Errors in Visual Identification of Juvenile Steelhead, Cutthroat Trout and Their Hybrids

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Project Background

- ESA Listing of Steelhead in Northern California "ESU"
- Coastal Populations of *O. mykiss* and *O. clarki clarki* sympatric
- Diver based surveys unable to reliably ID juveniles

In sympatric settings…Field ID's of Juveniles= Error Prone

- Phenotypic Plasticity
- Presence of Hybrids

Hybrid physical appearance varies… phenotype not always intermediate

Field Protocols

Two-phase field design coinciding w/ "Hankin-Reeves"* survey

Phase 1:

Collect large random sample of fish through electrofishing and visually classify into one of three categories: Steelhead, Unknown trout, Cutthroat trout.

Phase 2:

A systematic subsample of the first phase fish are selected for genetic analysis and phenotypic observations (correlate field ID with genetic identity)

*Modified Hankin and Reeves (1988)/ Hankin and Mohr (…2005…)

Phenotypic Observations

1) Categorical index scores

A. Slash Intensity (1, 2, or 3)

B. Maxillary Extension (1, 2, or 3)

2) Morphometric relationships

A. Maxillary Length : Forklength

B. Maxillary Length : Head Length

C. Head Length : Forklength

Slash score =1

Slash score =2

Slash score =3

Max score = 1

Max score =2

Max score =3

Visually Classify– All fish in hand
Phenotypic Index Scores- Same ^
Measurements- Systematic
Genetic samples- Systematic

Study Sites

McGarvey Creek, Lower Klamath River

O. clarki clarki abundant vs. *O. mykiss*

2002: n=188 sampled trout
2003: n=399 sampled trout

Freshwater Creek, Humboldt Bay tributary

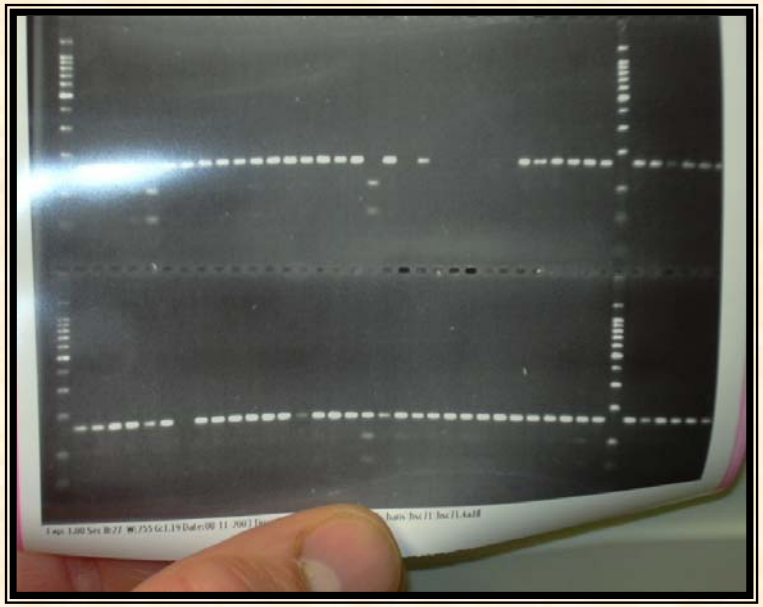
O. mykiss and O. clarki clarki both "abundant"

2002: n=341 sampled trout
2003: n~700 sampled trout; n=362 genetics

Genetic Analyses

Baker et al. (2002)- Developed Oligonucleotide Primers

- **Seven nuclear DNA loci and one mitochondrial DNA locus exhibit "fixed species specific" differences for steelhead vs. cutthroat**



Visual ID vs Genetic ID:

Freshwater Tributaries 2003 < 80 mm Fork Length

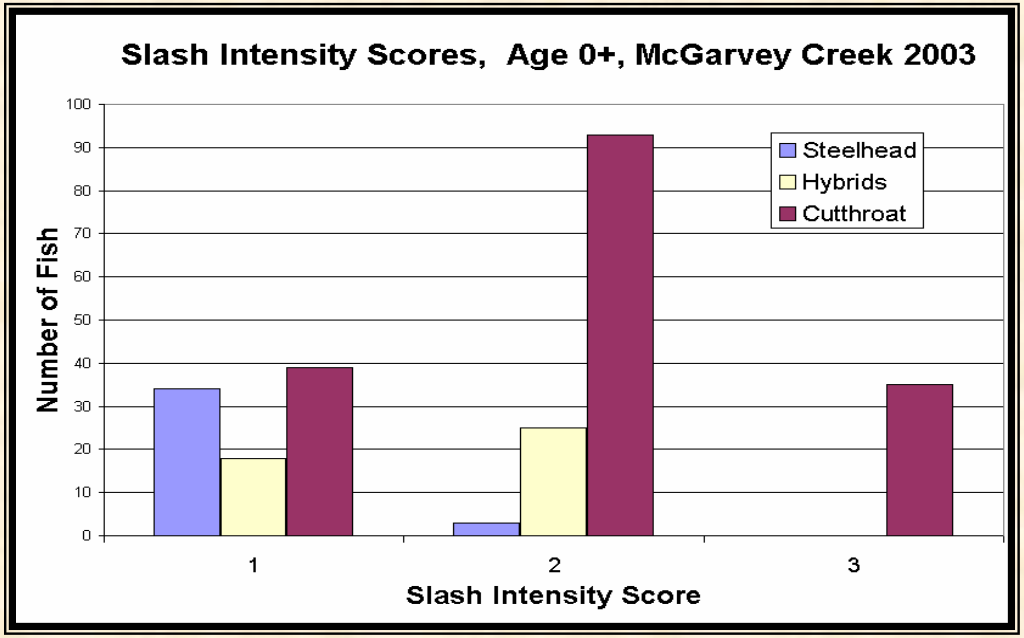
	GENETIC CATEGORIES		
	Pure Steelhead	"Hybrid"	Pure Cutthroat
VISUAL CATEGORIES: Steelhead	9	17	26
Hybrid/ Unknown	0	0	8
Cutthroat	0	14	31

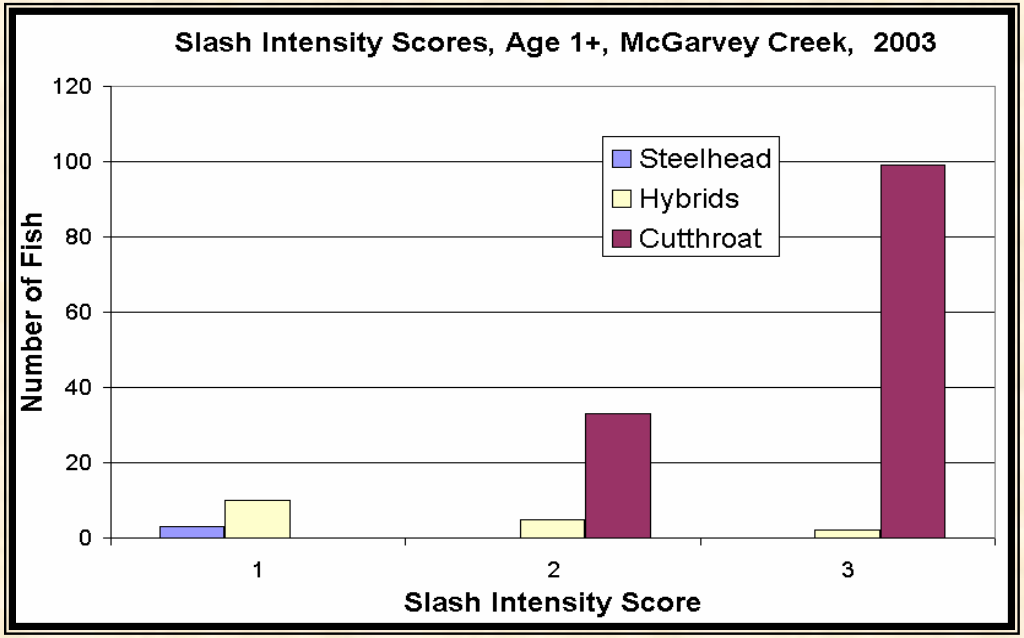
Visual ID vs Genetic ID:

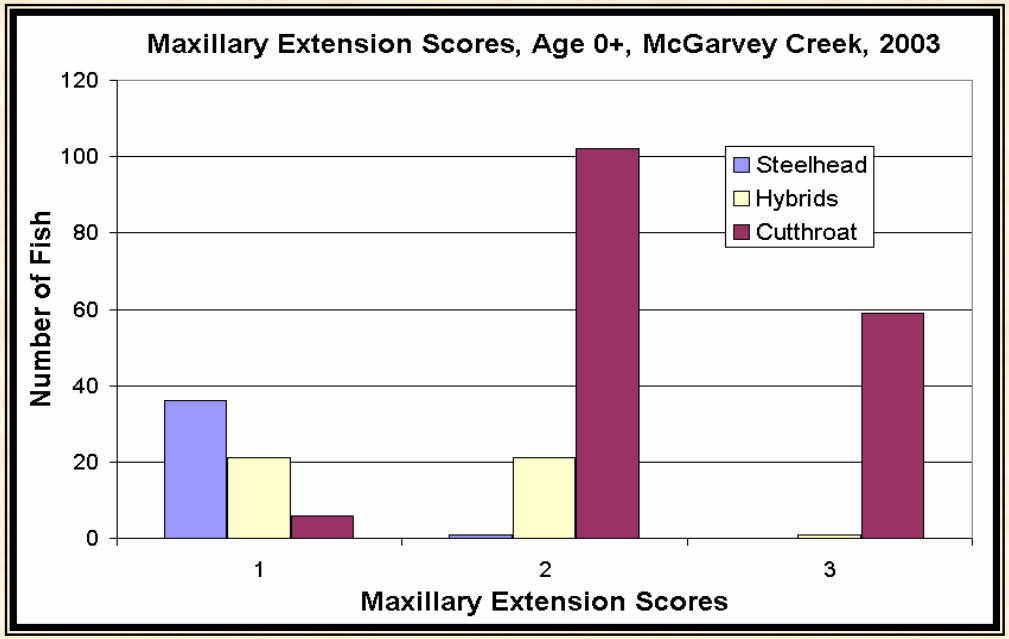
Freshwater Tributaries 2003 >= 80 mm Fork Length

