F.G.P. fluctuated within a narrow range until 2 years old (fig. 5). That is, the F.G.P. appeared first just after birth, and a rapid proliferation of them occurred at an early stage of life; they then remained constant in number through out life. The finding called to the author's minds the formation of the vascular network in cerebral tissue. According to Bär[8], and to Mato and Ookawara's[9] observations, the pial vessels elongated and penetrated into the cerebral cortex, and branched frequently for 3 weeks after birth. Further, it is also known that myelination of nerve fibers takes place at the maximal rate in the cerebral cortex of rats in the 3rd postnatal week.

Recently, the authors found a primitive form of F.G.P. adjacent to venules running in the vicinity of the cerebral surface, in rats just after birth. They contained a lot of fibrous bundles and immature inclusion bodies. It is postulated that the primitive F.G.P. are able to migrate along vascular walls by their own motility (unpublished data).

In SHR-SP rats, as reported elsewhere[7], a small number of degenerating F.G.P. were recognized under the electron microscope. However, as shown in the table, the total of F.G.P. in SHR-SP did not deviate much from the mean value for contraol Wistar rats. In other words, the number of F.G.P. kept a constant level not depending on species, age or food, and was unaffected by some pathological conditions; a loss of F.G.P. seemed to be compensated by a temporal proliferation of F.G.P. Further, these schemes also demonstrated regional differences in the numbers F.G.P. The numerical density of F.G.P. was high in cerebral cortex and thalamus, moderate in hippocampus and amygdaloid nucleus, and low in the corpus callosum and internal capsule. Such regional differences in F.G.P. might reflect the differences in vasculature, function and metabolism between the cerebral regions. The production of metabolic wastes was estimated to be more vigorous in gray matter than in white matter. It has not yet been determined whether the number of F.G.P. is enough for the segregation and digestion of waste products in the central nervous system or not.

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Electron microscopical studies of gonads in primary and secondary males of protogynous hermaphroditic fish *Coris julis* L. (Labridae, Teleostei)[1]

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Summary. Electron microscopical examinations of *Coris julis* (L.) showed that Leydig cells are definitely present in the gonads of primary, as well as of secondary males. During sex change the Leydig cells develop from the remnants of the ovary in the newly organized testes.

The sex of most vertebrates remains constant throughout their life (gonochoristic). However in the teleosts, especially of the order perciformes a number of species exists in which 2 types of hermaphroditism can be distinguished.

1. True (simultaneous or synchronous) hermaphroditism. Here male or female gonadal tissue is present and often eggs and spermatozoa reach maturity at the same time. Sex cells are released through different channels[3-5].

2. Spontaneous (protogynous or protandrous) hermaphroditism. The animals start their life either as males and change to females (protandrous hermaphroditism) or as females and change into males (protogynous hermaphroditism)[6-13]. In some protogynous species 2 different kinds of males occur. The first are born as males (primary males), the other develop into secondary males from females (diandrie; example, *Coris julis* L.). The 2 types of *Coris julis* male differ from each other in color and in the shape of the dorsal fins[14-20].

The primary males are identical in color to the females and are thus indistinguishable from them. 70% of the animals with smooth color are females and only 30% primary males. The number of secondary males is smaller, about 8% of the whole population. The color-difference between secondary males on one side and primary males and females on the other side is a result of sex-inversion from female to secondary male. This process is correlated to some extent with gonadal change from females to males.

Chromosomal studies have shown that there are 2 types of female in *Coris julis*. One is relatively rare and remains female throughout its life. The other undergoes sex change, as an adult, into a secondary male with the characteristic color and fin pattern[20,21].

In our previous investigations, somatic cells of *Coris julis* gonads of 1 primary and 1 secondary male were found to be H-Y antigen positive. Two females were H-Y antigen negative. From these results it seems that H-Y antigen negative somatic cells of the female gonads become H-Y antigen positive during sex inversion[22].

