ProBiota, FCNyM, UNLP
ISSN 1515-9329
Serie Técnica y Didáctica nº 21(53)

Semblanzas Ictiológicas
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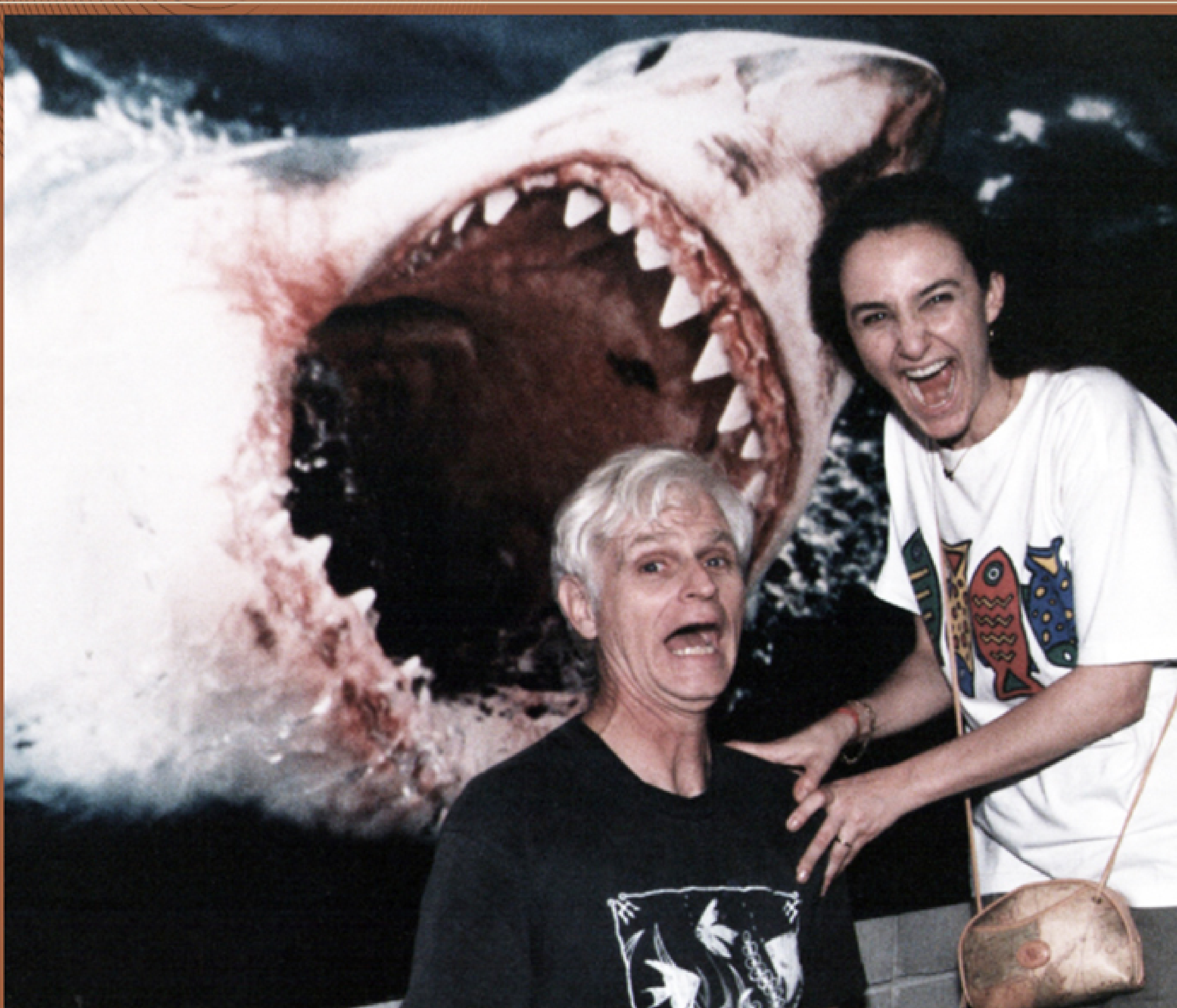

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y
Justina Ponte Gómez

Indizada en la base de datos ASFA C.S.A.
2014

Semblanzas Ictiológicas

Fabiana Laura Lo Nostro

Museo de la Ciencia Nemo, Amsterdam, 2013

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ProBiota
División Zoología Vertebrados
Museo de La Plata
FCNyM, UNLP

Octubre de 2014

Imagen de Tapa
Dr. Harry Grier y Fabiana Lo Nostro, The Florida Aquarium, USA, 2002

El tiempo acaso no exista. Es posible que no pase y sólo pasemos nosotros.