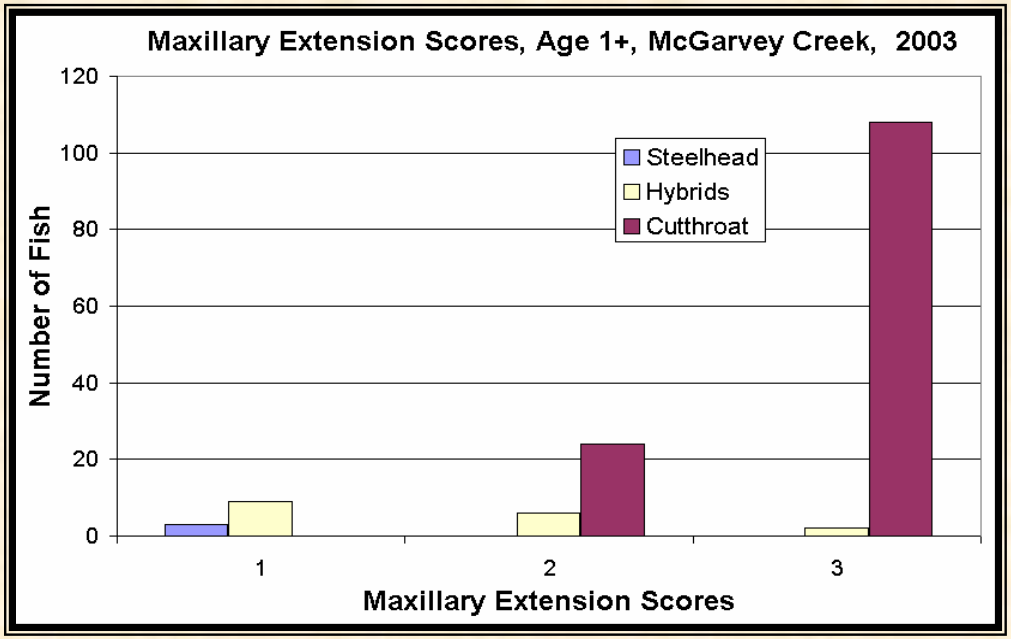
	GENETIC CATEGORIES		
VISUAL CATEGORIES	Pure Steelhead	"Hybrid"	Pure Cutthroat
Steelhead	35	4	1
Hybrid/ Unknown	1	4	4
Cutthroat	0	31	78