The sex-reversal normally starts from a relatively large ovary, continues by ovarian degeneration and ends with formation of a testis. In most females, clusters of male germinal cells are located peripherally within the ovary. The testicular tissue develops from the region where these clusters are located, after degeneration of the ovaries[14,17,19]. Reinboth in his light microscopical studies could not find any Leydig cells in testes of primary and secondary males[15].

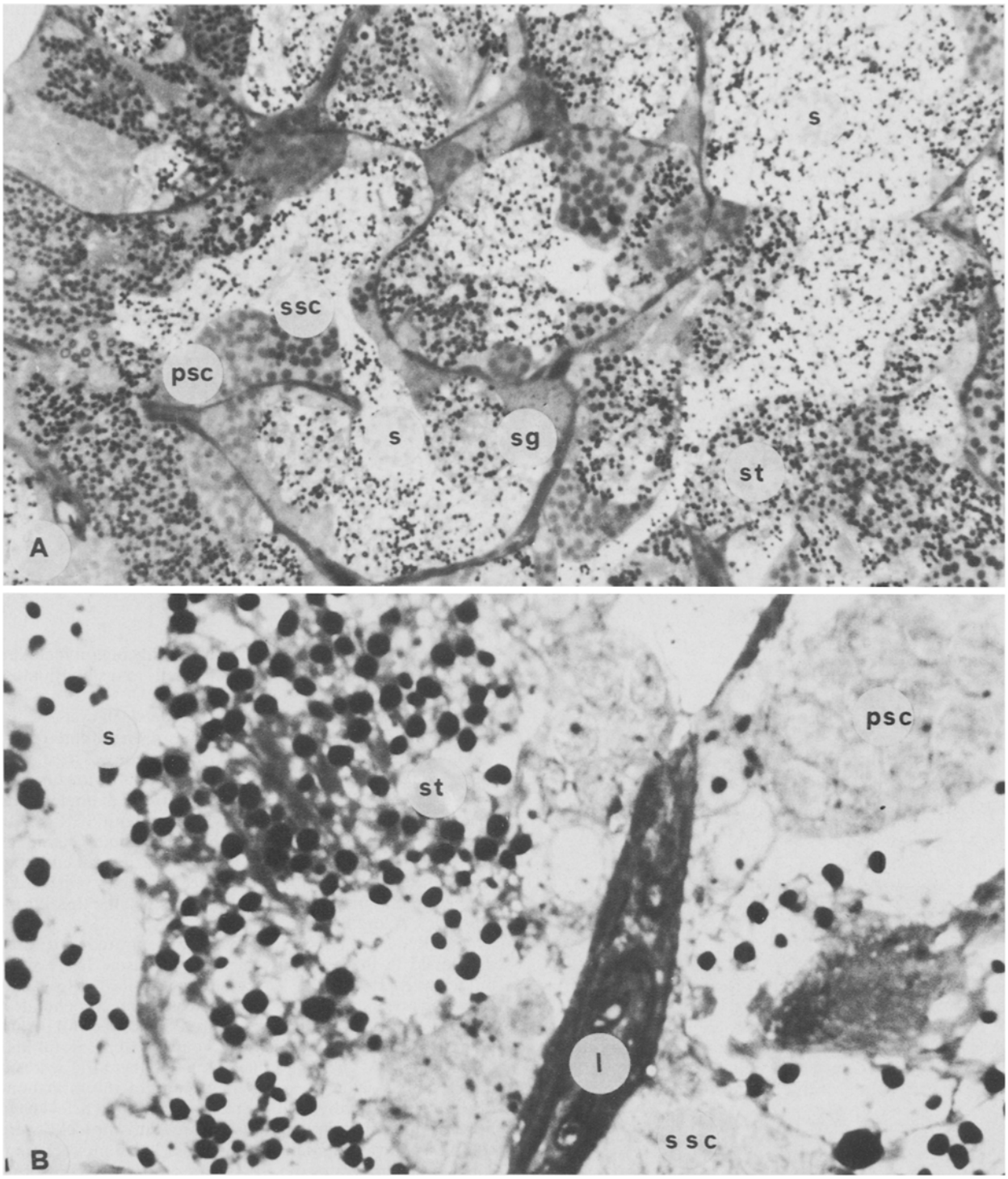
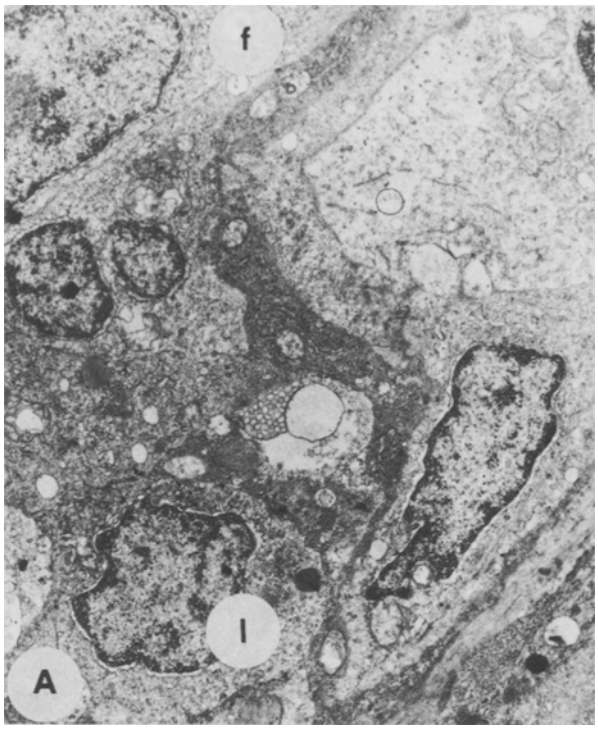
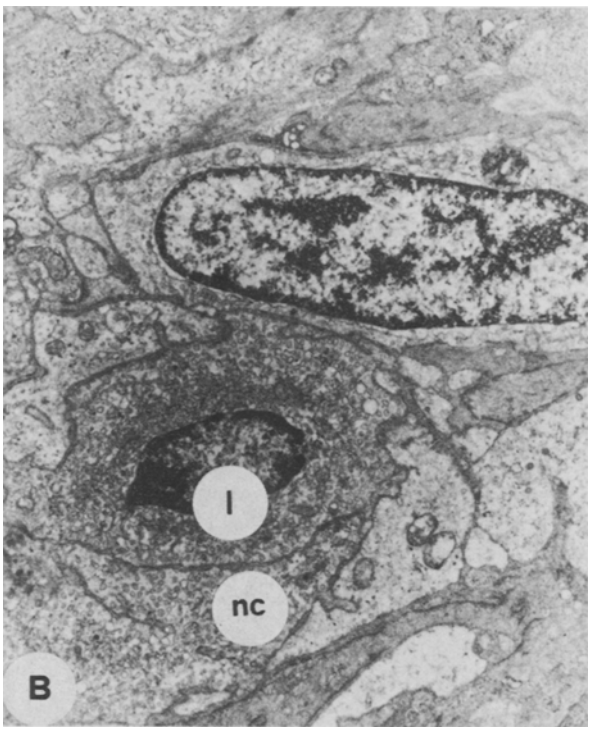


