

# Modeling the Brain-Pituitary-Gonadal Axis in Salmon

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## Abstract

The biological effects of many endocrine disruptors are diverse and may involve disturbances in normal pituitary and gonadal function. As an aid to better understand the complexity the brain-pituitary-gonadal axis (BPG) in fish and the effects of specific disturbances in sex steroid or gonadotropin secretion, we developed a biologically based pharmacodynamic model capable of accurately predicting the normal functioning of the BPG axis in salmon. This first-generation model consisted of a set of 13 equations whose formulation were published by published works and plasma concentrations of pituitary- (FSH, LH) and ovary- (estradiol, 17 $\alpha$ ,20 $\beta$ -dihydroxy-4-pregene-3-one) derived hormones measured in Coho Salmon over an annual spawning period. In addition, the model incorporated pertinent features of previously published mammalian models and indirect response pharmacodynamic models. Model-based equations include a description of gonadotropin releasing hormone (GnRH) synthesis and release from the hypothalamus, which is controlled by environmental variables such as photoperiod and water temperature. GnRH stimulated the biosynthesis of messenger RNA for FSH and LH, which were also influenced by estradiol concentration in plasma. The level of estradiol in the plasma was regulated by the oocytes, which moved along a maturation progression. Estradiol was synthesized at a basal rate and as oocytes matured, stimulation of its biosynthesis occurred. Oocytes progressed by a periodic function of time with basal levels eventually leading to final oocyte maturation and spawning. Applications of the fish BPG model include aiding risk assessments of endocrine disruptors by providing a systems biological approach for interpreting the significance of alterations in hormone synthesis on reproduction. The BPG model can be integrated with toxicogenomic data, allowing a whole fish perspective on altered gene expression and endocrine function.

## Introduction

A clear challenge with endocrine screening programs is the need to evaluate thousands of chemicals for which only a small percentage will be tested in-vivo. Computational toxicology in its various forms presents a practical approach to meet this challenge. Successful fish reproduction requires competent gametogenesis in both sexes of gonochoristic species to produce viable gametes and is summarized in Figure 1. Appropriate biotic and abiotic signals are critical for gametogenesis to properly proceed. Environmental signals (e.g., length of daylight) trigger brain production of gonadotropin releasing hormone (GnRH) that initiates a reproductive cascade leading to eggs in the female or sperm in the male. As in other vertebrates GnRH signals the pituitary gland in fishes to produce two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones enter the blood and travel to the gonads where they bind to specific receptors, which stimulates the gonads to synthesize the sex steroid hormones (estrogens, progesterins, and androgens). Sex steroids act within the gonads for proper gametogenesis in each sex and feedback upon the pituitary gland and hypothalamus to regulate gonadotropin secretion. Thus, a complex environmental and endocrine signaling network controls gametogenesis, regulates gamete maturation, and induces behavior that results in a spawning event. Underlying this complex network are key genes involved in the endocrine signaling and in the downstream events triggered by this hormonal cascade. In this study, we are developing a computational (mathematical) model of the fish brain-pituitary-gonadal (BPG) axis to aid in assessing the risk of endocrine disruptors towards fish reproduction. The BPG model is based on the biology of the hormone synthesis (GnRH, gonadotropins, sex steroids) and includes key steps in the signaling pathways.

## Methods: Female BPG Model Development

The model is based upon measured plasma concentrations of pituitary (FSH, LH) and ovarian (E2, 17,20DHP) hormones measured in coho salmon over the March-December period (Swanson 1991; Swanson et al. 2003). Model structure and linkages between parameters is shown in Figure 2 (red dashed lines indicate inhibitory or - feedback; solid blue lines indicate + feedback or stimulation of synthesis). The set of 8 differential equations plus the five equations that describe oocyte maturation (Box 1) were numerically integrated in WinNonlin software to simulate the temporal profile of each model variable. The simulated profiles (lines) and the experimentally measured values (points) for plasma concentrations of FSH, LH, E2, and 17,20-DHP in coho salmon are shown versus time in Figure 3, with January 1 being Day 1. Important features of the model are:

- GnRH (G in equations) was modeled at a constant level of 50 arbitrary units between days 75 and 250 of the calendar year, following the temporal profile of GnRH gene expression in rainbow trout (Gray et al. 2002).
- FSH & LH are synthesized in the pituitary gland and assumed to either immediately diffuse (FSH) or stored (LH) until later release. After secretion, FSH & LH distribute into a plasma-referenced volume of distribution, (V<sub>d,F</sub>, V<sub>d,L</sub>), and cleared at a rate controlled by plasma clearance (CL<sub>F</sub>, CL<sub>L</sub>).
- Oocytes progressed by a periodic function of time, with specified basal levels (O<sub>0</sub>(0), O<sub>2</sub>(0), O<sub>3</sub>(0), O<sub>4</sub>(0), and FOM(0) [Final Oocyte Maturation]), amplitudes (a<sub>1</sub>, a<sub>2</sub>, a<sub>3</sub>, a<sub>4</sub>, and a<sub>5</sub>), widths (W<sub>1</sub>, W<sub>2</sub>, W<sub>3</sub>, W<sub>4</sub>, and W<sub>5</sub>, days), and peak times (t<sub>1</sub>, t<sub>2</sub>, t<sub>3</sub>, t<sub>4</sub>, and t<sub>5</sub>, days).

- The concentration of 17,20DHP in the plasma (P<sub>n</sub>, ng/ml) was periodically regulated by the oocyte maturation process, with its basal synthesis level (P(0)) stimulated by biosignals (S<sub>o,p</sub> & S<sub>o,m</sub>) from oocyte subcompartment 4 (O<sub>4</sub>) and FOM.
- Spawning (SPWN) followed the FOM stage and was stimulated by 17,20-DHP (S<sub>p,spwn</sub>).

### Hepatic synthesis and secretion of VTG

The well-studied E2-stimulated synthesis of VTG permitted development of a mathematical model for VTG, which demonstrates the feasibility of modeling gene & protein expression in conjunction with BPG model. Model-based differential equations are shown at right in Box 2. The plasma profile of secreted VTG is described by a three-compartment PK model (Schultz et al. 2001). This model is then linked to the BPG model by the ovarian synthesis of E2 and its transport to the liver. E2 kinetics were characterized using a two-compartment model and values for systemic distribution and clearance obtained from rainbow trout (Baronier et al. 1987). Systemic clearance of VTG in female rainbow trout was based on Tyler et al. (1991).

