Final Report

Extent of Endocrine Disruption in Fish of Western and Alaskan National Parks

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I. INTRODUCTION

Understanding park ecosystems requires knowledge concerning quality of habitat for the biota using those habitats. It has recently become evident through the Western Airborne Contaminant Assessment Project (WACAP) that aquatic habitats of Western and Alaskan national parks (NPs) can be contaminated with anthropogenic substances, such as organochlorines (Landers et al. 2010). It is also important to have insight into possible individual and population-level effects of habitat conditions in the parks, particularly to key vertebrates such as trout that are important in their own right and cornerstones of higher food webs (Schwindt et al. 2009).

As part of the Western Airborne Contaminant Assessment Project (WACAP) (http://www.nature.nps.gov/air/studies/air_toxics/wacap.cfm) we (Schwindt et al. 2009) found that endocrine disruption has likely occurred in trouts of ecologically protected and remote environments in western NPs. Intersex salmonids, a gonochoristic taxon, and males that produce the female egg yolk precursor vitellogenin (Vtg) were identified in a majority of sub-alpine lakes in national parks of the Rocky Mountains. It has been well established that these effects can result from exposure to substances, anthropogenic or in rare cases natural, that act as or interfere with hormones; these are called endocrine disruptors. There are many contaminants that are endocrine disruptors; those that act as estrogens (female sex hormones) are widespread and known to be able to interfere with the sexual development of fishes. Exposure to such estrogenic substances during the time when the gonads are formed and normally differentiated into testes or ovaries can cause abnormal gonadal development in genetic males. This can lead to development of one or more female gametic cells amongst the testicular cells; we refer to such fish as "intersex males". Exposure of males to estrogenic compounds at other life stages can lead to feminization of other characteristics, such as the appearance of yolk precursor (Vtg) in their blood.

The thought is that the reproductive capacity of populations of fish containing feminized males is lower than in non-endocrine disrupted populations (Kime 1998). Further, the WACAP study showed that the proportion of intersex fish is significantly higher in samples obtained from 2003–2006 compared to 1871–1934, suggesting an increase in their frequency over time. We did not find similar effects in national parks of the Sierra Nevada, Cascades, Olympics, Brooks or Alaskan Ranges. Our ability to determine if the frequency of occurrence of intersex fish in far western parks is indeed similar to those of the Rocky Mountain parks was hindered by the low statistical power inherent in our data from the far western parks because of sample size. We were only able to obtain a limited number of fish from a few locations in the far western parks (2 from each park) compared to the Rocky Mountain parks where more extensive sampling was possible in terms of both sites and fish numbers. Hence, we were unable to rule out the possibility of false negatives regarding intersex condition. Similarly, we were unable to establish correlation of intersex condition with contaminant burden. Additionally, there is strong evidence that Vtg is related to the body-burden of some organochlorines (OCs) in fish from Lone Pine and Mills Lakes, Rocky Mountain NP (WACAP). While estrogenic responses in fish are well-documented in polluted areas (Garcia-Reyero et al. 2007), our recent research is the first demonstrating this phenomenon in some of the most ecologically protected environments.

Organochlorines are widespread (Simonich and Hites 1995; Ackerman et al. 2008), may be estrogenic (Garcia-Reyero et al. 2007), and accumulate in remote mountain ecosystems (Blais et al. 1998), including fish. Once incorporated into the foodweb, OCs can be directly measured and related to changes in biomarkers of contaminants in fish. Biomarkers of estrogenic chemical exposure provide insight into reproductive dysfunction (Harris et al. 1997) from which inferences of reproductive capacity can be made (Jobling et al.1998). Our results indicate that OCs are likely transported via the atmosphere (Hageman et al. 2006) to high elevation and remote environments where they accumulate in the food web and are associated with reproductive dysfunction in fish. There are other site-specific, non-toxicant related explanations regarding intersex occurrence such as genetic-founder effects, but they are less probable. We also found that the abundance of pigmented macrophages (blood cells that "eat" tissue debris and other particulates) appears to be a reasonable biomarker of contaminant load, in particular mercury. Some western parks and those in Alaska exhibit trends of elevated macrophages (Schwindt et al. 2008). Plasma levels of vitellogenin, an egg yolk precursor protein, are an excellent biomarker of estrogen exposure, but analysis of this protein requires centrifugation in the field and storage of frozen samples soon after collection. Therefore, while Vtg was an important indicator in the WACAP study, we could not measure it here. Our modeling efforts as part of the WACAP suggest that the vertebrate biotic communities (fish, birds, and mammals) that use the waters where endocrine-disrupted fish are present can have reduced birth rates and in some cases population abundances.

This report describes results of work done subsequent to WACAP to expand the range of assessments concerning intersex condition of fishes resident in western parks. In addition, the work also expands the number of sites in Rocky Mountain National Park so that the coverage is more extensive for that system.

The overall goal of this project was to assess the general health of fish from eleven western national parks to infer whether health impacts may be linked to contaminant health thresholds for animal and/or human health. This was accomplished by evaluating the presence of intersex (fish with eggs developing in male gonads or sperm developing in female gonads) using histology. In addition, endocrine disrupting compounds and other contaminants were quantified in select specimens. General histologic appearance of the gonadal tissue and spleen were observed to assess health. Specific objectives of this work included:

1. Establish the extent of endocrine disruption of fishes in National Parks/Monuments/Historic Preserves of the Western U.S. and Alaska, by assessing the presence of intersex condition in salmonids from these parks. Specifically, the parks involved are Crater Lake, Glacier, Grand Teton, Great Sand Dunes, Lassen Volcanic, Mount Rainier, North Cascades, Rocky Mountain, Sequoia & Kings Canyon, Wrangell-St. Elias, and Yosemite.

A secondary objective emerged during the course of this study, and that was to establish if not only intersex males could be present, but also if intersex females (fish with ovaries containing male gametes) could be present in waters of these parks. This was to demonstrate if apparent observations of intersex females were actually procedural artifacts (which turned out to be the case). This work is referred to subsequently as the "female diagnostic accuracy experiment."

Determine concentrations of contaminants in a minimum of 30 fish, including at least 7 fish from Rocky Mountain NP. Those fish "positive" for biological effects will be analyzed first as a step towards establishing cause-effect, however those fish "negative" for biological effects could also be used in order to broaden the interpretation. Results from this portion of the study are presented and discussed in a separate report (see Simonich et al. 2012); hence, only conclusions from that work are considered in the present report.

Disseminate findings of this work to the scientific community and general audience via presentations at scientific meetings and the peer reviewed scientific literature if feasible.

II. METHODS

The above objectives were met by analysis of fish specimens obtained from the parks. A sampling protocol (see Appendix A) was provided to each participating park that included detailed instructions for tissue sample collection, preservation, and shipping, and photographs for identification of organs to aid in collection. We also provided sample storage containers (cuvettes), plastic bottles for holding all collection cuvettes, and buffered formalin for fixing and storing the tissues. The sampling protocol also provided instructions for freezing and storing entire fish for contaminant analysis, if feasible, after the organs were collected. We coordinated the sampling efforts in both 2008 and 2009 with local NPS personnel, with the assistance the NPS project officers in Denver. In addition, we personally, with the assistance of NPS personnel, collected fish from Crater Lake National Park in 2010 for QA/QC purposes, what were called the female diagnostic accuracy experiment. While the collection methods for fish may have differed among water bodies sampled (e.g., gill netting or electrofishing), our analyses are not influenced by fish collection method. Park personnel provided consistently good samples across the parks and the field notes that accompanied the samples were professional and thorough. Our goal of obtaining juvenile fish well past the stage of gonadal differentiation and/or as adults of various ages was met.

Analytical protocols followed those developed for WACAP and described by Schwindt et al. (2009). Similar QA/QC standards were also followed. Gonads for histological analysis were fixed in buffered formalin and then sectioned for hematoxylin-eosin staining. Slides were read under a compound microscope. At a later date all slides were re-read to ensure accuracy of findings.

Female Diagnostic Accuracy Experiment

The ability to diagnose intersex males can be done with 100% assurance that testicular material that contains one or more female gametes came from an intersex male (see discussion in Schwindt et al. 2009). The analysis of the slides from female fish collected as part of this study suggested that numerous fish from numerous locations could be intersex females as evidenced by the presence of minute amounts of male gametic cells on slides of female gonad tissue. Given that this would be a novel and rather surprising finding, we sent representative photomicrographs of some of these slides to five international colleagues – experts in the area of sex differentiation in fish – for their interpretation. They all concluded that there were indeed male gametic cells present in or on the ovarian tissues, and that these fish could be intersex females. Such a

condition had heretofore been seen by only two of these experts, and that was in a single fish each collected from the exact same location but at a different time. However, histological artifacts can happen, where one cell type or tissue from one sample can contaminate another organ from the same or different individual during collection or histological processing. Spermatids or sperm could be a likely candidate for such an event, leading to a false positive diagnosis, which, after additional QA/QC, turned out to be the case here.

We conducted an experiment to shed light on the accuracy of our diagnosis of the intersex female condition. In 2010, we collected kokanee salmon and rainbow trout and from Crater Lake National Park using gill nets set by NPS personnel. For females that were collected, we placed one ovary from each fish into an individual cassette and then into the mass container of buffered formalin. We placed the other ovary from each of the same fish into an individual cassette and then into the mass container that contained the testes cuvettes that were sampled from all of the males obtained from the gill net. Samples from each species were kept separate. Equipment and solutions used for histology were cleaned between sample types; that is, between processing ovaries that had never been held together with testes and those that were placed in the same container of fixative with testes. That is not to say that our normal histological sectioning process is unclean, for we follow routine medical histologic techniques. Hence, blades and instruments that come in contact with our tissue samples were always wiped between samples; but they are not scrubbed in tissue processing solutions. We were thus able to perform histology and analysis on ovaries that could not have been and those that could have been contaminated with testicular material.

Because of the findings from the samples obtained in 2008–2010, we determined that it could be informative to reexamine the histological preparations for female collected during the WACAP project. Therefore, all of the slides representing females from that study were reinterpreted.

III. RESULTS

Table 1 provides a summary of the specimens provided and the histological interpretation of the results. Specifically, Table 1 lists the national parks that provided samples, the names of the bodies of water from which the fish were collected, the sampling year, the species collected, the number of samples that we analyzed, the number of males and females amongst those samples, and the number of intersex males or females found. Basically, we obtained 998 fish samples from 11 parks in 2008 and 2009 representing 43 different water bodies; some parks supplied specimens both years. In total we analyzed samples from fish representing 17 different taxa, plus one collection where the species name was not provided and one collection where tissues from two species were combined so that we could not distinguish between them. The aim of our original sampling design was for us to obtain a large number (~30) of fish of the same species from each collection site for assessment of biological effects. The parks did an excellent job of providing this number of fish. In addition, lesser numbers of some species from some water bodies were also provided. We analyzed every intact tissue specimen that we received. The very slight discrepancy between the total number of fish sampled by some of the parks (i.e., Crater Lake, Lassen Volcanic, Mount Rainier, Sequoia & Kings Canyon) and the number of

samples analyzed by us stems from the fact that a few cassettes were empty (did not contain gonads) or were deteriorated.

The presence of intersex male fish (Figure 1) in some of the parks can be seen in Table 1, where results are organized alphabetically by park. There appears to be a greater propensity for intersex fish in fish collected from parks located in the Rocky Mountains, and specifically in Rocky Mountain National Park, as can be seen in the following regional breakdown:

A. Rocky Mountains

Rocky Mountain National Park had one intersex male in each of two of the five lakes sampled. Cutthroat each from Hutcheson (1 of 9 males total) and Nanita (1 of 5 males total) lakes were intersex. No intersex males were found amongst the fish from the three other lakes sampled. In total, 2 of 52 male fish were intersex; a frequency of 3.8%. These findings are quite similar to those reported by Schwindt et al. (2009) for the WACAP project.

No intersex males were found amongst fish sampled from two **Glacier** (n=21 male fish), three **Grand Teton** (n=45 male fish), or two **Great Sand Dunes** (n=28 male fish) national park bodies of water. The sample sizes evaluated were quite robust, suggesting if intersex fish were present in the waters sampled, that their frequency is quite low to non-existent.

The frequency of male intersex fish in parks of the Rocky Mountains was 1.4%.

B. Sierra Nevada

One intersex male brook trout was found in the sample from Mildred in **Yosemite** National Park out of 18 fish sampled. No brook, rainbow or brown trout sampled from other locations in the park had intersex fish. In total, 1 of 42 male fish were intersex, a frequency of 2.4%.

Similarly, none of the two trout taxa sampled from three locations in **Sequoia & Kings Canyon** National Parks had intersex males (n=50 male fish). Between the two Sierra parks the sample sizes were quite robust, suggesting that the frequency – 1.1% – of male intersex fish is quite low.

C. Cascades

Lassen Volcanic National Park had one intersex of 20 male brook trout from Summit Lake. None of the other three locations sampled in this park had intersex fish. In total, 1 of 45 male fish were intersex, a frequency of 2.2%.

No intersex males were found amongst fish sampled from two **Crater Lake** (n=43 male fish), five **North Cascades** (n=57 male fish), and twelve **Mount Rainier** (n=48 male fish) national park sites. The very low frequency – 0.5% – of male intersex fish across all of the sites, numerous taxa, and years sampled in Cascade Mountain waters suggests that this condition is very uncommon.

D. Alaskan

Two of 20 male kokanee salmon collected in **Wrangell-St. Elias** National Park & Preserve from Copper Lake were intersex males. None of the other three taxa sampled from this site or fish from two other waters were intersex. In total, 2 of 89 male fish were intersex; a frequency of 2.3%.

Female Diagnostic Accuracy Experiment

It appears as though the presence of male gametic material observed in ovaries (Figure 2) is an artifact of the tissue collection or processing. As is apparent from Table 2, no male gamete(s) was observed in ovaries fixed and processed in the absence of male gonadal contamination. However, 8 of 10 of the contralateral ovaries stored with testis and processed for histology where contamination could have occurred had small amounts of male gametic material in or on the ovarian tissue.

Hence, while it is nearly impossible to prove a negative, we do not believe that we can conclude that there are intersex females present amongst any of the fish collected. The frequency of such false positives was actually quite high across the samples processed (denoted by an asterisk on Table 1).

Many of the ovarian tissue sections obtained during the WACAP study were similarly found to contain male gametic material. Hence, these samples are also false positives.

IV. DISCUSSION

Rocky Mountain National Park exhibited a higher frequency of male intersex fish (3.8%) than did the other parks. The frequency of this condition at the sites where it was found and the frequency of sampling sites with fish exhibiting this condition are similar to that found earlier in the WACAP study reported by Schwindt et al. 2009. Because we did not observe intersex fish in waters of the other parks in the Rocky Mountains sampled, the cause of this condition may be more common in Rocky Mountain National Park. We did find one intersex male fish in Glacier National Park in the WACAP study, but that was in a different species of fish from the two species sampled in 2008 or 2009. To the best of our knowledge no one has reported the presence of intersex lake whitefish or bull trout (fish taxa sampled at Glacier NP), but it is also likely that no one has looked. There is evidence of the intersex condition in another species of whitefish species (*Coregonus lavaretus*) from one Swiss lake.

Similarly, this is likely the first report of intersex male kokanee salmon, a condition we discovered in a few fish from Wrangell-St. Elias National Park & Preserve. The frequency of intersex kokanee salmon males from this park in Alaska, based on a small sample size, is 10%, a frequency similar to that for trout and charr of some waters in Rocky Mountain National Park. Interestingly, the frequency of bodies of water in the Alaskan national park containing intersex males is roughly equivalent to that of Rocky Mountain National Park. At this point there is insufficient information to allow us to speculate about the reproductive health of the fish population waters of Wrangell-St. Elias National Park & Preserve since only three bodies of

water were sampled, and without larger samples sizes of fish and waters, we cannot conclude if these results reflect the park as a whole or are due to sampling some random effects.

