



**Freshwater Fisheries Society of BC**

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*Development of All-Female Sterile Kokanee  
for recreational fisheries in British Columbia*

# Outline

- Background
- Potential effort response to stocking new lakes with kokanee (AF3n)
- Progress in the development of all-female sterile (AF3n) kokanee



# Background

- By 2000 the province cultured about 8 million fish of 7 species / 50 stocks annually
- Stock roughly 800 lakes annually
- Angling effort has declined by 30% over 15 years
- 50% of all freshwater fishing occurs on stocked lakes
- 51% of stocked lakes in the province now receive sterile or all-female sterile fish



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# *Development of Special Stocks in BC*



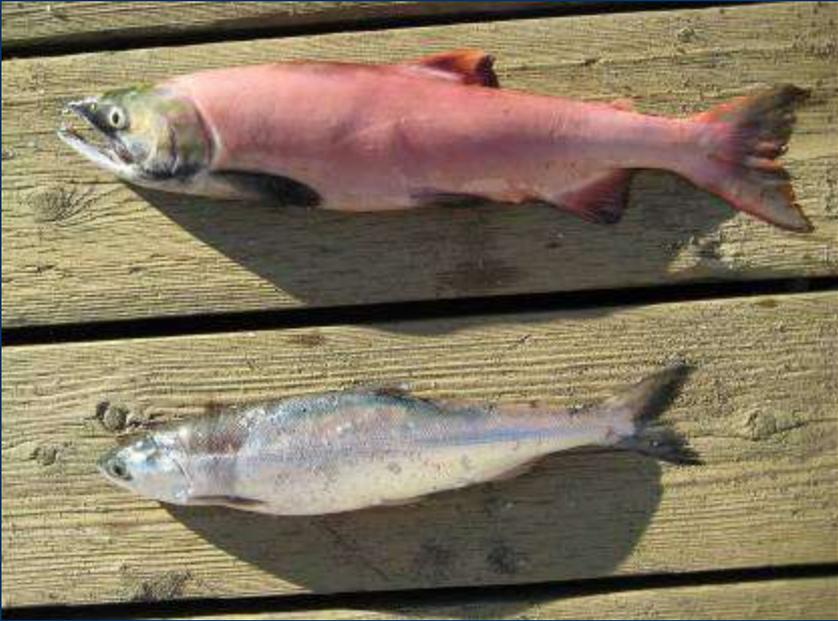
- **rainbow trout**  
(wild, domestic, 3n, AF, AF3n)
- **steelhead**  
(3n)
- **cutthroat trout**  
(3n)
- **eastern brook trout**  
(AF3n only)
- **kokanee**  
(3n, AF3n)



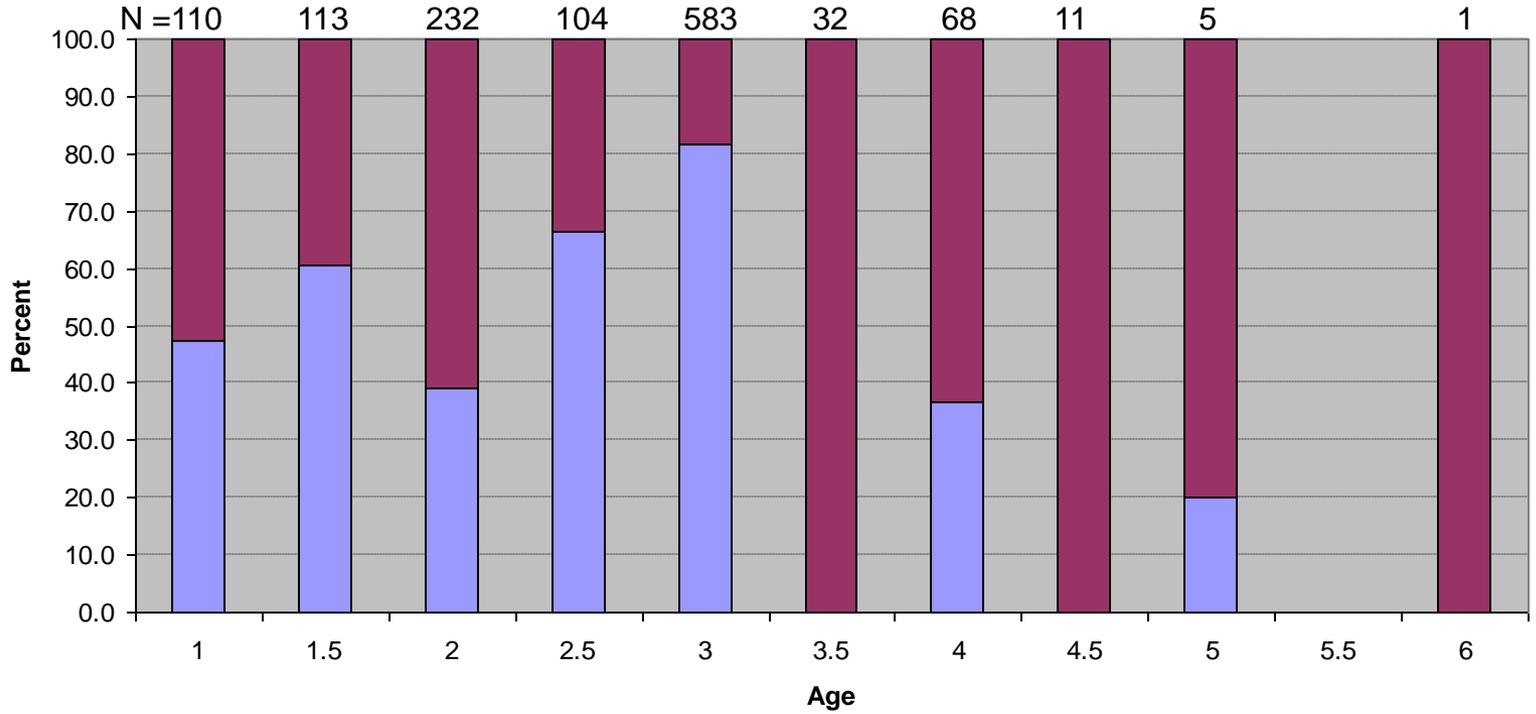
# *Post-stocking performance of 3n kokanee*

