COMPARISON OF FAMILY, SOURCE AND REARING TEMPERATURE EFFECTS ON EARLY GONAD DEVELOPMENT AND PHENOTYPIC SEX IN WILD AND HATCHERY STEELHEAD, *Oncorhynchus mykiss*

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UNIVERSITY OF HAWAI'I 1907 UA MAU KE EA O KA 'AINA I KA PONO

OREGON HATCHERY RESEARCH CENTER

OSU Oregon State UNIVERSITY

FACTORS AFFECTING EARLY DEVELOPMENT OF PHENOTYPICALLY PLASTIC SALMONIDS SUCH AS STEELHEAD/RAINBOW TROUT (*Oncorhynchus mykiss*)

ORIGINS: WILD vs HATCHERY

PARENTAL: FAMILY LINE

GENOTYPIC SEX: MALE vs FEMALE

ENVIRONMENT: FOOD AVAILABILITY, TEMPERATURE, pH

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STUDY QUESTIONS

TO WHAT EXTENT DO GENETIC FACTORS AND/OR REARING TEMPERATURES:

i) ALTER PHENOTYPIC SEX WITH RESPECT TO GENOTYPIC SEX?

ii) AFFECT PATTERNS OF GONADAL DEVELOPMENT?

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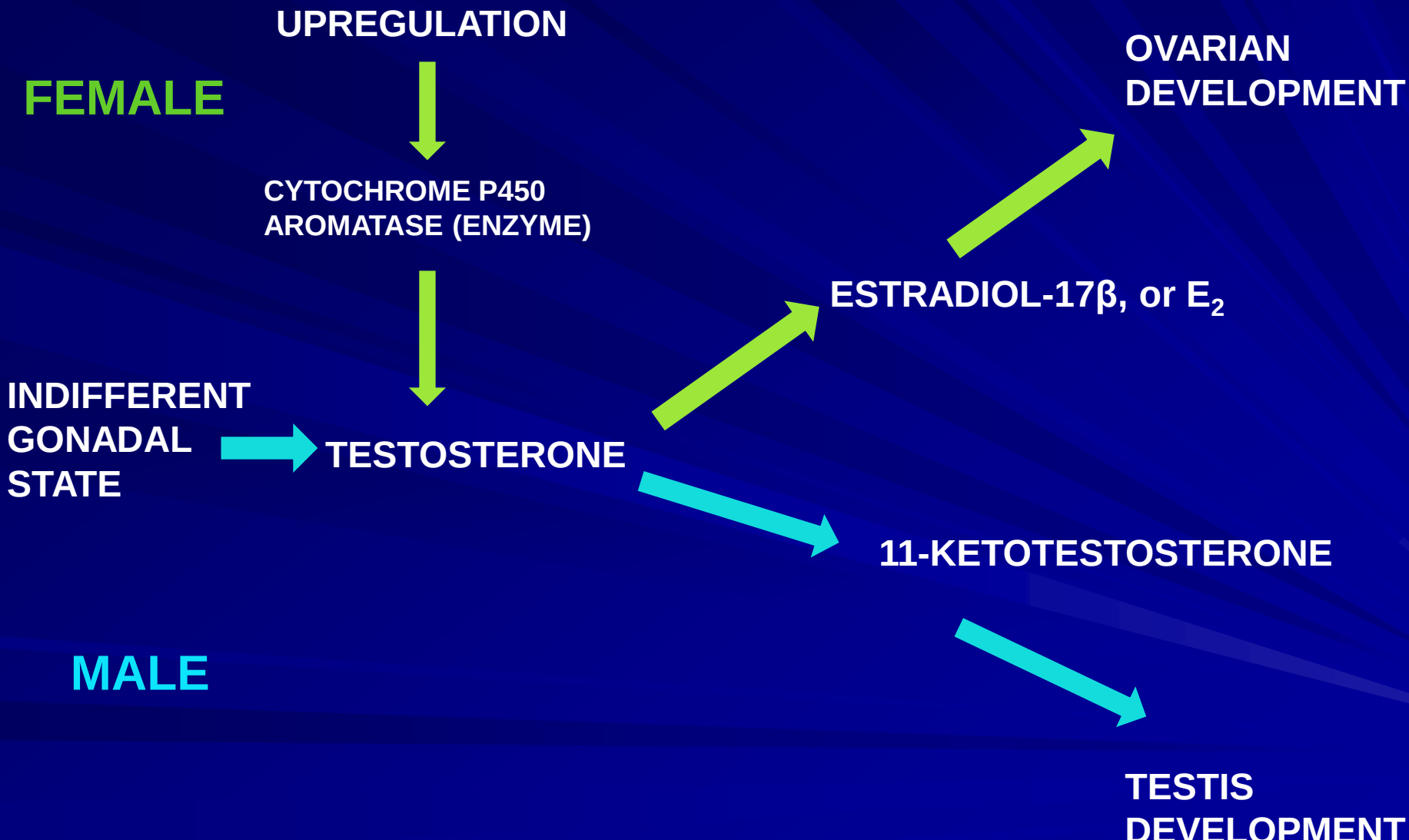
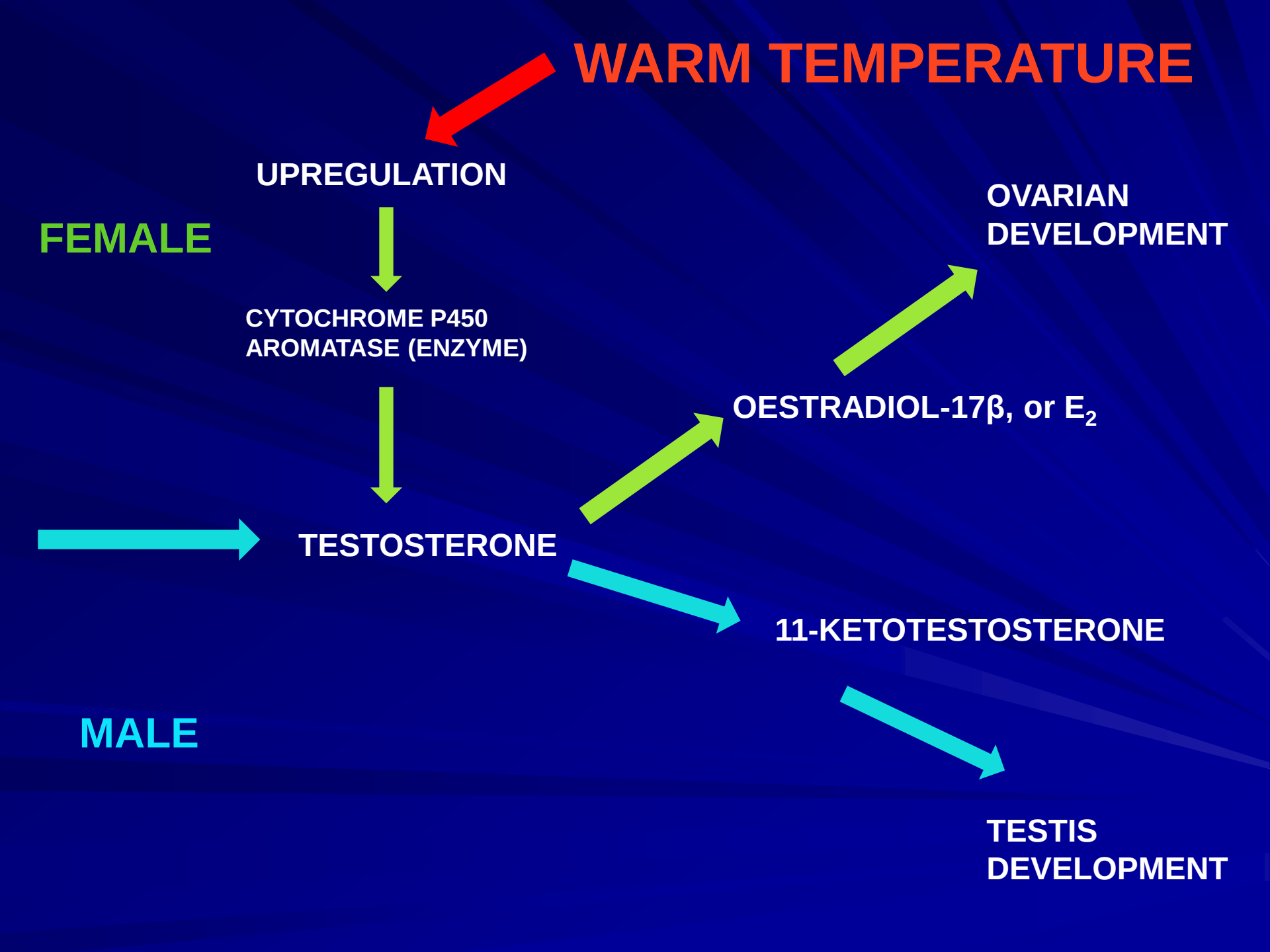
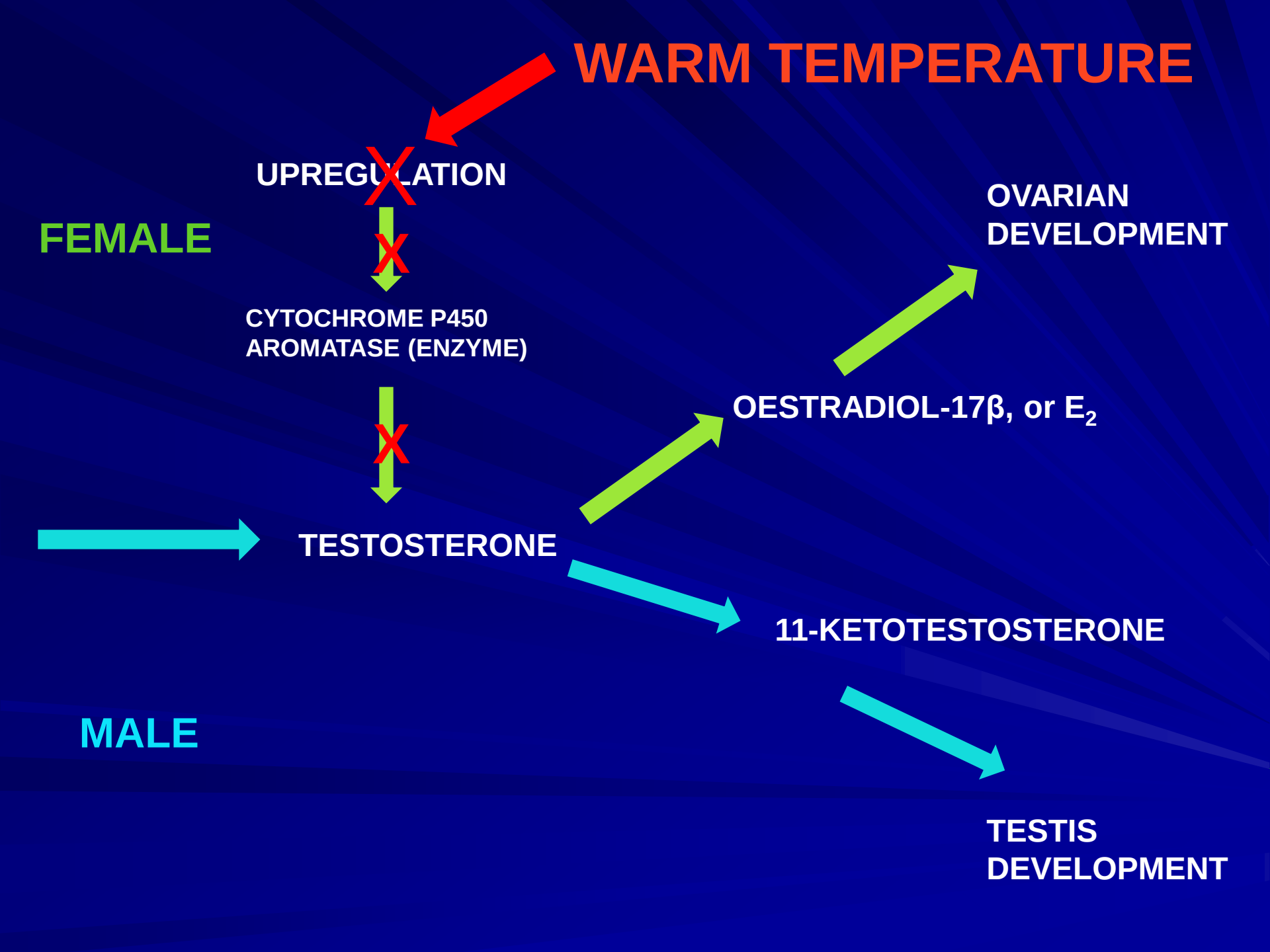
RATIONALE FOR WHY ELEVATED WATER TEMPERATURE MAY INFLUENCE PHENOTYPIC SEX