The frequency of intersex fish in park waters outside of the Rocky Mountain area and perhaps the Alaskan park is also similar to the original findings of WACAP where only one intersex individual was identified in total (from Mount Rainier National Park) (Schwindt et al. 2009). This finding suggests that for parks in the Sierra Nevada and Cascades that the risk of fish to this sort of aberrant reproductive development is more minimal. In thinking about the meaning of this, it is important to consider that it is likely more ecologically meaningful to think of this in terms of the number of sites having one or more intersex fish present rather than the frequency of intersex per population of fish in the parks of a region. That is because there are more than one species with considerably different reproductive biology represented in the samples from a park and region, the age structure may not be the same within the fish sampled, and the timing of exposure to whatever caused the intersex condition at the different sites may have been different. Based on frequency of sites with intersex males, it is possible that the drivers (e.g., perhaps the types of, concentrations of, and time periods of exposure to contaminants and/or mixtures of contaminants) for this condition in Rocky Mountain National Park, and perhaps Wrangell-St. Elias National Park & Preserve are either absent or considerably less potent elsewhere.

There is no way for us to ascertain the cause of the male intersex condition in those fish where it was observed. This dilemma was discussed by Schwindt et al. (2009), and it basically amounts to the fact that it is likely caused by some anthropogenic endocrine disrupting compound or compounds. Statistical comparison of contaminant burden of male intersex fish and a subsample of non-intersex individuals in from this study did not show any significant difference (Simonich et al. 2012). However, while we know that the fish are exposed to contaminants that could potentially result in such a condition (Simonich et al. 2012), the small sample sizes of fish analyzed, compounded by the large number of such compounds found in greatly varying concentrations in our samples, precludes the possibility of any statistically realistic interpretation of the data. In addition, we do not know the contaminant load of the fish at the time when fish became intersex. All we know is the contaminants present at the time when the fish were collected, and they may have become intersex well before that time. We do know that exposure to endocrine disruptors early in life (around the time of hatching to a few months thereafter) can induce intersex salmonids. The ability for this to happen later in life, as in the adult, had not been established (Liney et al. 2005).

V. CONCLUSIONS

Interpretation of results based on data such as that collected on intersex frequency within and among populations can be confounded by a variety of factors. In general, the frequency of intersex males within a population where it occurs is relatively low (perhaps less than 10%). Also, the bodies of water containing intersex fish appears to differ geographically, with parks in different regions apparently having different frequencies. Very low rates of the intersex condition is likely a natural phenomenon for a variety of reasons (Schwindt et al. 2009). The difficulty, then, is deciding when the presence of intersex fish signifies that the fish have experienced an anthropogenic endocrine disrupting event or not. In parks, such as Rocky Mountain, where the number of bodies of water with intersex fish is relatively high (~50%),

where the condition is found in more than one species, and where further sampling has demonstrated that the intersex fish can be consistently be sampled from a body of water, one has a sound basis for concluding that such events are not natural. However, in parks where no intersex fish have been found or where a low frequency of intersex has been seen over a fairly large number of sites sampled, one is much less secure in speculating as to whether or not this represents a problem. We cautiously speculate that a park like Mount Rainier probably does not have a contaminant-induced sex development issue, even though Schwindt (2009) found one intersex male there. Similarly, Sequoia & Kings Canyon National Parks may also not have such a problem when one looks at the present data in concert with those found by WACAP. Contrarily, a conclusion based on negative data or the presence of a single intersex individual for other parks is much more difficult to justify. It clearly would be worth examining other biomarkers of endocrine disruption in parks where one or two intersex individuals have been found. Circulating levels of vitellogenin could be one such biomarker; it proved useful in supporting the findings suggesting endocrine disruption of intersex fish in the lakes sampled as part of the WACAP project of intersex condition (Schwindt et al. 2009).

VI. RECOMMENDATIONS

The finding that many, if not all, of the female fish in our samples were likely false positives is a new discovery. The various stages of male gametic development (spermatogonia, spermatocytes, spermatids, and sperm) are extremely difficult to recognize because of their extremely small size and resemblance to other cell types such as many white blood cells that appear very similar and are present in great abundance. To be able to recognize such male cells deep within an ovary is exceptionally difficult, particularly since no one has described this before. By analogy, it would be like throwing a handful of sand (analogous to sperm) into a room (analogous to a gonad) full of beach balls (analogous to oocytes) that already has lots of other sand in it from a different source (analogous to white blood cells), taking a 1 cm thick slice through the room, and then being able to visually find a few grains of that sand in that slice. We believe that this is why others have not reported this condition. Figure 3 shows the largest nest of male gametic material within an ovary preparation that we observed in the hundreds of sections read. In most of the other cases the male cells where much fewer in abundance and far more dispersed. Thus, for future studies trying to establish if there are female intersex fish, we recommend using histological collection and preparation procedures that ensure that there can be zero chance for contamination of ovarian samples with testicular products. This could be achieved by fixing gonad samples in individual vials, thorough scrubbing between samples of any tools and surfaces that could come in contact with the tissues, and using fresh tissue processing solvents for each sex.

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VIII. FIGURES

Figure 1. Photomicrographs of two intersex male gonads from two fish each showing one oocyte within the testicular matrix. A = individual from Lassen Volcanic National Park, Summit L. B = individual from Yosemite National Park, Mildred L. Bars = 100µM.

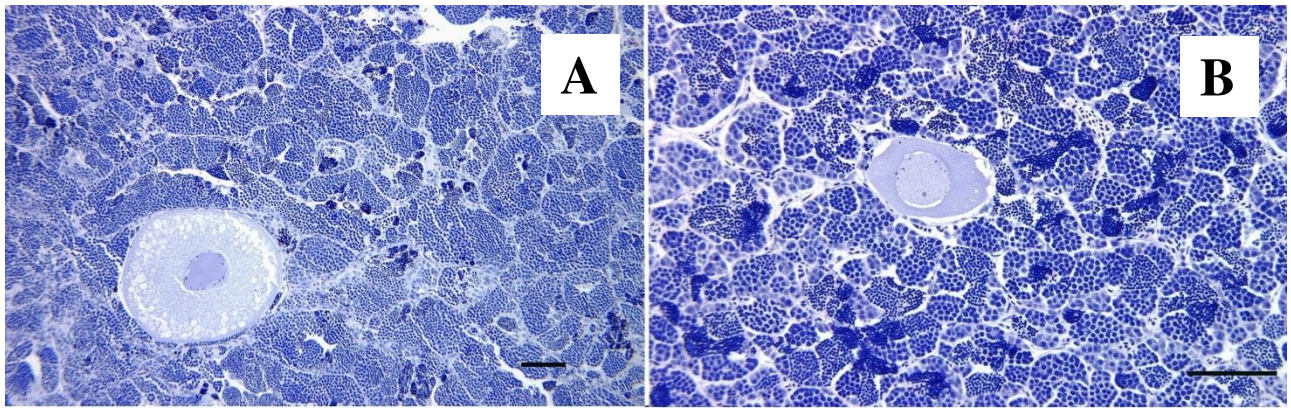


Figure 2. Photomicrographs of male germ cells (arrows) in or on ovarian tissue from four different fish. A = individual from Denali National Park, Wonder L. B = individual from Rocky Mountain National Park, Haynach L. C = individual from Wrangell-St. Elias National Park, Copper L. D= individual from Lassen Volcanic National Park, Ridge L. Bars = 50µM.

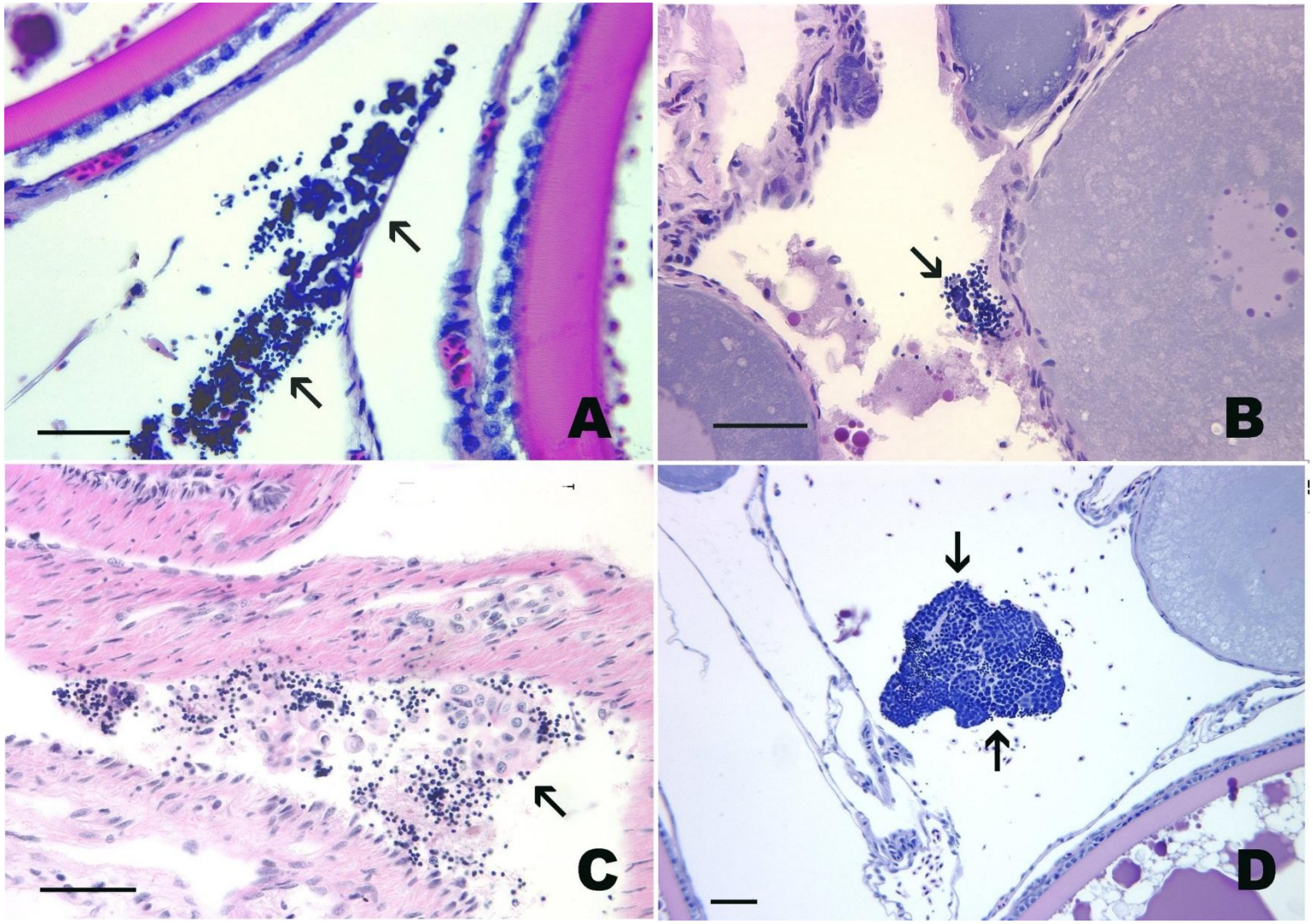
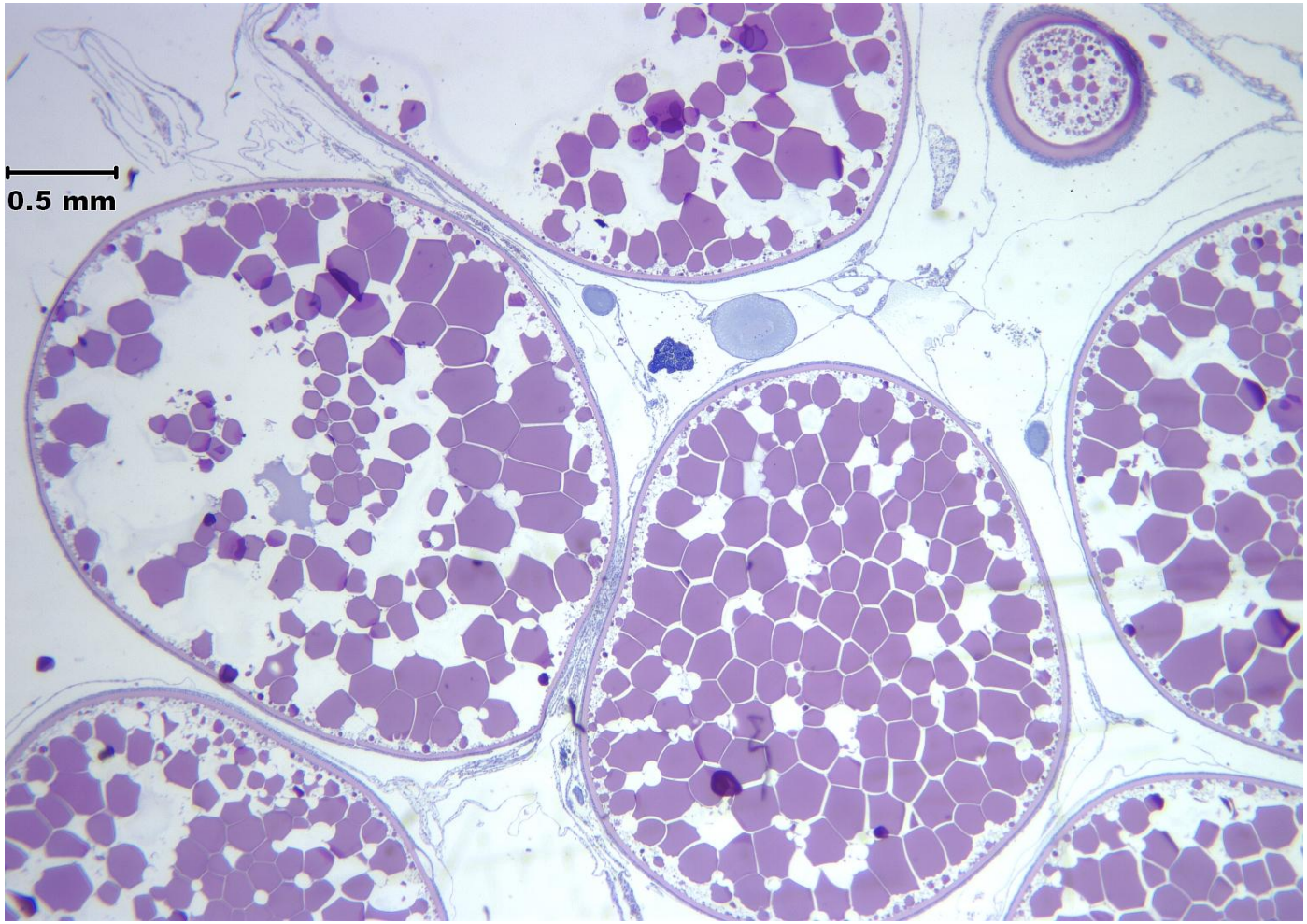


Figure 3. Lower figure is low magnification of the Panel D of Figure 2. The small dark blue item in the center represents a nest of male gametic representing hundreds of cells amongst large circular oocytes.



IX. TABLES

Table 1. National Parks that provided samples, the names of the bodies of water from which the fish were collected, the sampling year, the species collected, the number of samples that we analyzed, the number of males and females amongst those samples, and the number of intersex males or females found. L = Lake; Cr. = Creeks. An asterisk (*) indicates that one of more females in the sample had male gametic material in or on their ovaries, likely due to procedural issues.

