PITUITARY GLAND, GONADS AND INTERRENAL GLAND OF THE IMMATURE "PACU" *Piaractus mesopotamicus* HOLMBERG, 1887 (TELEOST, CHARACIDAE): MORPHOLOGICAL STUDY

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ABSTRACT

The gonads, the interrenal tissue and the pituitary gland of the immature "pacu", a teleost fish with great economic importance in South America, were studied using histological and histochemical techniques under light microscopic level. The male gonad presented dense conjuntive tissue, spermatogonia and Sertoli cells. Leydig cells were not observed. The ovary presented oogonia, chromatin-nucleolar and initial perinuclear oocytes. The interrenal tissue was found in the head kidney composing the wall of the main posterior cardinal vein branches. The chromaffin tissue was also found to be arranged in small clusters or isolated cells between the interrenal tissue. In the adenohypophysis the adrenocorticotropic-like cells and the acidophilic prolactin-like cells were found in the rostral pars distalis (RPD), both of them negative to Periodic Acid Shiff (PAS) and Alcian Blue (AB) histochemical methods. The first one type was arranged in cords which bordered the major neurohypophysial branches of the RPD. In the proximal pars distalis (PPD), two cianophylic cell types, the gonadotropic-like and the thyrotropic-like cells, were positive to PAS and AB. An acidophilic cell type, somatotropin-like, was found among the basophils in the PPD. In the pars intermedia (PI) were observed two acidophylic cellular types of different sizes, both negative to PAS and AB.

Key words: Immature "pacu" *(Piaractus mesopotamicus)*; Teleost; Pituitary Gland; Gonads, Interrenal Gland; Histology; Histochemistry.

RESUMO

HIPÓFISE, GÔNADAS E GLÂNDULA INTERRENAL DE PACU IMATURO *Piaractus*

mesopotamicus HOLMBERG, 1887 (TELEOSTEO, CHARACIDAE): ESTUDO MORFOLÓGICO.

As gônadas, o tecido interrenal e a hipófise de pacu, peixe teleósteo de importância econômica na América do Sul, foram estudados em animais imaturos utilizando técnicas citológicas e citoquímicas, sob microscopia de luz. O testículo apresentou tecido conjuntivo denso, espermatogônias e células de Sertoli, não sendo verificadas células de Leydig. Os tipos celulares germinativos observados no ovário foram oogônias, oócitos cromatina-nucleares e oócitos perinucleolares iniciais. O tecido interrenal foi encontrado no primeiro terço do rim cefálico, compondo a parede dos principais ramos das veias cardinais posteriores. O tecido cromafim foi observado em pequenos grupos ou células isoladas entre o tecido interrenal. Na adeno-hipófise, células levemente basófilas, semelhantes às adrenocorticotrópicas de outros peixes, e células acidofílicas, semelhantes às células prolactínicas, foram observadas na "rostral pars distalis" (RPD), ambas negativas aos métodos citoquímicos do ácido periódico-Schiff (PAS) e Azul de Alcião (AB). As primeiras apresentava-se dispostas em cordões ao redor dos grandes ramos da neuro-hipófise presentes nesta região. Na "proximal pars distalis" (PPD), foram observados dois tipos celulares cianofílicos, positivos ao PAS e AB, assemelhando-se às células gonadotrópicas e tireotrópicas da maioria dos teleósteos. Um tipo celular semelhante às células somatotrópicas foi observado entre as células basófilas na PPD. Na "pars intermedia" (PI), foram verificados dois tipos celulares cianofílicos de tamanhos diferentes, ambos negativos ao PAS e AB.

Palavras-chave: Pacu imaturo; *Piaractus mesopotamicus*; Teleósteo; Hipófise; Gônadas, Glândula Interrenal; Histologia; Histoquímica.