Tulio Carella

Cinco minutos bastan para soñar toda una vida, así de relativo es el tiempo.

Mario Benedetti

Semblanzas Ictiológicas

A través de esta serie intentaremos conocer diferentes facetas personales de los integrantes de nuestra "comunidad".

El cuestionario, además de su principal objetivo, con sus respuestas quizás nos ayude a encontrar entre nosotros puntos en común que vayan más allá de nuestros temas de trabajo y sea un aporte a futuros estudios históricos.

Esperamos que esta iniciativa pueda ser otro nexo entre los ictiólogos de la región, ya que consideramos que el resultado general trascendería nuestras fronteras.

Hugo L. López

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Especialidad o línea de trabajo: Reproducción en peces; Ecotoxicología acuática

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Un festejo de tantos, 1994
Laura López Greco, Alejandra Volpedo y Fabiana Lo Nostro

Delhi, India, 2011
F. Lo Nostro y la Prof. R. Guimarães Moreira de la Universidad de São Paulo, Brasil

Journal of Fish Biology (1996) **49,** 788–800

Presence of primary and secondary males in a population of the protogynous *Synbranchus marmoratus*

F. L. Lo Nostro and G. A. Guerrero

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(*Received 24 November 1995, Accepted 16 March 1996*)

Synbranchus marmoratus, the ' swamp eel ', is a protogynous diandric fish. The primary and secondary males can be distinguished from each other easily by differences in gonadal morphology. Primary males have lobular, unrestricted testes with central efferent ducts. The secondary male has a ' lamellar ' testis, efferent ducts are present in the ventral region (new formation), lateral supports and it is covered by the former ovarian capsule. The length of primary male varies from 13 to 88 cm while secondary males range from 56 to 91 cm. Transitional individuals vary between 45 and 60 cm in length. The swamp eel population studied is composed of 80% primary males and 20% secondary males. Although gonadosomatic indices are always higher in primary males, they increase in secondary ones as the newly acquired stage progresses. The lamellar organization of the gonad of secondary males, the absence of this kind of testis in individuals smaller than 56 cm, and the presence of hermaphroditic fish, is evidence for protogyny in this species.

Key words: hermaphroditism; protogynous; diandric; sex reversal; gonads; Synbranchidae.

INTRODUCTION

Teleosts exhibit various patterns of sexual ontogeny during which the gonads become functional ovaries or testes. The most remarkable expression of plasticity in sexual development is sex change, which occurs in a number of fish families (Francis, 1992). Even in the gonochoristic teleosts, there is a marked variation in the pattern of sexual determination and differentiation (Reinboth, 1983, 1988). Yamamoto (1969) described differentiated and undifferentiated species of gonochoristic fishes based upon the morphological sequence of sexuality and the occurrence of spontaneous intersexes and sex reversal. Natural sex reversal, the successive maturation of the male and female sex tissues at different stages of an individual's life history, occurs as a spontaneous phenomenon in various teleosts. The vast majority of ambisexual teleosts inhabit marine waters and the majority of these fishes live close to reefs (Reinboth, 1988). In sequential hermaphroditism, protogyny is especially common in warm-water marine species, such as the Gobiidae (Cole, 1983, 1989), Labridae (Ross *et al.*, 1983), Scaridae (Robertson & Warner, 1978), Serranidae (Hastings, 1981; Shapiro, 1981), and Sparidae (Hecht & Baird, 1977).

In some protogynous species, two types of males have been found: (1) primary males develop directly as males; (2) secondary males develop from functional females by sex reversal. Diandric species have both primary and secondary male

0022–1112/96/110788+13 $25.00/0

JOURNAL OF MORPHOLOGY 257:107–126 (2003)

Involvement of the Gonadal Germinal Epithelium During Sex Reversal and Seasonal Testicular Cycling in the Protogynous Swamp Eel, *Synbranchus marmoratus* Bloch 1795 (Teleostei, Synbranchidae)