Figure 1. *Coris julis (A, B):* section through the gonad of a primary male. Spermatogonia (sg), primary spermatocytes (psc), secondary spermatocytes (ssc), spermatids (st), spermatozoa (s), Leydig cells (l). Magnification A × 500, B × 2000.

However, this type of cell is necessary for the production of sex steroids. Therefore, this study was undertaken to show that there are indeed Leydig cells in the male gonads.

Material and methods. Five primary and 5 secondary males of *Coris julis* (L.) were caught, and their sex determined by dissection, during the summers of 1981 and 1982 at Elba (Italy). Pieces of the gonads (about 1 mm^3) were fixed in 3% glutaraldehyde (Serva, Heidelberg) and stored at 4 °C. For electron microscopical studies these tissues were washed in PBS (pH 7.5) and postfixed for 1.5 h at 4 °C in 2% osmiumtetroxide (Johnson-Matthey, London). After washing again in PBS, they were dehydrated in graded ethanol solutions (70%–95%–100%), cleared in propylenoxide (Merck) and impregnated with Epon/propylenoxide prior to being embedded in Epon (Merck). Sections were cut, using a LKB-ultrotome III, stained for 5 min with 5% uranyl





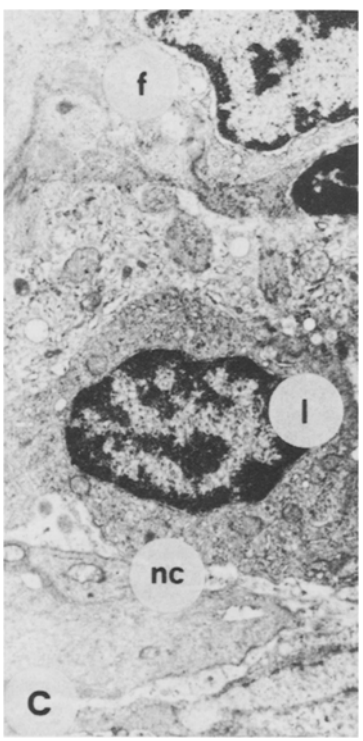


Figure 2. *Coris julis (A, B, C):* interstitial tissue of a secondary male with Leydig cells (l), fibroblasts (f), nerv cells (nc). Magnification A, B, C × 7740.

acetate (Merck) and 1 min with lead citrate (Merck). Examinations were made using a Philips 300 electron microscope.

Results. All gonads of primary and secondary males examined by electron microscopy contained cysts with germinal cells in different stages of development; spermatogonia, spermatocytes, spermatids and mature spermatozoa (fig. 1, A and B).

Leydig cells were normally concentrated in the triangular region formed by the juxtaposition of lobules, cysts and sperm ducts. Other cell types present in this region included fibroblasts, macrophages, pigmented cells and smooth muscle cells (fig. 2, A–C).

Leydig cells were mostly concentrated between cysts, which contain mature spermatids. There was a direct connection to blood vessels (gonadotrophic stimulation). These cells were ovoid to cubic in shape, up to about 10 µm in size, with a rather large nucleus, about 2 µm in diameter. The major portion of the cytoplasm was filled with SER, small, round to ovoid, secretory granules and tubular elements. Further, the cytoplasm of the Leydig cells contained many free ribisomes, polysomes, glycogen particles, lipofuscin pigment inclusions, Golgi-zones and often some lipid droplets (fig. 3, A and B).

The SER of the Leydig cells was concentrated and most developed in the periphery of the cytoplasm. There were only few RER vesicles. The cysternae of the Golgi-zones were mostly located near the nucleus. The mitochondria profiles of the Leydig cells measured up to about 4 µm in length. The cristae of the mitochondria were of tubular shape (fig. 3, A and B). Some tubuli of the SER were in direct contact with the outer membrane of the mitochondria. In some Leydig cells there were only few or even no lipid droplets, which probably means that the cells were completely differentiated and ready for hormone secretion. They were innervated and therefore under the control of the CNS, with respect to hormonal regulation. These characteristics indicate that the Leydig cells of *Coris julis* (L.). represent cells producing steroids.

