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EXTERNAL SEX DETERMINATION OF LAKE TROUT (Salvelinus namaycush), WHITE SUCKER (Catostomus commersoni) AND LAKE WHITEFISH (Coregonus clupeaformis) IN THE EXPERIMENTAL LAKES AREA, NORTHWESTERN ONTARIO

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Mohr, L.C. 1982. External sex determination of lake trout (Salvelinus namaycush), white sucker (Catostomus commersoni) and lake whitefish (Coregonus clupeaformis) in the Experimental Lakes Area, northwestern Ontario. Can. Tech. Rep. Fish. Aquat. Sci. 1114: iv + 14 p.

Characteristics of the anal and urogenital openings were used to successfully determine the sex of female and male lake trout (Salvelinus namaycush), white sucker (Catostomus commersoni) and lake whitefish (Coregonus clupeaformis) from June to October 1981, at the Experimental Lakes Area, northwestern Ontario. Fish were sexed using a) external morphological features, and b) extracting sexual products with a syringe. Dissections were used to confirm external sex determination. The success rate for the procedure was 92% (lake trout), 81% (white sucker), and 92% (lake whitefish). The sexing syringe did not always produce reproductive materials but was used successfully to test elasticity of the urogenital opening and penetrability of the urogenital tract. Distention or retraction of the anal and/or urogenital pores, formation of a genital papilla, coloration and accessibility of the urogenital pore were the most successful characteristics used in externally sexing the fish.

Key words: external sexing; urogenital opening; genital papilla; sexing syringe.

RESUME

Mohr, L.C. 1982. External sex determination of lake trout (Salvelinus namaycush), white sucker (Catostomus commersoni) and lake whitefish (Coregonus clupeaformis) in the Experimental Lakes Area, northwestern Ontario. Can. Tech. Rep. Fish. Aquat. Sci. 1114: iv + 14 p.

Nous avons étudié les caractéristiques des orifices anaux et urogénitaux pour déterminer le sexe du touladi (Salvelinus namaycush), du meunier noir (Catostomus commersoni) et du grand corégone (Coregonus clupeaformis), tant chez le mâle que chez la femelle, de juin à octobre 1981, dans la Région des Lacs Expérimentaux du nord-ouest ontarien. L'opération fut menée avec succès à l'aide a) des facteurs morphologiques externes, et b) de produits sexuels extraits au moyen d'une seringue spéciale. On eut recours à des dissections pour confirmer les résultats de l'opération. Le taux de réussite de l'expérience a été établi à 92% pour le touladi, à 81% pour le meunier noir et à 92% pour le grand corégone. La seringue à détermination de sexe n'a pas toujours réussi à extraire des agents de reproduction, mais est parvenue dans tous les cas à éprouver l'élasticité de l'orifice urogénital et la pénétrabilité du tractus urogénital. La distension et la rétraction des pores anaux et urogénitaux, la formation d'une papille génitale, la coloration et l'accessibilité des pores urogénitaux, furent les caractéristiques les plus valables pour la détermination externe du sexe des poissons.

Mots-clés: détermination externe du sexe; orifice urogénital; papille génitale; seringue à détermination de sexe.

INTRODUCTION

External sex determination of individuals from any fish population can be a very useful tool for the fishery biologist. For the hatchery manager, fingerling production can be increased by varying female to male stocking ratios (Parker 1971). To research and management biologists, external sex determination can provide greater detail for system production, population trends, and life table formation. Weatherley (1972) suggests that differences exist in relative condition between sexes as well as sex-linked differences in growth rates between males and females. A more obvious advantage of sexual differentiation is the greater insight into population fecundity patterns with which to evaluate recruitment or stock determination (Bagenal 1978). Robson and Spangler (1978) suggest that sex can be used as the primary variable in a "change-in-ratio" estimator of population size. There are also many published accounts of fishing gear bias due to behavioral differences between male and female fish, either as a function of season, location or condition and size (Lagler 1978).

Previously, sexual differentiation was possible for many species either by internal examination of gonads, which required sacrificing the fish, or by a manual milking procedure which was successful only close to spawning periods. Recently, several authors have suggested using external sexing techniques year round for species such as northern pike (Esox lucius) and largemouth bass (Micropterus salmoides) (Snow 1963; McComish 1968; Driscoll 1969; Flickinger 1969; Parker 1971; Casselman 1974). Other relatively new techniques include use of hematocrit values in largemouth bass (Streuke and Atherton 1965) and secondary sex characteristics such as pearl organs, ovipositors, gonopodiums and papillar processes (Yamamoto 1969). As early as 1935, ratios of various body measurements were used as a means of distinguishing between female and male white suckers (Catostomus commersoni) (Spoor 1935). Such sex-related external features as nuptial tubercles, breeding size and color are also valuable for distinguishing between sexes, although these characteristics vary seasonally.

The purpose of this study was to determine: (1) whether any external, secondary, sexual characteristics existed in lake trout (Salvelinus namaycush), white sucker and lake whitefish (Coregonus clupeaformis), and (2) whether these characteristics could be used successfully for separating sexes in these fish species. A technique for extracting reproductive products was also tested.

METHODS AND MATERIALS

Fish were taken in 1981 from lakes in and near the Experimental Lakes Area (ELA), northwestern Ontario (Johnson and Vallentyne 1971). Three species were originally chosen for close examination due to their relative abundance in this region and their importance in fisheries research and management: lake trout, white sucker and lake whitefish.

Several lakes were sampled to obtain data from different lake sizes and types, population structures and growth rates, and species abundances (Beamish et al. 1976). Almost all fish were caught using experimental gill nets (bar mesh sizes 19-45 mm) and commercial gill nets (stretched mesh sizes 64-102 mm). These were set for one night only. Several fish were obtained by angling and from trapnets. Once preliminary data were taken, the sex of each fish was determined by external characteristics, then determined by the syringe method (described below), and finally verified by visual examination of the gonads.

