FINAL
SUMMARY REPORT

Greenback Cutthroat Trout Genetics and Meristics Studies
Facilitated Expert Panel Workshop

REGION 6 OFFICE
US FISH AND WILDLIFE SERVICE

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Prepared for:

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(Courtesy Shiozawa Presentation at Workshop)

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Executive Summary

Subspecies of cutthroat trout in Colorado were believed to follow geographic boundaries within the state until recently. With the completion of a genetic study comparing mitochondrial DNA of extant Colorado cutthroat trout populations with museum specimens (Metcalf et al. 2012) and a meristic study of cutthroat trout collected from all major drainages in Colorado (Bestgen et al. 2013). The purpose of this review was to provide a thorough scientific review and evaluation prior to taxonomic, listing, and management decisions. Toward that purpose, the US Fish and Wildlife Service hosted an expert panel Workshop that included a group of experts in the field of native trout genetics, meristics, and taxonomy.

The Workshop was held July 30 – August 1, 2013 and was facilitated by Dr. Tom Turner from the University of New Mexico. The goals of the Workshop were to: 1) evaluate science of recent genetics and meristics studies and reach consensus about implications for management and 2) develop recommendations for future efforts, including both additional research and management activities. The participants included the expert panel, members of the Greenback Cutthroat Trout Recovery Team, agency representatives, and authors. Following the workshop, the expert panel and other attendees provided responses to 17 discussion questions. This report summarizes those responses, with Workshop material and complete responses provided in the appendices.

Panelists generally believed both studies were well-designed and informative, given limitations of sample size and available specimens. The studies both supported the same hypotheses and provided insight into the relationships among Colorado cutthroat trout and relative to historic specimens. Both studies support the existence of four extant and two extinct lineages of cutthroat trout in Colorado. Yellowfin cutthroat trout and the native cutthroat trout in the San Juan River (red lineage) appear to be extinct. Rio Grande cutthroat trout continue to stand as a distinct lineage, as in previous studies. Colorado River cutthroat trout (blue lineage) still occur on the West Slope but only on the northern end of it; East Slope populations appear to be a result of stocking. Greenback cutthroat trout could refer to two different lineages: green lineage and Bear Creek. Green lineage trout occur on both the West and East Slopes, but the reason for their presence on the East Slope is unclear. In addition, the Bear Creek population is a unique lineage and appears to represent the native South Platte River cutthroat trout, although that population does not occur in that drainage now.

While some conclusions were strongly supported, there remain topics that require further research. Additional genetics and meristics research is required to further clarify the status and relationship of the green lineage to other lineages. Additional genetics research is required to provide a more complete phylogeny among the lineages. Confirmation of the extinction of the San Juan River native cutthroat trout is needed. Taxonomic revisions are required to resolve issues associated with the green lineage and the Bear Creek population. Research is needed to evaluate a number of issues associated with the hatchery stock for the Bear Creek population. The Bear Creek population merits protection and additional management efforts. The individual responses provided a thorough and comprehensive review of these studies and their taxonomic and management implications.

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1.0 Background

Until recently, delineations of subspecies of cutthroat trout in Colorado were believed to follow geographic boundaries within the state, with greenback cutthroat trout (*Oncorhynchus clarkii stomias*) on the eastern side of the Continental Divide and Colorado River cutthroat trout (*O. c. pleuriticus*) on the western side. Rio Grande cutthroat trout (*O. c. virginalis*) occur within the Rio Grande drainage; their range and genetic identity does not appear to be in question. The recently published genetic study by the University of Colorado - Boulder genetics lab (Metcalf *et al.* 2012) compared mitochondrial DNA of extant Colorado cutthroat trout populations with cutthroat trout museum specimens collected in the late 1890s to early 1900s, thereby providing an understanding of the native ranges of cutthroat trout in Colorado prior to fish stocking efforts.

The conclusions of this genetic study have significant implications under the Endangered Species Act (ESA) and for management decisions as the data indicate that only one greenback cutthroat trout population remains in existence; this population is present in Bear Creek on Pikes Peak in the Arkansas River drainage. The other “greenback” streams that are present on the eastern side of the Continental Divide are cutthroat trout that had been stocked from the West Slope of the Continental Divide at earlier times. Another significant conclusion of the genetic study is the identification of two separate distinct lineages of the Colorado River cutthroat trout, which had previously been considered to consist of one lineage across the entire West Slope of Colorado.

The genetic study complements a concurrent meristic study of cutthroat trout in Colorado. The meristic study was conducted by the Larval Fish Laboratory at Colorado State University and included cutthroat trout specimens collected from all major drainages in Colorado, Wyoming, Utah, and New Mexico. Meristic and genetic analyses are being conducted on the specimens in a “double-blind” test in which neither group of researchers is aware of the origin of the specimens. The meristic study was completed in the spring of 2013, with the report available for the Workshop. Both of these studies were initiated and funded by the Greenback Cutthroat Trout Recovery Team, which is comprised of the following agencies: Colorado Division of Parks and Wildlife, US Forest Service (USFS), Bureau of Land Management (BLM), National Park Service (NPS), and the US Fish and Wildlife Service (FWS or Service).

The purpose of this review was to provide a thorough scientific review and evaluation prior to taxonomic, listing, and management decisions. Toward that purpose, the Service’s Colorado Field Office, in concert with our partners in the Greenback Cutthroat Trout Recovery Team, coordinated a facilitated expert panel Workshop that included a group of experts in the field of native trout genetics, meristics, and taxonomy.

(Courtesy Krieger Presentation at Workshop)

2.0 Expert Panel

Expert panel members were selected by the Service and were invited to attend a Workshop and provide answers to Discussion Questions. The panel included individuals with professional qualifications and experience related to as many as possible of the following areas: greenback and other cutthroat trout genetics, cutthroat trout meristics, taxonomy, and other related fields. In addition, the Service invited the authors of the two studies to attend and other agency representatives. The Workshop was facilitated by Tom Turner, PhD (University of New Mexico) in Lakewood, Colorado from 30 July – 1 August 2013 (see Section 3.0 for summary, Appendix A for agenda, and Appendix B for the meeting notes).

The independent expert panel members are all experienced, senior-level fish conservation biologists, fish geneticists, and/or fish taxonomists. There were four groups of attendees at the Workshop: expert panelists, members of the greenback cutthroat trout recovery team, agency representatives, and authors. Each panelist attended the Workshop and provided individual responses to the discussion questions (Appendix C) provided by the Service (Appendix D). In addition, other attendees were also given the opportunity to provide responses (Appendix E).

Facilitator

Dr. Tom Turner – University of New Mexico

Expert Panel Members

Dr. Marlis Douglas – University of Illinois
Dr. Richard Mayden - St. Louis University (presenter, public comment period)
Dr. Jeffrey Olsen - FWS Conservation Genetics Program
Mr. Bruce Rosenlund - retired, FWS fisheries
Dr. Dennis Shiozawa – Brigham Young University (presenter, cutthroat trout genetics generally)
Dr. Robin Waples – Northwest Fisheries Science Center, NOAA Fisheries
Dr. Andrew Whiteley – University of Massachusetts Amherst

Greenback Cutthroat Trout Recovery Team

Ms. Leslie Ellwood – FWS
Mr. Doug Krieger - Colorado Parks and Wildlife (presenter)
Mr. Jay Thompson - BLM
Ms. Mary Kay Watry - NPS
Mr. David Winters - USFS

Agency Representatives

Mr. Dirk Miller - Representative Colorado River Cutthroat Trout Conservation Team
Ms. Pam Sponholtz - Representative FWS Fisheries Program

Authors

Mr. Chris Kennedy – Fishery Biologist, FWS, Estes Park, Colorado (presenter)
Dr. Andrew Martin – University of Colorado, Boulder (presenter)
Dr. Jessica Metcalf – University of Colorado, Boulder (presenter)
Dr. Kevin Bestgen – Colorado State University, Ft. Collins (presenter)
Dr. Kevin Rogers – Aquatic Research Scientist, Colorado Parks and Wildlife

3.0 Workshop

The Workshop was held at the Service's regional offices in Lakewood, Colorado. The Workshop started at 8:00 am on Tuesday, July 30, 2013 and finished at 5:00 pm on Thursday, August 1, 2013. Dr. Tom Turner from the University of New Mexico facilitated the Workshop. The goals of the Workshop were to: 1) Evaluate science of recent genetics and meristics studies and reach consensus about implications for management and 2) Develop recommendations for future efforts, including both additional research and management activities.

3.1 Essential Reading

The Service provided the following documents as essential reading during the expert panel review (Appendix A contains full citations and the complete list of suggested reading):

BESTGEN, K.R., K.B. ROGERS AND R. GRANGER. 2013. Phenotype predicts genotype for lineages of native cutthroat trout in the Southern Rocky Mountains. Final Report to US Fish and Wildlife Service, Colorado Field Office, Denver Federal Center (MS 65412), Denver, CO. Larval Fish Laboratory Contribution 177.

BRUNELLI, J.P., J.M. MALLATT, R.F. LEARY, M. ALFAQIH, R.B. PHILLIPS, G.H. THORGAARD. 2013. Y chromosome phylogeny for cutthroat trout *(Oncorhynchus clarkii*) subspecies is generally concordant with those of other markers. Molecular Phylogenetics and Evolution 66:592-602.

HOUSTON, D. D., D. B. ELZINGA, P. J. MAUGHAN, S. M. SMITH, J. S. KAUWE, R. P. EVANS, R. B. STINGER, AND D. K. SHIOZAWA. 2012. Single nucleotide polymorphism discovery in cutthroat trout subspecies using genome reduction, barcoding, and 454 pyro-sequencing. BMC Genomics 13: 724- 740.

LOXTERMAN, J. L., AND E. R. KEELEY. 2012. Watershed boundaries and geographic isolation: patterns of diversification in cutthroat trout from western North America. BMC Evolutionary Biology 12:38.

METCALF, J. L., V. L. PRITCHARD, S. M. SILVESTRI, J. B. JENKINS, J. S. WOOD, D. E. COWLEY, R. P. EVANS, D. K. SHIOZAWA, AND A. P. MARTIN. 2007. Across the great divide: genetic forensics reveals misidentification of endangered cutthroat trout populations. Molecular Ecology 16:4445-4454.

METCALF, J. L., S. L. STOWELL, C. M. KENNEDY, K. B. ROGERS, D. MCDONALD, J. EPP, K. KEEPERS, A. COOPER, J. J. AUSTIN, AND A. P. MARTIN. 2012. Historical stocking data and 19th century DNA reveal human-induced changes to native diversity and distribution of cutthroat trout. Molecular Ecology 21:5194-5207.

PRITCHARD, V. L., J. L. METCALF, K. JONES, A. P. MARTIN, AND D. E. COWLEY. 2008. Population structure and genetic management of Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*). Conservation Genetics.

ROGERS, K. B. 2010. Cutthroat trout taxonomy: exploring the heritage of Colorado's state fish. Pages 152-157 *in* R. F. Carline and C. LoSapio, editors. Wild Trout X: Sustaining wild trout in a changing world. Wild Trout Symposium, Bozeman, Montana.

SHIOZAWA, D. K., R. P. EVANS, P. UMACK, A. JOHNSON, AND J. MATHIS. 2010. Cutthroat trout phylogenetic relationships with an assessment of associations among several subspecies. Pages 158-166 *in* R. F. Carline and C. LoSapio, editors. Wild Trout X: Sustaining wild trout in a changing world. Wild Trout Symposium, Bozeman, Montana.

3.2 Day One Summary

Mr. Doug Krieger presented on background information on behalf of the Greenback Cutthroat Trout Recovery Team. The attendees discussed the purpose and goal of the Workshop. Mr. Chris Kennedy presented the history of cutthroat trout stocking in Colorado. Dr. Jessica Metcalf and Dr. Andrew Martin presented on the genetics of cutthroat trout in Colorado and the Metcalf et al. 2012 study. Dr. Kevin Bestgen presented on the meristics study of cutthroat trout. All presentations were followed by discussion amongst the panelists.

3.3 Day Two Summary

Dr. Dennis Shiozawa presented on cutthroat trout genetics in general. Dr. Behnke's research was discussed. Dr. Tom Turner led a review and discussion of the different hypotheses. Dr. Richard Mayden presented on species concepts during the Public Comment session. One public comment was provided by a recreational off highway vehicle (OHV) group.

3.4 Day Three Summary

Attendees reviewed the Factsheet (Appendix C) and made revisions. Attendees then reviewed the Discussion Questions (Appendix C). Attendees discussed management implications of the studies.

The following section summarizes the terminology used for common and scientific names and geography throughout this report and the individual memoranda.

Lineage	**Common Name**	***Scientific Name***	***Notes***
Blue	Colorado River cutthroat trout	*O. c. pleuriticus*	May require revision of historical range
Green	Greenback cutthroat trout?	*O. c. stomias?*	May require taxonomic revision and new subspecies name
Purple	Greenback cutthroat trout?	*O. c. stomias?*	Bear Creek fish, see green lineage
Yellow	Yellowfin cutthroat trout	*O. c. macdonaldi*	Presumed extinct
Orange	Rio Grande cutthroat trout	*O. c. virginalis*	Not disputed
Red	none	none	San Juan River fish, presumed extinct, likely requires taxonomic revision and new subspecies name

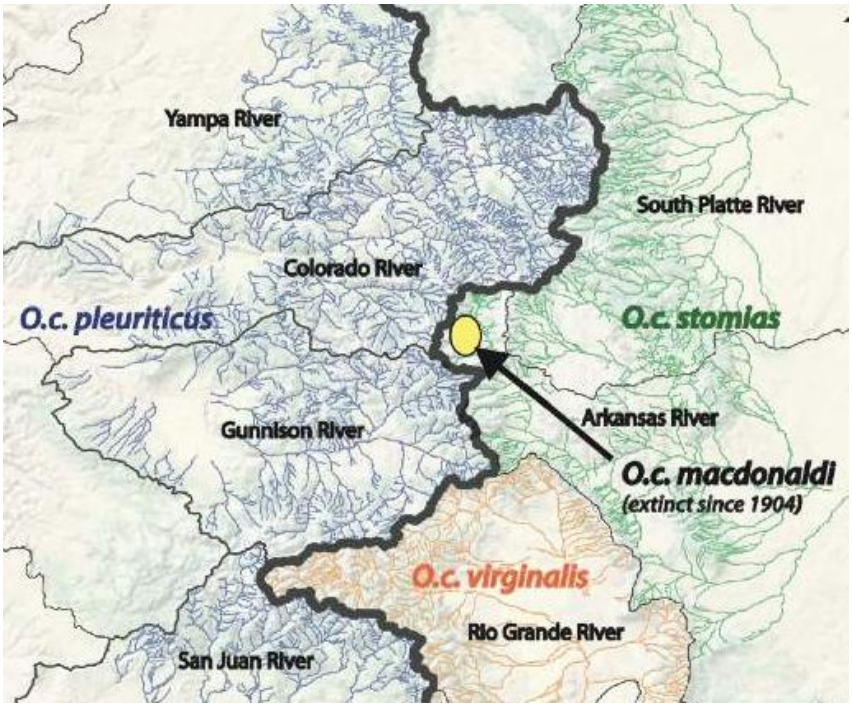

Figure 1. Previous hypothesis about interior cutthroat trout distributions (courtesy Krieger presentation at Workshop).

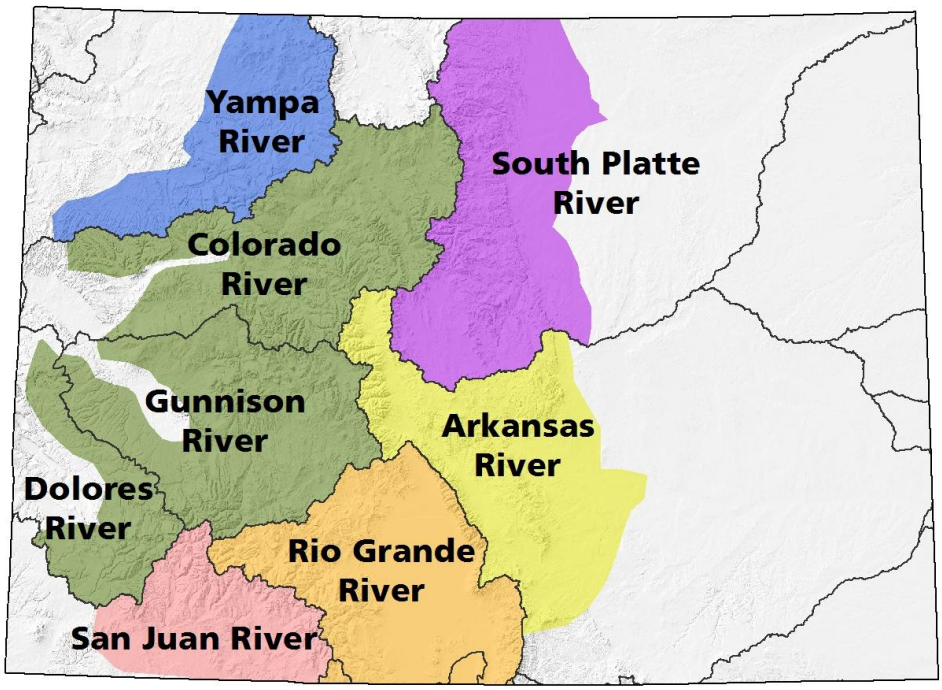

Figure 2. Revised hypothesis about interior cutthroat trout distributions (courtesy Metcalf et al. 2012).

4.0 Summary of Expert Panel Responses

The expert panel considered and responded to the Discussion Questions (Appendix C) provided during the Workshop. The following section summarizes their responses, with their full responses provided in Appendix D. Responses from the Recovery Team, agency representatives and/or authors are provided in Appendix E. Table 1 below provides a summary of whether a reviewer provided a response to a question and the total pages provided by the reviewer. Answers with 'No Response' indicate the reviewer did not feel themselves qualified to respond to that particular question. Expert Panel members are summarized separately from other attendees of the Workshop (i.e., Recovery Team Members, Agency Representatives and Authors).

After the table, summaries of the answers for each question are provided. These summaries focus on the expert panel responses (Panelists #1-8), while including highlights and relevant points from other attendees (Panelists #9-16).

Table 1: Reviewer Response to Each Question

Expert Panel	Evaluation of the Science					Biodiversity Implications						Management Implications						Total Pages
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Panelist #1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10
Panelist #2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	4
Panelist #3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	-	✓	10
Panelist #4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10
Panelist #5	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	5
Panelist #6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	12
Panelist #7	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	7
Panelist #8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	5
Other Attendees																		
Panelist #9	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	5
Panelist #10	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	-	6
Panelist #11	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	5
Panelist #12	✓	✓	✓	-	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	-	✓	4
Panelist #13	✓	✓	✓	-	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	4
Panelist #14	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	7
Panelist #15	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	4
Panelist #16	✓	✓	✓	-	✓	✓	✓	✓	-	✓	-	✓	✓	✓	✓	✓	✓	3

4.1 Evaluation of the Science

1. *Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat trout lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?*

- Panelist 1: The conclusions are plausible, but it should still be considered a working hypothesis and needs to be supplemented with additional research. Evidence supports blue lineage fish being stocked into the Arkansas and South Platte River basins and the yellowfin cutthroat trout association with the Arkansas River basin. The history of the green lineage in the Arkansas River basin is unclear with several possible explanations.

- Panelist 2: The conclusions reached by the Metcalf studies were not supported by the study. The gene flow analyses were not done to the best science available and their conclusions are only one set of many possible alternatives.

- Panelist 3: The Metcalf study does answer many questions, but does not completely answer questions pertinent to the green lineage fish. The study does support six lineages, mostly separated by major river basins (Also Panelist 6, 7, 8, 12, 14, 16). With respect to the green lineage fish, there are alternative explanations for the distribution of these fish on both the East and West Slopes.

- Panelist 4: The protocols to generate molecular data are sound, with caveats of low sample size and short sequences (Also Panelist 3, 6, 8, 10, 14). The historical research collection sites, curatorial practices, and record gaps provide some uncertainty. The study disregards the possibility of ancestral polymorphism and defaults to the stocking scenario to explain the distribution of green and blue lineage fish, when the scenario could be more complex.

- Panelist 5: The inferences were more plausible than other scenarios.

- Panelist 6: The study was careful regarding contamination and the validity of using DNA for historical references.

- Panelist 7: The study is the best available science on the historic and current distribution of cutthroat trout in the headwaters of the South Platte River. The unlikely alternative explanation of Metcalf et al. 2012 data would be that all the matching mtDNA museum samples represent the range-wide organized movement and introduction of a lineage of fish prior to 1871.

- Panelist 8: Further study is needed to test the six lineage hypothesis (using appropriate nuclear markers) and refine the geographic range of the lineages (examine more historical samples). There are alternative explanations for the origin of the Bear Creek population.

<u>Other Panelists</u>

- Panelist 9: The paper explains the patterns in the most parsimonious way, but other hypotheses cannot be ruled out. From museum specimens, it seems the South Platte River basin contained its own lineage of cutthroat trout consistent with those found in Bear Creek. However, few museum specimens were available for the Arkansas River basin to comfortably determine that the yellowfin were the native cutthroat trout there. Perhaps, it is

possible these fish were not founded from a stocking event, as suggested in the paper, but from an invasion from west of the Continental Divide in the late Pleistocene.

- Panelist 10: It is reasonable that Bear Creek fish were stocked from the South Platte River drainage based on the historical evidence. The native fishes of the Arkansas River drainage are not as transparent. The conclusions of the 2012 paper, that green lineage fish were not native to the Arkansas River basin, is somewhat uncertain. See Panelist 4.

- Panelist 14: The conclusion that Bear Creek is native to the South Platte River and not native to the Arkansas River is supported by the data presented but could be disputed. Bear Creek fish may have been historically present in the Arkansas River drainage and simply not represented in the museum samples. The conclusion that the blue lineage fish were historically restricted to drainage basins on the western slope is supported but the inference that the blue lineage was historically restricted to the Yampa and Green River drainages is not supported. The full historic distribution of blue lineage fish remains uncertain. I disagree with the interpretation that it is unlikely that green lineage was native to the Arkansas River.

- Overall

  Panelists generally agreed:

  - the study was sound (1, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16), within the limitations of low sample sizes, short sequences and available museum specimens (1, 3, 4, 6, 7, 8, 9, 10, 14)
  - the study made a strong case for six lineages in Colorado, with two of them extinct (3, 5, 6, 7, 8, 11, 12, 14)
  - the study did not provide a clear answer about the relationship between East Slope and West Slope green lineage fish (1, 2, 3, 4, 6, 10, 14)
  - further analysis is necessary to confirm the conclusions (1, 2, 3, 4, 6, 14)

  Other responses worth noting (most of these are elaborated on under other questions):

  - the study supported that the Bear Creek lineage represented fish from the South Platte River drainage (5, 6, 10, 11, 14)
  - the San Juan River (red lineage) and yellowfin (yellow lineage in Arkansas River basin) cutthroat trout are likely extinct (3, 6, 12)
  - the Rio Grande cutthroat trout is a distinct lineage (3, 4, 6)
  - the blue lineage ancestral watersheds were the Yampa and White Rivers (3, 6)
  - the blue lineage fish were stocked on the East Slope (1, 5, 6, 14)
  - the green lineage ancestral watersheds were the Colorado, Gunnison and Dolores Rivers (3, 6)

2. *Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?*

- Panelist 1: They generally do correlate and show difference among the lineages. In particular, the Bear Creek, Rio Grande, and blue lineages separate consistently in both studies (Panelist 6, 7, 10, 14, 16).

- Panelist 2: Yes, based on other genetic studies and at a wider scale, the morphological studies provide a clearer picture of the diversity.

- Panelist 3: The molecular hypothesis explains more variation than the geographical hypothesis, which is consistent with the results of the genetics study (Panelist 5). There was evidence that the meristic variation was at the scale of geographic management units (GMUs). Additional samples are needed to clarify some of the variation, in particular East Slope green lineage fish.

- Panelist 4: The studies both find there are four extant lineages (green, blue, Bear Creek, Rio Grande) (Panelist 5, 6, 9, 11, 12, 13). The meristics study provided a more nuanced perspective, demonstrating difference amongst GMUs.

- Panelist 5: See Panelist 3 and 4.

- Panelist 6: See Panelist 1 and 5.

- Panelist 7: The meristics study supports all six historical lineages and generally agrees with the genetics study.

- Panelist 8: See Panelist 3.

Other Panelists

- Panelist 10: The green lineage fish had the greatest trait variation. Either the East Slope green lineage fish represent native trout diversity from a West Slope to East Slope invasion or the geographical model is correct in supporting differentiation between extant green and blue fish on the West Slope. Also, see Panelist 1.

- Panelist 11: The populations of green lineage fish on the East Slope are best explained by either being out of place and originating from the West Slope or they are an admixture of the green and blue lineages (based on genetic and historical stocking data). It is possible founder effects caused the unique patterns found in small populations. An analysis of South Platte River lineage museum samples would help with understanding if Bear Creek is native to the South Platte River. Also, see Panelist 4.

- Panelist 14: The meristics study revealed more fine-scale differences in green lineage fish than the genetics study. The West Slope green lineage is intermediate between the blue lineage and Rio Grande cutthroat trout, whereas the East Slope green lineage had traits that were intermediary between the West Slope green lineage and Bear Creek fish. Also, see Panelist 8.

- Overall

  Panelists generally agreed:

  - the meristic study does correlate with the genetic study and supports the four extant lineages of cutthroat trout in Colorado (All panelists)
  - Bear Creek, Rio Grande and blue lineages consistently separate from other lineages (1, 3, 6, 7, 10, 11, 14, 16)

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3. *To what extent are historical spatial distributions of green and blue lineages known?*

- Panelist 1: The Colorado River basin is actually subdivided into two basins: the Upper Colorado River basin and the Lower Colorado River basin. It is presumed the blue lineage fish originate in the entire Upper Colorado River basin and its three subbasins (Panelist 9). Green lineage fish have been identified in the South Platte and Arkansas River basins (East Slope) and Lower Colorado River basin (West Slope). The distribution of the green lineage on both sides of the Continental Divide and in both the Arkansas and South Platte River basins is not typical for natural dispersal unless the event occurred recently (Holocene).

- Panelist 2: The historical distributions of the green and blue lineages are not well known.

- Panelist 3: Green lineage fish occurred historically in the Colorado, Gunnison, Dolores drainages on the West Slope and potentially in the Arkansas basin on the East Slope (Panelist 11, 13, 14). The blue lineage fish occur in the White, Yampa, and Colorado River drainages, but are likely in the Arkansas River from stocking (Panelist 8, 11, 12, 13, 14).

- Panelist 4: The complexity of the trout phylogeny and stocking history make it nearly impossible to infer with certainty, historical distributions. The actual process was likely much more complicated than we can predict. With the small sample size of specimens, we cannot say they are “proof” of historical populations.

- Panelist 5: They are not known with certainty (Panelist 15). One hypothesis is that much of the current overlap in blue-green distribution is the result of stocking. This hypothesis seems plausible given the available information but this issue has not been resolved conclusively.

- Panelist 6: Data suggesting the distinct blue lineage fish were historically confined to the Yampa/White drainage (Panelist 10) and East Slope blue lineage fish were a result of stocking are convincing (Panelists 12, 13). Green lineage fish are presumed to historically reside in the Colorado/Gunnison/Dolores drainages (Panelist 10, 11, 12, 14). The origin of East Slope green lineage fish can be explained in a few ways, but more research must be done to make a firm conclusion (Panelist 11, 14). There are some issues with the data used to evaluate green lineage fish; notably the mtDNA and nuclear DNA did not agree the assignment of individual fish to blue or green lineages in some cases.

- Panelist 7: There appears to be good evidence for a blue lineage in the Yampa River basin, with David Starr Jordan’s 1889 description of Trappers Lake fish consistent with modern day pure Trappers Lake fish. David Starr Jordan’s physical descriptions and figures indicate an

abundant East Slope green lineage as of 1889. Jordan's descriptions of West Slope fish are not consistent, but tend to support a West Slope green lineage as of 1889.

- Panelist 8: The evidence is at least as strong that the green lineage existed historically on the East Slope and more analysis will be needed to resolve the question of the origin the East Slope green lineage fish. As stated in my reply to question 1, this can best be resolved by additional study including examining more historical samples (if they exist for the area of interest) and applying appropriate nuclear markers. See Panelist 3 also.

Other Panelists

- Panelist 10: The idea that green lineage fish are not native to the East Slope is plausible and I would accept it as the best explanation until/if other information comes to light. If Bear Creek fish were actually native to the Arkansas River basin, then it is likely it would have been represented in the 1889 samples. Also, see Panelist 6.

- Panelist 13: Data are limited to clarify the origin and status of East Slope blue and green lineages. We need to proceed with caution when managing the fish on the East Slope, but neither the blue or green East Slope populations warrant protection under ESA. Also, see Panelists 3 and 6.

- Panelist 14: The Bear Creek haplotype matches five haplotypes in the South Platte River, which provides evidence that the purple lineage was native to the South Platte River. Also, see Panelists 3 and 6.

- Panelist 15: It may be more important to agree on the current "boundaries" of the lineages and move forward to restore robust populations. Also see Panelist 5.

- Panelist 16: The historical stocking records are remarkably intact and represent the best information we have to delineate historical distributions. We have a fairly strong concept of where these fish originated.

- Overall

Generally panelists agreed:

  - blue lineage fish are from the Upper Colorado Basin (White, Yampa Rivers) historically (1, 3, 6, 7, 8, 9, 10, 11, 12, 16), with Lower Colorado Basin (Gunnison, Dolores Rivers) and East Slope populations a result of stocking (3, 6, 7, 8, 10)

  - green lineage fish are from the Lower Colorado Basin (Colorado, Gunnison, Dolores Rivers) on the West Slope, with the reason for their occurrence on the East Slope unclear (1, 3, 6, 8, 10, 11, 16)

But there was a clear dissenting conclusion:

  - the historical spatial distributions of green and blue lineages are unclear (2, 4, 5, 13, 14, 15)

4. *How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or evolutionary significant units (ESUs) in other cutthroat trout?*

- Panelist 1: The relationship between genetic and meristic variation and subspecies designation in cutthroat trout is complex. The separation of both the green lineage and the Bear Creek population is significant and both populations are likely eligible for subspecies or ESU-level recognition. There is some complexity associated with the green lineage due to possible admixturing with the blue lineage.

- Panelist 2: This cannot be answered as comparable studies do not exist for other cutthroat trout.

- Panelist 3: Blue, green, orange and purple (Bear Creek) lineage fish are demonstrably monophyletic and exhibit significant divergence in meristic traits across lineages. Molecular and morphological evidence for diversification of lineages is comparable to, and in most cases, much better than evidence available for other named subspecies of cutthroat trout.

- Panelist 4: Comparing levels of divergence among studies is difficult, due to differences in methodologies, sample sizes, analytical protocols, scope, and focus of studies. Rather than comparing results of studies that focus on single groups, a better approach is to examine levels of divergence in broad-scale studies.

- Panelist 5: This is challenging to answer because genetic lineages within Colorado often do not follow geography, but in general, the levels of genetic variation found within populations of Colorado cutthroat trout seem comparable to those found in other areas. We were not shown enough data for other subspecies to comment on a comparison. Divergence between green, blue, Bear Creek, and Rio Grande constitute ESUs or subspecies. Divergence between East and West Slope populations within the green lineage equate to Management Units (MUs)

- Panelist 6: The divergence of Colorado cutthroat trout species seems relatively recent compared to other subspecies of cutthroat trout. An analysis of outbreeding depression among southern Rocky Mountain cutthroat trout would be highly useful.

- Panelist 7: The lineages appear to qualify as ESUs.

- Panelist 8: There is not enough published information to answer this question. Any comparison should be based on studies that ideally use the same markers (or marker types, e.g., mtDNA, microsatellites, SNPs). A study of cutthroat trout thought out the range is needed and factors such as effective population size, gene flow, and mutation rate, should all additionally be considered when analyzing diversity differences.

Other Panelists

- Panelist 9: Within species of cutthroat trout, other designated subspecies show similar amounts of molecular variation. Some strains of Pacific salmon currently managed as ESUs might show less differentiation in genotype and perhaps meristic characters than discussed in this situation.

- Panelist 10: Shiozawa's work on cutthroat trout phylogenies which concluded that the Bear Creek lineage is distinct from Colorado River and Rio Grande cutthroat trout is important.

- Panelist 11: The differences between the different lineages are similar in magnitude to differences between other trout species. Cutthroat trout are anomalous in that they are recognized at the subspecies level. Bear Creek should be assigned *O.c.stomias* along with the South Platte River fish. Green lineage should be recognized as a distinct subspecies. The native distribution of *O.c.pleuriticus* should be revised so it is not historically being recognized as being present in the Colorado, Gunnison, or Delores River basins. The San Juan lineage should be described as a new subspecies.

- Panelist 14: Multiple efforts indicate that green, blue, Bear Creek, and Rio Grande are separate from one another.

- Overall

  There was conflicting conclusions among the panelists:

  - genetic and meristic variation is similar to or greater than that seen between other cutthroat trout lineages (1, 3, 9, 11, 14)
  - comparisons cannot be made with other cutthroat trout lineages (2, 4, 8)
  - at least two lineages qualify for subspecies or ESU designation (1, 3, 5, 7, 11)

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5. *Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?*

- Panelist 1: Yes, although further testing will be needed to support the hypothesis.

- Panelist 2: No, the genetic studies should have included data from other studies in larger scale evaluations. The morphology study suffered from not examining historic specimens from the South Platte River.

- Panelist 3: Both studies cite and include all pertinent literature. There are areas in both studies where more data and study are warranted.

- Panelists 4 - 7: See Panelist 3

- Panelist 8: Yes, although further study is warranted to address historical distribution of the green lineage, particularly evidence of the East Slope populations, and testing the molecular hypothesis using appropriate nuclear markers must be conducted.

- Overall

  The majority agreed that the studies included the relevant literature (1, 3-9, 11-15)

4.2 Biodiversity Implications

6. *Do lineages identified in genetics and meristics studies rise to the level of a listable entity? (a. different subspecies? b. distinct population segments (DPSs)? c. other?)*

- Panelist 1: The blue and green lineages on the West Slope should be managed as two distinct entities and should be considered, at a minimum, two DPSs. To understand the status of the West Slope green populations, more research is needed of their origin through use of phylogenetic data to identify nuclear haplotypes. Once this is done, the abundance of "pure" green lineage populations in the West Slope should be identified, which could result in East Slope green lineage fish becoming important if it is determined they were the result of stocking since they would be candidate sources for reintroduction to the West Slope.

- Panelist 2: The lineages constitute different species when their differentiation is compared to other trout species not in North America.

- Panelist 3: The subspecies, *O.c.pleuriticus* should be more narrowly defined to the drainages historically occupied by the blue lineage (Yampa, White). The subspecies *O.c.virginalis* refers to fishes in the upper Rio Grande drainage, and should maintain management on the GMU level. *O.c.stomias* is not as clear and may require a newly elevated green lineage and a stricter definition of *stomias* will require taxonomic revision. It may be most defensible to propose a new lineage that encompasses green lineage that identifies East and West Slope lineages as 'DPSs' with the East Slope population having more protection than the West Slope.

- Panelist 4: Green, blue, Bear Creek, and Rio Grande approximate differences between ESUs and divergence between East and West Slope green lineage can be equated to MUs. *O.c.stomias* may be categorized as either the green or Bear Creek lineage (see comments under Questions 7 and 8). This situation has been encountered in other species groups, particularly the Boreal Toad.

- Panelist 5: Rio Grande populations are *O.c.virginalis* (Panelist 10). Blue lineage represents the contemporary distribution of *O.c.pleuriticus* and should continue to be treated as a subspecies (Panelist 10) with a revised distribution. Blue lineage presence on the East Slope is likely due to stocking. The green lineage is a second lineage that seems to be widely distributed in what was historically the range of *O.c.pleuriticus.* There are several scenarios for how to list the green lineage depending on what conclusions are drawn about the history of the species and what we define as a subspecies. Bear Creek is a distinctive population that can be listed under ESA. The taxonomy of the greenback trout and whether or not Bear Creek should be given the name *O.c.stomias* needs to be resolved by taxonomists. Bear Creek should either be given the designation of *stomias* solely for listing purposes under ESA or declared as a DPS of the species *O.clarkii* and be listed as such.

- Panelist 6: Addressing subspecies designations will require taxonomic revision and this lies outside of the purview of the review panel. The Bear Creek (Panelist 16), West Slope green, East Slope green, and West Slope blue lineages all warrant protection as separate DPSs.

- Panelist 7: Using Mayr and Ashlock (1991), all the lineages appear to be good subspecies defined within major river drainages, with limits to natural movement of genetic materials between river drainages.

- Panelist 8: It is unclear what actually constitutes a subspecies. Data suggests the four distinct lineages (blue, green, Rio Grande, Bear Creek) are listable DPSs or ESUs.

Other Panelists

- Panelist 9: Since the question posed simply asks whether the lineages are potentially listable, we are looking to address the first two criteria for a DPS: Discreteness and Significance. Regardless of how the USFWS decides to rule, both molecular and meristic data suggest these lineages should be managed as discrete entities even at the GMU level.

- Panelist 11: Bear Creek should be recognized as *O.c.stomias* and listed under ESA as endangered. The green and blue lineages do not satisfy the criterion for listing at this time.

- Panelist 14: Blue, green, Rio Grande, and Bear Creek should be listed as subspecies (Panelist 16). The East Slope green lineage should be considered for a listing as a DPS.

- Overall

  Panelists generally agreed:

  - Six lineages constitute separate, distinct lines (1, 3-12, 14-16) and taxonomic revision of Colorado cutthroat trout is likely warranted (3, 5, 6, 11, 15)

  - *O.c.virginalis* is limited to the Rio Grande populations (3, 4, 5, 10, 11)

  - *O.c.pleuriticus* should be more narrowly defined to the drainages historically occupied by the blue lineage (Yampa, White) (1, 3, 5, 10, 11)

  - Green lineage may be distinct, and the West Slope and East Slope populations may constitute DPSs or at least MUs (assuming East Slope is not stocked) (1, 3, 4, 6, 10, 14)

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7. *Is the Bear Creek population considered to be greenback cutthroat trout?*

- Panelist 1: The term “greenback” has a varied history, so it is difficult to make a conclusion. The name for the Bear Creek fish will depend on a decision on 1) whether or not to retain the common name greenback for the fish in Bear Creek, 2) the result of a petition to the International Commission on Zoological Nomenclature to change the type specimen for *stomias* and to retain the Arkansas River basin, 3) a decision on whether or not to separate the green lineage fish on the West Slope from those on the East Slope, and/or 4) a decision as to the relation of the Bear Creek fish to other interior cutthroat trout taxa. The common name, greenback, can be moved to the Bear Creek fish with less difficulty than the scientific name, *O.c.stomias*. Additionally, it is important to show independent support that the Bear Creek fish are the true original South Platte greenback.

- Panelist 2: This is unsure since the samples from the South Platte River basin were never examined for morphology.

- Panelist 3: This is difficult to answer because doing so, would potentially remove protection from green lineage fish previously classified as greenback cutthroat trout. *O.c.stomias* could continue to be defined how they are and all green lineages and Bear Creek can be named in the recovery plan as lineages that warrant special management and protection.

- Panelist 4: It may be a prudent solution to equate Bear Creek lineage with the taxon *O.c.stomias* to maintain a listing for the species. Bear Creek is a distinct genetic and morphological lineage and represents a distinct evolutionary lineage.

- Panelist 5: See answer to Question 6.

- Panelist 6: The common name seems to be meant for the cutthroat trout in the South Platte River and it would therefore seem appropriate to give the common name greenback cutthroat trout to Bear Creek fish (Panelist 7, 8, 10-13).

- Panelist 7: See Panelist 6. Additional work to review and document the phenotypes of the museum specimens in relationship to modern day Bear Creek and other lineages may help resolve questions and inconsistencies (Panelist 12).

- Panelist 8: Regardless of the name, it seems the cutthroat trout in Bear Creek are the only existing representatives of the South Platte River lineage (Panelist 15). Also see Panelist 6.

Other Panelists

- Panelist 9: If the yellowfin was the aboriginal fish of the entire Arkansas River basin, then the name greenback should default to those found in the South Platte River basin. This is not necessarily the case for the scientific name *stomias* since those were assigned to type specimens that appear to be Rio Grande cutthroat trout. Since the name *virginalis* predates *stomias*, the latter then technically becomes a synonym for Rio Grande cutthroat trout.

- Panelist 16: No. Dr. Shiozawa's data show separation between Bear Creek and "greenbacks" 1 million years ago. Greenback cutthroat trout are the fish from the South Platte River but maybe due to founder effects, Bear Creek fish, while the ancestor to the South Platte River, are no longer "greenback" cutthroat trout.

- Overall

Panelists generally agreed:

  - the Bear Creek population should/could retain the common name, greenback cutthroat trout (1, 4, 6-10, 11-13, 15)

  - the Bear Creek population is likely the only remaining population that was originally in the South Platte River, but this requires confirmation (1, 6, 7, 8, 10, 12, 14, 15)

Other responses worth noting:

  - the Bear Creek population probably should not retain the scientific name (*O.c.stomias*) (3, 9), although others felt it should (11)

- confusion has been caused by the type specimen likely belonging to another subspecies (1, 9, 10, 11, 12)
- modifying the name could remove protection from the Bear Creek fish and they definitely warrant protection (3, 5)

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8. *How do we describe the East Slope green lineage?*

- Panelist 1: The East Slope green lineage is a group that clades out within the West Slope green lineage. The East Slope lineage is not monophyletic with West Slope haplotypes, and the lack of this indicates the fish entered the region recently from a genetically diverse population or they were introduced by man from a population that no longer exists on the West Slope. The meristics study suggests that East Slope and West Slope green lineage fish are different.
- Panelist 2: The East Slope Green lineage is a distinct lineage (Panelist 6).
- Panelist 3: This will depend on the outcome of studies designed to ascertain the origin of East Slope haplotypes (Panelist 9). See answers to previous questions.
- Panelist 4: Whether the green lineage is equated with *O. c. stomias* or a new subspecies name, the West Slope and East Slope populations should be regarded as two seperate DPSs or MUs.
- Panelist 5: There are two hypotheses that must be resolved before being able to make a decision (Panelist 15). One is whether or not the green lineage is native to the East Slope (Panelist 9, 15). The second is whether the green lineage haplotypes in some East Slope populations are the result of stocking from West Slope sources (Panelist 15).
- Panelist 6: See Panelist 2. However, the divergence between East and West Slope green populations warrants further research (Panelist 9).
- Panelist 7: The East Slope green lineage is a group of four existing populations that physically and genetically assign to the West Slope green lineage. However, they have unique haplotypes not found within West Slope green lineages. There was originally only one known South Platte East Slope green lineage population (Como Creek), with the other South Platte East Slope green lineage population (Fern Lake) being a transplant of Como Creek stock.
- Panelist 8: The East Slope green lineage fish should be considered from an unknown origin until more research can clarify their relationship to West Slope fish.

Other Panelists

- Panelist 10: The relationship between the East and West Slope green lineage populations needs to be resolved further (Panelist 16). Until further research can be conducted, we should consider affording higher protection to the West Slope green lineage (within range) and some protection for the East Slope green lineage fish.

- Panelist 11: The green lineage is a distinct subspecies native to the Colorado, Gunnison, and Dolores basins. The whole species complex should be revised so the main lineages are considered species and the minor lineages are considered subspecies.

- Panelist 12 and 13: The East Slope green lineage fish is comprised of green lineage fish native to the West Slope that have been stocked east of the Continental Divide. It does not seem the East Slope lineage fish should be considered a separate listable entity; they should be considered the same as green lineage fish on the West Slope. East Slope green fish should not be eliminated, but do not warrant protection under the ESA.

- Panelist 14: The current research indicates that East Slope green lineage populations have unique haplotypes present only on the East Slope and geographic separation from other green lineage populations. In addition, meristically they appear a bit different than the West Slope green lineage. Although they are still clearly green lineage fish both genetically and meristically, they should be managed separately at this time.

- Overall

  Panelists generally agreed:

  - the East Slope green lineage is distinct from the West Slope green lineage (1, 2, 4, 6)

  - additional studies are needed to determine the relationship between the East Slope and West Slope green lineage, including whether the East Slope fish were stocked from the West Slope (1, 3, 5, 6, 8, 9, 10, 15, 16)

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9. *What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?*

- Panelist 1: This depends on whether the East Slope green lineage originated from stocking or from a recent natural invasion. If the East Slope green lineage trout can be shown to have originated from stocking from the West Slope, then their distinctness in morphology (especially spotting), is not of much importance. The populations then may be of importance for rehabilitation of West Slope populations. If the East Slope green lineage fish are shown to be native, the fact that they are imbedded in the West Slope green lineage with mtDNA should be verified with nuclear genes. Corroborating findings would suggest that they are a part of the West Slope green lineage. At that point I would manage them as DPSs or ESUs.

- Panelist 2: They suggest they are distinct.

- Panelist 3: East Slope green lineages that are not found in West Slope populations may comprise a DPS of the green lineage and GMU level management is most appropriate (Panelist 14).

- Panelist 4: The entire green lineage should be recognized as a distinct ESU or subspecies (Panelist 12), with several GMUs recognized as geographically isolated entities within this ESU/subspecies.

- Panelist 5: I see little basis from existing data to consider the East Slope green lineage populations a separate subspecies. Under some scenarios, these populations might meet the criteria to be considered a DPS, if it were concluded that their presence east of the divide was not the result of stocking.

- Panelist 6: East Slope green lineage should be considered a distinct ESU or DPS.

- Panelist 7: The existence of unique East Slope green haplotypes is not supported by the limited museum samples, but their uniqueness is not refuted by genetic markers from non-native populations.