INTRODUCTION

One of the main differences between adult and juvenile fishes is the funcional state of the endocrine system as in mammalians and other vertebrate. To understand the endocrine regulation of the reproduction, it is necessary to have a morphological basis of the organs envolved in all the process, as the glands and gonads of mature and immature individuals. The "pacu" *Piaractus mesopotamicus* is a South American fresh-water teleost that naturally lives in the rivers of Pantanal Matogrossense complex at Central Brazil, and in Paraná basin complex, in the Southeast. In nature, this fish migrates upstream for reproduction, and in captivity, the spawn does not occur without hormonal induction.

Recently we have shown the cells of the pituitary gland of adult "pacu" by histochemical and immunohistochemical methods (Borella et al., 1997), and described the interrenal gland (Gazola et al., 1995) and the chromaffin

cells (Borges et al., 1996). We have also reported the plasma level of gonadal steroids and corticosteroids in female and male adult "pacu" (Gazola et al., 1996; Gazola & Borella, 1997), and the primary structure of the GnRH of this specie was determined (Powell et al., 1997). As for the head kidney of South American fishes, there are few researches, despite the great number of species and their importance as knowledge and economical features. Gazola et al. (1995) studied the distribution and microscopic structure of interrenal gland of "pacu" during the reproductive cycle, but these authors did not compare mature and immature fishes.

Considering the scarcity of histophysiological information about juvenile individual of this species, the purpose of the present study is to describe important organs related to their reproductive physiology, namely gonads, pituitary and head kidney at light microscopical level. It could provide morphological basis to understand differences between juvenile and adult individuals, improving with the knowledge on reproductive endocrine aspects of South American freshwater teleosts.

MATERIAL AND METHOD

For this study we used immature male and female "pacu" *Piaractus mesopotamicus* Holmberg, 1887, less than one year old (n = 30), weigthing 200-400 g (males) and 180-400 g (females). The fishes were grown in captivity at Centro Nacional de Pesquisa de Peixes Tropicais - CEPTA-IBAMA (National Center for Research of Tropical Fish), in Pirassununga, SP, Brazil (subtropical zone, 22° 02' S, 47° 30' W). The fish were captured, desensibilized in cold water for 20 minutes, weighed and sacrificed by decapitation.

After sacrifice, the gonads, the pituitary gland and the cephalic kidney were carefully dissected and fixed in Bouin's solution for 18 h at room temperature. The organs were rinsed, dehydrated and embedded in paraffin or hydroxyethyl methacrylate (Historesin). Sections (5 or 2 mm) were obtained from each organ and stained with Hematoxylin-Eosin (HE) and Mallory's Trichrome (MT). For the pituitary gland, the histochemical methods of Periodic Acid-Schiff (PAS) for neutral polissacharids and Alcian Blue pH 2.5 (AB) for acid groups were employed.

RESULTS

Gonads
Females

The ovaries of all fishes collected were small elongated flat paired organs, poorly vascularized, and with a translucent light pink coloration. The main type of cells were oogonias and oocytes in early maturation (Fig. 1). The oogonia were relatively large and round cells, with a spherical central nucleus of euchromatin, isolated or arranged in small nests (Fig. 1). The chromatin-nucleolus oocyte is a little smaller than the oogonia and presented a large pale-staining central nucleus containing a prominent nucleolus (Fig. 1). For these two cell types, the cytoplasm was scarce, showing a weak basophilia. The perinucleolar initial oocytes were larger than the former, with an intense basophylic cytoplasm and a large round nucleus with various peripherical nucleolus (Fig. 1). A thin layer of follicular cells can be seen involving the oocytes more developed (perinucleolar oocytes). These cells have flattened nucleus and scarce cytoplasm (Fig. 1).

Males

The testes were pink elongated flat paired organs, poorly vascularized. At light microscopy, they were composed by interstitial and primitive lobular compartments. The interstitium between lobules consisted of a well developed connective tissue, with abundant collagen fibers, fibroblasts and blood vessels (Fig. 2). The lobular component contained two cell types: germ and somatic cells. The germ cells were composed by only one type of spermatogonia, the type I. They were relatively large cells with pale basophilic cytoplasm and a round central nucleus with one central and prominent nucleolus. The condensed chromatin is arranged in radial filaments and accumulations on the periphery of the nucleus (Fig. 2). They were seen either isolated or in small clusters of three or four cells near the lobular lumen. The somatic or Sertolli cells (Fig. 2) were found lining the periphery of the lobule, and close to the spermatogonia. Interstitial (Leydig) cells were not observed.