Overlap of qualitative phenotypic characteristics

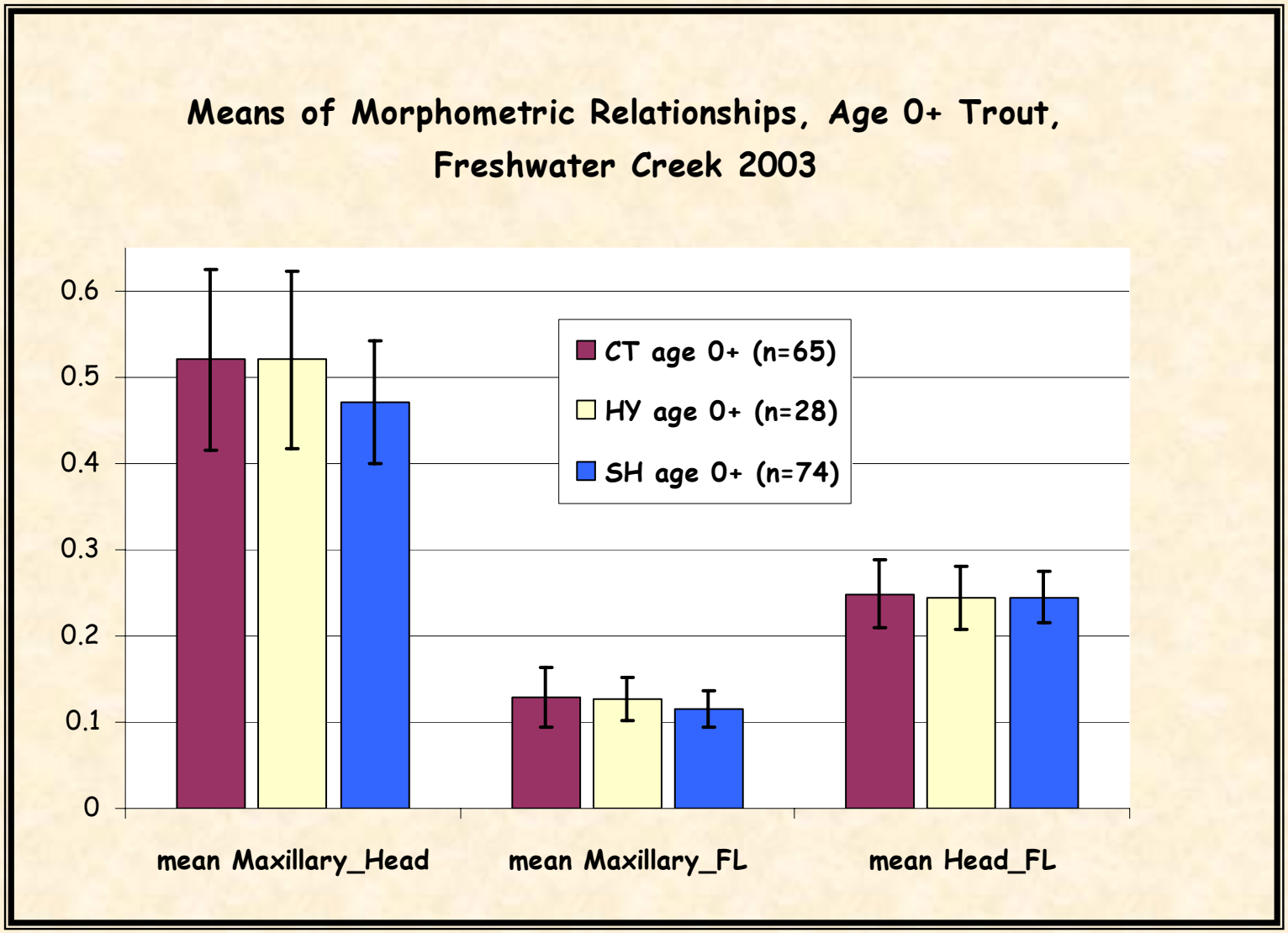


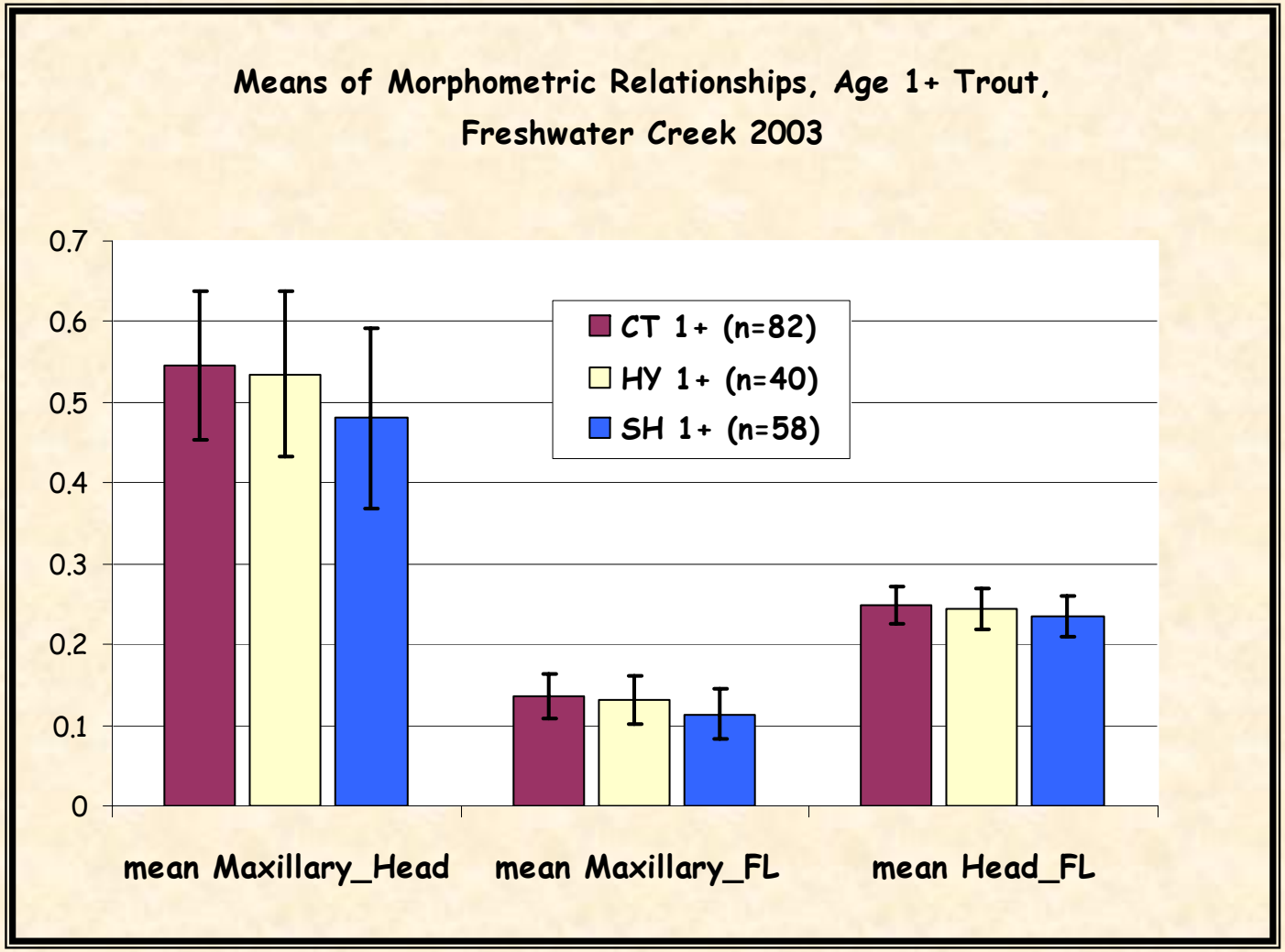



Maxillary Extension Scores, Age 0+, McGarvey Creek, 2003
Number of Fish
120
100
80
60
40
20
0
1
2
3