- Panelist 8: This question cannot be answered without further research (Panelist 15). There is not enough information to characterize East Slope green lineage fish (Panelist 9).

Other Panelists

- Panelist 9: Examination of meristic characters in the museum specimens and characterizing all green lineage population haplotypes would help inform this decision (Panelist 14).

- Panelist 10: The entire green lineage should be designated a DPS, with greater protection afforded to the West Slope populations.

- Panelist 14: An investigation of East and West Slope green lineage fish must be done to analyze the amount of admixture between green lineage fish and blue, rainbow or Yellowstone, and identify a "pure" population of green lineage fish. Also, additional exploration of all East and West Slope green lineage haplotypes is needed to see if they share haplotypes with those on the West Slope. Also, see Panelists 3 and 9.

- Overall

  Panelists agreed that the data suggest they are distinct lineages at the DPS or ESU level (1, 2, 3, 5, 6, 10, 14)

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10. *Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?*

- Panelist 1: No, these studies do not resolve the phylogenies enough to conclude probable routes of colonization (Panelist 4, 7, 8, 9). They do show strong support for geographically defined subspecies with the addition of Bear Creek. Additional sequence data for mitochondrial DNA and developing primer sets for nuclear DNA will help to clarify routes of colonization. The meristics study suggests overlap of West Slope green lineage fish with the Rio Grande cutthroat trout, but I believe it is too difficult for meristics studies to generate phylogeographic associations.

- Panelist 2: This question cannot be answered until more studies are done (Panelist 8).

- Panelist 3: Shiozawa's work provides the most likely scenario for colonization/evolution of interior cutthroat trout, with the ancestor coming from the northwest and diversifying on the West Slope (blue lineage). Then there was dispersal to the East Slope with subsequent evolution of the green/yellowfin/Bear Creek/Rio Grande lineages (Panelist 10, 13, 16). It is unclear whether or not East Slope green lineage fish are native to the East Slope or recently introduced. Blue lineage fish on the East Slope are likely there from stocking activities.

- Panelist 4: No, the two studies do not give probable routes of colonization for the lineages. Contradicting evidence from the two studies could be accommodated by a scenario that invokes an initial dispersal of *O. clarkii* into drainages on both sides of the Continental Divide, followed by divergence of lineages in subdrainages. Subsequent dispersal would have allowed spread of lineages into other drainages.

- Panelist 5: There are two hypotheses and currently available analyses do not allow one to distinguish between these hypotheses.

- Panelist 6: It is difficult to objectively test routes of colonization. It is not necessary to objectively weigh evidence of genetic and phenotypic divergence of extant lineages to make decisions about separate conservation designations such as ESUs or DPSs.

- Panelist 7: See Panelist 5.

- Panelist 8: See Panelist 1.

Other Panelists

- Panelist 11: The fact that the meristic study revealed the East Slope green lineage fish fall between the two main green and blue clusters and three out of four show evidence of genetic admixture supports the hypothesis that they are of hybrid descent.

- Panelist 14: It seems fairly accepted in the published phylogenies that all four lineages (Rio Grande, blue, green and Bear Creek) came from a common ancestral line. There are two potential routes of colonization. The first is that cutthroat trout entered Colorado from the NW and then the Rio Grande, green and Bear lineage moved south and eventually East over the Continental Divide diversifying along the way. The second route is two separate events, one from the NW establishing the blue lineage and one from the West/Southwest which moved East establishing the Rio Grande, green and bear lineages. In either case, the more established historical view that the green lineage descended directly from blue lineage has less current support than green lineage descending from Rio Grande.

- Overall

  Panelists generally agreed that these studies do not provide enough data to identify routes of colonization (1, 2-10, 14, 16). Almost all panelists refer to other research than these genetics and meristics studies to answer this question, in particular Dr. Shiozawa's research.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

- Panelist 1: The blue lineage fish are the Colorado River cutthroat trout that originated in the West Slope and were most likely stocked to the East Slope (Panelist 14). This is supported by the East Slope blue lineage fish matching a subset of haplotypes in Trapper's Lake (Panelist 14). The lack of genetic diversity in East Slope blue lineage fish could be due to stocking by man or a small founder population or bottlenecks (Panelist 8, 10). The green lineage fish can be interpreted as a single lineage polytomy containing all interior cutthroat trout except the Yellowstone cutthroat trout line. The Bear Creek lineage is then an independent line in the polytomy or the entire green lineage can be divided into two clades with the Bear Creek fish being one line branching off basally from the predominant green lineage. Morphological data separating the Bear Creek lineage from green lineage is less informative because Bear Creek is so highly inbred. Examination of South Platte River museum specimens will help to answer how much inbreeding influences their morphology.

- Panelist 2: The variation is significant and should be considered distinct lineages until they are tested and proven otherwise.

- Panelist 3: Addressed under questions 6, 9, 10.

- Panelist 4: The morphological and genetic patterns correspond with subdrainages and are consistent with variation among populations (MUs) within lineages (ESUs).

- Panelist 5: Addressed under questions 6 and 8.

- Panelist 6: Addressed under questions 1, 6, and 8 for the green lineage. Evidence suggests East Slope blue lineage fish are of recent hatchery origin from the West Slope (Panelist 8, 13).

- Panelist 7: Addressed under question 9 for the green lineage. Many East Slope and West Slope (outside of the Yampa River drainage) blue lineage populations were established from a unique lake population of Colorado River cutthroat trout within the blue lineage (Panelist 9, 14).

- Panelist 8: Green lineage fish likely historically existed on the East Slope, but more analysis will be needed to resolve the question of the origin of East Slope green lineage fish.

Other Panelists

- Panelist 9: See Panelist 7 for blue lineage fish. Green lineage fish show variation between East/West Slopes in both phenotype and genotype, which could be a result of natural invasions or from stocking west to east (Panelist 14).

- Panelist 11: The East Slope green lineage is likely a result of stocking and are an admixture between the green and blue lineages.

- Panelist 12 and 13: The East Slope green lineage is the result of small founding populations of stocked fish.

- Overall

The answers under question 6, 8 and 9 generally apply here. Panelists generally agreed:

- the blue lineage on the East Slope were stocked from Trapper's Lake (1, 3, 6, 7, 8, 9, 12, 13, 14)
- the green lineage has several possible relationships between East and West Slope populations (1, 3, 5, 6, 7, 9, 10, 11, 14, 15), as well as the Bear Creek population (1, 3)
- there is not enough evidence to separate East Slope and West Slope populations of both blue and green lineages within their respective lineage (1, 3, 6, 8, 15), although they may merit protection as DPSs or MUs for the green lineage (3, 4, 5, 6)

4.3 Management Implications

12. *The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green and blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?*

- Panelist 1: Bear Creek fish show less variability than the green lineage fish (Panelist 16). However, Bear Creek fish are a single, inbred population, subject to drift and founder effect, so it is difficult to say how representative of the morphology historically. The Harvard collection of fish should be examined. The population must be duplicated in the South Platte River basin, immediately. It may be useful to try genetic rescue (Panelist 6, 8, 9) with other green lineage fish in those replicated populations. The risk of population loss is too high with one small population.

- Panelist 2: The genetic variability in the Bear Creek lineage should not be altered by introducing other alleles from other lineages. The population should be maintained and protected in Bear Creek, habitat restoration should be done in the area. There is justification to relocate them to fishless streams in the South Platte River basin.

- Panelist 3: An unpublished MS thesis from Andrew Martin's lab suggested very limited genetic variability in the Bear Creek population exists (Panelist 14). There is also evidence of developmental abnormalities in the hatchery, which could be from environmental factors and selection pressures (Panelist 6, 16). The recovery of the Bear Creek population requires it to be replicated in South Platte River basin streams (Panelist 6, 12), in Bear Creek itself, and in the hatchery (Panelist 14).

- Panelist 4: Bear Creek only exhibits one mtDNA haplotype (Metcalf et al. 2012). Details on nuclear genetic data are not published. Morphological analyses (Bestgen and Rogers 2013) show a contracted morpho-space, although this lineage exists as a single population, and thus among-population variation is not available. Maintaining a large population in the wild (possible replicated in suitable habitats as outlined below) should be a priority.

- Panelist 5: There is not a consistent trend with Bear Creek meristic variability based on the meristic study. The most important issue for this population is whether it shows inbreeding depression. The experiment currently being conducted on Bear Creek fish should shed light on whether the abnormalities in hatchery-raised fish are because of inbreeding depression and/or environmental conditions associated with culture (Panelist 10). In addition, comparisons with other lineages to determine if Bear Creek has higher than average abnormalities would informative.

- Panelist 6: Metcalf et al. (2007) reports low genetic diversity for the four East Slope populations, including Bear Creek. Phenotypically, Bear Creek fish are divergent from other lineages but have maintained variation comparable to other lineages. Inbreeding effects should be examined. Duplicating the population within the South Platte River basin should be undertaken and if issues arise, genetic rescue of those populations may be merited. The Bear Creek population should not be impacted by these activities. See Panelist 1 and 3.

- Panelist 7: High levels of hatchery deformities may not limit the establishment of new wild Bear Creek lineage populations, but is assumed to reflect the lineages' overall fitness. Caution should be used with any outcrossing activities.

- Panelist 8: The immediate priority is to replicate the Bear Creek population (Panelist 14). Individuals should be transferred to a large enough location that can support a population size which minimizes loss of diversity due to a bottleneck. See Panelist 1.

Other Panelists

- Panelist 9: Studies should be conducted to determine if there are any actual inbreeding effects in the wild and, if there are, genetic rescue should be performed on a small number of individuals. Bear Creek and several replicate populations should be maintained.

- Panelist 10: Bear Creek fish were clearly different from other lineages but the there was no opportunity to compare Bear Creek fish to museum specimens. This is an investigation with broad support that would be important to our understanding of potential inbreeding that the Bear Creek fish might have experienced over the past 130 years. To ameliorate possible inbreeding in hatcheries, maximize the size of any replicated populations to include as many mature individuals in future spawning efforts. Also, see Panelist 5.

- Panelist 16: I do not advocate for introducing additional genetic material or "forcing hybridization" but instead translocating Bear Creek fish to a range of different habitats (gradient, temperature, aspect, etc) and letting genetic selection exert pressure on each of these populations. Mixing them then at a later period could then help preserve the extant genetics left in this population. Also, see Panelists 1 and 3.

- Overall

All panelists agreed this is an important and small population worthy of protection and that is currently at-risk.

There is no agreement on whether the Bear Creek population shows reduced variability.

- the Bear Creek population shows reduced variability compared to the green lineage fish, with respect to meristics (1, 4, 10, 16) and genetics (3, 4, 11, 14, 16)
- the Bear Creek population does not show reduced variability compared to other lineages (5, 6, 9, 15)

There are conflicting opinions about management actions:

- replicate Bear Creek fish into multiple populations, usually within the South Platte River basin (1, 2, 4, 6, 8, 10, 11, 12, 14, 16)
- undertake genetic rescue of the replicated populations along with extensive monitoring (1, 6, 8)
- do not interfere with the genetics, there is still too little information (2, 6, 7, 15, 16)
- evaluate hatchery stocks, inbreeding depression and outbreeding depression prior to any genetic rescue or population replication (3, 5, 6, 8, 9, 10, 15)

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13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat trout for the Arkansas River basin?

- Panelist 1: The likely native Arkansas River cutthroat trout is the extinct yellowfin (Panelist 3, 5, 7, 11, 12, 16). However if a recent natural invasion of the green lineage into the Arkansas River basin has occurred, then the green lineage in the Arkansas River basin would likely also contain genetic material from the Yellowfin. A series of good nuclear gene phylogenies would allow identification of those haplotypes.
- Panelist 2: The East Slope green lineage with its unique haplotype and distinctive morphology.
- Panelist 3: Yellowfin are presumed extinct, so the East Slope green lineage fish are the best candidates for reintroduction to the Arkansas River basin. It appears that there is less urgency to restocking the Arkansas River drainage at this time, so it will be best to await results of definitive studies to identify whether East Slope green lineage fish are native, and whether suitable (low to no introgression) populations exist as donors (Panelist 12).
- Panelist 4: Bear Creek is a poor candidate because of its small population size, low genetic variability, and propagation problems and may not be native to the Arkansas River basin. The best candidate for re-establishing native cutthroat trout across the Arkansas River basin is the green lineage from the East Slope (Panelist 6).
- Panelist 5: Green lineage fish are from an uncertain origin and other trout introduced could not be considered “native cutthroat trout of the Arkansas basin.” The basin could be used to further conservation goals by expanding Bear Creek and/or Rio Grande populations there.
- Panelist 6: See Panelist 4. Subsequent data and analyses may find that East Slope green lineage fish are introduced from the West Slope.

- Panelist 7: No reintroductions of “native Arkansas” fish should be conducted until more data is available to confirm what fish are native to the basin (Panelist 9, 10). However, Bear Creek fish could be reared in controlled reservoir sites within the Arkansas River basin for reintroduction to the South Platte River basin.

- Panelist 8: There is not enough information to determine what population to reintroduce in the Arkansas River basin. However, if sufficient habitat exists to replicate the Bear Creek population within the Arkansas River basin, this should be done to stabilize the Bear Creek population with the understanding Bear Creek may not be the native species of the basin.

Other Panelists

- Panelist 11: It makes sense to stock Bear Creek (Panelist 13) fish since the native cutthroat trout species to the Arkansas River have gone extinct. Alternatively, it is possible that South Fork Hayden Creek may represent a native Arkansas River drainage population, suggesting that decisions about whether to replace current populations of cutthroat trout inhabiting Arkansas River basin streams should be made on a case-by-case basis and populations without strong evidence of admixture or stocking should remain.

- Panelist 12: See Panelist 3. If it becomes apparent the East Slope green lineage fish in the Arkansas River basin are a result of stocking, then it may be appropriate to reintroduce Bear Creek cutthroat trout into the Arkansas River basin (Panelist 14).

- Panelist 14: Both the green lineage and the Bear Creek lineage could be native to the Arkansas River, although Bear Creek are more likely. If East Slope green lineage fish are found to be native through more research, they should be stocked. See Panelist 12.

- Panelist 15: I would be more concerned with disease introductions and/or potential human movement into the wrong drainages by the public. Most of our limited funding should be used for the study and introduction of the lineages for which we are confident.

- Overall

Panelists generally agreed:

  - the likely native Arkansas River cutthroat trout is the extinct yellowfin (yellow lineage) (1, 3, 5, 7, 11, 12, 16)

  - East Slope green lineage fish are the best candidate for reintroduction in the Arkansas River basin (2, 3, 4, 6)

  - additional studies (genetic and field) should be performed before reintroductions are done (1, 3, 5-12, 14)

14. How should next-generation DNA sequencing (NGS) approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

- Panelist 1: First, nuclear markers should be identified and verified (Panelist 12). Also, additional independent loci should be examined. Second, it could be used to generate longer sequence data (Panelist 9). Third, both SNP development and phylogenetic data generation can be accomplished with less cost per base pair of data than is currently achieved with Sanger sequencing. Finally, this technique can be used to obtain significantly more data from degraded DNA found in museum specimens.

- Panelist 2: They should be done at a larger scale to identify relationships and evidence of ancestral polymorphisms in genes that are not interpreted as originating from interbreeding.

- Panelist 3: Further development of nuclear DNA markers (Panelist 4) and a large suite of microsatellite DNA loci to fully characterize genetic diversity, relatedness, and levels of introgression is necessary. Also, further study of East Slope green lineage fish is warranted.

- Panelist 4: NGS can be used to assay a larger proportion of the genome and develop diagnostic markers. Despite suggestions, there is limited use of NGS for historic samples. Also, see Panelist 3.

- Panelist 5: NGS could be used to screen markers and identify genes associated with adaptations, identify nuclear data that would help to resolve the hypotheses surrounding East Slope populations with green lineage haplotypes, and provide more information about the distribution of genetic diversity across the Bear Creek genome.

- Panelist 6: Target single nucleotide polymorphisms (SNPs) only within the blue, green, Bear Creek, and Rio Grande lineages which would help to distinguish among lineages (Panelist 11). Another option would be to obtain a much larger set of SNPs for individuals from the four lineages in question (Panelist 13). This would provide more data and a greater resolution of genetic relationships than in the first option. Also, NGS could be used to reanalyze the museum fish from Metcalf *et al.* (2012) (Panelist 11).

- Panelist 7: Apply next-generation DNA sequencing on the cross section of known cutthroat trout lineages. Use NGS DNA to assist with identifying source populations for genetic rescue of Bear Creek and for monitoring recovery goals.

- Panelist 8: NGS should be used to address questions concerning the range of the green lineage fish (specifically, the origin of East Slope green lineage fish) and test the six lineage hypothesis generated from the mtDNA data. NGS should be applied to both museum and contemporary samples (although the application to museum specimens will likely be difficult due to lower quality of the DNA) (Panelist 11).

- <u>Overall</u>

  Panelists generally agreed that next generation DNA sequencing could be used:

  - to examine additional independent loci to develop better phylogenetic relationships (1, 2, 3, 5, 6, 8)

- to identify and verify nuclear markers and nuclear sequences that can be used for a variety of purposes, including documenting genetic exchanges and during monitoring of populations/broodstock (1, 2-7, 9, 12, 16)

Other responses worth noting:

- costs can be prohibitive when used at a broad scale (4, 6, 8)
- NGS could be used with museum specimens (1, 6, 8)

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15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

- Panelist 1: It is critical to continue to search the West Slope green lineage for mitochondrial DNA haplotypes matching the East Slope green lineage (Panelist 14). Non-conservation populations should be examined. SNPs should receive less emphasis until good nuclear DNA phylogenies are developed for numerous genes (Panelist 3). Nuclear DNA phylogenies must also be generated to strengthen the assessment of mitochondrial signals (Panelist 3). The status of populations must be determined as accurately as possible now.

- Panelist 2: A full scale morphological study with finer level of evaluation of differentiation within drainages should be conducted. Also, broader and more complete sampling of genes for all of the lineages of cutthroat trout with finer level of evaluation of differentiation within drainages.

- Panelist 3: Establish new populations of Bear Creek lineage in the South Platte River drainage as soon as possible and protect Bear Creek from human encroachment (Panelist 6). Sustain a viable fish hatchery program for Bear Creek lineage fish. Evaluate pure and experimental crosses of Bear Creek fish for inbreeding and outbreeding depression. Evaluate the genetic and meristic status of East Slope green lineage fish once nuclear markers are developed (Panelist 14). After identifying the status of East Slope green lineage fish, identify the appropriateness of stocking Arkansas River drainages. Initiate taxonomic revision of cutthroat trout in Colorado. Survey the San Juan GMUs for presence of red lineage fish (Panelist 12). Scope a program to produce red lineage fish through backcrossing. Assess threats to green lineage fish on the West Slope and maintain federal listing as necessary. Assess the population status of blue lineage fish in the West Slope drainages, continue demographic and genetic assessment, and prevent habitat degradation and hybridization. Also see Panelist 1.

- Panelist 4: A comprehensive morphological study of historic collections should be conducted including West Slope specimens (Panelist 7, 14).

- Panelist 5: See responses to other questions.

- Panelist 6: NGS analysis of museum specimens from Metcalf et al. (2012) should be a priority. Develop a panel of SNPs from Dr. Shiozawa's NGS data or from additional RAD-sequence data. Resolve the West Slope versus East Slope and natural colonization versus anthropogenic introduction theories surrounding green lineage fish. Quantify the inbreeding

of Bear Creek lineage fish (Panelist 7). Perform genetic rescue of Bear Creek fish if necessary. Increase the understanding of connectivity among populations of both blue and green lineages. Test the likelihood of outbreeding depression in crosses among lineages.

- Panelist 7: Apply NGS DNA sequencing on the cross section of known cutthroat trout lineages. Determine what existing cutthroat trout populations could be considered for mixing with Bear Creek to increase genetic diversity and reduce deformities, while maintaining the maximum Bear Creek genetic signature and meristics, based on DNA and physical characteristics. Determine the level of DNA and meristics deviation that could be allowed by the ESA under a beneficial Bear Creek hybridization program. See Panelist 4.

- Panelist 8: A broad phylogeographic look at cutthroat trout is needed. It should also be a priority to survey the San Juan lineage.

Other Panelists

- Panelist 9: We should continue to manage these lineages at the GMU level while protecting at least East Slope green fish until taxonomic uncertainty can be resolved. Research priorities should include examining museum specimens to further assess meristic traits among Bear Creek fish, green lineage fish and yellowfin cutthroat trout. More importantly, fitness studies should be conducted to evaluate what the consequences (if any) might result from limited genetic diversity in the Bear Creek population, and whether they are suitable for large-scale reintroduction efforts. These studies are critical to inform whether genetic rescue efforts are warranted.

- Panelist 11: The top priority is to establish some natural populations of Bear Creek fish in either the Arkansas or South Platte River basins. The second priority is to assess the degree of inbreeding depression in Bear Creek fish.

- Panelist 12: Meristic and morphometric studies of the San Juan and South Platte River museum specimens would provide insight into which of the current lineages they are most similar to and if they still exist (Panelist 14). Intensive inventories in the San Juan drainage should continue in an effort to find an extant population of the presumed extinct San Juan cutthroat lineage. Projects that eliminate non-native populations and replace them with pure lineages should continue. See Panelists 3 and 9.

- Panelist 13: Extend genetic and meristics analyses to Colorado River cutthroat trout in the rest of their historic range outside of Colorado. Also, use NGS with all intermountain cutthroat trout at a finer scale (6 digit HUC).

- Panelist 14: Analyzing more East Slope green populations to see if the meristic differences continue to consistently show the East Slope green populations as “different” will provide evidence for whether DPS or another listing is warranted. In addition, further study on the West Slope of both green and blue lineages is needed to inform their historic distribution, especially for the Colorado River drainage. Also, see Panelists 1, 4, 9, and 12.

- Panelist 15: Reintroducing fish into the South Platte River drainage may be difficult because of extreme population growth and past and current drought-related fire conditions. In order to reintroduce Bear Creek fish into this area, a very thorough knowledge of land use, productivity, and availability are needed. Criteria to identify recovery sites must be created

also. One prudent research topic that should be pursued is to better understand the phylogenetic relationships among different lineages. Also, given the variability in the Bear Creek fish, we should look at the effects of placing fish in new habitats.

- Panelist 16: Resolve the West Slope and East Slope green lineage confusion. Test the survival of different Bear Creek stocks overtime and differences in recruitment strength. Evaluate the success of Bear Creek fish translocation into different habitats.

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16. *Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado. What conclusions can you make from these findings and what inference to future management of the lineage can you predict? What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

- Panelist 1: The Bear Creek population is expressing a higher proportion of deformed fish that usually observed. It is possible that the deleterious genes causing deformities are in such high frequency that it is not rapidly removing defective genes from the population. It would be helpful to have the hatchery raised deformities quantified by conducting an experiment with other green lineages or interior cutthroat trout populations (Panelist 9).

- Panelist 2: You cannot make conclusions about this from hatchery fish because there are too many variables that cannot be controlled for.

- Panelist 3: The underlying causes of abnormalities warrant further study. Recovery of the Bear Creek lineage requires it to be replicated into South Platte streams (with renovation and barriers as needed) and existing fish in Bear Creek itself and in the hatchery program are the only possible sources.

- Panelist 4: Potential issues could arise due to low genetic diversity and high-relatedness (Panelist 16). Widespread propagation and stocking of Bear Creek should not occur. Instead, a few isolated habitats should be conserved and replicated and a monitoring plan should be put into place for population parameters and morphological characteristics.

- Panelist 5: Experiments to understand the deformities are warranted and important. If the studies underway provide evidence for significant inbreeding depression, then some sort of genetic rescue should be attempted, that involves controlled introductions over time.

- Panelist 6: Addressed under question 12. Further research into the deformities is warranted.

- Panelist 7: This was experienced in other cutthroat trout populations and was alleviated by establishing broodstocks and then fertilizing hatchery-reared eggs with milt from wild populations. Since Bear Creek fish are able to survive in current habitat limitations, they may be successful in reestablishing new stream and lake locations.

- Panelist 8: Inferences of the findings cannot be made because there has been no study that quantifies the abnormalities and relates them to the environment. To better understand how to produce viable populations, look for locations that share similar habitat features with the Bear Creek drainage, such as water temperature. Given the need (in my opinion) to replicate the population soon, it would be preferable to integrate the research into habit needs with the replication effort. Researchers should monitor closely the relationship between habitat features and the performance of the replicate populations.

Other Panelists

- Panelist 10: We should use caution when using this population for future production and restoration. We can use techniques to maximize genetic diversity and further fitness-related studies. Also, progeny of Bear Creek hatchery fish will be ready for stocking in the wild in 2014, and monitoring of the released fish will provide insight as to their survival in the wild.

- Panelist 11: This should be revisited after populations have been established to assess the success rate and fitness. It is important for all manipulations of Bear Creek fish to be studied so we can learn as much as possible about population establishment and gene pool changes over time.

- Panelist 13: Bottleneck events likely played a role in the Bear Creek population and reduced the genetic diversity of the population. Managers may also consider PIT tagging fish and taking a “stud book” approach to spawning, check the motility of sperm from males, or track the hatching success of each pairing.

- Panelist 14: It is still important to keep and replicate the Bear Creek population in several locations with its current genetic make-up to safeguard this population. The long–term management of this species likely cannot rest on the Bear Creek population alone but will need to include a plan to systematically diversify targeted replicate populations by adding one or more closely related individuals. The ongoing fitness study in the hatchery crossing Bear Creek and green lineage fish should be replicated in a field. In addition, a study to add diversity at a much lower introgression level, can be implemented and closely monitored.

- Panelist 16: These fish originated in a small founder population and future movements and management will be very difficult. Future research may include introducing them into different habitats to see where they survive best.

- Overall

  Panelists generally agreed that it would valuable to quantify hatchery raised deformities relative to other lineages or interior cutthroat trout populations and under a variety of environmental conditions (1, 3, 5, 6, 8, 9, 11, 15).

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17. Please provide other relevant comments not addressed in the above questions.

- Panelist 1: Geographic isolation is likely the primary evolutionary force in speciation of North American obligate aquatic organisms. The Arkansas and South Platte River basin lineages are confusing because they are similar but downstream connections were too far apart. This

suggests a recent, possibly Holocene, transfer across a low order stream system. Similar patterns are seen with other populations of cutthroat trout and other fishes in the west.

- Panelist 2: Overall, more data must be collected on all lineages in order to make sound, scientific conclusions. Without the necessary data, a decision should not be rushed or else the natural signature of the complex could be lost forever.

- Panelist 3: Several alternatives are available. First, nothing could be done and the Bear Creek and East and West Slope green lineages would be protected. Second, the East Slope green lineage could be designated as a DPS and could be managed as a separate population from the West Slope. Finally, the Service could await a taxonomic revision, and list the purple lineage as endangered under the ESA. Assuming the green lineage becomes a listed subspecies, the East Slope could be a DPS under the ESA.

- Panelist 4: An overarching conservation goal should be to identify entities that encapsulate biodiversity across the landscape. Many freshwater fishes of the northern hemisphere represent recently evolved, sympatric lineages of uncertain taxonomic status, with unrecognized taxonomic diversity perhaps greatest in salmonids.

Other Panelists

- Panelist 12: The current status of West Slope green lineage populations as “threatened” requires federal agencies to engage in formal Section 7 consultation on all actions that may affect one of these populations. The time and funding spent on consultation is a cause for concern for federal agencies. Re-examination of the number of green lineage populations may reveal that threatened status is no longer warranted. We need to generate a non-technical summary of the workshop findings that can be shared with the public and with managers in the various management agencies (state and federal). Agency managers who are charged with making land management decisions need to understand the basic findings from the workshop in simple terms. Specialists within each agency can then work with managers to provide technical details as needed.

- Panelist 13: Precaution should be taken, but action should not be paralyzed by uncertainty. We must act to conserve the Bear Creek population and replicate it in other watersheds. Rio Grande cutthroat trout management should continue as it has been.

- Panelist 15: Maintaining current lineages and improving rare lineages is of more concern to me than definitely understanding the historic connections.

4.4 Public Comments

A member of the OHV group attended the Workshop public session and expressed a general interest in the research and potential management implications to the recreational users along Bear Creek in El Paso County, Colorado. Written public comments were received from Trout Unlimited and are included in Appendix F.

5.0 Overall Summary

Unless otherwise stated, each conclusion was accepted by every panelist that mentioned this item (there are several items that were not mentioned by one or more panelists).

- Rio Grande cutthroat trout are a distinct lineage and there are no revisions to historic or current range, taxonomy or nomenclature.
- San Juan River drainage had a distinct lineage that is currently unnamed/unrecognized and is likely extinct. There seemed to be general agreement that some field effort should go toward confirming the lack of native (red lineage) cutthroat trout there now.
- Yellowfin cutthroat trout were the native trout to the Arkansas River and is now extinct. There are no revisions to historic range, taxonomy or nomenclature. Most panelists felt there were no other native trout to the Arkansas River drainage, but that was not a strong consensus.
- Colorado River cutthroat trout (blue lineage) historic range was on the West Slope and included the Yampa and White Rivers. The blue lineage fish on the East Slope were stocked from the West Slope (Trapper's Lake). There was less agreement about how far south the blue lineage fish occurred historically on the West Slope (i.e., Colorado River). Generally, panelists also did not feel the blue lineage merited any special protection.
- Bear Creek trout are a distinct lineage and merit protection and significant efforts to preserve the population. The majority of panelists did agree that the Bear Creek trout were the remnant population representing the original South Platte River basin cutthroat trout. There was not agreement about the priority management needs for this species, although most panelists agreed for the need for experiments to determine if the deformity rate is unusual for cutthroat trout generally and/or if they are result of hatchery conditions. There was also general agreement for stocking Bear Creek fish into more locations (with most agreeing that should be within the South Platte River basin).
- There was little consensus regarding the green lineage on any topic. All panelists agreed that Gunnison, Dolores and the Colorado River on the West Slope included the historic range of the green lineage. There was obvious disagreement about the green lineage on the East Slope. Some panelists viewed the data as supporting stocking on the East Slope; other panelists felt it supported complex evolutionary history, which may or may not include stocking. Some panelists suggested the green lineage could be native to the East Slope, most did not. All panelists agreed further study was needed to resolve this issue with larger sample sizes and better understanding of the separation between the blue and green lineages on the East Slope.
- There was not agreement on whether Bear Creek lineage or green lineage should be considered greenback cutthroat trout and/or *O.c.stomias*. Ultimately, this issue for both lineages cannot be resolved until pending taxonomic issues are resolved and a taxonomic revision undertaken, as pointed out by several panelists.
- Panelists generally felt that both studies were high quality research and the data was informative, but that the low sample sizes of the genetic study hampered drawing firm conclusions about specific issues.

6.0 Management and Research Recommendations

The panelists suggested a number of relevant research projects and management activities. The following include a short of list of suggested research and management that were suggested by multiple people and/or were suggested as priorities.

Suggested Priority Research Projects

- Taxonomic efforts to resolve common and scientific names including:
  - Revision of the historic range of *O.c.pleuriticus*
  - Establish common and scientific names for both the green lineage and the Bear Creek population (purple lineage), with one of them retaining the common name "greenback cutthroat trout". This would be in conjunction with clarifying the status of the scientific name *O.c.stomias*.
  - Establish common and scientific names for the San Juan river native cutthroat trout (red lineage)
- All panelists suggested additional research into the East Slope green lineage:
  - Additional assessment of current genetic status with larger sample sizes to determine level of introgression with the blue lineage and whether there was any introgression with yellowfin cutthroat trout prior to their extinction.
  - Additional sampling from West Slope green lineage fish to determine if there are matching haplotypes
- Nuclear DNA research relating to:
  - Developing phylogenies among these lineages
  - Develop additional genetic information to provide better resolution among interior cutthroat trout lineages
  - Develop nuclear DNA markers that can further clarify the relationship between East and West Slope green lineage fish
- Many panelists suggested research regarding Bear Creek (purple) lineage:
  - Assess hatchery deformity rate relative to other interior cutthroat trout
  - Assess hatchery deformity rate under a range of hatchery conditions
  - Assess fitness of hatchery stock in a range of field conditions
  - Assess inbreeding and outbreeding depression
- Conduct additional field efforts to identify any remnant San Juan cutthroat trout (red lineage) populations

Suggested Priority Management Activities

- Protect and replicate the Bear Creek population. There were several ways proposed for doing this but establishing additional populations within the South Platte River basin was the most frequent suggestion after understanding and protecting the hatchery stock.
- Coordinate protection with the taxonomic efforts so that the Bear Creek population, in particular, does not end up without protection.
- Coordinate ESA protection (via DPS or ESU designations) with the taxonomic revisions.
- Evaluate status of green lineage in particular after additional genetic research to determine merit for protection under ESA.

(Courtesy Krieger Presentation at Workshop)

APPENDIX A

Agenda, Attendees and Suggested Reading for the Greenback Cutthroat Trout Genetics and Meristics Expert Panel Workshop

30 July – 1 August 2013
Lakewood, Colorado

Greenback Trout Genetics and Meristics Studies – Facilitated Expert Panel Workshop
U.S. Fish & Wildlife Service
Denver, Colorado

Dates: July 30, 31, August 1, 2013

Location:
U.S. Fish and Wildlife Service Office
134 Union Blvd.
Lakewood, Colorado 80228

Workshop Goals

1) Evaluate science of recent genetics and meristics studies. Reach consensus about implications for management
2) Recommendations for future efforts
    a. Additional research
    b. Management activities

Workshop Agenda

Day 1 (July 30, 2013)

8:15	Welcome and introductions
8:30	Overview of the workshop objectives, agenda, and process
9:00	Presentation by Greenback Recovery Team – Context of Genetics and Meristic Studies
9:30	Break
9:45	Presentation of Genetics Study (Metcalfe et al. 2012)
10:45	Recitation of Genetics Study discussion questions
11:00	Discussion of Genetics Study
12:00	Lunch
1:00	Presentation of Meristics Study
2:00	Recitation of Meristics Study discussion questions
2:15	Discussion of Meristics Study
3:15	Break
3:45	(Continued) Discussion of Meristics Study
4:30	Compilation of notes, comments, and recommendations by Workshop Panelists
4:45	Review of Day 1 findings
5:00	End of Day 1

Greenback Trout Genetics and Meristics Studies – Facilitated Expert Panel Workshop
U.S. Fish & Wildlife Service
Denver, Colorado

Day 2 (July 31, 2013)

8:15	Brief review of objectives and agenda for Day 2
8:30	Presentation by Dr. Shiozawa on his recent genomic analysis work
9:30	Discussion on Dr. Shiozawa's presentation
10:00	Break
10:15	Overview and discussion of other relevant studies
11:30	Lunch
12:30	Sharing of comments from Dr. Behnke (Kevin Bestgen – presenting)
1:30	Discussion of Dr. Behnkes's comments
2:30	Discussion of questions regarding areas of agreement/disagreement between studies
3:30	Session Compilation of notes, comments, and recommendations by Workshop Panelists
3:45	Break
4:00	Public Input
6:00	Review of Day 2 findings
6:15	End of Day 2

Day 3 (August 1, 2013)

8:15	Brief review of objectives and agenda for Day 3
8:30	Discussion of questions regarding taxonomic and management implications of studies
10:00	Break
10:30	(Continued) Discussion on taxonomic and management implications of studies
11:30	Lunch
12:30	Discussion Questions
3:00	Break
3:15	(Continues) Discussion Questions
3:45	Compile notes, comments, and recommendations of Workshop Panelists
4:00	Review of Day 3 findings
4:15	Review of Workshop findings
4:30	Next Steps – Preparation of Individual Reports and Workshop Summary Report
5:00	End of Day 3 and Workshop

D*iscussion Questions for Panelists*

To discuss during workshop and to which each panelist will respond in writing after the workshop. The responses will then be compiled into a summary report.

1. Does the meristic study correlate with findings in the genetics study (i.e, does the meristics study show a difference in physical characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)? To what extent are current spatial distributions of GB, CR lineages known?
2. Do the lineages identified in the genetics and meristics studies rise to the level of a listable entity
    a. different subspecies?
    b. distinct population segments (DPS)?
3. Are the conclusions reached in the genetics study (Metcalf *et al.* 2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study?
4. How does genetic and meristic variation identified in the Studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout? Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?
5. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, Rio Grande cutthroat trout management?
6. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to GB, CR lineages? What are the research priorities for the species given the outcome of these studies?
7. Do our genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroats?
8. Are the east slope - west slope variation seen for green and blue lineages significant? What could lead to those differences and is there any taxonomic implications?
9. What do the rare haplotypes and morphological consistencies of east-green fish suggest in terms of subspecies or ECU distinctions?
10. How should we address the nomenclature for O. stomias?
11. Is the Bear Creek population considered to be greenback?
12. How do we describe the east slope green lineage?
13. Could the “unusual” east slope green populations be a factor of native populations plus stocked fish from Grand Mesa?

Management Discussion Topics

14. What approaches, if any, should be considered to manage genetic variability in the Bear Creek lineage to ameliorate potential or actual inbreeding effects?
15. What guidelines should be used to manage populations that show signs of introgression?
    - With non-native species, native species
16. Can existing blue or green restoration projects be converted to good representatives of Bear Creek by stocking over blue or green populations?
17. Which cutthroat lineage or subspecies should be considered for reintroduction in the Arkansas basin and for the San Juan basin?
18. In terms of population biology, what the implications of managing green and blue lineages that are not in their native drainages.
19. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.
    - What conclusions can you make from these findings and what inference to future management of the lineage can you predict?
    - What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the S. Platte drainage?

Documents for Distribution Prior to Workshop

Essential reading:

BESTGEN ET AL. In Prep. Meristics paper (draft available to Workshop members)

Brunelli, J.P., J.M. Mallatt, R.F. Leary, M. Alfaqih, R.B. Phillips, G.H. Thorgaard. 2013. Y chromosome phylogeny for cutthroat trout *(Oncorhynchus clarkii*) subspecies is generally concordant with those of other markers. Molecular Phylogenetics and Evolution 66:592-602.

HOUSTON. D. D., D. B. ELZINGA, P. J. MAUGHAN, S. M. SMITH, J. S. KAUWE, R. P. EVANS, R. B. STINGER, AND D. K. SHIOZAWA. 2012. Single nucleotide polymorphism discovery in cutthroat trout subspecies using genome reduction, barcoding, and 454 pyro-sequencing. BMC Genomics 13:724- 740.

LOXTERMAN, J. L., AND E. R. KEELEY. 2012. Watershed boundaries and geographic isolation: patterns of diversification in cutthroat trout from western North America. BMC Evolutionary Biology 12:38.

METCALF, J. L., V. L. PRITCHARD, S. M. SILVESTRI, J. B. JENKINS, J. S. WOOD, D. E. COWLEY, R. P. EVANS, D. K. SHIOZAWA, AND A. P. MARTIN. 2007. Across the great divide: genetic forensics reveals misidentification of endangered cutthroat trout populations. Molecular Ecology 16:4445-4454.

METCALF J. L., S. L. STOWELL, C. M. KENNEDY, K. B. ROGERS, D. MCDONALD, J. EPP, K. KEEPERS, A. COOPER, J. J. AUSTIN, AND A. P. MARTIN. 2012. Historical stocking data and 19th century DNA reveal human-induced changes to native diversity and distribution of cutthroat trout. Molecular Ecology 21:5194-5207.

PRITCHARD, V. L., J. L. METCALF, K. JONES, A. P. MARTIN, AND D. E. COWLEY. 2008. Population structure and genetic management of Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*). Conservation Genetics.

ROGERS, K. B. 2010. Cutthroat trout taxonomy: exploring the heritage of Colorado's state fish. Pages 152-157 *in* R. F. Carline and C. LoSapio, editors. Wild Trout X: Sustaining wild trout in a changing world. Wild Trout Symposium, Bozeman, Montana. Available online at http://www.wildtroutsymposium.com/proceedings.php

SHIOZAWA, D. K., R. P. EVANS, P. UMACK, A. JOHNSON, AND J. MATHIS. 2010. Cutthroat trout phylogenetic relationships with an assessment of associations among several subspecies. Pages 158-166 *in* R. F. Carline and C. LoSapio, editors. Wild Trout X: Sustaining wild trout in a changing world. Wild Trout Symposium, Bozeman, Montana. Available online at http://www.wildtroutsymposium.com/proceedings.php

Greenback Trout Genetics and Meristics Studies – Facilitated Expert Panel Workshop
U.S. Fish & Wildlife Service
Denver, Colorado

Short summaries:

FWS. 2012. FWS Position Paper on ESA consultations on greenback cutthroat trout, including the cutthroat referred to as Linage GB. Updated October 4, 2012.

ROGERS, K. B. 2012. Characterizing genetic diversity in Colorado River cutthroat trout: identifying Lineage GB populations. Colorado Division of Wildlife, Fort Collins.

ROGERS, K. B. 2013. Recent developments in cutthroat trout taxonomy: implications for Colorado River cutthroat trout. Colorado Parks and Wildlife, Fort Collins.

Historical perspective:

BEHNKE, R. J. 1992. Native trout of western North America. American Fisheries Society Monograph 6. (no PDF).

BEHNKE, R. J. 2002. Trout and salmon of North America. The Free Press. (no PDF).

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Greenback Cutthroat Trout Scientific Review Workshop – List of Attendees

Facilitators

Dr. Tom Turner – University of New Mexico

Ms. Melissa Greulich – AMEC

Panel Members

Dr. Marlis Douglas – University of Illinois

Dr. Richard Mayden - St. Louis University

Dr. Jeffrey Olsen - FWS Conservation Genetics Program

Mr. Bruce Rosenlund - retired, FWS fisheries

Dr. Dennis Shiozawa – Brigham Young University

Dr. Robin Waples – Northwest Fisheries Science Center, NOAA Fisheries

Dr. Andrew Whiteley – University of Massachusetts Amherst

Agency Representatives

Mr. Dirk Miller - Representative Colorado River Cutthroat Trout Conservation Team

Dr. Kevin Rogers – Aquatic Research Scientist, Colorado Parks and Wildlife

Ms. Pam Sponholtz - Representative FWS Fisheries Program

Ms. Leslie Ellwood – Greenback Cutthroat Trout Recovery Team, FWS

Mr. Doug Krieger - Greenback Cutthroat Trout Recovery Team, Colorado Parks and Wildlife

Mr. Jay Thompson - Greenback Cutthroat Trout Recovery Team, BLM

Ms. Mary Kay Watry - Greenback Cutthroat Trout Recovery Team, NPS

Mr. David Winters - Greenback Cutthroat Trout Recovery Team, USFS

Dr. Andrew Martin – University of Colorado, Boulder

Dr. Jessica Metcalf – University of Colorado, Boulder

Dr. Kevin Bestgen – Colorado State University, Ft. Collins

Mr. Chris Kennedy – Fishery Biologist, FWS, Estes Park, Colorado

APPENDIX B

Meeting Notes from the
Greenback Cutthroat Trout Genetics and Meristics
Expert Panel Workshop

30 July – 1 August 2013
Lakewood, Colorado

Greenback Trout Genetics and Meristics Studies – Facilitated Expert Panel Workshop
US Fish and Wildlife Service
Denver, CO

July 30, 2013

- 8:15 Introductions

- 8:20- 9:25 Doug Krieger: Presentation on the Purpose and Background of the Workshop
    - Greenback Trout
        - Extinct in 1937
        - Rediscovered in 1973 and then listed as endangered
        - Threatened in 1978: Arkansas drainage discovered, rendered downlisting
    - Greenback recovery team is comprised of:
        - USFWS
        - CDOW, now CPW
        - USFS
        - BLM
        - NPS
    - Original distribution of trout species in the state of Colorado:
        - West slope: Colorado River
        - East slope: Greenback
        - South: Rio Grande
    - Restoration of native cutthroat trout species:
        - Is difficult because many non-native species exist.
        - Involves restocking native species and then reevaluating progress.
        - Requires the sorting out non-native and native species. Yellowstone, Snake River, and rainbow trout are the main species that hybridized with native cutthroat species. Many techniques used to distinguish between different species – shown in PowerPoint but not discussed in detail.
    - Metcalf et al 2007: Molecular ecology discovered that native species were not necessarily isolated to their original locations.
        - Results found that out of 9 east slope populations only 4 were actually of GB lineage and 1 population in the west slope was of GB lineage.
        - The next step was to find out where populations were located pre-settlement.
    - CU Genetic Study
        - Museum specimens were studied to see how historic populations now line up with current population locations.
    - Meristics Study – Kevin Bestgen at CSU
        - Genetics may not be the only way to distinguish what species are present; meristics may also assist with distinguishing populations.
    - Current understanding:
        - Colorado cutthroat is now NW part of state
        - Greenback is now west slop in Colorado river, Gunnison, and Dolores
        - South Platte is in the Central North part of the state
        - ***Look at slides for before and after
- It is important to figure out where to restock certain species in the correct places, which should incorporate both historical data and current population locations.
- Comments and Questions:
    - Bruce Rosenlund speaks about the history of the program at US FWS. Historically, fish that represented distinct populations were collected, including invasive species, and then scientists blindly tested specimens and identified them. GB, Rio, and

Colorado River all formed a large cluster and were hard to distinguish while others, non-natives, were easily distinguished**.**

- Question: What does the science say, and what should we do? Are these both objectives of the workshop?
    - Response: The aim of the workshop is to come to a consensus of what the science says and how we should apply the results. Another important component of the workshop is to also identify the threats to cutthroat trout: hybridization, land use, etc.
- Comment: The question of what we should do does not really have a scientific response.
    - Response: Individual opinions and consensus of what we should do should be discussed. In the end it is up to FWS to make the final decisions about what species to list. What entities out there should the FWS look at while making that decision? That is the purpose of the workshop. Do any of these threats qualify the subpopulations to be listed under ESA? Is the FWS being overly protective? The aim is not to figure out what should be listed, but to provide information to make the decision.
- Comment: A participant attended a similar workshop before and panelists created a single, cohesive report so that attendees' responses to identified questions were not tied to them. This was a concern because they were worried about their jobs.
    - Comment: Doing the cohesive, single report is not a good approach for this workshop mostly because it is a violation of a law. We also would like to show the diversity in responses from the array of attendees that have been brought together to evaluate such a complex issue.
- Question: What parts of these outcomes are parts of the public record?
    - The meristics study is not complete and Dr. Bestgen would not like it to be distributed to the public until it is completely ready.
    - Comment: In previous reviews, a draft study has never been used, so this is unfamiliar territory for the FWS with regard of what to do with panelist responses to questions that could change when the draft meristics paper changes.
- Question: When we make the final report, will it be available to the public?
    - Response: Yes. There has been discussion of holding onto the summary report until the meristics study is complete.
    - Concern was expressed about when the report comes out. The answers and comments need to match the meristics study. What if the study changes and then does not match the comments?
    - Question: Can panelists be listed as #1, #2, etc?
- Comment: We should write a prelude to each section that describes the background of each panelist that writes a response to assist readers with understanding where a panelist is coming from in their interpretation and perspective.
    - Comment: We chose the variety of people here today because we wanted a variety of perspectives.
    - Comment: We have enough scientific integrity to make educated statements about the questions and that is why we were chosen. We do not need to state our qualifications before each question.
    - Comment: Each person should evaluate whether or not they have enough background to make educated comments on the questions. Don't make a comment if you do not feel comfortable with the subject matter.
- Comment: It is important to remember that most of all, the workshop was created to provide scientific expertise and disregard any politics of the situation.