Interrenal tissue

The interrenal tissue was located in the most anterior part of the kidney, named head kidney The head kidney presented one central region and two lateral lobes, rigth and left, located ventrolaterally to spinal cord, near the 2$^{nd.}$ 3st and 4th vertebra. They were composed by hemocytopoietic tissue, interrenal tissue and cromaffin tissue (Fig. 3 and 4).

The interrenal tissue was located around the principal branches of cardinal posterior veins, as a perivascular sheet (Fig. 3). The cells of this tissue were dispersed, arranged in small groups or isolated in a conective tissue layer, separated from the inner part of the vein wall by a layer of endothelial cells (Fig. 3). There were many colagen fibers of the conective tissue between the interrenal cells (Fig. 3). The cells were polyhedric in shape, with light acidophilic cytoplasm when stained by HE. The central nucleus was spherical

with clumps of heterochromatin and one prominent nucleolus.

The cromaffin cells, usually associated with the interrenal cells, were disposed in groups of one, two or three cells, near the endothelium of the vein wall. The cromaffin cells were bigger than the interrenal cells, globous in shape with a clear cytoplasm lightly acidophilic when stained by HE. The large nucleus was central, spherical and composed by euchromatin (Fig. 4).

PLATE 1

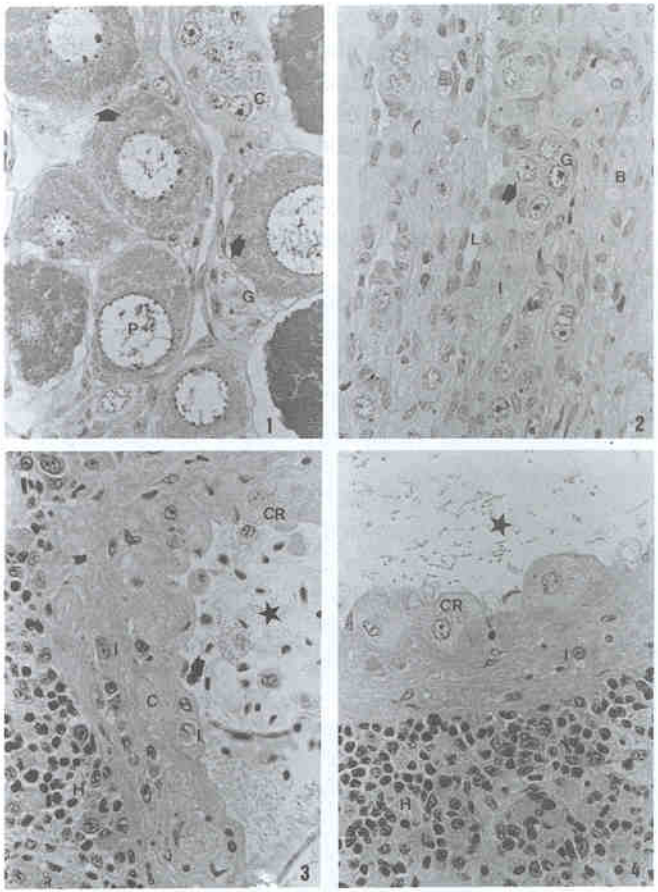

Fig. 1 - Ovary of immature "pacu". Oogonia (G); chromatin-nucleolus oocyte (C), perinucleolar initial oocyte (P), follicular cell (arrow). 1320x. HE.

Fig. 2 - Testis of immature "pacu". Lobule (L), interstitium of connective tissue (I), blood vessel (B), type I spermatogonia (G); Sertolli cell (arrow). 1320x. HE.

Fig. 3 - Head kidney of immature "pacu". Interrenal cell (I), connective tissue (C), cromaffin tissue (CR); endothelial cell (arrow), lumen of the vein (star), hemocitopoietic tissue (H). 1320x. HE.