Water Body	Year	Species	# of tissue samples	# of males	# of intersex males	# of females	# of intersex females
CRATER LAKE NATIONAL PARK							
Crater L	2008	Kokanee	24	16	0	8	0*
		Rainbow Trout	5	3	0	2	0
Sand Cr.	2009	Brook Trout	30	12	0	18	0*
Crater L	2010	Rainbow Trout	11	6	0	5	0*
		Kokanee	15	6	0	5	0*
GLACIER NATIONAL PARK							
L McDonald	2008	Lake Whitefish	11	3	0	8	0*
		Bull Trout	3	2	0	1	0
Quartz L	2009	Bull Trout	25	16	0	9	0
GRAND TETON NATIONAL PARK							
Death Canyon	2009	Cutthroat	32	17	0	15	0*
Grizzly Bear L		Cutthroat	24	6	0	18	0*
L Solitude		Cutthroat	31	22	0	9	0*

* one of more females in the sample had male gametic material in or on their ovaries, likely due to procedural issues

Table 1, *continued*

Water Body	Year	Species	# of tissue samples	# of males	# of intersex males	# of females	# of intersex females
GREAT SAND DUNES NATIONAL PARK & PRESERVE							
Medano Cr.	2009	Rio Grande Cutthroat	25	11	0	14	0*
Sand Cr.		Pikes Peak Cutthroat Hybrid	31	17	0	14	0*
LASSEN VOLCANIC NATIONAL PARK							
Horseshoe L	2009	Brook Trout	17	9	0	8	0*
Ridge L		Brook Trout	32	13	0	19	0*
Summit L		Brook Trout	37	20	1	17	0*
Left L		Unknown	6	3	0	2	0*
MOUNT RAINIER NATIONAL PARK							
Deadwood L	2008	Rainbow Trout	7	3	0	4	0
Mowich L		Kokanee	2	0	0	2	0
		Sculpin	5	1	0	4	0
L Ethel		Rainbow Trout	7	5	0	2	0
Unamed L		Cutthroat	21	6	0	15	0
Louise L		Brook Trout	31	16	0	15	0
Adelaide L	2009	Rainbow Trout	1	1	0	0	0
Blue L		Rainbow Trout	7	3	0	4	0
Upper Deadwood L		Rainbow Trout	3	2`	0	0	0
L Ethel		Rainbow Trout	7	2	0	5	0
Green L		Westslope Cutthroat	3	2	0	1	0
Mystic L		Cutthroat	3	1	0	2	0
Snow L		Westslope Cutthroat & Brook Trout	11	6	0	5	0*

***** one of more females in the sample had male gametic material in or on their ovaries, likely due to procedural issues

Table 1, *continued*

Water Body	Year	Species	# of tissue samples	# of males	# of intersex males	# of females	# of intersex females
NORTH CASCADES NATIONAL PARK							
Wilcox L[A]	2008	Westslope Cutthroat	12	5	0	7	0
Middle Blum L		Brook Trout	30	19	0	11	0
Diobsud L		Westslope Cutthroat	23	13	0	10	0*
Upper Triplet L	2009	Westsope Cutthroat	21	8	0	13	0
Lower Berdeen L		Westslope Cutthroat	30	12	0	18	0*
ROCKY MOUNTAIN NATIONAL PARK							
Hutcheson L	2008	Greenback Cutthroat	11	9	1	2	0*
Nanita L		Cutthroat	9	5	1	4	0*
Mirror L	2009	Brook Trout	30	12	0	18	0*
Poudre L		Brook Trout	29	16	0	13	0*
Ypsilon L		Colorado River Cutthroat	30	10	0	20	0*
SEQUOIA & KINGS CANYON NATIONAL PARKS							
Center Basin	2008	Rbt/Golden Trout hybrid	29	10	0	19	0*
Bench L		Brook Trout	28	12	0	9	0
	2009	Rbt/Golden Trout hybrid	21	12	0	9	0
Kern Pt. [B]		Rbt/Golden Trout hybrid	29	16	0	13	0*

[A] Upper Wilcox Lake and Lower Wilcox Lake are connected by a "short stretch of stream." 2 of the 12 sampled from Upper Wilcox Lake and 10 of the 12 sampled from Lower Wilcox Lake.
[B] This is the name of the mountain; the name of the water body was not specified.

***** one of more females in the sample had male gametic material in or on their ovaries, likely due to procedural issues

Table 1, *continued*

Water Body	Year	Species	# of tissue samples	# of males	# of intersex males	# of females	# of intersex females
WRANGELL-ST. ELIAS NATIONAL PARK							
Copper	2008	Lake Trout	29	16	0	13	0*
		Kokanee	29	20	2	9	0*
		Berbot	1	1	0	0	0
		Arctic Grayling	26	18	0	8	0*
Summit	2009	Rainbow Trout	25	22	0	3	0
Tanada		Lake Trout	19	12	0	7	0*
YOSEMITE NATIONAL PARK							
Eleanor Reservoir	2009	Rainbow Trout	2	2	0	0	0
Mildred L		Brook Trout	34	18	1	16	0*
Spillway L		Brook Trout	30	18	0	12	0*
Spillway L		Brown Trout	4	4	0	0	0

***** one of more females in the sample had male gametic material in or on their ovaries, likely due to procedural issues

X. APPENDIX

A. Sampling Protocol

The following protocol was sent to each of the parks that expressed interest in providing samples for this project:

General:

The sampling of fish depends on each park's particular situations. Basically, it really depends on the remoteness of the collection site and your ability to transport samples with and without dry ice. We will provide instructions as to how to collect and preserve the tissues. Below are the basics.

1. We would like to receive samples of up to 30 fish per collection site, and representing as many size-categories as possible (so that we get old as well as young fish). We are providing all sampling materials needed, except for coolers and dry ice. Save the box in which the materials are sent for returning the organ samples to us.

2. Data should be recorded in the "Rite in the Rain" notebook provided. Please label the book as to park and NPS contact person. Use pencil only as ink will wash off with some pens. Errors should be corrected by drawing a line through the incorrect entry, date and initial the crossed-out material and make the corrected as a new entry. **PLEASE FILL OUT THE BOOK FOR EACH SITE ACCORDING TO THE EXAMPLE PROVIDED IN THE FRONT OF THE BOOK.**

a. Collection information should include the name of the site collected, the date of collection, and the people involved in the collection.

b. Data on specific fish should include fish number by site, 1-XX running consecutively.

c. Other information that you feel might be useful.

d. It is good practice to make a photocopy of any entries in the data book as soon as you come back from the field. These should be stored in a building that is different from the place where the original data book is kept.

3. Samples would have to be 'fixed" in 10% buffered formalin soon after fish collection, depending on temperature or if you could store fish on regular ice before removing tissues. We would need the samples taken before the fish would spoil (such that you wouldn't consider eating them. The fresher the better).

4. Remove organs from the fish in the field and send these to us. Attached are figures for recognition of ovaries, testes, and spleens. We have provide small (matchbook-size) plastic cuvettes to place the tissues into (one per fish, the gonad and spleen sample go into the same cuvette). We have also provided a small (~ 1 Liter) Nalgene container for buffered formalin into

which the 30 cuvettes would then be placed and stored, per site. Additional sets of cuvettes and formalin containers should be used for each additional site to be sampled.

5. If you could not remove the organ samples for us in the field or shortly thereafter, would you have the capacity to deal with a larger container so that whole fish could be preserved in buffered formalin? The volume would depend on the size of the fish. This could be problematic as large volumes of formaldehyde are dangerous and not easy to handle.

6. If you have the capacity to deal with a cooler of dry ice at or near the collection site, the fish carcasses could be frozen **after** the organs are sampled. These would be used for potential chemistry to determine contaminant loads. We can not provide coolers, but we can return any coolers sent to us.

7. We will provide mailing labels for use in shipping the samples and specimens back to us. Use the original box in which you receive the supplies for sending the samples back to us. Contact us when you are ready to ship organ samples and/or fish, and we will provide shipping instructions at that time. There are some specific details that shipping requires, so we will need to do these on a case by case basis with each park.

Necropsy Protocol

Before necropsy
1. Label cassettes. Cassettes are labeled by code for park and lake, and fish are numbered 1 – 30 Eg. OLY/XXX – 1.

2. Label the nalgene container as to Park, collection site, date(s) of collection and name of NPS contact person.

3. Prepare 10% buffered formalin. Add all contents (concentrated formalin) of all small containers plus salt mix in zip lock bag to nalgene container and fill to line marked on bottle with water from collection site.

3. Record lake code, date, etc. in data book provided.

Necropsy

1. Euthanize fish with a blow to the head with a heavy instrument.

2. Place fish left side up, head to left.

3. Measure length of fish from snout to fork in tail (fork length, ruler provided).

4. Open body cavity with scalpel blade. Start just posterior of the pectoral fins and cut toward vent. Try to avoid cutting the gut, as this makes things messy. However, tissues are still O.K.

for histology. Extra scalpel blades are provided; take extreme care in removing old blades for scalpel handles; needle nose pliers are handy and safe for this. The used blades should be safely stored.

5. Remove gonad and spleen (see Figures below) using scalpel and forceps (provided). Immature gonads are thin, paired, and look like rubber bands. They can be mistaken for intestines. For mature gonads, collect a piece about 1 cm^3 from the middle of the gonad. For large fish a small piece of spleen, the size of your little finger nail, is all that is needed.

6. Place spleen and gonads tissues in labeled cassette.

7. Place cassettes in formalin container. Make certain that all cassettes are submersed in the formalin. Store at room temperature.

Freezing fish for chemistry

Where it is feasible to freeze fish for chemistry to correlate with histology:

1. After removing spleen and gonad, place fish on clean aluminum foil (provided).

2. Place label "Rite in the Rain" piece of paper (about 1 inch) with fish number and lake/park code. Use paper from back of data book provided.

3. Place label in fish's mouth.
4. Wrap fish in foil, place in a plastic bag, then place all fish from each lake in a large plastic bag with another label (page from back of notebook) giving the park, collection site, collection date(s) and responsible NPS contact person's name. The freeze on dry ice ASAP. Fish could be held in a cold environment (e.g., on ice) for up to 24 h, but it is much better to freeze immediately.

Large blocks or pieces of dry ice are desirable, as they do not melt as fast as smaller pieces. Five or more pounds of dry ice in a cooler should last a couple of days if insulated with newspaper and if the cooler is not opened very much. The more often a cooler is opened, the shorter the ice will last. Place fish samples on the bottom and do not insulate the fish from the ice. If the fish are large in size, you may need to layer the fish between dry ice.

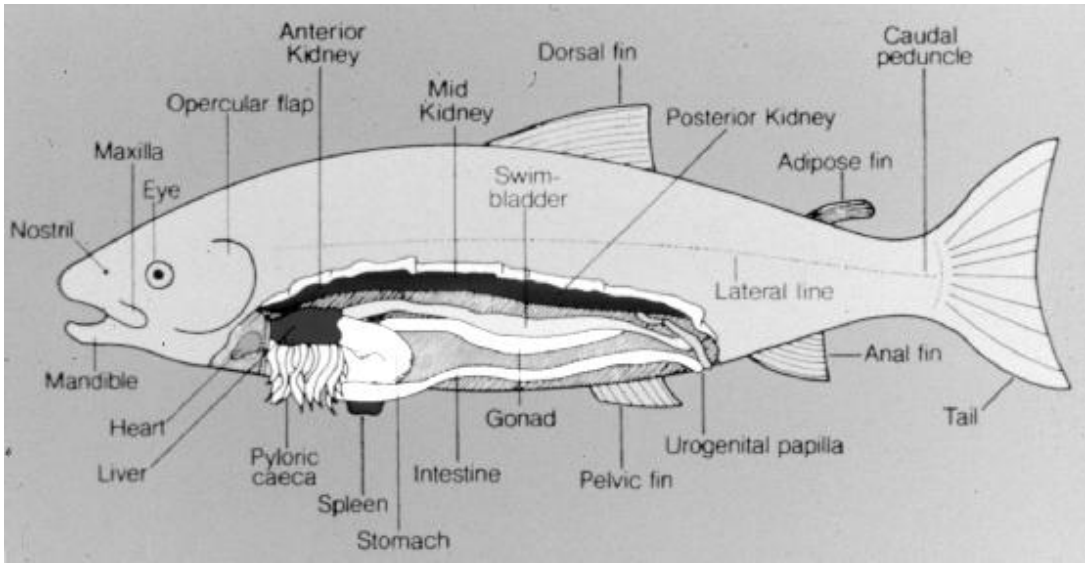
6. Frozen fish can be stored in a conventional freezer until shipping. They will need to be shipped on dry ice. **DO NOT FREEZE FORMALIN-FIXED TISSUES OR SHIPTHESE ON DRY ICE.** Only the whole fish should be shipped frozen.

Blood

The determination of intersex condition or contaminant concentration is NOT dependent on sampling blood. But, if your park had the capacity to collect blood samples from the fish in the

field, separate the plasma by centrifugation, and then immediately freezing the plasma samples on dry ice, we could run additional tests for determination of endocrine disruption (levels of vitellogenin and sex hormones).

If your park has this capability, please contact us for instructions. We would provide necessary materials other than a centrifuge. We have one hand-powered centrifuge that we could make available.



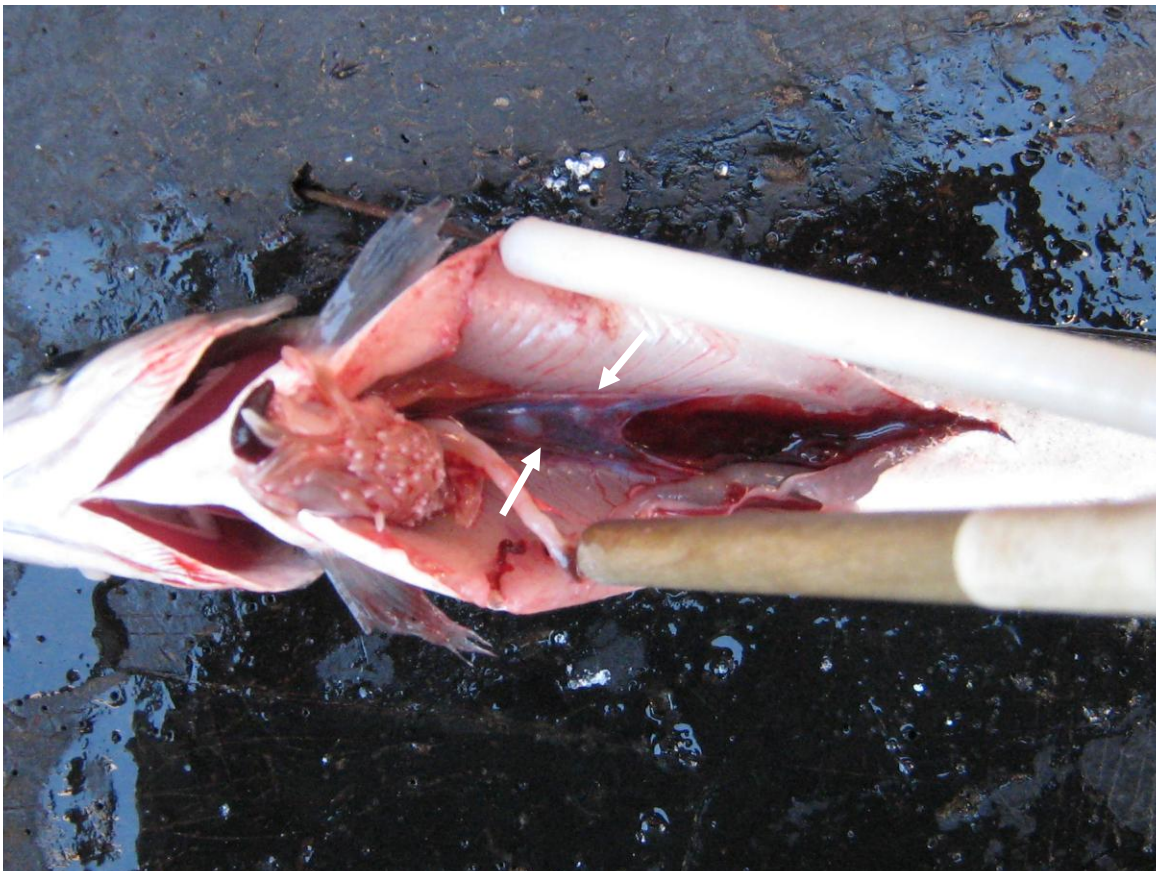


Figure 1. Testes are paired organs that run the length of the body cavity to the vent. The stomach and intestine have been pulled forward to reveal the two testes (white arrows) resting against the dorsal (top side) of the body cavity alongside the kideny (red organ running just underneath the spinal column. Note, the kidney may be obscured by the swim bladder if the swim bladder is inflated.