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ABSTRACT The swamp eel, *Synbranchus marmoratus*, is a protogynous, diandric species. During sex reversal, the ovarian germinal epithelium, which forms follicles containing an oocyte and encompassing follicle cells during the female portion of the life cycle, produces numerous invaginations, or acini, into the ovarian stroma. Within the acini, the gonia that formerly produced oocytes become spermatogonia, enter meiosis, and produce sperm. The acini are bounded by the basement membrane of the germinal epithelium. Epithelial cells of the female germinal epithelium, which formerly became follicle (granulosa) cells, now become Sertoli cells in the developing testis. Subsequently, lobules and testicular ducts form. The swamp eel testis has a lobular germinal compartment in both primary and secondary males, although the germinal compartment in testes of secondary males resides within the former ovarian lamellae. The germinal compartment, supported by a basement membrane, is composed of Sertoli and germ cells that give rise to sperm. Histological and immunohistochemical techniques were used to describe the five reproductive classes that were observed to occur during the annual reproductive cycle: regressed, early maturation, mid-maturation, late maturation, and regression. These classes are differentiated by the presence of continuous or discontinuous germinal epithelia and by the types of germ cells present. *Synbranchus marmoratus* has a permanent germinal epithelium. Differences between the germinal compartment of the testes of primary and secondary males were not observed. J. Morphol. 257:107–126, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: synbranchiformes; swamp eels; germinal epithelium; sex reversal; testis; annual cycle

Numerous descriptions of the stages of germ cell maturation and development that are observed in teleosts during annual reproductive cycles have been published (Ruby and McMillan, 1970; Billard and Breton, 1978; Scott and Sumpter, 1989; Ntiba and Jaccarini, 1990; Smith and Young, 1996). However, the mechanisms of testicular regression and of the cycling of cells between annual breeding seasons are known for only a few species (Grier, 1993; Grier and Taylor, 1998; Taylor et al., 1998; Brown-Peterson and Warren, 2001; Brown-Peterson et al., 2002). The germinal epithelium (GE), which is found in both the testes and the ovaries of fish, has only recently been defined (Grier, 2000, 2002; Grier and Lo Nostro, 2000). Histological changes in the GE's morphology have been used to document a sequence of five reproductive classes during the annual reproductive cycle of the male common snook, *Centropomus undecimalis* (Taylor et al., 1998; Grier and Taylor, 1998; Grier and Lo Nostro, 2000), and of the pelagic cobia, *Rachycentron canadum* (Brown-Peterson et al., 2002). These reproductive classes were based on the alternation of the GE between continuous and discontinuous types and the stages of germ cells present. Continuous and discontinuous GE refer to whether both Sertoli cells and germ cells form a continuum or whether the germ cells are scattered and separated by Sertoli cells, respectively (Grier, 1993; Grier and Lo Nostro, 2000). Recognition of these two different forms of GE made it clear that among the chordates, a permanent GE that persists throughout annual reproductive cycles first appears in the fishes (Grier and Taylor, 1998), not in amphibians, as originally reported (Lofts, 1987). However, description of the GE and its annual changes, which can be used to describe reproductive classes, is wholly confined to the perciform teleosts.

Synbranchus marmoratus Bloch, 1795, the New World swamp eel (Liem, 1963, 1968; Lo Nostro and Guerrero, 1996), is a protogynic, diandric, freshwa-

Contract grant sponsor: (to FLN, University of Buenos Aires) UBA; Contract grant numbers: TW/41, EX/157, EX/217; Contract grant sponsor: CONICET; Contract grant number: PIP4558.

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DOI: 10.1002/jmor.10105

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Aquatic Toxicology 63 (2003) 127–137

AQUATIC TOXICOLOGY

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Vitellogenin detection in surface mucus of the South American cichlid fish *Cichlasoma dimerus* (Heckel, 1840) induced by estradiol-17β. Effects on liver and gonads

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Received 19 March 2002; received in revised form 30 July 2002; accepted 16 September 2002

Abstract

During the last decade, special attention has been focused on the consequences of exposure to environmental estrogens on reproduction in wild fish species. For this reason, characterization of biomarkers of such exposures could result in useful tools for these studies. The detection of vitellogenin (Vtg), a precursor of yolk proteins, is being intensely studied since its synthesis in the liver is regulated by the estradiol-17β and is influenced by other estrogenic compounds. The aim of this work was to assess the presence of Vtg in the surface mucus of males of *Cichlasoma dimerus* (Teleostei, Perciformes), a typical South American freshwater cichlid, after hormonal treatment with estradiol-17β (intraperitoneal injections of 10 μg E_2/g fish). Plasma and mucus from females and treated males analyzed by Western blot revealed that different heterologous antisera against Vtg bind to putative protein of 180 & 120 kDa and 120 & 110 kDa, respectively, whereas no reaction was found in samples of untreated males. The same profile was observed in mucus samples using Dot blot, a very easy and direct technique. Using immunocytochemistry techniques, immunoreactive Vtg (ir-Vtg) producing cells in the liver of females and treated males were detected. Testes and liver tissues were also assessed by histological techniques. Marked alterations in both organs were observed, such as lower sperm production, presence of immature germ cells in the lobular lumen of testes, and some morphology changes in the hepatocytes due to the accumulation of Vtg. This is the first report about the effects of an estrogen in the Vtg synthesis and their consequences on liver and gonads of a South American fresh water cichlid. These results also support the possibility of using Vtg from surface mucus as a potential biomarker for estrogenic compounds through a noninvasive, useful and easy assay to monitor the presence of endocrine disrupting chemicals in the environment.

