

FY06 Pilot study: Vitellogenin monitoring of PPW Cutthroat female broodstock at LNFH and time of movement into chilled water units, Oct05-Jan06.

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Summary

1. Plasma vitellogenin (VTG) was a good indicator of the potential to spawn
VTG could be detected in plasma but not mucus (sensitivity of assay)
 - a. Method (ref.1) needs more validation work,
 - i. specific concentration questionable- relative data only
 - b. Alkaline inorganic phosphate method (Ref2) requires a minimum of 1-2 mL plasma, unsuccessful with extraction
2. Plasma Calcium > 13 mg/dL fair indicator of potential to spawn (ref 3 method).
There was modest correlation between high Ca values and VTG (Fig2).
Vtg ELISA needs further validation for quantification purposes, other indirect methods were not satisfactory

2. Data of the 15 females tracked (5/each in the Oct, Nov, and Dec Chill groups): See attached spreadsheet for individual female data
 - a. 9 spawned, 1 mort, 4 did not ovulate (these females could also be identified by low VTG)
 - b. % Eyeup ranged 0 -96%, 6 lots >50%
 - c. No. of females with egg lots >50% eye up
 - i. OCT =2
 - ii. NOV = 3
 - iii. DEC = 1
 - d. Spawning (6Mar – 1May) occurred 77 – 184 days after move to chilled water and neither VTG profile or eyeup was obviously related to time of chilling. VTG profile indicated that vitellogenesis was peaking after the last plasma samples were collected on 04Jan.

No strong relationship between start of chilling : VTG profile or eye-up (see Fig1).
It is recommended that movement to 7° system occur ~ 30d prior to expected spawn date (mid-December?)

Areas for further consideration

- e. Incubation temperature (narrow range? Fluctuating?), DO& TGS levels in stacks
- f. Final egg maturation period (~2-14d) temperature
- g. Female diet (nutrition – specific additives& lipids months in advance of vitellogenesis)
- h. Close monitoring of diurnal DO & TGS in female tanks

Objectives:

1. Examine effect of chilled water (7-8°C) entry on female VTG response and egg eye-up rate.
2. Determine a method to assay VTG in plasma and mucus
 - ATS VTG based ELISA
 - Extracted phosphate content (standardize by protein)
 - Plasma Calcium

Methods:

Plasma and mucus collection - Approximately 500 – 800 µL of blood was collected with a 1cc syringe (flushed with 250mg/mL lithium heparin solution) from 5 females of the October, November, and December groups placed into the chilled (7-8°C) system. The blood was placed into 1.5mL conical tubes and briefly centrifuged to allow for the collection of 200 – 500 µL of plasma. Plasma was stored frozen at -20C until first assayed in September 2007.

Pointe Scientific kits used for determination of plasma protein (ref2), calcium (ref3), and inorganic phosphate (ref2).

alkaline inorganic phosphate: (ref2)

Reference = **F Gagne and C Blaise. 2000. Technical methods section Organic alkali-labile phosphates in biological materials: A Generic assay to detect vitellogenin in biological tissues (book?), J. Wiley & Sons**

VTG ELISA: (ref1)

Biosense Laboratories AS- rabbit anti- Atlantic salmon VTG (V01402201-100) and purified 5-10µg VTG (V01002301-001)

Reference = **ND Denslow, MC Chow, KJ Kroll and L Green. 1999. Vitellogenin as biomarker of exposure for estrogen or estrogen mimics. Ecotoxicology 8: 385-398. Direct ELISA p394-395.**

1000x dil of plasma, mucus 100x, 2000x antiVTG (primary)& KPL peroxidase labeled anti-rabbit (secondary) , overnight incubation of samples and antibodies

Results; See attached Excel spreadsheet for plasma calcium and VTG ELISA values. Ignore protein data.

Note: Mucus samples did not contain enough VTG for the current ELISA

Figure 1 Relationship between days reared on chilled water and % eye-up. No strong relationship is apparent.