NORMAL SEXUAL DEVELOPMENT IN TELEOSTS




WARM TEMPERATURE
UPREGULATION
FEMALE
CYTOCHROME P450
AROMATASE (ENZYME)
OVARIAN
DEVELOPMENT
OESTRADIOL-17β, or E_2
TESTOSTERONE
11-KETOTESTOSTERONE
MALE
TESTIS
DEVELOPMENT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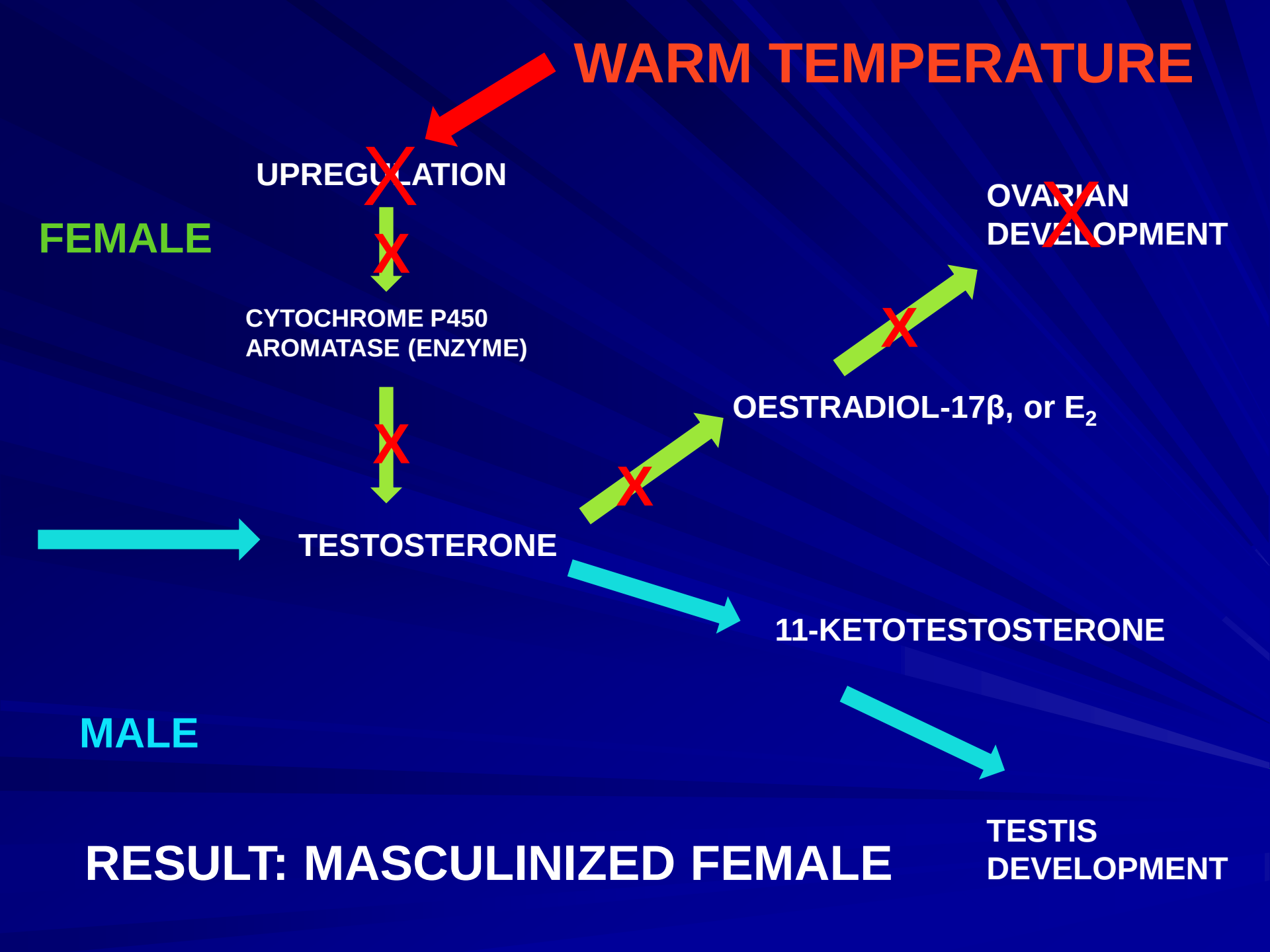
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DEVELOPMENT
OESTRADIOL-17β, or E_2
TESTOSTERONE
11-KETOTESTOSTERONE
MALE
TESTIS
DEVELOPMENT
RESULT: MASCULINIZED FEMALE

QUESTION

DOES EARLY EXPOSURE TO ELEVATED WATER PROMOTE THE DEVELOPMENT OF PHENOTYPIC MALES AMONG GENOTYPIC FEMALES IN *Oncorhynchus mykiss*?

AND IF YES, DO GENETIC FACTORS EXAMINED HERE INFLUENCE PHENOTYPIC REPONSE?

NOVEL APPROACH:

WE ADDRESSED THIS QUESTION BY EXAMINING BOTH PHENOTYPIC AND GENOTYPIC SEX

LOCATION:
OREGON HATCHERY RESEARCH CENTER

EXPERIMENTAL DESIGN

FISH SOURCE: WILD OR HATCHERY															
FAMILY 1				FAMILY 2				FAMILY 3				FAMILY 4			
AMBIENT		HEATED		AMBIENT		HEATED		AMBIENT		HEATED		AMBIENT		HEATED	
REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2

EXPERIMENTAL VARIABLES

Source (2): (i) wild

(ii) hatchery

Family (parental) lines within source (pair-spawnings): four

Temperature regimes (2) : (i) ambient

(ii) heated = ambient + 5°C applied from fertilization to swim up

Replicates: two

TOTAL OF 32 EXPERIMENTAL GROUPS

EXPERIMENTAL DURATIONS: 145-245 dpf

TREATMENT GROUP	DURATION RANGE (DAYS)	TOTAL TU RANGE
HATCHERY		
AMBIENT		
FAMILY 1	145	2898
FAMILY 2	145-237	2898-4918
FAMILY 3	145	2898
FAMILY 4	146-149	2924-3003
HEATED		
FAMILY 1	147-236	3263-5216
FAMILY 2	145-237	3263-5231
FAMILY 3	238-239	5246-5263
FAMILY 4	243	5317
WILD		
AMBIENT		
FAMILY 1	146	2924
FAMILY 2	146	2924
FAMILY 3	146	2924
FAMILY 4	147	2951
HEATED		
FAMILY 1	147-236	2289-4239
FAMILY 2	147-239	3263-5263
FAMILY 3	239	5263
FAMILY 4	243-245	5317-5351