At the start of this study, a few individuals of each species were used as study samples to develop external sexing criteria. The fish were a) examined externally to document any physical characteristics present (urogenital region only) and then b) dissected and sexed internally. The physical characteristics were then associated with the respective sexes to form the criteria used for sex determination throughout the study. As well, a "sexing syringe" was developed to aid in the sexing procedure (Fig. 1). This apparatus consisted of: (A) a 5 cc disposable Plaskipak syringe, (B) a detachable hypodermic needle with the tip ground smooth, and (C) a 20 cm length of Tygon tubing (2.4 mm outside diameter, 0.8 mm wall and opening). A second tube (D) (3.2 mm outside diameter, 0.8 mm wall and 1.6 mm opening) was also tried but the tube diameter was too large to be used successfully. The syringe apparatus was used by inserting the tubing into the urogenital tract and extracting reproductive material from the gonads. To observe insertion of the syringe into the gonad of the study samples, the abdomen of the specimen was opened without damaging the anal region. The tubing was then inserted into the urogenital pore and its path followed visually. Successful penetration of the gonads was achieved in all cases. Care must be taken however, in the insertion process, to avoid damaging the genital tract. Photographs of the urogenital region were also taken of approximately 100 fish of all maturity stages. These were found useful for demonstration purposes and detailed examination at a later date.

Several live fish were sampled to test whether or not insertion of the syringe into the urogenital tract would cause any damage to the fish. Approximately 30 to 40 white suckers from Lakes 223 and 302 were sampled between mid-June and mid-July in order to collect gonads from post spawned females for another study. Visual examination along with the syringe method were used to select the females needed.

Total samples for this study consisted of 261 lake trout and 324 white suckers both sampled from June 3 to October 28, 1981, and 364 lake whitefish sampled from July 8 to October 28, 1981.

As well as being sexed, the maturity state of each fish was determined. Immature fish were also sexed as often as possible. All results shown are sex and maturity state successes unless otherwise stated as only maturity state results. The Chi square test (X^2) was used to analyse the sexing success rates and $p<0.05$ was used as a significance level.

RESULTS

LAKE TROUT

Using external techniques for sex determination 92% of all trout sampled were sexed correctly. No significant differences were detected between times of year (X^2; P>0.05) and success of external sexing (Table 1). Mature females and males were equally easy to sex externally, although immature fish were sexed with slightly less success. A comparison in success rates and lakes sampled resulted in no significant differences (X^2; p>0.05). At no time were any fish assigned an incorrect maturity state (immature versus mature).

Using the syringe technique, males produced a white or cream colored milt; females released a reddish liquid containing small undeveloped eggs which were visible to the eye. As more fish were examined, the gonadal extraction technique became unnecessary. If the urogenital pore was found easily and penetration unhindered, the fish was sexed female. When location was difficult and penetration limited to the urogenital tract the fish was sexed male. When the urogenital opening was indistinguishable and impenetrable, the fish was immature.

Besides urogenital elasticity, several visual external characteristics in the anal region soon became readily discernable in separating the sexes. The urogenital opening of females was much larger and more obvious than that of males. It was deep red in color and allowed easy penetration, even if eggs were not extracted. The urogenital and anal pores were distinctly separate with the urogenital pore slightly extended, conical in shape and visible from a posterior-ventral view; mature females developed a permanent genital papilla. The anal pore was enlarged and fleshy, forming an oval to circular ridge around the opening itself (Fig. 2 and 3).

The urogenital region of male lake trout was smaller and much less conspicuous than that of females. The entire anal region appeared elliptical and was slightly indented (or flush) with the general ventral body surface. The urogenital tract was located medial to the anal pore (in the ventral view) and was never openly visible without moving the anal pore. The anal cavity and the urogenital pore were flesh white with the anal pore light red or pink in color. Only immediately prior to and following spawning did these characteristics change (mid-September to mid-October). At these times, the anal region became slightly extended with the urogenital pore becoming slightly more distinct. The anal region became scarlet in color while the urogenital pore and anal cavity remained predominantly white. Mature males developed a very small genital papilla just prior to and during spawning (Fig. 2 and 3).

During, immediately prior to, and following spawning, both sexes were easily milked by hand or the syringe method, and were distinguishable by body shape and color. By the end of October, most fish exhibited similar characteristics to resting fish sampled earlier in the study.

WHITE SUCKER

External sexing results using the white sucker were the poorest of all three species. A total of 81% of all suckers were sexed correctly. The least success was with males at 65% correct. Females and immature fish showed no significant differences in success rate (X^2; P>0.05) during the different sampling months (Table 1). Differences in success rates between lakes were minimal for all suckers (Table 2). Only one fish was given an incorrect maturity state, being sexed a mature male and verified as an immature male.

Male specimens caught in June and early July bore nuptial tubercles, present primarily on the anal fin, lower lobe of dorsal fin and caudal peduncle scales. Both sexes were milkable by hand, with males producing milt as late as July 20.

Use of the syringe extraction technique early in the summer produced a post-spawn mucus with a few undeveloped eggs in females, while males released their milt. Penetration into the female genital tract was very successful and unhindered until the end of August when specimens allowed only easy partial penetration. Entry into male urogenital tracts became more difficult after spawning and later became almost impossible. In general, a) easy penetration and location of the urogenital tract meant female; b) partial or poor entry meant male, and c) difficult location and no penetration of the urogenital pore meant an immature fish. At the same time, multiple visual characteristics emerged to aid in sexing this species.

Spawning female suckers developed a very large, distinct, fleshy anal "flap" which covered both the anal and urogenital pores. This flap was opaque white in color and triangular in cross section. From June through August, it extended almost to the anterior base of the anal fin. After this period it receded slightly. This flap had a very broad base and generally a rounded or fine tip. A genital papilla did not develop in either female or male suckers. The urogenital opening was located beneath the anal pore, recessed into the anal cavity (Fig. 4 and 5). The urogenital region and anal pore were colored deep red. The color gradually lightened late in the summer. The anal flap did decrease slightly in size but never disappeared entirely. It was never present on any immature fish.

Male suckers never exhibited the anal flap to the extent of females. They did possess an accented anal region with the anal pore exposed and directed towards the posterior of the fish. The urogenital opening was recessed in the anal cavity. The male anal flap that did exist was rather narrow and lacked a broad base. Its posterior tip was ended bluntly with the resulting posterior face perpendicular to the abdomen of the fish (Fig. 4 and 5). The anal pore was crimson in contrast to the cream colored urogenital pore and anal cavity. These colors remained consistent throughout the summer and fall. By the end of September, the anal pore had subsided slightly and was laying flush to the abdomen of the fish.

The success rate for the live white suckers tested was hard to determine. Eight fish were removed from the population after being sexed as females. Post-morten examination confirmed their sex with 100% accuracy. However, it was impossible to tell how many females had been overlooked and released. All fish released after syringe examination recovered normally and were returned to the lake.