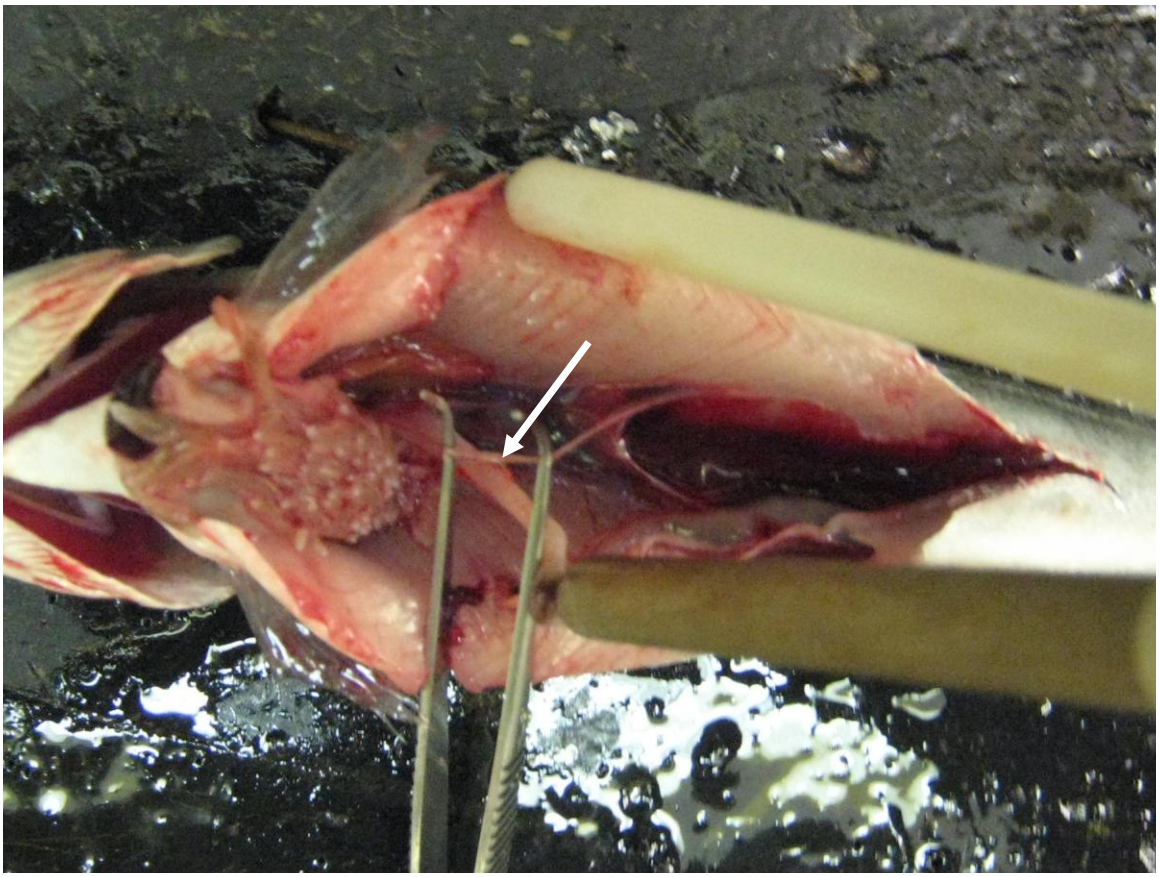


Figure 2. Testis of same fish resting on forceps. Note that they may have the width of a finely stretched fine rubber band. Mature testis are large and swollen with white sperm.

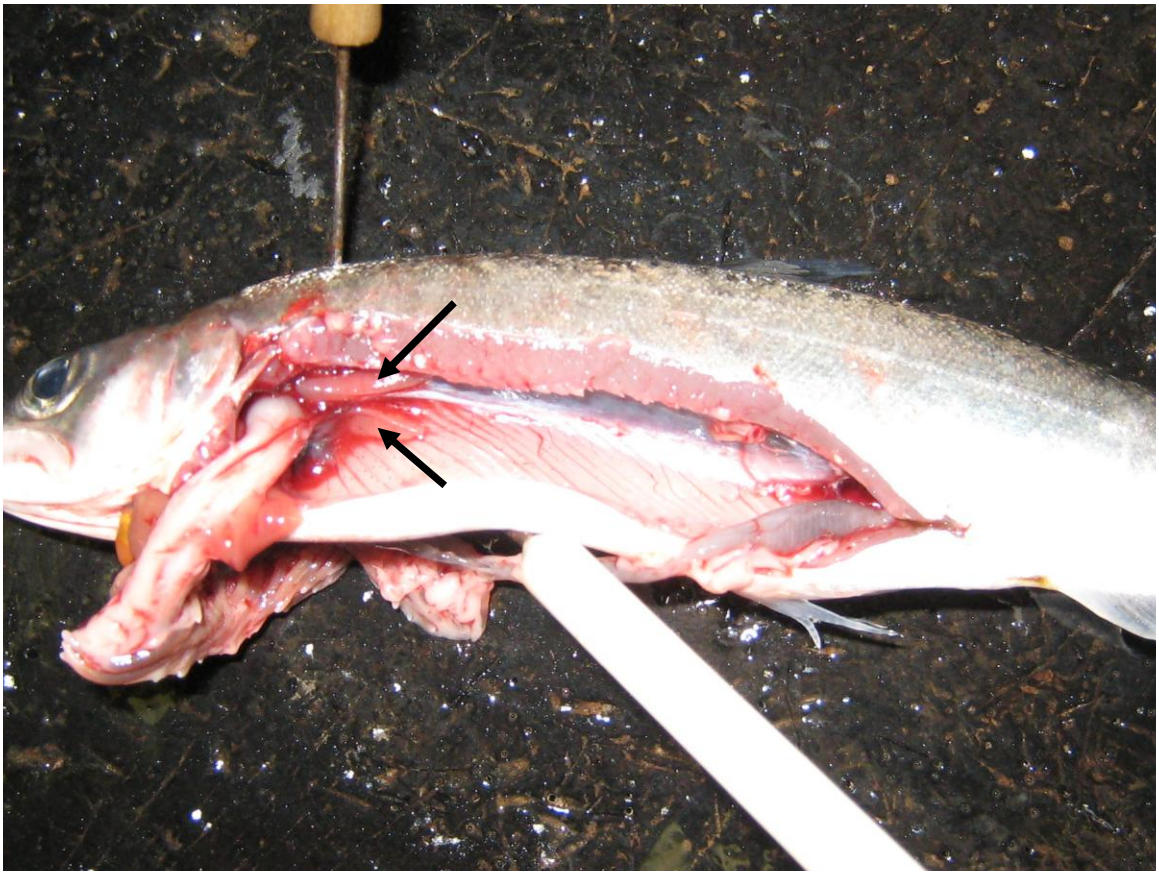


Figure 3. Ovaries are paired organs (black arrows) that are enlarged and triangular looking anteriorly (in front) and run the length of the body cavity towards the vent. Immature ovaries may have only a slight enlargement at the front end and have the width of a fine thread posteriorly. Eggs can readily be seen in maturing (appears granular) or mature ovaries.

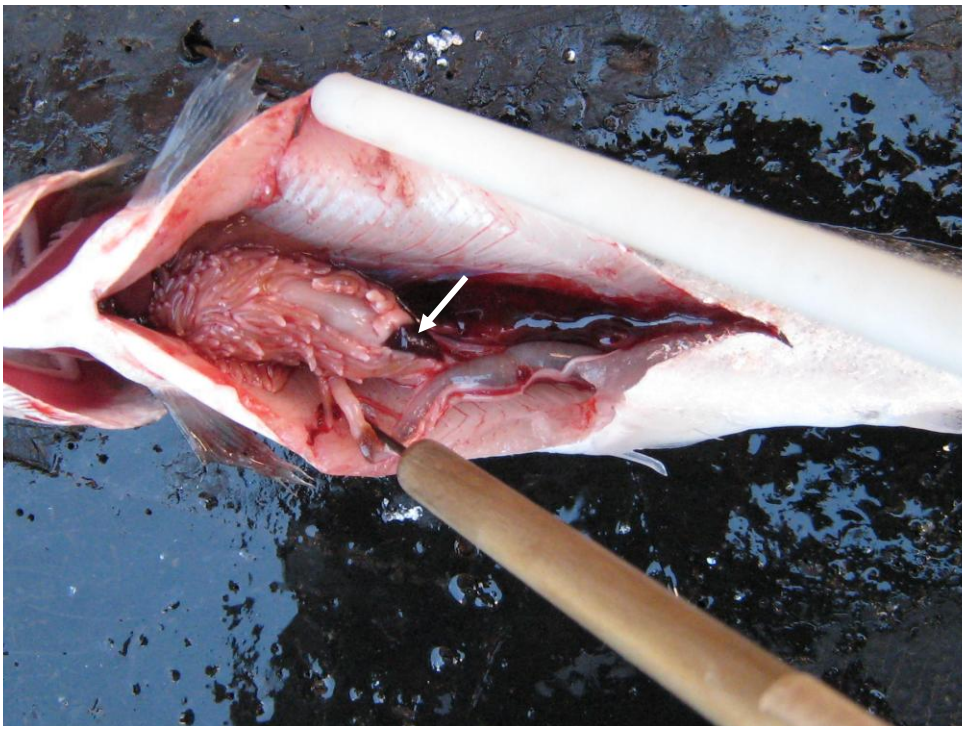


Figure 4. The spleen (tip of white arrow) is a reddish organ apparent when the body cavity is cut open and the viscera are not disturbed.

B. 2008 Fish Data

Histological interpretation of sex and other data for individual fish obtained from various western National Parks in 2008. "Block" refers to our histological section. * = Data provided by park; sex determined by histology. F = Female; M = Male. False + represents a sample of an ovary artificially contaminated with male gametes due to processing procedures.

CRATER LAKE NATIONAL PARK

LAKE: CRATER

Species: Kokanee & Rainbow Trout

Number of Samples: 30

Block #	Sex	Intersex	Fork Length (mm)	Weight (g)
Kokanee				
CRLA 3920	F	No	300	340
CRLA 3928	F	False +	332	410
CRLA 3933	F	False +	299	309
CRLA 3943	F	No	328	340
CRLA 3944	F	No	300	310
CRLA 3945	F	No	305	315
CRLA 3953	F	No	314	400
CRLA 3956	F	False +	295	320
CRLA 3919	M	No	299	467
CRLA 3921	M	No	280	322
CRLA 3922	M	No	299	349
CRLA 3923	M	No	327	471
CRLA 3924	M	No	298	383
CRLA 3926	M	No	314	344
CRLA 3927	M	No	291	350
CRLA 3930	M	No	305	377
CRLA 3931	M	No	294	362
CRLA 3932	M		275	291
CRLA 3946	M	No	300	340
CRLA 3947	M	No	305	388
CRLA 3948	M	No	330	448
CRLA 3949	M	No	293	336

CRATER LAKE NATIONAL PARK *continued*

LAKE: CRATER *continued*

Species: Kokanee & Rainbow Trout

Number of Samples: 30

Block #	Sex	Intersex	Fork Length (mm)	Weight (g)
CRLA 3951	M	No	315	420
CRLA 3954	M	No	303	380
CRLA 3955	M	No	305	400
Rainbow Trout				
CRLA 3950	F	No	232	140
CRLA 3929	F	No	222	140
CRLA 3925	M	No	465	1350
CRLA 3952	M	No	465	1148
CRLA 3942	M	No	435	450

GLACIER NATIONAL PARK

LAKE: MCDONALD

Species: Lake Whitefish

Number of Samples: 12

Block #	Sex	Intersex	Total Length (mm)*	Weight (g)*
MCLWF-1	F	No	505	1020
MCLWF-10	F	No	487	1078
MCLWF-4	F	False +	482	1026
MCLWF-6	F	No	477	822
MCLWF-8	F	No	465	843
MCLWF-2	F	No	462	853
MCLWF-3	F	No	455	860
MCLWF-11	F	No	451	856
MCLWF-9	M	No	470	850
MCLWF-5	M	No	458	802
MCLWF-7	M	No	443	793
MCLWF-12		No Gonadal Tissue	Not recorded	Not recorded

GLACIER NATIONAL PARK *continued*

LAKE: MCDONALD

Species: Bulltrout (BLT)

Number of Samples: 5

Block #	Sex	Intersex	Total Length (mm)*	Weight (g)*
MCBLT-5	F	No	432	673
MCBLT-4	M	No	564	1558
MCBLT-3	M	No	521	1180
MCBLT-2		No Gonadal Tissue	431	719
MCBLT-1		No Gonadal Tissue	499	1096

MOUNT RAINIER NATIONAL PARK

LAKE: DEADWOOD (LW32)

Species: Rainbow Trout

Number of Samples: 7

Block #	Sex	Intersex	Fork Length (mm)*	Weight (g)*
MORA LW32-6	F	No	353	440
MORA LW32-3	F	No	312	310
MORA LW32-4	F	No	292	240
MORA LW32-5	F	No	247	170
MORA LW32-1	M	No	342	390
MORA LW32-2	M	No	308	280
MORA LW32-7	M	No	239	140

LAKE: MOWICH (LM04)

Species: Kokanee

Number of Samples: 2

Block #	Sex	Intersex	Total Length (mm)*	Weight (g)*
MORA LMO4-1	F	No	197	60
MORA LMO4-2	F	No	181	66

MOUNT RAINIER NATIONAL PARK *continued*

LAKE: MOWICH (LM04)

Species: Sculpin

Number of Samples: 7

Block #	Sex	Intersex	Total Length (mm)*	Weight (g)*
MORA LMO4-3		No Gonadal Tissue	129	20
MORA LMO4-6	F	No	129	22
MORA LMO4-7	F	No	128	20
MORA LMO4-4	F	No	126	20
MORA LMO4-5	F	No	125	19
MORA LMO4-8	M	No	120	20
MORA LMO4-9		No Gonadal Tissue	118	20

LAKE: ETHEL (LFO4)

Species: Rainbow Trout

Number of Samples: 7

Block #	Sex	Intersex	Fork Length (mm)*	Weight (g)*
MORA LFO4-4	F	No	289	210
MORA LFO4-2	F	No	276	210
MORA LFO4-1	M	No	272	235
MORA LFO4-7	M	No	272	210
MORA LFO4-6	M	No	261	190
MORA LFO4-5	M	No	260	180
MORA LFO4-3	M	No	254	160

LAKE: UNKNOWN (LH15)

Species: Cutthroat Trout

Number of Samples: 21

Block #	Sex	Intersex	Fork Length (mm)*	Weight (g)*
MORA LH15-14	F	No	301	240
MORA LH15-6	F	No	295	160
MORA LH15-8	F	No	292	160
MORA LH15-7	F	No	280	140
MORA LH15-13	F	No	277	170
MORA LH15-3	F	No	274	160

MOUNT RAINIER NATIONAL PARK *continued*

LAKE: UNKNOWN (LH15) *continued*

Species: Cutthroat Trout

Number of Samples: 21

Block #	Sex	Intersex	Fork Length (mm)*	Weight (g)*
MORA LH15-12	F	No	266	150
MORA LH15-11	F	No	245	140
MORA LH15-17	F	No	245	110
MORA LH15-16	F	No	244	130
MORA LH15-20	F	No	229	110
MORA LH15-18	F	No	223	110
MORA LH15-21	F	No	220	120
MORA LH15-15	F	No	213	80
MORA LH15-1	F	No	210	80
MORA LH15-2	M	No	255	150
MORA LH15-4	M	No	255	170
MORA LH15-5	M	No	242	130
MORA LH15-10	M	No	240	120
MORA LH15-19	M	No	234	120
MORA LH15-9	M	No	322	290

LAKE: LOUISE (LZ21)

Species: Brook Trout

Number of Samples: 32

Block #	Sex	Intersex	Fork Length (mm)*	Weight (g)*
MORA LZ21-33	F	No	208	78
MORA LZ21-6	F	No	203	80
MORA LZ21-21	F	No	195	74
MORA LZ21-47	F	No	190	59
MORA LZ21-3	F	No	187	60
MORA LZ21-31	F	No	184	65
MORA LZ21-30	F	No	181	62
MORA LZ21-10	F	No	173	50
MORA LZ21-24	F	No	168	52