    - Question: Has the prohibition of any other groups from the discussion part of the workshop been legally approved?
        - Response: Yes. The public interest groups understood the need for scientific stakeholders to be the only ones present during discussions in order to make the most progress.
    - Question: Who is the public that will be invited?
        - Response: Some local advocacy groups, Trout Unlimited, etc.
        - Question: Aren't there more stakeholders that should be involved other than Trout Unlimited?
            - Response: Yes, there will be oil and gas and water groups that are interested once the listing process starts.
            - Response: The listing process is completely separate and the public will be included in that process as well. It will probably stimulate more involvement.
- Comment: Abbreviations and acronyms of different species should be cleared up before discussions begin:
    - Blue = CR, Colorado River
    - Green = GB, Greenback
    - Bear Creek = South Platte native GB, BC
- Question: In the Houston et al. paper, some of the graphs have "GR", should that be "GB"?
    - Response: Yes, it should be GB
- Comment: The discussion questions came together rather quickly because the meristics report came out close to the workshop. They were created prior to discussions and therefore may evolve with the process. The group organizers are open to additional questions and edits to existing questions.
- Comment: The definition of subspecies is very nebulous. Species complexes could also be discussed and considered for the GB trout situation.
    - Comment: Species complexes: hybrid species – 2 different species as parents. The FWS discussed looking at this as a species complex. Zuni blue sucker example was given.
    - Comment: There is no time on the agenda to discuss exactly what a subspecies is, and this is important for the topic. It is difficult to say if we could come to a decision without discussing what a subspecies is, however this could take days.
    - Question: Why doesn't the group discuss lineage, which can be identified, rather than species or subspecies?
        - Yes, the topic of lineage will be brought up many times
    - Comment: From the FWS perspective, the agency often talks about what lineage is. It doesn't have to be 100% of one population, if it is mostly 1 type, then the agency considers it that species. However, representatives of FWS would still like to hear what the group thinks about what a lineage is.
        - Comment: If local populations are reviewed, there is much variation. The concept of a lineage makes sense and most of the studies do discuss lineage.

- 9:45 History of Cutthroat Trout Stocking in Colorado – Chris Kennedy
    - Review of the Metcalf studies, but focused more on the 2012 paper.
    - The 2007 paper found that the distribution changes in the east and west slope primarily was from fish stocking.
    - Conducted research on fish stocking records from the State Fish Commissioner reports – 1889 & 1890

- 1899 – 1943 Colorado state archives
- 1942-1972 CDPW kardex files
- Ends up producing complete state archive of files
- Federal files more difficult to find.
- Overall, about 1.2 billion fish that were stocked in a 100+ period
    - About 245 million cutthroat trout, about 2.5 million/year
- Peak of stocking in mid 1900, in the 1930s
    - This coincided with state agencies acquiring cutthroat trout eggs.
- State cutthroat trout egg retrieval occurred between 1914-1940
    - Twin lakes – state had a hatchery, most of what was taken was put back into the Arkansas River drainage
    - Most important Areas:
        - Trappers and Marvine Lakes – White-Yampa
        - Grand Mesa Lakes – Colorado and Gunnison
        - Emerald lakes - Upper San Juan
- Federal – Leadville national fish hatchery 1899-1930
    - Most insignificant except for Grand Mesa Lakes, and Yellowstone
- Grand Mesa Lakes
    - Gunnison river drainage, had cutthroat trout historically
    - Operated by Leadville national fish hatchery 1899-1909
    - State also collected eggs
    - Over 50 million eggs collected. 29 million restocked.
    - 5 lakes primarily used for egg retrieval. Stocked rainbow trout into Ward lake.
        - Island
        - Ward
        - Eggleston
        - Barron lake
        - Alexander lake
    - Also stocked brook trout eggs to another lake – see ppt.
    - The private landowner, Radcliffe, gained control of the area to stock and maintain fish in the area that contained all 5 lakes.
    - State took control of operations in 1913 and stocked Alexander, Barren, and Island lake with rainbow trout.
    - Stocked trappers and Marvine lakes with cutthroat species later on.
    - Fish from Grand Mesa lakes area was stocked in almost every major drainage in Colorado at some point by the federal government. The state also stocked many of the drainages in the state with Grand Mesa eggs.
        - Also sold eggs to other states and countries.
- Trapper's Lake
    - White river drainage
    - Historically contained cutthroat trout
    - Eggs collected by the state in 1903.
    - 26 million eggs collected from 1914-1925
    - Late 1930s stocked Yellowstone cutthroats
- Marvine Lakes
    - White river drainage
    - Historically contained cutthroat trout
    - Egges collected 1907 to 1936
- Eggs from Marvin and Trapper's lakes
    - Almost every major drainage in CO received eggs.

- Emerald Lakes
  - San Juan River drainage
  - Historically fishless
  - Rainbow trout introduced before 1900, most fish suspect from these lakes. Not a significant source of native cutthroat
    - CPW looked at fish and none showed similarities with San Juan lineage.
  - Collected eggs 1898-1924
  - Records of Emerald Lakes egg distribution very vague and do not allow for anyone to make conclusions.
  - In the hatchery, eggs are not fertilized. Fertilized on site, then taken and distributed by the hatchery.
- New Mexico vs. Colorado stocking
  - CO started stocking about 20 years before.
  - CO had 14 hatcheries operating while NM only had 1.
  - A lot less stocking occurring in NM. NM had more of an appreciation for their native cutthroat trout species.
    - Read letter from game warden to fisheries resource operator at NPS (Yellowstone).
      - Letter from NM biologist stated they would only stock the native, indigenous species to the NM water ways that are more adapted to their waters and land.
- Bear Creek Greenback Population
  - Every taxonomist has stated this population is different.
  - Bear Creek near Pike's peak and CO Springs
  - In 1889, a scientist wrote that Bear Creek was fishless except for brook trout
  - 1882, writing indicates that a man named Jones had a fish hatchery.
  - Beaver Creek, De la Verge Hatchery, and Bell Hatchery were nearby and contained trout. Jones could have obtained his fish from these sites.
    - De la Verge Hatchery – had cutthroat trout. Newspaper article said the man who operated the hatchery personally went to the mountains to collect his trout.
    - Chris's opinion is that Jones got eggs from De la Verge Hatchery.
    - Comment – peaks to Beaver Creek and De la Verge hatchery very low and accessible by people, even in early 1900's.
- Summary:
  - CO leader in trout propagation.
  - See slides….
- Question: How much did you miss? There was a lot of data to go through.
  - Response: There was a lot of private hatchery work prior to State and Federal government work with fish distribution. However, these people were out to make money from the trout. Now most of the native cutthroat species exist at higher elevations, which indicates that they had to be stocked later when roads and accessibility existed by the government and not by private landowners.
- Question: Are there any waters in CO that have not been influenced by stocking?
  - Response: There may be some smaller, individual waters. CO stocking was massive. At one point, CO was the leading producing of the non-native species brook trout.

        - Comment: Stocking of eggs is so easy. Bestgen received bull trout eggs from Montana in the mail.
        - Comment: Even back then, people would send eggs abroad on steam ships and the eggs would survive.
        - Comment: It is nice to gain the historical opinion. New Mexico saw that native species did much better than non-native species. Maybe this is also why some of the southern CO pops are still native. Specifically, the native GB population and Rio Grande population.
    - Question: Have you found anything about attempts to choose certain eggs from fish hatcheries before dispersing them?
        - Response: No, they took whatever they could find.
    - Question: Is it safe to say there was a genetic bottleneck effect in the case of the BC?
    - Question: What is the average fecundity of a female?
        - About 500-600/female back in those days
    - Comment: The mindset of the late 1800's was more agriculturally focused. It is important to remember this when analyzing the history of why they stocked the way they did. There also weren't regulations, fishing licenses, etc.

- Jessica Metcalf – The Journey of Cutthroat Trout Genes in CO
    - Conservation genetics
        - Issues arise when a population is extirpated before genetic analysis is completed.
        - DNA analysis was conducted on museum specimens that predate events to help establish baselines.
        - Ancient DNA is from post-mortem specimens. DNA molecules often decayed: highly fragmented, scarce, chemically modified/mutated. Also easily contaminated with modern DNA.
    - Methods involve using DNA extraction and PCR in a facility that prohibits introduction of other DNA samples.
    - Purpose of the 2012 study:
        - Conducted a genetics survey of cutthroat trout species across CO to find a genetic pattern that represents the subspecies.
        - Found support for 2 separate types of subspecies. Mitochondrial DNA used to make 2 groups.
            - Question: Where is the Bear Creek population? Did you use K = 3?
                - Response: We did, but ended up at K = 2. Can't remember the data. There is data that shows K = 3 is divergent
            - Question: How high up did you go up for K = analysis.
                - Response: K = 10
            - Comment: If you do look at K = 3, the PDR could be a completely different population.
            - Comment: At K=2, the result is more robust than the K=3, which was much more variable between the 2 datasets (DNA).
        - Found 1 population with the green haplotype on the west slope.
            - Concluded that the locations are not likely due from natural movements, but from historical stocking.
        - Blue was CR and Green was GB from this study.
        - New hypothesis started to be questioned. Chris began to research to see if the natural locations could indeed be completely mixed up.

        - Started to look for museum samples that predated stocking and found some.
    - 2012 study
        - Sampling
            - Samples were from the CO and NM between 1857 and 1889.
            - Attempted to extract DNA from 44 individuals and successfully extracted it from 30 samples.
            - Samples were fixed in ethanol, which allowed for easier extraction than if specimens were in formalin.
            - Samples were from 5 geographically different areas of the S. Platte River Drainage.
        - Sequence data
            - Only sampled mitochondrial data
            - DNA highly fragmented
                - Subset of the modern DNA
                - Question: Were they contiguous?
                - Response: Somewhat contiguous. They were looking at areas where there was some variation between subspecies and also where primers would adhere easiest.
                - Sequenced 6 mtDNA fragments
                - Question: were you able to compare nucleotide sequences from individuals?
                - Yes. Answer to question later on
                - 430 base pairs analyzed
        - Replication
            - Many cases, multiple fish were sampled
            - For an individual specimen, multiple DNA samples were taken
            - For an individual specimen, multiple labs sampled the same DNA
            - For each sequence, multiple PCRs and sequencing were repeated.
        - Nine out of 14 samples from the S. Platte specimens had DNA that was amplifiable.
            - Two samples were able to obtain 4 DNA extractions – example
        - An additional way to confirm amplification and DNA validity.
            - Degraded DNA bps quantity maxes out at about 200 bps.
            - Amplification of smaller bp segments easier than the large segments for fragmented bps.
    - Results:
        - Most sequenced from S. Platte and Arkansas River Drainage.
        - Statistical parsimony network, 430 bps explanation
            - Eight fish from the S. Platte were the same
            - Question: Were the jars containing the specimens separate?
                - Response: The jars were actually separated by project site.
            - Question: Of the 5 jars, how many haplotypes came from the same jars?
                - Response: S. Platte – 4 jars. There is a chance the DNA could have contaminated other samples from the same jar. San Juan drainage specimens – 2 haplotypes and from the same jar.
            - Question: The network is not rooted, correct? How did the orange become its own lineage? Andy will discuss.

        - Results indicate that lineage can be explained by drainage.
        - S. Platte river drainage
            - Only 1 lineage found in the drainage
            - Represented as the “Bear Creek” lineage
        - Arkansas river drainage
            - Yellowfin and Greenback
            - Arkansas river – yellow, not found today, extinct
            - Green specimens in this area explanation:
                - Green lineage could have crossed the continental divide naturally OR
                - Could be a result of a stocking event even though Chris did not find evidence of this.
        - Underlying assumption of the research: historical specimens are from where they actually say they are from.
        - Question: how high is the continental divide by the S. Platte and Arkansas River drainage?
            - Response: Very high. Water diversions were also considered and evaluated. No diversion in the area until 1932.
    - Modern data as sampled the same amount of times as the historic data and performed AMOVA. Conclusion was that there has been a significant decrease in variation by drainage.
        - Only computed the unique permutations for the modern dataset.
    - Question: Could you get the same result if the historic sample was not a random sample? You focused the samples on certain places?
        - Response: No, we sampled everything that we could, but there could be a bias with the collection sites from the original collectors of the samples.
    - Question: Could it be that if more samples were taken from the historic specimens, that lineages are actually more similar than divergent? Relating to the network image, most of the black dots are much closer together than divergent with the exception of the San Juan.
- Andrew Martin – Addressed questions that are related to studies
    - Structure plot for the number of K values assessed.
    - K = 3
        - BC clusters closer to Como
        - Severy may be a combination
        - Lack of variation or complete genetic divergence could explain the east slope populations.
    - Data for phylogeny on cutthroat trouts
        - Loxtermand and Keeley
        - Metcalf et al. 2012 – The relationships between the putative subspecies are unsure. The larger triangles show the variation amongst a subspecies and area spanned by the subspecies spatially.
    - Uncertainty
        - The mitochondrial data from the 2012 dataset – mitochondrial DNA is maternally passed down and cannot reveal admixed ancestry.
    - Widespread stocking
        - It is possible that every population has been influenced by stocking, including the Bear Creek population.
        - If Bear Creek is from admixed ancestry, you would expect it shares ancestry with others

            - Some allele networks show that BC is fairly divergent from other pops. Preliminary data shows that BC is not an admixture of other pops. Still doing more research on this.
            - Bear Creek alleles are not strikingly different from other populations, but there are alleles that exist in the BC pop that do not exist in any other pop.
        - How many microsatellite data per locus
            - BC < 2
            - Stock populations < 1 allele
            - On average about 1.5 per locus
    - Taxonomic revision
        - What is a species and a subspecies?
            - Is there separation in morphological space?
            - Can they reproduce?
            - Genetic/developmental cohesion
- Comment: The relationship and overlap between mtDNA and nuclear DNA is very beneficial for this study. If there is any more nuclear DNA that can supplement the mtDNA 2012, it would be helpful.
- Comment: Brunelli et al. paper – it would be good to talk about this paper and discuss how the Y chromosome research is related.
- Was ND2 used for a certain reason?
    - Yes, used in Yellowstone studies before

- 1:00 Kevin Bestgen – Phenotype predicts genotype for lineages of native cutthroat trout in the southern Rocky Mountains
    - Metcalf et al 2007 and the antelope creek GB population created confusion.
    - Revisited Metcalf research and results about population locations and distribution
    - Purpose of Study:
        - Do the morphological characteristics support the genetic patterns found in Metcalf?
        - US law supports using morphological distinction of species to identify and subsequently protect species.
        - Can the three lineages be distinguished based on morphological characteristics?
        - Compare the Geographic Model and Molecular Model to explain lineage distributions.
    - Methods:
        - Stream selection protocol:
            - Picked 3 populations from each geographical pop. Unit
            - 24 or 12 fish per stream
            - Restricted to streams to reduce lake population influences that may affect morphology
            - Picked streams that had some molecular data available
            - Also made sure that streams sampled had >150 fish/mile to eliminate the possibility of reducing populations with low numbers
            - Conservation stream are those that have a certain percentage of trout purity and therefore do not have many non-native species.
                - No stocking history, etc.
                - Some conservation streams were found they shouldn't be included in the database following the molecular analysis.

        - Collection
            - Strict guidelines for protocols and samples taken (size limits)
            - 839 specimens from 49 streams
        - Sample preparation
            - Transferred from formalin to ethanol
        - Data collection
            - Many morphological traits analyzed, only important will be discussed
            - Spot patterns: divided into 6 zones and counted spots by zone
    - Analyses
        - Variation of GMU's could point out how the morphological traits are associated with the landscape.
        - Population means used frequently in the past
    - Morphological analyses
        - DFA classify individuals or populationss by geographic or molecular models and GMUs
        - DFA assumes groupings are equal prior to study
    - Molecular analyses
        - ND2 gene sequence used to assign pops to lineages
        - AFLP markers used for Yellowstone and rainbow
        - These used as screening tools to understand lineages and remove admixed species.
        - Question: What about mixing green and blue?
            - Response: There wasn't much contamination, but Kevin will look into this more.
            - Response: Putative admixed GB and CR fish were not excluded from the study, but unsure of how many pops.
            - Response: Some of the individuals were classified using the ND2 and some with the AFLP markers were used.
        - Question: Do the colors fade after preserving the fish?
            - Response: Yes, but the blacks stay and are very distinguishable even after preservation.
    - Results
        - Random selection was important – picking out the oddities to sample would have skewed the data and resulted in a bad sample
        - Geographic coverage was good and spatially spread out
        - Blind protocol ensured no bias – when assessing the fish, was not sure where the fish were from.
        - Replicated counts for the difficult traits
        - From the genetic information:
            - Only one blue lineage in the San Juan and Dolores
            - 2 green lineages in the Arkansas
        - Censored populations
            - 36 Bear Creek Fish
            - Irish Canyon Creek was eliminated – found to be all Yellowstone cutthroat
            - Abrams Creek (25) changed from blue to green
        - AFLP censored individuals, >0.5% admixture
        - Molecular model
            - Blue : 24 streams
            - Green 14 streams

        - RG 12 streams
        - BC 36 fish
    - Geographic
        - West 25 streams
        - East 10 streams
        - RG 12 streams
        - BC 36 fish
    - Variation for the sampled species extremely variable. Showed example of Rio Grande cutthroat, which is one of the least variable of the 4 species. Variation within species and between species.
        - Fin counts, spots are features that were found to be variable.
    - Trait comparisons
        - Lateral Series Scale Counts
            - Bear creek and Rio Grande have lower lateral series scale counts whereas Blue and Green have higher counts
            - Between east and west slope species, not much difference of lateral scale
        - Basibranchial tooth counts
            - 61% of Bear Creek do not have them at all.
            - Close behind, Rio Grande
            - Green and Blue typically have more
            - Still much variation within species, specifically in the Rio Grande
        - Pyloric ceaca counts
        - Posterior gill raker counts
            - Bear Creek – few
            - Blue lineage- relatively more than others
        - Trunk Spots
            - Green lineage and Rio Grande had lower numbers
            - Bear Creek had higher number of spots
        - East and West trunk spots
            - Relatively equal
        - Fore-caudal spots
            - Rio Grande and green low
            - Bear creek higher
        - Mid-caudal spots
            - Green and rio grande low
            - Blue high
        - Caudal peduncle spot size
        - % with spots on head
            - Rio grande few
            - Bear creek many
    - Multivariate data analysis
        - Geographical
        - Green and blue lineages very similar overall
        - Rio grande very different
    - Molecular model
        - Green lineage very broadly distributed and overlaps with the blue

    - 4 outliers of the green lineage populations are those that lie on the east slope, which indicates they are much different from the other pops on the west slope.
    - BC is very different, Blue different, Green and RG overlap
- Question: For the PCA, in Table 3, even spot number and spot size are correlated with length in every population. Does this have something to do with size? It may have something to do with shape and size and would be worthy of consideration.
    - Response: With the Bear Creek pop being smaller with less spots, would mean that the BC pop would pull away more. There is something else going on in PC1. The traits are not necessarily attributed to size, which are fairly stable once measured on an adult fish.
    - Comment: Size should not have an impact on PC1. A covariance was run, but a correlation may be useful to run.
- Question: Were the BC populations stunted at all?
    - Response: No they were just small, but not visibly stunted or skinny. Hatchery fish were also in good condition, those that were not in good condition were not used.
- Evaluated the 4 east slope populations and compared to the west slope fish
    - Lateral series scale counts and trunk spots very different
- Question: Meristics can be sensitive to temperature when developing. Is there a consistent temperature difference between east and west that would influence meristics?
    - Response: No climate data was taken.
    - Comment: A lot of the streams sampled are instrumented, so it is possible to take data on such environmental factors.
    - Comment: Could use temperature and a regression model. It would also be useful to integrate elevation too.
    - Comment: There have been studies that show temperature can alter morphology when genetics may match up. Larval studies also have shown that temperature can be an influence.
- Question: Are there any morphological characteristics that are more impacted by microhabitats, inbreeding, etc. Could you expect a broader phenotypic variation than in others?
    - All GB populations on the east slope have 2 haplotypes, all GBs on west side are different.
    - All the CR on east side have common trapper's lake haplotype, but the Blue in the west slope have another.
    - Multidimensional scaling could be used to assess environmental influences on phenotype. Would be a powerful tool to analyze data more.
- Comment: In Mexican trout species, there is no correlation between the amount molecular and morphological variation. Most species can be distinguished more often with genetic analyses, but is often supplemented with morphological data if available.
- Geographic model
    - Bear creek and Rio Grande classify well
    - West slope classified poorly
- Discriminant function analysis (DFA) - geographic

            - 100% of east slope fish classified correctly
            - About 56% of west slope classified correctly, which indicates there likely is another species that exists on the west slope.
        - Question: Why wasn't the Bear Creek population included in the East slope population?
            - They would not have classified as an east slope population because they are so different. They would have been excluded because they would have been seen as an admixed population. They would have never been tested.
        - Molecular Model
            - Green lineage had the most individuals being misclassified
            - Bear Creek classified correctly most
        - Discriminant function analysis – molecular
            - Green again misclassified most
        - DFA completed within a lineage- how did a lineage classify within their GMU?
            - There are unique attributes of the populations within the GMUs.
    - Discussion
        - Bear creek are the most distinct populations.
        - It is difficult to compare BC populations to historical specimens to find any differences and similarities.
        - The Rio Grande population is very distinct.
            - Morphologically closest to Green lineage fish
            - Populations are very structured around 3 major drainages
        - E and W slope populations:
            - Geographic models moderately support
            - East slope lineages, distinct between east and west slope pops
            - West slope – indicates there are at least 2
        - Blue lineage had the lowest variation among all traits and was also classified correctly the most.
    - There was a high classification rate among blue, green and RG pops within GMUs.
    - Recommendations
        - Assess museum specimens
        - Additional samples – blue and green especially.
        - Front range Green populations need more research
        - Phenotype structuring present at watershed/ GMUs
    - Comment: Nanita fish picture from the US National Museum from Trapper's Lake is very similar to a presumed blue lineage fish.
    - Comment: In the paper, ¾ of the strange east slope fish don't match up mtDNA. The best explanation for those is that they have some type of mixed ancestry. It may end up not giving any explicit answer of what type they are.
        - Question: Wouldn't you expect this for blue also?
            - Response: Not necessarily, because the stocking in those areas happened more recently, this would show more strongly in the genetic analysis.
    - Comment: The unique east slope haplotype is not seen in the historic S. Platte samples. Also, in the 1800's FWS, train car service moved east to west via train, including moving fish. Some correlation with the train lines and stocked waters close in proximity to the trains has been noted.
    - Question: Why is the story for the greens not as straightforward?

    - Comment: When doing a geographical model – greens and blues are combining on each slope.

- 3:45 Continued Discussion of Meristics Study
    - Question: Is there anything in the morphological data that shows BC data is variable?
        - You would expect a bottleneck to reduce variation in a population, but the data does not show the BC population is any less variable than other populations.
        - Sometimes, asymmetry may be evaluated to see if there is any evidence of inbreeding. Could this be done for the BC population?
            - You could do this for a small population; it is not out of the question.
    - Question: How do the meristics and the genetics data correlate with one another?
        - In order to understand that, the group needs a better understanding of why the genetics studies are showing different patterns?
            - The mtDNA and FLPs are showing different patterns
        - The biggest question is why the east slope shows so much variation. It comes back to the historical stocking data and the realization that stocking had a large influence on what we are seeing today. The mtDNA and nuclear DNA comparisons show that the current species are of an admix ancestry.
            - The east and west slope GB are different especially when the blue type are the same. Shouldn't there be some sort variation in the blue because they were also stocked?
            - Overall there is a lot more variation in the green lineage, in the trapper's haplotype, so this may be the reason why GB diverged.
        - An issue with the genetics study: we need to analyze alleles from the outgroups and delete those alleles from the group being analyzed – plesiomorphics traits.
    - Question: Are we talking about a phylogenetic unit that diverged but never connected again geographically? Or are we talking about a phylogenetic unit that is connected and related but are just different from each other? Would most populations be connected geographically or are there certain barriers that would completely inhibit their transport to areas?
        - There are three basins that have confluences that would allow connections, and unsurprisingly they have similar lineages.
        - QUESTION: When was the last time they could have crossed the continental divide?
            - There are some scenarios where they could have crossed with recent glaciation.
        - General consensus is that the events happened within the last 3 million years. The possibility of incestral polymorphism is there. The other issue is human induced changes. Gene flow amongst trout is not common. Could the key question really be whether or not we need todistinguish natural from man-made events?
        - There has also been a lot of tectonic movement that literally moved rivers. It is difficult to say when these events took place and how to separate this from human-induced change.
    - Question: Another issue is how do we explain what alleles have been selected for?
        - Discussion of supergenes.

- Question: There is a strong geographical signal related to the GMUs. This is a good indication that it is a natural signal. With there being so much similarity within GMUs, this is a signal that the environment is playing a larger role. Although humans did play a role, the environment prevails. Now that we have this diversity in the state, it is what is present and is what we should manage for now. The question should be, how do we manage what we have?
- Question: From the services' perspective, how important is a geographically confined entity for listing?
  - The ESA hierarchy is species, then subspecies.
  - Distinct populations segments also are looked at for ESA listings. Specifically, discrete populations and ecological significance (up to interpretation).
  - It depends on the scientific community to determine subspecies/species and the contribution of the population to the community as a whole – i.e. the BC population is unique when compared to all other populations and would afford a listing because it has scientifically been shown there are no other populations like it.
- Question: If the BC population is distinct morphologically, but you do not have any historical range data and it is up for protection, where do you conserve?
  - Metcalfe et al 2012 found that S. Platte is its historic range.
  - From the ESA perspective, you must not just show it is a unique population, but also that there is a threat to its existence.
- Question: Why do we only see a few mitochondrial haplotypes within the S.Platte?
- Comment: The BC population has been used in hatcheries and individuals have shown extreme incestral traits and are very difficult to cultivate. Many deformities, etc.
- Question: When talking about such abnormalities, it suggests the population is inbred and bottlenecked, which suggests it comes from a fairly large population. In a small population, you would not see such abnormalities. It suggests Jones' fish came from the S. Platte.
- Question: Isn't there some structure in the BC population? Lower and upper?
  - There are some very large waterfalls. Most of work has been done with the lower population. Fish are capable of moving downstream but not upstream.
- Question: How do the abnormalities in Como creek compare to the ones seen in BC hatchery raised fish?
  - In nanita and como fish, about 5% are abnormal in hatcheries, whereas the majority of BC fish are abnormal when raised in a fish hatchery.
  - Environmental conditions can produce impacts in fish morphology and could be to blame.
- Question: Are we following the BC progeny in the hatchery to see if they are dying off?
  - Some of the fish are not making it due to deformities, but majority are doing alright.

- The group decides there is a general consensus that the BC lineage is something different and actions need to be made to at least conserve this species.
- Where we are now and what do we need to discuss in order to address the GB issue?
  - Dennis Shiozawa: The difference between GB lineage between east and west slope needs more research.
  - Bruce Rosenlund: It was surprising how well the meristics study separated out the blues, rio grandes, and BC.
  - Doug Krieger: The quality of the studies is outstanding. It is interesting how we are back to meristics after 30 years.

- Leslie Ellwood: The meristics study is interesting. Looking forward to more discussions on the green and blue lineages on greenback.
- How many of the GB populations are linked to the Como broodstock?
- It would be worthwhile to consider restoring genetic diversity within the existing BC population to ensure stability and then distribute the population. We also have not even brought up the issue of climate change.
- The next question should be focused on what do we do about the science we discussed today?
- If BC is unique, are we coming to the consensus that it is the new GB cutthroat trout? We spent a lot of time on discussing the green lineage, is that what we truly need to discuss or should we redirect discussion towards the BC population?
- Keep the big picture in mind.

Greenback Trout Genetics and Meristics Studies – Facilitated Expert Panel Workshop
US Fish and Wildlife Service
Denver, CO

July 31, 2013

- 8:30 Dennis Shiozawa: Cutthroat genetics – mtDNA, nuclear markers, and marker development
    - Cutthroat made of 8 major species and 12-15 subspecies. Subspecies are thought to have started to diverge 12-15 million yrs. ago.
    - Review of studies:
        - Behnke 's hypothesis (2002). His view was developed by looking at drainages in order to classify species.
        - Smith et al. (2002) phylogeny study. Developed through an older DNA-based RFLP method. Shiozawa does not believe the *O.c. clarki*, *O.c. utah* are placed correctly.
        - Wilson and Turner (2009). Used ND4. Dr. Shiozawa agrees more with the phylogeny of this study.
        - Loxterman and Keeley (2012). Used ND2. Found "great basin" which is actually Bonneville. Also distinguished Bonneville – Yellowstone, which is actually Yellowstone.
        - Metcalf et al. (2012). Used ND2 and COI. More realistic with what the genes are able to tell us.
    - Compared mtDNA genes for *O. clarkii* vs *O. mykiss* and found differences in bps.
    - Created a phylogeny based whole mitochondrial genome. *O. mykiss* and *O. clarkii* were placed next to each other.
    - Shiozawa's study
        - 3649 bp of mtDNA at 1000 reps
        - ND1, ND2, ATPase, Cytb
        - Issues with some "bootstrap" values, so reran with more bps.
        - Found the 8 cutthroat trout subspecies form well supported clades
        - Bear River form of the Bonneville cutthroat trout is a sister taxon to the Yellowstone
        - Bonneville, CR, GB, and Rio Grande are sister taxa with strong separation
        - Two major cutthroat trout lines show strong separation
        - Two major polytomy
            - Can be explained in Benhke's phylogeny tree
            - *O. c. utah* location in Benhke's work is not supported by Shiozawa's.
            - BC lineage was found to break off near the GB lineage rather than the Rio Grande population.
    - Timing of the splits – Molecular clocks
        - Project divergence times with 0.5% per million years
        - 2.2 million Lahontan and west slope separate from each other
        - 1.94 million RG, GB separate from Yellowstone and Bear River
    - Hydrography history:
        - Lahontan and west slope were separated when Lake Idaho dried up subsequently creating two sister taxa. Lahontan ended up being concentrated near the Humboldt River and the West slope moved from the Snake River to the Bear River.
    - Split of Bear River from the Yellowstone taxa

        - 2400 bps of mtDNA genes ND1 and ND2
        - Some Bear River haplotypes are found in Snake River drainage
        - Bear River population was found to be much more divergent earlier than the estimated time period from the phylogenetic analysis.
    - GB species separation at around 1.15 million years ago, which means there should be more diversity since the divergence occurred longer ago than the Bear River and Yellowstone.
        - Good "bootstrap" values in phylogenetic tree ~ 92. Found GB and BC lineages diverged from each other
    - Question: Did you explore other modes of max likelihood assessments?
        - Response: Yes, I did maximum likelihood and neighbor joining.
    - Question: What did you use to calibrate the clock?
        - Response: The calibration was mainly going through and speaking with Jerry Smith. Before we publish this, we will go through and compare with fossil dates. Really, right now there is no calibration so I compared with the Crete-Lafreniere paper as calibration.
        - Question: Where did you get the 0.5% per million years?
            - Response: It was more of a visual measurement based on values.
    - Question: What are you using as your mechanism for the split between Bear River and Lahontan? Barriers?
        - Response: When you look at whitefish, they diverge much more slowly than others. Behavior could play a role, migration specifically. Whitefish migrate and move much more which limits the diversification and explains a lower % divergence time/year. Trout/Salmonids, however, move, but then remain in a location which would allow for more diversification. In the Bear River situation, once the population reached the Snake River they remained sedentary for a bit and diversified.
    - Question: There was a lot of diversification about a million years ago. What was happening geographically that would cause GB populations to diverge?
        - Response: There was a glaciation event around 600,000 years ago. It is not known exactly what was going on around that area. In the Great Basin area, there were a lot of headwater stream captures that allowed fish to come in, but not to leave. However, it is not known of what exactly happened in the Rocky Mountain region at that time.
    - Shiozawa believes the CR is not of concern – distinct population. The GB lineage is however not as straightforward.
    - Image of trout movement in the west.
        - Colored polygons represent species range.
    - Ribosomal DNA discussion
        - So specific to the organism, that one can almost identify the exact individual by looking at it.
        - Currently they are doing work on creating ribosomal markers to better use this method for identifying trout species.
    - Can Metcalf be tested independently?
        - Arkansas and S. Platte basins should have nuclear and mtDNA identical to some of the source CO river basin populations
        - The contemporary GB basin of origin should have a greater diversity of haplotypes.
            - Should look at CO River basin for haplotype diversity
        - Residual DNA from original lineages may exist in introgressed populations of cutthroat trout.

            - Can still look for this by doing quick scans with AFLPs. Then those populations with residual DNA can be used to generate sequences.
- Tools
    - Analyzed 8057 bp of CO River and Arkansas River basin
        - Co River basin divergent from Arkansas River except for South Prong, Hayden Creek population.
            - Is this a product of invasion or stocking?
            - This should be looked into, to see if anything does truly represent Arkansas River drainage fish.
        - Question: You are saying that there were 2 invasions of the Arkansas River Drainage?
            - Response: Shiozawa's sequences should be looked at to see if more sequences can be replicated in the preserved fish. Jessica should do this.
            - Comment: You are a suggesting that there were multiple invasions of different haplotypes?
- Limitations
    - Study based on mtDNA, which is technically a single marker. This may not reflect the entire history because the marker is only from one parent (females). Nuclear markers are needed.
- Development of New Markers
    - Next Generation DNA sequencing platforms used for a few methods:
        - Single nucleotide polymorphisms (SNPs)
            - Aimed to obtain more markers that could be used to classify trout.
            - Method looks at several taxa and a single nucleotide, then DNA is amplified and the nucleotide sequence is looked for.
            - Recent studies did not completely cover cutthroat trout, so Shiozawa lab decided to tackle this.
        - Transcriptomes using Illumina HiSeq 2000
- Shiozawa SNP study
    - 9 lineages of cutthroat and rainbow trout were reviewed.
    - Methods:
        - 36 individuals sampled
        - Used EcoR1 enzymes from bacterial cells to cut DNA at restriction sites, then removed smaller DNA segments by spinning DNA samples, then ligated at sticky ends back together. Separated DNA sequences according to MID barcodes.
        - Fluidigm – conducted over 9,000 reactions for 1 tray
            - Allowed for classification of homozygous and heterozygous individuals for a certain allele.
    - Results:
        - A total of about 29,000 SNPs were identified and 228 primers were developed and run on Fluidigm chips. About 100 SNPs were validated.
        - Separation of taxa
            - West slope, coastal, Lahontan, and Bonneville separated out easily
            - GB not as clean of a separation
            - Bear Creek fall in the same area as GB populations

            - A few GB populations fall in the CR populations
        - Question: What percentage of SNPs were variable within a subspecies, compared to percentage of variability within a group?
            - Unsure, will have to look into this.
        - Question: Can you track which individual produced a data point on the Fluidigm graphs?
            - Yes. Each point can be traced back to an individual.
        - Conclusions
            - This study has added diagnostic markers that can be used to determine levels of hybridization and introgression. The process used will be very helpful for management of natural resources when it is necessary to distinguish between species and subspecies.
        - Future Studies
            - Markers have often been chosen because of their availability rather than their ability to provide phylogenetic information.
            - Widely used genes have been developed using organisms that are hard to adapt to non-model organisms.
            - Whole genome studies would be helpful, but are extremely expensive and do not provide enough information to make management decisions.
        - Comment: S. Prong Hayden population looks as if it lines up more with the Rio Grande populations more than the GB population. How much of the data is dependent on the choice of SNPs?
            - Response: It could change the data, and is an interesting point. We chose SNPs that showed differences between the different groups. This issue goes back to us needing to screen more SNPs or do more research on the segments we chose. Additionally, we put in GB populations that were actually intergressed populations, so it is deceiving to look at them as pure GB populations.
    - Alternatives to SNPs study
        - Transcriptome: all of the RNA in a group of cells, i.e. muscle tissue.
            - Depends on the timing also because certain mRNA will be active at different times of the year.
            - mRNA degrades very quickly once an organism dies.
                - There are ways to counteract this process.
        - Results for the 3 cutthroat trout (Bear River, Bonneville, and Lahontan). A total of 288 genes are promising for higher level of phylogenetic relationships among Salmonids. 421 genes exhibit variation among cutthroat trout subspecies. Can use this data to develop more SNPs that are more promising for the previous methodology.
            - If the same species and populations are sampled at another time of the year, additional SNPs could be identified because other mRNA will be active.
    - Comment: The next generation sequencing is an extremely new method and the work being conducted in present day will really help to shape future hypotheses.
        - Comment: Obtaining as many bps from 96 samples with the next gen sequencing methodology ends up costing much less.
    - Comment: BC falls in between Rio Grande and Greenback, is this correct based on your results?

        - Response: Yes, that is what we concluded, but we would like to go back and reevaluate.
        - Comment: It does not contradict the genetic or the meristics studies.
    - Comment: It is important to remember with the nuclear DNA, that there is natural selection occurring, which does not occur with mtDNA. With the SNPs we have to remember that selection may be a part of the equation.
    - Question: Jessica, what is the possibility of using next gen sequencing with museum specimens?
        - Response: The genome is very fragmented, so the methods would have to be a little different. Contaminants in the preserved specimens could impact the results. It would be something that we would really have to plan and think about. We would have to identify what modern data we would need, etc.
    - Comment: The SNPs have much lower mutation rate, which could be useful. If you want fine scale structure within Colorado, then using 100 SNPs is providing less information than fewer microsatellites.
    - Question: Does anyone know how far along the rainbow trout genome is? Around 80% complete as of now?
    - Question: Dennis, what is your thought on why these 100 stamps did not distinguish coastal and rainbow?
        - Response: They may have been undersampled compared to the other groups. It would be nice in future studies to get a much more robust range of variation within groups.

- 1:20 Discussion of Dr. Behnke 's work (statements attributed to him were compiled from various sources for discussion)
    - Discuss how the molecular model fits with the geographical model.
    - Behnke : Reliance on ND-2 mtDNA unreliable and unstable, CO1 should be used.
        - Response: Unsure of why ND2 is not a valid tool for the GB trout purposes.
        - Comment: The rate of evolution for the time frame involved did not occur quickly, therefore using the ND2 segment should not be an issue.
        - Comment: Maybe, by more unstable he means it changes more rapidly, which would actually be more useful for our purposes.
        - Comment: General consensus that ND2 is fine to use in our issue.
    - Behnke : Examine all hypotheses
    - Behnke : Science is about confronting uncertainty with alternative hypotheses
    - Behnke : We have to be careful with the use of mtDNA because all the confusion with the DNA studies is bad PR for conservation of biodiversity. What does he mean by this?
        - Comment: He is speaking of the 2 Metcalf studies that have differing results and that the public perception of science may be tarnished because the science has changed. However, that is how science works, but it does look different to those who are not familiar with the process.
        - Comment: We should not rely solely on the newest data as the current truth; we should look at all the research as a whole.
        - Comment: We also have to consider that Behnke 's audience for decades was Trout Magazine, and he was responsible for relaying the scientific message to a huge audience. He also felt that the constant debate was holding up conservation efforts; something could have been done for conserving the species the whole time the debate has been going on.
    - Is there a serious public relations problem with science?

        - We must use the best science and management at the time. Mistakes have been made in the past, but it was the right decision at the time.
            - In ESA it states the best available knowledge must be used to make decisions.
    - On the ESA protection side of the issue, patience is wearing thin. GB on the east slope and GB west slope are being protected still temporarily until we come to a conclusion.
        - Which units of Cutthroat are listed?
            - Greenback listed
            - Rio Grande candidate
            - Colorado River, not warranted for protection
            - Lahontan listed
    - There has been talk of doing some research on the existing populations while they are still listed.
        - Practical management implications would be good to address tomorrow.
    - Behnke : We should shift from a typological species concept to a polytypic species concept. This does somewhat regress thinking.
        - Typological concept is the thought that a species was created and exists for a reason.
    - Discussion of the origin of subspecies and the complexity involved in identifying subspecies (Rick Mayden).
    - Comment: Behnke has been right so many times that it would be unwise to disregard his insight that has been developed from years of experience.
        - Comment: Bestgen's study did disprove some of Behnke 's results, and it is completely understandable that Behnke missed the distinctions from Bestgen's.
    - Question: What were some of Behnke 's comments with Metcalf 2007? Similar to 2012?
        - Yes, he stated molecular information isn't the entire story and that there was a lack of information.
        - He argued that with some population loss and ancestral diversity leads to the mosaic we see across Colorado.
        - Also, we have lost a lot of populations and diversity that is is difficult to determine what we are seeing today is not just an artifact of what was here previously.
    - When Behnke first diagnosed a GB, which populations did he originally use? What characteristics did he use to make this conclusion?
        - It isn't totally clear what specimens he used to draw his conclusions.

- 2:15 Review and Comparison of Hypotheses summarized by Tom
    - Geographic Hypothesis
        - Add: it is not just cutthroat trout that we can apply these hypotheses to, but also across taxa. Drainage divides have often been used to explain fish biodiversity in many species and regions.
        - This hypothesis also defines human impacts to the historical populations.
    - Molecular-Historical Hypothesis
        - Metcalf et al 2007 – cannot discern stocking and natural events through ancestral polymorphism.
        - The Bear Creek lineage is thought to only occur in the S. Platte and currently exists outside of its natural range.

            - How do we know that the Bear Creek lineage did not extend into the Arkansas River drainage from the S. Platte drainage?
            - If so, this would put 3 potential lineages in the Arkansas.
        - We are fairly confident that the green lineage originally was a west slope species, but whether or not it moved naturally or through stocking is the question.
            - Green on west slope have the same haplotypes as the east slope, but are a few bps different – Fact.
        - 1889 sample had the same haplotype as two lakes in the east slope. They could have naturally moved or could have been stocked. However, they are not found on the west slope, and never were, which gives weight to them being native to the east slope. Hayden is in the Arkansas drainage and is one of the areas these fish were found, which is in the same drainage as the BC population.
        - Green lineage on east slope discussion.
            - Despite admixture, these species have somehow have maintained their integrity.
            - Bestgen et al. Molecular data was stronger than the geographic range. However, the geographic distinctions still dictate the populations.
    - Geographic Management Units (GMUs) – Hierarchal Analysis of Variance
- These hypotheses gradually are divided into smaller pieces (Geographic to Historical/molecular to GMUs).
- Can we put together a list that summarized findings?
    - Facts that no one can dispute.
    - Generally accepted scientific findings that are not (yet) disputed.
    - What are the disputed scientific findings?
        - Interpretations of data that do not match
        - Findings that do not coincide between studies

- 4:35 Public Comment period/Richard Mayden : Species Concepts
    - Classes:
        - Artificial classes do not participate in natural processes
        - Natural classes participate in natural processes
    - Individuals vs. Classes
        - Individuals can only be described but not defined like classes. Classes have characteristics that define them.
    - Natural vs. Artificial classes
        - Natural classes have many definitions because they participate in natural processes that regularly alter their state.
    - Species: Individuals vs. Classes
        - Species category of monophyletic trees change with time because species evolve. Kingdom through Genus do not follow the same changes
    - If you treat species as a class, they do not change over time. If treated as individuals, they will show change with time.
    - Concepts: Evolutionary Species Concept is a theoretical concept that maintains lineage over time.
    - In order classify, multiple species concepts must be used to catch all biodiversity. Each concept individually misses species.
    - Question: What is the utility of lineage vs subspecies vs species?

        - Response: Under this paradigm, subspecies just do not exist. Lineages exist and are species. The suckers and trout are classified differently even though they have completely the same distribution. Suckers are species, trout are subspecies.
    - Question: A lot of management occurs at the population level, but the ESA addresses species. Distinct populations are more flexible than classifying entities as species. Working together and relaying correct information to the management agencies from the scientists in key. A lack of this communication has led to many mistakes in management history.
    - In ESU guidance:
        - Based on best scientific information
        - Sparingly use distinct population segments
            - DPS should add up to a species
            - Collectively, each one is a piece of diversity and if one is lost, the species is lost.
            - Aldo Leopold – if biodiversity is going to be altered, the pieces that make up the whole must be preserved.
            - However, how small do you go? What constitutes a DPS?
        - Conserving genetic diversity
- Final Discussion
    - Break management and scientific questions up.
    - Are there pressing management questions that are not on the list?
    - Any additional questions that need to be addressed should be sent to Leslie.

