

POECILIID RESEARCH

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Endocrine control of reproduction in the guppy fish (*Poecilia reticulata*)

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Abstract. *Poecilia reticulata* (guppy fish) exhibit several characteristic reproductive traits: intrafollicular gestation, ovoviviparity and multiple mating, either cooperatively or coercitively. Systemic hormones and endocrine-disrupting chemicals influence reproductive morpho-physiology and reproductive behavior in *Poecilia reticulata*. In female guppies, estrogens promote oocyte growth and vitellogenesis and facilitate parturition in conjunction to gonadotropins. In contrast, exposure to estrogens in males reduces courtship and alters spermatogenesis. The role of androgen hormones in reproductive physiology of guppies is not well-established, however, the implication of 11-ketotestosterone, an androgenic metabolic compound, in sexual recognition during courtship in poeciliids is discussed. In addition, hormone secretion was immunolocalized in poeciliids for luteinizing hormone (LH), LH-releasing hormone (LHRH), thyrotropin-releasing hormone (TRH), prolactin-releasing peptide (PRLrP), vasotocin, thyroxin etc. Further studies are needed to expand knowledge on endocrine regulation of reproduction in *Poecilia reticulata*.

Keywords: *Poecilia reticulata*, reproduction, neurohormones, sex steroids.

Rezumat. *Poecilia reticulata* (peștele guppy) prezintă o serie de particularități reproductive: gestație intrafolliculară, ovoviviparitate și împerechere poligamă sau de tip monogam cu masculi dominanți. Hormonii sistemici și o serie de compuși cu efect de disruptor endocrin influențează atât morfo-fiziologia reproductivă cât și comportamentul reproductiv la *Poecilia reticulata*. În cazul femelelor, estrogenii stimulează creșterea ovocitelor și vitelogeneza, iar în conjuncție cu hormonii gonadotropi, facilitează parturiția. Dimpotrivă, expunerea la estrogeni a masculilor diminuează manifestările nupțiale și influențează nefavorabil procesul de spermatogeneză. Rolul hormonilor androgeni în fiziologia reproducerii peștilor guppy nu este încă clar elucidat dar se discută implicarea 11-ketotestosteronului, un metabolit cu acțiune androgenică, în semnalizarea sexuală interindividuală din perioada de împerechere. Totodată, prin studii de imunolocalizare s-a demonstrat secreție la poeciliide a hormonului luteinizant (LH), a gonadoliberinei (LHRH), a tiroliberinei (TRH), a prolactin-releasing peptide (PRLrP), a vasotocinei, a tiroxinei etc. Cercetărilor viitoare le revine rolul de a aprofunda cunoașterea controlului endocrin al reproducerii la *Poecilia reticulata*.

Cuvinte cheie: *Poecilia reticulata*, reproducție, neurohormoni, steroizi sexuali.

Introduction. The small tropical fish, *Poecilia reticulata* Peters (guppy fish) represents one of the most valuable tools in the study of sexual selection and as a bio-indicator of effects of chronic irradiation (Woodhead et al 1977), heavy metals toxicity (Khunyakari et al 2001; Petrescu-Mag et al 2010; Oroian et al 2013) and, lately, endocrine disruptors (Georgescu et al 2005; Mousavi-Sabet et al 2012; Țălu et al 2012). Guppies are increasingly employed in bio-monitoring programs of lakes and freshwater contamination, being considered biomarkers of exposure (mortality/immobility of fish), gender balance, the gonadosomatic index, the hepatosomatic index, the gonadal histology, the vitellogenin level and vitellogenin gene expression as well as certain reproduction parameters (Cardinali et al 2004; Georgescu et al 2012). Apart from somatic effects, reproductive behavior studies in *Poecilia reticulata* are widely used in fish toxicology,

whereas non-reproductive behavior research in teleosts emerges as a more recent tool in eco-toxicology.

Brief description of reproductive anatomy and behavior in *Poecilia reticulata*.