MOUNT RAINIER NATIONAL PARK *continued*

LAKE: LOUISE (LZ21) *continued*

Species: Brook Trout

Number of Samples: 32

Block #	Sex	Intersex	Fork Length (mm)*	Weight (g)*
MORA LZ21-9	F	No	166	50
MORA LZ21-41	F	No	164	45
MORA LZ21-19	F	No	128	20
MORA LZ21-20	F	No	126	18
MORA LZ21-42	F	No	120	15
MORA LZ21-48	F	No	117	85
MORA LZ21-4	M	No	315	90
MORA LZ21-7	M	No	221	105
MORA LZ21-1	M	No	215	90
MORA LZ21-37	M	No	204	89
MORA LZ21-16	M	No	202	85
MORA LZ21-49	M	No	195	69
MORA LZ21-23	M	No	187	68
MORA LZ21-5	M	No	186	60
MORA LZ21-11	M	No	180	65
MORA LZ21-34	M	No	179	62
MORA LZ21-8	M	No	174	50
MORA LZ21-52	M	No	169	51
MORA LZ21-22	M	No	167	48
MORA LZ21-45	M	No	167	45
MORA LZ21-40	M	No	164	47
MORA LZ21-15	M	No	133	23
MORA LZ21-2		No Gonadal Tissue	Not Recorded	35

ROCKY MOUNTAIN NATIONAL PARK

LAKE: UPPER HUTCHESON

Species: Greenback Cutthroat Trout

Number of Samples: 11

Block #	Sex	Intersex	Length (cm)*	Weight (g)*
Romo Hutch-1	F	False +	22.1	Not Recorded
Romo Hutch-1B	Tissue missing			
Romo Hutch-3	F	False +	20.7	Not Recorded
Romo Hutch-11	M	No	24.5	Not Recorded
Romo Hutch-10	M	No	23.6	Not Recorded
Romo Hutch-8	M	No	22.5	Not Recorded
Romo Hutch-6	M	No	21	Not Recorded
Romo Hutch-2	M	No	20.3	Not Recorded
Romo Hutch-4	M	No	20	Not Recorded
Romo Hutch-5	M	Yes	19.9	Not Recorded
Romo Hutch-7	M	No	19.9	Not Recorded
Romo Hutch-9	M	No	18.4	Not Recorded

LAKE: NANITA

Species: Cutthroat Trout

Number of Samples: 10

Block #	Sex	Intersex	Length (mm)*	Weight (g)*
Romo Nan-9	F	False +	315	Not Provided
Romo Nan-7	F	False +	310	Not Provided
Romo Nan-8	F	False +	305	Not Provided
Romo Nan-6	F	False +	270	Not Provided
Romo Nan-10	M		281	Not Provided
Romo Nan-1	M	No	280	Not Provided
Romo Nan-5	M	No	243	Not Provided
Romo Nan-4	M	No	232	Not Provided
Romo Nan-3	M	Yes	228	Not Provided
Romo Nan-2	M	No	218	Not Provided

NORTH CASCADES NATIONAL PARK

LAKE: WILCOX

Species: Westslope Cutthroat Trout

Number of Samples: 12

Block #	Sex	Intersex	Fork Length (mm)*	Weight (g)*
Upper Wilcox				
NOCA EP-06-01-1	F	No	185	Not recorded
NOCA EP-06-01-2	M	No	195	Not recorded
Lower Wilcox				
NOCA EP-06-02-7	F	No	279	Not recorded
NOCA EP-06-02-1	F	No	274	Not recorded
NOCA EP-06-02-10	F	No	252	Not recorded
NOCA EP-06-02-5	F	No	242	Not recorded
NOCA EP-06-02-6	F	No	232	Not recorded
NOCA EP-06-02-8	F	No	230	Not recorded
NOCA EP-06-02-4	M	No	235	Not recorded
NOCA EP-06-02-2	M	No	216	Not recorded
NOCA EP-06-02-3	M	No	215	Not recorded
NOCA EP-06-02-9	M	No	204	Not recorded

NORTH CASCADES NATIONAL PARK *continued*

LAKE: MIDDLE BLUM

Species: Brook Trout

Number of Samples: 30

Block #	Sex	Intersex	Fork Length* (mm)	Weight (g)*
NOCA/M-11-01-25	F	No	182	Not recorded
NOCA/M-11-01-6	F	No	178	Not recorded
NOCA/M-11-01-14	F	No	178	Not recorded
NOCA/M-11-01-23	F	No	178	Not recorded
NOCA/M-11-01-17	F	No	157	Not recorded
NOCA/M-11-01-26	F	No	142	Not recorded
NOCA/M-11-01-28	F	No	136	Not recorded
NOCA/M-11-01-7	F	No	135	Not recorded
NOCA/M-11-01-15	F	No	127	Not recorded
NOCA/M-11-01-18	F	No	125	Not recorded
NOCA/M-11-01-4	F	No	120	Not recorded
NOCA/M-11-01-10	M	No	256	Not recorded
NOCA/M-11-01-3	M	No	184	Not recorded
NOCA/M-11-01-16	M	No	178	Not recorded
NOCA/M-11-01-1	M	No	175	Not recorded
NOCA/M-11-01-29	M	No	174	Not recorded
NOCA/M-11-01-9	M	No	170	Not recorded
NOCA/M-11-01-5	M	No	168	Not recorded
NOCA/M-11-01-27	M	No	165	Not recorded
NOCA/M-11-01-8	M	No	165	Not recorded
NOCA/M-11-01-12	M	No	161	Not recorded
NOCA/M-11-01-13	M	No	158	Not recorded
NOCA/M-11-01-24	M	No	155	Not recorded
NOCA/M-11-01-22	M	No	155	Not recorded
NOCA/M-11-01-21	M	No	151	Not recorded
NOCA/M-11-01-20	M	No	150	Not recorded
NOCA/M-11-01-19	M	No	149	Not recorded
NOCA/M-11-01-11	M	No	129	Not recorded
NOCA/M-11-01-2	M	No	126	Not recorded
NOCA/M-11-01-30	M	No	109	Not recorded

SEQUOIA & KINGS CANYON NATIONAL PARKS

LAKE: CENTER BASIN

Species: Rainbow/Golden Trout hybrid

Number of Samples: 30

Block #	Sex	Intersex	Length (mm)*	Weight (g)*
SEKI CB 16	F	No	176	Not Recorded
SEKI CB 13	F	False +	172	Not Recorded
SEKI CB 22	F	False +	169	Not Recorded
SEKI CB 24	F	False +	166	Not Recorded
SEKI CB 4	F	No	166	Not Recorded
SEKI CB 2	F	No	164	Not Recorded
SEKI CB 3	F	No	163	Not Recorded
SEKI CB 25	F	No	160	Not Recorded
SEKI CB 12	F	False +	155	Not Recorded
SEKI CB 30	F	No	153	Not Recorded
SEKI CB 26	F	No	151	Not Recorded
SEKI CB 15	F	False +	145	Not Recorded
SEKI CB 19	F	False +	145	Not Recorded
SEKI CB 18	F	Yes	144	Not Recorded
SEKI CB 29	F	No	140	Not Recorded
SEKI CB 9	F	False +	136	Not Recorded
SEKI CB 14	F	No	135	Not Recorded
SEKI CB 8	F	No	132	Not Recorded
SEKI CB 10	F	No	120	Not Recorded
SEKI CB 23	M	No	166	Not Recorded
SEKI CB 17	M	No	164	Not Recorded
SEKI CB 27	M	No	164	Not Recorded
SEKI CB 6	M	No	162	Not Recorded
SEKI CB 5	M	No	155	Not Recorded
SEKI CB 21	M	No	150	Not Recorded
SEKI CB 20	M	No	149	Not Recorded
SEKI CB 7	M	No	140	Not Recorded
SEKI CB 11	M	No	117	Not Recorded
SEKI CB 28	M	No	143	Not Recorded
SEKI CB 1		No Gonadal Tissue	148	Not Recorded

WRANGELL-ST. ELIAS NATIONAL PARK & PRESERVE

LAKE: COPPER

Species: Lake Trout

Number of Samples: 30

Block #	Sex	Intersex	Length (mm)*	Weight (g)*
WRST-COP LT2	F		620	Not Recorded
WRST-COP LT3	F	No	590	Not Recorded
WRST-COP LT17	F	No	550	Not Recorded
WRST-COP LT12	F	No	520	Not Recorded
WRST-COP LT7	F	No	515	Not Recorded
WRST-COP LT22	F	False +	510	Not Recorded
WRST-COP LT27	F	False +	510	Not Recorded
WRST-COP LT29	F	False +	490	Not Recorded
WRST-COP LT4	F	No	475	Not Recorded
WRST-COP LT9	F	No	460	Not Recorded
WRST-COP LT14	F	False +	460	Not Recorded
WRST-COP LT30	F	False +	445	Not Recorded
WRST-COP LT6	F	No	420	Not Recorded
WRST-COP LT25	F	False +	430	Not Recorded
WRST-COP LT28	M	No	540	Not Recorded
WRST-COP LT21	M	No	525	Not Recorded
WRST-COP LT13	M	No	505	Not Recorded
WRST-COP LT5	M	No	490	Not Recorded
WRST-COP LT15	M	No	490	Not Recorded
WRST-COP LT8	M	No	480	Not Recorded
WRST-COP LT10	M	No	475	Not Recorded
WRST-COP LT11	M	No	475	Not Recorded
WRST-COP LT16	M	No	470	Not Recorded
WRST-COP LT19	M	No	465	Not Recorded
WRST-COP LT24	M	No	460	Not Recorded
WRST-COP LT26	M	No	455	Not Recorded
WRST-COP LT20	M	No	445	Not Recorded
WRST-COP LT18	M	No	440	Not Recorded
WRST-COP LT23	M	No	440	Not Recorded
WRST-COP LT1	M	No	420	Not Recorded

WRANGELL-ST. ELIAS NATIONAL PARK & PRESERVE *continued*

LAKE: COPPER

Species: Kokanee

Number of Samples: 29

Block #	Sex	Intersex	Std Length (mm)*	Weight (g)*
WRST-COP K4	F	False +	330	Not Recorded
WRST-COP K6	F	False +	300	Not Recorded
WRST-COP K19	F	False +	300	Not Recorded
WRST-COP K30	F	False +	299	Not Recorded
WRST-COP K21	F	False +	298	Not Recorded
WRST-COP K7	F	False +	295	Not Recorded
WRST-COP K17	F	False +	295	Not Recorded
WRST-COP K26	F	False +	290	Not Recorded
WRST-COP K25B	F	No		Not Recorded
WRST-COP K8	M	No	335	Not Recorded
WRST-COP K14	M	Yes	325	Not Recorded
WRST-COP K10	M	No	322	Not Recorded
WRST-COP K9	M	No	320	Not Recorded
WRST-COP K22	M	No	320	Not Recorded
WRST-COP K13	M	No	320	Not Recorded
WRST-COP K24	M	No	318	Not Recorded
WRST-COP K11	M	No	316	Not Recorded
WRST-COP K3	M	No	315	Not Recorded
WRST-COP K12	M	No	314	Not Recorded
WRST-COP K16	M	No	313	Not Recorded
WRST-COP K28	M	No	313	Not Recorded
WRST-COP K20	M	No	312	Not Recorded
WRST-COP K29	M	No	312	Not Recorded
WRST-COP K27	M	No	311	Not Recorded
WRST-COP K5	M	Yes	310	Not Recorded
WRST-COP K15	M	No	310	Not Recorded
WRST-COP K23	M	No	303	Not Recorded
WRST-COP K18	M	No	295	Not Recorded
WRST-COP K25A	M	No		Not Recorded

WRANGELL-ST. ELIAS NATIONAL PARK & PRESERVE *continued*

LAKE: COPPER

Species: Burbot
Number of Samples: 1

Block #	Sex	Intersex	Length (mm)*	Weight (g)*
WRST-COP B1	M	No	330	Not Recorded

LAKE: COPPER

Species: Arctic Grayling
Number of Samples: 31

Block #	Sex	Intersex	Length (mm)*	Weight (g)*
WRST-COP AG6A	F	No		Not Recorded
WRST-COP AG5			390	Not Recorded
WRST-COP AG6B	M	No		Not Recorded
WRST-COP AG2	M	No	350	Not Recorded
WRST-COP AG3	M		340	Not Recorded
WRST-COP AG4A	M			Not Recorded
WRST-COP AG4B	M			Not Recorded
			Fork Length	
WRST-COP AG8	F	No	395	Not Recorded
WRST-COP AG9	F	False +	370	Not Recorded
WRST-COP AG28	F	False +	361	Not Recorded
WRST-COP AG21	F	False +	358	Not Recorded
WRST-COP AG19	F	False +	340	Not Recorded
WRST-COP AG30	F	False +	319	Not Recorded
WRST-COP AG27	F	False +	315	Not Recorded
WRST-COP AG12	F		313	Not Recorded
WRST-COP AG14	M	No	416	Not Recorded
WRST-COP AG29	M	No	404	Not Recorded
WRST-COP AG16	M	No	388	Not Recorded
WRST-COP AG18	M	No	383	Not Recorded
WRST-COP AG26	M	No	378	Not Recorded
WRST-COP AG10	M	No	378	Not Recorded
WRST-COP AG13	M	No	368	Not Recorded
WRST-COP AG11	M	No	365	Not Recorded
WRST-COP AG7	M	No	362	Not Recorded

WRANGELL-ST. ELIAS NATIONAL PARK & PRESERVE *continued*

LAKE: COPPER *continued*

Species: Arctic Grayling
Number of Samples: 31

Block #	Sex	Intersex	Length (mm)*	Weight (g)*
WRST-COP AG20	M	No	359	Not Recorded
WRST-COP AG23	M	No	354	Not Recorded
WRST-COP AG17	M	No	345	Not Recorded
WRST-COP AG25	M	No	345	Not Recorded
WRST-COP AG15	M	No	332	Not Recorded
WRST-COP AG22	M	No	304	Not Recorded
WRST-COP AG24	M	No	297	Not Recorded

C. 2009 Fish Data

Histological interpretation of sex and other data for individual fish obtained from various western National Parks in 2009. * = Data provided by park; sex determined by histology. "Block" refers to our histological section. F = Female; M = Male; m = Maturing; ming = Maturing. False + represents a sample of an ovary artificially contaminated with male gametes due to processing procedures. False + represents a sample of an ovary artificially contaminated with male gametes due to processing procedures.