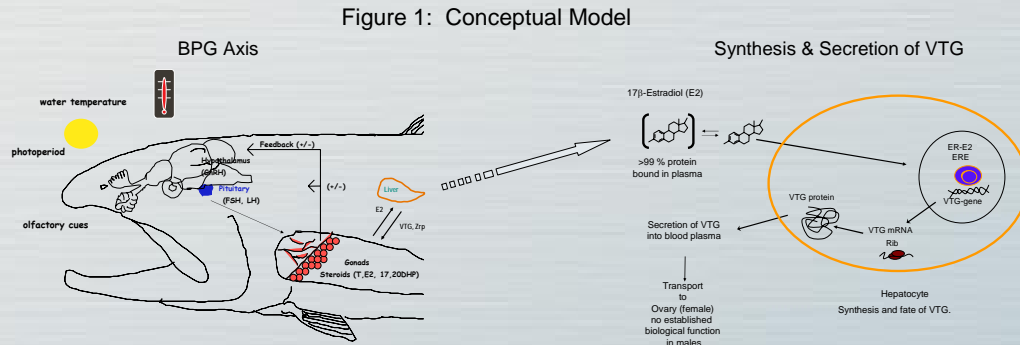


Figure 1: Conceptual Model

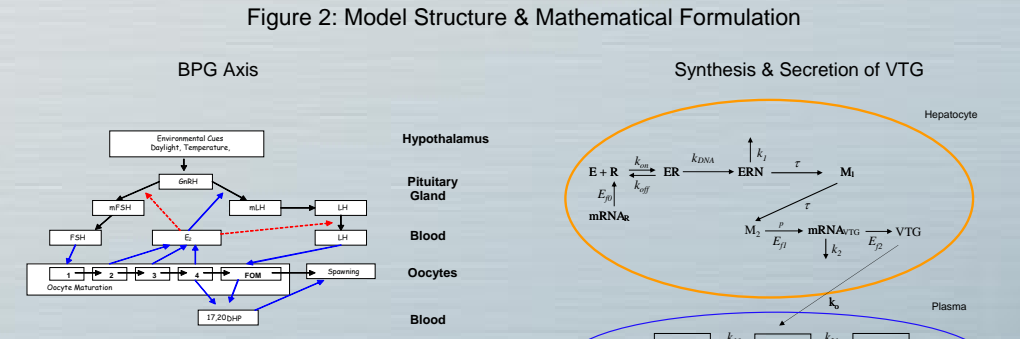


Figure 2: Model Structure & Mathematical Formulation

If  $75 \leq t \leq 250$ , then  $G = 50$  (Unitless), otherwise,  $G = 0$

$$\frac{d\text{mF}}{dt} = k_{i,\text{mf}} \cdot \left( 1 + \frac{I_{E,\text{mf}}}{E_{2,\text{mf}}} + S_{o,\text{mf}} \cdot G \right) - k_{d,\text{mf}} \cdot \text{mF} \quad (\text{Eq 1})$$

$$\frac{dF_p}{dt} = \frac{1}{V_{d,F}} \cdot (k_{i,F} \cdot \text{mF} - \text{CL}_F \cdot F_p) \quad (\text{Eq 2})$$

$$\frac{d\text{mLH}}{dt} = k_{i,\text{mLH}} \cdot (1 + S_{o,\text{mLH}} \cdot G + S_{E2,\text{mLH}} \cdot E2) - k_{d,\text{mLH}} \cdot \text{mLH} \quad (\text{Eq 3})$$

$$\frac{dLH}{dt} = k_{i,\text{LH}} \cdot \text{mLH} - k_{d,\text{LH}} \cdot \text{LH} - k_{i,\text{LH}} \cdot \left( \frac{I_{E,\text{LH}}}{E_{2,\text{LH}}} + (E2)_n \right) \cdot \text{LH} \quad (\text{Eq 4})$$

$$\frac{dLH_p}{dt} = \frac{1}{V_{d,L}} \cdot \left( k_{i,LH} \cdot \left( \frac{I_{E,\text{LH}}}{E_{2,\text{LH}}} + (E2)_n \right) \cdot \text{LH} - \text{CL}_L \cdot \text{LH}_p \right) \quad (\text{Eq 5})$$

$$\frac{dE2_p}{dt} = \frac{1}{V_{d,E}} \cdot (E2(0) + S_{O2,E2} \cdot O_2 + S_{O3,E2} \cdot O_3 + S_{O4,E2} \cdot O_4 - \text{CL}_{E2} \cdot E2_p) \quad (\text{Eq 6})$$

$$\frac{dP_n}{dt} = \frac{1}{V_{d,P}} \cdot (P(0) + S_{O4,P} \cdot O_4 + S_{FOM,P} \cdot \text{FOM} - \text{CL}_P \cdot P_n) \quad (\text{Eq 7})$$

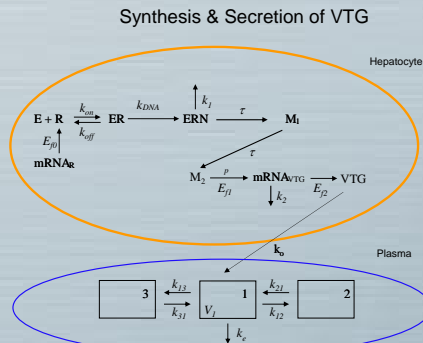
$$O_n = O_n(0) + a_n \cdot \exp\left(-\frac{t-t_n}{W_n}\right) \quad \text{where } n = 1, 2, 3, 4, \text{ and } 5 \text{ (FOM)} \quad (\text{Eq 8})$$

$$\frac{d\text{SPWN}}{dt} = k_{j,\text{spwn}} \cdot (\text{FOM} + S_{p,\text{spwn}} \cdot P_n) - k_{s,\text{spwn}} \cdot \text{SPWN} \quad (\text{Eq 9})$$

