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PROTANDROUS HERMAPHRODITISM IN AUSTRALIAN SILVER PERCH, *BIDYANUS BIDYANUS* (MITCHELL, 1836)

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Abstract

Gonadal development in two progenies of the Australian silver perch, *Bidyanus bidyanus*, was studied from the larval stage to 18.5 months. For approximately one month after hatching, it was impossible to visually or histologically determine the sex of the fish. Two to three months after hatching, anatomical and cytological sex differentiation occurred. At 4-5 months, the testis in most of the fish longer than 7-8 cm exhibited all stages of spermatogenesis including spermatozoa. Very few females were found among the 4 and 6.5 month fish. Up to 18.5 months, 7.1-23.5% were female (except in one batch). There were only 25 females, all at early stages of oogenesis, amongst 204 fish with differentiated sex gonads. In 10 of 17 histologically studied females, there were degenerating male cells (possibly spermatocytes) among a majority of developing oocytes. The predominance of phenotypic males, and the occurrence of females with ovaries and degenerating male cells, indicate that during the first years of life, this species is a protandrous hermaphrodite. The testis develops during ontogenesis in a direct manner, whereas the female gonad develops indirectly, passing through an intermediate masculine stage.

Introduction

The silver perch, *Bidyanus bidyanus*, a native Australian freshwater species, is a new introduction to Israeli aquaculture. This species inhabits the Murray-Darling River system of southeastern Australia and is a significant commercial species (Deering, 1996). Silver perch is a protandrous species and, in the wild, adults migrate upstream and require an increase in water level to induce spawning. Spawning usually occurs during the summer floods when the water temperature is above 20°C (Lake, 1968; Cadwallader, 1986). Female silver perch reach sexual maturity at 3 or 4 years of age and are approximately 340 mm in length, while males mature at 2 or 3 years of age and are approximately 233 mm.

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There is no sexual dimorphism except for the reproductive season when there is a difference in body contour in near-ripe females and, for at least one month before the water temperature is sufficiently high for spawning, milt can be forced from males (Lake, 1967).

During 1975-1995, techniques for large-scale hatchery production of silver perch were elaborated in Australia (Thurstan and Rowland, 1994; Lawrence, 1995). Fish for broodstock are captured in the wild, stocked into ponds for nearly 8 months and, when the temperature reaches 21°C in early summer, they are induced to spawn by a single injection of human chorionic gonadotrophin. Thus, the Australian technique for silver perch reproduction is based on maintenance of broodfish captured from nature.

Formation of silver perch broodstock in Israel started in 1997 when fingerlings of this species were brought from Australia. Establishing fish broodstock requires knowledge of the gonadal development of the fish under Israeli conditions. The aims of this investigation were to study sex differentiation in silver perch and determine the gonadal development during the first years of life.

Materials and Methods

Fish. Two progenies of fish were investigated. Both progenies were obtained from fish brought from Australia to Israel as fingerlings and reared in tanks and ponds at the Aquaculture Research Station Dor (Ministry of Agriculture). The first progeny hatched in June-July, 1998. Samples were taken from August 1998, when the fingerlings were 1-1.5 months old, until October 1999 when the fish reached 15-15.5 months. The second progeny hatched in May 1999. Samples were taken from the larval stage to the age of 18.5 months. For both progenies, 6-34 individuals of the same age were randomly chosen from the tanks or ponds every month or two months. Fish were killed by decapitation, then measured and weighed.

Histology. Samples were fixed in Bouin's fluid. Larvae and small individuals with a body length of 15-30 mm were fixed whole. In larger fish, the whole gonad was fixed. When the gonad was quite large, a small piece of tissue was cut from its middle and fixed. All samples were dehydrated, embedded in paraplast and sectioned into 4-5 μm pieces. The sections were stained with alcian blue (pH 3.0; Herlant, 1960) in combination with azocarmine G, alum hematoxylin and eosin, Heidenhain's iron hematoxylin and Heidenhain's azan (Romeis, 1953). The diameters of cells and nuclei of different cell types were measured. Forty-five larvae and 242 fish at different ages were studied for determination of gonadal state. All larvae and 208 fishes were studied histologically.

Results

Five phases of gonad development were distinguished: indifferent period, anatomical differentiation, cytological differentiation, period preceding sexual maturity (prepuberty) and sexual maturity.

