Reproductive Biology and

Maturation Control of Brook Trout

(S*alvelinus fontinalis*, Mitchill) in Tasmania

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Resource Sustainability
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Declaration

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of the my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Bio safety Committees of the University.

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Statement of Co-authorship

Following publication is part of this thesis as chapter 2.

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Dedicated

To

My Lord

Allah the Almighty

&

My Family

Abstract

Pilot scale commercial production of brook trout (*Salvelinus fontinalis*) in Tasmania encountered a high incidence of early sexual maturation particularly in males. Attempts by commercial growers to produce all-female populations (by neomale–female crosses) using standard industry techniques were unsuccessful. As maturation has a deleterious effect upon flesh quality, growth and immunocompetency, commercial production was aborted until this issue could be resolved. This study firstly aimed to describe early gonadal development, sex differentiation, developmental and endocrine changes during puberty and throughout the annual reproductive cycle to establish baseline information pertinent to the species reproductive strategies under Tasmanian environmental conditions. Once determined, the results from that study facilitated an experiment investigating the most appropriate timing and approach for the production of neomales. Photoperiod manipulation in mixed populations of brook trout was likewise investigated as a further means of reducing the incidence of early maturation.

Sex differentiation and gonadal development in brook trout was studied from 8 degree days post-hatch (ºdph) until the age of 27 months. Gonadal development was histologically studied while plasma profiles of testosterone, estradiol-17β and 11-KT were measured by RIA and ELISA. Gonadal development began during pre-hatch period and undifferentiated gonads directly developed into testes and ovaries at 3354 ºdph. Males had attained puberty by the age of 14 months. However, females did not achieve maturation during their first year. During the second year, maturation was observed during March and May for males and females respectively. This study determined that pre-hatch period might be the appropriate time for sex inversion treatment as development of undifferentiated gonads commenced during this time period. Moreover, variations in gonadal development and profiles of sex steroids were

controlled by seasonal changes in photoperiod during second year thus suggesting the possible inhibition of maturation under manipulated photoperiod conditions.

Neomale production was undertaken by immersion and in-feed treatment (and a combination of both) with 17α-methyltestosterone (MT). Sex inversion treatment targeted the pre-hatch, hatch and post-hatch period to determine the most sensitive window of time. Treatment given during 4 to 6 days pre-hatch (at 400 µg/L MT for 4 hours) was found to be the most successful producing a population containing 75% of male fish without the occurrence of any sterile fish. Feeding fish a MT supplemented diet (3 mg/kg) for 60 days from first feeding did not affect the normal sex ratio and MT oral treatment combined with immersion treatments resulted in high percentages of sterile fish. In the present study, 4 to 6 days pre-hatch was found to be the most sensitive window of time to produce the highest male population with only two immersions thus significantly reducing the dose of MT and treatment duration.

Three photoperiod regimes over a 10 month period during the second reproductive year were tested. The regimes were simulated natural photoperiod (NP), advanced photoperiod accelerated by 8 weeks (AP) and continuous illumination (CP). Fish exposed to advanced photoperiod corrected their maturation cycle by an advanced phase shift of their endogenous rhythm. Advanced photoperiod inhibited maturation by 6% and 8% in males and females, respectively, relative to natural photoperiod treated fish in which 100% maturation occurred. However, most of the fish successfully recruited for maturation presumably because the threshold of growth during the "critical phase" of photoperiod treatment was surpassed. Similarly, treatment of continuous photoperiod failed to inhibit the onset of maturation however continuous photoperiod did inhibit the final stage of maturation.

Overall, the present study provided the baseline data about gonadal differentiation, puberty and annual reproductive cycle of brook trout under Tasmanian climate conditions, reported for first time for the southern hemisphere. Furthermore, this PhD project provided the most efficient and commercially applicable sex inversion protocol to masculinize genetic brook

trout females, the most critical step of indirect feminization. Progeny testing by crossing these neomales and normal females can be conducted as a future study to produce all-female population. Furthermore, this protocol may produce 100% male population if applied to gynogenetic females instead of mixed sex culture which needs to be investigated. Moreover, this study suggested the possible success of photoperiod manipulation to control maturation in brook trout but further refinement of photoperiod regimes is required before its commercial trial.