LAKE WHITEFISH

External sexing of lake whitefish proved very successful (Tables 1 and 2). A total of 92% of all lake whitefish sampled, were sexed correctly. Males, females and immature fish all presented the same amount of difficulty in sexing. Monthly success rates were statistically similar for female and immature fish (X^2; P>0.05). Male success rates were not as consistent, but still similar between months (X^2; P>0.10). Success rates from all lakes sampled were similar for all whitefish sampled. Only one fish was classified an incorrect maturity state (mature versus immature).

Initial use of the sampling syringe produced egg samples from females. In males, however, the urogenital opening was difficult to locate and usually impossible to penetrate fully. Penetration of the female urogenital opening remained relatively easy throughout the summer and fall, while penetration of male openings varied from no entry in July to only partial entry by late October. No reproductive material was ever extracted from males. Once the spawning season approached (early October) females' eggs were inaccessible due to their size, while entrance into the urogenital tract remained unhindered. Males were soon producing milt upon hand milking, though syringe penetration was still very difficult.

Morphologically, both females and males showed change throughout the study. The urogenital pore of females collected early in the study (July) was set flush in the anal cavity but was still readily discernible. Later, a genital papilla developed and became more and more visually pronounced. By mid-August the papilla (urogenital pore) was protruding from the anal cavity (Fig. 6 and 7). The anal pore and urogenital papilla were originally a light red color. The anal region retained this color, becoming more robust and fleshy by late October. Once the urogenital papilla was extruding from the anal cavity, it became a deeper red color especially on the tip, with the anal cavity an opaque white color. Abdominal body extension due to egg production occurred just prior to and during spawning (late October).

The characteristics of the male whitefish urogenital opening remained quite consistent throughout the whole sampling period. As mentioned previously, the urogenital pore was difficult to locate. Entry into the urogenital tract was virtually impossible although several specimens allowed difficult penetration. The anal pore was a deep crimson color and yet less conspicuous than the females due to its small size. Only when the spawning season approached, did the males exhibit any changes. The anal pore became slightly more prominent and fleshy and became an even darker crimson (Fig. 6 and 7). The urogenital pore became visible in the anal cavity though still not allowing penetration even though milt could be manually released. A genital papilla never did develop in males.

Immature whitefish were those that exhibited neither anal nor urogenital discoloration or extension. The urogenital pore could not be distinguished and the general anal region remained flush to the abdomen, in no way conspicuous. Size of fish (fork length, weight, etc.) also helped to separate immature from mature fish.

DISCUSSION

At the beginning of this study, it was hoped that by using the syringe technique alone to extract reproductive material from all fish, a practical and efficient method of external sexing would be possible. It soon became clear that extraction was not possible in all cases. At the same time accurate sexing could be achieved by relying solely on penetrability or elasticity of the urogenital opening. Driscoll (1969) had similar results when sexing largemouth bass with an otoscope. He found that he could distinguish sexes successfully not only by observing gonads but by comparing plasticity and ease of insertion of a 1.5 mm diameter tube into the gonads.

In this study a definite relationship existed between the sex of the fish and the ease and depth of penetration into the urogenital opening. The 1.6 mm diameter tube proved most successful in that penetration of any sort classified the fish as sexually mature while no penetration meant an immature fish. The larger 3.2 mm diameter tube while allowing larger reproductive samples to be extracted, did not penetrate the urogenital opening of most fish. Furthermore, the flexibility of the tubing allowed for easier penetration into the gonad of females (complete penetration) while in non-spawning males, penetration was limited to just the urogenital tract and not into the gonad (partial penetration). A rigid tube would probably be too restrictive in allowing full penetration although Driscoll (1969) and Streuke and Atherton (1965) inserted the tip of an otoscope into the urogenital opening far enough to view the gonads with no apparent damage done.

External morphological features were also useful in correct sex determination of fish. Distention or retraction of the anal and/or urogenital pores, formation of a genital papilla, coloration and even accessibility of the urogenital pore were reliable indicators of sex (Table 3). Casselman (1974) used many of these characteristics successfully in his study of external sexing of northern pike (91% males and 94% females). He also incorporated stripping the fish for reproductive materials, which at peak spawning times increased external sexing accuracy.

Using a combination of syringe penetrability and urogenital examination, acceptable success rates were achieved for all fish with perhaps the exception of mature male white suckers and immature lake trout. Only 65% of all male white suckers examined were sexed correctly. This varied from a high of 100% in October to a low of 43% in July. This variation does not seem to be related to different sucker populations, since lake to lake success rates are relatively the same. The most common mistake with the mature males was to classify them as immature females; external urogenital characteristics were very similar. With more skill and closer examination, these similarities disappear and better results probably can be achieved. The other inconsistent group of fish was immature lake trout of which only 78% were sexed correctly. This was almost totally due to mistaking immature males for immature females. This may not be too critical because sexing of immature fish is of less importance than identifying them as immature. The techniques used in this study are probably applicable to other fish populations although initial and periodic internal examination of gonads after external sexing, would be a necessary safeguard.

Month to month success rates using the syringe technique and external characteristics were consistent which would suggest that these techniques could be useful year round. However, winter sampling should be conducted to verify this assumption. Sexing the three different species of fish simultaneously tended to cause confusion as to which characteristics should be used for each species. Working on one species at a time is suggested so that no confusion occurs. A table similar to Table 3 as a field version also aids in separating species-specific external sexing characteristics. Casselman (1974) also suggests that slight magnification of the urogenital region for difficult specimens can increase accuracy of the urogenital technique. External characteristics observed at spawning, can be used in sexual differentiation only if their use is limited to the peak spawning period itself.

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Table 1. Monthly summary of success rates for external sex determination of lake trout, white sucker and lake whitefish caught in the Experimental Lakes Area.