The indifferent period. During this period, gonads formed and primordial germ cells migrated and concentrated. The indifferent period of gonadal development continued for approximately one month after hatching, during which it was impossible to determine the sex of the fish either visually or histologically. Single germ cells were first found in 4-day larvae at the site of the presumptive gonad, i.e., between the wall of the gut and the pronephric ducts (Fig. 1). The largest diameter of the gonocytes was 12.0±0.41 μm and the smallest 7.3±0.37 μm; the nuclei measured 6.1±0.20 by 4.8±0.19 μm. Germ cells were round or oval and their boundaries were not always clearly defined. Light cytoplasm formed a narrow layer surrounding a clearly delineated nucleus. The nuclei were stained considerably more intensively than the cytoplasm and appeared "dusty" due to the profusion of small fragments of chromatin randomly scattered throughout the karyoplasm. The nucleoli were not seen. There were many cells with polymorphic nuclei. The gonocytes were similar in their morphological features to the primordial germ cells (PGC) of other vertebrates including fish (Persov, 1975; Bruslé and Bruslé, 1978; Nieuwkoop and Sutasurya, 1979; Moiseyeva, 1983; Moiseeva et al., 1988).

The gonads in 12 and 14-day larvae took the shape of interrupted threads along the

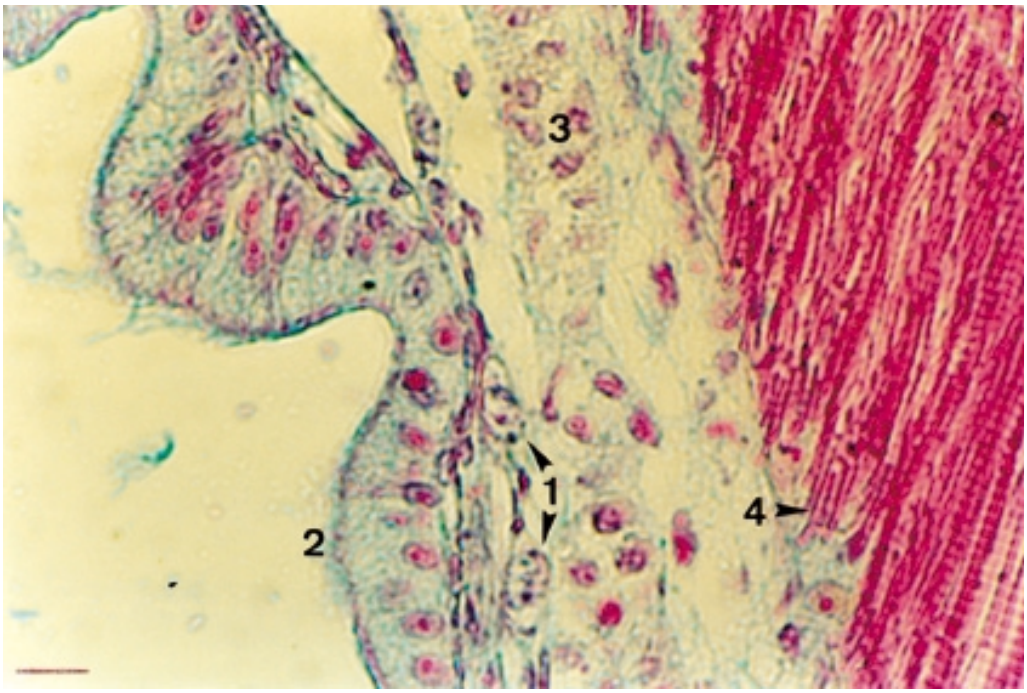


Fig. 1. Primordial germ cells in 4-day-old larvae: (1) primordial germ cells, (2) wall of intestine, (3) pronephric duct, (4) muscle. Stained by alcian blue, azocarmine. Bar scale: 50 μm.

dorso-lateral peritoneal wall and, in cross-sections, they looked like sexual folds or toruli, in which many somatic elements and sparse single germ cells could be seen. These were genital ridges. PGCs were in the state of migration and accumulation into the primordial gonad (Fig. 2). The cells and their nuclei were smaller than in younger larvae, 11.4±0.47 by 7.6±0.32 μm for cells and 5.7±0.32 by 4.6±0.15 μm for nuclei. In 18 and 22-day larvae, blood capillaries and several types of somatic cells were present in the gonad. As in the earlier phase, germ cells were not abundant and the somatic elements formed the major mass. Morphological features of the germ cells were like those in the 4-day larvae, but cells and nuclei decreased to 9.2±0.26 by 6.3±0.25 μm and 4.7±0.17 by 3.9±0.12 μm, respectively.