The gonopodium, a modified tubular anal fin located behind the ventral fin, is used by male guppy fish as an intromittent organ to deliver spermatozoa during copulation. This structure exhibits significant variability in size and shape within populations but there is no clear evidence of male genital morphology to influence mating success (Gasparini et al 2011).

Guppies are livebearers with internal fertilization. Oocyte maturation is followed by intrafollicular fertilization and gestation. The fully developed embryos are ovulated by the female at term just prior to parturition. The gestation period of a guppy is about 21-30 days (28 days on average), slightly varying according to water temperature.

Hormones play a key role in vertebrate mating behavior. One trait of the reproductive behavior in *Poecilia reticulata* is that during the sexual receptivity phase in the month, females choose their partners in reproduction according to several male phenotype characteristics such as skin pigmentation, body size and courtship display intensity, showing preference for a larger body size and larger ornaments of males (Rodd & Sokolowski 1995; Pilastro et al 2002) and the orange color polymorphism. The complex pigmentation polymorphism in guppy males represents a genetically determined trait (Mag & Bud 2005, 2006), as for example the half-black guppy fish phenotype, which is characterized by posterior half of the body black, is related to a dominant genotype of *Nigrocaudatus* gene (Petrescu-Mag et al 2011). Likewise, the polymorphism in the melanocortin-1 receptor (MC1R) gene is linked to black pigmentation, *i.e.* animals with a 963-bp (*Del*) MC1R genotype tend to exhibit less black pigmentation than those with a 969-bp (+) MC1R genotype (Tezuka et al 2011). Aside to genetic factors, a complex endocrine regulatory network described in *Poecilia reticulata* intervenes in reproduction control in these animals.

It is well-described that *Poecilia reticulata* exhibit multiple mating, meaning that a female will mate with two or more male partners. It appears that multiple mating is beneficial to the offspring and characterized by an increase in offspring quantity and an improvement in offspring quality. Using a polymorphic microsatellite marker to determine paternity, Evans & Magurran (2000) showed that multiple mating is associated with shorter gestation times and larger broods; offspring born to multiply mated females appear to gain several benefits as juveniles, such as better developed schooling abilities.

Aside to cooperatively copulation between males and females, females may be coerced into mating by the less desirable males. Interestingly, the type of copulation may impact on the parturition process in guppy fish as the majority of females that copulated coercively failed to give birth to offspring; however, copulation type had no influence on brood size which was dependent on female body size and male orange spot area (Sato et al 2011). Recent studies propose 11-ketotestosterone to be involved in sexual recognition during courtship in poeciliids (Gabor et al 2010).

Reproduction regulation by steroids and androgen-to-estrogen aromatase activity in *Poecilia reticulata*.

Estrogens play an important role in vitellogenesis to promote oocyte growth. In addition, estrogens may facilitate parturition. Measurement of estradiol and testosterone concentration in female guppies during gestation showed a two-phase pattern with hormone levels increasing during the vitellogenic oocyte development and declining after fertilization, thereafter increasing gradually to reach prefertilization levels at periparturition (Venkatesh et al 1990). Cortisol levels decrease during fertilization followed by an increase during gestation until periparturition when cortisol declines abruptly (Venkatesh et al 1990). Corticoids and progestins delay while 17 β -estradiol induces premature parturition in *Poecilia reticulata* (Venkatesh et al 1991).

In males, exposure to environmental estrogens may affect body size and color, alter spermatogenesis with reduced sperm count (Kristensen et al 2005) and increase the number of Sertoli cells (Kinnberg et al 2003). In addition, estrogen exposure leads to a reduction in the rate and intensity of sexual display in male guppies. Nonetheless, some

authors rather consider guppies as not sensitive enough to be used as test organisms for the detection of environmental estrogens effects but responding only to high concentration exposure (Nielsen & Baatrup 2006). On the other hand, it was reported that males subjected to environmentally relevant concentrations of 17 α -ethinylestradiol, a synthetic estrogen, exhibited besides reproductive effects, increased freezing and bottom-dwelling which suggest an anxiogenic behavior induced by xenoestrogens (Hallgren et al 2011).