MAINTENANCE AND REARING TEMPERATURE CONDITIONS

FOR 32 EXPERIMENTAL GROUPS

HEATED OR AMBIENT

AMBIENT

INDOOR

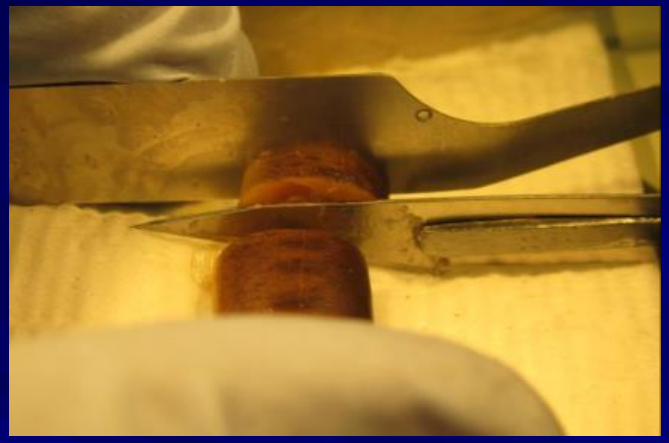
OUTDOOR

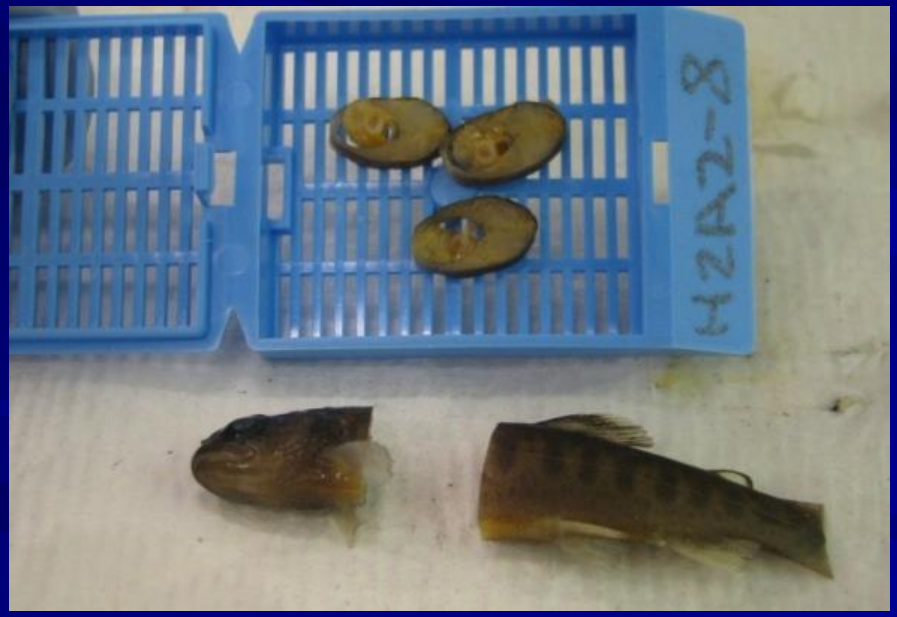
AMBIENT

INITIAL FISH PROCESSING

FISH WERE EUTHANIZED, WEIGHED (g), MEASURED (mm, TL) , TISSUE-SAMPLED FOR FUTURE GENOTYPING, AND PRESERVED INDIVIDUALLY IN SEPARATE VIALS OR TISSUE CAPSULES, IN 10% BUFFERED FORMALIN.

HISTOLOGY FISH PROCESSING

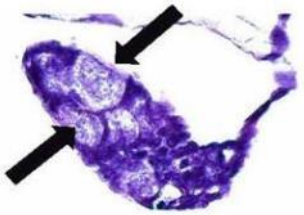




Histology protocols followed Cole (2008, 2011).

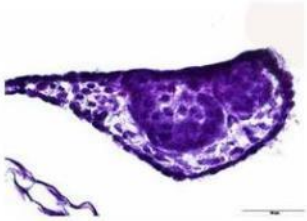
FOUR DEVELOPMENTAL CLASSES OF GONAD MORPHOLOGY FOR *Oncorhynchus mykiss* RECOGNIZED FOR THIS STUDY

INDIFFERENT GONAD



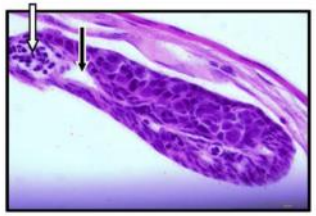
A. Indifferent stage gonad with primordial germ cells (black arrows) conspicuous by their larger size, surrounded by less well-defined somatic cells. 102 dpf. 1185 TUs. Scale bar, 50 µ.

B. Indifferent stage gonad starting to assume a club-like shape triangular in cross-section (frequently typical of a future testis. 102 dpf. 1185 TUs. Scale bar, 50 µ.

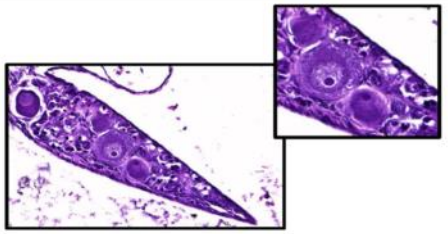


C. Indifferent stage gonad with primordial germ cells and surrounding somatic cells. 102 dpf. 1185 TUs. Scale bar, 50 µ.

DEVELOPING OVARY

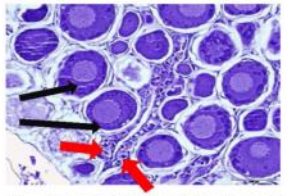


A. Genotyped female, indifferent stage gonad showing blood vessel lumen (white arrow), and gonadal lumen (black arrow). 237 dpf. 4918 TUs. Scale bar, 20 µ.



B. Detail and inset of genotyped female early stage ovary with chromatin nucleolus stage oocytes surrounded by stromal somatic cells. 147 dpf. 3263 TUs. Scale bar, 100 µ.

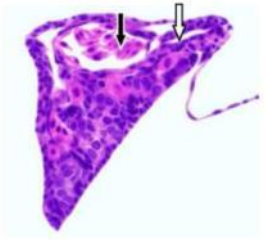
C. Genotyped female, developing ovary showing early lamellar folding and perinucleolus oocytes . 147 dpf. 3263 TUs. Scale bar, 500 µ.