Anatomical differentiation. In 30-day fry, the gonads were anatomically formed. They were attached to the swimbladder by mesenteries (Fig. 3). Within the gonad there were two large blood vessels (artery and vein), and somatic cells forming the lobular structure. The number of germ cells did not increase but cell and nucleus sizes increased to 11.8±0.41 x 9.3±0.41 μm (cell) and 6.1±0.19 x 5.5±0.19 μm (nucleus). The morphology of the germ cells also changed. The nuclei became vesicular and did not stain as intensively as before. The nucleoli were visible and, in some nuclei, the nucleoli occupied a central position. No mitosis of the sex cells was observed. It was impossible to determine the sex of the 30-day fish. However, in the two largest fish of the first progeny (body lengths 7.0 and 7.3 cm), the gonads had triangular cross-sections with cells arranged in a lobular structure with a cleft-like cavity (Fig. 3b). These features indicated the initial structure of a testis but no signs of cytological sex differentiation were observed.

Cytological sex differentiation. The first

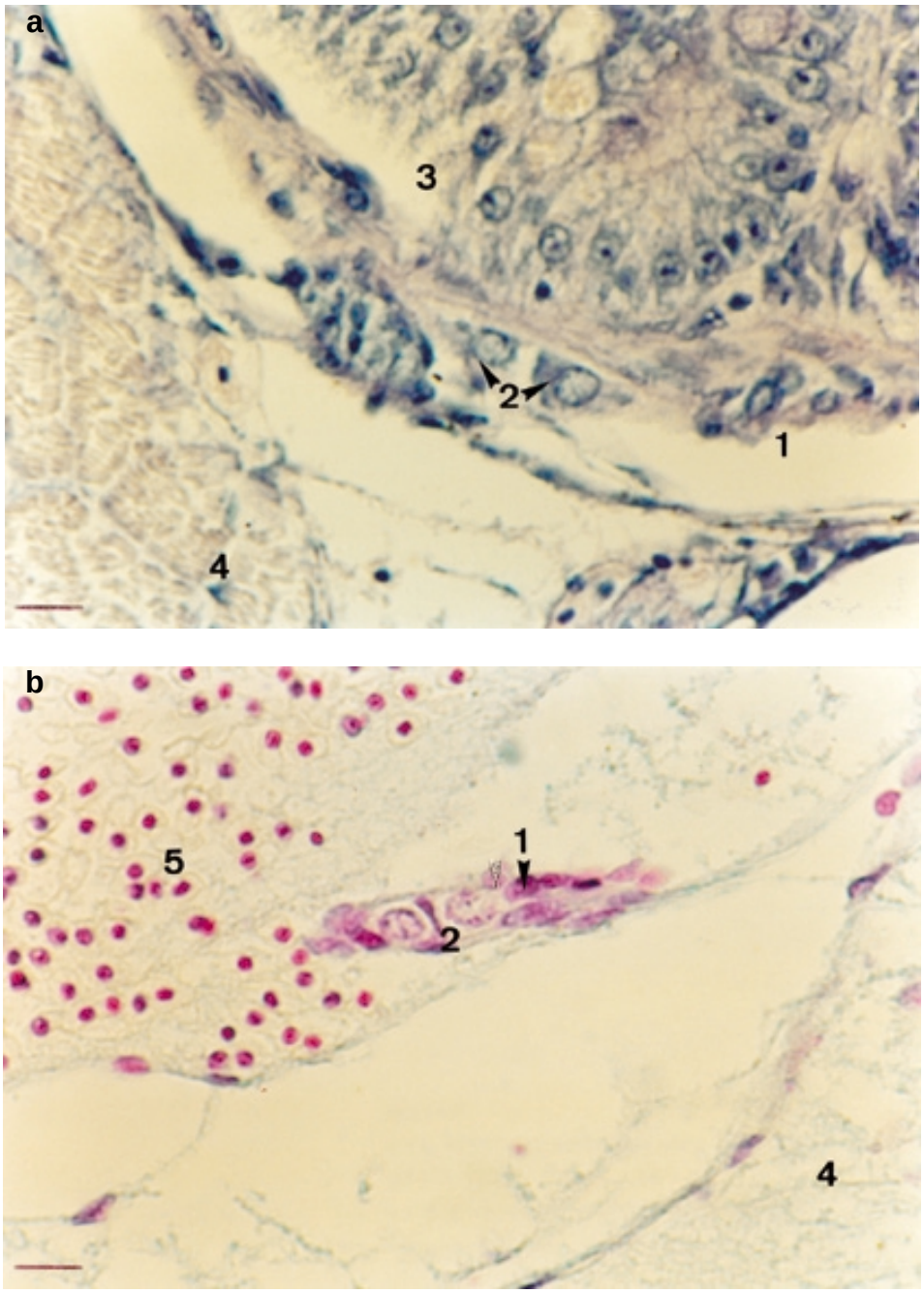


Fig. 2. Gonocytes of the 12-day-old larvae: (a) transverse section - primordial germ cells in process of migration; stained by alum hematoxylin, eosin, (b) longitudinal section - germ cells populate the gonad; stained by alcian blue, azocarmine. (1) gonad, (2) germ cells, (3) intestine, (4) muscle, (5) blood cells in the adipose tissue. Bar scale: 50 µm.