Greenback Trout Genetics and Meristics Studies – Facilitated Expert Panel Workshop
US Fish and Wildlife Service
Denver, CO

August 1, 2013

- Revision of "Factsheet"
  - Facts
    - Subspecies of cutthroat trout historically have been defined largely by geography—major drainage basins.
    - Extensive transfer of cutthroat trout through stocking occurred for more than a century.
      - Transfers primarily occurred from West to East. Primary sources were centered around the White River at Trapper's/Marvine Lakes (blue lineage) and on the Grand Mesa (green lineage) at the headwaters of the Gunnison.
    - Como creek, Hutcheson Lake and Como creek/Hunter's/South Fork Poudre crosses were historically used to stock recovery populations in the S. Platte drainage to establish new pops of presumed greenback.
    - Cascade creek was used to stock recovery populations in the Arkansas River drainage.
    - Yellowfin declared extinct by 1904.
    - Greenback declared extinct in 1937. From the 1950's to the 1980's, populations thought to be greenback cutthroat trout were found. Listed as endangered in 1973. Downlisted to threatened in 1978.
    - Bear Creek haplotype matches the museum specimens from 5 locations within the S. Platte drainage.
    - As the first reviser, Jordan coined the term "greenback" for native trout in the S. Platte and the Arkansas drainages.
    - Historic reference materials were collected from 1856-1889 and are available to past and current researchers.
    - Morphological variation of historical specimens is largely unknown.
    - All lineages on the east slope are currently listed as threatened, green lineage on the west slope are threatened, and blue lineage populations on the west slope are not protected under ESA.
    - Rio Grande cutthroat trout are a candidate species for listing under ESA.

  - Generally accepted (or at least not yet disputed) findings
    - Habitat quality at Bear Creek has been compromised by sedimentation.
    - Bear Creek and Como Creek fish appear normal in the wild, but exhibit serious deformities in the hatchery.
    - Current data supports at least 4 extant lineages of cutthroat trout in Colorado.
      - mtDNA and meristics/morphometric studies are congruent in supporting this.
    - Bear Creek is upstream what appears to be a one-way barrier to upstream migration of cutthroat and was presumed to historically be fishless.
    - In samples analyzed so far, green lineage mtDNA haplotypes are found on both sides of continental divide. The two most common east slope haplotypes are not found on the west slope.
    - Blue lineage mtDNA haplotypes are found on both sides of the continental divide. East slope haplotypes are a subset of what is found on the West slope and are limited to haplotypes currently found in Trapper's Lake.

        - Most green and blue lineages are separated by barriers. Most barriers are natural, but some are man-made. Populations above natural barriers are likely present from stocking.
        - Green lineage fish are not found in the Yampa or the Green River drainage.
    - The type specimens for O. c. stomias have a haplotype found in Rio Grande lineage.
        - Hammon's collection notes suggest that the type specimens were likely collected from the Rio Grande drainage.
    - Geological and other evidence indicates stream-capture events across the continental divide occurred during the Pleistocene.
    - Meristic/morphometric analyses show consistent differences between groups defined by mtDNA haplotypes. Patterns were stronger when individuals were grouped according to genetic lineage than when grouped according to geographical hypothesis. Meristic differences were also apparent at the level of GMUs.
    - The green lineage East slope fish show meristic/morphometric differences from the West slope green lineage fish.
    - The blue lineage fish on the East slope show meristic/morphometric differences from the West slope blue lineage fish.
    - The blue lineage fish currently present on the west slope are presumed to have originated on the west slope.
    - Some conservation populations show evidence of introgression from Yellowstone cutthroat trout and rainbow trout. Conservation populations are populations that count towards recovery.

- Key Questions to Resolve:
    - Can ancestral polymorphism be distinguished from historic stocking influences on populations with our existing analyses? If so, how? IMA, other programs, or evaluation of nuclear genes?
    - The appropriateness of using ND2 and CO1.
    - Are the green lineage fish on the East side of the divide are a result of stocking or natural processes?
    - What is the level of inbreeding in the Bear Creek population?
    - Is Bear Creek representative of historic populations in the S. Platte drainage?
        - See point in the Facts section.
        - Use of next generation sequencing of museum samples and the meristics study would help to verify this.
    - The green lineage fish currently present on the west slope are presumed to have originated on the west slope.
    - What is the phylogenetic relationship among cutthroat trout lineages?
        - Additional character information should be obtained.

- Review of Questions:
    - Management Question 14:
        - K. Rogers: Costs of the bottleneck were assessed. 4 female BC and 4 green lineage eggs were fertilized. Offspring were evaluated for survivorship, morphology, movement patterns, etc. Lab data are still being collected and evaluated. This should help to determine if there is a problem with the bottleneck and will help to guide what actions are taken to manage the BC population. It will help determine if we should disperse BC individuals in the S. Platte and risk possible inbreeding with green lineage fish.

- Comment: Cannot answer this until we know what the level of inbreeding is in the population. It is premature to try and answer this until we know if there is indeed inbreeding depression in the BC population.
- Comment: Currently, data suggests that this population
- Question: So is this the *O.c.stomias* population then? What is the "green" lineage population?
    - Yes, BC=stomias, green population is unnamed.
- Answer: Whitely: If the BC is indeed the last and there is no sign of inbreeding, I propose that we replicate the population over the landscape. Tom agrees.
- Comment: Another thing to consider is that the hatchery fish are very different from the wild population: hatchery population had different avg. spots and had basalbrachio teeth, whereas the natural populations had no teeth. We could use wild, hatchery, or a combination of the two to create wild populations. The hatchery fish are F1 generation, so they are not too removed from the wild populations.
- Question: Are the temperatures in the hatchery comparable to the wild populations?
    - Yes, they are almost identical.
    - So there must be some other factor affecting the BC hatchery fish.
- Question: Is the BC wild population suitable on its own to propagate with the populations low genetic diversity?
    - Response: Given that BC only represents a small fraction of what was actually present historically, do we want to use the current population or try and back up the genetic clock to try and diverse the population.
- Question: Do we want to keep the wild BC population "pure"?
    - It would be smart to replicate the existing population in several streams and other waterways to have it present, but also create a population that is more robust and diverse, then compare.
- Question: are there candidate streams?
    - Yes.
    - The FS is working on quantifying suitable habitat for the BC pop.
- Question: Is there concern with putting a stream fish in a lake? Selection issues?
    - Yes, in the long term, but with our current situation it wouldn't really affect the population other than the size of fish. A priority is to replicate the populations soon, and the use of a lake would allow for this; potentially use of Zimmerman Lake.
    - Monitoring of inbreeding impacts in new wild populations would be a priority. Comparison of any signs of inbreeding in both wild and hatchery populations would be very valuable lesson.
- Comment: The morphology of the BC are so different that it would not take long to compare samples to historic samples in order to conclude whether or not BC was the historic cutthroat trout in the S. Platte.
- Question: Is a recovery plan in place?
    - No, the taxonomic classification must happen first, but there will definitely be a recovery plan.
- Question: Should there be some consideration of artificial connectivity to help disperse the BC populations? I'm not a fan of anthropogenic/unnatural connections, but it is a consideration.

        - Question: Concerning climate change, are you more concerned with waterways being altered in the north?
            - Yes, it is a big concern with Rio Grandes because they are so high up and waterways at higher elevations are expected to decrease the most.

- **Management Question: What guidelines should be used to manage populations that show signs of introgression?**

    - **With non-native species, native species**

    - Changed question to ask about populations rather than lineages.
    - Added question about introgression with non-native and native species introgression with the native species.
    - Question: How many populations are left that are considered to have introgression with non-native species (rainbow and Yellowstone)?
        - A lot. More on the west slope, east slope has less.
    - Comment: A lot of populations reflect the history of people affecting the landscape. Managing species for the historical stocking history is an interesting concept and I would not completely wipe out what was created from stocking.
        - Would you completely wipe out the Yellowstone and rainbow then?
        - We should not wipe out admixed populations until we know whether or not they were indeed created by stocking or natural processes.
    - Question: What is known about the historic life history of these species?
        - Yellowstone have an extensive life history
        - If we look at the populations we have now, we have relatively sedentary populations and that is why you see some gene flow between populations that are mobile.

- **Management Question 16: Can existing blue or green restoration projects be converted to good representatives of Bear Creek by stocking over blue or green populations?**
    - Does anyone have a proposed hypothesis to explain the blue and green distribution the west slope (Figure 2 from Rogers 2013)?
        - The upper Colorado basin is a place where both the blue and the green lineages could have both naturally have been present.
        - This hypothesis is consistent with saying blue and green lineages were split between southern (green) and northern (blue) in the west slope, with a separate population in the San Juan drainage and possible admixing in between in the Colorado drainage.
    - Another option would be to stock upstream and let individuals trickle downstream and naturally let mixtures occur, but maintain natural populations upstream.
    - A few ESA deliberations:
        - Should the BC be listed as endangered?
        - If the blue and green lineages are separate entities, should they be listed?
            - Must look at threats to the smaller green lineage group.
    - How much does the ESA stick to taxonomy? This will determine how specific we are with management and listing of a species.
        - The blue and the green are more different than the blue and the west slope greens are alike. It would make more sense to list the east and west slope

greens as a package and the blue separately rather than separate the greens and blues by east and west slopes.

- **Management Question: Which cutthroat lineage or subspecies should be considered for reintroduction in the Arkansas basin and for the San Juan basin?**
    - Arkansas River drainage:
        - Currently, BC are present, but thought to originate in the S. Platte and there is no evidence showing that BC were historically present in the Arkansas.
        - It is much harder to remove cutthroat and replace with other cutthroat down the road than to replace brook with cutthroat. We should be absolutely sure that the Arkansas is BC range before we restore it, otherwise down the road we will be in the same predicament we are in today.
        - Green lineage should be considered for the Arkansas because there are historic populations that were present. This could however be a result of stocking because they were found at Twin lakes, which was heavily stocked in 1889.
        - Our priority should maybe turn immediately be turned to the S. Platte, where we know what populations should be there – BC. Maybe we should hold off on the Arkansas drainage until we find more information before we make any decisions about the Arkansas. Be cautious.
            - Could this impact the green lineage populations in the S. Platte if we find out later that they are unique?
    - San Juan drainage:
        - How confident are we that we have sampled enough to determine that the San Juan lineage is not present?
            - Not confident.
            - Then, the plausible solution to this would be to sample first to see what is there and then determine what *should* be there.
        - Are reintroductions being occurred in the San Juan?
            - Yes, by Durango there is a blue reclamation that is occurring and has been going on for a few years. It has halted now until further developments are made.
        - Does the upper San Juan near the Lower Colorado basin, have suitable habitat?
            - Yes, there is a lot of suitable habitat.
            - It was suggested that the area should be extensively sampled to be sure there is not a population that drifted up there.

- Action Item: It would be helpful to have a 1-2 page document that can be understood by managers to explain what was discussed and the decided conclusions. For the USFS and BLM. This would maybe be completed by the US FWS.

- Meristics Study and Workshop Summary Report Issue:
    - Kevin and Kevin will talk and estimate how long it will take to finish their study and report.
    - The summary report should not be shared with anyone outside of the working group until it is finalized and published.
- Trout Unlimited may push the report and the listing.

    - We are certain the BC population needs to be listed, but unsure about the green and blue lineages. TU needs to be talked to and told a better “package” will be developed if given enough time.
    - There is interim protection for the green lineages and many oil and gas projects are underway and consulting with FWS currently. There is some urgency because of this issue.
- Biodiversity Implications: Listable Entities?
    - Do lineages identified in the Genetics and Meristics studies rise to the level of a listable entity? Different subspecies, distinct population segments, other?
        - Currently, all listings are to the level of subspecies. We did not hear anything new at this workshop regarding listing subspecies. Now, all we can do is compare subspecies.
        - For DPS, the framework is very similar. We could go through the framework and discuss in this context, but currently, cutthroat trout are discussed by subspecies.
            - Significance to the taxa, range, must occur in a unique ecological setting, differs markedly in genetic characteristics.
        - The blue and green lineage distinction is validated by both studies, can they be thought of as two separate species?
            - How different are the green and blue from other cutthroat species?
                - If they are different lineages, they are different lineages.
            - Listing the east and west slope green lineages could be listed as two separate DPS – suggested by Susan Linner.
            - West slope blue is a monophyletic individual entity, strongly defined by meristic and genetic data.
            - The difference with the blue and green issue is that the green needs taxonomic revision because it is not *pleuriticus*.
        - In the end, does it really matter that we call BC *stomias*? The point is not what we call it, but that we protect it.
    - Where we currently stand is 3 distinct lineages: BC, Blue, and Rio Grande.
- Is the BC population considered to be the Greenback cutthroat trout?
    - Jordan originally named *stomias* as “greenback”, which means large spots. It seems to reference the Rio Grande lineage, however the museum specimens of *stomias* and *virginalis* were mixed up. Although he may have been naming a lineage that did not originate in the area, the name should stay with the lineage that it was associated with by the first reviewer, which is Jordan.
- How do we describe the East Slope Green lineage?
    - It at least should be a DPS.
- What do rare haplotypes and morphological consistencies of east-green fish suggest in terms of subspecies or ESU distinctions?
    - There is sufficient evidence to say that a DPS that can represent the east green fish.
    - There are 2 haplotypes on the east slope that are not found on the west slope, although only a few bps off. The meristics study suggested they were much more different.
- From an ESA perspective, Steve Chambers suggested it is easier to list more, then delist, rather than try to additionally list something later.
    - Susan Linner responded that it is not easier from a work load perspective.
- Common name reference that should be used for populations:
    - Green
    - Blue

    - Rio Grande
    - Bear Creek
- In responses to the questions, panelists will create a small glossary of the terminology used.
- Evaluation of the Science questions
    - Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in the study? Are there other alternative interpretations?
        - What are the historical distributions?
        - What is the stocking history?
        - What do people think of the “fishless” inference of Bear Cr?
    - How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?
- Additional questions or topics
    - Could post on the Greenback cutthroat trout recovery page that the panel was held and is finished. Also post an estimated timeline. Same goes for the CR cutthroat recovery team.

APPENDIX C

Greenback Cutthroat Trout Genetics and Meristics
Expert Panel Review
Discussion Questions and Fact Sheet

Discussion Questions for Panelists

Evaluation of the Science

1. Are the conclusions reached by Metcalf *et al.* (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

3. To what extent are historical spatial distributions of green, blue lineages known?

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

BiodiversityImplications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?
    a. different subspecies?
    b. distinct population segments (DPS)?
    c. Other?

7. Is the Bear Creek population considered to be greenback cutthroat trout?

8. How do we describe the East Slope green lineage?

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroats?

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

-What conclusions can you make from these findings and what inference to future management of the lineage can you predict?

-What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?

17. Please provide other relevant comments not addressed in the above questions.

Fact Sheet (for use with Discussion Questions)

Historical Summary

- Subspecies of cutthroat trout historically have been defined largely by geography—major drainage basins.
- Extensive transfer of cutthroat trout through stocking occurred for more than a century.
    - Transfers primarily occurred from West to East. Primary sources were centered around the White River at Trapper's/Marvine Lakes (blue lineage) and on the Grand Mesa (green lineage) at the headwaters of the Gunnison.
- Como Creek, Hutcheson Lake and Como Creek/Hunter's/South Fork Poudre crosses were historically used to stock recovery populations in the S. Platte drainage to establish new populations of presumed greenback cutthroat trout.
- Cascade Creek was used to stock recovery populations in the Arkansas River drainage.
- Yellowfin cutthroat trout declared extinct by 1904.
- Greenback cutthroat trout declared extinct in 1937. From the 1950's to the 1980's, populations thought to be greenback cutthroat trout were found. Listed as endangered in 1973. Downlisted to threatened in 1978.
- Bear Creek haplotype matches the museum specimens from 5 locations within the S. Platte drainage.
- As the first reviser, Jordan coined the term "greenback" for native trout in the S. Platte and the Arkansas drainages.
- Historic reference materials were collected from 1856-1889 and are available to past and current researchers.
- Morphological variation of historical specimens is largely unknown.
- All lineages on the east slope are currently listed as threatened, green lineage on the west slope are threatened, and blue lineage populations on the west slope are not protected under ESA.
- Rio Grande cutthroat trout are a candidate species for listing under ESA.

Generally Accepted Findings (not currently in dispute)

- Habitat quality at Bear Creek has been compromised by sedimentation.
- Bear Creek and Como Creek fish appear normal in the wild, but exhibit serious deformities in the hatchery.
- Current data supports at least 4 extant lineages of cutthroat trout in Colorado.
    - mtDNA and meristics/morphometric studies are congruent in supporting this.
- Bear Creek is upstream what appears to be a one-way barrier to upstream migration of cutthroat and was presumed to historically be fishless.
- In samples analyzed so far, green lineage mtDNA haplotypes are found on both sides of continental divide. The two most common east slope haplotypes are not found on the west slope.
- Blue lineage mtDNA haplotypes are found on both sides of the continental divide. East slope haplotypes are a subset of what is found on the West slope and are limited to haplotypes currently found in Trapper's Lake.

    - Most green and blue lineages are separated by barriers. Most barriers are natural, but some are man-made. Populations above natural barriers are likely present from stocking.
    - Green lineage fish are not found in the Yampa or Green River drainages.
- The type specimens for *O. c. stomias* have a haplotype found in Rio Grande lineage.
    - Hammon's collection notes suggest that the type specimens were likely collected from the Rio Grande drainage.
- Geological and other evidence indicates stream-capture events across the continental divide occurred during the Pleistocene.
- Meristic/morphometric analyses show consistent differences between groups defined by mtDNA haplotypes. Patterns were stronger when individuals were grouped according to genetic lineage than when grouped according to geographical hypothesis. Meristic differences were also apparent at the level of GMUs.
- The green lineage East slope fish show meristic/morphometric differences from the West slope green lineage fish.
- The blue lineage fish on the East slope show meristic/morphometric differences from the West slope blue lineage fish.
- The blue lineage fish currently present on the west slope are presumed to have originated on the west slope.
- Some conservation populations show evidence of introgression from Yellowstone cutthroat trout and rainbow trout. Conservation populations are populations that count towards recovery.

Key Questions to Resolve:

- Can ancestral polymorphism be distinguished from historic stocking influences on populations with our existing analyses? If so, how? IMA, other programs, or evaluation of nuclear genes?
- The appropriateness of using ND2 and CO1.
- Are the green lineage fish on the East side of the divide are a result of stocking or natural processes?
- Is Bear Creek representative of historic populations in the S. Platte drainage?
    - See point in the Facts section.
    - Use of next generation sequencing of museum samples and the meristics study would help to verify this.
- The green lineage fish currently present on the west slope are presumed to have originated on the west slope.
- What is the phylogenetic relationship among cutthroat trout lineages?
    - Additional character information should be obtained.
- What is the level of inbreeding in the Bear Creek population?

APPENDIX D

Expert Panel Reviewer's Responses
to the
Greenback Cutthroat Trout Genetics and Meristics
Discussion Questions

Panelist #1 Responses to Discussion Questions

Evaluation of the Science

1. *Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study?Are there alternative interpretations?*

They appear to be generally supportable, especially the identification of the linkage between Bear Creek and the South Platte River Basin. But this is still to be viewed as a strong working hypothesis, not an absolute proof. Additional corroborating evidence should be sought. For example the matching of identical blue lineage cutthroat trout haplotypes from the Yampa River Basin with haplotypes in the Arkansas and South Platte river basins is strong secondary evidence that those fish were stocked from the Green River Basin into the Arkansas and South Platte river basins.

The association of the Yellowfin cutthroat trout with the Arkansas River Basin is also plausible. These data make sense relative to what one would expect for a fish lineage that naturally invaded a separate basin – they should show divergence from their ancestral source because of the isolation provided by the basins.

But two other factors should also be considered. First the existence of museum specimens from the late 1800s in the Arkansas River Basin which are part of the 'green lineage' raises some question as to the nature of the trout lineages originally present in the Arkansas River Basin. Clearly the museum specimens could have been stocked. But these and modern green lineage haplotypes in the Arkansas River Basin do not match known haplotypes from the green lineage in the Colorado River Basin. As with the blue lineage fish, you would expect to see very similar or identical haplotypes in the Arkansas River Basin if the fish were stocked from a Colorado River Basin source. It is important to note that: 1) the full diversity of the Colorado River Basin green lineage is not yet known 2) several green lineage haplotypes in the South Platte River Basin match those in the Arkansas River Basin 3) green lineage haplotypes in the Colorado River Basin and the Arkansas River Basin do not show clear separation into two discrete groups within TCS networks, which would be expected if sufficient time had passed for lineage extinctions and subsequent diversification. The mismatch of the green lineage haplotypes in the Colorado River Basin and the green lineages in the Arkansas River Basin means we cannot rule out a second natural invasion into the Arkansas River Basin occurring more recently in geological time. The matching of the green lineage fish in the Arkansas and South Platte River Basins suggest a common origin, potentially stocking, for one or both basins.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Creek, and Rio Grande)?

They generally do, especially with the principal components analysis where multiple data sets are combined. The Bear Creek population consistently separates from the other populations sampled. The Rio Grande and blue lineages also tend to separate relatively consistently. Addition of spotting measures generates better separation of the blue and green lineages. Yet the separation is not so distinct that it can be used in place of molecular data. In fact you would expect that morphological data would be the result of multiple factors – multiple genes, environmental influences, potentially even epigenetic processes. So the lack of distinct separation is not surprising.

3. To what extent are historical spatial distributions of green, blue lineages known?

To discuss the regions in which these two lineages are found it is useful to also clarify their presumed basins of focus. Use of the term upper Colorado River may work for our workshop, but on a broader scale it could lead to some confusion.

For water management purposes the Colorado River Basin is divided at Lee's Ferry, located just below Glen Canyon Dam, into the Upper Colorado River Basin (upstream of Lee's Ferry) and the (Lower Colorado River Basin) downstream of Lee's Ferry. All waters of the Colorado River Basin considered in this discussion are in the Upper Colorado River Basin. Within the Upper Colorado River Basin are three major subdivisions, the Green River Basin (including the Yampa and White Rivers), the Colorado River Headwaters (including the Colorado River upstream of Moab, Utah, which thus includes the Gunnison River and the Dolores River basins), and the San Juan River Basin.

The entire Upper Colorado River Basin has traditionally been assumed to contain the Colorado River cutthroat trout. The type specimen for Colorado River cutthroat trout was collected in Wyoming in the Green River Basin. The mitochondrial haplotypes that correspond to the cutthroat trout in the type locality region (Green River Basin) are the blue lineage. By default the definition of the entire Upper Colorado River Basin as the native range of the Colorado River cutthroat trout implies that the blue lineage is expected to be found throughout the three subbasins of the Upper Colorado River Basin. From broader studies of the Colorado River Basin cutthroat trout, the blue lineage is well established in the Green River Basin and is also found in the Colorado River Headwaters and the San Juan River Basin. This distribution was assumed to reflect the ancestral distribution of the blue lineage fish.

The South Platte River Basin and the Arkansas River Basin on the east slope of the Rocky Mountains have traditionally been considered the native range of the greenback cutthroat trout. Robert Behnke identified populations of cutthroat trout in the two basins as remnant greenback populations. Mitochondrial DNA studies conducted for Colorado Parks and Wildlife included these populations and identified a separate mitochondrial lineage that appeared to belong to the greenback cutthroat trout. This mitochondrial lineage was found in scattered populations in the Arkansas and South Platte River drainages. This is the green lineage.

Green lineage fish have also been identified in the Colorado River Basin and these have been confined to the Colorado River Headwaters as defined above. The distribution of the green lineage fish on both sides of the continental divide and in both the Arkansas and South Platte River basins is not what would be expected under natural dispersal hypotheses unless the dispersal event was very recent (Holocene). The Bear Creek population which appears to either form a polytomy with the other interior cutthroat trout (Bonneville, Colorado River, Rio Grande and green lineage greenback cutthroat trout), or to be basal to the greenback (green lineage) cutthroat trout is more concordant with patterns expected from ancient dispersal events, especially if it is representing the South Platte greenback which would have had to invade from an Arkansas Basin ancestor which in turn had to invade from the Colorado River Headwaters (West Slope) or Rio Grande basins.

The dilemma that exists is while the difference between the Bear Creek population and the other green lineage fish is real, we cannot yet rule out a recent natural invasion for the origin of the green lineages in the Arkansas River Basin. Since the greenback was traditionally designated as the native trout of the Arkansas and South Platte River Basins, this implies that the native trout should be inclusive of a broader range of genetic lineages.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

The designation of subspecies is generally based on geography and that is backed by clear genetic separation. But notably the Paiute cutthroat trout (*O. c. seleniris;* federally listed as endangered) was described based on the lack of spots on the body. This subspecies is only found in a single stream system in the Lahontan Basin on the east slope of the Sierra Nevada. Morphological and genetic studies have not shown significant separation from the Lahontan cutthroat trout (federally listed as threatened). Likewise the Snake River finespot (tentatively *O. c. behnkei*) has very distinct spotting differences from the 'sympatric' Yellowstone cutthroat trout, but again genetic markers have not been able to distinguish any differences. Behavioral and spotting differences between finespot and Yellowstone cutthroat trout has resulted in their being managed as separate entities. Essentially they are treated as separate subspecies. Conversely, the Bonneville cutthroat trout in the Bear River of the Bonneville Basin are genetically significantly closely linked to the Yellowstone cutthroat trout, not the Bonneville cutthroat trout in the main Bonneville Basin. Their morphological difference with Bonneville cutthroat trout in Snake Valley of the western Bonneville Basin was felt to be due to divergence induced by isolation of the Snake Valley region following dessication of Lake Bonneville.

So the degree of genetic separation of both the green lineage and the Bear Creek population is quite significant. Both entities are likely eligible for either subspecies or ESU-level recognition. What is not yet clear relative to the green lineage on the West Slope is how much of the admixture with the blue lineage is due to stocking and how much may be due to fish from the Green River Basin naturally infusing genes into the population through infrequent or rare dispersal events. Given that they are in the same major river basin, some exchange is likely to have occurred naturally. That may be decipherable by studying blue lineage haplotype diversity in the Colorado River headwaters since the work on the Arkansas and South Platte River Basins show Trappers Lake haplotypes being the stocked lineage.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Generally I think they did. I did not extensively examine the literature cited by the authors. However in publications the authors are limited by editors in the number of citations they can include. So it is not possible to evaluate the literature they cited relative to the total number of papers they considered. I did get the feeling that at least one of the authors wanted their paper to be the final answer to the problem. I agree that their interpretation does appear to follow the logical pattern I would expect to find with these fish. However if it does, then it should stand up to additional tests of their hypothesis. That is how science works. Once a hypothesis is posed others should be able to test it and either refute or support it. By multiple testing of the hypothesis we get closer to the true answer.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?
a. different subspecies?
b. distinct population segments (DPS)?
c. other?

The blue and green lineages on the West Slope should be managed as two distinct entities. The blue lineage is probably in good condition given the populations in both Utah and Wyoming as well as those in northwestern Colorado. The green lineage may suffer from introgression with stocked blue lineage fish, and the first step to understanding its status will be to evaluate the nature of the nuclear genomes in the West Slope green populations, especially relative to that from the blue lineage Trappers Lake fish. This means that it will be important to generate phylogenetic data with multiple nuclear genes, and the nuclear genes need to have sufficient variability to generate a good phylogenetic signal isolating Trappers Lake trout from other blue lineage haplotypes. The general prediction is that if the blue lineage nuclear haplotypes in the Colorado River headwaters region (the green lineage drainages) match those in the Trappers Lake fish, then the likelihood of introgression with introduced Trappers Lake fish is high. This does require phylogenetic datasets and not AFLPs, microsatellites, or SNPs because lines of descent need to be known. It is possible that the green lineage has historically had some introgression induced by natural movement of blue lineage fish from the Green River Basin. Again phylogenetic data should help identify the region of origin of those nuclear haplotypes.

If the green lineage is found to have little or no blue lineage influence other than that attributed to stocking, then the challenge will be to assess the abundance of relatively 'pure' green lineage populations in the West Slope drainages. This situation could raise the green lineage to listable status. This could also make the East Slope green lineage fish important if it is determined that those were the result of stocking since they would be candidate sources for reintroduction to the West Slope.

At minimum the blue and green lineages should be considered distinct population segments.

7. Is the Bear Creek population considered to be greenback cutthroat trout?

This is a difficult question because the greenback cutthroat trout has such a varied history. The incorrect designation of a type specimen begins the problem – were the characters used to define the type specimen significantly different than the actual characters in the South Platte River cutthroat trout? Was Behnke's decision to resurrect an assumed extinct species based on characters gleaned from introgressed populations? The DNA markers were initially developed based on populations designated as pure by Behnke. Many of the markers developed were informative, not diagnostic, and other than mtDNA, no markers were used to generate phylogenetic information relative to other cutthroat trout subspecies and rainbow trout. Most markers in widespread use in the state of Colorado are based on AFLPs and microsatellite data analyzed in Structure, where lineages are determined by their assignment into set groups. This does not generate lineage of descent data.

So the history of misidentification in greenback cutthroat trout has significantly confounded the issue. Technically the subspecific epitaph, *O. c. stomias*, is invalid, if the search of the historical records is correct. Further Behnke's morphological and meristic work will be incorrect since his populations were likely introgressed with multiple lineages of cutthroat trout. Thus the name of the Bear Creek fish will depend on 1) a decision on whether or not to retain the common name greenback for the fish found in Bear Creek, 2) the result of a petition to the International Commission on Zoological Nomenclature to change the type specimen for *O. c. stomias* and to retain the Arkansas River drainage designation (and of course it will require the designation of a new type specimen which should either be from a historic

collection – i.e., Harvard University's specimens or a Bear Creek specimen), 3) a decision on whether or not to separate the green lineage fish from the Colorado River Basin from the green lineage in the Arkansas and South Platte River basins, and 4) a decision as to the actual phylogenetic association of the Bear Creek fish with other interior cutthroat trout taxa. The common name, greenback, can be moved to the Bear Creek fish with less difficulty than can the scientific name, *O. c. stomias*. However given the significance of this subspecies a solid case for an exception does exist.

It will be very important to show independent support for classifying the Bear Creek cutthroat trout as a true remnant of the original South Platte River greenback cutthroat trout. This should be possible with next generation sequencing.

8. How do we describe the East Slope green lineage?

The East Slope green lineage fish are a group that, based on mtDNA, clades out within the West Slope green lineage. From data generated to date, the green lineage on the East Slope is imbedded within that West Slope clade. The East Slope lineage is not monophyletic within the West Slope haplotypes. The lack of monophyly indicates that either the fish entered the region recently or from a major transfer event of a genetically diverse population (and lineage extinction has not taken place but potentially haplotype divergence has) or that they were introduced by man from multiple sources which no longer exist (or have not yet been sampled) on the West Slope. As noted elsewhere the East Slope haplotypes, while clearly imbedded in the West Slope clade, are neither identical to nor nearly identical to known West Slope haplotypes. This should be further investigated.

The morphological/spotting data suggest that the East Slope green lineage fish are different from the West Slope green lineage. This could be due to a founder effect, drift, environmental differences, or introgression with a pre-existing cutthroat trout. It would be very useful to have similar morphological data for the museum fish from the green lineage in the Arkansas River Basin, the yellowfin cutthroat trout, and the South Platte greenback collection from Harvard's Museum of Comparative Zoology.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

This depends on whether the East Slope green lineage originated from stocking or from a recent natural invasion. If the East Slope green lineage trout can be shown to have originated from stocking from the West Slope, then their distinctness in morphology (especially spotting), is not of much importance. The populations then may be of importance for rehabilitation of West Slope populations.

If the East Slope green lineage fish are shown to be native, the fact that they are imbedded in the West Slope green lineage with mtDNA should be verified with nuclear genes. Corroborating findings would suggest that they are a part of the West Slope green lineage. At that point I would manage them as distinct population segments or ESUs.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

Currently published papers on cutthroat trout genetics do not resolve the phylogenies of the interior cutthroat trout well enough to answer this question since they all generate polytomies or weakly supported nodes separating the Bonneville, Colorado River, greenback, Bear Creek, and Rio Grande lineages. It is important to note that while these analyses fail to show resolution in line of descent, they do show strong support for each 'geographically defined' subspecies with the addition of the Bear Creek lineage as a separate branch on the polytomy.

Additional sequence data from the mitochondrial genome will generate more strongly supported clades, which will help answer that question. In addition the development of primer sets for nuclear genes will allow the establishment of multiple gene phylogenies, which can be examined both individually and as a concatenated data set. These will generate a consensus phylogeny which should establish the most likely pathways of colonization of the interior west by cutthroat trout.

The meristic data suggest an overlap of the West Slope green lineage with the Rio Grande cutthroat, but I do not put much weight in the ability of meristics to generate phylogeographic associations because the range of variability is too great with any individual character to separate founder effects from pleisiomorphic states.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

Blue lineage – the wide distribution of the blue lineage in the Green River Basin indicates that it is an endemic lineage of cutthroat trout and, given that the type specimen is from Wyoming, it is the subspecies currently identified/recognized as the Colorado River cutthroat trout. The blue lineage populations on the East Slope appear to be introduced from stocking activities in the late 1800s and early 1900s. This is strongly supported by the east slope blue haplotypes being identical to several found in the Trapper's Lake region of the White River Drainage. Genetically the blue lineage fish also appear to be less variable in the East Slope basins. This could be interpreted as supporting their originating from stocking activities by man, although a small founder population or bottlenecks could also be invoked to generate low haplotype diversity. The most likely cause is stocking.

Green lineage – this lineage is found in the Colorado River Headwaters (defined in question 3 above), the Arkansas River Basin, and the South Platte River Basin. It appears to be absent from the Green River Basin. Depending on the analysis, the green lineage fish can be interpreted to be a single lineage in a polytomy containing all of the recognized interior cutthroat trout except the Yellowstone cutthroat trout line. The Bear Creek lineage, in this case, is an independent lineage in the polytomy. Or the green lineage can be divided into two clades, with the Bear Creek fish being one line branching off basally from the main (predominant) green lineage.

Depending on how one designates the basic relationships of the Bear Creek fish (unresolved member of a polytomy or a resolved clade branching from within the green lineage), the Bear Creek trout would be treated as either a separate but equal entity to the green lineage (thus both definitely appear to deserve subspecies status), or the Bear Creek population would be a highly unique offshoot of the green lineage, making the Bear Creek population's status as an independent subspecies less clear. This needs to be answered, but published data do not have the resolution to give a definitive (high bootstrap support) picture of the relationships. The use of the early museum specimens resulted in 430 base pairs of sequence data, too few to generate a robust phylogenetic signal. Even as many as 3600 bp of mtDNA data fail to give much additional resolution (Shiozawa et al 2010). Yet this question is basal to the determination of the degree of uniqueness in the Bear Creek population relative to the green lineage

populations being examined across the various drainages. The Bear Creek population is clearly unique, but if it is a member of a true irresolvable polytomy, showing equivalent separation from the other interior cutthroat trout subspecies, management decisions are easier to make than if it is an offshoot of the green lineage, or any other interior lineage.

The separation of the Bear Creek fish from the green lineage – the difference between the Bear Creek and other green lineage fish is significant. The morphological data are less informative simply because the Bear Creek population is so highly inbred. It is not clear what effect that will have on the morphological differences. The examination of the South Platte museum specimens will help answer that question.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

The meristics report shows that the Bear Creek fish have less variability than the green lineage fish for many measured characters. However since this is a single inbred population which has clearly been subject to drift and founder effect, it is not clear how strongly this represents what these characters might have been in the Arkansas River Basin. The fish in Harvard's collection should be examined.

It is imperative that the population be duplicated in multiple isolated streams, in the South Platte River Basin, as quickly as possible. While this is being done, it would be useful to attempt genetic rescue in additional duplicated populations with limited, say 2-5%, introgression with other green lineage fish. Of concern is the possibility that current inbreeding is so high that some deleterious genes are approaching fixation. It is much better to continue with both population rescue and population duplication while other concerns such as genetic status, fry deformities, etc. are being assessed because the risk of population loss is too high with just the one small population that is known to exist.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

At this point the most likely native Arkansas River cutthroat trout is the extinct Yellowfin. However if a recent natural invasion of the green lineage into the Arkansas River Basin has occurred, then the green lineage in the Arkansas River Basin would likely also contain genetic material from the Yellowfin. These hidden haplotypes should be seen in trout from within the basin in either mitochondrial or, most likely, nuclear DNA sequences. The information could be retained in fish from highly introgressed populations. A series of good nuclear gene phylogenies would allow identification of those haplotypes.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

A number of possibilities exist with next generation sequencing. The first priority with this procedure should include development and verification of nuclear markers capable of producing phylogenetic signals. While it is highly likely that mtDNA studies underway will help resolve questions of lineage of descent, mtDNA is a single locus. Additional independent loci should be examined so that the phylogenies are robust.

Second, this technology can be used to generate sequence data (skipping Sanger sequencing altogether). Such procedures will shift the processing of data into the bioinformatics realm and is the logical next step to generating large nuclear DNA sequence data sets.

Third, both SNP development and phylogenetic data generation can be accomplished with less cost per base pair of data than is currently achieved with Sanger sequencing.

Finally, next generation sequencing provides a way to obtain significantly more information from museum specimens. Given that some of the next gen sequencers are optimized for fragment sizes found in degraded DNA, it is possible to generate significantly more genomic information from the museum fish DNA.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

Most of the priorities are scattered throughout my responses.

It is critical to continue searching the West Slope green lineage for mitochondrial DNA haplotypes matching the East Slope green lineage. This can be aided by using the proper nuclear DNA markers that have a phylogenetic signal.

Non-conservation populations should be examined as well, since residual genetic information should remain in introgressed populations. This can be sorted out with phylogenetic approaches.

SNPs suffer from the same limitations as AFLPs and microsatellites and probably should receive less emphasis until good nuclear DNA phylogenies are developed for many genes (from which diagnostic SNP loci can be obtained).

It is critical to generate nuclear DNA phylogenies so that the strength of the mitochondrial signal can be assessed and so that additional genes are available for forensic investigations of the drainage associations of the various trout populations.

It is important that the status of the populations be determined as accurately as possible. The technology that is rapidly developing with next generation sequencing will provide answers to many of the questions about this complex group which could not be breached just a few years ago.

One factor to keep in mind with all of the turmoil associated with the current state of the greenback cutthroat trout is that, had preservation not begun until we had the genetic tools in use today, we would likely have lost many of the populations that can now potentially serve as source populations for recovery of fish on both slopes of the Rocky Mountains. The effort to this point has not been in vain.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*
- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

Anecdotally it appears that the Bear Creek fish are expressing a higher proportion of deformed fish than is usually observed. Highly inbred populations risk problems with deleterious genes that are expressed in

the homozygous state. In small inbred populations these problem genes may increase in frequency by genetic drift enhanced by low population size. That could be the case in this population. The process of domestication involves continued breeding to eliminate (purge) such genes from the population. The lack of expression of these traits in the adult fish is likely due to a failure of deformed fry to survive. Yet, whether this is due to a series of single genes, each causing different deformities, or a number of polygenic complexes causing such deformities, it is likely that the deleterious genes are in high enough frequency within the population that the mortality of deformed fish is not rapidly removing the defective genes from the population.

It is not clear what the frequency is for similar deformities in other small, isolated populations of trout. Hatchery rearing is not attempted on most of those populations because they are often not critical enough to management that hatchery rearing is needed. It would be useful to have this quantified as # of deformities /1000 swim-up fry (or other easily quantified age class), in replicates, and with multiple other green lineage (or interior cutthroat trout) populations including both large meta-populations and isolated, potentially bottlenecked populations. The experiment would need to be done under fixed temperature and chemical conditions. This would establish baseline data for understanding the significance of this level of inbreeding and the apparent normal appearance of the adult fish. Further, fluctuating asymmetry can be examined in the fish used for the meristic study to determine if greater asymmetry exists in the Bear Creek population than is seen in other populations. Parallel genetic studies could be conducted to determine overall heterozygosity of the population, especially in comparison with other potentially bottlenecked populations as well as populations in streams with robust metapopulation structure.

Such studies, comparing multiple populations with the Bear Creek population, would allow a better understanding of the degree of concern that one should have with the fry deformities. If other populations show similar deformity frequencies when reared in a hatchery environment, then the deformities would be less of a concern, even if the Bear Creek fish show higher homozygosity and asymmetry than other populations.

17. Please provide other relevant comments not addressed in the above questions.

In reality, geographic isolation is likely the primary evolutionary force driving speciation processes within western North American obligate aquatic organisms. So with cutthroat trout the general hypotheses put forth by Behnke are rational. What he did not anticipate is the potential for reticulation (re-coalescing of populations) during repeated Pleistocene climate oscillations and the ability of phylogenetic approaches to tease these events apart. It is becoming more apparent that even within basins, large subbasins can have their own evolutionary trajectory especially if the connections between the subbasins include sections with less favorable habitat which can act as partial barriers to gene flow.

In the case of the trout in the Arkansas and South Platte River systems, one recurring question to me relative to data I had seen prior to the 2012 paper was why were the fish in both basins so similar to one another when their downstream connections were obviously too far apart to be likely paths of colonization. This coupled with the lack of native trout in the North Platte River Basin suggested that a very recent, possibly Holocene age, transfer event across a low order stream system must have occurred. Behnke had hypothesized such a recent transfer of Colorado River cutthroat trout as being the origin of the greenback cutthroat trout. But that did not explain the similarity between the two East Slope basins. A recent event could have happened but would be unlikely. Two recent events, one from the West Slope of the Rocky Mountains to the Arkansas River Basin and one from the Arkansas River Basin to the South Platte River Basin would be even less likely. The findings of Metcalf et al. 2012 do fit better with what is known for trout dispersal and isolation patterns.

Similar patterns are seen with other populations of cutthroat trout as well as other fishes in the west. An example occurs in the Bonneville Basin where fish in the Bear River Basin appear to have entered that Basin at a different time than the existing fish (cutthroat trout - Loudenslager and Gull 1980, Martin et al 1985; redside shiner - Houston et al.2010; leatherside chub - Johnson et al 2004; Utah chub - Johnson 2002; speckled dace – Billman et al 2010). Two points can be seen in this transfer event – first species were transferred and then isolated from the basin of origin and second they had contact with resident fish in the receiving basin. The populations retained relatively high separation between the invading taxa and the resident taxa – generating a relatively narrow hybrid zone. Thus in Utah, streams of the Bear River contain the Northern leatherside chub, a recognized species while those streams from approximately Utah Lake south contain the Southern leatherside chub. Utah chub show a similar pattern as does the redside shiner.

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Panelist #2 Responses to Discussion Questions

Evaluation of the Science

1. *Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?*

This reviewer is not convinced by the conclusions presented in either Metcalf *et al.* (2007) or Metcalf *et al.* (2012). The analyses regarding gene flow via introductions were not done to the best science available and as a result cannot distinguish between ancestral polymorphisms of alleles (falsely interpreted as introductions and breeding of stocks or introgression). Their conclusions are only one of a few others.

2. *Does the meristic study correlate with findings in the genetics study (i.e, does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?*

Yes, based on other genetic studies and at a wider scale by others than Metcalf *et al.* the morphological studies provide a much clearer picture of the diversity. Studies of this nature should be continued and funded to resolve the taxonomy of the cutthroat lineage. These studies should not be done by anyone that does strictly genetics or morphology of any other organisms. They should be done by a professional taxonomist and systematist for proper data collection and interpretation of analyses.

3. *To what extent are historical spatial distributions of green, blue lineages known?*

Not very well, frankly.

4. *How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?*

This cannot be answered as comparable studies do not exist for other cutthroat trout.

5. *Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?*

No, the genetic studies should have included data from other studies in larger scale evaluations. The morphology study suffered from not examining historic specimens from the South Platte River.

Biodiversity Implications: Listable Entities?

6. *Do lineages identified in genetics and meristics studies rise to the level of a listable entity?*

   a. different subspecies? At least.

b. distinct population segments (DPS)?

c. other?

Frankly these lineages constitute different species when one compares the differentiation of these lineages with that of other trout species not in North America (those that have been historically studied by the same people in the US and have conservative views). If the diversity of characters were to be interpreted for Eurasian trout species the cutthroat lineages would be called different species.

7. *Is the Bear Creek population considered to be greenback cutthroat trout?*

I cannot say as the samples from the South Platte River were never examined for the morphology.

8. *How do we describe the East Slope green lineage?*

It is a distinct lineage.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

They are distinct.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

This question can in no way be answered as there has not been enough detail done in any studies thus far. Anything proposed otherwise is simply guesswork and not science.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

Yes, they are significant. They have unique morphologies and genetics. There are many reasons for historic transfer and differentiation that do not include human activities. Taxonomically they should be considered distinct lineages until such time they are tested to interpret otherwise.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Yes, there may be limited variability in this population but this may be natural and should not be changed through the introductions of other alleles from other trout. This species should be maintained and protected in Bear Creek and there should be habitat restoration done in this system. Otherwise, there could be justification to relocate individuals to some known fishless streams in the South Platte River Basin with good habitat to see if there is increase in genetic variability.