		June		July		August		September		October	
		% Correct	N	% Correct	N	% Correct	N	% Correct	N	% Correct	N
Lake Trout	♀	93	14	100	5	96	83	100	7	91	10
	♂	75	16	100	10	93	68	100	10	100	2
	Imm	100	2	80	5	82	22	50	2	60	5
	Total	84	32	95	20	93	173	95	19	82	17
White Sucker	♀	82	17	92	12	83	57	78	36	71	7
	♂	80	5	43	7	63	38	56	27	100	12
	Imm	100	12	91	42	91	21	96	27	100	4
	Total	88	34	85	61	78	116	77	90	91	23
Lake Whitefish	♀	-	-	100	4	91	117	100	36	100	2
	♂	-	-	100	6	88	113	100	40	89	9
	Imm	-	-	100	2	94	33	100	2	-	-
	Total	-	-	100	12	90	263	100	78	91	11

"-" - indicates no fish sampled

♀ - symbol for female

♂ - symbol for male

Table 2. Lake population success rates for external sex determination in 10 ELA lakes.

		Lake										Total			
		122	222	223	226NE	258	305	382	467	468	634	Male	Female	Imm	Sample
Lake Trout	N	-	-	10	-	17	154	31	13	36	-	106	119	36	261
	% Correct	-	-	90	-	82	94	94	100	89	-	92	96	78	92
White Sucker	N	-	2	22	-	26	69	32	38	76	59	89	129	106	324
	% Correct	-	100	96	-	73	83	86	76	78	80	65	81	93	81
Lake Whitefish	N	76	-	-	3	56	203	-	10	16	-	168	159	37	364
	% Correct	100	-	-	100	96	88	-	100	94	-	91	93	96	92

"-" - indicates no fish sampled from this lake

Table 3. Summary of external characteristics used to sex lake trout, white sucker and lake whitefish.

	Male	Female	Immature
Lake Trout	-produce sperm when stripped -June extraction produced milt -Urogenital pore difficult to locate -tube penetration difficult and partial (into urogenital tract 0.5 to 1.0 cm) -anal region elliptical in shape and flush with ventral body surface -very slight anal extension-pre-spawning -urogenital tract under anal pore in anal cavity -genital papilla very small and present only pre-spawning -anal cavity and urogenital pore white -anal pore pink-crimson only pre-spawning	-produce eggs when stripped -June produced undeveloped eggs in extraction -urogenital pore easily located -tube penetration easy and complete (into urogenital tract and often gonad: up to 5 cm or more) -anal pore enlarged, circular in shape, extended from ventral body surface -extreme anal pore extension-pre-spawning -urogenital pore distinctly separate, conical in shape, slightly extended -genital papilla permanent, visible in different degrees year-round -anal pore crimson red -urogenital pore and anal cavity pink to red -pre-spawning - genital papilla extruding from anal cavity, crimson red in color	-no product when stripped -no extraction -urogenital pore indistinguishable -no tube penetration -anal region flush to ventral body surface -anal pore and urogenital pore both inset in very small anal cavity -no genital papilla -anal and urogenital pores both cream white
White Sucker	-nuptial tubercles prominent pre and post-spawning -sperm produced when stripped -post-spawn milt produced in syringe extraction -partial or poor urogenital entry and location -very small anal extension or flap -anal flap narrow, ended bluntly, not extending completely over anal cavity -anal pore exposed facing anal fin -urogenital pore recessed in anal cavity -no genital papilla -anal pore crimson, urogenital pore and anal cavity white or cream	-nuptial tubercles seldom present pre-spawning -eggs produced when stripped -post-spawn-undeveloped eggs in red liquid produced in syringe extraction -easy penetration and location of urogenital pore -fleshy anal flap present predominantly -anal flap with broad base, rounded tip, triangular in cross-section and extending completely over anal region -urogenital and anal pores under anal flap -no genital papilla -anal and urogenital pores red in color -anal cavity white	-nuptial tubercles seldom present -no result from stripping -no syringe extraction -difficult location and no penetration of urogenital pore -no anal flap -no genital papilla -total anal region creamy white
Lake Whitefish	-sperm produced when stripped; pre and post-spawn -no results from syringe extraction -urogenital opening difficult to locate and only partial entry -genital papilla never present -urogenital pore inconspicuous set flush in anal cavity -anal pore small; slightly extended in pre-spawn period -anal pore deep crimson -urogenital pore and anal cavity white -no abdominal extension	-eggs produced when stripped; pre-spawn -syringe extraction in spring produced eggs -urogenital pore easy to locate and penetration unhindered -genital papilla present majority of sampling period, very distinct -urogenital pore easily discernable, set in anal cavity, extending out during pre-spawn -anal pore extended, robust and very visible -anal and urogenital pores red in color -anal cavity white -abdominal extension; pre-spawn	-no results from stripping -no extraction -no penetration and very poor location -no genital papilla -no anal or urogenital distention or discoloration