Keywords: Cichlids; *Cichlasoma dimerus*; Vitellogenin; Mucus; Estradiol; Liver and testes pathology

1. Introduction

During the last decades there has been a significant effort to determine the effects of endocrine disrupting chemicals on reproduction,

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PII: S0166-445X(02)00175-3

Tissue & Cell 36 (2004) 221–231

Tissue&Cell

www.elsevier.com/locate/tice

Testicular interstitial cells, and steroidogenic detection in the protogynous fish *Synbranchus marmoratus* (Teleostei, Synbranchidae)

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Received 28 May 2003; received in revised form 27 November 2003; accepted 3 March 2004

Abstract

The swamp eel, *Synbranchus marmoratus*, is a freshwater protogynic diandric species. Primary males develop directly as males while secondary males arise from the sex reversal of females. Fishes from Argentine and Brazil inland waters were collected, examined and compared for this study. In order to characterize the interstitial testicular compartment, light and electron microscopy techniques and an enzyme histochemical examination for steroidogenic cells detection were used. The interstitial compartment of *S. marmoratus* is composed of Leydig and myoid cells, collagen fibers blood cells, macrophages,and amyelinic nerves. At the ultrastructural level, no differences were observed in the interstitial tissue, either between specimens from the different sampling sites or between primary and secondary males. Leydig cells are present in all testes examined throughout the year. A cytoplasmatic reaction of 3β-HSD was detected only in Leydig cells during sex reversal and in both type of males, mainly during the regressed and early maturation classes (autumn and winter). Leydig cells possess the typical fin structural characteristics associated with steroidogenesis. Furthermore, in both type of males, during sex reversal and after the spawning period, the number of granulocytes and macrophages present in the testes increased, suggesting that they could be involved in phagocytosis and resorption of damaged cells.

Keywords: Synbranchiformes; Sex reversal; Protogynous; Interstitial compartment; Leydig cells; Blood cells

1. Introduction

During the last 50 years, numerous studies focused on testicular interstitial tissue morphology have been performed in different teleost species (Billard et al., 1982; Nagahama, 1987; Dodd and Sumpter, 1984; Grier, 1993). The presence of Leydig cells in the testis appears to be typical of teleosts as in the rest of the vertebrate lineage (Nagahama, 1983). However, in testes of primary and secondary males of the protogynous wrasse *Coris julis*, Reinboth (1962) reported a lack of Leydig cells. The same absence of Leydig cells was reported in the protogynous Caribbean labrids (Roede, 1975). However, Bruslé (1987) demonstrated the presence of Leydig cells in *C. julis* using an ultrastructural approach. Histochemistry, electron microscopy (Hurk van den et al., 1978a; Grier, 1981; Nagahama, 1987; Grier et al., 1989), and in vitro techniques (Loir, 1990a,b; Cinquetti, 1994; Hoar and Nagahama, 1978) have demonstrated interstitial Leydig cells in the testes of many fis species and confirme that they are the main source of gonadal steroids.

Together with Leydig cells, the interstitial tissue contains fibroblasts collagen fibers myoid cells, and blood cells (Reinboth and Bruslé-Sicard, 1997; Cinquetti and Dramis, 2003). Grier et al. (1989), using electron microscopy to study testes of two species of pike, demonstrated a contractile network of myoid boundary cells in the interstitial compartment. The ultrastructure of myoid cells has been described in carp and trout (Timmermans et al., 1993; Cauty and Loir, 1995). In general, the information related to blood cells is scarce in fishes The lymphohemopoietic cells with phagocytic capacity are scattered randomly throughout the stroma (Quesada et al., 1990; Press et al., 1994) and sinusoidal capillaries (Zapata, 1979; Zapata et al., 1996).