Don't mess with the genetics of this species. We do not know enough about it morphologically or genetically to justify any management changes that involves introductions of alleles from other trout. This will only make the matters worst in the future.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

The current lineage there with the unique haplotype and distinctive morphology.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

These data provide good information on the phylogenetic relationships of these trout lineages and should be done at a broader scale to identify close relationships to determine evidence of ancestral polymorphisms in genes that are not interpreted as originating from interbreeding. Otherwise the data gathered from this will provide a large library of genes that can be monitored to examine historical and recent exchanges and relationships.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

Full scale morphological study with finer level of evaluation of differentiation within drainages

Broader and more complete sampling of genes for all of the lineages of cutthroat with finer level of evaluation of differentiation within drainages

Once these are done by consortium of researchers for best available science interpretation then a sound basis will be available to make management decisions but only then.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*

You cannot make any general conclusions from this from hatchery materials. There are too many variables that cannot be controlled for. This lineage may simply be unique relative to others and in an evolutionary and developmental transition.

- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the S. Platte drainage?*

See above about comments for South Platte River.

17. Please provide other relevant comments not addressed in the above questions.

There needs to be significantly more data collected on these fishes across the cutthroat lineages and at a finer scale than what has been done to date in order to make sound scientific conclusions. The data that exists today, much of it unpublished or not in complete focus on the questions at hand, do not provide a sound basis for making significant management decisions at this time. Until such time that the science exists, and funding should be provided to do this, the best available science to address these questions is just not there at this time. Too much rushed and preliminary findings can further complicate the picture–possibly to the point that the natural signature of the complex will be lost forever. Some researchers involved in this seem to be too rushed and have done very incomplete analyses and interpretations to be considered sound.

Panelist #3 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

I will begin my answer by stating that the Metcalf et al. 2012 paper, published in the high-quality scientific journal *Molecular Ecology*, was a carefully conducted piece of work that was enormously helpful in identifying and understanding historical distributions of cutthroat trout lineages in Colorado. These researchers obtained considerable historical material and used state-of-the-art ancient DNA techniques and practices to avoid problems with DNA sample contamination. The resulting data set is well analyzed and conclusions appropriate. That said, there were few samples available for analysis (roughly 30 were successfully isolated and amplified) and only short DNA fragments from the mtDNA could be analyzed. In my view, small sample sizes limit some inferences that can be conclusively drawn from the data, and the study does not definitively rule out alternative hypotheses. Some alternatives are more important than others from a management perspective, and I will follow up on those below.

A major finding of the paper was that six distinct (i.e., demonstrably monophyletic) mitochondrial DNA (combined ND2 and COI gene sequences) lineages were identified from historical samples – three distinct lineages occurred on the East Slope of the Rocky Mountains including purple (South Platte drainage – represented only in Bear Creek in modern samples), orange (Rio Grande drainage), and yellow (Arkansas River, now presumed extinct); and three lineages on the West Slope including blue (Yampa and Colorado Rivers), green (Colorado, Gunnison, and Dolores Rivers), and red lineage (San Juan River – presumed extinct). The green lineage also occurred in the Arkansas River Basin in historical samples, and as such, was the only lineage that occurred on both sides of the continental divide. Metcalf et al. hypothesized that purple lineage fish were *O. c. stomias* (*sensu stricto* – limited to the South Platte River and introduced into Bear Creek), yellow lineage fish were *O. c. macdonaldi*, orange lineage fish were *O. c. virginalis*, blue lineage fish were *O. c. pleuriticus*, and that green and red lineage fishes were unnamed lineages. Of unnamed lineages, extant green lineage fish could meet criteria to be named a distinct subspecies (geographic isolation, distinctive meristic features).

At this time, it is unknown whether red lineage fish is nameable or a listable entity, and the issue may be moot unless one or more native population of fishes is found in the San Juan River drainage. It is possible that introgressed individuals will be identified that have the red lineage mtDNA haplotype or something closely related to that haplotype. In this case, it may be possible to 'resurrect' a relatively genetically pure strain through careful brood stock management and genetic-marker assisted backcrossing, if deemed important from a conservation standpoint. A successful marker-assisted program necessitates further development of co-dominant nuclear DNA markers (i.e., microsatellites or SNPs) to fully characterize the degree of introgression of each individual in the brood stock. It appears likely that yellow lineage fish (*O. c. macdonaldi*) are extinct. Population surveys along with genetic analysis of Arkansas River drainage populations may identify some fishes that bear yellow lineage haplotypes, but this seems unlikely.

A second important finding was that four of six historical lineages were distributed solely in single major river drainages. A number of researchers, dating back to some of the first ichthyologists to describe cutthroat trout subspecies (e.g., David Starr Jordan), have hypothesized that major drainage divides are important geographic features that isolate distinct cutthroat trout lineages (a more recent example is Loxterman and Keely 2012), although gene flow across drainage divides can and does occur (through

stream capture). Metcalf et al. 2012 present historical data that are consistent with the interpretation that major drainage divides are the predominant structuring feature for cutthroat trout diversity in Colorado. Two important and notable exceptions to this finding were observed for the green lineage that occurred on both sides of the continental divide and in the Colorado, Gunnison and Dolores R. drainages on the West Slope, and the blue lineage that occurred in both the Yampa and Colorado R. drainages on the West Slope. Modern samples analyzed in Metcalf et al. 2007 also indicated that blue lineage fish were present in large numbers in East Slope drainages. In cases where lineages occur in multiple drainages, Metcalf et al. hypothesized that historical stocking is predominately the cause. Chris Kennedy, a co-author of the paper and a presenter at the Greenback Cutthroat Trout (GBCT) workshop, offered compelling analysis of historical stocking records that indicated that stocking was conducted on a massive scale (hundreds of millions of fish), stocked predominately from West Slope to East Slope drainages. This would serve to mix lineages and obscure historical patterns of isolation that corresponded with distinct watersheds. Most compelling was the evidence that blue lineage fish were stocked into the S. Platte and Arkansas River drainages and subsequently were established in previously fishless habitats, or introgressed with existing fishes (Metcalf et al. 2007, 2012, Kennedy presentation). This was supported by the presence of identical haplotypes (e.g., common alleles found at Trapper's and Marvine Lakes that served as a source for stocked fish) on both sides of the divide.

The origin of green lineage fish on the East Slope is less certain. There are several alternative hypotheses based on the data in hand:

1) The green lineage is native to the West Slope and was introduced into East slope drainages by stocking.
2) The green lineage is native both to the West and East Slopes.
3) The green lineage is native to the East Slope and was subsequently reintroduced to the West slope either by natural events (i.e., stream capture) or by stocking.

Hypothesis 1 is favored by Metcalf et al. 2012, and although these authors raise the possibility that Hypothesis 2 is correct in the paper, they do not discuss it further. The strongest support for Hypothesis 1 would be if all East Slope green lineage populations contained haplotypes that were identical to West Slope haplotypes (i.e., East Slope haplotypes are a nested subset of West Slope). This is not observed with the samples in hand, but it is possible that more extensive geographic sampling may reveal this outcome. Hypothesis 3 seems least likely and is not supported by the data in hand as stocking rates from East to West Slope drainages were much lower than from West to East.

With regards to Hypothesis 2, a gene tree based on ~8000 bp mtDNA sequence data (presented by D. Shiozawa at the workshop) suggested the following relationships with good levels of bootstrap support: a) monophyly of a clade that contains all green lineage and Bear Creek haplotypes (96% support) b) monophyly of Taylor Creek, Como Creek, and Severy Creek haplotypes (100% support), and c) a sister relationship of Rio Grande (orange lineage) plus green lineage (with 98% bootstrap support). One green lineage haplotype found in the S. Prong of Hayden Creek, Arkansas River drainage did not fall within the monophyletic lineage in item (b) above. This tree suggests that Hypothesis 2 cannot be rejected at this time and that the East Slope contained individuals with a divergent green lineage haplotype(s).

A potentially complicating factor is that Hypotheses 1 and 2 are not necessarily mutually exclusive, and it is possible that they are both correct. In other words, it is possible that West Slope green lineage fish were stocked into populations that already contained genetically distinct native East Slope green lineage fish and then subsequently introgressed. It is also possible that West Slope fish were stocked into fishless creeks to create a mosaic pattern of West and East Slope haplotypes in the Arkansas (and perhaps S. Platte) River drainages. The gene tree presented by Shiozawa suggests that S. Prong of Hayden Creek

may be one such population where West Slope green lineage fish have introgressed, because haplotypes there do not share most recent common ancestry with other East Slope green lineage fishes in Taylor, Como and Severy Creeks. Another complication is that incomplete lineage sorting (i.e., haplotypes have not achieved monophyly) have caused this result. Incomplete lineage sorting occurs when insufficient time has passed to 'sort' ancestral haplotypes (through genetic drift, mutation, and possibly local selection) into monophyletic lineages, even though the two gene pools have been isolated for several thousand years or more.

It should be noted here that Shiozawa's work is unpublished and has not been peer-reviewed. However, the methods appear to be sound and the dataset is impressive with respect to the number of base pairs examined, but few individuals were sequenced. I should also note that the Metcalf et al. 2012 gene tree was unresolved at key nodes that would have indicated the relative timing of certain events with regard to the evolution of green lineage haplotypes. Without larger and more geographically widely distributed sampling, it may not be possible to assess alternative hypotheses. More research on this topic is necessary and warranted.

In summary, the Metcalf et al. 2012 paper, while an excellent piece of work, leaves some unanswered questions that pertain mostly to green lineage fish. Among these are: 1) What is the most probable origin and process that gave rise to green lineage haplotypes on the East Slope? 2) Are East Slope green lineage fish 'native' to the Arkansas River or were they recently introduced? 3) Are East Slope green lineage haplotypes a nested subset of West Slope haplotypes? 4) What are the effects of incomplete geographic sampling and/or incomplete lineage sorting as it pertains to reconstructing the origin of East Slope/West Slope green lineage fish? 5) Are there genomic remnants of yellow lineage fish or native East Slope green lineage fish in present-day populations of the Arkansas River drainage? These questions are germane to what should lineage(s) should be restored to the Arkansas River drainage.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

The meristic study by Bestgen et al. examined ten meristic traits (plus standard length) in 744 fish distributed in 49 distinct localities and 14 Geographic Management Units (GMUs) across relevant drainages. Meristic traits matched four that were traditionally used by ichthyologists and added six more, including a quantitative analysis of spotting patterns. It was one of the tasks of this workshop to critically evaluate this work, but it has not been subject to peer review otherwise. Fin clips were obtained from each individual for genotyping to link mtDNA lineage to individual meristic counts explicitly. A strict blind protocol was employed to minimize bias due to investigator pre-conceptions about a particular collecting locality. In the final study design, it was possible to evaluate variation in meristic traits attributable to major drainage divides, ND2 haplotype, and Geographic Management Units. Two hypotheses were examined: (1) the Geographical Hypothesis states that the majority of variation in meristic traits across individuals and populations could be explained by grouping individuals into West Slope, East Slope, and Rio Grande categories, and (2) the Molecular Hypothesis states that most variation could be explained by grouping individuals and populations by mtDNA-ND2 lineage. Principal Components Analysis (PCA) and Discriminant Function Analysis (DFA) indicated that the Molecular Hypothesis explained more variation than the Geographical Hypothesis – consistent with the results of Metcalf et al. 2012. Thus, the answer to the question posed above "are meristics and genetics correlated?" is yes. Moreover, the Molecular Hypothesis provided a better fit to the meristic data than the Geographical Hypothesis (based on multivariate analyses), although it should be noted that these hypotheses are related -- the Molecular Hypothesis essentially partitions the Geographical Hypothesis into more drainages. However, a critical

difference between the two hypotheses is that the Molecular Hypothesis allows for cross-drainage stocking (because individuals are assigned to lineage based on ND2 haplotype, independent of collection locality), but the geographical hypothesis does not.

Univariate and multivariate analyses of meristic data based on mtDNA-ND2 lineage demonstrated that blue and green lineage fish could be separated primarily on the basis of the number of trunk spots and fore- and mid-trunk spotting ratios. There was strong support for historical stocking as a factor that explained the distribution of blue lineage fish on the East Slope. Other strongly supported findings were that Bear Creek was significantly distinguishable from all other cutthroat trout examined and did not overlap with any other species in PC space. The number of basibranchial teeth, number of anterior gill rakers, and mean spot size were important traits that distinguished Bear Creek from other lineages. Rio Grande cutthroat trout were also significantly distinct, but some characters were shared across different lineages (usually green and Bear Creek lineages, although different subsets of characters overlapped between lineages). Similarities for subsets of characters among green, purple (Bear Creek), and orange (Rio Grande) lineage fish is not surprising given that they share recent most common ancestry compared to the blue lineage fish that are more distantly related (based on ND2 tree in Bestgen's presentation and Shiozawa's mtDNA gene [~8000 bp] trees).

There was evidence that a substantial portion of meristic variation could be explained at a geographic level that is finer than implied by either the Geographic or Molecular Hypotheses. This is the Geographic Management Unit scale (there are 14 GMUs throughout the region). Variation attributable to GMU was especially evident for Rio Grande cutthroat trout and green lineage when classification via DFA was conducted at the population level. This is the *de facto* level that management activities are currently being conducted.

Another important finding was that East Slope green lineage fish were distinguishable from West Slope green lineage fish based on lateral line scale counts, basibranchial tooth counts, and trunk spot counts. This is consistent with the idea raised above (in Question 1) that East Slope green lineage fish may have deeper and more complicated origins on the East Slope than would be predicted if they were introduced more recently by stocking. Inter-lineage variation probably contributed to relatively high misclassification rates in the green lineage. However, some differences between East and West Slope blue lineage fish were also identified, for example, lateral line scale counts and fewer spots. This result may also suggest complex introgression histories and local environmental conditions that may account for at least some of the East Slope/West Slope differences in the green lineage. At the very least, examination of meristics in historical material (if possible) and extant populations, and more genetic study of East Slope green lineage fish is warranted.

With regards to quality, the Bestgen et al. study is among the most comprehensive meristic datasets ever compiled for cutthroat trout. Sample and data collection was sufficiently geographically dense to address all hypotheses laid out in the objectives of the study. Any limitations in sample size were imposed by the lineage examined, for example, Bear Creek had only two "populations" one in the wild and the other from the hatchery. There are also relatively few East Slope green lineage populations. The analyses are appropriate and well executed. It should be publishable in a peer-reviewed scientific journal after peer-review and some revision. Future research should focus on extensive surveys of cutthroat trout populations throughout Colorado, with collection of sufficient specimens (where possible) to conduct additional meristic and molecular analyses. Specimens and tissues should be carefully archived in a well-curated natural history collection to create a resource base for future conservation efforts.

3. To what extent are historical spatial distributions of green, blue lineages known?

The preponderance of evidence suggests that green lineage fish occurred historically in the Colorado, Gunnison, Dolores River drainages on the West Slope, and potentially in the Arkansas River Basin on the East Slope (Metcalf et al. 2012). Interestingly, the green lineage does not fit the "single drainage – single lineage" paradigm that appears to be the general observation in cutthroat trout. Likewise, blue lineage fish occur in the White, Yampa, and Colorado River drainages. It appears clearer that blue lineage fish were introduced into the Arkansas River by stocking and historically were limited to the White and Yampa River drainages. Geological and/or climatic events can facilitate headwater capture across major divides – detailed study across drainage divides is warranted to evaluate the presence of major lineages across numerous drainages.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

Blue, green, orange and purple lineage fish are demonstrably monophyletic and exhibit significant divergence in meristic traits across lineages. Molecular and morphological evidence for diversification of lineages is comparable to, and in most cases, much better than evidence available for other named subspecies of cutthroat trout.

5. Did genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

I have commented on the high quality of both the Metcalf et al. 2012 and Bestgen et al. (unpublished) studies in my responses to Questions 1 and 2, respectively. These are well researched, well executed, and clearly written studies that, to my knowledge, cite and include all pertinent literature including a lot of unpublished stocking records and other literature that is difficult to access. I have identified areas in both studies where additional data and study are warranted, but both stand alone as seminal contributions to understanding the processes responsible for variation in present-day cutthroat trout lineages in Colorado.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?

The Metcalf et al. 2012 study identified, and Shiozawa's mtDNA and the Bestgen et al. meristic studies confirmed, that there is previously unrecognized biodiversity within currently named subspecies of cutthroat trout in Colorado. Taxonomic revision of cutthroat trout in Colorado is warranted and justified based on the new understanding of diversity afforded by molecular and meristic studies. There is some subjectivity about subspecific designation (the classic "lumpers" vs. "splitters" debate among taxonomists), but the evidence for additional biodiversity in this complex is clearly demonstrated by the data. However, any taxonomic revision will take considerable time.

The preponderance of molecular and meristic data indicates the following:

- The subspecies name *O. c. pleuriticus* currently refers to fishes in the White, Yampa, Colorado, Gunnison, Dolores (and San Juan?) River drainages. Metcalf et al. 2012 suggested that the name *pleuriticus* should be more narrowly defined to the drainages historically occupied by the blue

lineage (Yampa, White) – a stricter definition than is currently recognized. Molecular and meristic data suggest that this is appropriate, and indicate that the previous definition was too broad and did not recognize biodiversity contained in green lineage fish. This change would leave open the taxonomic status of the green lineage to revision.

- The subspecies name *O. c. virginalis* currently refers to fishes in the upper Rio Grande drainage (Rio Grande, Pecos, Canadian Rivers) and this designation is well supported by molecular and meristic data. There is also considerable molecular and meristic variation across GMUs, which suggests that management should continue at the GMU level.

- The subspecies name *O. c. stomias* currently refers to all cutthroat trout on the East Slope excluding fish in the Rio Grande drainage. Metcalf et al. 2012 suggested that the name *stomias* be restricted to purple lineage fish. The purple lineage is demonstrably monophyletic and exhibits large differences in meristic traits from all other East Slope fish, and so this is justifiable. However, one important question is distinctiveness of East Slope green lineage fish – whether they should be "lumped" into *O. c. stomias* or not? Jordan clearly intended fishes in the South Platte *and* the Arkansas River to be included in *stomias*. According to Shiozawa's tree, purple and green lineage fish (East and West slope inclusive) share most recent common ancestry with respect to other named subspecies. This situation will require additional careful study, and a newly elevated green lineage (perhaps to a new subspecies) and a stricter definition of *stomias* will require taxonomic revision.

- In the long run, it may be most defensible to propose a new entity that encompasses green lineage fish (perhaps as a new subspecies) with East and West Slope lineages identified as 'distinct population segments'. The East Slope DPS would probably require protection and intensive management. The West Slope DPS is probably much more secure.

b. *distinct population segments (DPS)?*

See my reponse in the 3rd bullet above.

c. Other?

7. Is the Bear Creek population considered to be greenback cutthroat trout?

This is a difficult question to answer, and it depends on the evolutionary origins and taxonomic status of East Slope green lineage fish. From a pragmatic standpoint, restricting the name *O. c. stomias* to Bear Creek would remove protection from green lineage fish on the East Slope (and the West Slope). In the short run, it may afford more protection to keep the current taxonomic status in place, and adopt a model currently employed for Gila Trout, that is, to name relict lineages in the recovery plan and then manage those separately. Thus, if *O. c. stomias* is retained as it is currently defined, and Bear Creek, East Slope, and West Slope green lineages are named in the recovery plan as lineages that warrant special management and protection. This would allow intensive management that circumvents the need for immediate taxonomic revision as a precursor to conserve green lineage fish on the East Slope. Recovery goals can be written in a way that emphasizes the critical status of Bear Creek fish, while not removing protection from the East Slope green lineage until its origin can be ascertained.

8. How do we describe the East Slope green lineage?

This will depend on the outcome of studies designed to ascertain the origin of East Slope haplotypes – see my comments above.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

Haplotypes that are (thus far) unique to the East Slope green lineage (i.e., not found in West Slope populations) appear to be relatively common in Como, Severy, and Taylor Creeks, and possibly in the S. Prong of Hayden Creek. This observation, along with unique meristic variation suggests that certain East Slope populations may comprise a distinct population segment (DPS) of the green lineage, given that they are discrete from other populations and significant in relation to the green lineage. It may be difficult to manage green lineage populations on the East Slope because they may have different introgression histories and repeated backcrossing events to green lineage fish more recently introduced via stocking – perhaps the GMU level of management is most appropriate for this purpose.

A few caveats are that mixed ancestry in these populations may complicate ESU designation. Likewise, the green lineage may need to be elevated to a subspecies to allow for DPS status to be assigned under the endangered species act (ESA).

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

The most likely scenario for the historical route of colonization comes from Shiozawa's work, because trees are nearly fully resolved with regards to the relative timing of ancestry of cutthroat trout lineages in Colorado. This work suggests that colonization occurred from the northwest, with the common ancestor of blue and green/purple/orange/yellow/red lineage fish diversifying on the West Slope. An ancient colonization event occurred via an ancestor of green/purple/orange/yellow/red lineage to the East slope. The earliest divergence among extant lineages (yellow/red excluded) in this group was Rio Grande cutthroat trout, and then purple and green. What is not clear is if East Slope green lineage fish are native to the East Slope or recently introduced. This is a critical issue to be addressed by future research. Blue lineage fish on the East Slope almost certainly occur there via stocking activities.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

I have addressed this question in my responses to questions 6, 8, 9 and 10.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

The Bear Creek (purple) lineage is critically endangered, given that it resides only in a single creek with probably fewer than 500 fish. Andrew Martin presented research from an unpublished MS thesis that suggested limited genetic variability in this lineage compared to other cutthroat trout lineages in Colorado. There is evidence of developmental abnormalities in the hatchery, which may arise in high frequencies in a controlled environment because of relaxed natural selection pressures in captivity. It is also possible that abnormalities occur for environmental reasons such as water quality issues, lack of essential minerals or nutrients, and others. The underlying causes of abnormalities warrant further study.

Recovery of the Bear Creek lineage requires it to be replicated into South Platte streams (with renovation and barriers as needed) and existing fish in Bear Creek itself and in the hatchery program are the only possible sources. This leads to the possibility of inbreeding or an unintentional founder event that could influence local genetic diversity. Under ideal conditions, it is most prudent to stock fish directly from a donor stream into a recipient stream to avoid hatchery-induced "domestication" selection. However, given low numbers in Bear Creek and the possibility for demographic fluctuations due to sedimentation and other environmental changes, it will be critical to use hatchery fish as founders to new populations. Thus, careful evaluation of hatchery stocks for genetic diversity and evidence of viability loss due to inbreeding is essential. It may be prudent to develop a brood stock management plan that stipulates mating designs, refreshment and retirement rates of brood stock, and steps to reduce domestication selection (e.g., supplying wild feed, and naturalized conditions in captivity). A monitoring program for newly established populations should be implemented immediately following restocking.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

The Metcalf et al. 2012 study showed that Bear Creek (purple lineage) fish were found only in the South Platte River, and that yellow lineage fish (presumably native to the Arkansas River) are probably extinct. In my estimation, that leaves East Slope green lineage fish (Como, Severy, Taylor, and perhaps Hayden Creek) as candidates for donor populations for reintroduction into the Arkansas River drainage. It is essential to identify suitable streams and assess what trout populations exist there. Judicious stream renovation, barriers, and stocking from donor populations may be the best approach to repopulation Arkansas River streams. It appears that there is less urgency to restocking the Arkansas River drainage at this time, so it will be best to await results of definitive studies to identify whether East Slope green lineage fish are native, and whether suitable (low to no introgression) populations exist as donors. Otherwise, a carefully designed and genetic marker assisted breeding program could be established to 'restore' genetic purity through backcrossing. This would necessitate a large suite of nuclear DNA markers that could be assessed non-destructively and careful tagging and recording of mating schemes in the hatchery. A general guideline should be to use wild populations as donors when possible. Once new populations are established, a monitoring program should be implemented to assess stocking success and demography of stocked fish.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

*S*uccessful marker-assisted breeding programs necessitate further development of co-dominant nuclear DNA markers (i.e., preferably a large number of single nucleotide polymorphic markers [SNPs]) and perhaps a large suite of microsatellite DNA loci to fully characterize genetic diversity, relatedness, and levels of introgression. High-throughput characterization of nuclear DNA markers will be absolutely essential as all fishes in the Bear Creek lineage should be genotyped to assist with setting goals for genetic diversity, assessing new populations for founder effects, and designing brood stock management plans. Further study of East Slope green lineage fish is also warranted, with similar goals and objectives as indicated for Bear Creek.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

Management, Highest Priority:

a. Establish new populations of Bear Creek lineage fish in the South Platte River drainage as soon as possible.
b. Protect Bear Creek from human encroachment.
c. Continue and sustain efforts to establish a viable hatchery program for Bear Creek lineage.
d. Carefully evaluate pure and experimental crosses of Bear Creek fish for inbreeding and possible outbreeding depression.
e. Establish a large set of co-dominant nuclear DNA markers that are set up for high-throughput analysis. I suggest SNPs should be developed from the cutthroat trout transcriptome (using East Slope and West Slope green, blue, and purple lineage fish as sequencing templates with barcoding) and mapped to the rainbow trout genome to identify genes that are potential targets of natural and hatchery-induced selection, as well as neutral variation. AFLPs are dominant markers and are not as well suited to exploring backcrosses and variation linked to known genes.
f. Once item e. is complete, evaluate the genetic and meristic status of East Slope green lineage fish.
g. Based on the outcome of item f., identify appropriateness of stocking Arkansas River drainages.
h. Initiate taxonomic revision of cutthroat trout in Colorado. This is desperately needed to recognize biodiversity that was revealed by molecular and meristic study, and to pave the way for protection of East Slope green lineage fish if warranted by further molecular study, and for increased protection of Bear Creek fish.
i. Survey San Juan GMUs for presence of red lineage, conduct simultaneous demographic surveys.
j. Depending on the outcome of item i., scope a program to produce ‘red lineage’ fish through backcrossing.

Continuing Activities:

k. Assess threats to green lineage on the West Slope. Explore (or maintain) federal protection as necessary and warranted. Continued management at the GMU level is appropriate.
l. Assess population status of blue lineage fish in West Slope drainages, continue demographic and genetic assessment, prevent habitat degradation and opportunities for hybridization with non-native species. Management at the GMU level is appropriate.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*
- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

I answered this question above (see response to Question 12).

17. Please provide other relevant comments not addressed in the above questions.

There seems to me to be several alternatives with regards to listing and federal protection. There is a possibility that the discovery of new biodiversity within cutthroat trout in Colorado could lead to reduced protection of some new biodiversity. The options as they appear to me are to:

1) Do nothing. In this case, Bear Creek and East and West Slope green lineages are protected. The disadvantage will be challenges to protection of West Slope green lineage fish based on Metcalf et al. 2012.
2) As an alternative to the 'do nothing' scenario, it may be possible to designate East slope green lineage as a DPS under the current scenario and manage it separately (and more intensively) from West Slope. However, the Service should monitor impacts to West Slope green lineage populations closely to assess impacts to West Slope green lineage fish.
3) The Service could await much need taxonomic revision, list the purple lineage as the only remaining greenback cutthroat and uplist it to Endangered under ESA. Assuming that the green lineage becomes a listed subspecies, the East Slope could be a DPS under ESA.

There will almost certainly be challenges to scenarios 1 and 2, but they could be employed as a stopgap until taxonomic revision is complete.

Unresolved question:

1) How did Rio Grande cutthroat trout maintain distinctiveness as a lineage despite historical stocking? The record indicates that New Mexico favored native fish over stocked fish, and that stocking was much less than occurred in Colorado, but some stocking did occur. Given the importance and emphasis on massive stocking in Colorado, why weren't more blue and green lineage fishes stocked into the Upper Rio Grande basin in Colorado? Was there evidence of massive stocking there, and if so, why is there no evidence of introgression in Rio Grande cutthroat trout? This question speaks to expected outcomes of stocking in the South Platte River – it may be that stocking could be relatively ineffective as an agent of gene flow in some circumstances. This could be because stocked fish fail to thrive in a novel environment, or hybrids are selected against and backcrosses are favored. It may be most appropriate to address this question if Rio Grande cutthroat trout is listed in the near future.

Panelist #4 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

A **major limitation is the small number (N=30) of historic specimens** used in Metcalf et al. (2012: Table 1). Obtaining and analyzing DNA for museum specimens is no easy accomplishment and the data the study generated contribute to our understanding of mtDNA diversity in historic populations of *O. clarkii*. However, the small sample size that represents historic diversity warrants special caution in interpretation of the data.

Uncertainty surrounds collection localities, and thus, basin/drainage of origin of the historic specimens used in Metcalf et al. (2012). Historic records appear ambiguous and some information is based on inferences made from anecdotal information (as summarized in Kennedy 2010). Contributing to this uncertainty are historic acquisition of museum specimens (i.e., actual routes of surveys or expeditions), curatorial practices (i.e., re-packaging/ shipping of fishes post-collection) and likely gaps in stocking records (i.e., undocumented moving of fishes prior to the collection of historic specimens).

Generally, methodologies and analytical protocols employed to generate the molecular data in Metcalf et al. (2012) are **technically sound, with caveats** of low sample size (as above) and short sequences (partial ND2 gene), limiting statistical power of analysis and inferences that can be made (e.g., AMOVA Fig. 6).

One shortcoming of the Metcalf et al. (2007) paper was that stocking history was considered the only likely scenario explaining the pattern, while an evolutionary scenario explaining these patterns as retention of ancestral polymorphism was disregarded. This led to the conclusions that "the wrong fish were stocked in the wrong places." Rogers (2010) showed that green and blue lineage populations were genetically distinct and native to West Slope drainages, underscoring that evolutionary patterns are often complex and not easily explained with simplistic scenarios. The subsequent study by Metcalf et al. (2012) considered the green lineage native to the West Slope, but **still favored stocking as likely scenario for occurrence of green lineage populations on the East Slope.**

I **disagree with stocking as the best explanation** for occurrence of green-lineage fishes on both sides of the Continental Divide. An **alternative scenario that assumes the green lineage to be native to both East and West Slope drainages is likely**, because (a) green-lineage haplotypes unique to East Slope drainages are not replicated among natural populations on the West Slope (data unpublished); and (b) morphological distinctness of East versus West Slope green lineage populations (Bestgen and Rogers 2013).

Two additional reservations: (1) **Data (sample sizes) are insufficient to make the assertion** that the Bear Creek population was **native to South Platte River only**, but not the Arkansas River basin (Metcalf et al. 2012). (2) **Alternative biogeographic scenarios**, most notably hypotheses by Behnke (1992, 2002) are **inaccurately presented**; Behnke (1992) noted close similarity between Colorado River, greenback and Rio Grande Trout and invoked derivation of Rio Grande from greenback cutthroat trout as one among several alternative biogeographical scenarios.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

The **morphological (Bestgen and Rogers 2013) and genetic (Metcalf et al. 2007, 2012) data agree that there are four distinct extant lineages** (blue, green, Bear Creek and Rio Grande). Other studies with a broader focus also identified the same lineages [e.g., Loxterman and Keely 2012, Housten et al. 2012 and Wilson and Turner (although the latter study did not include a sample representing the green lineage)].

Morphological analyses by Bestgen and Rogers (2013) further provided a **more nuanced perspective**, demonstrating differences among populations in subdrainages (referred to as **GMUs: Geographic Management Units**). Methodologies of this study are sound and include: a broad geographic coverage, random population selection, blind protocols and replicate counts for difficult traits.

Results show frequency differences across meristic and qualitative (spotting pattern) traits, which translated in a multivariate framework (ordination and cluster analyses) into distinct groups with some overlap in multidimensional morphospace. However, complete separation based on morphological diversity, particular in meristic characters, is unlikely for closely related lineages as the ones examined; traits were selected in part on what was historically used in cutthroat trout taxonomic studies (e.g., Behnke 1992), so as to facilitate comparisons with published data. Classification rates in Discriminant Function Analysis (DFA), both of individuals and populations to GMU of origin, were surprisingly high for intra-specific variation, underscoring morphological distinctness of the blue and green lineages.

3. To what extent are historical spatial distributions of green, blue lineages known?

The complexity of phylogeographic (evolutionary) patterns in combination with an extensive stocking history makes it **impossible to infer with certainty historical distributions**. However, available morphological and genetic data are likely a **good approximation of phylogeographic patterns** as long as **alternative hypotheses** are fully considered. The "truth" is likely more complex than what we anticipate (or would like). Natural populations exist as dynamic entities over space and time, distributed across a heterogeneous landscape and subjected to a multitude of processes (e.g., climate fluctuations, stochastic events) that influence their genetic and morphological diversity in unexpected ways (Douglas et al. 2003).

Genetic analyses of historic museum samples (Metcalf et al. 2012) documented the blue lineage in West Slope drainages and the green lineage in West and East Slope drainages. Failure to detect lineages in specific subdrainages cannot be taken as "proof" that they indeed did not occur there historically; sample size was simply insufficient to encapsulate historic diversity.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

Comparing levels of divergence among studies is difficult, due to differences in methodologies, sample sizes, analytical protocols, scope and focus of studies. Even a simple measure, such as percent sequence divergence, is dependent on the evolutionary rate of the marker examined (e.g., ND2 in vs ND4). Rather than comparing results of studies that focus on single groups, a better approach is to examine **levels of divergence in broad-scale studies** (i.e., those including more taxa and/or a larger geographic area).

For example, results in Wilson and Turner (2009) indicate genetic differences derived from mtDNA sequence analysis among *O. clarkii* subspecies are similar to differences among *O. gilae* subspecies. A caveat of broad-scale studies is that each taxon is only represented by a few individuals. Within-group

diversity is thus difficult to evaluate, and some groups may not be represented (e.g., green lineage is missing in Wilson and Turner 2009).

Studies of *O. clarkii* involving nuclear genetic data (e.g., microsatellite loci, AFLP) have mostly aimed at evaluating levels of introgression by introduced subspecies, and analyses focus on clustering, rather than genetic divergence (Metcalf et al. 2007, Pritchard et al. 2008, Rogers 2010, 2012, 2013). Furthermore, due to the uncertainty of homology of AFLP fragments (a particular band is present or absent, different character states unknown), distance estimates are not meaningful.

As stated above, morphological characters commonly used in cutthroat trout studies were selected by Bestgen and Rogers (2013) to facilitate comparison among studies and details on congruence between their study and earlier works are summarized therein.

Without a detailed assessment (which goes beyond this review), I would offer as a general observation: level of **divergence between the four lineages** (green, blue, Bear Creek and Rio Grande) approximate differences between **ESUs or subspecies**, respectively; divergence **between East and West Slope populations within the green lineage** can be equated with **Management Units** (MUs).

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Pertinent literature appears to be included. See comments under (1) re: conclusions in Metcalf et al. (2012).

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?
- *a. different subspecies?*
- *b. distinct population segments (DPS)?*
- *c. Other?*

Based on my review of the available data, levels of divergence between the **four lineages** (green, blue, Bear Creek and Rio Grande) approximate differences **between ESUs**; divergence **between East and West Slope populations within the green lineage** can be equated to **Management Units (MUs)**. It is important to note that divergence among the four lineages is quite similar (also discussed under 10).

The existing taxonomy recognizes three distinct subspecies, creating a dilemma how to partition four lineages among three taxa. Based on geography, the blue lineage could be equated with *O. c. pleuriticus*, and the Rio Grande lineage with *O. c. virginalis*, leaving *O. c. stomias* as available category for either the green or Bear Creek lineage with the remaining one having to be described as a new subspecies (see comments under questions 7 and 8).

Taxonomic re-arrangement would influence conservation of subspecies/lineages similar to the *Anaxyrus boreas* species group (Goebel et al. 2009). Interestingly, the Boreal Toad has a similar distribution to *O. clarkii* subspecies and it also exhibits "many highly divergent and isolated lineages at the southern edge of its distribution" (Goebel et al. 2009: 223), a reflection of shared biogeographic histories. As recommended by Goebel et al. (2009) resource agencies should be encouraged to consider phylogeographic patterns in their management of aquatic and terrestrial species in the region (see also comments under question 10).

7. Is the Bear Creek population considered to be greenback cutthroat trout?

As Behnke points out (2002:195) there is much confusion regarding what represents a greenback cutthroat trout, mainly due to inadequate and erroneous early descriptions (as summarized in Behnke 1992). Behnke (2002) recognized the distinct phenotype of Bear Lake (Bear Creek) lineage; it is prominently depicted (page 195) in addition to a stream resident form (page 197).

As outlined under (1) the available data are inconclusive regarding Bear Creek population as the "true" representative of the taxon *O. c. stomias*. However, since the Bear Creek population represents a distinct mtDNA lineage, exhibits morphological distinctness, and exists as a small isolated population on one hand, whereas the taxon *O. c. stomias* is a listed entity on the other hand, it **may be a prudent solution for management to equate the Bear Creek lineage with the taxon *O. c. stomias***. The Bear Creek population is **genetically and morphologically unique and represents a distinct evolutionary lineage (ESU)**. Due to its **rarity**, extensive management focus will be needed to prevent it from becoming extinct. Listing under the ESA would provide this level of attention and protection.

8. How do we describe the East Slope green lineage?

Molecular genetic markers have aided tremendously in detecting cryptic biodiversity, or alternatively, reducing inflated taxonomic diversity (Douglas et al. 2006). Since species are still described morphologically, once new molecular clades (evolutionary lineages) are discovered, a sound morphological evaluation is necessary to guarantee taxonomic recognition of evolutionary diversity (as per Douglas et al. 2007).

If the Bear Creek lineage is equated with the taxon *O. c. stomias*, a new subspecies would have to be described (morphologically) to accommodate the green lineage (including both West and East Sope populations), with the East Slope populations being regarded as a **distinct population segment (DPS) or management unit (MU)** within this new *O. clarkii* subspecies. Similarly, if the green lineage is equated with *O. c. stomias*, the East Slope lineage would also have be designated as an MU or DPS within *O. c. stomias* to accommodate its genetic and morphological distinctness from West Slope populations of the green lineage.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

The four lineages (green, blue, Bear Creek and Rio Grande) are separated by several nucleotide differences from each other (Metcalf et al. 2012:Fig. 4); there is some additional minor variation within lineages (Metcalf et al. 2012:Fig. 2) but a detailed overview of haplotype distribution by drainages/populations is lacking. If my recollection of presented/discussed data is correct, these minor variations correspond to geographic patterns and consequently represent additional variation among drainages. This is consistent with the morphological data (Bestgen and Rogers 2013) that showed a subtle, but clear signal of geographic variation among different qualitative and meristic characters, leading the authors to **distinction of GMUs (Geographic Management Units)**.

The **entire green lineage should be recognized as a distinct ESU or subspecies**, **with several GMUs/ recognized as geographically isolated entities (MUs) within** this ESU/subspecies; the East Slope green lineage populations would represent one such MU. Genetic and morphological differences between East

and West Slope populations of the green lineage are not sufficient to warrant recognition of each as a distinct ESU.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

Evolution of cutthroat trout in the West likely occurred as a series of reticulate events, rather that a single-non-reversible event. While headwater transfers/stream captures are rare, **climate oscillations (and precipitation) during Pliocene/Pleistocene were driving forces** in shaping diversity in the arid Southwest (see Douglas et al. 2006, Hopken et al. 2013).

Pleistocene climate was characterized by increasing variability (oscillations) providing potential **biogeographic hypotheses for diversification in cutthroat trout in spite of apparent geographic barriers** (as summarized in Minckley et al. 1986). Pleistocene climate fluctuations have been shown to correspond with intra-specific diversification (Douglas et al. 2009).

The range-wide study of *O. clarkii* by Loxterman and Keely (2012) showed that **genetic diversity largely correspond with basins**; smaller drainage connections added complexity to the overall generalized patterns. Similarily, Shiozawa et al. (2010) provided a detailed analysis of mtDNA divergence across *O. clarkii* and associated major splits with vicariant events; drainage divides were likely major barriers to gene flow. In addition, biogeographic hypotheses have generally broad support across diverse taxa (e.g., Boreal Toad as mentioned under question 6).

Metcalf et al. (2007) rejected an interbasin transfer of fishes in the Rockies of Colorado "because it has not been recorded," although it is unclear what this statement referred to (I agree – nobody was there to observe it and lived to tell about it). Several studies (Loxterman and Keely 2012, Turner and Wilson 2009, Shiozawa et al. 2010, Metcalf et al. 2012) have shown that relationships among the four lineages are unresolved. This reflects recent divergence of these lineages, and stands in contrast to the assertion by Love Stowell (2011, unpublished) that the *O. c. pleuriticus/O.c. stomias* split is much older than proposed. However, mid-Pleistocene fossils document presence of potential ancestors of *O. clarkii* in the Rio Grande basin, suggesting a deeper split between cutthroat trout in East and West Slope drainages. Behnke (1992) regarded this scenario as less plausible, because given the high morphological similarity between West and East Slope populations, he assumed a more recent split between Colorado River and greenback cutthroat trout. The morphological analyses by Bestgen and Rogers (2013) showed co-occurrence of two morphologically distinct forms in these basins, once genetic data were used to distinguish between populations representing each lineage.

Contradicting evidence could be accommodated by a **scenario that invokes an initial dispersal of *O. clarkii* into drainages on both sides of the Continental Divide, followed by divergence of lineages in subdrainages**. **Subsequent dispersal would have allowed spread of lineages into other drainages. When and where basin-transfers occur is way beyond the scope of this review**.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

The **morphological (and to some extent genetic) patterns correspond with subdrainages** and are consistent with variation among populations (MUs) within lineages (ESU).

Genetic data have been primarily generated and analyzed to distinguish lineages or detect hybridization among distinct subspecies (Metcalf et al. 2007, 2012, Rogers 2010, 2012, 2013). Detailed information on variation within each lineage was not presented.

The morphological study by Bestgen and Rogers (2013) specifically examined variation within and among populations in both lineages. Results demonstrate variation by subdrainage within each lineage, leading the authors to **identify GMUs**.

Management Implications

*12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects***?**

Bear Creek only exhibits **one mtDNA haplotype** (Metcalf et al. 2012). Details on nuclear genetic data are not published. Morphological analyses (Bestgen and Rogers 2013) show a **contracted morpho-space**, although this lineage exists as a single population, and thus among-population variation is not available.

In small populations, genetic diversity is lost due to genetic drift. Maintaining a large population in the wild (possible replicated in suitable habitats as outlined below) should be a high priority.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

The **Bear Creek lineage, albeit currently in the Arkansas River drainage, is a poor candidate** due to its small population size, low genetic variability and apparent problems in propagation environments. It is also questionable if indeed it is or ever was indigenous to the Arkansas River basin (but see comments above under questions 1, 6 and 7).

If the goal is to re-establish native cutthroat trout across Arkansas River drainages, green lineage fishes would be the best candidates. It is a likely scenario that the green lineage is native to both East and West Slope based on: (a) morphological data by Bestgen and Rogers (2013), and (b) unpublished information provided during the workshop [specifically, distribution of distinct green-lineage haplotypes unique to East Slope drainages, and patterns in nuclear DNA (albeit the latter data was sparse and presentation lacked context)].

Green lineage population as many replicated populations (Rogers 2010, 2012, 2013); preferably, **green lineage fish from the East Slope localities should be selected for establishment of additional populations in the Arkansas River basin**, since they form a distinct MU (see comments under questions 9, 10, 11)

14. How should next-generation DNA sequencing (NGS) approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

NGS approaches can be powerful tools to assay a larger proportion of the genome, and consequently of the genetic variation within an individual, population or species. However, costs are prohibitive to generate genome-scale data for studies at the population level with conservation implications (i.e., assay variation within and among populations in various drainages). Furthermore, NGS approaches can be employed to develop diagnostic markers, such as SNPs (Single Nucleotide Polymorphisms as per Houston et al. 2012) that distinguish different species, subspecies, lineages or even populations and will make screening methodologies (e.g., introgression, purity assessment) more reliable and cost-effective.

Caution is warranted. NGS appears to be the panacea for all the limitations of previous genetic methods (as the next technology "on the PCR block" always has) but we are only starting to understand the bioinformatics tools that will be needed to mine the massive amounts of data generated by these approaches, the best practices to generate, manage and archive such enormous data sets, and how to assure data are accurate and reflecting biological diversity and not methodological artifacts (e.g., RG90712, RG90714 and RG90732 in Houston et al. 2012).

Despite suggestions during the workshop, I see **limited use of NGS approaches for historic samples**. The limitations and uncertainties listed under (question 1) will not magically disappear, simply because fancier technologies are used. Poor quality of DNA in historic samples, combined with low DNA concentration (yield) will amplify genotyping errors common in some NGS technologies (i.e., they are very efficient, but also very "sloppy" methods compared to more traditional approaches with much lower accuracy). Before agencies step forward to provide funding for NGS studies, they should make sure that the results are not just of academic interest, but will be applicable in managing natural resources.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

A **comprehensive morphological study of historic collections** would be informative and insightful. Questions that could be addressed are:

(1) How widespread (or restricted) were the morphological patterns characteristic for the extant Bear Creek population?

(2) Which extant green and blue lineage populations are morphologically most similar to historic specimens found in Colorado, Arkansas and South Platte River drainages?

The morphological study by Bestgen and Rogers (2013) detected fine-grained variation that corresponds to sub-drainages and reflects a natural pattern either induced by slight differences in habitat among the different basins/drainages (i.e., ecophenotypic variation) or due to evolutionary differences (ecotypic differences).

Because they first used molecular diagnosis for separating green versus blue lineage individuals, they then were successful in using ordination and clustering algorithms to detect subtle morphological variation that exhibits large overlap in character trait distribution and thus was puzzling to Behnke (1972) who unfortunately did not have advanced molecular tools available at the time of his seminal studies. The **morphological study by Bestgen and Rogers (2013) forms a very solid foundation against which future population changes can be gauged**; thus, future monitoring should continue collection of morphological data.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*
- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

Evolutionary and conservation concepts suggest potential issues due to low genetic diversity and high-relatedness (genetic similarity) in breeding parents. Perhaps fishes in the wild can recognize related individuals and avoid becoming "kissing cousins;" more technically expressed: mate choice might reduce inbreeding.