Maxillary Extension Scores
Steelhead
Hybrids
Cutthroat

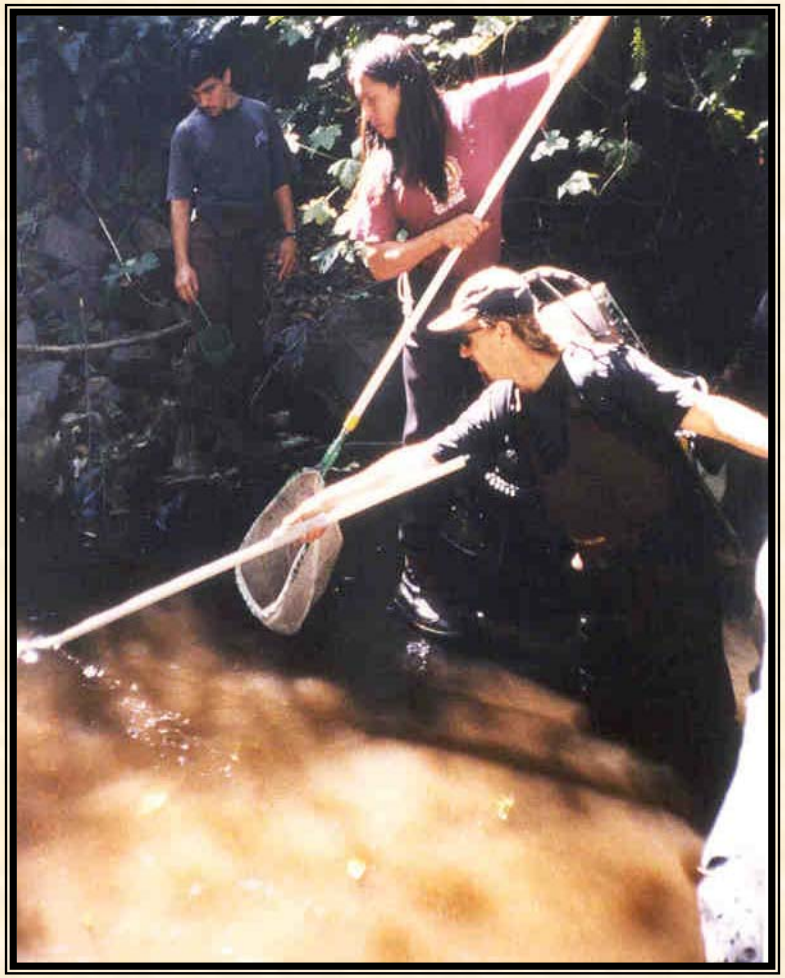

Maxillary Extension Scores, Age 1+, McGarvey Creek, 2003
Number of Fish
120
100
80
60
40
20
0
1
2
3
Maxillary Extension Scores
Steelhead
Hybrids
Cutthroat