CRATER LAKE NATIONAL PARK

LAKE: SAND CREEK

Species: Brook Trout

Number of Samples: 32

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
CRLA-SAND-1	F ming	No		138	*	
CRLA-SAND-2	F m	No		150	*	
CRLA-SAND-3	M m	No		138	*	
CRLA-SAND-4	F m	No		154	*	
CRLA-SAND-5	M m	No		145	*	
CRLA-SAND-6	F m	False +		201	*	nest of sperm and spermatagonia deep in stroma
CRLA-SAND-7	M m	No		149	*	
CRLA-SAND-8	F m	No		196	*	
CRLA-SAND-9	F m	No		171	*	
CRLA-SAND-10	F m	No		111	*	
CRLA-SAND-11	M m	No		180	*	
CRLA-SAND-12	M m	No		208	*	
CRLA-SAND-13	F m	No		135	*	
CRLA-SAND-14	M m	No		114	*	
CRLA-SAND-15	F m	No		191	*	
CRLA-SAND-16		spleen only		94	*	
CRLA-SAND-17		spleen only		88	*	
CRLA-SAND-18	M m	No		220	*	
CRLA-SAND-19	F m	No		155	*	
CRLA-SAND-20	M m	No		143	*	
CRLA-SAND-21	M m	No		156	*	
CRLA-SAND-22	F m	No		130	*	
CRLA-SAND-23	F m	No		143	*	

CRATER LAKE NATIONAL PARK *continued*

LAKE: SAND CREEK *continued*

Species: Brook Trout

Number of Samples: 32

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
CRLA-SAND-24	F m	No		146	*	
CRLA-SAND-25	F m	No		138	*	
CRLA-SAND-26	M m	No		196	*	
CRLA-SAND-27	M m	No		163	*	
CRLA-SAND-28	F m	No		149	*	
CRLA-SAND-29	M m	No		148	*	
CRLA-SAND-30	F m	No		137	*	
CRLA-SAND-31	F m	No		156	*	
CRLA-SAND-32	F m	No		149	*	

GLACIER NATIONAL PARK

LAKE: QUARTZ

Species: Bull Trout

Number of Samples: 24

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
GLAC-01	F m	No	501	476	*	
GLAC-02	M? Im	No	254	241	*	
GLAC-03	M? Im	No	363	342	*	
GLAC-04	? Im	No	363	344	*	
GLAC-05	F ming	No	530	504	*	
GLAC-06	F ming	No	419	395	*	
GLAC-07	F ming	No	335	317	*	
GLAC-08	F ming	No	430	402	*	
GLAC-09	M? Im	No	525	505	*	
GLAC-10	M ning	No	610	578	*	
GLAC-11	M im	No	549	524	*	
GLAC-12	M im	No	520	493	*	
GLAC-13	M? Im	No	451	425	*	
GLAC-14	F ming	No	474	447	*	
GLAC-15	M? Im	No	435	408	*	
GLAC-16	M? Im	No	380	356	*	
GLAC-17	No slide		202	198	*	tissue not provided by park
GLAC-18	M? Im	No	605	578	*	
GLAC-19	F ming	No	370	348	*	
GLAC-20	M? Im	No	420	396	*	
GLAC-21	M? Im	No	554	521	*	
GLAC-22	F ming	No	420	396	*	
GLAC-23	F ming	No	445	422	*	
GLAC-24	M ming	No	535	505	*	
GLAC-25	M im	No	293	276	*	

GRAND TETON NATIONAL PARK

LAKE: DEATH CANYON

Species: Cutthroat Trout

Number of Samples: 32

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
GT-DC-01	F ming	False +	*	*	*	aggregate of sperm and spermatogonia near edge
GT-DC-02	F ming	No	*	*	*	
GT-DC-03	M ming	No	*	*	*	only ducts with sperm visibile in section
GT-DC-04	F ming	No	*	*	*	only ducts with sperm visibile in section
GT-DC-05	M ming	No	*	*	*	
GT-DC-06	F ming	No	*	*	*	
GT-DC-07	M ming	No	*	*	*	
GT-DC-08	M ming	No	*	*	*	
GT-DC-09	M ming	No	*	*	*	
GT-DC-10	F ming	No	*	*	*	
GT-DC-11	F ming	No	*	*	*	
GT-DC-12	M ming	No	*	*	*	only ducts with sperm visibile in section
GT-DC-13	M ming	No	*	*	*	
GT-DC-14	M ming	No	*	*	*	
GT-DC-15	F ming	No	*	*	*	
GT-DC-16	F ming	No	*	*	*	
GT-DC-17	M ming	No	*	*	*	
GT-DC-18	M ming	No	*	*	*	
GT-DC-19	F ming	No	*	*	*	
GT-DC-20	F ming	False +	*	*	*	aggregate of sperm and spermatogonia near edge
GT-DC-21	M ing	No	*	*	*	
GT-DC-22	M	No	*	*	*	only ducts with sperm visibile in section
GT-DC-23	M	No	*	*	*	only ducts with sperm visibile in section
GT-DC-24	F ming	No	*	*	*	
GT-DC-25	F ming	No	*	*	*	
GT-DC-26	??	No	*	*	*	
GT-DC-27	F ming	No	*	*	*	
GT-DC-28	M ming	No	*	*	*	
GT-DC-29	M ming	No	*	*	*	
GT-DC-30	F immature	No	*	*	*	
GT-DC-31	M	No	*	*	*	only ducts with sperm visibile in section
GT-DC-32	F immature	No	*	*	*	

GRAND TETON NATIONAL PARK *continued*

LAKE: GRIZZLY BEAR

Species: Cutthroat Trout

Number of Samples: 31

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
GT-GBL-01	M m	No	*	*	*	
GT-GBL-02	F m	False +	*	*	*	testis in ovary PHOTO RECUT
GT-GBL-03	F m	No	*	*	*	
GT-GBL-04	M m	No	*	*	*	
GT-GBL-05	M m	No	*	*	*	
GT-GBL-06	F m	No	*	*	*	
GT-GBL-07	M m	No	*	*	*	
GT-GBL-08	F m	No	*	*	*	
GT-GBL-09	F m	No	*	*	*	
GT-GBL-10	F m	No	*	*	*	
GT-GBL-11	M m	No	*	*	*	
GT-GBL-12	F m	No	*	*	*	
GT-GBL-13	F m	No	*	*	*	
GT-GBL-14	F m	No	*	*	*	
GT-GBL-15	F m	No	*	*	*	
GT-GBL-16	F m	False +	*	*	*	large piece of putative testis in ovary RECUT
GT-GBL-17	F m	No	*	*	*	
GT-GBL-18	F m	No	*	*	*	
GT-GBL-19	F m	False +	*	*	*	possible aggregate of mature sperm
GT-GBL-20	F m	No	*	*	*	
GT-GBL-21	F m	No	*	*	*	
GT-GBL-22	F m	No	*	*	*	
GT-GBL-23	F m	No	*	*	*	
GT-GBL-24	M m	No	*	*	*	
GT-GBL-25	*	No	*	*	*	
GT-GBL-26	*	No	*	*	*	
GT-GBL-27	*	No	*	*	*	
GT-GBL-28	*	No	*	*	*	
GT-GBL-29	*	No	*	*	*	
GT-GBL-30	*	No	*	*	*	
GT-GBL-31	*	No	*	*	*	

GRAND TETON NATIONAL PARK *continued*

LAKE: SOLITUDE

Species: Cutthroat Trout

Number of Samples: 33

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
GT-LS-01	M m	No	*	*	*	
GT-LS-02	M ming	No	*	*	*	
GT-LS-03	F immature	False +	*	*	*	sperm deep in ovary
GT-LS-04	Spleen only	No	*	*	*	
GT-LS-05	M m	No	*	*	*	
GT-LS-06	M ming	No	*	*	*	
GT-LS-07	F m	False +	*	*	*	sperm in aggregates deep in tissue, 2nd areas with possible spermatagonia
GT-LS-08	F m	False +	*	*	*	sperm present
GT-LS-09	F m	False +	*	*	*	RECUT sperm and putative spermatagonia
GT-LS-10	M m	No	*	*	*	
GT-LS-11	M m	No	*	*	*	
GT-LS-12	M m	No	*	*	*	
GT-LS-13	M ming	No	*	*	*	
GT-LS-14	M m	No	*	*	*	
GT-LS-15	M ming	No	*	*	*	
GT-LS-16	Mm	No	*	*	*	
GT-LS-17	Mm	No	*	*	*	
GT-LS-18	Fm	No	*	*	*	
GT-LS-19	Fm	False +	*	*	*	nest of sperm
GT-LS-20	M m	No	*	*	*	
GT-LS-21	M m	No	*	*	*	
GT-LS-22	F m	False +	*	*	*	nest of sperm
GT-LS-23	M m	No	*	*	*	
GT-LS-24	Spleen only	No	*	*	*	
GT-LS-25	M m	No	*	*	*	
GT-LS-26	F m	No	*	*	*	
GT-LS-27	F ming	False +	*	*	Photo	sperm scattered deep in tissues, 1 region of putative spermatagonia PHOTO
GT-LS-28	M m	No	*	*	*	
GT-LS-29	M m	No	*	*	*	
GT-LS-30	M immature	No	*	*	*	
GT-LS-31	M ming	No	*	*	*	
GT-LS-32	M ming	No	*	*	*	
GT-LS-33	M ming	No	*	*	*	

GREAT SAND DUNES NATIONAL PARK & PRESERVE

MEDANO CREEK

Species: Rio Grande Cutthroat Trout

Number of Samples: 31

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
GRSA/MED-01	M ming	No	175	*	52	
GRSA/MED-02	M m	No	205	*	70	
GRSA/MED-03	M m	No	180	*	52	
GRSA/MED-04	No slide	No	75	*	4	tissue not provided by park
GRSA/MED-05	No tissue	No	107	*	11	
GRSA/MED-06	Spleen	No	218	*	109	
GRSA/MED-07	F m	No	160	*	40	
GRSA/MED-08	M m	No	153	*	28	
GRSA/MED-09	F m	No	145	*	20	
GRSA/MED-10	M m	No	250	*	148	
GRSA/MED-11	F m	No	190	*	60	
GRSA/MED-12	Fm	No	190	*	75	
GRSA/MED-13	Tapeworm, n	No	181	*	62	section of a tapeworm, not gonad
GRSA/MED-14	F m	No	171	*	50	
GRSA/MED-15	Fm	No	225	*	134	
GRSA/MED-16	Spleen only	No	155	*	39	
GRSA/MED-17	Spleen only	No	145	*	28	
GRSA/MED-18	F m	False +	204	*	84	putative testis with sperm RECUT PHOTO
GRSA/MED-19	F m	No	162	*	39	
GRSA/MED-20	M m	No	129	*	22	
GRSA/MED-21	M m	No	221	*	104	
GRSA/MED-22	M m	No	205	*	86	
GRSA/MED-23	M m	No	227	*	117	
GRSA/MED-24	M m	No	186	*	57	
GRSA/MED-25	F im	No	156	*	35	
GRSA/MED-26	F m	No	175	*	51	section of pancreas present with sperm float on
GRSA/MED-27	M m	No	191	*	58	
GRSA/MED-28	F m	No	207	*	82	
GRSA/MED-29	F ming	No	174	*	48	
GRSA/MED-30	F m	False +	139	*	25	areas of testis with sperm, located at edge of section so could be float-on RECUT
GRSA/MED-31	F ming	No	142	*	29	
GRSA/MED-32	F ming	No	179	*	52	

GREAT SAND DUNES NATIONAL PARK & PRESERVE *continued*

SAND CREEK

Species: Pikes Peak Cutthroat Trout Hybrid

Number of Samples: 32

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
GRSA/SAND-01	F m	No	268	*	207	
GRSA/SAND-02	M ming	No	259	*	196	
GRSA/SAND-03	F m	No	183	*	57	
GRSA/SAND-04	F m	False +	241	*	134	aggreate of sperm and spermatogonia RECUT
GRSA/SAND-05	M m	No	268	*	229	
GRSA/SAND-06	M m	No	218	*	118	
GRSA/SAND-07	M m	No	281	*	263	
GRSA/SAND-08	M m	No	183	*	60	
GRSA/SAND-09	M m	No	247	*	161	
GRSA/SAND-10	M m	No	256	*	144	
GRSA/SAND-11	F m	No	190	*	73	
GRSA/SAND-12	Fm	False +	223	*	113	sperm in on area, putative testis in another
GRSA/SAND-13	M m	No	216	*	96	
GRSA/SAND-14	M m	No	190	*	77	
GRSA/SAND-15	M m	No	225	*	119	
GRSA/SAND-16	F m	No	189	*	67	
GRSA/SAND-17	M m	No	237	*	134	
GRSA/SAND-18	F im	No	150	*	28	
GRSA/SAND-19	F m	No	205	*	84	
GRSA/SAND-20	F im	No	164	*	41	
GRSA/SAND-21	M m	No	180	*	61	
GRSA/SAND-22	F minh	No	255	*	158	
GRSA/SAND-23	M ming	No	228	*	131	
GRSA/SAND-24	M m	No	215	*	115	
GRSA/SAND-25	F m	No	207	*	102	example of necrotic cells at edges of section that could be confused with sperm
GRSA/SAND-26	M m	No	302	*	279	
GRSA/SAND-27	F m	False +	276	*	218	sperm present PHOTO
GRSA/SAND-28	M m	No	207	*	92	
GRSA/SAND-29	M m	No	260	*	224	
GRSA/SAND-30	F m	No	200	*	72	
GRSA/SAND-31	F m	False +	225	*	108	RECUT
GRSA/SAND-32	Spleen only	No	110	*	12	tissue not provided by park

GREAT SAND DUNES NATIONAL PARK & PRESERVE *continued*

SAND CREEK *continued*

Species: Pikes Peak Cutthroat Trout Hybrid

Number of Samples: 32

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
GRSA/SAND-33	*	No	115	*	14	tissue not provided by park
GRSA/SAND-34	*	No	110	*	12	

LASSEN VOLCANIC NATIONAL PARK

LAKE: HORSESHOE

Species: Brook Trout

Number of Samples: 18

Block #	Sex & Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments
LAVO-HL-01	F m	No	*	224	*	
LAVO-HL-02	F m	False +	*	276	*	sperm
LAVO-HL-03	M m	No	*	338	*	
LAVO-HL-04	F m	No	*	262	*	
LAVO-HL-05	M m	No	*	314	*	
LAVO-HL-06	F m	No	*	224	*	minimal tissue
LAVO-HL-07	M m	No	*	230	*	
LAVO-HL-08	M m	No	*	316	*	
LAVO-HL-09	F m	No	*	303	*	
LAVO-HL-10	M m	No	*	266	*	
LAVO-HL-11	*	No	*	283	*	missing block # 11
LAVO-HL-12	M m	No	*	242	*	
LAVO-HL-13	F m	No	*	231	*	
LAVO-HL-14	F m	False +	*	354	*	Sperm and spermatogonia
LAVO-HL-15	F m	No	*	256	*	
LAVO-HL-16	M m	No	*	305	*	
LAVO-HL-17	M m	No	*	320	*	
LAVO-HL-18	M m	No	*	314	*	

LASSEN VOLCANIC NATIONAL PARK *continued*

LAKE: RIDGE

Species: Brook Trout

Number of Samples: 38

Block #	Sex	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LAVO-RL-01	M m	No	*	229	*	
LAVO-RL-02	M m	No	*	217	*	
LAVO-RL-03	M m	No	*	226	*	
LAVO-RL-04	F m	No	*	217	*	
LAVO-RL-05	M m	No	*	228	*	
LAVO-RL-06	M m	No	*	206	*	
LAVO-RL-07	F m	False +	*	211	*	`sperm and spermatogonia RECUT
LAVO-RL-08	M m	No	*	192	*	
LAVO-RL-09	F m	No	*	225	*	
LAVO-RL-10	F m	No	*	225	*	
LAVO-RL-11	F m	No	*	215	*	
LAVO-RL-12	No slide	No	*	227	*	missing block # 12
LAVO-RL-13	M m	No	*	218	*	
LAVO-RL-14	F m	False +	*	204	*	Testis deep in ovary PHOTO
LAVO-RL-15	M m	No	*	215	*	
LAVO-RL-16	F m	False +	*	211	*	small nest of sperm
LAVO-RL-17	M m	No	*	243	*	
LAVO-RL-18	F m	False +	*	209	*	small nest of sperm
LAVO-RL-19	F m	False +	*	226	*	small nest of sperm
LAVO-RL-20	F m	No	*	218	*	
LAVO-RL-21	F m	No	*	224	*	
LAVO-RL-22	*	No	*	199	*	missing block # 22
LAVO-RL-23	*	No	*	228	*	missing block # 23
LAVO-RL-24	*	No	*	216	*	missing block # 24
LAVO-RL-25	*	No	*	226	*	missing block # 25
LAVO-RL-26	*	No	*	211	*	missing block # 26
LAVO-RL-27	F m	No	*	223	*	
LAVO-RL-28	F m	No	*	217	*	
LAVO-RL-29	F m	No	*	210	*	
LAVO-RL-30	M m	No	*	217	*	
LAVO-RL-31	F m	No	*	221	*	
LAVO-RL-32	M m	No	*	191	*	
LAVO-RL-33	M m	No	*	230	*	