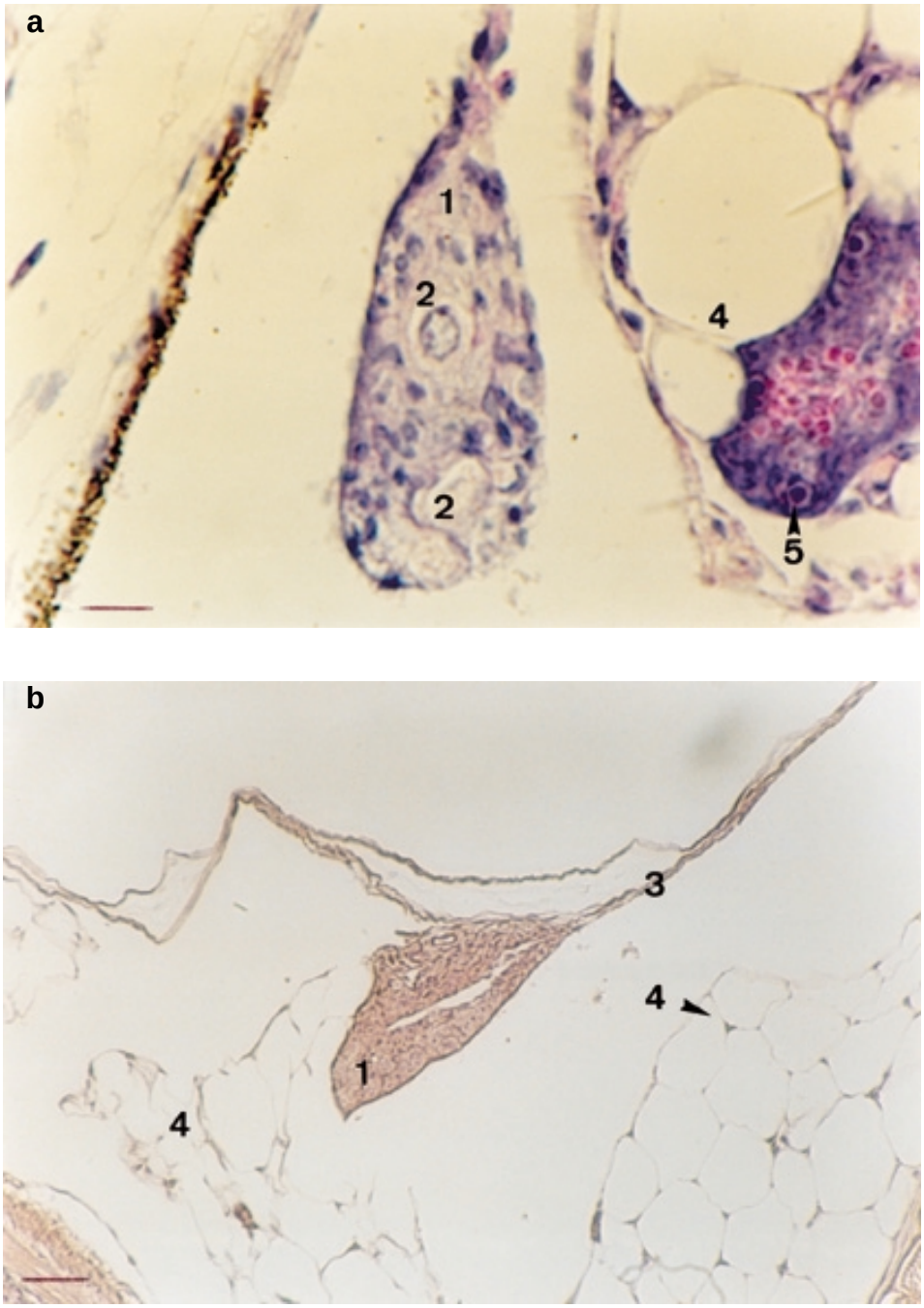


Fig. 3. Gonad of the 30-day-old fry during the indifferent phase of gonadal development: (a) stained by alcian blue, azocarmine; bar scale: 50 mμ; (b) stained by iron hematoxylin; bar scale: 250 mμ. (1) gonad, (2) germ cells, (3) mesorchium, (4) adipose tissue, (5) pancreas.

signs of cytological sex differentiation were found in 2-month silver perch in 77.8% and 20% of the fish from the first and second progenies, respectively (Tables 1,2). All were male. Germ cells were presented by spermatogonia of different generations, spermatocytes I and II (primary and secondary), spermatids and spermatozoa (Fig. 4, Tables 1,2). In the 4-month fish, the number of fish with a differentiated gonad reached 91% in the first progeny and 100% in the second progeny.