Overlap of quantitative phenotypic characteristics




Means of Morphometric Relationships, Age 1+ Trout,
Freshwater Creek 2003
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
CT 1+ (n=82)
HY 1+ (n=40)
SH 1+ (n=58)
mean Maxillary_Head
mean Maxillary_FL
mean Head_FL

Muddy Waters



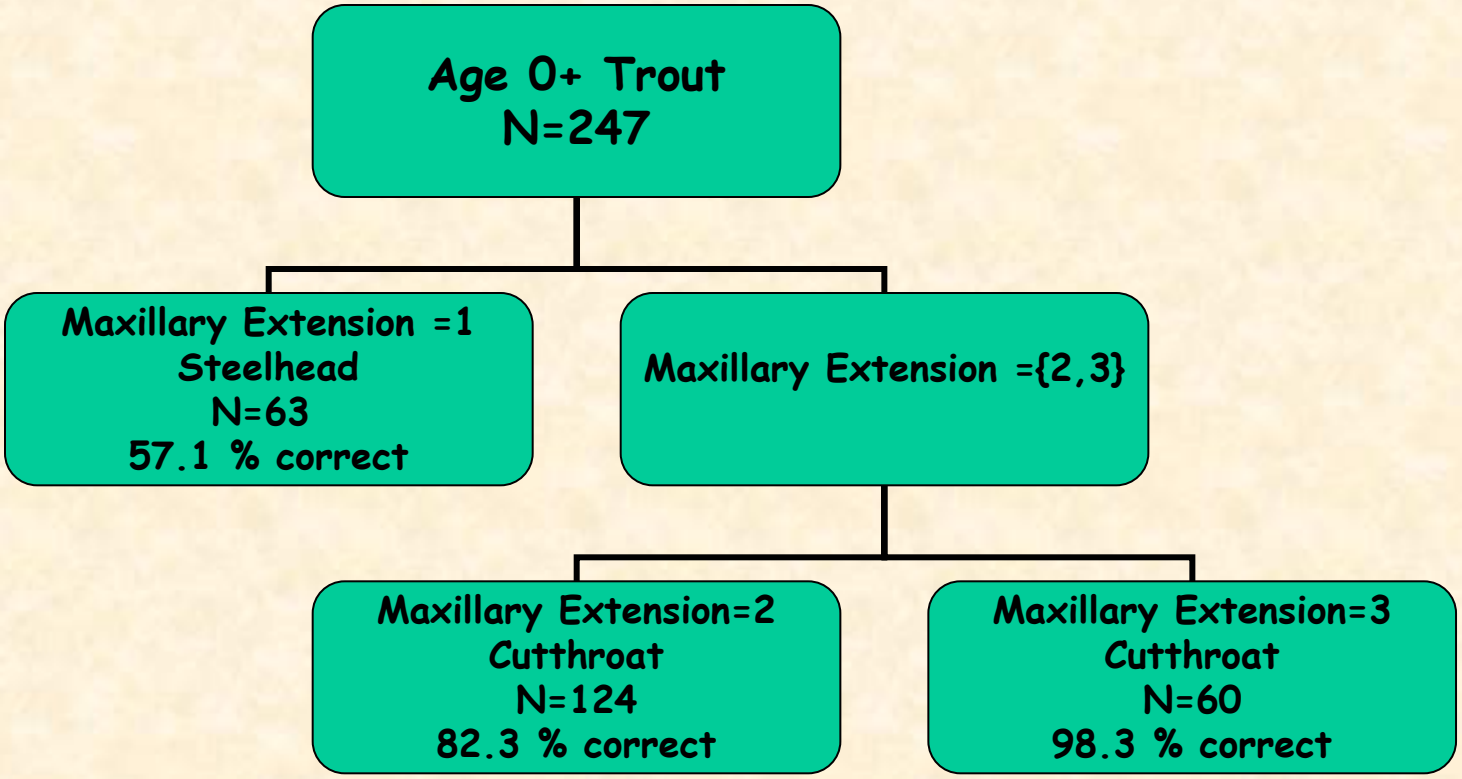
Given that misclassifications DO occur

Can field methods be developed to replace or minimize the need for costly genetics???