As in synbranchid species, *Monopterus albus* (Liem, 1963; Chang and Phillips, 1967; Nagahama, 1983), sex reversal is a natural life history process. *Synbranchus*

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0040-8166/$ – see front matter
doi:10.1016/j.tice.2004.03.001

JOURNAL OF MORPHOLOGY 271:1399–1406 (2010)

Reproductive Histology of *Tomeurus gracilis* Eigenmann, 1909 (Teleostei: Atherinomorpha: Poeciliidae) With Comments on Evolution of Viviparity in Atherinomorph Fishes

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ABSTRACT *Tomeurus gracilis* is a species long considered pivotal in understanding the evolution of livebearing in atherinomorph fishes. *Tomeurus gracilis* is a zygoparous or embryoparous poeciliid: internal fertilization is followed by females laying fertilized eggs singly or retaining fertilized eggs until or near hatching. *Tomeurus* was hypothesized as the sister group of the viviparous poeciliids until it was proposed as a close relative of a derived viviparous poeciliid, *Cnesterodon*, hence nested among viviparous taxa rather than near the root of the tree. Here, we describe and compare reproductive morphological characters of the little-known *Tomeurus* with those of representative atherinomorphs. In *Tomeurus* and *Cnesterodon*, sperm are packaged in naked sperm bundles, or spermatozeugmata, in a configuration considered here diagnostic of viviparous poeciliids. Testes are single and free sperm are stored in the ovary in both taxa in contrast to oviparous atherinomorphs in which testes are paired and sperm are not packaged and not stored in the ovary. Efferent ducts in *Cnesterodon* testes and other viviparous poeciliids have a PAS-positive secretion demonstrating presence of a glycoprotein that inactivates sperm or prevents final sperm maturation. No PAS-positive staining secretion was observed in *Tomeurus* or oviparous atherinomorphs. *Tomeurus* shares apomorphic reproductive characters, such as sperm bundle and testis morphology and a gonopodium, with viviparous poeciliids and plesiomorphic characters, such as a thick zona pellucida with filaments, with oviparous taxa. We do not postulate loss or reversal of viviparity in *Tomeurus*, and we corroborate its phylogenetic position as sister to the viviparous poeciliids. J. Morphol. 271:1399–1406, 2010.

KEY WORDS: spermatozeugma; zona pellucida; egg morphology; embryoparity/zygoparity; testis types

INTRODUCTION

Atherinomorph fishes, with an estimated 1,552 species classified in three orders, Atheriniformes, Cyprinodontiformes, and Beloniformes, have long been featured in studies of reproductive biology (see Parenti, 2005; Nelson, 2006). Atherinomorph monophyly is well-supported by a range of morphological characters (Rosen, 1964; Rosen and Parenti, 1981; Parenti, 1993, 2005) and has been recovered in molecular analyses of bony fish phylogeny (e.g., Setiamarga et al., 2008). Two diagnostic characters of atherinomorphs are explicitly of reproductive morphology: a testis with spermatogonia restricted to the distal ends of testis lobules rather than distributed along the length of the lobule and a relatively large egg with fluid rather than granular yolk (Grier et al., 1980; Rosen and Parenti, 1981; Grier, 1993; Parenti and Grier, 2004). Internal fertilization and viviparity have evolved multiple times within atherinomorphs, as interpreted from the most parsimonious distribution of morphological and molecular characters, many of which are not related directly to reproduction (see Rosen, 1964; Parenti, 1981, 1993, 2005; Rosen and Parenti, 1981; Meyer and Lydeard, 1993; Grier et al., 2005; Mank and Avise, 2006; Hrbek et al., 2007; Reznick et al., 2007a).

Tomeurus gracilis Eigenmann (1909) (Fig. 1A) is a diminutive, zygoparous or embryoparous atherinomorph poeciliid fish that lives in northeastern South America. A simple, straightforward classifi-

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Received 5 March 2010; Revised 27 May 2010; Accepted 19 June 2010

Published online 22 September 2010 in Wiley Online Library (wileyonlinelibrary.com)
DOI: 10.1002/jmor.10886

Arch Environ Contam Toxicol (2011) 61:300–310
DOI 10.1007/s00244-010-9621-3

Effect of the Organochlorine Pesticide Endosulfan on GnRH and Gonadotrope Cell Populations in Fish Larvae

Yanina G. Piazza · Matias Pandolfi · Fabiana L. Lo Nostro

Received: 8 June 2010 / Accepted: 22 October 2010 / Published online: 26 November 2010

Abstract Endocrine-disrupting chemicals can influence the hypothalamus–pituitary–gonad axis and possibly affect reproduction in vertebrates. We analyzed the effect of 30-day endosulfan (ES) exposure in sexually undifferentiated larvae of the cichlid fish *Cichlasoma dimerus*. The number, area, mean cytoplasmic and nuclear diameter, and mean cytoplasmic optical density of gonadotropin-releasing hormone (GnRH) I, II, and III immunoreactive (ir-) neurons and β follicle-stimulating hormone (βFSH) ir-cells were measured. Animals exposed to the highest ES concentration (0.1 μg/l) showed a decrease in GnRH I nucleus/cytoplasm area ratio upon exposure. Nuclear area and mean nuclear diameter of βFSH ir-cells was higher in ES treated fish. βFSH nucleus/cytoplasm area ratio was high in exposed animals, and animals exposed to 0.1 μg/l ES showed smaller mean cytoplasmic optical density. These findings suggest that ES affects GnRH I and βFSH protein synthesis/release. However, these responses seem to be insufficient to affect gonadal differentiation at this stage of development.