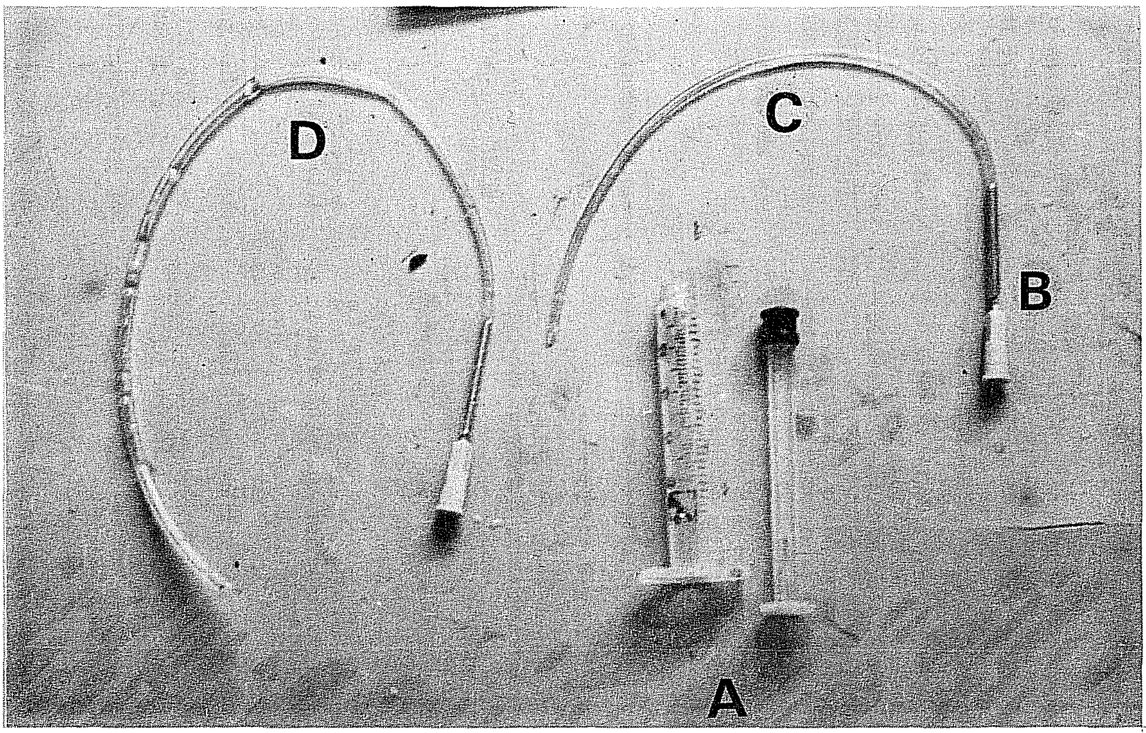

Fig. 1. Syringe sexing apparatus used to extract reproductive materials from gonads of male and female fish: (A) 5 cc disposable syring, (B) detachable hypodermic needle, (C) 0.8 mm opening Tygon tubing, (D) 1.6 mm opening Tygon tubing.

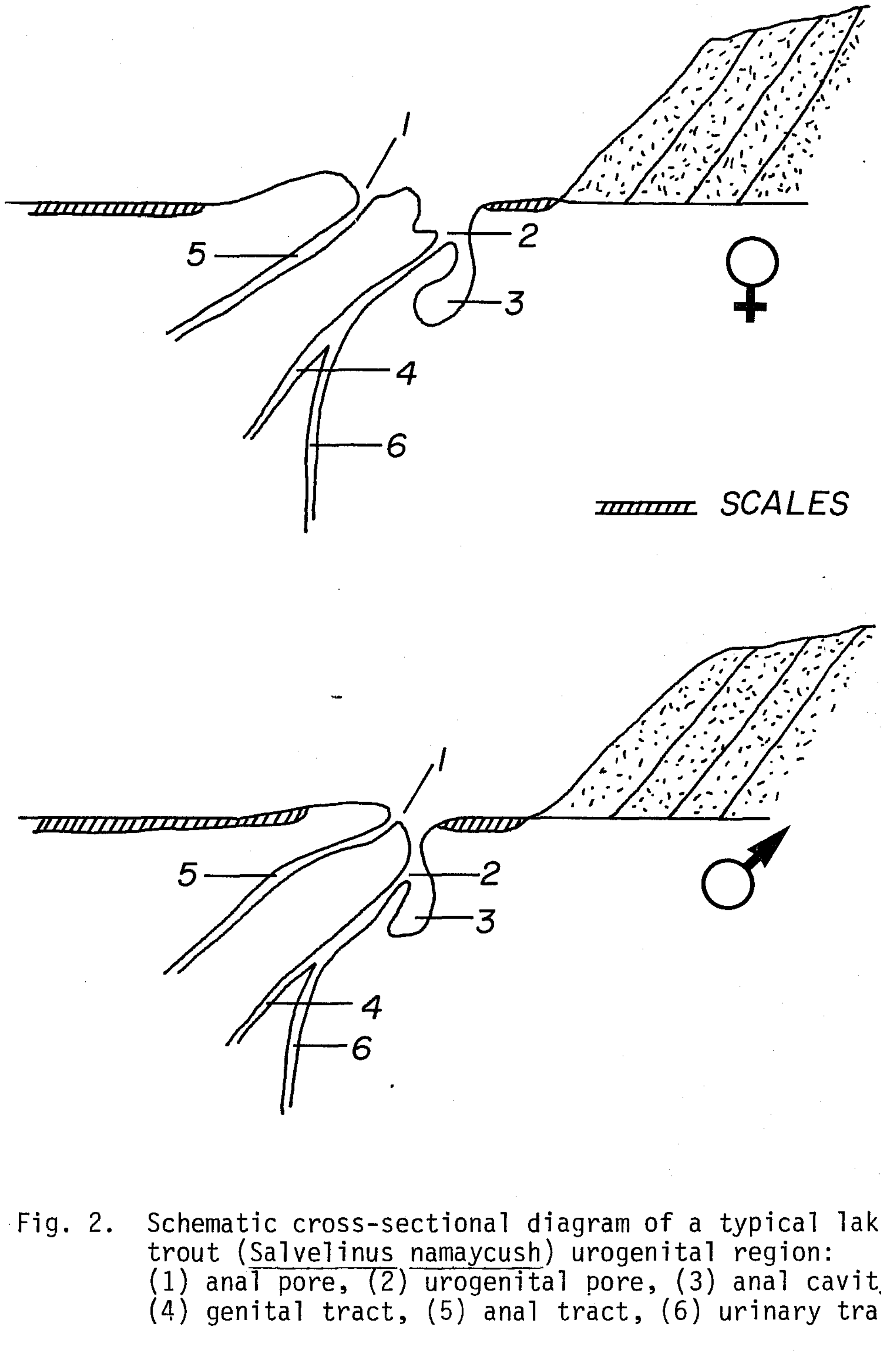

Fig. 2. Schematic cross-sectional diagram of a typical lake trout (Salvelinus namaycush) urogenital region: (1) anal pore, (2) urogenital pore, (3) anal cavity, (4) genital tract, (5) anal tract, (6) urinary tract.