Although in several species, androgens, in particular testosterone, appears to be relevant to body growth and body size, in a recent study no relationship was found between androgen hormones (i.e. 11-ketotestosterone, testosterone) and the condition index of male guppies (Gupta & Kahlbach 2011).

The aromatase is a tissue enzyme converting androgen hormones into estrogens which is highly expressed in humans and several other species, including teleosts. In comparison to other species, teleosts, express 100-1000-fold higher aromatase levels in particular in the brain (i.e. the hypothalamus, the pre-optic area and the optic tectum) which appears to be involved in regulation of the sexual behavior in fish. Indeed, in *Poecilia reticulata*, the aromatase is distributed in areas of the brain that are linked to reproductive control, and aromatase activity suppression by aromatase inhibitors treatment results in reduced sexual behaviors sigmoid display and reduced gonopodium swinging (Hallgren et al 2006). This suggests a specific role of brain estrogens in reproductive behavior of male guppies and in addition to the high sensitivity of the enzyme to estrogens (Hallgren & Olsén 2010) it may suggest a potential use of aromatase expression in *Poecilia reticulata* as an indicator of endocrine disruption at low, typically environmental concentrations.

The pituitary hormones activity and reproduction in *Poecilia reticulata*. Pituitary hormones participate in gonadal development and gametogenesis and are responsible for the development of secondary sex characteristics in guppies. Gonadotropins participate in the process of parturition. In juvenile guppies (2-3 weeks old) hypophysectomy resulted in lack of gonadal development; even though methyltestosterone treatment stimulated the differentiation of gonadal ducts and secondary sex characteristics in hypophysectomized animals, it had no effect on gametogenesis (Pandey 1969), an effect that resembles gonadal physiology regulation in human.

Immunocytochemical expression of neurohormones (e.g. TRH-like, CRH-like and somatostatin) and adenohypophyseal hormones has been reported in the teleost species *Poecilia latipinna* and control of luteinizing hormone (LH) levels by LH-releasing hormone (LHRH) and vasotocin has been demonstrated (Groves & Batten 1986ab). In addition, peripheral steroids intervene in LH regulation, with estradiol and testosterone stimulating gonadotropin secretion by active gonadotropins but inhibiting it in inactive cells, and progesterone acting in a suppressive manner (Groves & Batten 1986ab). In *Poecilia reticulata*, especially in juvenile animals and less in adult guppies, LHRH- and LH-immunoreactive cells were described and distinctively identified in the pituitary even though in close proximity (Zentell et al 1987).

Prolactin-releasing peptide (PRL-rP)-secreting neurons have been immunolocalized in the posterior part of the hypothalamus in adult guppy fish, possibly involved in organ development and neuromodulation (Amano et al 2007).

Thyroid hormones effects on reproductive behavior and activity in *Poecilia reticulata*. Bromage & Sage (1968) studied thyroid activity during gestation and identified a peak in thyroid activity during the first third of the gestation cycle, in a time of intense reproductive activity, followed by a decline and a second less significant increase during the last third of the cycle. Experimental thyroxin delivery to *Poecilia reticulata* individuals accelerates vitellogenesis and reduces the duration of gestation. Treatment of female guppies at 1-day postpartum with thyroid hormones results in shortened brood interval but without affecting the brood weight or the size of the brood (Lam et al 1985). As in other species, in the guppy fish, thyroid hormones may promote organ development and maturation.

Intra-peritoneal injection of UV- or gamma-irradiated thyroid cells results in another poeciliid, *i.e.* *Poecilia formosa* in development of an extensive pretumorous growth of the thyroid gland which is used as a research model in thyroid cancer studies (Achey et al 1979; Woodhead et al 1979).

Concluding remarks. Reproductive traits in *Poecilia reticulata* represent a stepping stone in its use as a model organism in the study of sexual selection and eco-toxicology. Both reproductive physiology and behavior are subjected to a tight control by the complex endocrine network in guppies, in particular by steroid and pituitary hormones. Further studies on the hormone regulation and activity in *Poecilia reticulata* are warranted.

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