The observed problems do not bode well for "Bringing back *O. c. stomias*" by extensive propagation/widespread stocking of progeny of the Bear Creek population as eluded to in Metcalf et al. (2012). The genetic and morphological distinctness exhibited by Bear Creek individuals should be conserved and replicating the existing population in a few natural, isolated habitats would serve as an assurance against extinction of this lineage should the population crash or be lost due to stochastic events. A monitoring plan should accompany these replication efforts, with standard population parameters being recorded to assure life history characteristics remain similar to those of the original population and also as a gauge for population well-being. In addition, morphological characteristics should be monitored, since they will be good indicators if the population experiences evolutionary changes (due to drift/small population size or environmental forces). Genetic monitoring on a 3-5-year interval would be informative, too, to detect any introgression/admixture by accidentally introduced cutthroat trout individuals.

17. Please provide other relevant comments not addressed in the above questions.

Our overarching conservation goal should be to identify entities that encapsulate biodiversity across the landscape. To manage biodiversity, groups with independent evolutionary histories must first be identified (Mayden and Wood 1995), and these may be categorized traditionally in taxonomic categories such as ''species'' and ''subspecies'', or, alternatively, as ''evolutionary significant units'' and "management units" (summarized in Douglas et al. 2005, Hopken et al. 2013). The latter categories provide efficient means of characterizing intra-specific diversity and have been particularly applicable to conservation of salmoniform fishes (e.g., Waples 1995).

Many freshwater fishes of the northern hemisphere represent recently evolved (often sympatric) lineages of uncertain taxonomic status (Douglas et al. 1999), with unrecognized taxonomic diversity perhaps being greatest in salmonids (Behnke 1972).

Systematists make taxonomic decisions on the basis of quantitative and qualitative traits (Douglas et al. 1989). The former are measurable and can thus be subjected to statistical analysis with repeatable outcomes. Other important traits that distinguish biological entities, such as pattern and extent of spotting patterns and shape or configuration of body parts, are intentionally or intuitively scored qualitatively. Visual recognition of subtle similarities and differences in appearance is a major component of "seeing well, or of noticing and distinguishing with accuracy the objects which we perceive" (Rafinesque 1820). No doubt, subtle differences exist and have been used to identify different evolutionary entities in cutthroat trout (Behnke 2002).

The '**art of seeing well**,' was defined by Rafinesque (1820) as ''the art . . . **of noticing and distinguishing with accuracy the objects which we perceive** . . . [It] is a **high faculty of the mind, unfolded in a few individuals, and despised by those who can neither acquire it nor appreciate its results**.'' (Doulas et al. 1998)

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Panelist #5 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

The identification of six different lineages, two of which have no contemporary representatives (identified so far, at least) seems to be robust. Great care was taken in preparing the historical DNA samples, so identification of museum specimens should be reliable. In general, the inferences about stocking seem more plausible than alternative hypotheses. Conclusions regarding nomenclature of *O.c.stomias* are speculative, although not necessarily implausible.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

The meristic study generally supports the conclusions of Metcalf et al. (2012) regarding the major lineages of cutthroat trout in Colorado. Statistically significant differences in several morphometric/ meristic traits were found between groups of samples, and these differences were stronger when the groups were based on mtDNA lineage than when they were based on geography.

3. To what extent are historical spatial distributions of green, blue lineages known?

They are not known with certainty. The historical study of Metcalf et al. (2012) focused on East Slope collections. Figure 2 in Rogers (2013) shows that the green and blue lineages currently have broad overlap in many West Slope drainages. One hypothesis is that much of the current overlap in blue-green distribution is the result of stocking. This hypothesis seems plausible given the available information but this issue has not been resolved conclusively.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

This is challenging to assess because previous studies have been based on the geographic subspecies hypothesis, and genetic lineages within Colorado often do not follow geography. However, in general the levels of genetic variation found within populations Colorado cutthroat trout seem comparable to those found in other areas. Some previous studies have shown that the geographically defined subspecies in Colorado are distinctive as a group but not as strongly differentiated among themselves as some of the other named subspecies.

I am not an expert in cutthroat trout morphometrics/meristics, and we were not shown enough comparative data for other subspecies to comment on this comparison. Given the recent demonstration that genetic lineages of *O. clarkii* within Colorado do not always follow geography, it would be important to revisit old meristic/morphometric studies that might have evaluated collections of mixed lineages and hence obscured underlying differences.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Both studies appropriately cited the published literature.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?
a. different subspecies?
b. distinct population segments (DPS)?
c. Other?

Four extant lineages were identified in these studies and they are considered separately

Rio Grande: Populations in this lineage are genetically and morphologically distinct and occur within the nominal historic range for *O.c.virginalis.* Therefore, it seems reasonable to retain that subspecies designation for these populations. Ideally, this would be tested by genotyping the type specimen(s).

Blue lineage: This appears to represent the contemporary distribution of *O.c.pleuriticus*. The most plausible explanation for appearance of some blue lineage haplotypes east of the continental divide is stock transfers from the west. Co-occurrence of many green lineage populations west of the divide indicates that the historical distribution of the blue lineage is not as broad as originally thought. However, this lineage appears to be the best representation of *O.c.pleuriticus* so it could continue to be treated as a subspecies with revised distribution, at least until a formal taxonomic revision is conducted. However, it would be important to confirm whether the type specimen(s) of *O.c.pleuriticus* actually does carry a blue lineage haplotype.

Green lineage: This is a second lineage that appears to be broadly distributed within what was historically considered to be the range of *O.c.pleuriticus*. Complications related to green-lineage haplotypes east of the divide are discussed below under Question 8. Several possibilities might be considered for placing this lineage within the ESA concept of 'species.' 1) If the blue lineage remains a subspecies and retains the name *pleuriticus*, the green lineage might be considered another (new) subspecies, with the two subspecies having largely non-overlapping distributions (after accounting for stock transfers) within the historical range of Colorado River cutthroat trout. Whether this is reasonable or not depends on one's concept of subspecies; because this issue was not discussed at any length in the workshop, it is difficult to draw any conclusions in that regard. 2) The blue and green lineages might both be considered to be DPSs within the subspecies *O.c.pleuriticus*. Genetic and morphological (and perhaps ecological) differences could be used to support such a designation under the joint DPS policy. However, at least some genetic analyses suggest that the green and blue lineages are not sister taxa. That argues against this approach, unless perhaps the other taxa were also included as DPSs of a larger, more inclusive species or subspecies. 3) The green lineage might be considered a DPS of another taxonomic unit (species or subspecies). It is not clear what this larger unit might be unless it is the entire species *O. clarkii*. Under this scenario, the species cutthroat trout would include a number of subspecies and one or more DPSs that are not nested within the subspecies. This might be reasonable, reflecting the reality that a species could be composed of various subspecific units that have achieved different degrees of isolation and divergence (some distinctive enough to be considered subspecies, others only DPSs). However, I can't think of a species for which this type of arrangement has been formally proposed.

Bear Creek: It seems clear that this population is distinctive enough to be a listable entity under the ESA. Others have made the argument that this population should get the name *O.c.stomias* and be considered to be true descendants of greenback cutthroat trout. I don't necessarily disagree but think that is an issue that taxonomists need to resolve. In the meantime, two options seem feasible: 1) declare that BC will be considered to be *stomias* and greenback for purposes of the ESA, at least until the nomenclature is formally resolved. This would provide for the most continuity in ESA listing status. 2) declare that, at a minimum, BC qualifies as a DPS of the species *O. clarkii* and can be listed as such. This would presumably require some legal maneuvering to accomplish.

7. Is the Bear Creek population considered to be greenback cutthroat trout?

See answer to previous question.

8. How do we describe the East Slope green lineage?

I am not sure that currently available information allows us to reliably distinguish between two competing hypotheses:

1) The green lineage is native to the East Slope.
2) Green lineage haplotypes in some East Slope populations are the result of stocking from West Slope sources.

My hunch is that the second hypothesis is more likely to be true, but I could be wrong. This is a situation where policy makers have to determine how precautionary one wants to be. Conserving these East Slope green lineage populations would help preserve future options, but would come at some cost to society. Under the assumption that #1 is true, FWS could consider these East Slope populations to be a DPS of the larger green lineage (if it becomes a new subspecies). If instead the green lineage is considered a DPS of *O. clarkii*, it is not clear how the East Slope population could have a separate listing status.

With more nuclear data it might be possible to distinguish between these hypotheses. Programs like Jody Hey's computer program IMa can potentially distinguish between hypotheses that involve long-term isolation and those that involve more recent gene flow. Collecting enough nuclear data to adequately test this should be a priority.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

Although (as noted above) the concept of subspecies is fuzzy and was not discussed much at the workshop, I see little basis from existing data to consider the East Slope green lineage populations a separate subspecies, under any subspecies definitions one can find in the literature. Per Question 8, under some scenarios these populations might meet the criteria to be considered a DPS, if it were concluded that their presence east of the divide was not the result of stocking.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

The two competing hypotheses are laid out in the MS thesis by Love Stowell (2011): Behnke's hypothesis of multiple, relatively recent stream-capture events across the continental divide, or Metcalf's hypothesis of an older and one-time crossing of the divide, followed by diversification among subbasins. I don't believe that currently available analyses allow one to distinguish between these hypotheses.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

See responses to Questions 6-9.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Based on Table 12 in the draft Bestgen report, Bear Creek fish have higher than average levels of morphometric/meristic variability at some traits and lower than average at others—no really consistent trend. Some of the variation could be influenced by environmental differences between hatchery and wild fish from Bear Creek.

For Bear Creek, the most important issue is not the level of (presumably neutral) genetic variation at traditional markers, but whether the population shows evidence of inbreeding depression. Reports of high frequencies of major abnormalities in hatchery Bear Creek fish are alarming in this regard, but they could be due partially or entirely to environmental conditions associated with culture. The common-garden experiment currently being conducted should shed light on this issue. This experiment should also provide an opportunity to evaluate the basis for the small size of Bear Creek fish. If it is genetically based it provides additional support for distinctiveness of this lineage; if it turns out to be largely environmental, the meristic/morphometric results should be re-evaluated with this in mind, because several of the characters used were positively correlated with size. It would also be useful to expand the meristics work to assess fluctuating asymmetry in Bear Creek and see how it compares with levels in putatively healthy populations.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

By all accounts, yellowfin are extinct. The two East Slope populations with green-lineage haplotypes are of uncertain origin; I would not recommend spreading them unless it can be conclusively demonstrated that they are indigenous to the basin. Therefore, native cutthroat from the Arkansas River basin could well be extinct. Trout introduced from other areas could not be considered 'native cutthroat of the Arkansas River Basin.' However, such introductions might serve general conservation goals by expanding the range of current lineages of Bear Creek and/or Rio Grande populations.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

Next generation sequencing (NGS) is a broad range of approaches that already are providing vast amounts of new genetic data for non-model species. This is not a silver bullet that will solve all of the challenging questions about cutthroat trout in Colorado. Nevertheless, these approaches might be useful in several regards:

- Screening large numbers of markers might identify outliers that are linked to genes associated with adaptations.
- More nuclear data might help resolve competing hypotheses regarding East Slope populations with green lineage haplotypes (see Q 8).
- NGS data could provide more detailed information about the distribution of genetic diversity across the Bear Creek genome.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

See responses to other questions

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than, some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*
- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

See response to Question 12. If the studies underway provide evidence for significant inbreeding depression, then some sort of genetic rescue should be attempted. As was the case for the Florida panther, this could involve controlled introductions of a small number of individuals over time with careful monitoring of founder contributions. This could be done in several new, experimental populations without affecting the base population. The most plausible sources for immigrants might be contemporary populations in the Rio Grande drainage or green lineage populations.

17. Please provide other relevant comments not addressed in the above questions.

A few minor suggestions to consider in finalizing the meristics report:

- Table 5-8: include sample size under the name of the collection
- Table 9 and elsewhere: show results for at least one more principal component, since the first 2 only capture a bit over 50% of the total variation.
- It would be good to see a more rigorous evaluation of the effects of size, since Bear Creek fish are very small and several traits are positively correlated with size.
- Table 10: exactly which groups of samples are compared here?
- Discriminant function analyses: clarify that jackknife/leave-one-out procedures were used in assessing classification accuracy.

Panelist #6 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

In my opinion, the Metcalf et al. (2012) study was very well performed. The data themselves were difficult to collect, given the degraded nature of the DNA and small quantities they were able to obtain. The measures they took, in collaboration with the Australian Center for Ancient DNA (ACAD), satisfy doubts about contamination and the validity of using DNA for historical inferences.

Metcalf et al. (2012) provides evidence for six, and possibly seven, distinct lineages of cutthroat trout in Colorado waters historically. I will start with discussing inferences that are more supported from the evidence and then progress towards inferences that have less support.

First, the historical presence of a distinct cutthroat trout lineage (I will use the term lineages as it has been used in Metcalf et al. (2012) and as we decided to focus on this term during our panel meeting) in the South Platte River (purple lineage) is supported by the data. The presence of two haplotypes in the nine individuals sequenced and the divergence of these haplotypes from other cutthroat trout historical and modern haplotypes makes a convincing case. The modern samples make a convincing case that this historical South Platte River lineage now is known only to occur in Bear Cr (Arkansas drainage; I will use the term drainages as it has been used in Metcalf et al. (2012) and other papers related to this issue, though it was also used interchangeable with 'basin'). The Bear Cr. lineage is genetically divergent from other southern Rocky Mountain cutthroat trout lineages (Love Stowell 2011; Metcalf et al. 2012).

Second, the presence of a distinct lineage in the Rio Grande drainage is clear and does not seem to need further discussion for this report.

Third, the existence of distinct blue lineage is well supported by the evidence presented in Metcalf et al. (2012). It needs to be recognized that only one individual was sequenced from the White/Yampa by Metcalf et al. (2012). When combined with modern data from the White/Yampa presented in Metcalf et al. (2012) but also in conjunction with the much broader survey summarized by Rogers (2013; Recent developments in trout taxonomy) a convincing case is made for the distinction of this lineage from others. Further, the combination of these studies makes a convincing case that the blue lineage is native to the White/Yampa drainage. Metcalf et al. (2012) found evidence of the blue lineage in the Colorado drainage. The data on historical stocking presented by Metcalf et al. (2012), Rogers (2013), and the presentation by Chris Kennedy that we saw during the panel meeting suggest that the presence of this blue lineage in the Colorado is due to historical stocking from nearby operations in the Yampa/White drainage.

Fourth, the existence of a distinct green lineage is well supported by the evidence presented in Metcalf et al. (2012). Again, it should be acknowledged that a limited number of historical samples were examined (six individuals from four locations in the Colorado and Gunnison drainages) but combined with the modern data from Metcalf et al. (2012) and in conjunction with broader geographic surveys (Rogers 2010; Rogers 2013), a convincing case is made that the green lineage is native to the Colorado/Gunnison/Dolores drainages.

Fifth, the historical presence of a distinct cutthroat trout lineage in the San Juan drainage is supported by the data. Two distinct haplotypes were observed. However, the use of one locus and limited historical samples dictate that care is used regarding this particular conclusion. Additional historical samples would help to substantiate this observation, as would fortuitous sampling of possible extant populations of this apparently distinct lineage.

Sixth, the distinct yellow lineage in the Arkansas drainage is well supported. The eight individuals sequenced from four locations make a good case that this represents the extinct *O. c. macdonaldi*.

Seventh, green lineage fish on the East Slope present the greatest challenge and harbor the most uncertainty. Two individuals from Twin Lakes (collected in 1889) in the Arkansas River drainage had green lineage haplotypes based on historical samples. This can be explained by two hypotheses. First, undocumented historical stocking could have moved green lineage fish from west to east of the divide. Second, green lineage fish may have naturally colonized the Arkansas River drainage at an earlier point (likely earlier than they would have been anthropogenically moved). Modern data, summarized by Rogers (Rogers 2010; Rogers 2013) reveal green lineage haplotypes in additional Arkansas River drainage sites. Further, the most common East Slope haplotypes are not found west of the Divide (see 'Facts' sheet). As discussed below, there is evidence of morphological divergence of green lineage fish on opposite sides of the Divide (Bestgen et al. 2013). Thus, the most contentious finding in Metcalf et al. (2012) relates to the East Slope green lineage fish.

Additional considerations: all historical inferences from Metcalf et al. (2012) are based on 30 individuals and one maternally inherited locus (a fact fully acknowledged by these authors). As was discussed during the panel meeting, next-generation sequencing (NGS) approaches may be useful to reveal patterns at many loci in the nuclear genome and/or a larger portion of the mitochondrial genome. NGS can work for small fragments of DNA so it may be very useful in this regard. More genetic data could help resolve the relationships in question and I recommend that this step occur. However, it should also be kept in mind that, unless additional museum specimens are found (from which usable DNA can be obtained), historical inferences will be limited by sample size. Caution should therefore be exercised and over-interpretation of historical data should be guarded against.

2. Does the meristic study correlate with findings in the genetics study (i.e, does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

The meristic/morphometric study performed and report written by Bestgen, Rogers, and Granger was very well done (Bestgen et al. 2013). Molecular and morphological data were generally concordant. The molecular and phenotypic data sets provide independent sources of information for the evaluation of taxonomic hypotheses. That these two sources agree in this case allows us to greatly strengthen the inferences we can make. The meristics/morphometrics report finds morphological differences among the blue, green, Bear Cr., and Rio Grande lineages. The Bear Cr. (purple) lineage was particularly divergent. The blue and green lineages were generally divergent once spotting pattern data were included. However, green lineage fish from the East Slope were more similar to blue lineage fish along PC1 than they were to West Slope green lineage fish.

The general concordance between molecular and phenotypic data sets provides highly valuable information for Colorado cutthroat trout. Phenotypic variation like that examined, which likely has a highly polygenic basis, can be influence by natural selection, gene flow, and genetic drift, and mutation (likely to a lesser extent over the time periods considered). Environmental factors could also influence the phenotypic data. However, the traits examined are likely to have low environmental sensitivity relative to many other traits. Further, the report found consistent differences between rainbow trout and cutthroat trout from the same location. Environmental induction would not be consistent with this observation. Thus, the potentially confounding effect of similar environments leading to similar phenotypes in highly divergent lineages seems to be minimized in this data set.

3. To what extent are historical spatial distributions of green, blue lineages known?

I review the evidence regarding historical spatial distribution of the green and blue lineages above (Rogers 2010; Metcalf et al. 2012; Bestgen et al. 2013; Rogers 2013). The data that indicate that the

genetically and morphologically distinct blue lineage was historically confined to the Yampa/White drainage are convincing. The data that suggest that blue lineage fish east of the Divide are due to past stocking are convincing (based on the distribution of blue lineage haplotypes and the phenotypic similarity of east slope blue lineage fish with the most likely hatchery west slope blue lineage hatchery source). The combined data that suggest the green lineage was historically present in the Colorado/Gunnison/Dolores drainages are also convincing. The lack of detection to date of the green lineage haplotypes in the Yampa/White drainage is particularly compelling. The case for the presence of blue lineage haplotypes throughout the Colorado/Gunnison/Dolores due to hatchery stocking is also convincing.

The most difficult pattern to explain remains the green lineage individuals/populations east of the Divide. Morphological as well as genetic divergence suggests that hatchery stocking alone may not explain these patterns. Further, of the four east slope blue lineage populations examined in the morphological study (Bestgen et al. 2013), three were green lineage for mtDNA haplotypes but their nuclear genome assigned largely to the blue lineage. I suspect that this may be due to the AFLP data used and the way the program STRUCTURE was implemented. AFLPs are dominant markers; band presence indicates that an individual has either two copies of the allele at a locus (homozygote) or one (heterozygote). Band absence indicates that an individual is homozygous recessive. STRUCTURE was used with the data to assign individuals from test populations to either the blue or green baseline data sets (K. Rogers pers. comm.). When forced to assign the individuals in question (from the east slope green mtDNA lineage), it did not assign to the green lineages in terms of their nuclear DNA. Instead they assigned largely to the blue lineage. However, their assignment to the blue lineage could also indicate that these populations are divergent in their nuclear allele frequencies from other west slope green linage populations. If a third choice had been present, that is, if east slope green lineage nuclear allele frequencies were well characterized and represented a third baseline group, assignment of the populations in question may have been made to this third group. It is difficult to distinguish between these alternatives with the data in hand. However, use of STRUCTURE with no location prior may provide some insight. Further, as I will elaborate upon in question 14, use of additional SNP data may help.

Further complicating this issue is the possibility that the East Slope green lineage could be native to the East Slope (that is, it naturally colonized the Arkansas River drainage at some point prior to human stocking activities) and subsequent hatchery stocking of west slope green and or blue lineage fish may have obscured the historical signal. It will be difficult to tease apart the historical *versus* anthropogenic hypotheses if both have occurred.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

It seems most likely that the radiation of cutthroat trout in Colorado (defined as the six or seven lineages defined in question 1 above) has been relatively recent compared to divergence of other subspecies of cutthroat trout. This radiation appears to have occurred on the order of 200,000 to 1 million years ago, though it is possible it was slightly longer (Love Stowell 2011). Other cutthroat trout lineages, particularly West Slope cutthroat trout (*O. c. lewisi*) and Yellowstone cutthroat trout (*O. c. bouvieri*) are more evolutionarily divergent (Allendorf and Leary 1988; Metcalf et al. 2012).

It should be noted that an analysis of outbreeding depression among southern Rocky Mountain cutthroat trout would be highly useful. Hybrid offspring between rainbow trout and other cutthroat trout lineages such as the West Slope cutthroat trout (*O. c. lewisi*) have been shown to have reduced fitness in the wild (Muhlfeld et al. 2009). The Rocky Mountain cutthroat trout lineages discussed in this report have

diverged more recently than rainbow trout and West Slope cutthroat trout. It is possible that hybridization among these southern Rocky Mountain lineages exhibit little to no outbreeding depression, but this needs to be tested. Crosses have been intentionally or unintentionally performed in hatcheries and in the wild. However, without performing these crosses in a controlled manner or quantification of their fitness effects, conclusions regarding outbreeding depression cannot be drawn. I recommend that experimental quantification of outbreeding depression be performed, as is already underway for the Bear Cr. lineage (Kevin Rogers pers. comm).

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions

Yes, Metcalf et al. (2012) and the Bestgen et al report (Bestgen et al. 2013) were both well written and draw from pertinent literature well.

BiodiversityImplications: Listable Entities?

6. *Do lineages identified in genetics and meristics studies rise to the level of a listable entity?*

a. different subspecies?

I agree with the argument made by one of the panelists that subspecies designation lies outside of the purview of the review panel. Addressing subspecies designations will require taxonomic revision. This does not preclude the need to determine whether distinct lineages warrant protection as DPS's.

b. *distinct population segments (DPS)?*

In my opinion, the purple lineage (Bear Cr.), West Slope green lineage, West Slope blue lineage, and East Slope green lineage could warrant protection as separate DPSs.

c. *Other?*

7. Is the Bear Creek population considered to be greenback cutthroat trout?

A great deal of discussion occurred regarding what should be called greenback cutthroat trout. This common name appears to have been meant for cutthroat trout in the South Platte River, according to David Starr Jordan. Outside of the controversy about type specimens and what should receive the name *stomias*, it seems appropriate to give the common name greenback cutthroat trout to the Bear Cr. purple lineage.

The origin of fish in Bear Cr. remains uncertain. Kennedy (2010) provides a detailed report about Bear Cr. and how cutthroat trout may have gotten there. He makes a convincing case that a) Bear Cr. was historically fishless and b) the fish likely came from a private hatchery either near Colorado Springs (De La Vergne hatchery) or in Manitou Park (Bell Hatchery). For the historically fishless point (a), there is a substantial barrier downstream of the extant Bear Cr. population and Kennedy (2010) uses some historical references to support a lack of fish in Bear Cr. The historically fishless Bear Cr. inference seems valid to me. For the point about the origin of Bear Cr. fish (b), fish at these two hatcheries at that time (roughly 1882) apparently came from either Trout Cr. (South Platte Drainage) or Beaver Cr. (Arkansas Drainage).

Kennedy (2010) and in his presentation at the panel meeting argues that Trout Cr. would have been the more likely source, based on geographic distance and ease of travel. While it appears that uncertainty will always remain regarding this issue, the analysis of historical records provides support for the current hypothesis, based on genetic data, that a cross-basin transfer occurred and that Bear Cr. is the last known remnant of the South Platte River-native purple lineage

8. How do we describe the East Slope green lineage?

The East Slope green lineage should be considered a distinct lineage until further information is available. The mtDNA and morphological data are concordant. I also describe above (question 3) how there may be nuclear genomic divergence of the East Slope green lineage from the West Slope green lineage. Together, the data in hand indicate that separate consideration is the more conservative conservation action.

It should be distinctly recognized that the divergence between East and West Slope green populations warrants further research. If historical divergence (separate invasion of West Slope green lineage into the Arkansas River drainage prior to human fish movement) has been followed by more recent anthropogenically induced hybridization with West Slope green lineage fish (through stocking) then it should be considered whether the East Slope green linages are a hybrid taxon worthy of protection (see Allendorf et al. 2001; Trends in Ecology and Evolution 16 (11): 613-622).

It is possible that future data and analyses will reveal that East Slope green lineage populations are solely of hatchery origin (founded from West Slope green lineage fish from hatcheries). Then the decision will have to be made regarding the continued conservation of a hatchery-origin lineage. It could be argued that divergence over approximately 100 years could be ecologically and evolutionarily significant and that continued conservation at that point is warranted. However, this would be a much more tenuous argument to make than the one outlined in the paragraph immediately above.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

As mentioned above, I believe the current evidence in hand suggests that the East Slope green lineage should be considered a distinct ESU or DPS.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroats?

It is very difficult to objectively test possible routes of colonization. Many routes are consistent with the data currently in hand. A scenario that involves separate invasion of the Colorado and White/Yampa rivers to form the green and blue lineages respectively seems possible. Further cross-Divide movement is necessary to explain the purple lineage and the East Slope green lineage. How these historical movements occurred and whether they occurred one or multiple times is beyond my expertise and current knowledge of this geographic region. Further, it is not necessary to fully understand colonization routes to objectively weigh evidence of genetic and phenotypic divergence of extant lineages and make decisions about separate conservation designations such as ESUs or DPS's.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

Please see my answers above (Questions 1, 6, and 8) for the green lineage. Regarding the blue lineage, I find the argument that blue lineage fish found east of the divide are of recent hatchery origin convincing. The East Slope blue lineage fish have the common mtDNA haplotypes found in the West Slope blue lineage hatchery source. Further, there was no evidence for morphological divergence of East Slope blue lineage populations from the West Slope blue lineage hatchery source.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Metcalf et al. (2007) report low genetic diversity for the four East Slope populations (green lineage and Bear Cr. included). They report a mean observed heterozygosity of 0.23 (sd = 0.11) for these four sites. However, data for Bear Cr. alone are not available in that paper. Metcalf et al. (2012) report that 'the population (Bear Cr.) harbours [*sic*] little genetic variation for loci that are typically variable in cutthroat trout populations' and cite Metcalf et al. (2007). Thus, I am not able to report heterozygosity or allelic diversity metrics for the Bear Cr. population relative to other populations. We have been told that Bear Cr. has low genetic diversity from the Martin group. It would be helpful to see a table of genetic diversity metrics for populations that have been analyzed to date at both microsatellites and AFLPs (would have to assume Hardy-Weinberg proportions for AFLPs to estimate expected heterozygosity).

Phenotypically, Bear Cr. fish were divergent from other lineages but have maintained variation in the traits examined (Bestgen et al. 2013). Fish from the blue lineage had consistently lower variation in traits examined, in terms of the reported coefficient of variation (CV; Table 12 Bestgen et al. 2013). If Bear Cr. went through a population bottleneck in the recent past (upon founding or later), we might expect less phenotypic variation in this population than in other populations. However, it is difficult to compare phenotypic variation among populations. Many factors influence phenotypic variation. If we assume there are many loci underlying the variation at each trait, then we are dealing with quantitative genetic variation. This variation will be influenced by variation at underlying loci as well as environmental variation. If we assume the traits examine have relatively low environmental sensitivity, then we are assuming the observed phenotypic variation is largely due to underlying genetic variation. In general, we might expect a close relationship between molecular genetic variation (as determined by nuclear markers such as microsatellites, AFLPs, or SNPs) and quantitative genetic variation, more specifically, with the additive genetic variation for the traits in question. When the effects of alleles are additive, they influence the phenotype in the same way regardless of what other alleles are present. The alleles act independently of each other and therefore, as a result, differences in phenotype resulting from additive effects of alleles get transmitted from parents to offspring. Dominance and epistatic effects are not transmitted in the same way. It is additive effects of alleles that contribute to evolutionary responses to selection. Further, when we speak of a relationship between molecular genetic variation and quantitative genetic variation, we are speaking of relationships between measures of heterozygosity at neutral markers and estimates of additive genetic variation of the phenotypic traits examined.

Importantly, bottlenecks can cause dominance genetic variation to be converted to additive genetic variation. If this occurs, the relationship between neutral genetic variation at molecular genetic markers and additive genetic variation is weak. With respect to Bear Cr., additive genetic variation for the traits measured may have been exposed during a bottleneck. This could explain the relatively large estimates of

phenotypic variation in this population we suspect went through a bottleneck. Importantly for the persistence of the Bear Cr. population, this population could still carry many recessive deleterious genes that may have drifted to high frequency or fixation during a bottleneck. Therefore, this population may still have a high genetic load. Thus, while it is promising that individuals from Bear Cr. have as much phenotypic variation as they do, we cannot make any conclusions about genetic variation underlying this phenotypic variation without controlled crosses and the implementation of a quantitative genetic study design. We also cannot draw conclusions about the likelihood of inbreeding effects on fitness (inbreeding depression) from the phenotypic data.

Inbreeding effects should be quantified in the Bear Cr. population. This could involve quantification of abnormalities in hatchery reared fish and estimates of fluctuating asymmetry (Roff and Reale 2004). An even more thorough assessment could be accomplished through controlled laboratory crosses. For example, a series of inbred crosses could be used to compare inbred and outbred offspring. If full-siblings were mated once, followed by the mating of a first-generation female offspring with her first-generation male brother, all second-generation offspring would be highly inbred (inbreeding coefficient (F) = 0.375). The same female could be mated to an unrelated male to obtain offspring with an inbreeding coefficient (F) of zero. Inbred and outbred offspring from replicate crosses could then be compared. Traits such as length, weight, growth rate, early survival, and fluctuating asymmetry would all be useful to examine.

Beyond quantification of inbreeding, I believe genetic rescue should be considered as a management option for the Bear Cr. population. Genetic rescue is an increase in mean fitness in a population owing to immigration of new alleles (Tallmon et al. 2004). A genetic rescue effect, if observed, indicates that inbreeding has negatively affected fitness in the focal isolated population. This is a major issue in conservation genetics. There is a wide variety of data that indicate that inbreeding depression (inbreeding with subsequent negative effects on fitness) can have major effects on population persistence, through extinction vortex dynamics. However, inbreeding effects are environmentally sensitive, condition-specific, and likely to be species-specific (Thrower and Hard 2009). Small population can become purged of deleterious recessive alleles by natural selection (Crnokrak and Barrett 2002). However, a vast amount of empirical data now make it clear that purging is not an effective mechanism to reduce inbreeding depression in most plants and animals (Leberg and Firmin 2008; Allendorf et al. 2013). On the other hand, many small headwater trout populations seem to persist for potentially long periods of time despite very small effective population sizes. Greater efficacy of purging over long time periods (hundreds to thousands of generations) under continuous inbreeding might help explain the persistence of naturally isolated trout populations.

Experimental data for a genetic rescue effect come from various taxa. The idea seems simple: introduce genetic variation through translocation of individuals and restore fitness. The likely mechanism is through masking (making heterozygous) deleterious alleles that negatively effect fitness when homozygous. Examples include prairie chickens (Westemeier et al. 1998), Swedish adders (Madsen et al. 1999), Scandinavian wolves (Vila et al. 2003), among others. However, theoretically, we may expect a fitness decline in the F2 generation and beyond (outbreeding depression). The likelihood of outbreeding depression is a function of genetic similarity of introduced and extant individuals: we expect increased outbreeding depression with increased genetic distance (Frankham et al. 2011). Few studies look beyond the F1, so this has not been well explored. Further, few studies to date have replicated their results or controlled for environmental conditions (Tallmon et al. 2004).

We have reason to be worried about inbreeding effects for the Bear Cr. population. In addition to the apparent effects on hatchery-reared individuals, this population also appears to have been introduced relatively recently. Therefore, purging of deleterious recessive alleles is unlikely to have occurred in this population.

I recommend the following with respect to the Bear Cr. population: I believe this population itself should not have individuals introduced to it. As the last remnant of the South Platte River lineage (to the best of current knowledge), it should be monitored closely and for now, not have attempts made to introduce individuals. However, the Bear Cr. population needs to be replicated across the landscape. One extreme event such as a fire or flood could cause extinction. Therefore, I recommend that Bear Cr. individuals, as the last remnant of the South Platte River lineage, should be introduced to a number of sites in the South Platte River drainage. Once established, these populations should be monitored closely, under the rationale explained by Schwartz et al. (2007). At a minimum, population growth rate should be monitored. It would be preferred if genetic metrics such as mean expected heterozygosity, mean number of alleles, and mean allelic richness were monitored yearly. Further, I recommend that the effective number of breeders that give rise to each cohort (N_b) be estimated yearly (Whiteley et al. 2012). N_b is estimated when single sample effective population size estimators (e.g. LDNe; Waples and Do 2008) are applied to a single cohort (young-of-the-year). N_b is an indicator of the number of families produced by the parents of that cohort, the variance in reproductive success among those parents, and early family-dependent survival of the offspring produced (Waples and Do 2010; Christie et al. 2012).

If multiple metrics in any of these replicated populations point towards population decline, loss of genetic diversity, or reduced numbers of effective breeders (as a surrogate for reproductive success and output), genetic rescue should be considered. This would entail the introduction of a small number of individuals to the replicated populations. The source of those individuals will need to be carefully considered. It is possible that East Slope green lineage individuals would be most appropriate. I would hope that more resolution about East Slope green lineage fish is available by the time this transfer may be necessary. Careful consideration should be used to decide the number and sex of introduced fish. Population genetics theory dictates one-migrant-per-generation among populations equally linked by gene flow is enough to keep the same neutral alleles segregating in the populations and that the relative strength of gene flow and selection would determine the fate of non-neutral alleles (Wright 1931; Wright 1940). Whitlock and colleagues (Ingvarsson and Whitlock 2000; Whitlock et al. 2000) have shown that the "effective immigration rate" of new alleles in small isolated populations should be elevated over that expected under the neutral theory because immigrant alleles can increase rapidly in frequency due to selection. The Florida panther could be used as an important case study for South Platte cutthroat trout. In this example, Hedrick (1995) used population genetic models to show that a brief period of high gene flow followed by subsequent generations of low gene flow should reduce the frequency of deleterious alleles without substantially reducing the frequency of locally adaptive alleles.

It is important to recognize that genetic rescue comes with risks. F2 individuals may have reduced fitness due to outbreeding depression (Edmands 2007). I recommend close monitoring to determine if genetic rescue is having a positive effect in this particular circumstance. This would allow an assessment of whether genetic rescue is a good option for additional populations. This monitoring would need to include an estimation of the fitness of individuals in the rescued populations. This can be done through genetic identification of fish to cross type (resident x resident, resident x transplant, or transplant x transplant) and estimation of fitness as a function of cross type via capture-mark-recapture (CMR) experimentation. Fish could be individually identified via genetics or with passive integrated transponder (PIT) tags.

A final note for this question, it came up during the panel meeting whether addition of fish from Bear Cr. to an existing population of blue and or green lineage fish in the South Platte River was a viable option. In my opinion, this is far less preferable than the type of controlled genetic rescue experiment I outline above. This alternative would be highly uncontrolled and it would be difficult to understand if there was a positive or negative effect of the introduction. It is much more likely that this would result in swamping the genome of Bear Cr. fish, compared to rescue where a small amount of gene flow is meant to alleviate inbreeding without swamping the genome.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

This question is directly related to the extant genetic data with the greatest uncertainty (East Slope vs. West Slope green lineage). Above, I describe the alternative explanations for the extant genetic data for the East Slope green lineage. Given that I conclude that these data suggest that the most prudent alternative is to consider the East Slope green lineage as a distinct entity worthy of separate conservation (an ESU or DPS), and given the data that the East Slope green lineage was present in Twin Lakes in the Arkansas River drainage in 1889 (Metcalf et al. 2012), then it follows that the East Slope green lineage should be considered for reintroduction as the native cutthroat trout of the Arkansas River drainage. However, I reiterate, subsequent data and analyses may find that East Slope green lineage fish are introduced from the west slope.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

Single Nucleotide Polymorphism (SNP) markers obtained from next generation sequencing (NGS) approaches could be highly useful for cutthroat trout from the blue, green, and Bear Cr. lineages. I see two possible options, which echo the comments made by one of the panel members during our meeting. The first option would be to use the data generated by Dr. Shiozawa's group but to target SNPs only within the blue, green, Bear Cr., Rio Grande lineages. This could provide a moderate (on the order of 100's) amount of SNPs that are highly informative for distinguishing among the lineages in questions. This relatively small set of SNPs could be efficiently run on future samples with the use of a platform such as the Fluidigm system, which can assay 96 SNPs in 96 individuals in a short amount of time.

The second option is to obtain a much larger set of SNPs. For example, the RAD-seq (Hohenlohe et al. 2011) approach could be used to obtain on the order of 10,000 SNPs for individuals from each of the four lineages in questions. This would provide more data and even greater resolution of genetic relationships than described in first option. A limitation is that RAD-seq remains relatively expensive and would be more difficult to perform on subsequent fish than the Fluidigm approach described above. Thus, the RAD-seq approach does not provide an efficient diagnostic tool moving forward. Given the need to screen large numbers of individuals from many populations, I recommend the first option.

AFLPs are useful and have provided valuable information regarding cutthroat trout in Colorado. They have greatly helped in unraveling the story as we understand it currently. However, due to issues related to the one I describe above (related to the East and West Slope green lineage; question 3), AFLPs will continue to provide results with multiple interpretations and that are less clear than those that could be obtained with an informative set of SNPs.

During the meeting, the utility of the application of NGS to museum-obtained DNA was discussed. NGS could be used to re-analyze the museum fish from Metcalf et al. (2012) at many loci in the nuclear genome and/or a larger portion of the mitochondrial genome. NGS can work for small fragments of DNA so it may be very useful in this regard. I reiterate my point, however, about the limitation of inferences based on 30 fish, no matter how much data per fish are ultimately obtained.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

I will summarize points I have made regarding future research in my answers above

1) NGS analysis of museum specimens from Metcalf et al. (2012)
2) Development of panel SNPs from Dr. Shiozawa's NGS data or from additional RAD-seq obtained data. This panel of SNPs should be used to help further resolve the relationship among lineages and patterns of hybridization.
3) Resolve the green lineage issue (West Slope vs. East Slope; natural colonization vs. more recent anthropogenic introduction)
4) Quantify inbreeding in the Bear Cr. lineage
5) Replicate the Bear Cr. lineage across the landscape, perform genetic rescue if necessary (as determined based on genetic monitoring)
6) Increase understanding of connectivity among populations of both the blue lineage (Yampa/White drainage) and green lineage (Colorado, Gunnison, Dolores drainages) west of the Divide. Quantify risk of extinction for isolated populations.
7) Test likelihood of outbreeding depression in crosses among these various lineages

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*
- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the S. Platte drainage?*

Please see my answer to question 12. The initial observation of high rates of physical abnormalities in Bear Cr. fish raised in captivity warrants further research on the potential effects of inbreeding in this small, isolated, and valuable population.

17. Please provide other relevant comments not addressed in the above questions.

All comments are contained within my answers to questions 1 – 16.

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Panelist #7 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

The ancient mtDNA work suggests the existence of six geographic lineages based upon the limits of museum samples available and the smaller number of museum samples that mtDNA could be recovered from. However, additional support for the existence of geographic based lineages is also supported by the Dr. Rogers AFLP data in Table S3, resulting in a correlation between museum samples, modern DNA samples and their locations within geographic locations.

Although limited, the small 430-bp subset of mtDNA data ties the mtDNA sequence of the existing Bear Creek (Colorado Springs) to nine mtDNA museum samples collected within the South Platte River drainage between 1871-1889. It is the best available science on the historic and current distribution of cutthroat trout native to the headwaters of the South Platte River drainage. The nine mtDNA museum samples, representing five separate populations, are reasonably well distributed and represent two collections separated by 18 years.

The unlikely alternative interpretation to the Metcalf (2012) data would be that all the matching mtDNA museum samples represent the range-wide organized movement and introduction of a lineage of fish prior by 1871. Although brook trout were present at the site of the 1889 Jordan collection in Bear Creek (Denver area), and documents the movement of fish from thousands of miles away by 1889, the ability to move a uniform population of fish into four separate sites within the South Platte River drainage would appear to be limited by 1871. However, ranching and mining were present with the South Platte River drainage by the 1860s (SPNHA), and Metcalf et al. (2012) indicates fish propagation and movement by the early 1870s. As indicated by the Jordan collections, the railroad made the wide spread movement of any fish species a possibility. The Denver, South Park and Pacific Railroad reached into the headwaters of the South Platte River (Como) by 1879 (SPNHA), and the headwaters of the Arkansas River (Leadville) by 1880 (Clark). As an indication of how fast the widespread movement of non-native fish became, the first fish distributed from the Leadville National Fish Hatchery were brook trout to Colorado, South Dakota and Nebraska in 1890 (Rosenlund, 1989).

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

Based upon a random selection of AFLP assigned lineage fish populations and blind passes of meristic and genetic materials, the Bestgen and Rogers study supports physical and genetic differences between major river drainages. As stated in the study, "individual traits and discriminant function analysis also showed substantial structuring within lineages, organized by drainage basin (GMU's)." Overall, the meristic study supports the assignment of the blue lineage within the Yampa River drainage, green lineage within the Dolores, Gunnison, Upper Colorado River drainages, and Rio Grande cutthroats within the Rio Grande River drainage. Metcalf (2012) and Bestgen (2013), and the Arkansas River collections of Jordan (1889) provide some indication of a green lineage within the Arkansas River, and possibly the South Platte River drainage. However, movement of non-native fish into Twin Lakes is documented by 1889, and may explain the existence of green lineage fish collected in 1889. However, the existence of the

unique East Slope green haplotypes is not supported by the limited museum samples, but not refuted by known genetic markers from non-native populations.

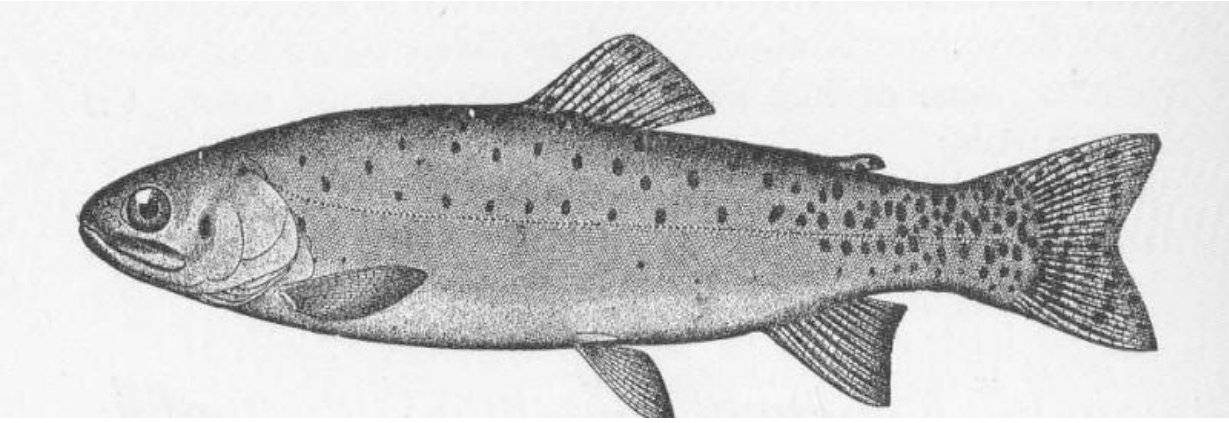
The meristic study also supports the evidence that the yellowfin and San Juan lineages are extinct, or currently at population levels below detection, based upon the AFLP screening of conservation populations of Colorado River cutthroat trout.

3. To what extent are historical spatial distributions of green, blue lineages known?

Although limited to less than 30 pages within the Bulletin of the United States Fish Commission, Vol. IX, for 1889, the work of David Starr Jordan appears to provide limited formal (for the time) documentation of the presence and distribution of native fish species within Colorado. Plus, some interesting insights into politics and loss of aquatic habitats. Jordan also documented the presence of non-native fish across the landscape by 1889, with brook trout, rainbow trout and Atlantic salmon present in Twin Lakes (headwaters Arkansas River drainage) by 1889 (page 6 of 1889 report). Although the documentation of non-native fish makes any speculation on the distribution of native fish difficult by 1889, some information may be inferred:

Blue lineage. Jordan describes fish from Trappers Lake, and states that several fine examples were obtained from anglers. Although it is not clear if he visited Trappers Lake, his description of the Trappers Lake fish appears consistent with known modern day specimens (page 29). Based upon Jordan's historic information, museum samples, Metcalf et al. (2012) and Bestgen (2013), there appears to be good evidence for a blue lineage in the Yampa River basin.

Green Lineage East. Jordan states that "*stomias*" is a "small trout with very large spots and small scales". "The black spots are larger than in any other of our trout."



GREEN-BACK Trout, Jordan 1889

Jordan also states that *stomias* is "very common in all the upper tributaries of the Arkansas River and Twin lakes." For the Platte basin, Jordan states that *stomias* is "abundant in the Park Range and in mountain streams generally." He also states that "the trout taken by us in the tributaries of the Platte seems to be identical with the "green-back trout" of the Arkansas." Thus, the observation of Jordan, suggests a common/abundant large spotted trout existing within the South Platte and Arkansas River drainages that Jordan visited, or had gained knowledge of by 1889. Although Metcalf obtained DNA from the 1800s collection, there is no reference to the physical appearance of the 1871-1889 museum

collections. Without impacting the museum samples, a limited meristic review of the 1871-1889 museum collections should be conducted.