Assess quantitative phenotypic data as potential solution

1. Perform simple (binary) logistic regression with target variable = "steelhead" or "non-steelhead" Use Beta Pr. Chi. Sq values to identify significant predictor variables (e.g.: slash scores)
2. Develop Classification Tree models to evaluate performance of selected predictor variables in various combinations (Weigel et al. 2001).
3. Compare overall error rates of "Visual ID's" vs. "Model classifications"

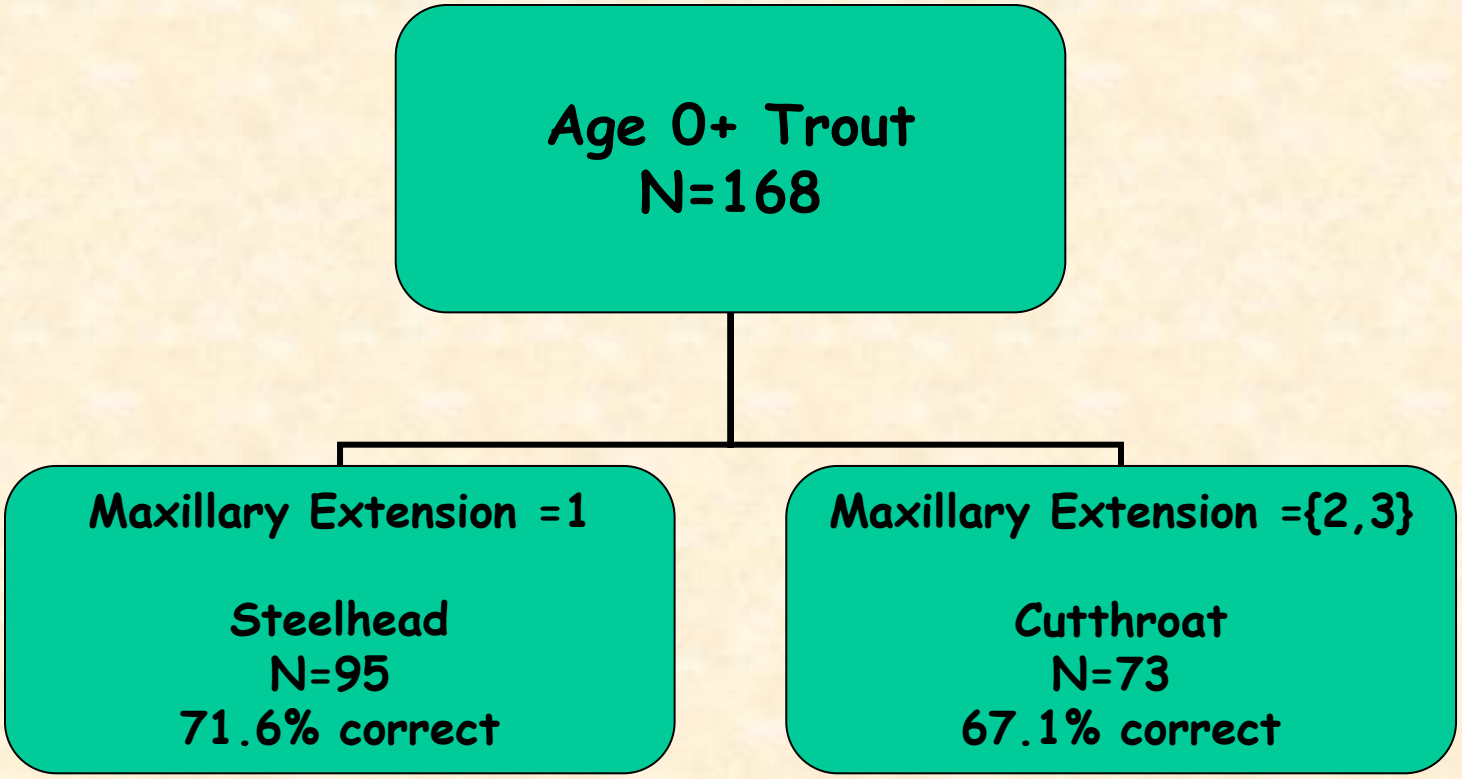
Using only "Maxillary Extension Score"
McGarvey Creek -mainstem and west fork 2003