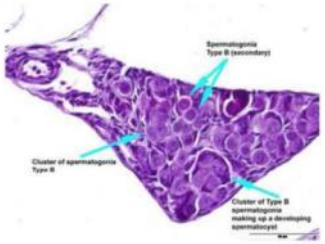
D. Genotyped female, developing ovary. showing perinucleolar oocytes (black arrows) and oogonia (red arrows). 147 dpf, 3263 TUs. Scale bar, 500 µ.

DEVELOPING TESTIS

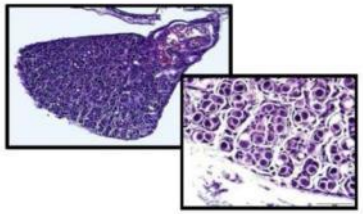
A. Genotyped male, indifferent gonad. Detail showing blood vessel lumen (white arrow), and gonadal lumen (black arrow). 237 dpf. 4918 TUs. Scale bar, 50 µ.



B. Genotyped male, indifferent gonad. Detail showing blood vessel lumen (white arrow), and gonadal lumen (black arrow). 237 dpf. 4918 TUs.

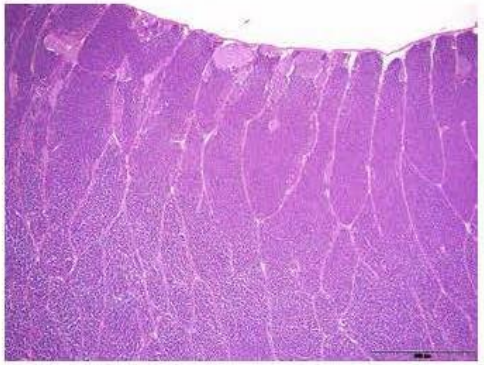


C. Early testis. Spermatogonia Type B single stage predominating. 115+17=132 dpf. 1092° TUs. Scale bar, 50 µ.



D. Early developing testis of genotyped male showing spermatogonia Type B spermatocysts acquiring a cord-like arrangement prior to seminiferous tubule formation. Scale bar in lower right image, 50 microns. 147 dpf. 3263 TUs.

MATURE TESTIS



Mature (precocious) testis. Wild, heated. 236 dpf. 5216 Tus. Scale bar 500 µ.

HISTOLOGY

16 FISH RANDOMLY SELECTED FROM EACH TREATMENT GROUP (N=16) IN TWO WILD FAMILIES (W1, W2) AND TWO HATCHERY FAMILIES (H1,H2)

….. NUMBER OF FISH = 256

279 ADDITIONAL FISH WERE RANDOMLY SELECTED ACROSS THE REMAINING TWO WILD FAMILIES (W3, W4) AND HATCHERY FAMILIES (H3, H4), IN SIMILAR NUMBERS PER TREATMENT GROUP.

TOTAL NUMBERS OF FISH PROCESSED

TOTAL NUMBER OF FISH EXAMINED HISTOLOGICALLY (i.e., FOR GONADAL (OR PHENOTYPIC) SEX) = 535

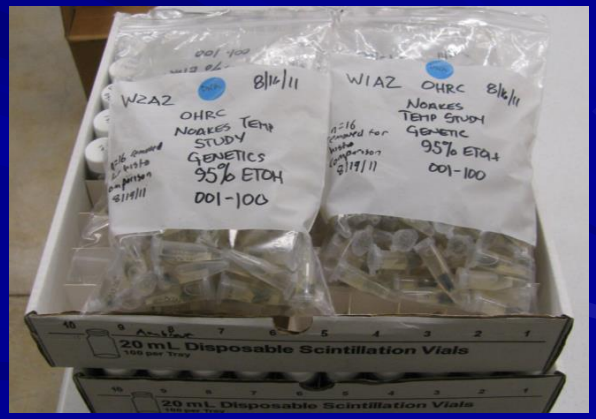
GENOTYPING (GENOTYPIC OR CHROMOSOMAL SEX)

GENOTYPING PROTOCOL

GENOTYPING PROTOCOL FOR DETERMINING GENOTYPIC SEX IN *Oncorhynchus mykiss* USED THE *OMYY1* MARKER (Brunelli et al. 2008) FOLLOWING Rundio et al. (2012), AS SUMMARIZED IN Thompson et al. (2015).

321 FISH WERE GENOTYPED AND ASSIGNED A CHROMOSOMAL SEX

BASED ON HISTOLOGY, 283 (88%) OF GENOTYPED FISH HAD A SEXUALLY DIFFERENTIATED GONAD AND COULD ALSO BE ASSIGNED A PHENOTYPIC SEX.



CONCORDANCE RESULTS

AMONG THE 283 FISH THAT WERE ASSIGNED A SEX USING HISTOLOGY AND GENOTYPING, ALL BUT FOUR FISH SHOWED THE SAME GONADAL AND GENETIC SEX

CONCORDANCE BETWEEN PHENOTYPIC AND CHROMOSOMAL SEX WAS 99%

NON-CONCORDANT FISH: n=4

NON-CONCORDANT FISH (n=4)		
	HATCHERY	WILD
AMBIENT	1	1
HEATED	1	1

NON-CONCORDANT FISH (n=4)		
	HATCHERY	WILD
AMBIENT	♂ GENO, ♀ PHENO	♀ GENO, ♂ PHENO
HEATED	♀ GENO, ♂ PHENO	♂ GENO, ♀ PHENO

QUESTION 1

DOES EXPOSURE TO ELEVATED WATER TEMPERATURE DURING EARLY DEVELOPMENT ALTER PHENOTYPIC SEX WITH RESPECT TO GENOTYPIC SEX?

NO

THERE IS NO EVIDENCE THAT EXPOSURE TO ELEVATED WATER TEMPERATURE OF 5 C ABOVE AMBIENT, FROM FERTILIZATION TO SWIMUP, RESULTED IN GENOTYPIC FEMALES DEVELOPING AS PHENOTYPIC MALES

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QUESTION 2

TO WHAT EXTENT DO GENETIC FACTORS AND/OR REARING TEMPERATURES:

i) ALTER PHENOTYPIC SEX WITH RESPECT TO GENOTYPIC SEX?

ii) AFFECT PATTERNS OF GONADAL DEVELOPMENT?

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GONAD DIFFERENTIATION ISSUES

535 FISH WERE EXAMINED HISTOLOGICALLY TO ASSIGN GONADAL SEX

40 FISH (7.5%) HAD AN INDIFFERENT GONAD

ALL BUT ONE FISH WERE OF HATCHERY ORIGIN.