Box 1: BPG Model Equations

### Abbreviations

G = GnRH F = FSH LH = LH P = 17,20-beta-DHP E2 = Estradiol  
 mF = FSH mRNA mLH = LH mRNA  
 $k_{i,\text{mf}}$  &  $k_{i,\text{mLH}}$  = Zero-order synthetic rate for individual mRNAs (mF & mLH)  
 $k_{d,\text{F}}$  &  $k_{d,\text{LH}}$  = First-order synthetic rate constant for individual proteins (F & LH)  
 $k_{i,F}$  = First-order elimination rate constant for F ( $k_{i,F}$ ,  $k_{i,LH}$ ,  $k_{i,LH}$ )  
 $k_{i,LH}$  = First-order release rate constant for LH  
 $k_{i,E}$  = Zero-order synthetic rate for individual proteins (E2 & DHP)  
 $X_p$  = Plasma (blood) concentrations of proteins (FH, LH, E2, P)  
 $V_{d,F}$  = Volume of distribution for F ( $V_{d,F}$ ,  $V_{d,LH}$ ,  $V_{d,E2}$ ,  $V_{d,P}$ )  
 $\text{CL}_F$  = Plasma clearance for F ( $\text{CL}_F$ ,  $\text{CL}_L$ ,  $\text{CL}_E$ ,  $\text{CL}_P$ )  
 $k_{s,\text{spwn}}$  &  $k_{j,\text{spwn}}$  = Zero-order formation rate ( $k_{s,\text{spwn}}$ ) and first-order elimination rate constant ( $k_{j,\text{spwn}}$ ) for spawning  
 $S_{X,Y}$  = Stimulatory factor from X to Y ( $S_{O2,E2}$ ,  $S_{O3,E2}$ ,  $S_{O4,E2}$ ,  $S_{O4,P}$ ,  $S_{p,\text{spwn}}$ )  
 $I_{E,F}$  = Inhibitory factor from X to Y ( $I_{E,\text{mf}}$ ,  $I_{E,\text{LH}}$ )



## Results

Figure 3: Model Predictions

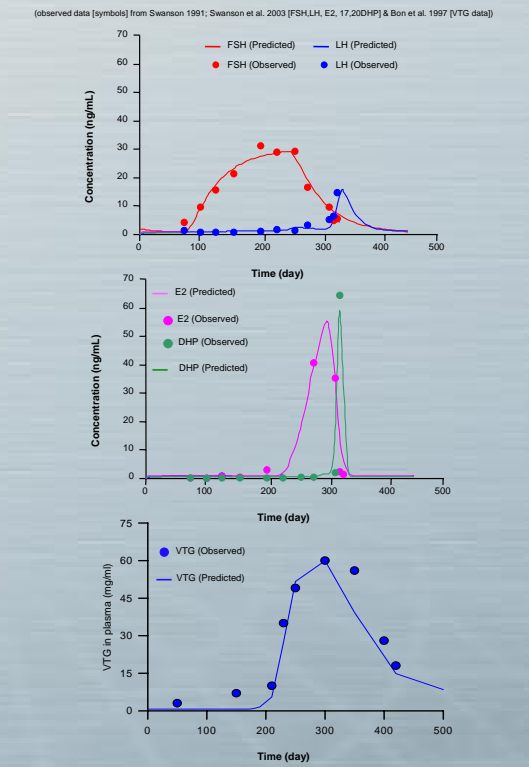


Figure 3: Model Predictions

Model predictions accurately reflected measured profiles for FSH & LH (Figure 3 top), E2 and 17,20DHP (Figure 3 middle) and VTG (Figure 3 Bottom). This provides strong support that the modeling approach described in this study can accurately characterize the synthesis and secretion of BPG hormones regulating reproduction in fish. The accurate description of the synthesis of an E2 regulated protein (VTG) also suggests the model can be expanded to describe the synthesis of other proteins which are regulated by BPG hormones.

The model can provide insight into the complex working of the reproductive endocrine system of a gonochoristic fish, and the interplay of genes involved in the sensing and implementation of hormonal signals. The present model was developed for synchronous annual spawning fish (e.g. trout & salmon). After modification to model equations describing oocyte maturation, the model can also be used with asynchronous spawning fish such as fathead minnows and Japanese medaka. Ultimately, these models will be a useful tool for exploring the effects on reproduction of BPG system perturbations caused by exposure to endocrine disruptors.

