, 1954: 278-282.
- 1955a. The osteology of Mylocheilus caurinus x Ptychocheilus oregonense, a cyprinid hybrid, compared with its parental species. J. Morph. 96: 333-358.
- 1955b. Three new intergeneric hybrids of cyprinid fishes from Western Montana. Am. Midl. Nat. 53: 396-411.
- Wolfe, K. and C.E. Dunbar, 1958. An explanation of the principles and methods of tissue culture. Progve. Fish Cult. 20: 3-7.
- Wolfe, K., M.C. Quimby, E.A. Pyle and R.P. Dexter, 1960. Preparation of monolayer cell cultures from tissues of some lower vertebrates. Science N.Y. 132: 1890-1891.