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Table of Contents

Declaration i
Authority of Access ii
Statement of Ethical Conduct iii
Statement of Co-authorship iv
Abstract v-vii
Acknowledgements viii
Table of Contents ix-xi
List of Figures and Tables xii-xviii
List of Abbreviations xix-xx
Thesis Format xxi

CHAPTER 1 – General Introduction 1

1.1 Commercial Importance of Brook Trout in Tasmanian Aquaculture 1
1.2 Gonadal Development and Sex Differentiation 2
1.3 Puberty 6
1.4 Circannual Rhythm of Maturation after Puberty 9
1.5 Endocrine Sex Reversal to Control Maturation by Production of all-Female Population 13
1.6 Control of Maturation by Manipulation of Photoperiod 16
1.7 Aim and Objectives of Research 18
1.8 References 19

CHAPTER 2 – Histological Study of Gonadal Development and Sex Differentiation in *Salvelinus fontinalis* under Tasmanian Climate Conditions 39

2.1 Abstract 39
2.2 Introduction 41
2.3 Materials and Methods 48
2.3.1 Sampling 50
2.3.2 Histological Study 51
2.4 Results 53
2.4.1 Histological Development of Gonadal Differentiation 53
2.4.1.1 Histological Development during the Undifferentiated Phase (8 - 2013°dph) (0.04 ± 0.05 - 2.47 ± 0.01 g) 53
2.4.1.1.1 Stage I (8 °dph) (0.04 ± 0.05 g) 53
2.4.1.1.2 Stage II (66 – 115 °dph) (0.04 ± 0.05 - 0.05 ± 0.01 g) 54
2.4.1.1.3 Stage III (172 °dph) (0.05 ± 0.01 g) 54
2.4.1.1.4 Stage IV (589 – 2013 °dph) (0.48 ± 0.06 - 2.47 ± 0.01 g) 54
2.4.1.2 Histological Development during the Differentiated Phase (2769 – 3354 °dph) (6.20 ± 0.72 – 12.25 ± 0.92 g) 55
2.4.1.2.1 Stage V (2769 °dph) (6.20 ± 0.72 g) 55
2.4.1.2.2 Stage VI (3354 °dph) (12.25 ± 0.92 g) 56
2.5 Discussion 59

2.6 References 66

CHAPTER 3 – Annual Variations in the Profiles of Sex Steroids and Gonadal Development of Brook Trout (*Salvelinus fontinalis*) during the Puberty and the Annual Reproductive Cycle in Captivity **70**

3.1 Abstract 70
3.2 Introduction 71
3.3 Materials and Methods 78
3.3.1 Sample Collection 78
3.3.2 Histology 80
3.3.3 Total Fecundity 80
3.3.4 Whole-Body Steroid Extraction 81
3.3.5 Hormone Analysis 81
3.3.6 Quantitative Estimation of Activity of 3β-HSD and 17β-HSD in Gonads 82
3.3.7 Determination of Crude Lipids in Liver 84
3.3.8 Meteorological Data 84
3.3.9 Statistical Analysis 84
3.4 Results 85
3.4.1 Photoperiod and Water Temperature 85
3.4.2 First Year (age = 8 – 15 months) 85
3.4.3 Second Year (age = 16 - 27 months) 95
3.5 Discussion 117
3.6 References 125

CHAPTER 4 – Sex Reversal of Brook Trout (*Salvelinus fontinalis*) by 17α-methyltestosterone Immersion **140**

4.1 Abstract 140
4.2 Introduction 142
4.3 Materials and Methods 145
4.3.1 Methyltestosterone (MT) Immersion Treatment 146
4.3.2 Methyltestosterone Feeding Treatment 146
4.3.3 Sampling 149
4.3.4 Statistical Analysis 151
4.4 Results 153
4.4.1 Non-immersed Controls (C-1 and C-2) 153
4.4.2 Immersed Treatments (I-1 – I-11) 154
4.4.3 Non-immersed – MT Fed Controls (C-1F and C-2F) 153
4.4.4 Immersed – MT Fed Treatments (I-1F - I-11F) 154
4.5 Discussion 157
4.6 References 164

CHAPTER 5 – The Effect of Photoperiod Manipulation upon Maturation and Sex Steroids Profiles of Brook Trout (*Salvelinus fontinalis*) **170**

5.1 Abstract 170

5.2 Introduction 171
5.3 Materials and Methods 176
5.3.1 Experimental Design 177
5.3.2 Sampling 177
5.3.3 Histological Study 178
5.3.4 Determination of Crude Lipid in Liver 179
5.3.5 Total Fecundity 179
5.3.6 Hormone Analysis 179
5.3.7 Statistical Analysis 180
5.4 Results 182
5.4.1 Condition Factor 182
5.4.2 Gonadosomatic Index (GSI) 182
5.4.3 Liver Weight, HSI and Liver Crude Lipids 183
5.4.4 Profile of Testosterone 184
5.4.5 Profile of Estradiol-17β 185
5.4.6 Histological Changes during Ovarian Development 186
5.4.7 Histological Changes during Testicular Development 187
5.5 Discussion 197
5.6 References 204

CHAPTER 6 – General Discussion **210**

6.1 References 222

Appendix I 231

Appendix II 240

Appendix III 250

Appendix IV 257