Fig. 4. Head kidney of immature "pacu". Cromaffin cell (CR), interrenal cell (I), lumen of the vein (star), hemocitopoietic tissue (H). 1320x. HE.

Pituitary gland: general morphology

The pituitary gland of *Piaractus mesopotamicus* consisted of neurohypophysis and adenohypophysis. The latter was divided into three segments: rostral pars distalis (RPD), anterior, proximal pars distalis (PPD), median, and pars intermedia (PI), posterior. The numerous processes of neurohypophysis, which penetrate the pituitary gland cranially by means of a stalk of nervous tissue, were interdigitated and differently distributed among the three adenohypophysial regions. In the RPD the interdigitations were large and had few branches, and as it penetrates the PPD the branches appeared thinner and closer to the cells. In the PI, the neurohypophysis occupies almost the totality of the region, arranged in smaller branches between the cells.

Cell types of the Adenohypophysis: Histological and Histochemical Reactions

Rostral Pars Distalis (RPD)

Acidophilic Prolactin (PRL) - like cells

These acidophilic cells were polyhedric in shape and occupied the major part of the RPD. These cells were histologically differentiated by their light red staining with HE and purple with MT staining. The nucleus was generaly spherical and central. They were negative for PAS and AB (Fig. 5).

Cyanophilic Adrenocorticotropic (ACTH) - like cells

They were prominent light blue stained cells, arranged in one or more layers of cell cords bordering the neurohypophysial projections in the RPD. Most of the ACTH cells were polyhedric or prismatic in shape with a round nucleus of euchromathin and a prominent nucleolus. The fine granules were negative to PAS and AB methods (Fig. 5).

Proximal Pars Distalis (PPD)

Acidophilic Somatotropin (STH) - like cells

These acidophilic cells were restricted to PPD, occupying the major part of this region, located adjacent to the neural projections or arranged in cords (Fig. 6 and 7). They were polyhedric or elongated in shape and stained red with eosin (HE) and orange G (MT). The nucleus is round and central or eccentric, turned to the basal portion when the cell was adjacent to a branch of neurohypophysis (Fig. 6). These cells were negative to PAS and AB methods.

Cyanophilic Gonadotropic (GTH) - like cells

These cells were gathered between the acidophilic cells of the PPD, mainly in the lateral and ventral parts of this region. They were large, round in

shape; the cytoplasm showed light cyanophilic granules and the round nucleus was eccentric (Fig. 6 and 7). This cell type was PAS and AB positive.

Cyanophilic Thyrotropic (TSH) - like cells

This cell type was restricted to the dorsal and lateral parts of the PPD, in the middle of the gland. They were fusiform in shape, with cyanophilic cytoplasm and eccentric nuclei. They reacted positively with PAS and AB. The TSH-like cells were rarely seen under light microscopy, due to their relatively small size and quantity, and the scattered distribution among the other cells in PPD (Fig. 7).

Pars Intermedia (PI)

Two cell types were found in the PI differing only by the size (Fig. 8). They were polyhedric in shape, weakly stained, and showed no positivity to any employed histochemical methods.