Green Lineage West. Jordan states that "trout are very abundant in all the headwaters of the Colorado and its tributaries, wherever the waters are clear and cold". He also states "As a whole, the trout from the Colorado approach most nearly to those from the Rio Grande." And on page 22, he states that the "Rio Grande trout have the dark spots rather large and more or less confined to the dorsal and caudal fins and the region between them." However, the Jordan illustrations of Rio Grande cutthroat (Plate III, Fig. 7-8) show a posterior fine spotted adult fish, and a larger spotted "young" fish. Interestingly, Jordan states that the Eagle River is "very well stocked with trout" and the "Eagle River show more resemblance to the yellow-fin of Twin Lakes in the small size of the spots and the plain coloration." Apparently, based upon his Eagle River observations, he concludes that "the nearest relative of the yellow-finned trout is *"pleuriticus,* from which I think it is descended."

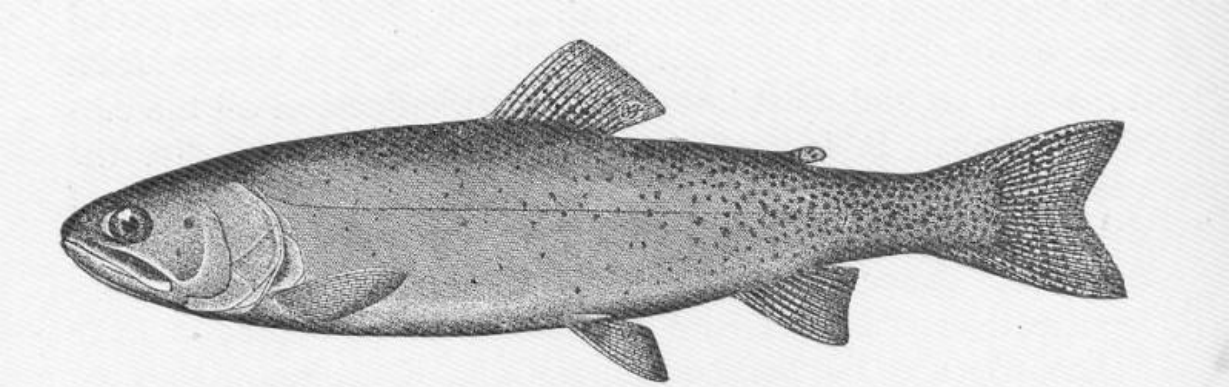


FIG. 237.—Yellow-fin Trout of Twin Lakes, *Salmo macdonaldi* Jordan & Evermann. Twin Lakes, Colo.

Thus, Jordan's physical descriptions and figures appear to support a large spotted cutthroat trout throughout the Arkansas and South Platte Rivers that would support a green lineage east in 1889. His Eagle River observations of fine spotted fish would not support a green lineage west. But, his written description of a Rio Grande cutthroat and his statement that, as a whole, trout from the Colorado resemble Rio Grande cutthroats, would support a green lineage west in 1889. Overall, Jordan's 1889 observations for Colorado were limited from 19 July when he arrived in Pueblo, to 2 August when he appears to depart Delta for Utah.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

I am unable to address with any degree of expertise the differences that have been published for other studies to either support or refute the degree of differences needed to support lineages, races, subspecies or species.

However, Metcalf et al. (2012) and Bestgen (2013) document landscape level genetic and meristic variation with the data and conclusions based upon blind passes of materials and protocols to limit

variation due to human error. The final report clearly shows that landscape boundaries have shaped physical and genotypic differences to where individuals and populations can be assigned to landscape boundaries within a known level of accuracy. Although Metcalf et al. (2012) and Bestgen (2013) have documented the exchangeability of lineages between major river drainages, Crandall (2000) would seem to support the Metcalf lineages as ESU's, since both genetic and physical differences can be demonstrated between the original physically isolated populations.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

In my experience as a management biologist, both studies reference a wide range of published and agency reports that support their conclusions.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?
- *a. different subspecies?*
- *b. distinct population segments (DPS)?*
- *c. Other?*

This is an area where I feel that I have limited abilities to address the question. However, based upon Mayr and Ashlock (1991), who defined subspecies as "a collection of populations occupying a distinct breeding range and diagnosably distinct from other such populations" all the lineages appear to be good subspecies defined within major river drainages, with limits to natural movement of genetic materials between river drainages. In addition, Bestgen and Rogers demonstrate that the lineages can be "diagnosed" to geographic sites by both physical and genetic methods.

7. Is the Bear Creek population considered to be greenback cutthroat trout?

Based upon the limited genetic data, it is reasonable that Bear Creek (Colorado Springs) represents the best known existing population of native trout that occurred within the South Platte River drainage between 1871-1889. If it is appropriate to continue to use "greenback" to describe the salmonid native to the South Platte and Arkansas River drainages, Bear Creek should be considered to be greenback cutthroat trout. However, there appears to be much confusion between the large spotted and small spotted forms of cutthroat trout collected and described within the South Platte, Arkansas, and Colorado River drainages. Additional work to review and document the phenotypes of the museum collections, in relationship to modern day Bear Creek (Colorado Springs) and other described lineages (Metcalf, Bestgen), may help resolve questions and inconsistencies within historic accounts.

8. How do we describe the East Slope green lineage?

The East Slope green lineage is a group of four existing populations found within the South Platte (2) and Arkansas (2) Rivers that physically and genetically assign to the West Slope green lineage. However, they have unique haplotypes not found within West Slope green lineages, and I assume, within no other known fish populations. Please note that there was originally only one known South Platte East Slope green lineage population (Como Creek), with the other South Platte East Slope green lineage population (Fern Lake) being a transplant of Como Creek stock.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

There may have been a large spotted form of cutthroat trout native to the South Platte and Arkansas River drainages that are now represented or partially represented by up to four existing populations. As previously stated, the existence of unique East Slope green haplotypes is not supported by the limited museum samples, but their uniqueness is not refuted by genetic markers from non-native populations.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

As of this time, no.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

My understanding is that there is no difference between the East and non-Yampa West Slope populations of Trappers Lake (blue lineages) which supports the fact that many East Slope and West Slope blue populations were established from a unique lake population of Colorado River cutthroat trout within the blue lineage. My position on the variation of east/west green populations is stated in question 9.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Hatchery reared fish founded from small adult cutthroat populations, such as Bear Creek and other "greenbacks" (Como Creek), exhibit high percentages of bi-lateral asymmetry and other obvious physical deformities. Although the wild Bear and Como Creek populations do not exhibit the high deformity rates observed in their hatchery off spring, it is assumed that it is a reflection of the overall fitness of the wild populations.

Milt from other "greenback" wild populations was used in past broodstock programs to increase genetic diversity and reduce deformities with some success. Since there appears to be no other living population that conforms to the genetics of the South Platte museum samples, past examples of mixing populations is not available. Hopefully, other panelists can provide recommendations on how to increase genetic diversity and reduce deformities (Florida panther example), but limit the dilution of what is unique within the remaining Bear Creek (Colorado Springs) population.

Based upon past experience with mixing populations to improve diversity, caution should be used for selecting candidates for out-crosses. Future science may not support the purity of the populations used for out-crosses.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

Based upon previous work, Metcalf (2012), and Bestgen (2013), there are no remaining yellowfin cutthroat trout known within the Arkansas River drainage. Although there may be a relationship between

green lineage large spotted greenbacks and the Arkansas River drainage, it is not well defined at this time. No reintroductions of "native Arkansas" basin fish should be conducted until more supporting data is available. However, controlled reservoir sites within the Arkansas River drainage may be well suited for the rearing of Bear Creek fish for reintroductions within the South Platte River drainage.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

Please see Question 15 for research issues.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

- Apply next-generation DNA sequencing on the cross section of known cutthroat trout lineages.
- Conduct experiments on the fitness of the existing Bear Creek fish.
- Based upon DNA and physical characteristics, what existing cutthroat trout populations could be considered for mixing with Bear Creek to increase genetic diversity and reduce deformities, while maintaining the maximum Bear Creek genetic signature and meristics?
- Determine the level of DNA and meristics deviation that could be allowed by the ESA under a beneficial Bear Creek hybridization program.
- Continue to search for museum specimens and conduct additional research to review and document the genetics and meristics of applicable museum collections in order to help resolve questions and inconsistencies within historic accounts.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*

This issue has been experienced in other cutthroat populations/broodstocks. In the early part of the greenback recovery program, the genetic limits of the remaining pure fish were uncertain, but direct transfers of approximately 60 adult Como Creek fish did result in new reproducing populations. Later in the greenback recovery program, broodstocks were established, and milt from other wild pure populations was used to fertilize hatchery reared eggs and reduce deformities. Both methods of re-establishing wild populations were successful in establishing new lake and stream populations from a subset of a small stream population(s). To some extent, the same can be said for the Trappers Lake strain of fish currently found in Lake Nanita, and the use of these fish for management purposes by Colorado Parks and Wildlife.

Since Bear Creek fish are able to survive within their current habitat limitations, I would think they would be as successful in re-establishing new stream and lake reproducing populations as the Como Creek stock was. However, research into the fitness and performance of these fish in the wild could improve the long-term chances for recovery success over a wide range of habitats, and possibly their performance within the presence of other native fish species. Please see Question 15 for suggested research topics.

-What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?

Please see Question 15 for suggested research topics.

17. Please provide other relevant comments not addressed in the above questions.

No response

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SPNHA. South Park National Heritage Area, southparkheritage.org

Bulletin of the United States Fish Commission, Vol. IX, for 1889. Pages 1-29

Panelist #8 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

The Metcalf et al. (2012) study is well designed and provides evidence for a new hypothesis regarding the spatial distribution of genetic diversity of cutthroat trout in Colorado prior to large scale transfers. The results supporting six lineages (4 extant) are compelling. In my opinion, however, there are two caveats regarding the data set that should be considered when interpreting the results (the authors acknowledge these caveats). First, the inferred spatial distribution of each lineage (historically in Colorado) is derived from a relatively small sample (n=30). This small sample size makes it challenging to define the geographic range of each lineage and leads to some uncertainty regarding the origin of some contemporary populations, notably Bear Creek and some green lineage fish on the East Slope. The authors contend Bear Creek cutthroat trout represent *O. c. stomias* and are outside of their native range (South Platte River drainage) due to an early 20th century transfer. This is a reasonable hypothesis but it also possible (perhaps not as likely) that *stomias* could have existed in the Arkansas River drainage and the Bear Creek area prior to stocking. Additional historical samples from the Arkansas River (if they exist) would be needed to examine this alternative explanation. Second, the mitochondrial DNA sequence data is effectively a single gene and thus represents one possible genetic outcome of the combined forces (e.g., gene flow, genetic drift, mutation, assuming no selection) that influence intra-specific genetic diversity. Mitochondrial DNA has advantages for this type of analysis but support for the new "molecular" hypothesis would be strengthened if similar results were found using appropriate nuclear markers.

Bottom line: I feel the conclusions by Metcalf et al. (2012) are consistent with the data in the study, supporting a hypothesis of six mitochondrial lineages in Colorado cutthroat trout (4 extant and 2 extinct). Further study is needed to test the six lineage hypothesis (using appropriate nuclear markers) and refine the geographic range of the lineages (examine more historical samples).

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

Yes, the meristic study does show that meristic variation is consistent with the molecular hypothesis. On the other hand, the meristic variation is also consistent with the geographic hypothesis, although the correlation does not appear to be as strong as for the molecular hypothesis. I found the combination of the meristic study (Bestgen et al. unpublished) and molecular study (Metcalf et al. 2012) showed that major diversity (lineages) in Colorado cutthroat trout is/was strongly influenced by major drainage basins as well as continental divide.

3. To what extent are historical spatial distributions of green, blue lineages known?

The results from Metcalf et al. (2012) and Bestgen et al. (unpublished) suggest the blue lineage was historically confined to the West Slope of the continental divide and limited mostly to the Yampa River drainage. This data includes contemporary samples (Bestgen et al. unpublished) that show blue lineage fish on the East Slope have lower genetic diversity and a subset of the haplotypes compare to blue lineage

fish on the West Slope – as would be expected from transfers that occurred in the late nineteenth and early twentieth centuries.

The historical distribution of the green lineage fish is less clear from the available data. The results from Metcalf et al. (2012) suggest the green lineage occurred on the West Slope south of the blue lineage in the Colorado and Gunnison River drainages. However, the historical collection also included two green lineage samples from the East Slope. The authors suggest these samples may have been influenced by transfers (that occurred prior to 1889) of West Slope green lineage fish. However, the East Slope collections contain unique haplotypes not found on the West Slope which is inconsistent with a west-to-east transfer. In my opinion, the evidence is at least as strong that the green lineage existed historically on the East Slope and more analysis will be needed to resolve the question of the origin the East Slope green lineage fish. As stated in my reply to question 1, this can best be resolved by additional study including examining more historical samples (if they exist for the area of interest) and applying appropriate nuclear markers.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

As a geneticist I'll focus my response mostly on the genetic variation. This is not an easy question to answer and I don't feel I have adequately considered the literature to address the question. First of all, any comparative assessment would have to account for the impacts of transfers and hybridization such as was done in the two studies above. Second, any comparison should be based on studies that ideally use the same markers (or marker types, e.g., mtDNA, microsatellites, SNPs). I am not aware of published studies of cutthroat trout that would allow this type of comparison. Ideally, what is needed is a single study of cutthroat trout throughout the range. A couple examples were presented during the workshop; however to my knowledge most of the information is unpublished. Finally, differences in diversity can be influenced by a number of factors (e.g., effective population size, gene flow, mutation rate) that should be considered when comparing and interpreting levels of genetic variation.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Both studies contained adequate literature support. What is needed, in my opinion, is further study to 1) address the historical distribution of the green lineage, particularly the evidence of east slope populations, 2) test the molecular hypothesis (based on mtDNA) using appropriate nuclear markers. Also, it is not clear if the molecular hypothesis describes the meristic variation significantly better than the geographic hypothesis. Perhaps this could be tested statistically.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?

a. different subspecies?

It is not clear to me what a subspecies is and therefore I have trouble evaluating the study conclusions in this context.

b. distinct population segments (DPS)?

I think the combination of the meristic and genetic studies suggest the four extant lineages are potentially listable entities as DPSs or evolutionarily significant units. Additional information concerning life history variation and ecological differences, if they exist among the lineages, would further support defining the lineages as the listable entities.

c. Other?

7. Is the Bear Creek population considered to be greenback cutthroat trout?

This make sense and is supported by the fact that the Bear Creek ND2 haplotype matches the museum specimens from the South Platte River drainage and the fact that the term "greenback" was assigned to native fish in the South Platte and Arkansas River drainages. Regardless of the name, it appears that the cutthroat trout in Bear Creek are the only existing representatives of the purple (South Platte) lineage.

8. How do we describe the East Slope green lineage?

Given that the East Slope populations possess haplotypes not found on the West Slope it is harder to argue that the East Slope fish are the result of transfers. They could represent part of the natural range of the lineage. In my opinion, the East Slope green lineage fish should be considered as of unknown origin until further study can clarify their relationship to the West Slope fish. Clarifying the distribution of the green lineage should be a top priority for future research

9. What do rare haplotypes and morphological consistencies of east-green lineage fish suggest in terms of subspecies or ESU distinctions?

In my opinion, this question cannot be resolved without further research. There is not enough information in the present studies to characterize the East Slope green lineage fish. Here is where additional genetic markers, preferably nuclear loci, could provide some insight.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

In my opinion they do not. The genetics and meristic (Bestgen et al. unpublished) studies I've seen were not designed to answer this question. The question should be addressed by evaluating alternative hypotheses and examining a broader geographic scale like some of the ideas presented by Dennis Shiozawa in the workshop.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

This question relates to question 3. With respect to the blue lineage, the East Slope fish appear to be non-native; the result of transfers in the late 1800's and early 1900's. The East Slope fish have less diversity and possess a subset of the haplotypes of the West Slope fish, consistent with transfers. This East Slope – West Slope variation most likely reflects founder events / bottle necks that occurred during and after the period of transfers.

Regarding the green lineage, the evidence is at least as strong that fish existed historically on the East Slope. That is, the East Slope fish possess haplotypes not found on the West Slope. This East Slope – West Slope variation could have taxonomic implications but more analysis will be needed to resolve the question of the origin the East Slope green lineage fish. See also the answers to questions #3, 8, and 9.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

The greatest concern regarding this lineage is that only a single population, Bear Creek, exists. The immediate priority must be to replicate the population to reduce the risk of loss of the lineage due to any catastrophic event in Bear Creek. Sufficient individuals should be used to transfer the extant genetic diversity to other locations sufficiently large to support a population size that minimizes loss of diversity due to a bottleneck. The replicate populations should be closely monitored and evaluated to assess changes in diversity.

Genetic rescue (where gametes from one or more other lineages are mixed with Bear Creek lineage to increase genetic diversity in Bear Creek) should be considered but only after a more detailed evaluation of the risk of inbreeding and loss of extant diversity.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

I don't believe the present studies provide sufficient information to fully answer this question. More study is needed, including a more detailed evaluation of the origin the East Slope green lineage fish using additional genetic markers and evaluation of more museum specimens from the Arkansas River Drainage (if they exist) to establish if it contained purple lineage (Bear Creek) populations. Having said that, if a high quality location for a replicate Bear Creek population exists in the Arkansas River drainage, then the Bear Creek lineage should be considered as one source for reintroducing native cutthroat trout in the Arkansas River drainage. Such an approach would have to weigh the risks associated with losing the Bear Creek population against potentially occupying non-native territory.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

This approach is not cheap. In my opinion, NGS should be used judicially to address questions concerning the range of the green lineage fish (specifically, the origin of East Slope green lineage fish) and test the six lineage hypothesis generated from the mtDNA data. NGS should be applied to both

museum and contemporary samples (although the application to museum specimens will likely be difficult due to lower quality of the DNA).

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

I think a broad phylogeographic look at cutthroat trout is needed. It should also be a priority to survey the San Juan drainage for existing populations that may represent the presumed extinct San Juan lineage. Beyond that, further research needs should be determined once the next study results are complete (e.g., question 14).

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*

I hesitate to draw any conclusions from this information because as far as I know there has been no study to quantify the abnormalities and relate them to the environment (hatchery vs wild). Therefore, I would first conduct a study to assess the extent and type of abnormalities in different environments using proper controls.

- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

I would suggest looking for locations that as much as possible share similar habitat features to the Bear Creek drainage. Specifically, consider factors such as water temperature that influence development and growth rate. It will be important to know what habitat features constrain population growth and influence survival. However, I would not wait on studies to replicate the population if the information is not easily acquired. Given the need (in my opinion) to replicate the population soon, it would be preferable to integrate the research into habitat needs with the replication effort. Researchers should monitor closely the relationship between habitat features and the performance of the replicate populations.

17. Please provide other relevant comments not addressed in the above questions.

No response.

APPENDIX E

Other Attendee's Responses
to the
Greenback Cutthroat Trout Genetics and Meristics
Discussion Questions

Panelist #9 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

In general, the paper describes the most parsimonious explanation for the patterns seen given the data. Some conclusions are better supported by the limited data than others. However, some alternative hypotheses cannot be ruled out. For example – with five widespread museum collections from the South Platte River basin, it certainly appears that it harbored its own native cutthroat lineage consistent with those currently found in Bear Creek. Unfortunately, few museum collections were available from the Arkansas River Basin to comfortably determine that the yellowfin was the native trout there – particularly since the collections were restricted to the headwaters of the Arkansas River basin, and that two specimens collected in 1889 from Twin Lakes showed mitochondrial signatures consistent with green lineage fish. Though perhaps unlikely, it is possible that these fish were not founded by a stocking event as suggested in Metcalf et al. 2012 but rather by a natural invasion from west of the Continental Divide perhaps in the late Pleistocene.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

The meristics study correlates surprisingly well with the genetic work – a finding not at all anticipated at the outset of the study.

3. To what extent are historical spatial distributions of green, blue lineages known?

Collections of blue and green lineage fish are unfortunately sparse in the museum study, making it difficult to nail down the distribution of these fish. However, when combined with information on extant populations, confidence in the ranges provided in the Metcalf study becomes more compelling. For example, not only have green lineage fish not been detected in the White and Yampa River basins despite intensive sampling, but the Green River basin in Utah and Wyoming also harbor blue lineage fish. It would be difficult to make the case that the Yampa and Green River basins were not part of the aboriginal range of blue lineage cutthroat trout.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

Within the species cutthroat trout, other designated subspecies show similar amounts of molecular variation (see Loxterman and Keeley 2012, Houston *et al.* 2012). I am not aware of ESU designations in cutthroat trout, but my impression is that some strains of Pacific salmon currently managed as ESUs might show less differentiation in genotype and perhaps meristic characters than we are discussing here. Homing to discrete natal streams has justifiably led them to be protected as ESUs.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Not sure about all, but certainly adequate to support their conclusions

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?
a. different subspecies?
b. distinct population segments (DPS)?
c. Other?

Although different species concepts will yield different interpretations for these questions, I think it is instructive to consider the position released by the USFWS in 1996 (USFWS 1996) since that will ultimately form the foundation for any listing decision. That document states that the "authority to list a species…extends to subspecies, and for vertebrate taxa, to distinct population segments" but that the authority to list DPS should be implemented sparingly. Conservation efforts under the Act should be taken to avoid important losses of genetic diversity (particularly those in which a population segment whose loss would produce a gap in the native range of a species). The National Marine Fisheries Service instituted a policy in 1991 for Pacific salmonids that suggests a stock should be considered an ESU if it is substantially reproductively isolated from other conspecific population units and represents an important component in the evolutionary legacy of the species – and it was the spirit of the ESU that was used to develop the USFWS policy on DPS. In that document, three elements are considered in a decision regarding the status of a possible DPS as threatened or endangered under the Act: 1) Discreteness of the population segment in relation to the remainder of the species to which it belongs; 2) The significance of the population segment to the species to which it belongs; and 3) The population segment's conservation status in relation to the Act's standard for listing. Since the question posed simply asks whether the lineages are potentially listable, we are really just looking to address the first two criteria:

Discreteness – the policy maintains a population segment may be considered discrete if it "is markedly separated from other populations of the same taxon as a consequence of physical, physiological, ecological, or behavioral factors. Quantitative measures of genetic or morphological discontinuity may provide evidence of this separation". Given the historically isolated nature of the various drainage basins (for fish) and the results of both the molecular and meristic work, it would be hard to argue that the extant lineages are not discrete. While the yellowfin cutthroat specimens also appear to be discrete, additional work should be conducted on the San Juan lineage to determine if they meet the above criteria.

Significance – three of the four criteria outlined in the policy under significance are relevant for the extant lineages of cutthroat trout. Loss of these lineages would result in a significant gap in the range of the taxon. In the case of the South Platte native, it appears that the Bear Creek fish represent the only surviving natural occurrence of that lineage, and there is evidence that the lineages differ markedly from each other in their "genetic characteristics" (as well as phenotypic traits). Again, it is hard to argue that the extant lineages are not significant.

Regardless of how the USFWS decides to rule, both molecular and meristic data suggest these lineages should be managed as discrete entities even at the GMU (4 digit HUC) level. That is presently the current practice for Colorado Parks and Wildlife, and should continue to be the case.

7. Should the Bear Creek population considered to be greenback cutthroat trout?

It is clear from David Starr Jordan's writings that he intended the name "greenback" to be applied to the fish of the Arkansas and South Platte River basins excluding the large yellowfin cutthroat trout found in Twin Lakes. If Metcalf et al. (2012) are correct in assuming that the yellowfin was the aboriginal fish of the entire Arkansas River basin, then the name greenback should default to those found in the South Platte River basin. This is not necessarily the case for the scientific name *stomias* since those were assigned to type specimens that now appear to be Rio Grande cutthroat trout. Since the name *virginalis* predates *stomias*, the latter then technically becomes a synonym for Rio Grande cutthroat trout.

8. How do we describe the East Slope green lineage?

It depends – I think more research is necessary to try and elucidate if they indeed are aboriginal to the East Slope, or if they merely display uncommon haplotypes and meristic characters by virtue of anthropogenic activities that may have subjected them to extreme genetic bottlenecks that could fix rare genotypic and phenotypic characters in their populations

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

Again, confirmation of whether these traits are an artifact of their past or represent real native diversity needs to occur. Examination of meristic characters in the museum specimens and characterizing all green lineage population haplotypes would help inform this decision.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

They certainly provide some compelling evidence for probable invasion routes, but not much resolution at this point.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

Blue lineage – there is no east/west variation in either the phenotype or genotype in these specimens when the relevant comparisons are made. Blue lineage fish on the East Slope are mildly different phenotypically from blue lineage west of the Divide only if the entire range is considered (includes fish from Utah and Wyoming) – but that is not the relevant comparison. Since the current paradigm suggests that all blue lineage fish on the East Slope were derived from spawn operations conducted at Trappers and Marvine Lakes, they should be the ones to compare to. There are no phenotypic differences between East Slope blue fish and fish derived from Trappers Lake. In addition, all East Slope blue populations share the common haplotypes found in Trappers Lake fish.

Green lineage – there is east/west variation in both phenotype and genotype in these fish, and compelling arguments can be made for how those differences could have arisen either through natural invasions from west to east across the Divide some 10,000 years ago or by stocking from west slope sources that harbored rare haplotypes that were easily fixed in the population through genetic bottlenecks that could have certainly manifested themselves through small founding populations in marginal habitats. Additional research is necessary to evaluate the significance of these differences and what the taxonomic implications should be.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Although molecular evidence suggests limited genetic variability, studies should first be conducted to determine if there are any actual inbreeding effects in the wild. If there are indeed fitness consequences, then genetic rescue should be considered – but only with a small number of individuals. In addition, Bear Creek and several replicate populations should be maintained even in their inbred state. Repatriated populations could be used to manage genetic diversity. Meristic variability did not appear to be much different than what was seen in blue and green lineages.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

We need to resolve the status of East Slope green fish before that can be answered. If indeed the yellowfin is the only native of the Arkansas River basin, then additional flexibility could be considered in Arkansas River basin repatriation efforts.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

Much longer sequence reads in both the nuclear and mitochondrial genomes will greatly improve our confidence in results and will likely reveal additional structure that will assist in addressing some of the questions above such as native routes of invasion and similarities between East and West Slope fish.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

We should continue to manage these lineages at the GMU level while protecting at least East Slope green fish until taxonomic uncertainty can be resolved. Research priorities should include examining museum specimens to determine if Bear Creek fish represent the meristic diversity present in the museum specimens well, and if the green lineage fish and yellowfins labeled as “greenbacks” in the museum collections show different meristic traits, and whether those meristic traits are also different from West Slope green lineage fish. More importantly, fitness studies should be conducted to evaluate what the consequences (if any) might result from limited genetic diversity in the Bear Creek population, and whether they are suitable for large-scale reintroduction efforts. These studies are critical to inform whether genetic rescue efforts are warranted.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*

The potential for fitness consequences may exist in this population that appears to have gone through a substantial bottleneck. Controlled studies should be implemented to evaluate the potential fitness consequences to inform future management

- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

Studies evaluating the general fitness of these fish compared to others and their hybrids should be considered in both lab and field settings.

17. Please provide other relevant comments not addressed in the above questions.

No response

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Panelist #10 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

For the most part, I believe that their conclusions are well supported. It is of value to have 5 museum collections for the South Platte River which provided more specimens than any other drainage on which to compare modern collection from Bear Creek. The possibility of the Bear Creek populations also being native to the Arkansas River basin is intriguing, but the preponderance of evidence does not suggest that that is a likely scenario. Newspaper accounts (all except one), Fish Commission reports, and the Jordan surveys suggest that Fountain Creek drainage was devoid of trout, and with the amount of fish husbandry occurring in the Pikes Peak region at the time – stocking of Bear Creek seems to be a reasonable assumption, and that the trout stocked came from the closest source (Trout Creek?) which is South Platte River in origin.

Further confusing the issue of what cutthroat was native to the Arkansas River drainage is the unique haplotype found in two of the fish from the Twin Lakes and Lake Creek 1889 collections that is also only exhibited in the S. Prong Hayden fish (and nowhere on West Slope). In addition, Jordan is his 1891 report states that the "green-back trout" was very common in the tributaries of the upper Arkansas River, and were also identical to those that he collected in the South Platte River.

This adds some uncertainty to the conclusion from the 2012 paper that green fish were likely not native to the Arkansas River basin. On the other hand, numerous (and consistent) Platte River museum specimens make it abundantly clear that the green fish (found in two of the upper Arkansas River museum collections) were not the same as the native trout to the Platte River, as Jordan suggested.

The low number of museum specimens actually evaluated, particularly for the West Slope, has been of concern. However, that is only based on a "more is better" bias, and I have not heard that this was considered a study weakness by the reviewers of the 2012 paper, or by our panel.

There was some concerned voiced that STRUCTURE should have been run with K set at more than 2. Doing so may have provided some better insight and may have altered some of the results in the Metcalf et al. (2007) paper. Some additional work with STRUCTURE (or SNP) may also be necessary to assist in sorting out the mixed AFLP vs mtDNA results for the three East Slope green fish populations.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

Using the molecular model, I believe that there is strong inference in support of the meristic work for Bear, RG, and blue; with less certainty for the green fish. Phenotypic traits, PCA and DFA all suggested that Bear Creek were substantially different morphologically. But, of course, there was no opportunity to examine museum specimens and conduct necessary morphological counts.

Blue fish grouped well phenotypically and via DFA, not surprising given their similarity (both morphologically and genetically) and appearance of "Trappers-like" attributes. Lowest CVs for 8 or 10 traits was indicative of the low variation in appearance.

Conversely, the green fish had the greatest trait variation and had highest CV values for 4 of 10 traits. The range of the widely varying characteristics was intermediate between blue and RG, which muddied the PCA results. The disconnect between mtDNA (basis for molecular model) and AFLP results for three out of the four unique east slope green populations is also a source of incertitude for the meristic study.

Bestgen suggests a couple of hypotheses that might explain the wide variation and also the distinctive morphology of the East Slope greens. One of those – that the East Slope greens may represent native trout diversity from a West Slope to East Slope invasion – was in part derived from Metcalf-identified rare haplotypes. On the other hand, using the geographic model lent support to differentiation between extant green and blue fish on the West Slope.

3. To what extent are historical spatial distributions of green, blue lineages known?

Metcalf et al. (2012) found that the later collection dates of the blue and green fish and their occurrence outside of their putative native range made the picture less clear for those two lineages, however there is more evidence in support of blue fish representing Colorado River cutthroats restricted to the White/Yampa drainages. No museum collections or extant populations of green fish in those drainages are defining.

See above (questions #1 & 2) for discussion about uncertainty in green fish historic range that is applicable here. Martin sent an email to the group on 7/31 with his views on the two alternatives to the complex green fish picture. I believe that his points suggesting that green fish are not native to the east slope (through a second wave colonization) are more plausible, and I would accept that as the best explanation until/if other information comes to light. S. Hayden and its unique haplotypes (matching two of the Arkansas River museum specimens, but also haplotypes not found on west slope) present a particularly untidy picture. But again, the preponderance of evidence suggests that the Fountain drainage was fishless including Severy Creek.

The possibility of Bear Creek fish being native to Platte AND Arkansas Rivers was suggested at the workshop, as an alternative to Bear Creek being stocked with South Platte River fish. Admittedly, there is not a smoking gun in terms of the actual stocking event of Bear Creek. However, it would stand to reason that if the Bear Creek fish were actually native to the Arkansas River that lineage would have been represented in the 1889 samples.

Although the East Slope picture is clouded for the green fish, there is more substantive support, particularly for morphology, to suggest that the green and blue were distinct on the west slope. Classification rates of these two groups were low under the geographic analysis.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

I do not have a working knowledge of other genetic cutthroat studies. However, I believe that Shiozawa's work that he explained in his presentation is of particular importance. Those investigations using over 8000 BP from numerous mtDNA genes (ND1, 2, 4L, 4, 5, 6, CytB) provides powerful resolution on cutthroat phylogenies. He concluded that Bear Creek lineage is distinct from Colorado River cutthroat trout and Rio Grande cutthroat trout.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

I can't offer anything on this question.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?

> *a. different subspecies?* Based on the evidence I have seen so far, I would feel comfortable with subspecies status for Rio Grande, Colorado River and Bear Creek (greenback).
>
> *b. distinct population segments (DPS)?* The green lineage is most in question, but mostly likely should be considered a DPS.
>
> *c. other?*

7. Is the Bear Creek population considered to be greenback cutthroat trout?

Bear Creek haplotype matches the museum specimens from 5 locations within the South Platte River drainage and provides strong inference that the Bear Creek lineage represents the native fish of the South Platte River. Since early taxonomists intended "*stomias*" to apply to fish from the Platte River (even though the type specimens are Rio Grande cutthroat trout), it seems reasonable to consider Bear Creek trout as greenbacks. I believe that this is the salient point. What that cutthroat is actually named, is of less concern to me and I will yield to those better schooled in deliberations of fish nomenclature.

8. How do we describe the East Slope green lineage?

The group generally accepted that "Meristic/morphometric analyses show consistent differences between groups defined by mtDNA haplotypes. Patterns were stronger when individuals were grouped according to genetic lineage than when grouped according to geographical hypothesis. Meristic differences were also apparent at the level of GMUs." This applies to the green lineage as well. Waples stated that the green lineage fish should not be considered as a portion of *pleuriticus*, and Martin emphatically stated that greens were not greenbacks.

But the East Slope green lineage fish issues still remains unresolved from my perspective. The disagreement between AFLP (blue) and mtDNA (green) for three of the East Slope populations needs to be resolved to further define the relationship between West Slope and East Slope green fish. As mentioned in my responses to previous questions, the shared haplotypes between the S. Prong and the museum specimens (but not West Slope green) may be a need for more investigation (SNPs?).

I did like the suggestion voiced by one of the panel members that until such time as we can further research the west/east green lineage questions, that we might consider managing the green lineage fish differently on West and East Slope. I believe that the point of that discussion was to consider affording a higher level of protection for the West Slope fish (within range), than those on the East Slope – but still with some overall ESA protection.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

With current knowledge, I favor arguments made by Metcalf that it is most likely that the East Slope green fish are a result of stocking. But phenotypic differences between east and west do add an element of uncertainty. Bestgen provides two possible explanations; the first that Grand Mesa derived stocking may have been replaced or "swamped" with existing or remaining native fish on the West Slope, but the same may not have occurred on the more sparsely populated (fishless?) East Slope; or second, that the East Slope green fish represent archetypal native cutthroat trout diversity from reinvasion from the west.

In terms of ESA issues, refer to my answer for questions #6 and 8. Likely that DPS is most appropriate for the green lineage, but with some greater protection afforded the West Slope (Colorado and Gunnison native) populations.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

Based on genetics, perhaps Shiozawa's analysis provides a reasonable hypothesis for phylogeny along with his "molecular clock". Generally, his progression supports the morphology of similarities of yellowfin, greenback and Rio Grande. The main difference between the genetic phylogeny as suggested by Shiozwa and the morphology is with blue fish (Colorado River cutthroat trout) and Bear Creek, where the Shiozawa phylogeny tree shows early divergence between Colorado River cutthroat trout and the link to Bonneville/Bear Creek and the yellowfin/Rio Grande cutthroat trout/greenback branch.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

There are perhaps more implications for taxonomy of the green lineage, rather than the blue, in part complicated due to the presence of green lineage museum specimens on the East Slope (Arkansas River). The DFA for the molecular model evaluated within the meristic report indicated all populations of blue lineage grouped together using phenotypic characteristics. The geographic model by reason of low classification rates for the west suggests distinction between green and blue, but, since the east fish were composed of nearly equal number of blue and green lineage, this poses the question of uniqueness for the East Slope blue fish. Those East Slope blues had physical characteristics intermediate between West Slope green lineage and blue lineage. Loss of genetic and morphological characteristics due to bottlenecking within East Slope populations was discussed at the workshop as a possible explanation. But generally, it is still reasonable to conclude that blue fish are represented by Colorado River cutthroats with a historic range restricted to the White/Yampa River basin.

Bestgen provides two hypotheses to explain the variation seen between East and West Slope green fish. As discussed in question #9 above, the uncertainty concerning green lineage makes taxonomic (and listing) questions more difficult without further research.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Metcalf concluded that Bear Creek fish exhibited "…*little genetic variation for loci that are typically variable in cutthroat trout populations*". Phenotypically, the Bear Creek trout were clearly different morphologically compared to other lineages in the meristic study, based on physical traits and PCA and DFA techniques. But there was no opportunity to compare Bear Creek fish to museum collections used for DNA studies by Metcalf. This is an investigation with broad support that would be important to our understanding potential inbreeding that the Bear Creek fish might have experienced over the past 130 years.

Deformities in hatchery produced progeny are common and suggest inbreeding.

One strategy for ameliorating possible inbreeding impacts that was discussed during the workshop was to maximize the size of any replicated populations and to include as many mature individuals in future spawning efforts to increase genetic recombination diversity and improve fitness.

The fitness research currently being conducted by Rogers should also provide valuable information to see if infusing green genetics provides biological benefits that would improve the ability to tolerate and adapt to other habitats.

A question came up during the workshop if some morphological characteristics are more susceptible to environmental or inbreeding impacts. It was suggested that multidimensional scaling could be used to assess environmental influences on phenotype.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

Before that decision could be made, some additional research (using SNPs) to investigate the unique haplotypes of Arkansas River green fish (S. Hayden and Severy), and the disagreement between mtDNA and AFLP results for other green populations (besides S. Hayden) should be evaluated.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

I can't offer anything on this question.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

It was noted during discussion of the Shiozawa SNP study that this line of investigation using next generation DNA sequencing has added diagnostic markers that can be used to determine levels of hybridization and introgression. It is hoped that the process used will be very helpful for management of natural resources when it is necessary to distinguish between species and subspecies. However, I am not qualified to suggest if that will be a "prudent and reasonable" research direction, nor how those might affect management.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*

Indicates a level of inbreeding that is uncommon, compared to other native cutthroat populations that have been used in hatchery production. Metcalf et al. (2012) suggested caution when using this population for future production and restoration, but suggested utilizing techniques to maximize genetic diversity and further fitness-related studies. I would agree with this approach and efforts are currently underway to do consider both of these strategies.

- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

Spawning efforts at CPW and USFWS hatcheries in 2013 will produce progeny which are planned for stocking to the wild in 2014. Monitoring and evaluation of those plants will provide our first assessment of how the Bear Creek trout fitness to survive and function in a new environment. Hopefully, on a parallel track, we will also be able to evaluate cutthroats that were purposely hybridized between Bear X green (Carr Ck) trout in both laboratory and field.

17. Please provide other relevant comments not addressed in the above questions.

No response

Panelist #11 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

Two things are worth pointing out. First, the Metcalf et al (2012) work involved many dedicated scientists and an immense effort to bring together data about the historical stocking record, modern DNA assessments of the distribution of lineages across the landscape, and painstakingly difficult and detailed analysis of museum samples. Each person had a different perspective about trout and biodiversity and we reached a consensus among the diverse group of scientists on the meaning of the data. Second, the work was published in a leading journal. Molecular Ecology is widely recognized as the best venue for publishing original research in the field of conservation genetics (see http://www.molecularecologist.com/2013/06/2012-impact-factors/). The work was subjected to rigorous peer-review, a process that improves the veracity of the work and requires absolute transparency and allows claims only when based on evidence.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

The meristics work has not been subject to peer review and so should be viewed as preliminary; nonetheless, there is clear evidence for phenotypic differentiation between green and blue lineage trout and between these two lineages and the trout in Bear Creek. Concordance between the phylogenetic and meristics results supports recognition of four distinct taxa in Colorado. The four populations of green lineage fish on the East Slope that are anomalous are best explained by 1) being out of place (the inferred ancestry of the green lineage traces to the West Slope) and 2) admixture between the green and blue lineages (based on genetic data and evidence of stocking from the West Slope to the East Slope) (see Bestgen et al. 2013, Table 2). It is possible that founder effects may have caused some of the unique patterns in the meristic data found in small populations such as South Prong Hayden and Bear Creek. A similar analysis of South Platte River lineage museum samples housed at Harvard's Museum of Comparative Zoology would help us understand if contemporary Bear Creek is morphologically similar to historic, native South Platte River cutthroat trout.

3. To what extent are historical spatial distributions of green, blue lineages known?

Given the work of Rogers and others (Martin and Metcalf, Loxterman, Shiozawa), the geographic distribution of the two lineages is well known and is unlikely to change in any significant way. The current distribution data coupled with the stocking data support the view that the green lineages' ancestral range was the Gunnison, Colorado and Dolores River drainages, and the blue lineage was restricted to the Green, Yampa and White River drainages. Nonetheless, there is some uncertainty surrounding the presence of the green lineage in the Arkansas River drainage. Although the most likely explanation is that stocking or mislabeled specimens explains the presence of the green lineage in the Arkansas River drainage in 1889, genetic and meristic analysis of additional museum specimens will shed more light on this issue.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

The taxonomy of cutthroat trout is somewhat anomalous because in many other genera, when there are clear groups based on phenotype, genes and geography, the groups would be recognized as distinct species. There is precedent for elevating some subspecies to species status. For instance, the divergence between *Oncorhynchus gilae*, *O. mykiss* and *O. apache* is equivalent to what is observed among the major subspecies of cutthroat trout, including between the green, blue, Bear Creek and Rio Grande lineages.

At the very least, 1) the Bear Creek fish should be assigned the subspecies name *O. c. stomias* and a new holotype designated that is a *bona fide* South Platte River fish (one from the Harvard collection); 2) the green lineage should be recognized as a distinct subspecies with an appropriate name; 3) the native distribution of *O. c. pleuriticus* should be officially revised so that it is not recognized as being historically present in the Colorado, Gunnison or Delores River basins; 4) the San Juan lineage should be described as a new subspecies (to emphasize its extinction).

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Yes, to our knowledge. Additionally, one aspect of the peer review process is an evaluation of this criterion; consequently, for the Metcalf et al. (2012) paper we can say that this is true emphatically. Were the citations exhaustive (meaning all relevant papers were cited): probably not.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?
- *a. different subspecies?*
- *b. distinct population segments (DPS)?*
- *c. Other?*

There are compelling data supporting recognition of *O. c. stomias* (Bear Creek) as deserving recognition as an endangered species because 1) it is rare, 2) its range is only a fraction of what it once was, and 3) there are significant threats. The green lineage and *O. c. pleuriticus* do not appear to satisfy the criteria for listing at this time because they are widespread and locally abundant on the West Slope.

7. Is the Bear Creek population considered to be greenback cutthroat trout?

Type specimen of *O. c. stomias* is actually *O. c. virginalis* from the Rio Grande basin. The common name greenback was first coined by Jordan for fish in the South Platte River, so technically, based on the data from Metcalf et al. (2012), Bear Creek fish are greenback cutthroat trout. Because greenbacks are referred to as *O. c. stomias*, it makes sense to recognize Bear Creek fish as representative of *O. c. stomias*.

8. How do we describe the East Slope green lineage?

As described earlier, the green lineage should at least be considered a distinct subspecies native to the Colorado, Gunnison and Dolores River basins. All available information should be included when formally describing the subspecies. Alternatively, the whole species complex could be revised so that the

main lineages are recognized as species and minor lineages considered subspecies. In this, we can imagine a single West Slope species (*O. pleuriticus*) that consists of three subspecies (*O. p. pleuriticus* restricted to the Green, Yampa, and White River basins; *O. p. gunnisoni* restricted to the Colorado, Gunnison, and Dolores River basins, and *O. p. sanjuani* restricted to the San Juan River). If this taxonomy is adopted, then the East Slope fish should also be correspondingly revised.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

See response to question #8.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

Our best explanation based on the stocking and genetic data is that all green lineages on the East Slope were stocked and many are likely descendents of admixture between the green and blue lineages. The fact that the meristic study revealed the East Slope green lineage fish fall between the two main green and blue clusters and three out of four show evidence of genetic admixture supports the hypothesis that they are of hybrid descent.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

Before we accept the pattern as real, I'd like to see a randomization test instead of a parameter test of the data. In other words, for the green lineage, remove the admixed populations and then randomly sample the population so that the sample sizes per slope are the same as the observed data and generate a non-parametric test. This also needs to be done for the blue lineage. This type of analysis is similar to what Metcalf et al. (2012) did for the AMOVA analysis. Importantly, when the meristics study is published, all of the raw data should be published as well, perhaps using the Dryad database as Metcalf et al. (2012) did for the data associated with the museum-based work.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Although the data were not presented at the meeting, the data are published. Most of the loci show that Bear Creek has one or sometimes two alleles whereas there are always multiple alleles present in GB or CR (see example below). Another indication of reduced genetic diversity and inbreeding depression is the high frequency of phenotypic abnormalities of Bear Creek fish when monitored in hatchery settings (see *Tiira et al. 2006. Evidence for reduced genetic variation in severely deformed juvenile salmon. Can. J. Aquat. Fish. Sci. 63: 2700-2707*).