- Sterility rates are not 100% (95 – 100%) so we are limited where we can stock them.
- Have been testing them in lakes for 8 years now (5 lakes not previously stocked with kokanee)
- Survival of sterile kokanee in the 1<sup>st</sup> season in “harsh” conditions is generally lower than non-sterile counterparts but no different when conditions are normal.
- Growth of sterile kokanee is similar to 2n kokanee, at least in less stressful environments.
- 3n males still “mature” and die off (especially in productive lakes). Females seem to live longer and stay bright but some do mature at ~age 5. >80% of fish older than age 3 are bright sterile females.





### Relative Sex Ratios



# *Advantages of AF3n kokanee for fisheries management*

- Addresses the loss to the fishery due to 2n and 3n male drop out and poor flesh quality
- Takes advantage of potential longevity and flesh quality of non-maturing females (provides more large bright fish for all angler types)
- Creates a functionally non-reproductive population
- Provides a diversity of fishing opportunity
- Provides a fishery at times when new/lapsed anglers are able to fish (summer/winter)



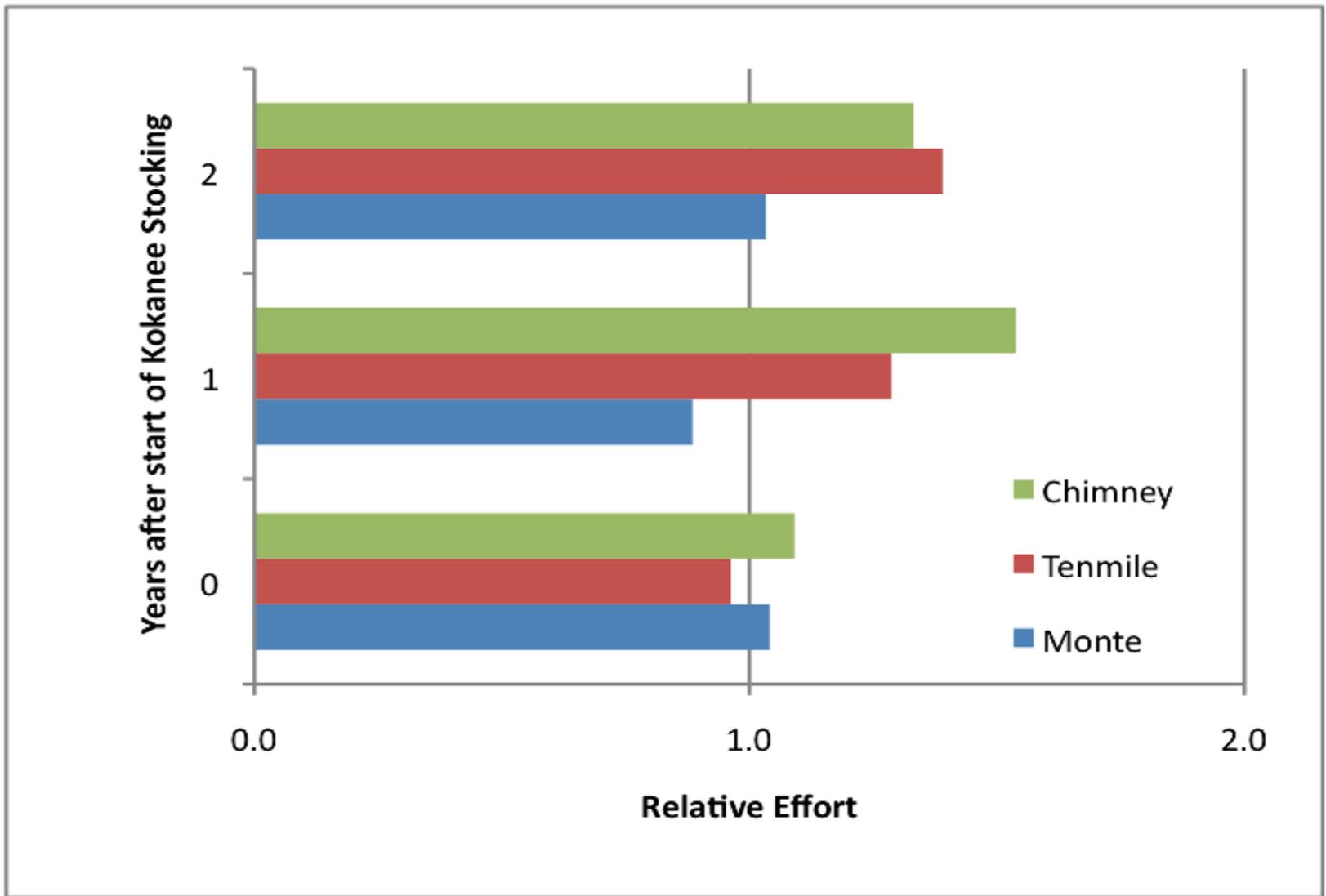


Figure 1. Relative summer effort 0, 1, and 2 years after kokanee introduction on three lakes (adjusted for weather and daytime effects).

# *Effort measurements*

- Camera counts





07/31/2009 09:00



## Angler effort before and after 3n kokanee introduction (AD)

LAKE	Prior to 3n kokanee stocking			2 years after stocking			% AD increase after KO stocking
	Winter	Summer	Total	Winter	Summer	Total	
Monte	0	3512	3512	7112	2944	10056	186%
Dutch	100	1000	1100	2000	2000	2000	82%
Ten Mile	low	3258	3258	4400	5224	9624	195%