The first females were found among 6-6.5-month fish of the first progeny and 4-month fish of the second. The ovaries consisted of oogonia, oocytes of early meiotic prophase and oocytes of the cytoplasmatic growth period (Fig. 5, Tables 1,2). Often, eosinophilic granular leukocytes and lymphocyte-like cells could be observed among the germ cells. In some ovaries, clusters of degenerating male cells could be seen side by side with female cells. In addition, there was a large amount of detritus in the ovarian lumen, probably resulting from degeneration of testicular cells (Fig. 5b). At the same time, in the testes, cells of all spermatogenic stages were found. Spermatozoa occupied the lumen of seminiferous ducts (Fig. 4b). No morphological signs of ovarian tissue were observed in male gonads.

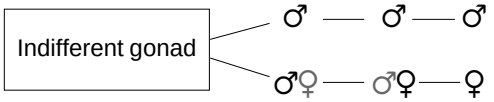
High variability of size was observed in all fish groups (Tables 1,2). No relationship was found between fish size and the beginning of cytological sex differentiation. However, in both progenies, the majority of fish with a body length of more than 7-8 cm were already physiological males.

Discussion

In teleosts, there are three basic forms of hermaphroditism: simultaneous hermaphroditism, protandry and protogyny (Atz, 1964; Reinboth, 1970; Sadovy and Shapiro, 1987). In simultaneous hermaphroditism, individuals function both as male and female at the same time. In protandry, some or all fish function first as males and later in ontogenesis as females. In protogyny, the sex of individuals changes from female to male during their life history.

According to Sadovy and Shapiro (1987), "the strongest indicators of protandry are transitional individuals whose gonads contain degenerating testicular tissue and developing ovarian tissue". The present histological study found remnants of testicular tissue in 10 of 17 silver perch females. The presence of degenerating testicular tissue simultaneously with a large number of developing oocytes in the same gonad strongly suggests protandrous hermaphroditism in *Bidyanus bidyanus*. No relationship was found between fish size and sex inversion.

In the ovaries of the silver perch, groups of spermatogenic cells were scattered among predominating oocytes but did not occupy a distinct position in the gonad as in Spariids (Atz, 1964; Sadovy and Shapiro, 1987; Micale and Perdichizzi, 1994; Bruslé-Sicard and Fourcault, 1997). Predominance of males in young fish groups and the presence in these groups of females with remnants of degenerating male tissue suggest that, in silver perch, male gonads develop in a direct way while female gonads develop in an indirect way. Hence, sex differentiation in this species according to Persov (1975) can be presented as follows:



After the indifferent period of development, gonads in some of the fish develop only to the male stage, while the rest of the population passes through an intermediate male stage, then the testicular tissue is replaced by oocytes. In older individuals, remnants of tissues of the opposite sex were not observed in the gonads, and the fish functioned gonochoristically. Such a situation was also observed in adult silver perch broodstock (data not shown).

No data regarding the sex ratio in young or adult silver perch in the wild or in hatchery broodstocks are available. Among the oldest fish (18.5 months), the number of males was considerably higher than females, as it was in fish of younger ages (Table 2). To determine the final sex ratio in cultured populations, older fish should be studied.

Gordon (1995) noted a stunting of growth and precocious maturation of silver perch