The neuroendocrine system of the hypothalamus–pituitary–gonad (HPG) axis regulates reproduction in vertebrates and can be influenced by chemicals, therefore affecting the reproductive system. Neurotoxic environmental contaminants recognized as endocrine-disrupting chemicals (EDCs) have aroused considerable interest in the field of neuroendocrinology (Gore 2000; Pillai et al. 2003; Panzica et al. 2005; Gore 2008a, b). Among these pollutants, organochlorine pesticides are considered to be hazardous because they are very persistent, are nonbiodegradable, and are ubiquitously found in the environment (Palmer and Palmer 1995; Donohoe and Curtis 1996). After international recognition of their long-term negative impacts on the global environment, the use of organochlorines in global agriculture has been largely banned (RAP-AL 2008; United Nations 2009). However, endosulfan (ES) remains as one major exception. ES (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3benzo-dioxathiepin-3-oxide) is a cyclodiene organochlorine insecticide used for the control of insects and mites in crops of high commercial value (RAP-AL 2008). ES is slightly soluble in water, but it dissolves in most organic solvents (Harding 1979). Given that organic solvents are commonly used in commercial formulations, they might contribute to the overall effect of ES on the dysfunction of the endocrine system (Hutchinson et al. 2006; Mortensen and Arukwe 2006). Exposure of *Thalassoma pavo* to ES decreased feeding behavior related to neuronal degeneration in the mesencephalon and the hypothalamus (Giusi et al. 2005). Cytological and structural oogonia and oocyte malformations, an important decrease in gonadotropins (GtHs) neurosecretory activity, as well as damage of the axons that innerve the pituitary were observed in adults of *Sarotherodon mossambicus* after chronic exposure to ES (Shukla and Pandey 1986). In *Oryzias latipes*, acute exposure to ES caused alterations in development, sexual behavior, and reproductive physiology (Gormley and Teather 2003).

The decapeptide gonadotropin-releasing hormone (GnRH) is mainly synthesized in the central nervous system, and its

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Physiology & Behavior 106 (2012) 193–200

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Aggressive behavior and reproductive physiology in females of the social cichlid fish *Cichlasoma dimerus*

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ARTICLE INFO

Article history:
Received 13 August 2011
Received in revised form 31 January 2012
Accepted 1 February 2012
Available online 9 February 2012

Keywords:
Reproduction
Cichlids
Pituitary
Neuroendocrinology
Steroids
GnRH

ABSTRACT

The South American cichlid fish *Cichlasoma dimerus* is a freshwater species that presents social hierarchies, a highly organized breeding activity, biparental care and a high frequency of spawning. Spawning is followed by a period of parental care (about 20 days in aquaria conditions) during which the cooperative pair takes care of the eggs, both by fanning them and by removing dead ones. The different spawning events in the reproductive period were classified as female reproductive stages which can be subdivided in four phases, according to their offspring degree of development: (1) female with prespawning activity (day 0), (2) female with eggs (day 1 after fertilization), (3) female with hatched larvae (day 3 after fertilization) and (4) female with swimming larvae (FSL, day 8 after fertilization). In Perciform species gonadotropin-releasing hormone type-3 (GnRH3) neurons are associated with the olfactory bulbs acting as a potent neuromodulator of reproductive behaviors in males. The aim of this study is to characterize the GnRH3 neuronal system in females of *C. dimerus* in relation with aggressive behavior and reproductive physiology during different phases of the reproductive period. Females with prespawning activity were the most aggressive ones showing GnRH-3 neurons with bigger nuclear and somatic area and higher optical density than the others. They also presented the highest levels of plasma androgen and estradiol and maximum gonadosomatic indexes. These results provide information about the regulation and functioning of hypothalamus–pituitary–gonads axis during reproduction in a species with highly organized breeding activity.

1. Introduction

Aggression is a common behavior in the context of competition for limited resources such as food or mating [1]. The behavior observed during the reproductive season, either defending a territory, during breeding, or during the protection of the offspring, is critical for the reproductive success.