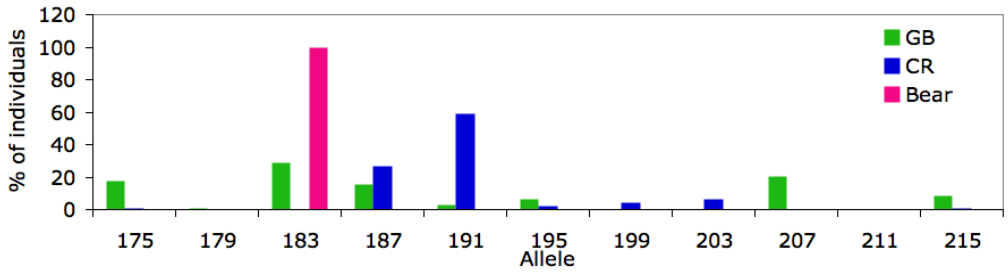

Figure 1. Histogram of allele frequencies (% of alleles sampled) for GB, CR and Bear Creek fish. This result is typical for all loci surveyed to date.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

Given that the native Arkansas River Basin fish appears to have gone extinct (based on Metcalf et al. 2012 and surveys), it makes sense to stock the native South Platte River fish (*O. c. stomias*, known as Bear Creek fish) throughout the Arkansas River simply because *O. c. stomias* is so rare. Both green lineage and *O. c. pleuriticus* are widespread and locally abundant on the West Slope (their native range) so there is no compelling reason to use East Slope habitats for propagation of lineages that are relatively abundant and native to the West Slope. Alternatively, it is possible that South Fork Hayden may represent a native Arkansas River drainage population, suggesting that decisions about whether to replace current populations of cutthroat inhabiting Arkansas River basin streams should be made on a case-by-case basis and populations without strong evidence of admixture or stocking should remain.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

The question should really not be how should NextGen be used for management, but rather, what are the most relevant questions for which we need answers? If the question is: What is the closest sister taxon to *O. c. stomias*, then NextGen can be used to sequence a large number of independent genes for the purpose of inferring phylogenetic relationships. If the question is: How much of the historic variation of *O. c. stomias* is still present in the modern Bear Creek population, then NextGen approaches can be used to sequence large fractions of the genomes of museum samples from the South Platte River and modern *O. c. stomias* from Bear Creek. Ms. Love-Stowell is currently pursuing study of modern *O. c. stomias* genomes at CU Boulder.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

The top priority, at this moment, is to establish some natural populations of *O. c. stomias* in either the Arkansas or South Platte River basins. Importantly, when this is done, the stocking and establishment of the population should be studied. This is a fertile area of research for a graduate student who is interested in combining field-based studies of ecology and population biology with genetic monitoring.

The second priority is to assess the degree that *O. c. stomias* suffers from inbreeding depression. Assessment of inbreeding depression is best accomplished using crosses and measuring the dependence of individual fitness on F (see *Jimenez et al. 1994. An experimental study of inbreeding depression in a natural habitat. Science 266: 271-273*).

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

-What conclusions can you make from these findings and what inference to future management of the lineage can you predict?
-What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?

This is a question that should be re-visited after several populations have been established to assess whether establishment is successful or not. In addition, it is important to assess the results of the fitness experiment. Overall, it will be important for all manipulations of *O. c. stomias* to be studied so that we can learn as much as possible about how populations become established and how the gene pool changes over time. This is another way NextGen sequencing can be applied to answer questions. In this case the question is: How does the gene pool of *O. c. stomias* change during establishment?

17. Please provide other relevant comments not addressed in the above questions.

We have been committed to doing the best possible science to aid the management of endangered species. We hope that we can continue to work productively together so that our efforts lead to the successful establishment of populations in nature AND provide opportunities for the next generation of scientists, teachers and ecologically informed citizens to participate in the science that should accompany the management of trout.

Panelist #12 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

I found the approach taken and the conclusions reached in Metcalf et al. (2012) to be logical and well supported by a significant amount of evidence. As a non-geneticist, the existence of six (6) historic lineages of cutthroat trout in Colorado as well as the current existence of four (4) extant lineages is well supported by the evidence presented in the study.

It appears that there are other interpretations, but none are as logical and well supported as the interpretations put forth by Metcalf et al. (2012). As Helen Neville from Trout Unlimited stated in her review of Metcalf et al. (2012), their conclusions "make sense".

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

The meristics study correlates well with the "Molecular Classification Model" proposed by Metcalf et al. (2007, 2012). The meristics study showed much less correlation with the traditional "Geographic Classification Model" that is based on separate sub-species evolving in isolated river basins on different sides of the Continental Divide.

3. To what extent are historical spatial distributions of green, blue lineages known?

Metcalf et al. (2012) provides strong evidence that the green lineage was historically distributed in the upper Colorado, Gunnison, and Dolores River basins in southwestern Colorado and east-central Utah. Green lineage populations found outside of these three basins can be logically traced back to stocking events.

Metcalf et al. (2012) provides strong evidence that the blue lineage was historically distributed in the Yampa, White, Green, and lower Colorado River basins in northwestern Colorado, southwestern Wyoming, and eastern Utah. Blue lineage populations found outside of these four basins can be logically traced back to stocking events.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

I lack the expertise to answer this question.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Both of these studies included all of the pertinent literature that I am aware of.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics & meristics studies rise to the level of a listable entity?

The preponderance of evidence suggests that there are four extant listable cutthroat trout entities in Colorado: Green, Blue, Rio Grande, and Bear Creek. I do not have the taxonomic knowledge to state whether these entities are subspecies, DPSs, or something else.

7. Is the Bear Creek population considered to be greenback cutthroat trout?

It appears that the Bear Creek fish are representatives of the native trout of the South Platte River basin. If the South Platte native trout are considered greenback cutthroat trout, then the Bear Creek population should be considered greenback cutthroat trout as well. Taxonomists need to sort out the confusion regarding the type specimen that was collected for greenback and determine which lineage should be assigned the name "greenback".

8. How do we describe the East Slope green lineage?

The East Slope Green lineage likely is comprised of green lineage fish native to the West Slope that have been stocked east of the Continental Divide. The East Slope green lineage fish originated on the West Slope and appear to have developed slightly different meristics and spotting patterns during their isolation on the East Slope (perhaps as a result of small population size, genetic inbreeding/ bottlenecking). It doesn't appear that the East Slope green lineage fish should be considered a separate listable entity (they should be considered the same as green lineage on the West Slope).

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

I don't believe that the East Slope green lineage fish should be considered a separate listable entity under ESA -- they should be considered the same entity as green lineage on the West Slope. Additional genetic testing/sequencing of West Slope green lineage fish should help clarify whether the East Slope green lineage fish possess a unique haplotype.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

I lack the expertise to answer this question.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

Again, I am not a geneticist, but it seems to me that the variation seen in the East Slope and West Slope fish is a function of genetic isolation of the East Slope fish for the past 100+ years since they were stocked from West Slope hatcheries. I do not believe that the green and blue lineage fish on the East Slope should be considered separate listable entities from their West Slope counterparts.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Not sure about the first part of the question, but logically the main short-term goal for the Bear Creek fish is to establish several self-sustaining wild populations. As these wild populations become established, gametes from different year classes and locations can be gathered to improve the genetic variability in captive reared populations.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

The evidence suggests that yellowfin cutthroat trout were the native cutthroat in the Arkansas River drainage. Since the yellowfin is extinct, it is unclear which lineage should be reintroduced into the Arkansas River drainage. Additional genetic studies of green lineage populations on the East Slope might clarify which lineage should be reintroduced in the Arkansas River basin. Further genetic analysis of the South Fork Hayden Creek cutthroat trout population might help determine if their unique haplotype is the appropriate lineage for reintroduction in the Arkansas River basin. If it becomes apparent that the East Slope green lineage fish currently in the Arkansas River basin were founded by stocking from the West Slope, then it may be appropriate to reintroduce Bear Creek cutthroat into the Arkansas River and thereby establish Bear Creek populations in both their native range (South Platte) and the other major drainage (Arkansas) on the East Slope.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

Though not my area of expertise, it sounds like nuclear DNA testing is needed to clarify the degree of introgression in many populations.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

--Conducting meristic and morphometric studies of the museum specimens from the South Platte River drainage in comparison to the Bear Creek fish should help clarify whether the Bear Creek fish are indeed native to the South Platte River.

--Meristic and morphometric studies of the San Juan museum specimens would provide insight into which of the current lineages they are most similar to.

--Intensive inventories in the San Juan drainage should continue in an effort to find an extant population of the presumed extinct San Juan cutthroat lineage.

--Projects that eliminate non-native populations and replace them with pure lineages should continue. The recent findings also re-emphasize the importance of using extreme caution when renovating/eliminating populations that include presumably admixed cutthroats – it is important not to lose important genetic material.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

-What conclusions can you make from these findings and what inference to future management of the lineage can you predict?

-What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?

No response.

17. Please provide other relevant comments not addressed in the above questions.

Hopefully the results from this workshop will allow the FWS to re-evaluate the ESA status of green lineage populations on the West Slope (and East Slope too). The current status of West Slope green lineage populations as "threatened" requires federal agencies to engage in formal Section 7 consultation on all actions that may affect one of these populations. The time and funding spent on consultation is a cause for concern for federal agencies. Re-examination of the number of green lineage populations may reveal that threatened status is no longer warranted.

We need to generate a non-technical summary of the workshop findings that can be shared with the public and with managers in the various management agencies (state and federal). Agency managers who are charged with making land management decisions need to understand the basic findings from the workshop in simple terms. Specialists within each agency can then work with managers to provide technical details as needed.

Panelist #13 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

I am not a geneticist but I am convinced that the findings of the study are sound and there is sensible and logical support for the findings.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

The findings of both studies are well correlated. I believe the conclusions of both studies. The data presented by Bestgen et al. support the Metcalf et al. findings. The results are most ambiguous for the green lineage but the statistical inferences in the paper are sound.

I also agree with Bestgen et al.'s recommendation that "Preservation of that genetic diversity, regardless of where it resides on the landscape, should be a guiding principle for future management."

3. To what extent are historical spatial distributions of green, blue lineages known?

I believe the blue and green lineages were native to the western slope and introduced on the eastern slope. But the data are limited to clarify the status of the blue and green fish on the eastern slope. We need to proceed with caution when managing the fish on the eastern slope but I do not think the blue or green lineages on the eastern slope require protection under ESA.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

I don't know.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Yes, I believe they are robust studies and well supported by the literature.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?

I think the Bear Creek fish should be protected as the fish native to the eastern slope. I do not think the blue and green lineages should be treated differently on the western slope. Additional recognition by managers to conserve and protect the blue and green lineages within their native GMUs (western slope) is warranted.

a. different subspecies?

No

b. distinct population segments (DPS)?

Possibly for the blue/green, but I'm not sure the status of those lineages on the western slope is in sufficient jeopardy to warrant listing.

c. other?

No

7. Is the Bear Creek population considered to be greenback cutthroat trout?

Yes

8. How do we describe the East Slope green lineage?

These are West Slope fish that were introduced to the eastern slope. They should not be eliminated but given the number of populations in their native range on the western slope the green fish on the eastern slope do not warrant protection under ESA.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

I'm not sure.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

I think Shiozawa's presentation is the most plausible.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

It is my opinion that those variations could be explained by small founding populations of stocked fish that were subsequently moved around on the eastern slope. The variation could have arisen from a mutation in the founding population that was then replicated.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

The approach that CPW has taken thus far seems appropriate. Introducing some variability is appropriate but a cautious approach should be taken (which is what CPW has done).

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

I would use the Bear Creek fish.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

It would be valuable to increase the sample size of fish run through that analysis. Adding different populations from each GMU (perhaps 6 digit HUC) across all subspecies would add greatly to our understanding of cutthroat phylogeny. That would of course be expensive and I'm not sure where that money would come from.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

-Extending genetic and meristic analyses to Colorado River cutthroat trout in the rest of their historic range outside of Colorado.

-Next generation sequencing of all intermountain cutthroat at a finer scale (6 digit HUC was suggested in response to question #14 above).

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*

There were likely one or more bottleneck events where much of the genetic diversity within the population was lost. The abnormalities are a concern since the overall fitness of the population is compromised. It will create a management challenge but there are brook trout populations throughout the west that were founded with just a few fish and seem to be thriving. So we should not over-react and tinker too much with the fish that have survived in Bear Creek.

- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

The work that CPW is undertaking to introduce some variation has merit. Managers may wish to PIT tag every fish in the population and then take a "stud book" approach to spawning such that individual

pairings are known and can be controlled. Perhaps split each batch of eggs and fertilize it with several individual males. It may also be desirable to check the motility of the sperm from each male and/or use more than one male with each female/batch so that the risk of failed fertilization is reduced. May also want to track hatching success of each pairing to see if there are some fish that are not contributing or have a higher level of success/failure.

17. Please provide other relevant comments not addressed in the above questions.

We need to take a precautionary approach to conservation of diversity on the landscape. But we should not be paralyzed by uncertainty. We simply must act to conserve and replicate the Bear Creek population. It needs to be replicated in other watersheds and steps must be taken to understand and manage the limited genetic diversity that exists in that population.

The blue and green lineage fish on the eastern slope were likely the result of fish stocking. In the short term I would continue to conserve them. If the Bear Creek fish can be saved they should be used to found additional populations and treated as the native cutthroat of the eastern slope.

Rio Grande cutthroat trout management should continue as it has been. Recognition of the diversity between GMUs should be incorporated into the next revision of the Rio Grande cutthroat trout rangewide conservation strategy.

Panelist #14 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

Yes and No. Of the conclusions that are supported there are some limitations that must be acknowledged. A very low number of samples were available (30 total to represent all Colorado lineages). Only mtDNA from 2 genes (ND2, CO1) totaling 430 base pairs were used in analysis. These limitations do not mean that conclusions are "wrong" but indicate a richer picture of lineages and interrelationships may exist that are unrecognized with this study.

Hypothesis One: The prevailing view of 4 divergent lineages is not supported. The conclusion of 6 distinct lineages is supported. Four of these lineages are confirmed through evidence presented in other studies (meristics).

Hypothesis Two: The prevailing view regarding the distribution of lineages (*pleuriticus* – western CO, *stomias* – South Platte and Arkansas, *macdonaldi* – Twin Lakes and *virginalis* – Rio Grande) was not supported. There is evidence for the distribution of historic lineage by drainage or adjacent drainage (but see additional comments below).

Hypothesis Three: The current distribution of cutthroat lineages differs markedly from the historic distribution could not be refuted.

The author's conclusion that there is support for 11 North American cutthroat trout clades is supported by the data presented but may be misleading. Colorado contained 6 of those 11 but 2 of the 6 have only been revealed through examination of museum samples. Based on this result, if such an effort was taken across the west many additional clades may be discovered.

The conclusion that the amount of genetic variation distributed among drainages has declined significantly is supported.

The conclusion that the Bear Creek population is native to the South Platte River and not native to the Arkansas River is supported by the haplotype data presented but could be disputed. It is true that in the analysis the purple, yellow, and green lineages do clearly separate and all historic purples samples are located in the South Platte River; however, the Arkansas River drainage is clearly more complex with two historic lineages present. I do not think we can rule out the possibility that the purple lineage was also historically present in the Arkansas River drainage and simply not represented in the museum samples.

The conclusion that the blue lineage fish were historically restricted to drainage basins on the western slope is supported.

The inference that the blue lineage was historically restricted to the Yampa and Green River drainages is not supported. This hypothesis is based on museum samples including only one fish from the Yampa River drainage which is contradicted by a single blue lineage fish collected from the Colorado River drainage. The authors' are silent regarding the blue lineage sample in the Colorado River drainage. The full historic distribution of blue lineage fish remains uncertain.

The conclusion that there was an extinction event in the San Juan River system is supported by the data presented; however, modern samples from the San Juan River drainage have not undergone systematic or extensive sampling (per Kevin Rogers) and this conclusion seems premature.

The conclusion that there was an extinction event in the Arkansas River of *O. c. macdonaldi* is well supported.

The conclusion that modern distribution is largely tied to stocking is supported in the blue lineage by matching haplotypes from stocking sources (Trapper's/Marvine Lakes) to modern blue lineages that appear "out of place" and the clear documentation of stocking events from state and federal sources. The green lineage does not fit this pattern as there is no connection between haplotypes from stocking sources (Grand Mesa Lakes) to modern "out of place" populations. Explaining modern distribution by stocking alone in the green lineage therefore could be disputed and alternative hypotheses should be explored.

I disagree with the interpretation that it is unlikely that green lineage was native to the Arkansas River. Alternative lines of evidence that support the native hypothesis include the unique haplotypes of the green lineage on the east side, matching SNP haplotypes between museum and a modern sample in the Arkansas River drainage and intermediary meristic characteristics.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?

Yes the two studies correlate in several ways. The meristic study found more support of the molecular model (Metcalf et al. 2012) rather than the traditional geographic model. It also supported a population and lineage structure organized by drainage basin. Additionally, there was support for two lineages on the West Slope. Bear Creek fish (purple lineage) and the Rio Grande cutthroat were clearly distinct from other lineages under all classification models. Blue lineage fish were also distinct from other lineages under the molecular classification model. Additionally, all blue lineage fish from the East Slope in the study had a single Trapper's Lake haplotype and when compared specifically to Trapper's Lake fish were indistinguishable meristically. This lends clear support to the stocking hypothesis of blue lineage fish from the West Slope to the East Slope.

The difference in the two studies arises in the green lineage fish where the meristic study revealed more fine-scale differences. In the meristics study the East Slope and West Slope green lineage fish differed. The West Slope green lineage is intermediate between the blue lineage and Rio Grande cutthroat. Whereas, the East Slope green lineage had traits (lateral series scale counts, basibranchial tooth counts, trunk spot counts, fore-spot and mid-spot ratios) that were intermediary between the West Slope green lineage and Bear Creek. In analyses this resulted in the green lineage fish overlapping with the blue lineage. However, a confounding factor is that three of the four East Slope green lineage populations were classified as blue lineage when nuclear markers (AFLPs) were used. The pattern of mtDNA classification in one lineage and nuclear DNA classification in another may indicate that these populations are admixed native cutthroat (blue and green lineage). This would explain why these populations could not be sorted easily into either green or blue lineages. Despite these differences the meristic data clearly show that most traits have non-overlapping 95% confidence limits between the green lineage and the blue, Rio Grande and Bear Creek lineage fish. Although more study is clearly warranted, at this time the green lineage separates as unique in this study from the other three lineages in accordance with the Metcalf et al. 2012 findings.

3. To what extent are historical spatial distributions of green, blue lineages known?

It is clear that early stocking has obscured the historic spatial distributions of green and blue lineages and there are still many questions. Based on the Metcalf *et al.* 2012 study and additional published and unpublished work it appears that the blue lineage was native and limited to the West Slope. All East Slope

blue lineage populations examined to date have an identical ND2 haplotype that is characteristic of Trapper's Lake. In addition, many of these East Slope blue lineage populations have clear stocking records. I personally feel relatively confident based on these various lines of evidence that the blue lineage is indeed a West Slope native.

The actual historic distribution of the blue lineage on the West Slope is less clear. Current evidence from museum and modern samples suggest that the blue lineage was limited to the Yampa, Green and perhaps Colorado River drainages. More work is needed to better inform the historic distribution on the West Slope.

The green lineage is more complex. Current evidence from both the museum and modern samples suggest that this lineage may be native to both the East and West slopes in the Gunnison, Dolores, Upper Colorado and Arkansas River drainages. There are also modern samples in the South Platte River drainage. Additional work is needed to clarify this species distribution and I am hesitant to make any clear statements about historic distribution based on the current evidence.

The Bear Creek haplotype matches five haplotypes in the South Platte River . This provides good evidence that the purple lineage was native to the South Platte River. It is less clear as to whether it was native to the Arkansas River.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

To my knowledge there are no comparable studies for cutthroat trout that have used meristic data. Dr. Shiozawa's most recent comprehensive phylogeny of cutthroat trout using eight mtDNA genes and 8057 base pairs provides clear evidence that the green, blue, and Rio Grande lineages are as separate from one another as are other cutthroat trout subspecies. Many additional published phylogenies lead to the same conclusion (Brunelli et al. 2013, Loxterman and Keeley 2012, Pritchard et al. 2008 and Shiozawa et al. 2012). The Bear Creek lineage is also clearly separated on Shiozawa's most recent phylogenetic tree with an estimated divergence time from the green lineage over one million years ago. This distinctness of the Bear Creek lineage from the other lineages (blue, green, Rio Grande) is at least as great as the separation between the named subspecies.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

To my knowledge yes.

Biodiversity Implications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?

- *a. different subspecies?*
- *b. distinct population segments (DPS)?*
- *c. other?*

Yes, the four lineages (blue, green, Rio Grande and purple-Bear Creek) do rise to the level of a listable entity. It seems consistent to list them as different subspecies based on the current listing of some of these

lineages and in comparison to other cutthroat trout listings. In addition the East Slope green lineage populations should be considered for listing as a DPS given the unique haplotypes present only on the East Slope and geographic separation from other green lineage populations.

7. Is the Bear Creek population considered to be greenback cutthroat trout?

Yes, based on the museum samples the historic South Platte River native fish is matched today to the modern Bear Creek fish.

8. How do we describe the East Slope green lineage?

The current research indicates that East Slope green lineage populations have unique haplotypes present only on the East Slope and geographic separation from other green lineage populations. In addition, meristically they appear a bit different than the West Slope green lineage. Although they are still clearly green lineage fish both genetically and meristically, they should be managed separately at this time.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

If a decision had to be made today based on the current evidence (and invoking the precautionary principle) I would describe the East Slope green lineage as a DPS of the overall green lineage. In mtDNA and nuclear DNA studies the green lineage populations including those of the East Slope clearly separate from the blue lineage and Rio Grande cutthroat. In the meristic study green lineage fish taken as a group had 95% confidence levels separating most traits between it and the other lineages. For these reasons the green lineage as a whole appears to warrant the classification of a separate subspecies. The East Slope green lineage fish have unique haplotypes not seen on the West Slope. Their characteristics are more intermediary with other lineages but they still seem to fall within the green lineage clade. For these reasons DPS seems an appropriate conservative designation to recognize and conserve those geographic and genetic differences within the green lineage.

A better approach would be to look at East Slope green lineage fish in more detail. In particular meristic data from green lineage populations (East and West Slope) that have no evidence of possible admixture with blue lineage, rainbow or Yellowstone is needed to see how similar or different these are from one another. The current meristic study unknowingly used “green” lineage populations that sort as blue lineage in program STRUCTURE. This may indicate admixture or simply the difficulty STRUCTURE had in assigning these populations. If admixed, this likely explains the meristic overlap between green and blue lineages and it would be informative to see if these lineages will clearly separate when “pure” populations are used. Secondly, additional exploration of all East and West Slope green lineage population haplotypes is needed to determine if any shared haplotypes exist with the West Slope. This knowledge could be used to further refine a DPS description for the East Slope and potentially include only those populations with unique haplotypes.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

Yes to an extent. There are multiple differing phylogenies in the literature but several recent publications have converging phylogenies. It seems fairly accepted in the published phylogenies that all four lineages

(Rio Grande, blue, green and Bear Creek) came from a common ancestral line (Brunelli et al. 2013, Loxterman and Keeley 2012, Metcalf et al. 2007, Pritchard et al. 2008, and Shiozawa et al. 2010). The blue lineage appears the first to separate which explains its positioning in many phylogenetic trees on a separate branch from that of Rio Grande, green lineage and Bear Creek (Loxterman and Keeley 2012, Metcalf et al. 2007, Pritchard et al. 2008, Rogers 2012, and Shiozawa et al. 2010). Rio Grande and green lineage then separate and it appears that Bear Creek descended from green lineage. This leads to two potential routes of colonization. The first is that cutthroat entered Colorado from the NW and then the Rio Grande, green and Bear lineage moved south and eventually East over the Continental Divide diversifying along the way. The second route is two separate events, one from the NW establishing the blue lineage and one from the West/Southwest which moved East establishing the Rio Grande, green and Bear Creek lineages. In either case the more established historical view that green lineage descended directly from blue lineage has less current support than green lineage descending from Rio Grande.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

The East Slope-West Slope variation in the blue lineage is not significant. All East Slope blue lineage populations to date correspond to a common Trapper's Lake haplotype. Many of these populations also have a clearly documented stocking history. When meristic data are used to compare East Slope blue lineage fish with fish of the same haplotype found on the West Slope they are indistinguishable from one another. The reason the East Slope blue lineage fish may separate from the rest of the blue lineage is because all East Slope populations have identical haplotypes and therefore present a narrower and more similar appearance to each other than the blue lineage as a whole.

The green lineage variation does show significant variation between the East and West Slope with no clear explanation. The current research indicates that East Slope green lineage populations have unique haplotypes present only on the East Slope. Phenotypically the East Slope green lineage fish have more total spots and higher fore-spot and mid-spot ratios. These characteristics are more similar to the blue lineage whereas the West Slope green lineage fish were more similar to Rio Grande (less spots, lower ratios). There are several potential explanations for these differences. 1) Stocking hypothesis – the East Slope green lineage fish were stocked by West Slope sources. Over time the haplotypes were lost on the West Slope and now only remain on the East Slope. 2) Native expansion hypothesis – The green lineage naturally expanded its range and crossed the Continental Divide. Founding effects resulted in only a small number of haplotypes becoming established on the East Slope and these were subsequently lost in West Slope populations. 3) Potential admixture hypothesis - A potential confounding factor is that three of the four East Slope green lineage populations studied for the meristic research were classified as blue lineage by program STRUCTURE when nuclear markers (AFLPs) were used. The pattern of mtDNA classification in one lineage and nuclear DNA classification in another may indicate that these populations are admixed native cutthroat (blue and green lineage) which could obscure the analysis. It also could simply be a STRUCTURal problem. More research is needed to more clearly delineate which of these hypotheses may be more likely. Until then the East Slope green lineage should be managed cautiously to protect the populations and unique haplotypes present.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

Genetic variability is clearly lacking as shown by A. Martin. As recovery efforts move forward it is first important to replicate the existing Bear Creek population in several areas to insure their protection in the future. Once that step is complete additional populations can be established with Bear Creek fish and adding some diversity from another population, probably of green lineage, which appears to be most closely related. These efforts will need to be carefully planned and monitored and the details go beyond the scope of the workshop and my personal knowledge.

Surprisingly the range of phenotypic variation in the Bear Creek fish is similar to the green and blue lineages for the traits measured. Despite this, attempts at hatchery rearing have been challenging compared to green and blue lineage fish. This may indicate that genes driving fitness have less variability and therefore Bear Creek fish are less able to survive under a variety of circumstances.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

Based on the information presented both the green lineage and Bear Creek lineage may be appropriate to the Arkansas River basin. There is a possibility that the green lineage is native to the Arkansas River basin. If additional information continues to support this hypothesis then the East Slope green lineage would be most appropriate for reintroduction. Conversely, if evidence supports that the East Slope green lineage populations are much more likely to be the result of stocking and are indeed native to the West Slope then Bear Creek fish would be preferred. Although there is no evidence in museum samples that Bear Creek fish are native to the Arkansas River basin it is still possible that they were and simply were not preserved historically into the museum collections. The barriers between the South Platte and Arkansas River drainages are far less than crossing the Continental Divide which is why the Bear Creek fish would be preferred over green or blue lineages for reintroduction.

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

I have limited knowledge on next-generation sequencing. This technique shows promise and may be useful in answering the remaining research questions outlined in question 15.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

Several key research priorities have come to light. One overarching goal is to make sure that all museum samples within the range of any of the Colorado lineages have been found. The focus for the Metcalf et al. 2012 study was greenback. Although it may be unlikely that more museum samples exist, a thorough search for specimens from West of the Continental Divide would be helpful in identifying the historic range and distribution of haplotypes present for all lineages.

<u>Green Lineage</u>: This scientific review process revealed that a key priority must be exploring the green lineage, especially the East and West Slope differences. Specific research may include 1) analyzing all extant green lineage populations on both the East and West Slope to identify the distribution of

haplotypes; this will help inform the stocking vs. native hypotheses for the East Slope and specifically the Arkansas River basin. 2) Sequencing the East Slope green populations used in the meristic analysis is important to determine if admixture is of concern for the current results and conclusions. Additionally, analyzing more East Slope green populations to see if the meristic differences continue to consistently show the East Slope green populations as "different" will provide evidence for whether DPS or another listing is warranted. In addition, further study on the West Slope of both green and blue lineages is needed to inform their historic distribution, especially for the Colorado River drainage.

Blue Lineage: As stated above the key question for blue lineage is identifying historic distribution. One of the blue lineage museum samples was in the Colorado River drainage and more research will help determine if this is likely the result of stocking or if some native blue lineage populations naturally expanded into this drainage.

San Juan River: For the San Juan River, searching extant populations to see if any San Juan fish do still exist is critical. In addition, examining museum specimens to look at meristics such as spotting patterns on the San Juan River fish would be of interest.

Bear Creek: With Bear Creek as the only extant South Platte River lineage population it would be useful to examine the South Platte River museum samples to determine the original variability for meristic characters studied. Using these data in comparison to Bear Creek will help determine if the Bear Creek fish are representative of the South Platte as a whole or phenotypically unique due to small population size, population bottleneck and genetic drift. Fitness studies as mentioned in question 16 (below) will also be important for robust recovery of the subspecies.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

-What conclusions can you make from these findings and what inference to future management of the lineage can you predict?
-What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?

The paucity of genetic variation in the Bear Creek population may explain these differences. It is still important to keep and replicate the Bear Creek population in several locations with its current genetic make-up to safeguard this piece of the cutthroat puzzle. The long –term management of this species likely cannot rest on the Bear Creek population alone but will need to include a plan to systematically diversify targeted replicate populations by adding one or more closely related individuals.

There is an ongoing fitness study in the hatchery crossing Bear Creek and green lineage fish. This study should be replicated in a field. In addition, a study to add diversity at a much lower introgression level, such as one individual per generation, can be implemented and closely monitored. This would elucidate whether a much lower level of crossing between Bear Creek and green will be effective at lowering or eliminating deleterious effects.

17. Please provide other relevant comments not addressed in the above questions.

I feel I have answered and commented on all areas pertinent to the workshop.

Panelist #15 Responses to Discussion Questions

Evaluation of the Science

1. *Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?*

The conclusions reached in the Metcalf et al. paper represents a logical progression in the genetic studies conducted during the last two decades. Improvements in techniques and an accumulation of new information lead to their conclusions related to cutthroat trout lineages in Colorado. Historical stocking data as well as meristic/morphometric studies support their findings. The identification of 4 populations on the East Slope of Colorado that do not follow the normal "pattern" leads to some speculation. Were there multiple invasions? Could the current major drainage locations possibly been altered over time through natural stochastic events and influenced contraction and expansion of different lineages? It would appear possible that further "refinement" may not be realistically possible. If further refinement is possible then further separation of the Metcalf results could result in historic habitats for a given lineage that we could manage over time. From that standpoint, their results provide a geographic area that is large enough that individual lineages and their populations could be managed.

2. *Does the meristic study correlate with findings in the genetics study (i.e., does the meristic study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Cr, and Rio Grande)?*

Yes, although I would have thought initially that the green and blue lineage would be more closely related than the Rio Grande and Bear Creek. Thus it still could explain that the green lineage as well as front range lineages moved into the current geographic areas at a different time period than the blue lineage, or are from stocking.

3. *To what extent are historical spatial distributions of green, blue lineages known?*

I don't think we understand the temporal migration of all the lineages, and unless we locate fossil or bone remnants probably won't. We can speculate. However, it may be more important to understand (agree) on the current "boundaries" of the lineages and move forward to restore robust populations.

4. *How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?*

The current results appear to be significant and explain the recent extent of the lineages. The studies we have now are as robust as I have seen. I believe they are as sound as any others available.

5. *Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?*

Yes

Biodiversity Implications: Listable Entities?

6. *Do lineages identified in genetics and meristic studies rise to the level of a listable entity?*

To be consistent with other lineages, it appears that there are 4 living subspecies and 2 extinct. However, socio/political influences could have an influence on nomenclature that should be avoided. One comment I overheard from a panel member was "based on their involvement the separation both genotypically and phenotypically are so far removed from the other lineages they should considered a new species". While I have no experience addressing nomenclature issues, this level of classification should not be totally ruled out.

7. *Is the Bear Creek population considered to be greenback cutthroat trout?*

Yes, although the range has been changed to the South Platte River drainage only.

8. *How do we describe the East Slope green lineage?*

One speculation was that it was part of multiple movements over time, with these populations being left. We also can't rule out human movement of these fish. This is one of the questions to be answered. It appears to me that they are a result of some stocking to me.

9. *What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?*

This question is too vague to answer. The term "subspecies" is a name under ESA. If the question relates to the 4 East Slope green lineage populations I think we know too little to make these type of conclusions.

10. *Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroats?*

The studies focused primarily on differences between the different lineages. To me, the introduction of the green lineage presents new information that may help answer this question. However, there are still questions and there are never easy answers to these questions. Even within a given basin, it seems that there would be many areas devoid of trout. It would seem that these trout would have continued to disperse into new habitats, except for the influence of European settlers.

11. *Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?*

I don't completely understand this difference at the moment but it definitely seems to point out that we don't understand all the movement that occurred, either through multiple climatic events or through human stocking. There are probably always going to be some taxonomic implications we are not confident with. This could be a question for future research.

Management Implications

12. *The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?*

The results of the current studies show that phenotypically as well as genotypically the Bear Creek trout are indeed very distinct from other lineages, but appear to be the same as early collections from the 1800's. While these fish are different from other lineages, they have fairly high phenotypic variability within the population. In addition, the progeny in one generation in a hatchery situation revealed basibranchial teeth not present in the wild. There could be more diversity than we think if placed in new habitats.

I believe that reintroducing new genes or hybridization studies would be premature, based on these findings. It would seem that understanding what adaptations within the Bear Creek trout when placed in new environments would be a better first start. I also would suggest that this situation would provide an opportunity to examine the potential for recovering from a strong "bottleneck" situation when placed in new habitats.

13. *Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin*?

At this point, I would be more concerned with disease introductions and/or potential human movement into the wrong drainages by the public. Most of our limited funding should be used for the study and introduction of the lineages we are more confident in.

14. *How next-generation DNA sequencing approaches should be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?*

I think the most important aspects are "firming" the information and data already used to make conclusions with. Anything that helps show the spatial movement over time with reference to the evolution of the lineages would be very helpful.

15. *What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?*

Management first – The South Platte River drainage is going to be far more difficult to find high quality habitat if for no other reason than socio/political reasons. The extremely high population growth and past and current drought related fire conditions will no doubt influence the ability to restore streams. In order to make a compelling argument for reintroducing Bear Creek fish a very thorough knowledge of land use, productivity and availability are needed. Decision makers are going to need information that relieves political and social pressures if we are going to be successful in getting permissions to work there. The recovery team will also have to be responsible in identifying "criteria" for identifying recovery sites. Priorities for recovery should not be made on "convenience", "lack of conflict", or "short term time frames".

Research – Obviously there are a number of avenues that could be researched. One prudent research topic I suggested previously discussed is understanding better the evolutionary pathway for these different lineages. This type of research would help understand more clearly the final nomenclature of the taxa. I

also do not agree that research on hybrid "vigor" should be our first step in restoration. Given the variability in the Bear Creek fish, we should look at the effect that placing populations in new habitats would have. Understanding habitat, as well as other physio/chemical parameters would be important for restoration. Currently we are biased by our own limited experience. Possibly research on social acceptance and what the public is willing to consider if a restoration project was introduced in an area they were familiar with or visited.

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

> *- What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*

After hearing similarities in other cutthroat trout rearing efforts I believe the abnormalities could be attributable to conditions in hatcheries probably more than in the wild. Different water quality, temperature, feed and growth could all attribute to these abnormalities as least as much as any genetic homogeneity.

> *- What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

I think the research that Kevin Rogers is proposing would help. I also think that studying the fish that are put in the wild in different environments would give us useful information on their plasticity physically as well as their adaptability.

17. Please provide other relevant comments not addressed in the above questions.

This effort is extremely interesting and represents as much art as science. While we would like to predict how these fish evolved and moved, I'm not sure we aren't following our own biases for some of our answers. There are so many confounding issues that face us that it is difficult to come to a very confident conclusion. For now I'm comfortable going with the group consensus. Maintaining current lineages and improving rare lineages is of more concern to me. While hopeful, I am not convinced that social values will allow us to be very successful

Panelist #16 Responses to Discussion Questions

Evaluation of the Science

1. Are the conclusions reached by Metcalf et al. (2012), including the identification of distinct cutthroat lineages and inferences based on historical stocking, logical and supported by the evidence provided in this study? Are there alternative interpretations?

Yes, I believe that based on the evidence presented at the workshop (including supporting stocking records and historical information) and in their paper that their conclusions are supported relative to the distinct 6 lineages as identified therein.

The only question I have is about resolution of the West Slope and East Slope green lineage fish and if the South Fork Hayden Creek fish are native to the Arkansas River drainage rather than the West Slope.

2. Does the meristic study correlate with findings in the genetics study (i.e., does the meristics study show a difference in phenotypic characteristics between blue lineage, green lineage, Bear Creek, and Rio Grande)?

Yes the studies are remarkably consistent and reinforce the differences between lineages.

3. To what extent are historical spatial distributions of green, blue lineages known?

I believe that the historical stocking records are remarkably intact and represent the best information/ evidence we have to delineate the historical distributions of these fish. Inaccuracies aside which we have no basis for evaluating, I believe we have a fairly strong concept of where these fish originated from (supported by genetic data) and where they were moved to within a reasonable margin of error.

4. How does genetic and meristic variation identified in the studies compare with variation in other cutthroat trout studies? Are levels of variation consistent with differences observed across species, subspecies or ESUs in other cutthroat trout?

I cannot comment.

5. Did the genetic and meristic studies include all the necessary and pertinent literature to support their assumptions/arguments/conclusions?

Not familiar enough with the current/past literature to comment.

BiodiversityImplications: Listable Entities?

6. Do lineages identified in genetics and meristics studies rise to the level of a listable entity?

a. different subspecies?

Yes, blue and green, Rio Grande and Bear Creek fish are different subspecies per genetics and meristics data.

b. distinct population segments (DPS)?

The Bear Creek fish may fit the designation of discreteness and significance for a DPS. It is also markedly separate from other populations of the same taxa.

c. other?

7. Is the Bear Creek population considered to be greenback cutthroat trout?

No. Dr. Shiozawa's data show separation between Bear Creek and "greenbacks" 1 million years ago. Greenback cutthroat trout are the fish from the South Platte River but maybe due to founder effects, Bear Creek fish, while the ancestor is from the South Platte River, are no longer "greenback" trout.

8. How do we describe the East Slope green lineage?

Unclear on this issue, more data may have to be analyzed to answer this question more fully and their relative differences with the West Slope green lineage. Both genetic and meristic data suggest low variation and that these two green fish lineages are different.

9. What do rare haplotypes and morphological consistencies of East Slope green lineage fish suggest in terms of subspecies or ESU distinctions?

I cannot comment.

10. Do genetic and meristic studies provide any resolution to probable routes of colonization for green, blue, greenback and Rio Grande cutthroat trout?

Yes, Dr. Shiozawa's SNP data allows for more genes to look for variation and, as such, provided a compelling argument for how these fish diverged and became isolated over time.

11. Is the East Slope - West Slope variation seen for green and blue lineages significant? What could lead to those differences and are there any taxonomic implications?

I cannot comment.

Management Implications

12. The Bear Creek lineage exists as a single small population. What is the evidence for limited genetic and meristic variability compared to green, blue lineages? What approaches, if any, should be considered to manage genetic variability in this lineage to ameliorate potential or actual inbreeding effects?

AFLP data and basiobranchial counts (meristics) both indicate low variability in Bear Creek fish. Also difficulties encountered in hatchery (deformities) also suggest a more homogeneous population when compared to green and blue lineages.

I do not advocate for introducing additional genetic material or "forcing hybridization" but instead translocating Bear Creek fish to a range of different habitats (gradient, temperature, aspect, etc) and

letting genetic selection exert pressure on each of these populations. Mixing them then at a later period could then help preserve the extant genetics left in this population.

13. Which lineage or subspecies should be considered for reintroduction as the native cutthroat for the Arkansas River basin?

Since the yellowfin is considered extinct, there is no native cutthroat for the Arkansas River Basin

14. How should next-generation DNA sequencing approaches be used in Colorado River, Bear Creek, and Rio Grande cutthroat trout management?

I think these new techniques should be used to determine which fish are stocked in which drainage and be used to double check all hatchery stocks and wild populations to ensure that the same mistakes aren't made again.

15. What are other prudent and reasonable management and research priorities for the species given the outcome of these studies?

1. Resolution of West Slope green vs. East Slope green fish
2. Survival of different stocks over time and differences in recruitment strength
3. Translocation success of fish into different habitats

16. Bear Creek trout sampled in the wild do not appear to have physical abnormalities, while fish from eggs collected in the wild and reared in a hatchery often have noticeable abnormalities; similar to, but potentially greater than some other stream and lake spawning attempts east of the Continental Divide in Colorado.

- *What conclusions can you make from these findings and what inference to future management of the lineage can you predict?*

That these fish originated from a small founder population and moving them to different habitats will be challenging and potentially problematic as they have little environmental plasticity in which to weather variation.

- *What steps or research could you take to better understand how these trout could successfully produce viable populations if replicated in streams in the South Platte River drainage?*

I would introduce them into a variety of habitats to see where they do best and survive.

17. Please provide other relevant comments not addressed in the above questions.

No response.

Appendix F

Public Comments
Received on the
Greenback Cutthroat Trout Genetics and Meristics

July 26, 2013

Leslie Ellwood
USFWS/ES/Colorado Field Office
134 Union Blvd, Suite 670
Lakewood, CO 80228

Re: Greenback Cutthroat Trout Scientific Review Workshop

Dear Leslie:

We write on behalf of Trout Unlimited and appreciate this opportunity to provide comment. As you know, we have long been involved in conservation work on Bear Creek and Colorado TU and its Cheyenne Mountain Chapter were financial contributors toward the Metcalf et al. study that is a part of the information you will be reviewing through this workshop.

Our ability to provide meaningful comments to this type of workshop is very limited – both by the nature of the process in which we cannot interact with your reviewers and raise questions, and by the fact that one of the critical studies – the meristic analysis from Dr Bestgen – has not yet been made available to the public. We hope that study will soon be published to help inform the interested public. If the study's publication is delayed – even as the Fish and Wildlife Service takes up important Endangered Species Act issues for Colorado's native cutthroat trout – then its results should be released to the public, so that stakeholders can have informed involvement.

While our ability to offer meaningful input is limited, we asked Dr. Helen Neville, a research scientist and geneticist with Trout Unlimited, to prepare some basic comments on the Metcalf et al. study drawing from her own expertise in the field. Those comments are attached.

We appreciate the importance of having a frank discussion among various experts in the field to help provide the Fish and Wildlife Service with a scientific basis for decisions on classification and listing of native cutthroat trout. At the same time, we ask that you maintain as open of a process as possible. In the interest of "sunlight" on agency efforts, we urge you to promptly release a list of workshop participants and information on what areas of scientific agreement and disagreement are identified. Without such information, the public cannot engage in a meaningful dialogue. We encourage you to follow a reasonable sequence – developing technical information through processes like this workshop, providing results to the public, and then using the combination of public input and technical data to inform development of a draft listing rule. Given the important partnerships that have been developed on Bear Creek, a specific briefing for the Bear Creek Roundtable would also be appropriate.

We appreciate the chance to offer these comments, and look forward to more extensive agency engagement of the public as your review of Colorado's native cutthroat trout proceeds.

Sincerely,

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To Whom It May Concern,

I would like to provide brief comments on the recent genetics study by Metcalf et al (2012) which is to be reviewed in the upcoming Scientific Review Workshop planned by the U.S. Fish and Wildlife Service (FWS) and the Greenback Cutthroat Trout Recovery Team.

My own genetic work on native (e.g., Neville et al. 2006) and non-native (Neville and Dunham 2011; Neville and Bernatchez 2013) trout in the West confirms the complex nature of factors contributing to modern diversity, and has emphasized to me the need to incorporate information from powerful genetic tools *and* historical perspectives on human activities when interpreting patterns of biological diversity. Earlier work by Dr. Metcalf et al. (2007) had already indicated our understanding of the historical distribution of Colorado and Greenback cutthroat trout had been clouded by the large-scale and intensive stocking practices initiated by the late 1800s, even if the authors were unable to resolve relationships fully. In this more recent study, the evaluation of museum samples provides important clarity on this issue and uncovers a surprising template of native diversity in southwestern cutthroat trout.

Concerns over technical matters will undoubtedly be raised in light of such controversial findings. Indeed, in any genetic study involving museum samples, degradation of ancient DNA and the potential for contamination from more robust modern DNA pose serious challenges. However, Dr. Metcalf and colleagues undertook extensive and appropriate measures to ensure the quality of their data by providing laboratory containment measures, amplification controls, and cross-validation from two different well-respected labs. The small number of samples available from museum specimens is certainly a limitation in this study, but these few samples likely represent the limits of knowledge we will ever gain as more extensive historical samples are unlikely to be uncovered; though it may not be fully representative of all previous variation, this small set of samples certainly sheds important light on historical patterns in cutthroat trout. Overall, the statistical analyses in the study were valid and the conclusions drawn were appropriate and 'make sense' in light of major hydrologic basins and the known stocking history of Colorado.

It is unfortunate that two trout lineages are already apparently lost, but equally fortuitous that other diversity was uncovered at the same time. Perhaps even more remarkable is the fact that these newly-uncovered biological resources are still in existence today, and may provide an opportunity for future recovery of significant evolutionary diversity in cutthroat trout. Lahontan cutthroat trout provide an interesting parallel, where fish from an early 20th Century out-of-basin transplant to Utah are now being used for Lahontan cutthroat trout reintroductions to Pyramid Lake, Nevada, after genetic confirmation of this lake and river system as their origin (see recent New York Times article cited below). Results thus far are promising in this effort to re-establish the world's largest cutthroat trout, with the reintroduced strain demonstrating unusually fast growth rates, and emphasize the importance of recognizing and maintaining critical genetic diversity in management of native trout.