PER CENT OF ALL HATCHERY FISH NOT GONAD-DIFFERENTIATED = 15%

GENOTYPED FISH ACCORDING TO GONADAL PHENOTYPE

SOURCE	GONADAL STATE	FEMALE	MALE	NON-CONCORDANT	Σ
HATCHERY	DIFFERENTIATED	66	69	2	137
	UNDIFFERENTIATED	18	20	na	38
WILD	DIFFERENTIATED	76	68	2	146
	UNDIFFERENTIATED	0	0	na	0
	Σ	160	157	4	321

GONAD DIFFERENTIATION ISSUES

39 OF 40 FISH HAVING AN INDIFFERENT GONAD WERE OF HATCHERY ORIGIN

38 OF 40 FISH HAVING AN INDIFFERENT GONAD OCCURRED IN TWO OF THE FOUR HATCHERY FAMILIES EXAMINED (H1 AND H2).

HATCHERY FAMILY 1	HATCHERY FAMILY 2
= 13 INDIFFERENTS	= 25 INDIFFERENTS
19%	33%

AN INDIFFERENT GONAD OCCURRED WITH SIMILAR FREQUENCY AMONG:

- **MALES AND FEMALES**
- **GROUPS HELD FOR SHORTER (145-147DPF) AND LONGER (236-237 DPF) TIME PERIODS**
- **GROUPS HELD AT LOWER (2898-3263) AND HIGHER (4918-52316) TU's**
- **HEATED (PINK) AND AMBIENT (BLUE) GROUPS**

Fish with indifferent gonads among genotyped and ungenotyped fish

Exp Group	Group n	No. Fish Genotyped	Male Genotyped	Female Genotyped	Unknown genotype	TUs	Days
H1H1	16	16	2	0	na	3263	147
H1H2	16	16	2	0	na	5216	236
H1A1	18	18	5	0	na	2897.5	145
H1A2	17	17	3	1	na	2897.5	145
H2H1	20	19	1	10	na	3263	147
H2H2	17	15	1	0	0	5231	237
H2A1	15	15	0	4	na	2897.5	145
H2A2	23	22	6	3	na	4918	237
∑	142	140	20	18	0		

QUESTION 2

TO WHAT EXTENT DO GENETIC FACTORS AND/OR REARING TEMPERATURES:

ii) AFFECT PATTERNS OF GONADAL DEVELOPMENT?

TEMPERATURE – NO

SEX - NO

STOCK SOURCE – YES

PARENTAL (FAMILY) - YES

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SUMMARY

EXPOSURE TO ELEVATED WATER TEMPERATURES DURING EARLY DEVELOPMENT DOES NOT ALTER PHENOTYPIC SEX

DELAYED OR BLOCKED GONAD DEVELOPMENT MAYBE WIDESPREAD AMONG SOME HATCHERY LINES

ACKNOWLEDGEMENTS

FINANCIAL SUPPORT:

OREGON DEPARTMENT OF FISH AND WILDLIFE
OREGON HATCHERY RESEARCH CENTER
FISHERIES AND WILDLIFE DEPARTMENT
OREGON STATE UNIVERSITY
USGS
BONNEVILLE POWER AUTHORITY
ONTARIO MINISTRY OF NATURAL RESOURCES
COLLEGE OF NATURAL SCIENCES, UNIVERSITY OF HAWAII AT MĀNOA

TECHNICAL SUPPORT:

JOYCE MAHR.

FACILITIES AND EQUIPMENT:

OREGON DEPARTMENT OF FISH AND WILDLIFE
OREGON HATCHERY RESEARCH CENTER.

SEX RATIOS

WAS THERE ANY EVIDENCE THAT SEX RATIOS DIFFERED AMONG TREATMENTS?

QUESTION: DID SEX RATIOS OF ALL SEXED FISH DIFFER BY SOURCES, OR BY TREATMENT?

SEX RATIOS, ALL FISH (N=529), BY SOURCE			
	FEMALE	MALE	∑
HATCHERY	130	142	272
WILD	121	136	257
∑	251	278	529

SEX RATIOS, ALL FISH (N=529), BY TEMPERATURE TREATMENT			
	FEMALE	MALE	∑
HEATED	112	150	262
AMBIENT	139	128	267
∑	251	278	529

NO

ADD STATS RESULTS

NO

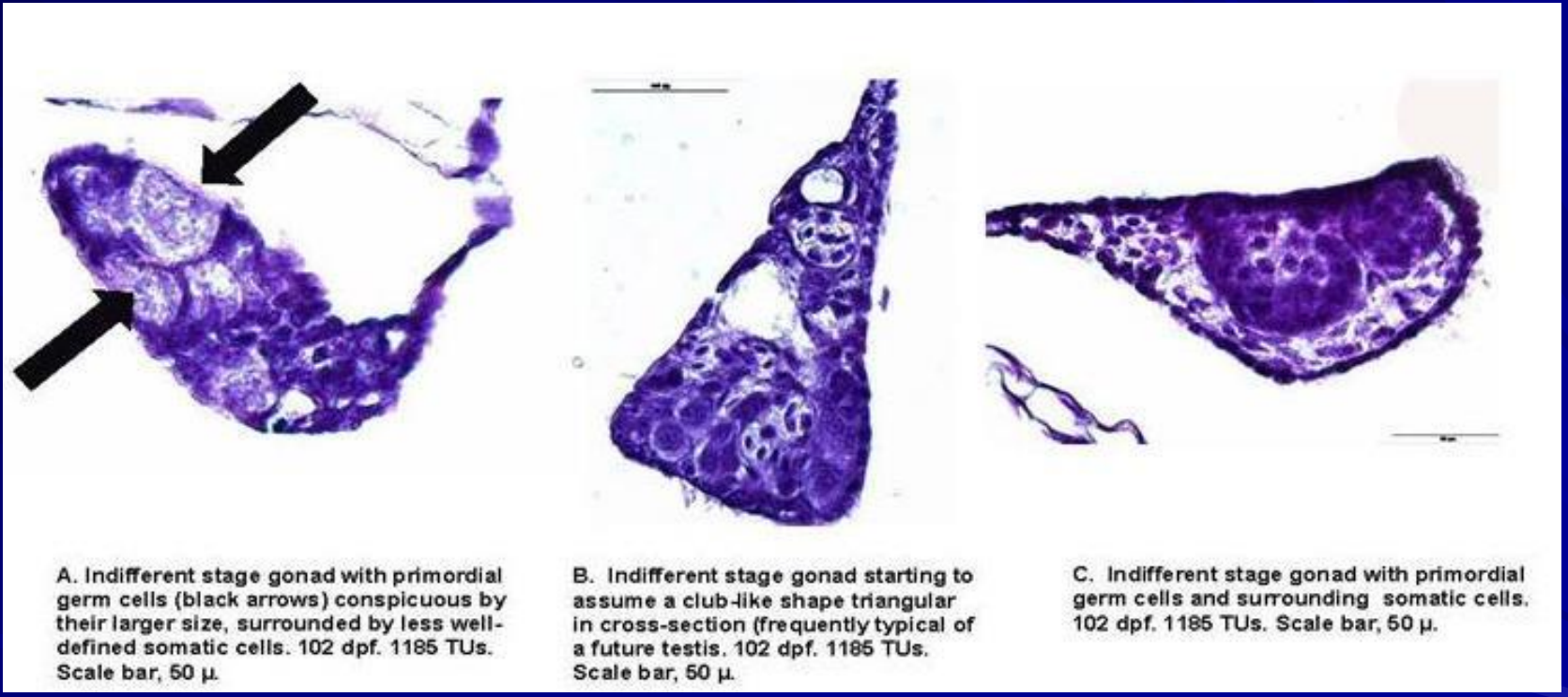
AMONG ALL FISH IDENTIFIED AS MALE OR FEMALE EITHER BY HISTOLOGY, GENOTYPING, OR BOTH (n=529) , SEX RATIOS WERE NOT SIGNIFICANTLY DIFFERENT FROM 1:1