LASSEN VOLCANIC NATIONAL PARK *continued*

LAKE: RIDGE *continued*

Species: Brook Trout

Number of Samples: 38

Block #	Sex	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LAVO-RL-34	Fm	No	*	213	*	
LAVO-RL-35	F m	No	*	168	*	
LAVO-RL-36	M m	No	*	217	*	
LAVO-RL-37	F m	No	*	223	*	
LAVO-RL-38	Fm	False +	*	196	*	nest of sperm

LAKE: SUMMIT

Species: Brook Trout

Number of Samples: 38

Block #	Sex	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LAVO-SL-01	M m	No	*	218	150	
LAVO-SL-02	M m	No	*	232	175	
LAVO-SL-03	M m	No	*	194	100	
LAVO-SL-04	M m	No	*	398	1000	
LAVO-SL-05	F m	False +	*	203	100	a few mature sperm seen
LAVO-SL-06	M m	No	*	235	200	
LAVO-SL-07	F m	No	*	306	450	pseudocapsule on spleen surface
LAVO-SL-08	M m	No	*	295	350	
LAVO-SL-09	M m	No	*	335	500	
LAVO-SL-10	M m	No	*	320	500	
LAVO-SL-11	**F m**	False +	*****	**420**	**1200**	**a few mature sperm seen**
LAVO-SL-12	F m	No	*	242	250	
LAVO-SL-13	F m	No	*	213	150	
LAVO-SL-14	F m	No	*	292	400	
LAVO-SL-15	M m	No	*	187	100	
LAVO-SL-16	F m	No	*	390	1000	
LAVO-SL-17	M m	No	*	190	200	
LAVO-SL-18	F m	No	*	194	200	
LAVO-SL-19	M m	No	*	290	600	
LAVO-SL-20	F m	False +	*	203	600	mature sperm present, oophoritis
LAVO-SL-21	F m	False +	*	299	400	sperm and spermatagonia RECUT
LAVO-SL-22	F m	No	*	305	425	

LASSEN VOLCANIC NATIONAL PARK *continued*

LAKE: SUMMIT *continued*

Species: Brook Trout

Number of Samples: 38

Block #	Sex	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LAVO-SL-23	Mm	No	*	219	150	
LAVO-SL-24	M m	No	*	199	100	
LAVO-SL-25	M m	No	*	215	300	
LAVO-SL-26	F m	No	*	195	300	
LAVO-SL-27	M m	No	*	286	375	
LAVO-SL-28	M m	No	*	202	100	
LAVO-SL-29	M m	No	*	202	150	
LAVO-SL-30	F m	No	*	344	950	
LAVO-SL-31	F m	False +	*	288	600	sperm and spermatagonia RECUT
LAVO-SL-32	M m	No	*	272	590	
LAVO-SL-33	F m	No	*	282	350	
LAVO-SL-34	M m	No	*	229	590	
LAVO-SL-35	F m	No	*	197	100	
LAVO-SL-36	F m	No	*	316	550	
LAVO-SL-37	M m	False +	*	213	15	egg in testis RECUT
LAVO-SL-38	*	No	*	390	800	

LAKE: UNKNOWN

Species: Unknown

Number of Samples: 6

Block #	Sex	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
blank 1	M m	No	*	*	*	
blank 2	F m	False +	*	*	*	small nest of spermatogonia
blank 3	M m	No	*	*	*	
blank 4	F m	False +	*	*	*	sperm
blank 5	M m	No	*	*	*	
blank 6	``````````````````````````````````````		*	*	*	

MOUNT RAINIER NATIONAL PARK

LAKE: ADELAIDE

Species: Rainbow Trout

Number of Samples: 1

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LF-01 (MORA-AD)	/MA	No	364	352	460	

LAKE: BLUE

Species: ONMY

Number of Samples: 7

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LZ-35-01 (MORA-BLU)	/IMM	No	211	202	120	
LZ-35-02 (MORA-BLU)	/MA	No	367	352	390	
LZ-35-03 (MORA-BLU)	/MA	No	363	342	385	
LZ-35-04 (MORA-BLU)	/MA	No	384	365	440	
LZ-35-05 (MORA-BLU)	/MA	No	409	384	485	
LZ-35-06 (MORA-BLU)	/MA	No	374	362	465	
LZ-35-07 (MORA-BLU)	/MA	No	223	208	110	

LAKE: DEADWOOD (upper portion)

Species: ONMY

Number of Samples: 3

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LW-32-01 (MORA-DW)	/MA	No	363	348	430	
LW-32-02 (MORA-DW)	/MA	No	347	330	340	
LW-32-03 (MORA-DW)	/IMM	No	212	202	80	

MOUNT RAINIER NATIONAL PARK *continued*

LAKE: ETHEL

Species: ONMY

Number of Samples: 6

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LF-04-01 (MORA-ET)	/MA	No	338	327	350	
LF-04-02 (MORA-ET)	/IMM	No	216	209	120	
LF-04-03 (MORA-ET)		No				
LF-04-03* (MORA-ET)		No				
LF-04-04 (MORA-ET)	/MA	No	303	294	270	
LF-04-05 (MORA-ET)	/MA	No	294	284	260	
LF-04-06 (MORA-ET)	/MA	No	334	316	340	

LAKE: GREEN

Species: Westslope Cutthroat

Number of Samples: 3

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LC-07-01 (MORA-GR)	/MA	No	350	336	462	
LC-07-02 (MORA-GR)	/IMM	No	159	152	38	
LC-07-03 (MORA-GR)	/MA	No	314	300	346	

LAKE: MYSTIC

Species: ONCL

Number of Samples: 3

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
Mystic 1	/MA	No	288	280	176	
Mystic 2	/MA	No	301	290	210	
Mystic 3	/MA	No	236	230	102	

MOUNT RAINIER NATIONAL PARK *continued*

LAKE: SNOW

Species: West Slope Cutthroat & Brook Trout

Number of Samples: 11

Block #	Sex/Maturity (Species)	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LZ-29-01 (MORA-SNO)	/IMM (WCT)	No	158	150	45	
LZ-29-02 (MORA-SNO)	/IMM (WCT)	No	207	200	103	
LZ-29-03 (MORA-SNO)	/MA (BRK)	No	233	230	138	
LZ-29-04 (MORA-SNO)	/IMM (WCT)	No	162	157	49	
LZ-29-05 (MORA-SNO)	/MA (WCT)	No	160	154	45	
LZ-29-06 (MORA-SNO)	/MA (WCT)	No	239	235	152	
LZ-29-07 (MORA-SNO)	/IMM (BRK)	No	159	150	38	
LZ-29-08 (MORA-SNO)	/MA (WCT)	No	273	262	75	
LZ-29-09 (MORA-SNO)	/MA (WCT)	No	221	218	191	
LZ-29-10 (MORA-SNO)	/MA (WCT)	No	201	195	108	
LZ-29-11 (MORA-SNO)	/MA (BRK)	No	259	253	183	

NORTH CASCADES NATIONAL PARK

LAKE: DIOBSUD

Species: Westslope Cutthroat Trout

Number of Samples: 24

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
LS-02-01-01	M m	No	*	226	*	
LS-02-01-02	F m	No	*	170	*	
LS-02-01-03	F im	No	*	119	*	
LS-02-01-04	M m	No	*	123	*	
LS-02-01-05	M m	No	*	128	*	
LS-02-01-06	F m	No	*	222	*	
LS-02-01-07	F ming	No	*	136	*	
LS-02-01-08	M ming	No	*	105	*	
LS-02-01-09	M ming	No	*	135	*	
LS-02-01-10	F ming	No	*	118	*	
LS-02-01-11	M m	No	*	203	*	
LS-02-01-12	F ming	No	*	135	*	
LS-02-01-13	No gonad	No	*	115	*	
LS-02-01-14	M m	No	*	154	*	
LS-02-01-15	M m	No	*	194	*	
LS-02-01-16	F m	No	*	265	*	
LS-02-01-17	F ming	False +	*	120	*	sperm deep in tissues
LS-02-01-18	F ming	No	*	110	*	
LS-02-01-19	M m	No	*	226	*	
LS-02-01-20	M m	No	*	200	*	
LS-02-01-21	F ming	No	*	116	*	
LS-02-01-22	M ming	No	*	126	*	
LS-02-01-23	M ming	No	*	180	*	
LS-02-01-24	M ming	No	*	105	*	

NORTH CASCADES NATIONAL PARK *continued*

LAKE: LOWER BERDEEN

Species: Westslope Cutthroat Trout

Number of Samples: 30

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
NOCA-LOBE-01	M m	No	*	250	*	
NOCA-LOBE-02	M m	No	*	214	*	
NOCA-LOBE-03	F m	?	*	235	*	sperm at edge of section, contamination
NOCA-LOBE-04	F m	No	*	210	*	
NOCA-LOBE-05	F im	False +	*	140	*	sperm deep in ovary
NOCA-LOBE-06	F ming	No	*	154	*	
NOCA-LOBE-07	F ming	No	*	206	*	
NOCA-LOBE-08	F ming	No	*	197	*	
NOCA-LOBE-09	M m	No	*	199	*	
NOCA-LOBE-10	F ming	?	*	137	*	sperm at edge of section, contamination
NOCA-LOBE-11	M m	No	*	159	*	
NOCA-LOBE-12	F m	No	*	220	*	
NOCA-LOBE-13	F m	No	*	231	*	
NOCA-LOBE-14	F m	No	*	216	*	
NOCA-LOBE-15	M m	No	*	169	*	
NOCA-LOBE-16	M ming	No	*	220	*	
NOCA-LOBE-17	F m	No	*	206	*	
NOCA-LOBE-18	M m	No	*	199	*	
NOCA-LOBE-19	F m	No	*	132	*	
NOCA-LOBE-20	F m	False +	*	162	*	small nest of sperm and testis RECUT PHOTO TAKEN
NOCA-LOBE-21	M m	No	*	214	*	
NOCA-LOBE-22	M m	No	*	155	*	
NOCA-LOBE-23	F m	No	*	215	*	
NOCA-LOBE-24	M m	No	*	261	*	
NOCA-LOBE-25	M m	No	*	163	*	
NOCA-LOBE-26	F m	No	*	177	*	
NOCA-LOBE-27	F m	No	*	182	*	
NOCA-LOBE-28	M m	No	*	273	*	
NOCA-LOBE-29	F ming	No	*	197	*	
NOCA-LOBE-30	F ming	No	*	218	*	

NORTH CASCADES NATIONAL PARK *continued*

LAKE: UPPER TRIPLET

Species: Westslope Cutthroat Trout

Number of Samples: 25

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
SM-02-01-01	M m	No	*	200	*	very mature
SM-02-01-02	M m	No	*	258	*	very mature
SM-02-01-03	M m	No	*	152	*	very mature
SM-02-01-04	F m	No	*	250	*	
SM-02-01-05	F m	No	*	158	*	
SM-02-01-06	F ming	No	*	153	*	
SM-02-01-07	F immature	No	*	146	*	
SM-02-01-08	F ming	No	*	213	*	
SM-02-01-09	Spleen only	No	*	146	*	
SM-02-01-10	F immature	No	*	148	*	
SM-02-01-11	F immature	No	*	135	*	
SM-02-01-12	F m	No	*	250	*	
SM-02-01-13	Spleen only	No	*	113	*	
SM-02-02-14	F immature	No	*	187	*	
SM-02-02-15	M m	No	*	154	*	
SM-02-01-16	M m	No	*	261	*	
SM-02-02-17	M ming	No	*	148	*	
M-02-02-18	M m	No	*	156	*	
M-02-02-19	F immature	No	*	138	*	
M-02-02-20	M m	No	*	151	*	
M-02-02-21	Spleen only	No	*	140	*	
M-02-02-22	Spleen only	No	*	94	*	
M-02-02-23	F m	No	*	225	*	
M-02-02-24	F m	No	*	240	*	
M-02-02-25	F ming	No	*	218	*	

ROCKY MOUNTAIN NATIONAL PARK

LAKE: MIRROR

Species: Brook Trout

Number of Samples: 30

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
ROMO-MIR-01	F m	False +	*	253	*	cluster of sperm
ROMO-MIR-02	F m	No	*	253	*	
ROMO-MIR-03	M m	No	*	252	*	
ROMO-MIR-04	M m	No	*	248	*	
ROMO-MIR-05	F m	No	*	259	*	
ROMO-MIR-06	F m	No	*	181	*	
ROMO-MIR-07	F m	No	*	250	*	
ROMO-MIR-08	F m	No	*	241	*	
ROMO-MIR-09	F m	No	*	268	*	
ROMO-MIR-10	F m	No	*	247	*	
ROMO-MIR-11	F m	No	*	258	*	
ROMO-MIR-12	F m	No	*	258	*	
ROMO-MIR-13	F m	No	*	260	*	
ROMO-MIR-14	M m	No	*	247	*	
ROMO-MIR-15	F m	No	*	250	*	
ROMO-MIR-16	M m	No	*	275	*	
ROMO-MIR-17	F m	No	*	253	*	
ROMO-MIR-18	F m	No	*	242	*	
ROMO-MIR-19	F m	No	*	238	*	
ROMO-MIR-20	F m	No	*	242	*	
ROMO-MIR-21	M m	No	*	232	*	
ROMO-MIR-22	M m	No	*	258	*	
ROMO-MIR-23	Mm	No	*	240	*	
ROMO-MIR-24	M IM	No	*	198	*	
ROMO-MIR-25	M	No	*	262	*	
ROMO-MIR-26	F m	No	*	210	*	
ROMO-MIR-27	M m	No	*	266	*	
ROMO-MIR-28	M m	No	*	244	*	
ROMO-MIR-29	M m	No	*	173	*	
ROMO-MIR-30	F m	No	*	264	*	sperm float on

ROCKY MOUNTAIN NATIONAL PARK *continued*

LAKE: POUDRE

Species: Brook Trout

Number of Samples: 30

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
ROMO-POUD-01	F m	No	*	211	*	
ROMO-POUD-02	M IM	No	*	208	*	
ROMO-POUD-03	F m	No	*	226	*	
ROMO-POUD-04	F ming	No	*	282	*	ming = maturing
ROMO-POUD-05	F ming	No	*	251	*	
ROMO-POUD-06	M m	No	*	267	*	
ROMO-POUD-07	Mm	No	*	262	*	
ROMO-POUD-08	Mm	No	*	220	*	
ROMO-POUD-09	M mig	No	*	267	*	
ROMO-POUD-10	F m	No	*	270	*	
ROMO-POUD-11	Fm	No	*	291	*	
ROMO-POUD-12	M ming	No	*	260	*	
ROMO-POUD-13	F m	No	*	211	*	
ROMO-POUD-14	F m	No	*	208	*	
ROMO-POUD-15	F m	No	*	258	*	
ROMO-POUD-16	M ming	No	*	208	*	
ROMO-POUD-17	F m	No	*	172	*	sperm float ons
ROMO-POUD-18	M m	No	*	153	*	
ROMO-POUD-19	M m	No	*	150	*	
ROMO-POUD-20	F m	No	*	263	*	
ROMO-POUD-21	M m	No	*	265	*	
ROMO-POUD-22	M m	No	*	285	*	
ROMO-POUD-23	M m	No	*	270	*	
ROMO-POUD-24	F m	False +	*	227	*	Recut spermatocytes and sperm
ROMO-POUD-25	*	No	*	270	*	
ROMO-POUD-26	M ming	No	*	260	*	
ROMO-POUD-27	F m	No	*	245	*	
ROMO-POUD-28	M IM	No	*	222	*	
ROMO-POUD-29	M ming	No	*	256	*	
ROMO-POUD-30	M ming	No	*	202	*	