Discussion. Before the sex-reversal-process begins, from female to male or male to female, germinal cells of the opposite sex are found in the gonads. In protandrous species such as *Gonostoma gracile, Cobitis taenia* and protogynous species such as *Thalossoma bifasciatum* and *Coris julis* the germ cells of the opposite sex are always easily found[16]. In protandrous species the gonadal tissues of both sexes occur separately within the gonads, but in protogynous species they are interspersed with each other. These and other distinctions observed in gonadal structure suggest

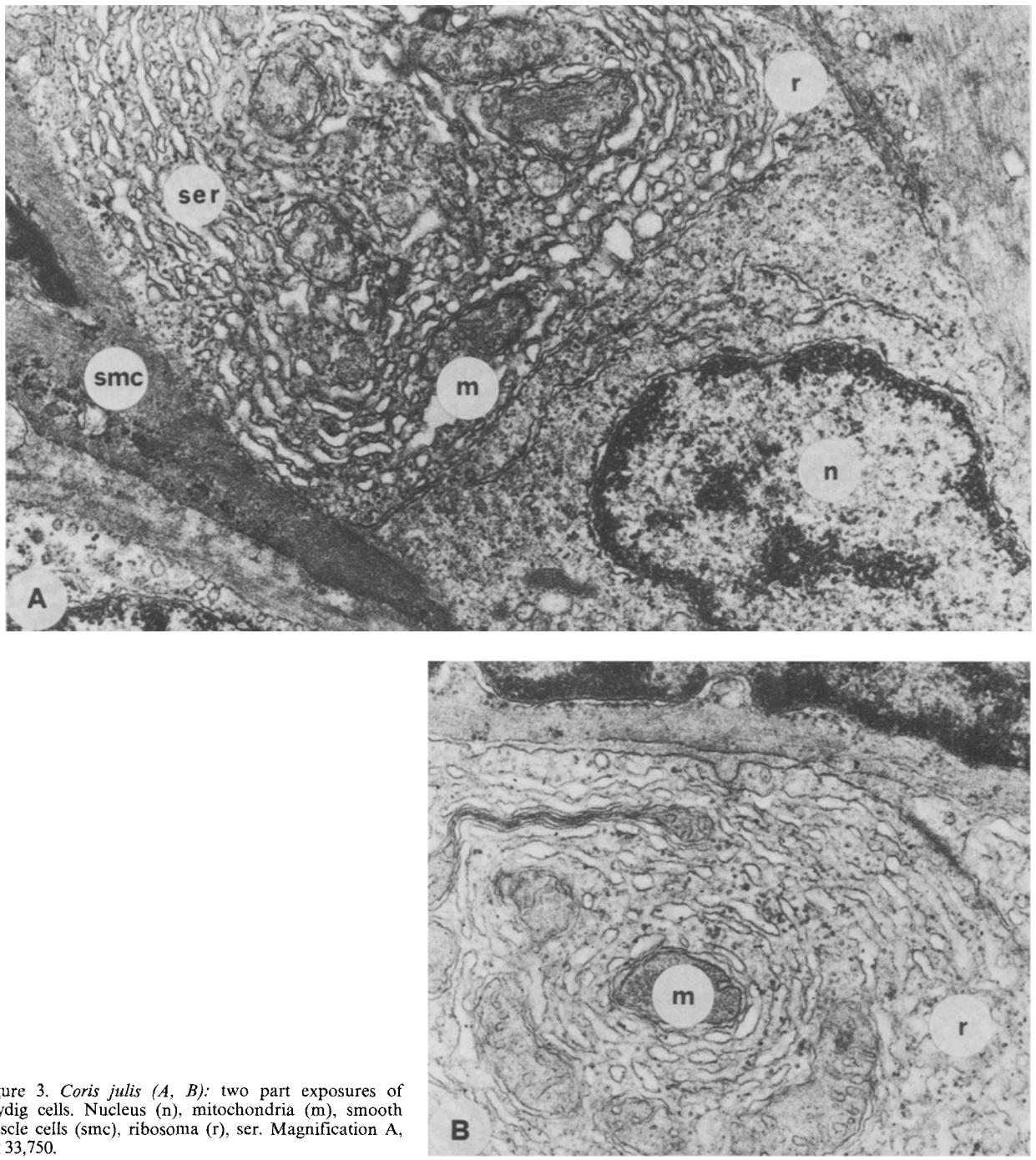


Figure 3. *Coris julis (A, B):* two part exposures of Leydig cells. Nucleus (n), mitochondria (m), smooth muscle cells (smc), ribosoma (r), ser. Magnification A, B × 33,750.

that hermaphroditism occurring in different groups of Teleost fishes is evolutionarily independent.