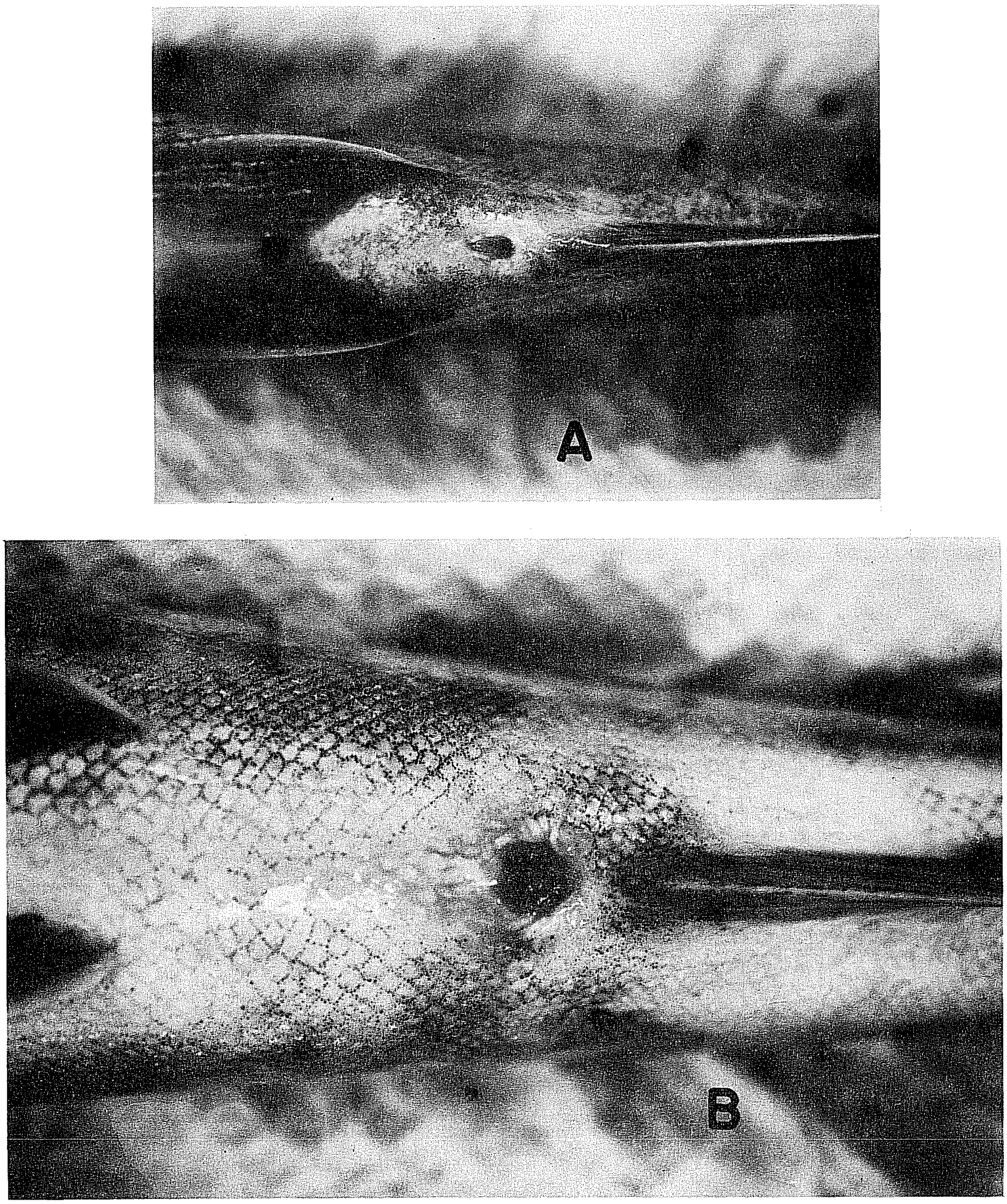

Fig. 3. Anal region of male (A) and female (B) lake trout (*Salvelinus namaycush*).

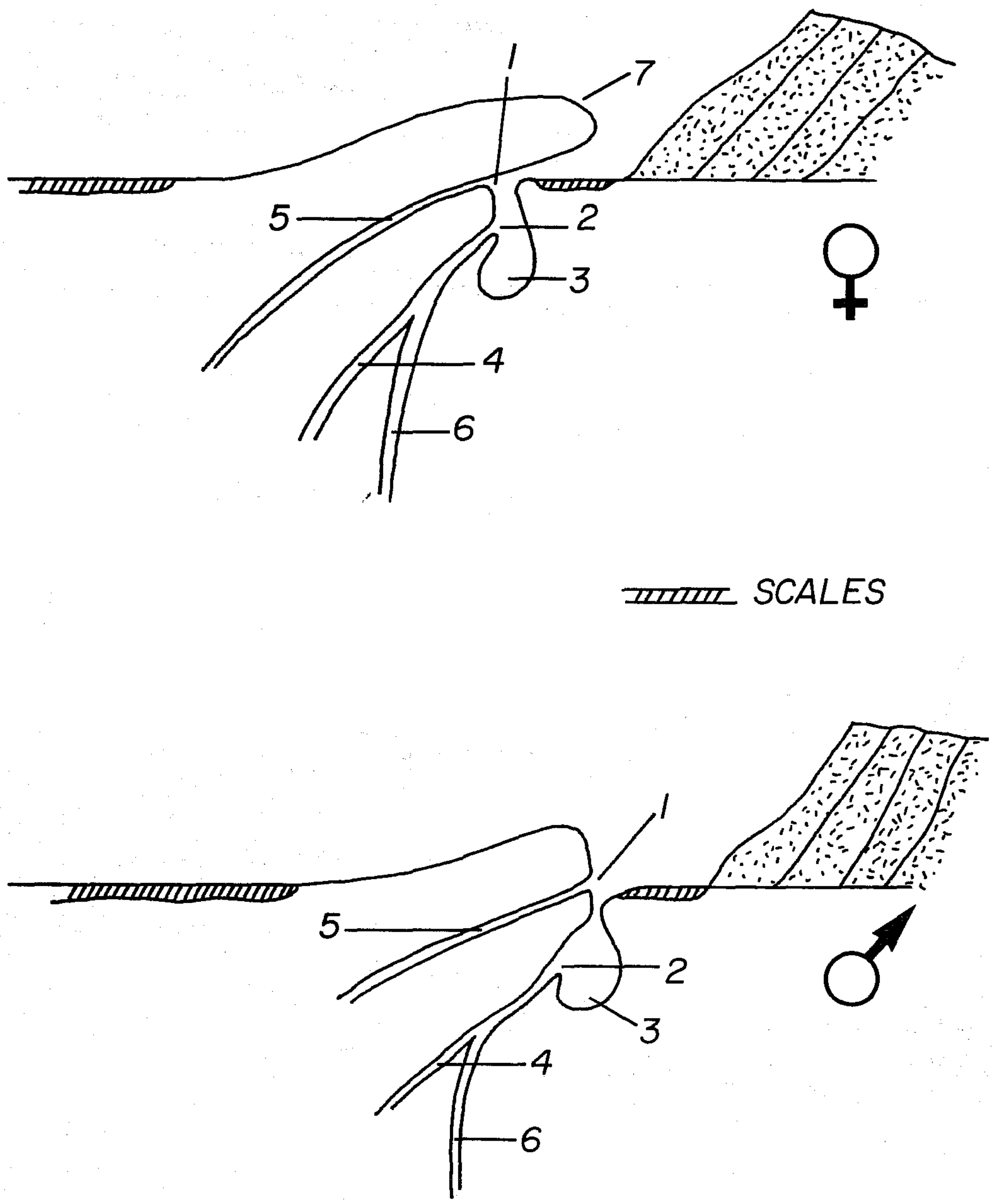

Fig. 4. Schematic cross-sectional diagram of a typical white sucker (Catostomus commersoni) urogenital region: (1) anal pore, (2) urogenital, (3) anal cavity, (4) genital tract, (5) anal tract, (6) urinary tract, (7) anal "flap".