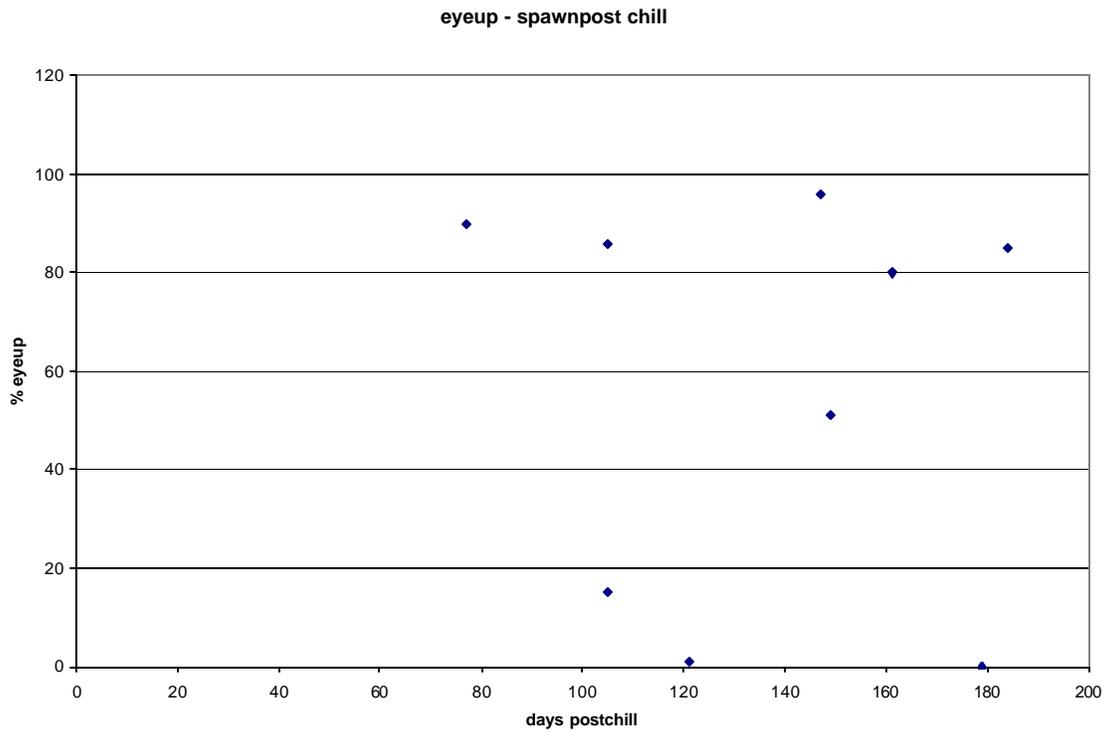


Figure 2 Relationship between plasma calcium and vitellogenin for all females. Modest correlation for samples with Ca > 13.

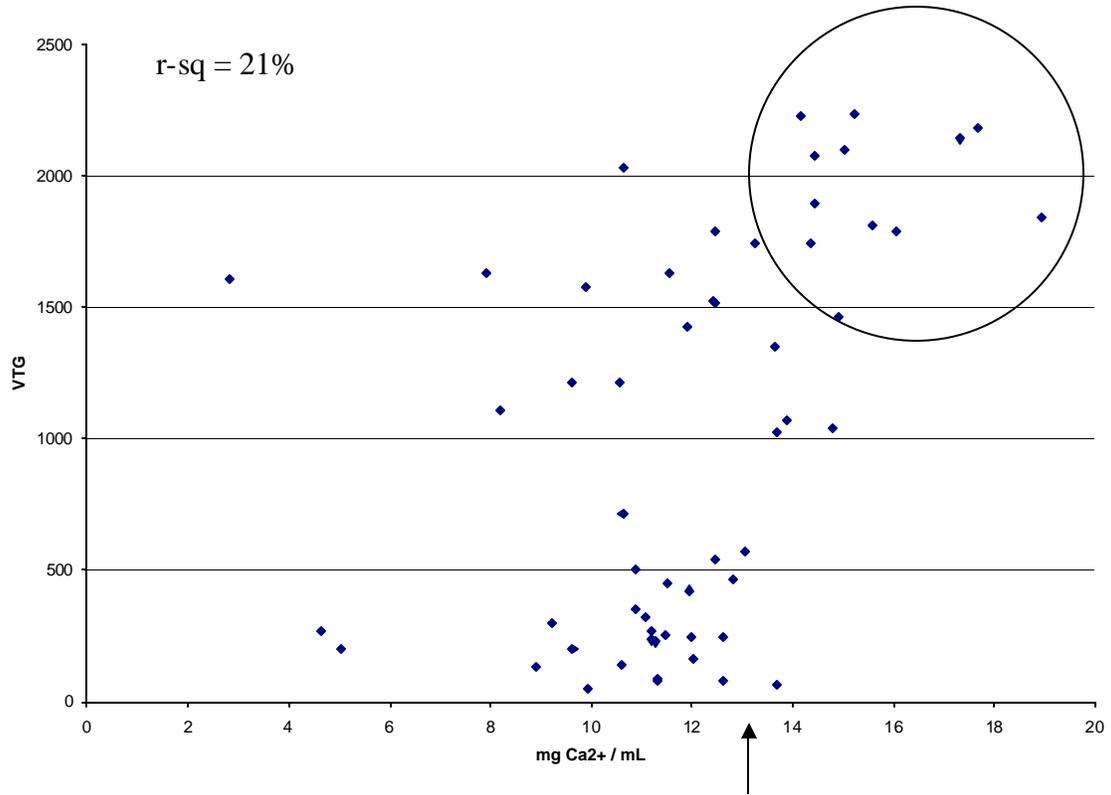


Figure 3. Calcium – Vtg relationship for female moved to 7°C water in October and spawned 13 March with 96% eye-up.