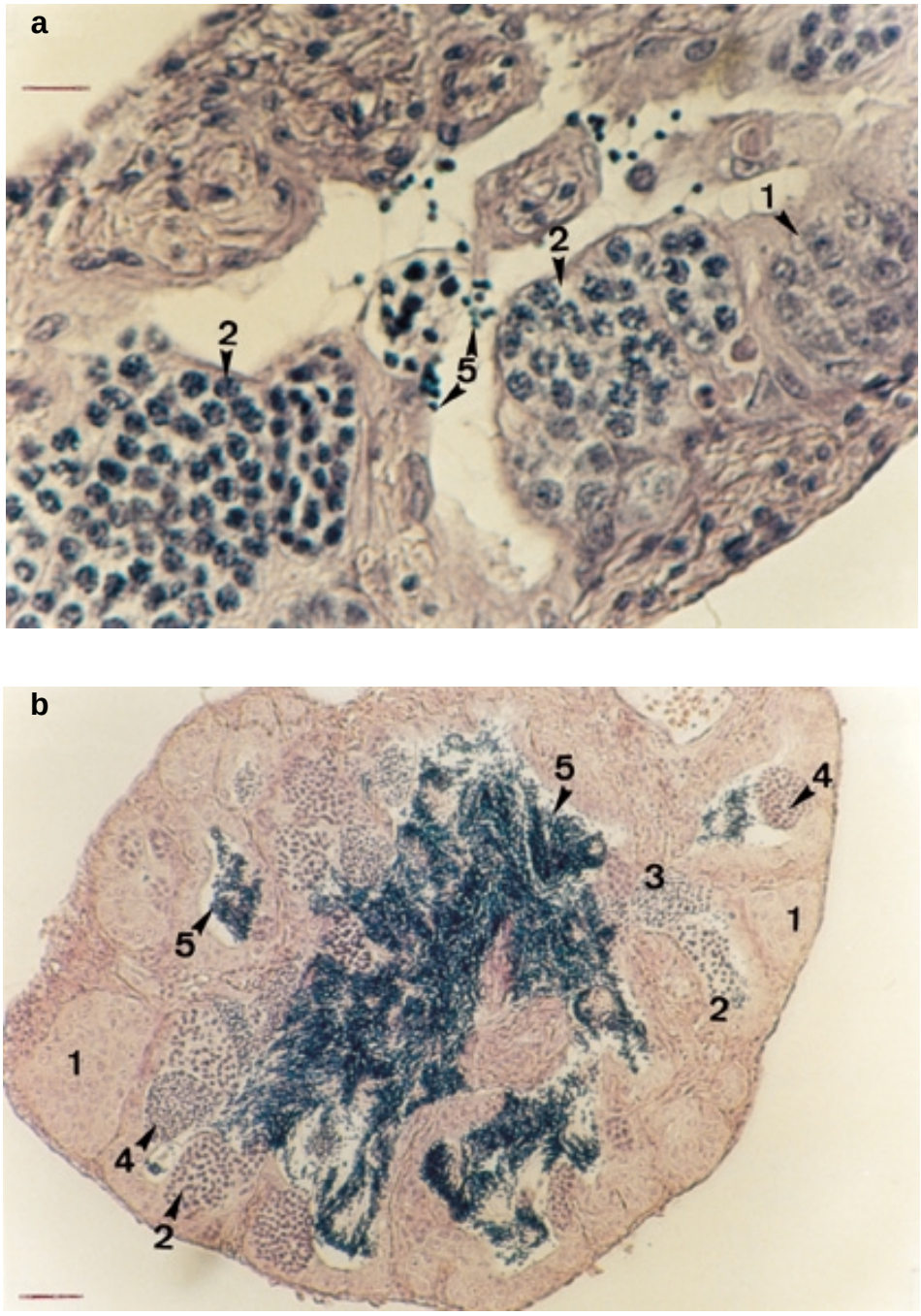


Fig. 4. Testis of (a) 2-month-old (body length 7.8 cm; bar scale 50 µm) and (b) 3.5-month-old (body length 10.8 cm; bar scale 250 µm) fish. (1) spermatogonia, (2) primary spermatocytes, (3) secondary spermatocytes, (4) spermatids, (5) spermatozoa. Stained by alum hematoxylin, eosin.