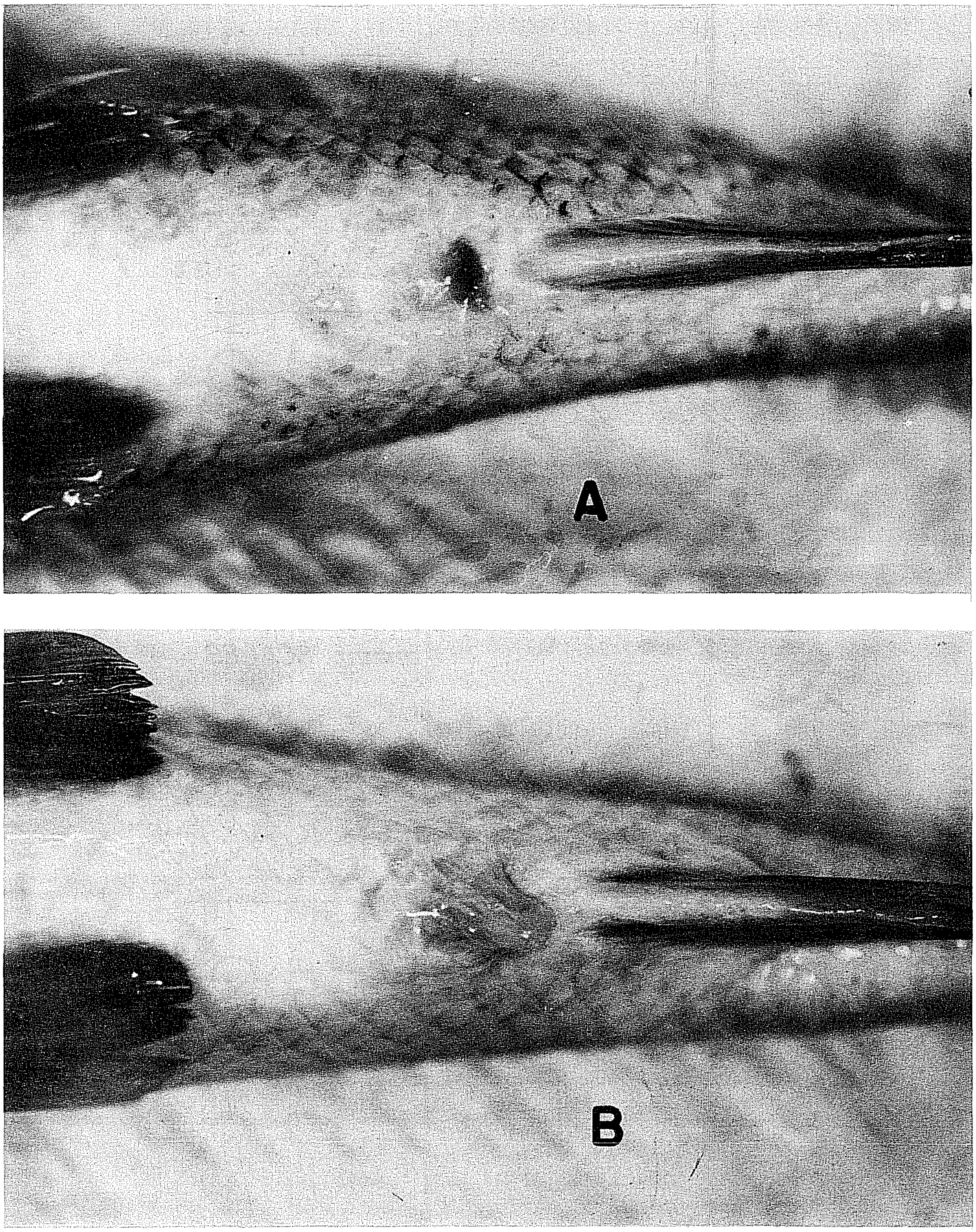

Fig. 5. Anal region of male (A) and female (B) white sucker (Catostomus commersoni).