Other applications for the BPG model are:

- Providing a framework to focus QSAR modeling & actions of suspect endocrine disruptors on specific areas in the fish reproductive system. For example, QSAR predictions of E2 receptor affinity can be integrated with our model to assess the effect on pituitary function and oocyte maturation.
- Allows integration of toxicogenomic / proteomic data into a model which can predict population level consequences (effects on reproduction).

## Discussion

Baronier JF, Foster A, Zohar Y, and Marozzi O (1987). The metabolic-clearance rate of estradiol-17 beta in rainbow-trout, *Salmo gairdneri* R., estimated by both single injection and constant infusion methods. Increase during oocyte maturation. *General and Comparative Endocrinology* 66: 85-94.  
 Bon E, Baber L, Rodriguez AI, Cussac B, Patisson C, Sempere JF, Lallemand E. (1997). Plasma vitellogenin levels during the annual reproductive cycle of the female rainbow trout (*Oncorhynchus mykiss*): Establishment and validation of an ELISA. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 117 (1): 75-84.  
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### Box 2: Liver-VTG Model Equations

$$\frac{d(R)}{dt} = -k_{off} \cdot (E) \cdot (R) + k_{on} \cdot (ER) + E_{in} \cdot (mRNA_E) \quad (\text{Eq 1})$$

$$\frac{d(ER)}{dt} = k_{on} \cdot (E) \cdot (R) - (k_{off} + k_{DNA}) \cdot (ER) \quad (\text{Eq 2})$$

$$\frac{d(ERN)}{dt} = k_{DNA} \cdot (ER) - k_1 \cdot (ERN) \quad (\text{Eq 3})$$

$$mRNA_E = mRNA_{E_0} \cdot e^{-(k_{degradation}) \cdot t} + mRNA_{E_{new}} \cdot (1 - e^{-(k_{degradation}) \cdot t}) \quad (\text{Eq 4})$$

$$\frac{dM_1}{dt} = \frac{(ERN - M_1)}{\tau} \quad (\text{Eq 5})$$

$$\frac{dM_2}{dt} = \frac{(M_1 - M_2)}{\tau} \quad (\text{Eq 6})$$

$$\frac{d(mRNA_{VTG})}{dt} = E_{j1} \cdot (M_2)^j - k_2 \cdot (mRNA_{VTG} - mRNA_{VTG_0}) \quad (\text{Eq 7})$$

$$\frac{V_d dVTG}{dt} = k_3 \cdot X_3 + k_{31} \cdot X_1 - (k_{12} + k_{13} + k_4) \cdot V_1 \cdot VTG + E_{j2} \cdot (mRNA_{VTG}) \quad (\text{Eq 8})$$

$$\frac{dX_2}{dt} = k_{12} \cdot V_1 \cdot VTG - k_{21} \cdot X_2 \quad (\text{Eq 9})$$

$$\frac{dX_3}{dt} = k_{13} \cdot V_1 \cdot VTG - k_{31} \cdot X_3 \quad (\text{Eq 10})$$

**Abbreviations**  
 $k_{on}$  &  $k_{off}$  = rate constants describing E2 binding to ER  
 $E_{in}$  = relates the ER production rate to the concentration of receptor messenger RNA (mRNA<sub>E</sub>)  
 $ERN$  = the activated ER-E2 complex  
 $mRNA_{E_0}$  &  $mRNA_{E_{new}}$  = the mRNA<sub>E</sub> concentrations initially and after up-regulation  
 $k_{DNA}$  &  $k_1$  = rate constants for binding & dissociation of ERN from DNA.  
 $M_1$  &  $M_2$  = Transfer compartments for transport of mRNA<sub>VTG</sub> to ribosome.  
 $\tau$  = avg. time for eqs 5-6.  
 $E_{j2}$  = relates translation of VTG mRNA (mRNA<sub>VTG</sub>) into VTG protein.  
 $k_{12}$  &  $k_{13}$  = First-order rate constants for VTG distribution & elimination.  
 $V_1$  = apparent volume of central compartment.  
 $X_2$  &  $X_3$  = the amounts of VTG in peripheral compartments.