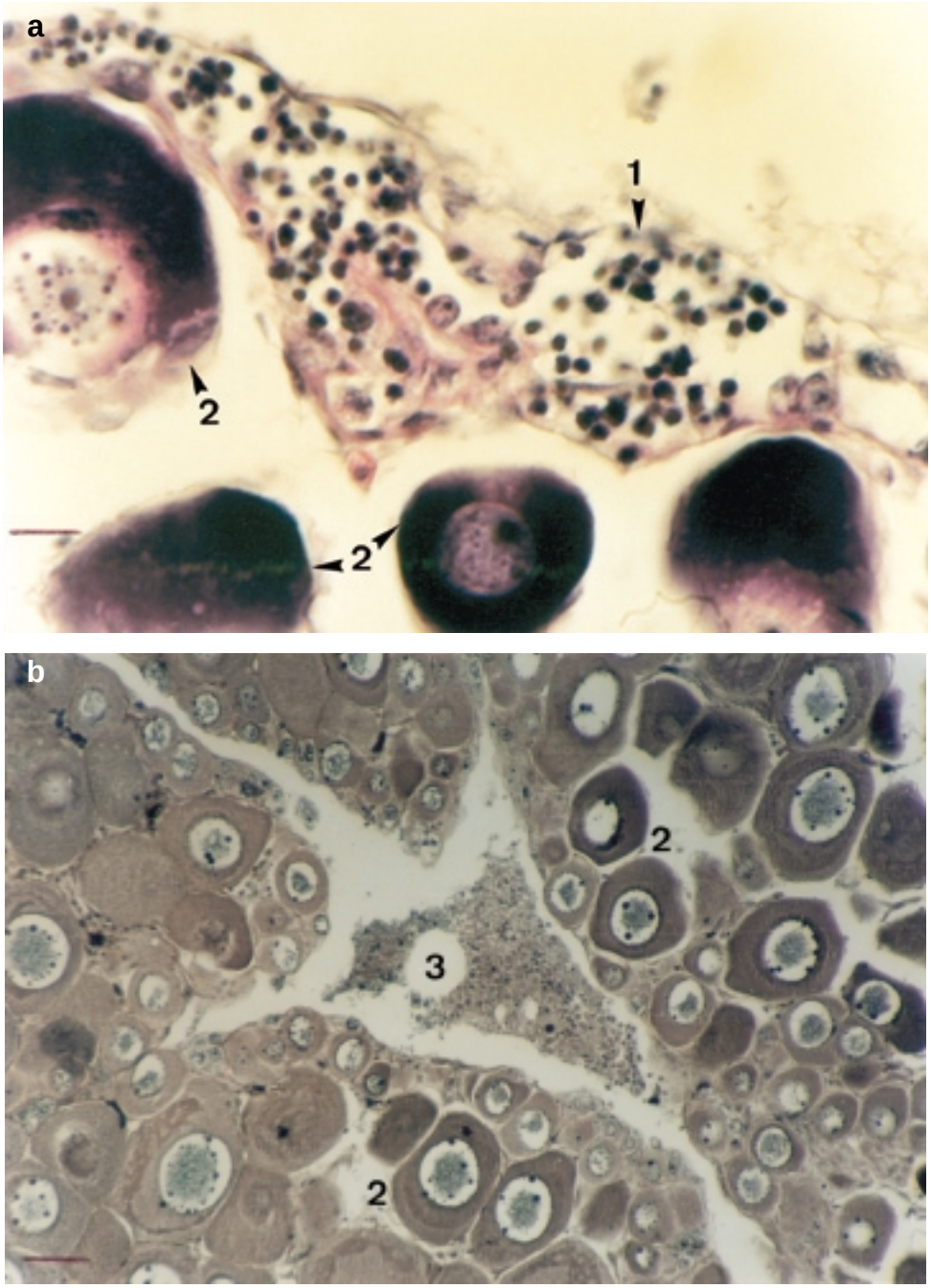


Fig. 5. Sections of developing ovaries of (a) 4-month-old (stained by alum hematoxylin, eosin; bar scale 50 µm) and (b) 8-month-old individuals (stained by iron hematoxylin; bar scale 250 µm). (1) degenerating male cells, (2) oocytes, (3) detritus in ovarian lumen.

Table 1. Size characteristics and the sex gland state in silver perch hatched in 1998 (the first progeny).

Age (months)	*Date*	*No. fish*	*Avg. length cm ± S.E. (range)*	*Avg. weight g ± S.E. (range)*	*Sex ratio, % (no.)*			*Composition of sex cells in gonad*
					male	*female*	*IG**	
1-1.5	28.08.98	6	5.1±0.67 (3.5-7.3)	2.2±0.81 (3.0-6.3)	0	0	100 (6)	Primordial germ cells (PGC), gonia
2-2.5	25.09.98	9	8.0±0.69 (5.2-10.5)	-	77.8 (7)	0	22.2 (2)	In males: spermatogonia, primary spermatocytes spermatids In IG: PGC, gonia
3-3.5	26.10.98	12	9.1±0.63 (6.8-12.5)	13.1±2.53 (4.1-28.7)	83.3 (10)	0	16.7 (2)	In males: cells of all stages of spermatogenesis In IG: gonia
4-4.5	26.11.98	11	11.7±0.85 (6.5-15.0)	21.4±4.60 (3.9-50.4)	90.9 (10)	0	9.1 (1)	Same as in 3-3.5-month-old fish
5-5.5	29.12.98	6	11.8±1.15 (8.0-15.0)	27.8±6.98 (7.3-52.1)	100 (6)	0	0	Cells of all spermatogenous stages
6-6.5	25.01.99	9	11.6±0.76 (8.2-14.7)	25.6±5.16 (7.1-50.4)	88.9 (8)	11.1 (1)	0	In males: cells of all spermatogenous stages In female: oogonia, oocytes of early stages of meiosis prophase, and oocytes of cytoplasmatic growth period
7-7.5	25.02.99	8	11.6±1.05 (8.2-16.0)	29.6±8.16 (8.9-66.5)	87.5 (7)	12.5 (1)	0	Same as in 6-6.5-month-old fish
9-9.5	26.04.99	15	12.4±0.62 (9.2-16.0)	33.9±4.98 (12.5-65.8)	100 (15)	0	0	Cells of all spermatogenous stages
10-10.5	25.05.99	8	13.3±0.67 (9.4-15.5)	41.2±4.48 (13.6-59.6)	87.5 (7)	12.5 (1)	0	Same as in 6-6.5-month-old fish
15-15.5	31.10.99	12	21.5±0.56 (17.5-24.5)	151.5±11.5 (84.2-215.4)	91.7 (11)	8.3 (1)	0	Same as in 6-6.5-month-old fish