The South American cichlid fish *Cichlasoma dimerus* is a freshwater species that presents social hierarchies, a highly organized breeding activity, biparental care and a high frequency of spawning. The dominant pair will strongly defend the prospective spawning site. Spawning is followed by a period of parental care (about 20 days in aquaria conditions) during which the cooperative pair take care of the eggs, both by fanning them and by removing dead ones. At a 26 °C water temperature larvae hatch at the beginning of the third day after fertilization (DAF) and are transferred by both parents to a previously dug pit. Larvae spend five days in the pit until they swim freely [2]. Aggressive behaviors include biting, mouth holding, chasing, fin erection, while submissive displays include escape and fin retraction [3].

Gonadotropin-releasing hormone type-3 (GnRH3) neurons are associated with the olfactory bulbs and they project axons to the retina and pineal organ which suggest a role in the light and photoperiodic behavioral and physiological responses [4,5]. In male tilapia *Orechromis niloticus* it was shown that GnRH3-immunoneutralization significantly decreases nest-building ability, nest size and aggressive behavior [6]. In male dwarf gourami *Colisa lalia* GnRH3 modulates reproductive behaviors related to nest building [7]. It has been proposed that GnRH3 could be acting on the visual and olfactory system coordinating sensory inputs with reproductive requirements [8]. Those results provided evidence that GnRH3 is a potent neuromodulator of reproductive behaviors in males, but no studies have been performed in females.

Aggression has also been associated with androgen levels. Manipulation experiments have demonstrated that androgen removal decreases aggression while androgen treatment rescues or increases aggression [9–11]. In the male cichlid *Orechromis mossambicus*, androgen treatment increases aggression and territorial defense [12]. These observations, paired with demonstrations that individuals

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doi:10.1016/j.physbeh.2012.02.002

Aquatic Toxicology 126 (2013) 299–305

Contents lists available at SciVerse ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Endocrine disruptive potential of endosulfan on the reproductive axis of *Cichlasoma dimerus* (Perciformes, Cichlidae)

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ARTICLE INFO

Article history:
Received 16 July 2012
Received in revised form
17 September 2012
Accepted 22 September 2012

Keywords:
Endosulfan
Cichlid fish
Endocrine disruption
Testes pathology
Gonadotropins
Sex steroids

ABSTRACT

Endosulfan (ES), a persistent organochlorine pesticide, is widely used despite its toxicity to non-target animals. Upon reaching water bodies, ES can cause negative effects on aquatic animals, including disruption of hormonal systems. However, the action of ES on fish reproductive axis has been hardly studied thus far. The aim of the present work was to assess the endocrine disruptive potential of endosulfan on the pituitary gonadotropins levels and on the testes function due to ES in the South American freshwater fish *Cichlasoma dimerus*, using *in vitro* and *in vivo* approaches. *In vitro* experiments showed that ES inhibited the LH-stimulated steroidogenesis in gonads; no change was observed in gonadotropins release from pituitaries in culture. Laboratory waterborne ES (0.1, 0.3 and 1 μg/L) exposure for two months caused decrease in βFSH pituitary content and γGT activity in the testes (Sertoli cell function marker). Testicular histology revealed pathologies such as scarce intermediate stages of spermatogenesis, release of immature germ cells into the lobular lumen, presence of foam cells and interstitial fibrosis. As FSH and FSH-mediated steroidogenesis regulate spermatogenesis and Sertoli cell function, the effect of ES on FSH could be responsible for the morphological alterations observed in testes. *In vitro*, ES disrupted steroidogenesis in gonads, therefore similar effects *in vivo* cannot be ruled out. Based on this evidence, ES exhibits an endocrine disruptive action on the reproductive axis of *C. dimerus*, causing disruption at the pituitary and/or at the gonad level. These effects could acquire ecological significance under prolonged exposure to the pesticide in nature.

1. Introduction

Water ecosystem pollution is, together with loss of habitat, one of the main factors endangering wildlife species (Wilcove et al., 1998). As pesticide runoff constitutes a significant contribution to aquatic pollution, their use in agriculture is under constant screening to ensure non-target animals' welfare, leading to the ban and restriction of a large number of products. The use of the organochlorine pesticide endosulfan (ES; 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) has been limited or discontinued in recent years in many European and North American countries due to its persistence in the environment and toxic effects (Sutherland et al., 2004). Endosulfan has been recently classified as a Persistent Organic Pollutant (POP) by the Stockholm Convention on POPs, supporting the ban on its use and production (POPRC, 2010). However, it is still widely used, particularly in developing countries, as a broad spectrum insecticide to control insects and mites in crops of high commercial value (soy, cotton, tea, coffee, maize, fruits) (Capkin et al., 2006). Following application, ES can reach non-target aquatic animals through groundwater, surface runoff and air drift from nearby agricultural fields (Miglioranza et al., 2002). Half life in water and soil for α isomer has been reported as 7–75 days, whereas for β isomer and endosulfan sulfate, the equally toxic main metabolite of ES degradation, half lives can exceed 300 days, depending on environmental conditions (Singh et al., 2000; Weber et al., 2010). Isomers have been detected in surface and groundwater in concentrations ranging from 0.05 to 2.5 μg/L (Dalvie et al., 2003; Leong et al., 2007) as well as in fish at