PLATE 1

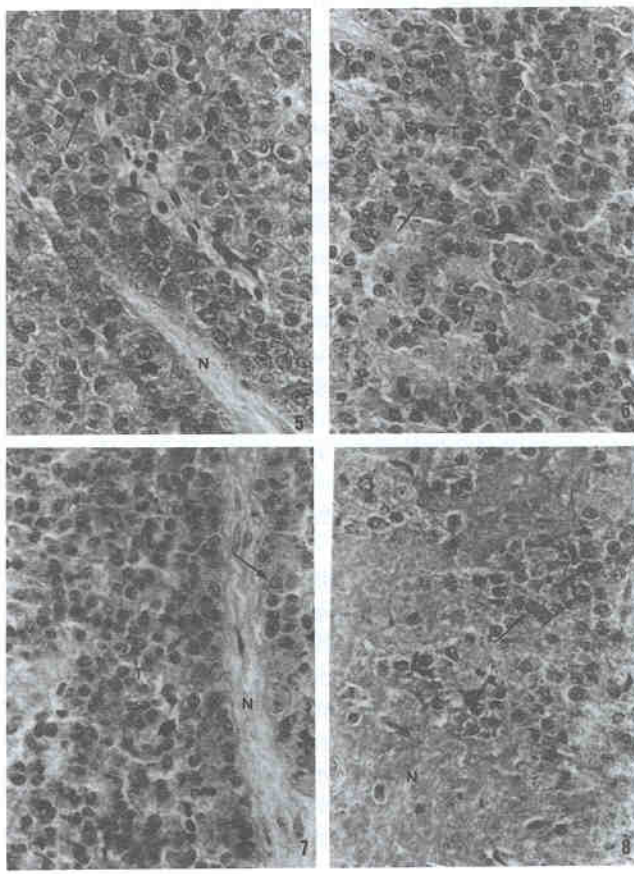

Fig. 5 - Pituitary gland of immature "pacu". Rostral Pars Distalis (RPD). Acidophilic prolactin (PRL) -like cell (long arrow), basophilic adrenocorticotropic (ACTH) -like cell (short arrow), neurohypophysial branch (N). 1320x. HE.

Fig. 6 - Pituitary gland of immature "pacu". Proximal Pars Distalis (PPD). Acidophilic somatotropic (STH) -like cell (long arrow), basophilic gonadotropic (GTH) -like cell (short arrow), basophilic thyrotropic (TSH) -like cell (T). 1320x. HE.

Fig. 7 - Pituitary gland of immature "pacu". Proximal Pars Distalis (PPD). Acidophilic somatotropic (STH) -like cell (long arrow), basophilic gonadotropic (GTH) -like cell (short arrow), basophilic thyrotropic (TSH) -like cell (T), neurohypophysial branch (N). 1320x. HE.

Fig. 8 - Pituitary gland of immature "pacu". Pars Intermedia (PI). Two acidophilic cell types of different size (arrow), neurohypophysial branches (N). 1320x. HE.

DISCUSSION

The bulk of scientific papers about fish reproduction concerns mature animals, and information about juvenil and/or immature individual who could contribute to the understanding of posterior events is still missing. Transitions along the reproductive life normally can not be followed, so important events that contribute to the recruitment of new groups into reproductive phase cannot be used as basic information and useful tools in aquacultural practices. As for South American freswater species, there are particularly few papers. In this study, we describe some histological features of organs that have endocrine functions, the pituitary, the gonads and interrenal gland of immature "pacu", comparing them with similar studies done on this or other teleost species.

In the current study, the cell types described for the ovaries of immature fish are similar to those observed in the adult "pacu" during the inithial phases of reproductive cycle and classified as cystovarian type (Lima et al., 1991). The inithial cells of the germinative lineage, oogonia, chromatin nucleolus oocyte and initial perinucleolar oocyte showed in the immature ovary the same morphological features described for resting phase ovaries of the adult. These cells are maintained in these stages waiting for the recruitment triggered by hormonal action.

According Romagosa et al. (1993), the testes of "pacu" can be included in the first classification standard described by Grier & Linton (1980), the "tubular unrestrict", when the spermatogonia are distributed along all the seminifer tubule. The cell types observed in the male gonad of immature "pacu" are adequate with the initial stage of development of the testes, and predominates mainly in immature individual and mature animals in the resting phase of their reproductive cycle,even though they could occur during all the sexual cycle (Romagosa et al., 1993; Borges Filho, 1987). The absence of Leydig cells in the analyzed sections suggests the immature status of the

fish, considering the endocrine reproductive function of this cell.

Fishes do not have typic adrenal gland as tetrapods. Two homologue tissues are found in the head kidney, medullar and cortical. The cortical portion is named interrenal tissue or interrenal gland, and the medullar cromaffim tissue. The head kidney, where these tissues are found in teleosts, is composed mainly of hemocytopoietic, linfoid tissue, and some thyroid follicles (Matty, 1985; Jones & Phillips, 1986).