The most important functions of the gonads are the production of mature gametes and secretion of sex-hormones. Study of the gonad-maturation aspect, as well as the hormone secretion in the gonads, revealed that the gonads of the gonochoristic and the hermaphroditic fish look alike[23].

The Leydig cells play a primary role in synthesis of androgenous hormones in gonochoristic fish[24–33]. However, in hermaphroditic fish the precise function of the gonads, especially with regard to sex steroids, in the beginning and during sex-reversal, is not well documented to date.

Experimental treatment with testosterone of *Anthias squamipinnis, Coris julis, Thalossoma bifasciatum* and *Halichoeres bivittatus* can produce a sex change, whereas administration of testosterone to *Monopterus albus* does not produce sex reversal[10,34–38].

Tang et al.[39] showed that in *Monopterus albus,* the interstitial cells develop before any other testicular tissue. Changes in the endocrine secretion of the gonads probably initiate natural sex-reversal. A favorable environment may promote the development of Leydig cells and maturation of germ cells of the opposite sex.

Our electron microscopical examinations show that the Leydig cells are definitely present in the gonads of primary

as well as of secondary males. They are located between triangular, interglobular spaces and mainly characterized by a rather large nucleus, well developed SER and many free ribosomes, as well as mitochondria with tubular cristae. A high heterochromatin content probably charactizes the cells in the quiescent phase and the high euchromatin concentration high-lights hormone synthesis. Some Leydig cells contain a few lipid droplets and poorly developed SER. These can be interpreted as being developing but not yet fully differentiated cells.

During sex change, the inactive germ cells are located peripherally in the ovary and develop into male germ cells in the newly organized testes. These germ cells are stimulated to grow and develop into mature spermatocytes. Probably before this, the other male gonadal specific cells, such as Leydig and sertoli cells, develop from the remnants of the ovary, and the external appearance changes; the fish develops the characteristic color and fin pattern.

The effect of androgenous hormones on the color and shape of the dorsal fin (elongated fin rays), as an accompanying phenomenon of the sex change from female to secondary male, would suggest that these are secondary sex characteristics. But the fact that there are no exterior differences between females and primary males seems to deny this suggestion. Influence of testosterone on the external morphology of the males may not be a direct one. Additional factors are probably involved in the development of the 'secondary' characteristics in secondary males.

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Effect of sublethal concentrations of sumithion on limb regeneration of fresh water field crab *Oziotelphusa senex senex*

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Summary. The initiation and progress of regeneration following the removal of the left 4th walking leg were altered in the crab *(Oziotelphusa senex senex)* by exposure to sumithion. Depending on the concentration used, sumithion caused a complete inhibition of regeneration, a delay of initiation of limb bud development or a reduction of limb bud growth rate. Crustacean limb regenration can also be used as a sensitive bioassay for studying the effects of environmental pollutants.

Sumithion, an organophosphorus pesticide, which is widely used in this area to control the rice stem borer *Tryporhyza sps.* is known to pollute the aquatic environment. Not much attention has been given until now to the consequences of sumithion in non-pest aspects of the ecosystem. Determination of acute toxicity levels has little relevance in the estimation of ecologic consequences. There is little data available on toxic effects of sublethal concentrations, possibly because no standardized tests exist. A sensitive parameter would be crustacean limb regeneration. This has already been applied for measuring sublethal effects on fiddler crabs[2-4] and shrimps[5].

We have tested the effects of sumithion (fenitrothion; O-O-dimethyl-O-(3 methyl-4-nitrophenyl) phosphorothioate) on limb regeneration of *Oziotelphusa senex senex* at concentrations of 0.01-0.1 mg/l.