Abbreviations: γGT, gamma-glutamyl transpeptidase; ES, endosulfan; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

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0166-445X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquatox.2012.09.015

Aquatic Toxicology 156 (2014) 30–40

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Nonmonotonic response of vitellogenin and estrogen receptor α gene expression after octylphenol exposure of *Cichlasoma dimerus* (Perciformes, Cichlidae)

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ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 20 July 2014
Accepted 24 July 2014
Available online 1 August 2014

Keywords:
Cichlid fish
Vitellogenin gene expression
Estrogen receptors
Octylphenol
Nonmonotonic dose–response

ABSTRACT

In oviparous vertebrates, vitellogenin (VTG) is mainly produced by the liver in response to estrogen (E_2) and its synthesis is traditionally coupled to estrogen receptor alpha induction. Even though VTG is a female-specific protein, chemicals that mimic natural estrogens, known as xenoestrogens, can activate its expression in males causing endocrine disruption to wildlife and humans. Alkylphenols such as nonylphenol (NP) and octylphenol (OP) are industrial additives used in the manufacture of a wide variety of plastics and detergents, and can disrupt endocrine functions in exposed animals. For more than a decade, the freshwater cichlid fish *Cichlasoma dimerus* has been used for ecotoxicological studies in our laboratory. We recently found an up-regulation of VTG gene expression in livers of male fish exposed to OP, from a silent state to values similar to those of E_2-induced fish. To better understand the underlying mechanisms behind the action of xenoestrogens, the aim of this study was to analyze the dose–response relationship of *C. dimerus* VTG and estrogen receptors (ERs) gene expression after waterborne exposure to 0.15, 1.5, 15, and 150 μg/L OP for up to 1 month (0, 1, 3, 7, 14, 21, and 28 days). At the end of the experiment, histological features of exposed fish included active hepatocytes with basophilic cytoplasm and high eosinophilic content in their vascular system due to augmented expression of VTG. In testis, high preponderance of sperm was found in fish exposed to 150 μg/L OP. A classic dose–response down-regulation of the expression of Na^+/K^+-ATPase, a "non-gender specific gene" used for comparison, was found with increasing OP concentrations. No VTG and very low levels of ERα were detected in control male livers, but an up-regulation of both genes was found in males exposed to 0.15 or 150 μg/L OP. Moreover, VTG transcripts were significant as early as day 3 or day 1 of exposure to these OP concentrations, respectively. Nearly no response was detected in 1.5 and 15 μg/L OP exposed-fish. Data was curve-fitted evidencing a nonmonotonic dose–response curve. Interestingly, ERβ2 mRNA expression was augmented above baseline levels only when males were exposed to the lowest OP concentration. We speculate that genomic control of vitellogenesis is under control of multiple steroid receptors with different affinities for ligands. ERβ isoform, only up-regulated with very low concentrations of ligand, would act as a sensors of OP (or E_2) to induce ERα and VTG. With high OP concentrations, the expression of ERα isoform is promptly augmented, with the concomitant VTG transactivation.

Abbreviations: APE, alkylphenol polyethoxylates; Ctrl, control; E_2, 17β-estradiol; OP, 4-tert-octylphenol; VTG, vitellogenin; NP, nonylphenol; AP, alkylphenols.

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http://dx.doi.org/10.1016/j.aquatox.2014.07.019
0166-445X/

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Grupo “Fish Physiology”, Portugal, 2014
De izquierda a derecha: Denisse Vizziano, Penny Swanson, Matias Pandolfi, Fabi Lo Nostro y Berta Levavi-Sivan; atrás, Fransesc Piferrer.

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Esta publicación debe citarse:

López, H. L. & J. Ponte Gómez. 2014. Semblanzas Ictiológicas: *Fabiana Laura Lo Nostro. ProBiota*, FCNyM, UNLP, La Plata, Argentina, *Serie Técnica y Didáctica* 21(53): 1-21. ISSN 1515-9329.

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Versión electrónica, diseño y composición

Justina Ponte Gómez

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Indizada en la base de datos ASFA C.S.A.