The head kidney evolves from the following embryonic sources: the nephron, hematopoietic tissue, and interrenal cells originate from mesoderm, whereas the chromaffin cells originate from ganglion cells of neuro-ectoderm that migrate from the neural crest (Jones & Phillips, 1986).

The interrenal gland of teleost is homologous to the mammalian adrenal cortex and is the source of cortical steroids. Several papers have been published on the head kidney of fishes. The earliest ones were reported by Nandi (1962, 1965) based in the morphology of head kidney of some cyprinidae, labridae and scaridae fishes. Others researchers reported the significance of the interrenal gland in head kidney of fishes, in the adaptation to stressors (Balm et al., 1995). In South American fishes, however, there are few researches on head kidney, despite the great number of species and their importance as knowlege and economic features (Farias et al., 1989; Gazola et al., 1995; Borges et al., 1996).

The amount of interrenal tissue observed in the head kidney of fishes vary among species. In *Puntius phutunio* this tissue is scarce, ocurring as a unique cell layer surrounding the principal branches of the posterior cardinal veins (Nandi, 1965). On the other hand, in *Heteropneustes fossilis* this tissue is well developed, occupying three thirds of the head kidney, being a kind of gland surrounded by hemocytopoietic tissue (Banerji, 1971).

According to Robertson & Wexler (1960), the morphological changes observed in the interrenal cells during the reproductive period are strongest in migratory fishes. Gazola et al. (1995) do not observe hypertrophy or hyperplasy of interrenal gland in adult female "pacu" during all the phases of the reproductive cycle, but putative variations of the cytoplasmic acidophilia and vacuolization. They worked with migratory adult female reared in captivity and submitted to hormonal indution to spaw.

The portion of interrenal tissue observed in immature "pacu" do not presente the extreme features and variations observed among the adult fish studied. In some cardinal vein branches isolated or small groups of interrenal cells were detected sparcelly distributed in the connective tissue. The interrenal cells from the adult "pacu" are more acidophilic and more vacuolizated, depending on the gonadal stage of the fish. The connective tissue is abundant in the immature, but not in the adult "pacu".

The identification and the distribution of different cell types in the pituitary

gland of nonmammalian vertebrates have been established by histochemical, cytophysiological, and immunocytochemical techniques (Fantodji et al., 1990; Joss et al., 1990; Koide et al., 1992; Borella et al., 1997), providing information to detect the cytological origin of the various pituitary hormones.

The teleost pituitary gland is located in the diencephalic basis, behind the optic chiasm, and can be anatomically (1) linked to this encephalic region through a nervous peduncle, a feature common to the majority of fish species or (2) linked directly to the diencephalic floor, as observed in primitive species (Rao, 1969).

The "pacu" pituitary presents a morphology similar to phylogenetically more evoluted teleosts. A hypophysary peduncle can be seen from the diencefalic basis penetrating in the gland. This feature was used by Val-Sella et al. (1986) to classify the hypophysis of immature "pacu" as "dorso-leptobasic" type, according to Duyvené de Wit apud Val- Sella et al. (1986). In the immature "pacu", as in the majority of studied teleost, two distinct portions can be microscopically identified, the glandular adenohypophysis and the neurohypophysis, composed by nervous tissue. The neurohypophysis penetrates and interdigitates through branches inside all the glandular portion. In this part of the hypophysis, the adenohypophysis, three regions can be identified, considering differences among morphological features and tintorial affinities of the cells: the "rostral pars distalis" (RPD), the "proximal pars distalis" (PPD) and the "pars intermedia" (PI), the posterior portion of the gland (Ball & Baker, 1969).

On the basis of present cytological and histological study, seven cell types have been characterized in the adenohypophysis of immature *P. mesopotamicus*: two types in the RPD, three types in the PPD and two types in the PI.