* IG = indifferent gonad

Table 2. Size characteristics and the sex gland state in silver perch hatched in 1999 (the second progeny).

Age (months)	*Date*	*No. fish*	*Avg. length cm ± S.E. (range)*	*Avg. weight g ± S.E. (range)*	*Sex ratio, % (no.)*			*Composition of sex cells in gonad*
					male	*female*	*IG**	
1	13.06.99	14	1.7±0.07 (1.3-2.1)	0.07±0.09 (0.03-0.16)	0	0	100 (14)	Primordial germ cells (PGC), gonia
2	14.07.99	15	3.9±0.96 (3.4-4.7)	0.93±0.06 (0.61-1.50)	20 (3)	0	80 (12)	In males: spermatogonia (SG), primary spermatocytes (SC-I), spermatozoa (SZ) In IG: PGC, gonia
3	12.08.99	10	7.1±0.44 (5.2-9.5)	6.04±1.16 (1.85-11.72)	90 (9)	0	10 (1)	In males: SG, SC-I, secondary spermatocyte (SC-II), spermatid, SZ In IG: gonia
4	15.09.99	13	9.5±0.36 (6.7-11.3)	13.3±1.36 (4.3-21.06)	92.3 (12)	7.7 (1)	0	In males: Same as in 3-month-old fish In female: oogonia, oocytes of early stages of meiosis prophase and oocytes of cytoplasmatic growth period
6	14.11.99	14	10.5±0.76 (8.0-15.5)	19.6±4.42 (4.3-21.06)	92.9 (13)	7.1 (1)	0	In males: Same as in 3-month-old fish In female: Same as in 4-month-old female
8	17.01.00	10	11.7±0.97 (8.2-17.6)	29.8±8.36 (7.1-89.6)	50 (5)	50 (5)	0	In males: Same as in 3-month-old fish In females: Same as in 4-month-old female
9	14.02.00	11	11.7±0.77 (8.2-15.5)	25.8±5.57 (7.10-64.4)	81.8 (9)	18.2 (2)	0	In males: Same as in 3-month-old fish In females: Same as in 4-month-old female
10	15.03.00	10	12.1±0.86 (7.3-17.8)	27.7±6.75 (4.6-85.3)	80 (8)	20 (2)	0	In males: Same as in 3-month-old fish In females: Same as in 4-month-old female
11	12.04.00	15	14.5±1.41 (7.4-23.5)	62.7±17.7 (4.4-206.7)	86.7 (13)	13.3 (2)	0	In males: Same as in 3-month-old fish In females: Same as in 4-month-old female
18.5	30.11.00	34	16.1±0.80 (10.0-25.0)	87.6±13.43 (11.0-262)	76.5 (26)	23.5 (8)	0	Not examined histogically

IG = indifferent gonad

males which were not dependent on fish-rearing conditions. Additionally, the author noted a wide range of fish sizes. Other authors also reported a wide range of silver perch sizes in the course of their rearing (O'Sullivan, 1992; Rowland, 1994; Rowland et al., 1994). Size-age characteristics of silver perch reared under the conditions of the Dor Experimental Station are similar to those of fish reared in the different aquaculture conditions of Australia (O'Sullivan, 1992; Rowland et al., 1994, 1995; Gordon, 1995; Walker and Clymo, 1995). In our study, there was considerable variability in size characteristics, as was marked by the Australian scientists.

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