Alleyne	125	1000	1125	365	1000	1365	21%





## *Potential of added Angler Days (AD)*

Increase in AD / ha when adding kokanee		Total Increase in AD if 8000 ha are stocked with kokanee	If 1 AD = \$25, and 20% are new anglers	If 1 AD = \$100, and 20% are new anglers
If add 4 AD/ha	4	16,000	\$160,000	\$640,000
If add 15 AD/ha	15	60,000	\$600,000	\$2,400,000
If add 30 AD/ha	30	120,000	\$1,200,000	\$4,800,000

# *Steps in producing AF3n stocks*

- 1 Change females into males to produce XX males
- 2 Cross XX males with females (XX) to produce all-female (AF) progeny
- 3 Pressure shock AF progeny (eggs) shortly after initial fertilization to produce sterile all-female fish (AF3n)
- 4 MT treat some AF progeny to maintain XX male brood fish



# *XX Male Kokanee Development*

- We've developed an immersion protocol to sex reverse female kokanee
- At 75% hatch, immerse the mixed sex eggs/alevins in MT baths for 2 hours, repeat 1 week later
- Rear them until you are able to sex them. If you are successful at converting females to males, sex ratios will be greater than 1:1 (>50% Males).



## *Kokanee – 2005 Brood*

<b>MT Immersion Treatment (<math>\mu\text{g}\cdot\text{l}^{-1}</math>)</b>	<b>% Males</b>		
	<b>Rep 1</b>	<b>Rep 2</b>	<b>Rep 3</b>
<b>0 (Control)</b>	<b>48.8</b>	<b>50.0</b>	<b>59.4</b>
<b>200</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>400</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>800</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>200 @ 100% hatch</b>	<b>100</b>	<b>--</b>	<b>--</b>
<b>400 @ 100% hatch</b>	<b>100</b>	<b>--</b>	<b>--</b>
<b>200, 3 immersions</b>	<b>100</b>	<b>--</b>	<b>--</b>
<b>400, 3 immersions</b>	<b>100</b>	<b>--</b>	<b>--</b>