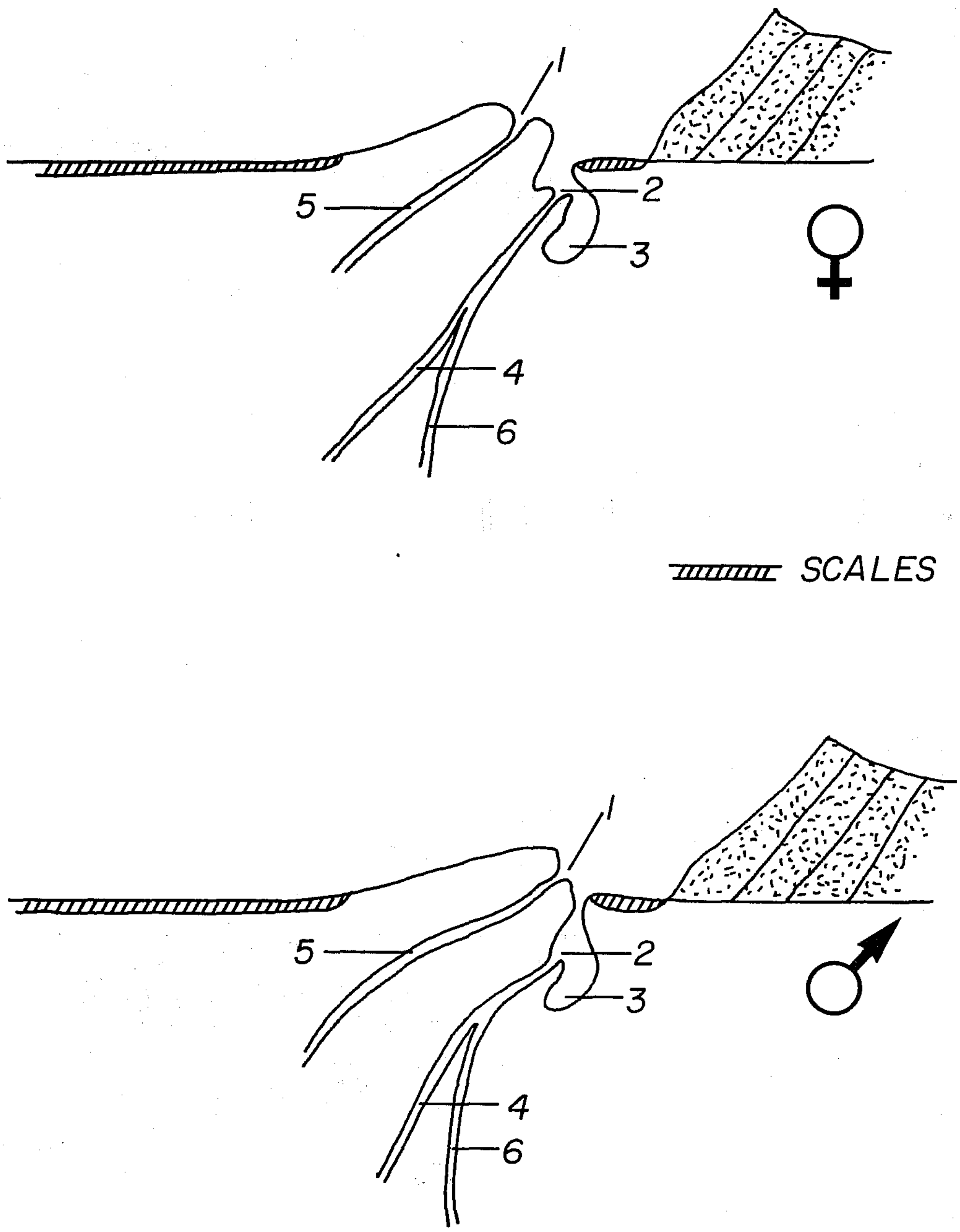

Fig. 6. Schematic cross-sectional diagram of a typical lake whitefish (Coregonus clupeaformis) urogenital region: (1) anal pore, (2) urogenital pore, (3) anal cavity, (4) genital tract, (5) anal tract, (6) urinary tract.

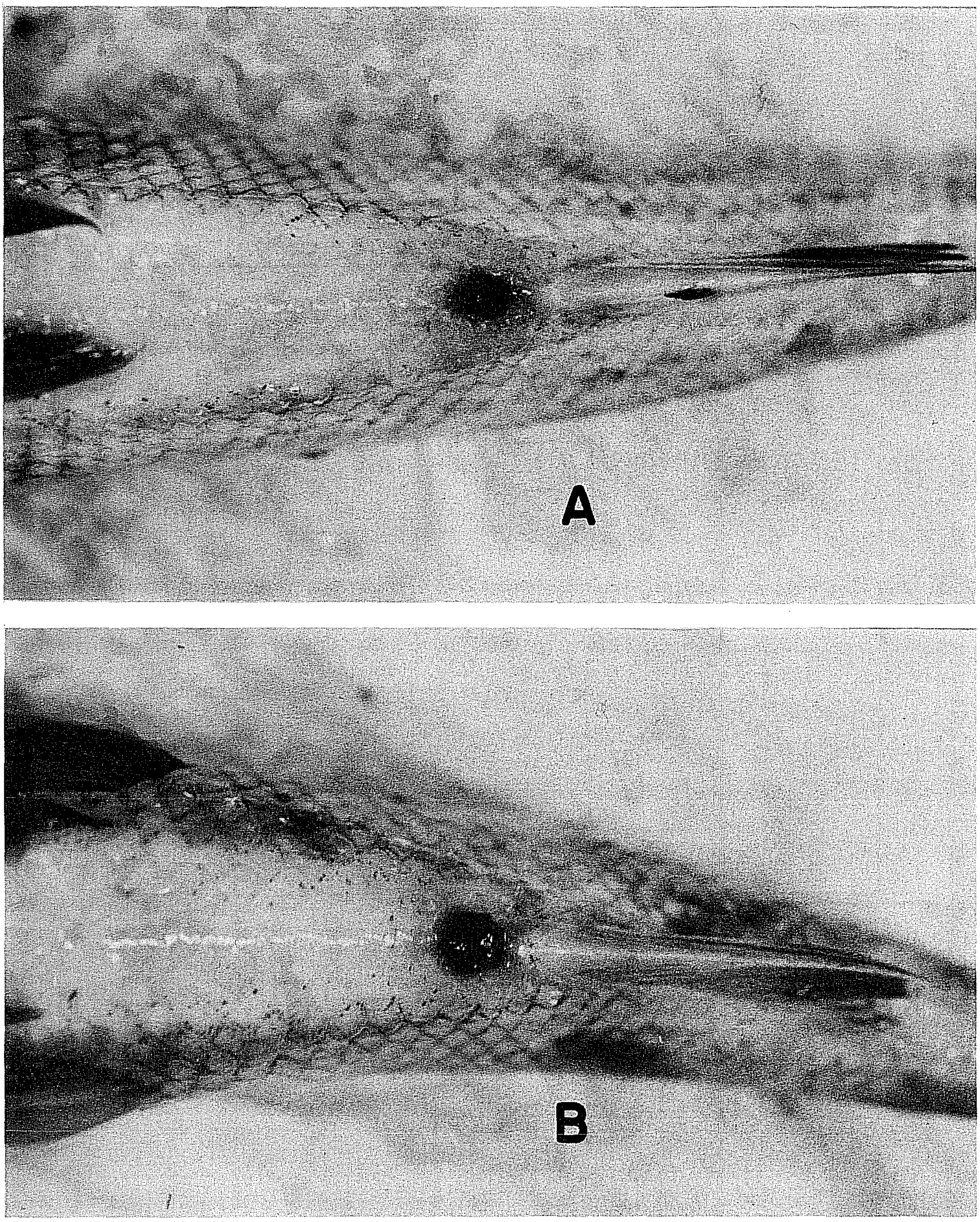

Fig. 7. Anal region of male (A) and female (B) lake whitefish (Coregonus clupeaformis).