AMONG THE 329 GENOTYPED FISH, THE SEX RATIO WAS ~ 1:1

	FEMALE	MALE
HATCHERY	82	91
WILD	83	73
	165	164

FOUR DEVELOPMENTAL CLASSES OF GONAD MORPHOLOGY FOR *Oncorhynchus mykiss* RECOGNIZED HERE

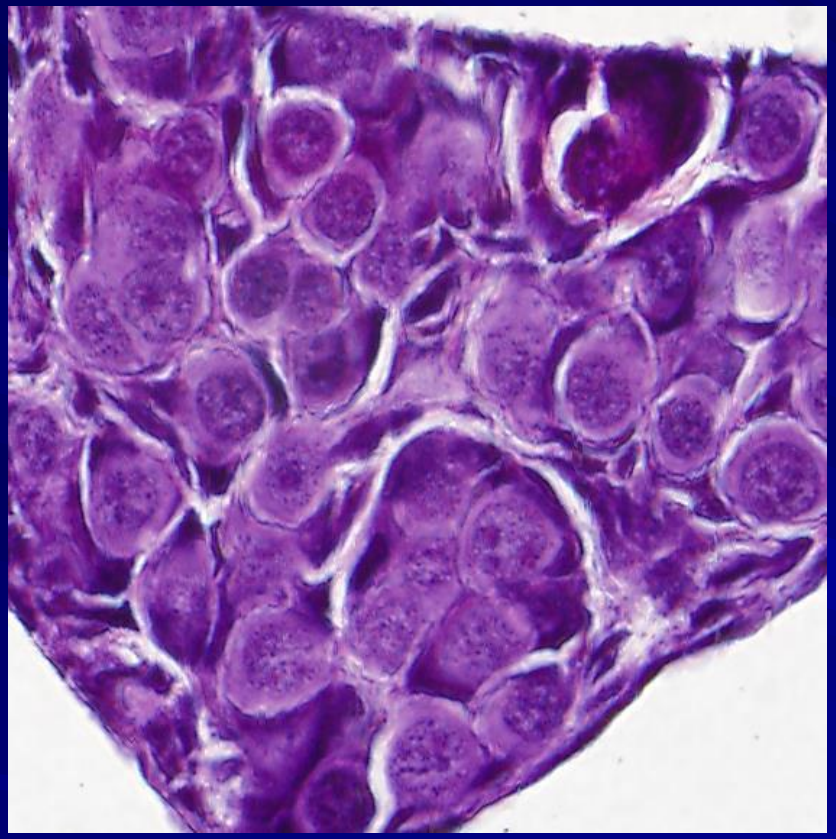
INDIFFERENT GONAD (i.e., prior to sexual differentiation)



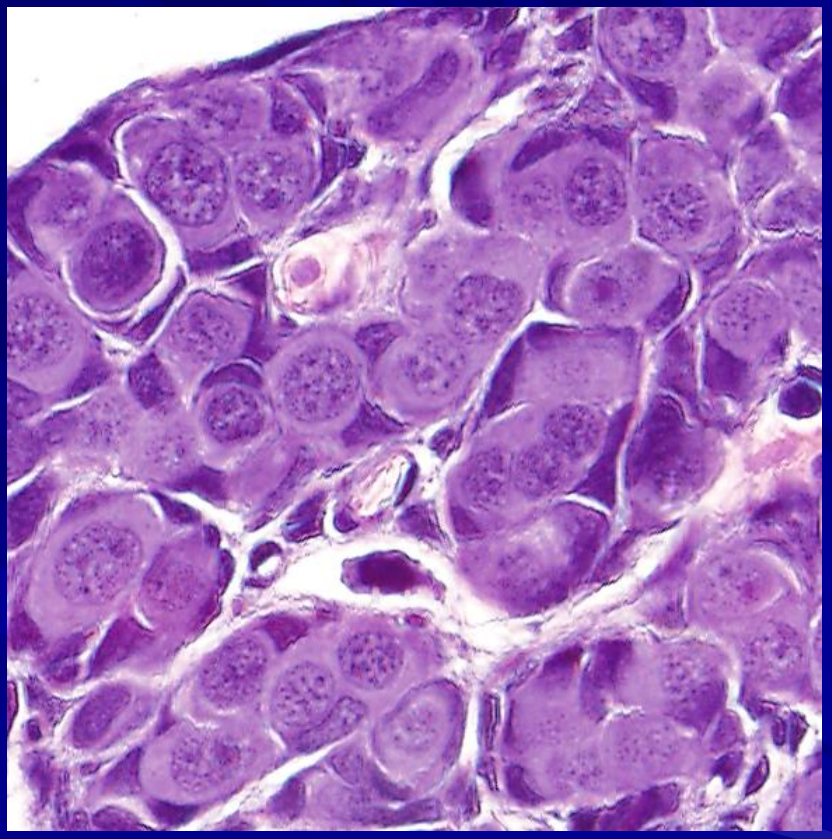
A. Indifferent stage gonad with primordial germ cells (black arrows) conspicuous by their larger size, surrounded by less well-defined somatic cells. 102 dpf. 1185 TUs. Scale bar, 50 µ.

B. Indifferent stage gonad starting to assume a club-like shape triangular in cross-section (frequently typical of a future testis. 102 dpf. 1185 TUs. Scale bar, 50 µ.