Visual classification error rate: 27.1%

Model classification error rate: 20.2 %

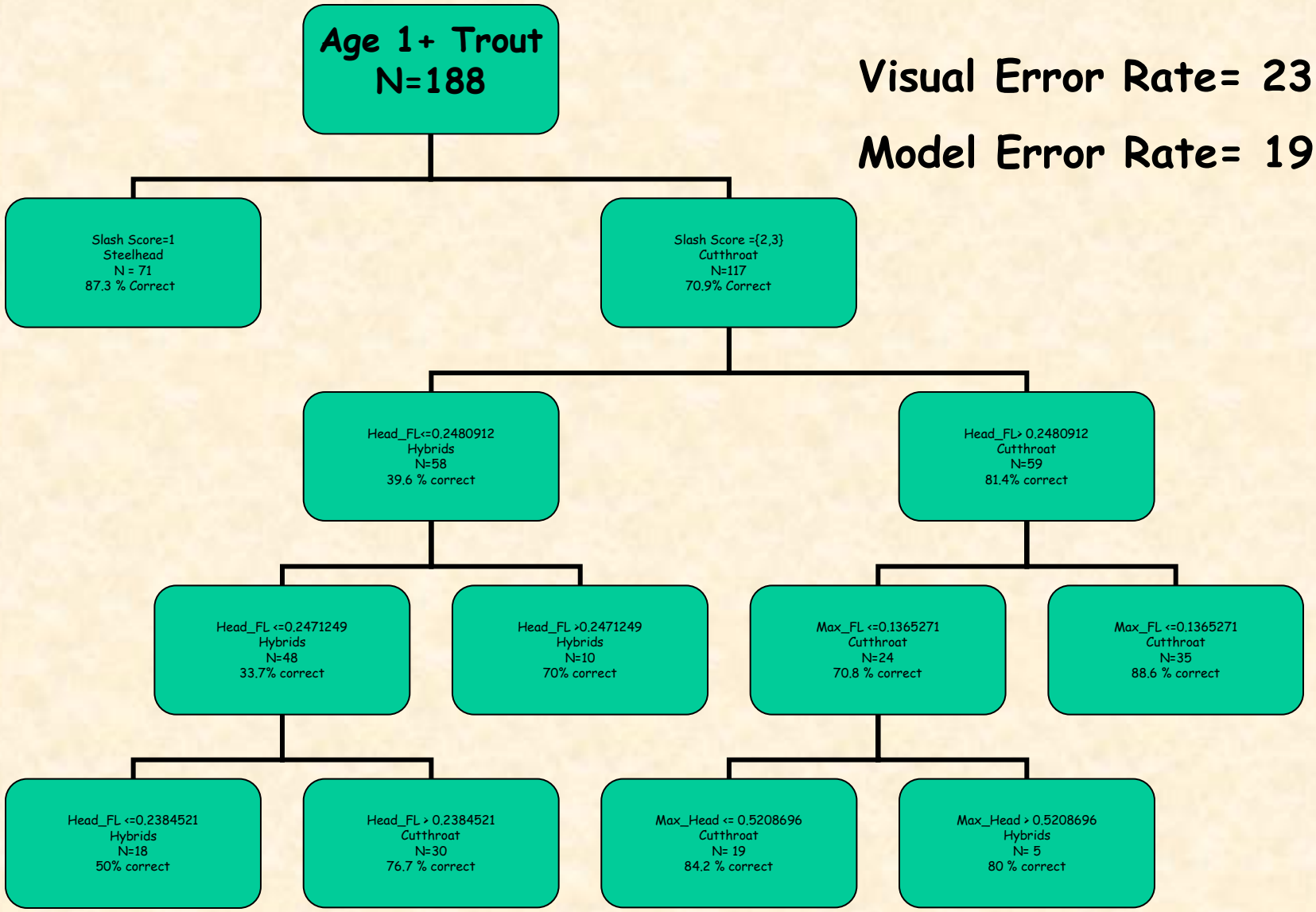
Using only "Maxillary Extension Score" Freshwater Creek -mainstem and tributaries 2003



Visual classification error rate: 37.5%

Model classification error rate: 30.4%

Using All Five Phenotypic Variables
Freshwater Creek mainstem and tributaries 2003



Visual Error Rate= 23.4%

Model Error Rate= 19.2%

Summary of *a posteriori* Classification Tree Models vs. Overall Visual Identification Errors (age class/location)

Drainage		Sample Size	Decision Tree Model Specification	Model ID Error	Visual ID Error
McGarvey	0+	N = 247 trout	{max_extens}	**20.2%**	27.1%
McGarvey	1+	N = 152 trout	{slash_intensity}	29.6%	**8.6%**
McGarvey	1+	N = 152 trout	{max_extens}	23.0%	**8.6%**
McGarvey	1+	N = 152 trout	{slash_intensity, max_extens., max_head, max_FL, head_FL}	10.5%	**8.6%**
Freshwater	0+	N = 168 trout	{max_extens}	**30.4%**	38.4%
Freshwater	1+	N = 188 trout	{slash_intensity}	23.4%	23.4%
Freshwater	1+	N = 188 trout	{slash_intensity, max_extens.,}	24.5%	**23.4%**
Freshwater	1+	N = 188 trout	{slash_intensity, max_extens., max_head, max_FL, head_FL}	**19.1%**	23.4%

Summary and Conclusions

- Visual ID error rates complicate unbiased estimation in sympatric settings
- Overlap of hybrid phenotypes with both parent species necessitates some level of genetics
- Visual ID errors higher for age 0+ fish (vs. age 1+) in both streams, and higher overall when SH and CT are similarly abundant (Freshwater)
- Classification tree models formed with phenotypic data improved error rates for age 0+ trout in both streams (age 1+ less conclusive improvement)
- Additional analyses using ordinal regression techniques (proportional odds) may help to improve on existing models

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