In the adenohypophysis of immature "pacu", as in other teleosts, the basophilic cells of the RPD form a defined zone bordering the neurohypophysary branches. Borella et al. (1997) have observed Mc Conaill's lead hematoxilin (HPb) staining reaction and anti-human adrenocorticotropic hormone (ACTH) antiserum positive immunohistochemical reaction of these cell type in adult "pacu". Considering the characteristic distribution, morphological features and stain reactions, we consider this cell type as ACTH cells in immature "pacu" also.

The other cell type of the RPD, the acidophilic one, is densely arranged in this region, forming compact cell cords. These cells are easily seen in histological preparations since they appear light red after staining with haematoxylin-eosin (HE) and Mallory's trichrome (MT) stains. These cells occupy the major part of the RPD in various teleost species (Cambré et al., 1986; Toubeau et al., 1991; Yan & Thomas, 1991). In teleosts as the *Anguilla anguilla* and in *Anchoviella lepidentostole* (Arana et al., 1997) these cells are

arranged in follicles, that are considered primitive feature (Ball & Baker, 1969). Despite of the fact that these cells do not present positive immunoreaction with anti-human prolactin (PRL), they are considered PRL cells in adult "pacu" (Borella et al., 1997) because of their anatomical and cytological characteristics.

In the PPD of immature "pacu" one acidophilic and two basophilic or cyanophilic cell types are seen. The basophils are PAS and AB positive because of the glicidic and strong acid groups present in the glycoproteic hormones of the storage granules. The acidophilic cell type that generally is in close contact with neural projections, have been described as somatotropic for many teleost species (Cambré et al., 1986; Yan & Thomas, 1991; Power, 1992), and in adult "pacu" also by morphological features, but not by immunohistochemical methods using anti-human STH (Borella et al., 1997). These cells are the main cell type in the PPD of juvenile fish (Ball & Baker, 1969), as we and Val-Sella et al. (1986) have seen in immature "pacu".

The basophilic fusiform cell type present in the dorsal and lateral middle PPD of "pacu" has been described as thyrotropic cells in many teleost species (Cambré et al., 1986; Yan & Thomas, 1991), as in adult "pacu", becoming evident by immunohistochemistry using anti-human TSH antiserum (Borella et al., 1997). These cells are not easily found in immature "pacu", probably due to of their small size and their scarce quantity and scattered distribution among the other cell types of the PPD. Val-Sella et al. (1986) described similar cells in immature "pacu" in the RPD, adjacent to the neurohypophysial branches. It is possible that some cells are in RPD and other in PPD along the course of the neural tissue.

The other basophilic cell type PAS and AB positive seen in the PPD of immature "pacu", has been refered as a gonadotropic cell in teleost fishes (Olivereau & Nagahama, 1983; Val-Sella et al., 1988). Borella et al. (1997) have demonstrated the positive immunoreaction of these cells with anti-human LH and FSH antiserum in adult "pacu". As it was expected, these cells, which are granulated and frequently seen in adult fishes near the reproductive stage of gonadal maturation, are not so developed and frequent in juvenile individuals.

The two cell types are found in the PI of immature "pacu" vary only in size, but not in staining or histochemical reactions. These results are different from those found by Val-Sella et al. (1986) and Borella et al. (1997). In the immature "pacu" studied by Val-Sella et al. (1986) the major cell type was anfiphilic. In adult "pacu", one type, acidophilic and bigger than the other, shows cross-reaction with anti-human ACTH (Borella et al., 1997). Yan & Thomas (1991) performed immunohistochemical studies in sciaenid species showing the presence of MSH in similar cells of the PI, and the binding of ACTH antiserum to the MSH cells has also been reported in other teleost fishes (Cambré et al., 1986; Joss et al., 1990; Yan & Thomas, 1991). This

positive reaction with the ACTH antiserum is possible due to the presence of ACTH in the cells as a precursor of MSH (Follénius & Dubois, 1980). For the other PI cell type, negative for all the histochemical and immunohistochemical methods employed in adult "pacu" (Borella et al., 1997), some functions have been attributed in teleost fish, as gonadotropic secretion (Batten, 1986) or somatolactin secretion (Planas et al., 1992).

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