C. Indifferent stage gonad with primordial germ cells and surrounding somatic cells. 102 dpf. 1185 TUs. Scale bar, 50 µ.



Early testis. Spermatogonia Type B single stage predominating. 115+17=132 dpf. 1092+?=? TUs. Scale bar, 50 µ. Chilled treatment, hatchery parental source. (5EH-06)



Developing ovary. Gonad populated by chromatin nucleolar stage oocytes. 115+17=132 dpf. 1092+?=? TUs. Scale bar, 50 µ. Chilled temperature, hatchery parental source. (5EH-79)

SUMMARY OF FINDINGS FOR FISH WITH AN INDIFFERENT GONAD

AMONG SEXUALLY GENOTYPED AND PHENOTYPED FISH, NO WILD-SOURCED FISH EXHIBITED AN INDIFFERENT GONAD.

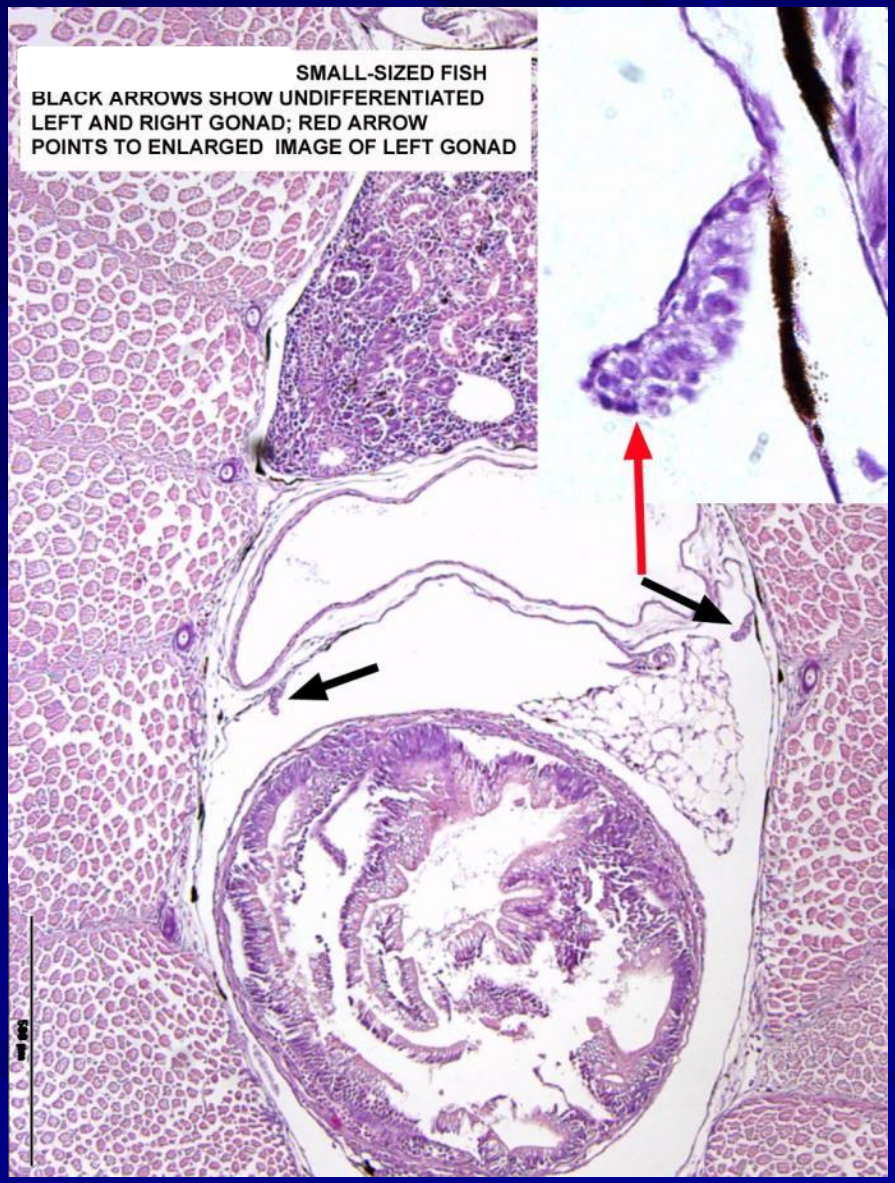
THE RESTRICTED OCCURRENCE OF DELAYED DIFFERENTIATION SUGGESTS THAT SOURCE AND FAMILY LINE HAVE A MAJOR INFLUENCE ON GONADAL DIFFERENTIATION PROCESSES

DELAYED GONADAL DIFFERENTIATION WAS NOT INFLUENCED BY REARING TEMPERATURE

Wild and hatchery-sourced *Oncorhynchus mykiss*, using a number of steelhead pairs as a parental source of embryos, were established to evaluate how genotype (stock source, parental line, chromosomal sex) and differing temperatures interact to influence phenotypic sex and gonad development. Embryos were reared from fertilization to swim up under ambient or heated (5 C^{o} above ambient) temperatures, then maintained under ambient conditions for a total of 145-245 days post-fertilization. There was no evidence that exposure to elevated temperature affected phenotypic sex. Sex ratios based on histological examination of gonadal tissue of 329 fish were not significantly different from 1:1, regardless of rearing temperature. Subsequent genotyping analysis of a subsample (n=292) showed that phenotypic sex based on histology was 99% concordant with genotypic sex. Histological examination further revealed that while all wild fish had normal gonad development, 21% of hatchery fish had an undifferentiated gonad. This phenomenon was independent of rearing temperature or genotypic sex.

AFS 2015 POSTER ABSTRACT

We compared wild and hatchery-sourced *Oncorhynchus mykiss,* using a number of steelhead pairs as a parental source of embryos, to evaluate to what extent genotype (stock source, parental line, chromosomal sex) and differing temperatures interact to influence phenotypic sex and gonad development. Among fish of all treatments raised at 5 C^{o} above ambient from fertilization to swim up, sex ratios based on histological examination of gonadal tissue were not significantly different from 1:1. Subsequent genotyping of a subsample showed near total concordance between genotypic and phenotypic sex indicating no effect of elevated temperature on sexual development. 21% of hatchery fish (but 0% of wild fish) had an undifferentiated gonad; lack of differentiation was independent of temperature or genotypic sex.


SMALL-SIZED FISH
BLACK ARROWS SHOW UNDIFFERENTIATED
LEFT AND RIGHT GONAD; RED ARROW
POINTS TO ENLARGED IMAGE OF LEFT GONAD