## *Kokanee – 2008 Brood*

<b>MT Immersion Treatment (<math>\mu\text{g}\cdot\text{l}^{-1}</math>)</b>	<b>% Males</b>		
	<b>Rep 1</b>	<b>Rep 2</b>	<b>Rep 3</b>
<b>0 (Control)</b>	<b>62.2</b>	<b>57.5</b>	<b>64.8</b>
<b>25</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>50</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>150</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>175</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>200</b>	<b>100</b>	<b>100</b>	<b>100**</b>
<b>400</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>800</b>	<b>100</b>	<b>100</b>	<b>100</b>

# Kokanee – 2009 Brood

<b>MT Immersion Treatment (<math>\mu\text{g}\cdot\text{l}^{-1}</math>)</b>	<b>% Males</b>		
	<b>Rep 1</b>	<b>Rep 2</b>	<b>Rep 3</b>
<b>0 (Control)</b>	<b>50%</b>	<b>50%</b>	<b>67%</b>
<b>0.1</b>	<b>66%</b>	<b>64%</b>	
<b>1</b>	<b>94%</b>	<b>92%</b>	
<b>5</b>	<b>100%</b>	<b>100%</b>	
<b>10</b>	<b>100%</b>	<b>100%</b>	
<b>25</b>	<b>98%</b>	<b>100%</b>	<b>100%</b>
<b>25*</b>	<b>94%</b>	<b>100%</b>	
<b>50</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>
<b>100</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>
<b>150</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>
<b>175</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>
<b>200</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>
<b>200*</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>

# *XX Male Kokanee Development*

- We've developed an immersion protocol to sex reverse female kokanee
- At 75% hatch, immerse the eggs/alevins in MT baths for 2 hours, repeat 1 week later
- Tested MT concentrations between 0.1 and 800  $\mu\text{g}\cdot\text{l}^{-1}$
- We successfully convert females to males using doses as low as 1  $\mu\text{g}\cdot\text{l}^{-1}$  (92-94%) and 50  $\mu\text{g}\cdot\text{l}^{-1}$  (100%) = 0.00005  $\text{g}\cdot\text{l}^{-1}$



# *Steps in producing AF3n stocks*

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(XX)

X



(XX)



AF3n

100%  
XX ♂  
(brood)

Pressure  
Shock  
after  
fertilization



Immerse  
in MT at  
75% Hatch



# *If you started with mixed sex eggs.....*

1 X 1 cross



(all XX)

1 X 1 cross



X

X

(XY)



(XX)



$\frac{1}{2}$  ♀ &  $\frac{1}{2}$  ♂ progeny

All ♀ progeny



&



# *Development of 100% XX males from mixed sex eggs (groups from 1\*1 crosses)*

Potential brood – MT  
treat all families at hatch

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24
25	26	27	28		

Control groups for all  
MT treated families –  
No MT treatment

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24
25	26	27	28		



# Development of 100% XX males from mixed sex eggs (groups from 1\*1 crosses)

Potential brood – MT  
treat all families at hatch

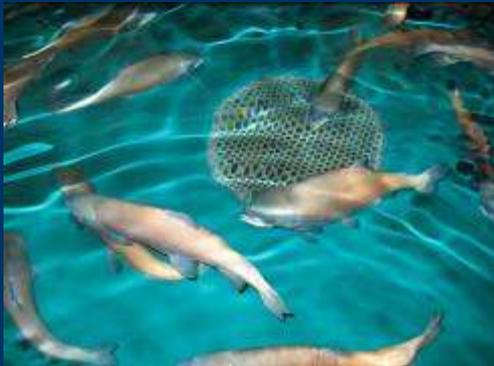
1	×	3	×	×	6
×	8	×	×	×	12
13	×	×	×	×	×
×	×	×	×	×	×
×	×	×	×		

Control groups for all  
MT treated families –  
No MT treatment

1XX	2XY	3XX	4XY	5XY	6XX
7XY	8XX	9XY	10XY	11XY	12XX
13XX	14XY	15XY	16XY	17XY	18XY
19XY	20XY	21XY	22XY	23XY	23XY
24XY	25XY	26XY	27XY	28XY	

# *Biostandards for XX male brood*

- Survival from ge to fry ranges from 0 to 83%
- Maturation rates at age two – 48 to 85%
- Maturation rates at age three – 100%
- XX male kokanee have functional gonaducts making the spawning or production numbers very easy



# *Time Line for Brood Development*

- Fall 2012 – Our first brood of 100% XX males (63 fish) will be mature.
  - Brood propagation crosses (MT treat family groups at hatch)
  - AF3n production (cross XX males with groups of pooled eggs and then pressure shock soon after fertilization)
- Spring 2013 – First stocking of production numbers of AF3n kokanee if all goes well
- Fall 2013 to Fall 2015 – Monitor the in-lake performance and fishing effort changes



*Huge Thank-You to all the  
Clearwater Hatchery Staff !!*



*Questions?*



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