ROCKY MOUNTAIN NATIONAL PARK *continued*

LAKE: YPSILON

Species: Colorado River Cutthroat Trout

Number of Samples: 30

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
ROMO-YSP-01	F m	False +	*	246	*	sperm contamination?
ROMO-YSP-02	F m	False +	*	298	*	sperm contamination?
ROMO-YSP-03	F m	False +	*	242	*	
ROMO-YSP-04	F m	False +	*	235	*	sperm contamination?
ROMO-YSP-05	F m	False +	*	258	*	sperm contamination
ROMO-YSP-06	M ming	No	*	250	*	
ROMO-YSP-07	F m	False +	*	287	*	sperm contamination?
ROMO-YSP-08	F m	False +	*	248	*	sperm contamination?
ROMO-YSP-09	F m	False +	*	273	*	sperm contamination?
ROMO-YSP-10	F m	No	*	238	*	
ROMO-YSP-11	M m	No	*	249	*	
ROMO-YSP-12	F m	No	*	321	*	sperm contamination
ROMO-YSP-13	F m	False +	*	278	*	spematids present
ROMO-YSP-14	F m	False +	*	298	*	spermatids present
ROMO-YSP-15	F m	False +	*	253	*	spermatids present
ROMO-YSP-16	F m	False +	*	294	*	spermatids present
ROMO-YSP-17	M m	No	*	237	*	spermatids present
ROMO-YSP-18	F m	False +	*	272	*	spermatids present
ROMO-YSP-19	F m	False +	*	263	*	spermatids present
ROMO-YSP-20	F m	False +	*	278	*	spermatids present
ROMO-YSP-21	F m	False +	*	308	*	spermatids present
ROMO-YSP-22	M m	No	*	190	*	
ROMO-YSP-23	M m	No	*	175	*	
ROMO-YSP-24	M m	No	*	233	*	
ROMO-YSP-25	F m	False +	*	202	*	spermatids present
ROMO-YSP-26	M m	No	*	246	*	
ROMO-YSP-27	M m	No	*	238	*	
ROMO-YSP-28	F m	False +	*	290	*	Good example of atric follicle with sperm clump
ROMO-YSP-29	M m	No	*	247	*	
ROMO-YSP-30	M m	No	*	220	*	

SEQUOIA & KINGS CANYON NATIONAL PARKS

LAKE: BENCH

Species: 1-29: Brook Trout & 30-40: Golden/Rainbow Trout Hybrid

Number of Samples: 40

Block #	Sex/Maturity	Intersex	Length (mm)	Weight (g)	Comments:
SEKI-BL-01	No gonad	No	125	*	
SEKI-BL-02	M m	No	182	*	maturing
SEKI-BL-03	F m	No	120	*	maturing
SEKI-BL-04	No gonad	No	121	*	
SEKI-BL-05	No gonad	No	120	*	maturing
SEKI-BL-06	F m	No	101	*	
SEKI-BL-07	M im/IMM	No	175	*	
SEKI-BL-08	F m	No	150	*	maturing
SEKI-BL-09	M im/IMM	No	154	*	
SEKI-BL-10	M im/IMM	No	154	*	
SEKI-BL-11	M im/IMM	No	128	*	
SEKI-BL-12	F im	No	109	*	
SEKI-BL-13	M m	No	178	*	
SEKI-BL-14	M im	No	165	*	
SEKI-BL-15	M im	No	166	*	
SEKI-BL-16	F m/MA	No	148	*	
SEKI-BL-17	F m	No	151	*	maturing
SEKI-BL-18	No gonad	No	104	*	
SEKI-BL-19	M im	No	104	*	
SEKI-BL-20	No gonad	No	122	*	
SEKI-BL-21	M im	No	166	*	
SEKI-BL-22	No gonad	No	109	*	
SEKI-BL-23	M m	No	165	*	
SEKI-BL-24	F m	No	148	*	maturing
SEKI-BL-25	No gonad	No	126	*	
SEKI-BL-26	M m	No	145	*	maturing
SEKI-BL-27	No gonad	No	105	*	
SEKI-BL-28	F m/IMM	No	120	*	maturing
SEKI-BL-29	F m	No	110	*	maturing
SEKI-BL-30	M im	No	112	*	
SEKI-BL-31	F m	No	157	*	maturing
SEKI-BL-32	M m	No	169	*	maturing
SEKI-BL-33	M m	No	185	*	maturing

SEQUOIA & KINGS CANYON NATIONAL PARKS *continued*

LAKE: BENCH *continued*

Species: 1-29: Brook Trout & 30-40: Golden/Rainbow Trout Hybrid

Number of Samples: 40

Block #	Sex/Maturity	Intersex	Length (mm)	Weight (g)	Comments:
SEKI-BL-34	F m	No	189	*	maturing
SEKI-BL-35	M m	No	165	*	maturing
SEKI-BL-36	F m	No	176	*	maturing
SEKI-BL-37	M m	No	183	*	maturing
SEKI-BL-38	M m	No	169	*	maturing
SEKI-BL-39	F m	??	159	*	spawned out, probably testis float on
SEKI-BL-40	M m	No	150	*	maturing

LAKE: KERN POINT

Species: Golden/Rainbow Trout Hybrid

Number of Samples: 32

Block #	Sex/Maturity	Intersex	Length (mm)	Weight (g)	Comments:
SEKI-KP-01	M m	No	211	*	
SEKI-KP-02	F m	No	213	*	
SEKI-KP-03	F m	No	191	*	
SEKI-KP-04	M m	No	216	*	
SEKI-KP-05	F m	No	123	*	ectopic kidney
SEKI-KP-06	F m	No	154	*	
SEKI-KP-07	M m	No	195	*	good example of Sertoli cells
SEKI-KP-08	Fm	No	202	*	
SEKI-KP-09	F m	No	217	*	
SEKI-KP-10	F m	No	192	*	
SEKI-KP-11	M m	No			
SEKI-KP-11*		No			
SEKI-KP-12	F m	No	234	*	
SEKI-KP-13	M m	No	181	*	
SEKI-KP-14	M m	No	194	*	
SEKI-KP-15	M m	No	155	*	
SEKI-KP-16	M m	No	189	*	
SEKI-KP-17	/Imm	No	133	*	no gonads in section
SEKI-KP-18	M m	No	190	*	
SEKI-KP-19	F/IMM	False +	132	*	small area in duct with sperm
SEKI-KP-20	Mm	No	204	*	

SEQUOIA & KINGS CANYON NATIONAL PARKS *continued*

LAKE: KERN POINT *continued*

Species: Golden/Rainbow Trout Hybrid

Number of Samples: 32

Block #	Sex/Maturity	Intersex	Length (mm)	Weight (g)	Comments:
SEKI-KP-21	F m	No	268	*	
SEKI-KP-22	F m	No	226	*	sperm at edge of ovary, contamination
SEKI-KP-23	M m	No	238	*	
SEKI-KP-24	M m	No	212	*	
SEKI-KP-25	M m	No	260	*	
SEKI-KP-26	M m	No	153	*	
SEKI-KP-27	F m	No	179	*	
SEKI-KP-28	F m	No	199	*	
SEKI-KP-29	M m	No	198	*	
SEKI-KP-30	F m	No	190	*	
SEKI-KP-31	M m	No	215	*	
SEKI-KP-32	*	No	212	*	no slides?

WRANGELL-ST. ELIAS NATIONAL PARK & PRESERVE

LAKE: SUMMIT

Species: Rainbow Trout

Number of Samples: 30

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
WRST-SUM-01	M/m	No	*	187	*	
WRST-SUM-02	M m	No	*	345	*	
WRST-SUM-03	F m	?	*	347	*	spermatozoa only
WRST-SUM-04	M m	No	*	256	*	
WRST-SUM-05	M m	No	*	450	*	little
WRST-SUM-06	F m	?	*	449	*	spermatozoa only
WRST-SUM-07	M m	No	*	312	*	
WRST-SUM-08	M m	No	*	223	*	
WRST-SUM-09	No gonads	No	*	113	*	
WRST-SUM-10	M m	No	*	223	*	
WRST-SUM-11	No gonads	No	*	171	*	
WRST-SUM-12	M m	No	*	216	*	
WRST-SUM-13	M m	No	*	279	*	
WRST-SUM-14	M m	No	*	235	*	
WRST-SUM-15	M m	No	*	354	*	
WRST-SUM-16	M m	No	*	270	*	
WRST-SUM-17	M m	No	*	420	*	
WRST-SUM-18	M m	No	*	148	*	
WRST-SUM-19	M m	No	*	227	*	
WRST-SUM-20	F m	?		382	*	free sperm
WRST-SUM-21	No gonads	No	*	164	*	
WRST-SUM-22	M m	No	*	310	*	
WRST-SUM-23	M m	No	*	239	*	spawned out
WRST-SUM-24	No gonads	No	*	140	*	
WRST-SUM-25	No gonads	No	*	150	*	
WRST-SUM-26	M m	No	*	157	*	
WRST-SUM-27	M m	No	*	262	*	
WRST-SUM-28	M m	No	*	352	*	spawned out
WRST-SUM-29	M m	No	*	148	*	
WRST-SUM-30	M m	No	*	142	*	

WRANGELL-ST. ELIAS NATIONAL PARK & PRESERVE *continued*

LAKE: TANADA

Species: Lake Trout

Number of Samples: 19

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
WRST-TAN-01	M m	No	*	489	*	good example of all stages
WRST-TAN-02	F m	False +	*	570	*	various stages of spermatogenesis
WRST-TAN-03	M m	No	*	515	*	maturing - all below males are similar
WRST-TAN-04	M m	No	*	573	*	
WRST-TAN-05	M m	No	*	464	*	
WRST-TAN-06	M m	No	*	515	*	
WRST-TAN-07	F m	No	*	504	*	
WRST-TAN-08	M m	No	*	558	*	
WRST-TAN-09	M m	No	*	605	*	
WRST-TAN-10	M m	No	*	478	*	
WRST-TAN-11	F m	No	*	520	*	minimal tissue to evaulte
WRST-TAN-12	F m	False +	*	500	*	intersex, early spermatogonia? Present
WRST-TAN-13	F m	No	*	519	*	
WRST-TAN-14	M m	No	*	464	*	
WRST-TAN-15	M m	No	*	448	*	
WRST-TAN-16	F m	No	*	526	*	
WRST-TAN-17	F m	False +	*	499	*	sperm? Spermatagonia?
WRST-TAN-18	M m	No	*	514	*	
WRST-TAN-19	M m	No	*	565	*	

YOSEMITE NATIONAL PARK

LAKE: ELEANOR RESERVOIR

Species: Rainbow Trout

Number of Samples: 2

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
YOS-70675-01	M m	No	*	385	*	
YOS-70675-02	M m	No	*	275	*	

LAKE: MILDRED

Species: Brook Trout

Number of Samples: 38

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
YOS-ML-01	M m	No	*	300	*	
YOS-ML-02	No gonad	No	*	285	*	
YOS-ML-03	No gonad	No	*	167	*	
YOS-ML-04	M m	No	*	162	*	
YOS-ML-05	M m	False +	*	265	*	numerous immature ova
YOS-ML-06	F m	No	*	270	*	
YOS-ML-07	F m	No	*	218	*	
YOS-ML-08	F m	No	*	273	*	
YOS-ML-09	F m	No	*	275	*	
YOS-ML-10	F m	No	*	280	*	
YOS-ML-11	Mm	No	*	292	*	
YOS-ML-12	Mm	No	*	300	*	regions of necrosis in testis
YOS-ML-13	F m	No	*	307	*	
YOS-ML-14	F m	No	*	290	*	severe cestode larva infection in gonads and spleen - few eggs present
YOS-ML-15	M m	No	*	265	*	
YOS-ML-16	F m	No	*	221	*	
YOS-ML-17	Mm	No	*	239	*	
YOS-ML-18	Mm	No	*	309	*	
YOS-ML-19	F m	No	*	255	*	
YOS-ML-20	M m	No	*	307	*	
YOS-ML-21	F m	No	*	252	*	
YOS-ML-22	F m	No	*	244	*	

YOSEMITE NATIONAL PARK *continued*

LAKE: MILDRED *continued*

Species: Brook Trout

Number of Samples: 38

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
YOS-ML-23	M m	No	*	199	*	
YOS-ML-24	F m	No	*	269	*	
YOS-ML-25	No gonad	No	*	249	*	
YOS-ML-26	F m	False +	*	305	*	possible nest of spermatogonia
YOS-ML-27	M m	No	*	270	*	egg float on
YOS-ML-28	Mm	No	*	284	*	
YOS-ML-29	Mm	No	*	189	*	
YOS-ML-30	Mm	No	*	185	*	
YOS-ML-31	Mm	No	*	235	*	
YOS-ML-32	Mm	No	*	227	*	
YOS-ML-33	F m	No	*	178	*	
YOS-ML-34	Spleen only	No	*	284	*	
YOS-ML-35	F m	No	*	148	*	
YOS-ML-36	F m	No	*	139	*	
YOS-ML-37	M m	No	*	169	*	
YOS-ML-38	M m	No	*	187	*	

LAKE: SPILLWAY

Species: Brooke Trout

Number of Samples: 31

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
YOS-SL-01	M m	No	*	275	*	
YOS-SL-02	Spleen only	No	*	115	*	
YOS-SL-03	M m	No	*	235	*	
YOS-SL-04	M m	No	*	258	*	
YOS-SL-05	F m	No	*	225	*	
YOS-SL-06	Fm	False +	*	260	*	aggregates of mature sperm
YOS-SL-07	M m	No	*	275	*	
YOS-SL-08	M m	No	*	143	*	
YOS-SL-09	F immature	False +	*	148	*	sperm and possible spermatogonia
YOS-SL-10	F m	No	*	250	*	

YOSEMITE NATIONAL PARK *continued*

LAKE: SPILLWAY *continued*

Species: Brooke Trout

Number of Samples: 31

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
YOS-SL-11	M m	No	*	275	*	
YOS-SL-12	F m	No	*	253	*	
YOS-SL-13	Fm	False +	*	270	*	sperm and possible spermatogonia
YOS-SL-14	F m	No	*	180	*	
YOS-SL-15	F m	No	*	172	*	
YOS-SL-16	M m	No	*	293	*	
YOS-SL-17	M m	No	*	202	*	
YOS-SL-18	M m	No	*	295	*	
YOS-SL-19	M m	No	*	205	*	
YOS-SL-20	F m	No	*	265	*	
YOS-SL-21	F m	No	*	272	*	
YOS-SL-23	M m	No	*	150	*	
YOS-SL-24	M m	No	*	280	*	
YOS-SL-25	M m	No	*	265	*	
YOS-SL-26	M m	No	*	240	*	
YOS-SL-27	F m	No	*	237	*	
YOS-SL-28	M m	No	*	227	*	
YOS-SL-29	M m	No	*	268	*	
YOS-SL-30	M m	No	*	232	*	
YOS-SL-33	M m	No	*	260	*	
YOS-SL-36	F m	No	*	240	*	

LAKE: SPILLWAY

Species: Brown Trout

Number of Samples: 5

Block #	Sex/Maturity	Intersex	Length (mm) Total	Length (mm) Fork	Weight (g)	Comments:
YOS-SL-22	Spleen only	No	*	165	*	
YOS-SL-31	M m	No	*	237	*	
YOS-SL-32	M m	No	*	290	*	
YOS-SL-34	M m	No	*	285	*	
YOS-SL-35	M m	No	*	143	*	

D. Female Diagnostic Accuracy Experiment

Data from the Female Diagnostic Accuracy Experiment showing false positives (intersex females) for many fish. One ovary from each fish was placed into a container of buffered formalin and equipment and solutions were cleaned to ensure that there could be no contamination with testicular material (ovaries alone). We placed the other ovary from each fish into a container that contained the testes that were sampled from all of the males obtained at the same time; these were histologically processed along with the testes (ovaries and Testes). "recut" refers to others histological preparations from the same Block (our histological sample). False + represents a sample of an ovary artificially contaminated with male gametes due to processing procedures.

CRATER LAKE NATIONAL PARK

Contact: Samples collected by Carl Schreck, Julia Unrein, Kristin Berkenkamp July 20 2010

LAKE: CRATER

Species: Kokanee

Number of Samples: 15

Block #	Sex & Maturity Determined by gross examination	Sex & Maturity determined by histology	Intersex	Total Length (cm)	Fork Length (cm)	Weight (g)	Initial Slide Females only	Initial Slide Mixed	additional slide recuts females only			additional slide recuts mixed			Comments
									recut 1	recut 2	recut 3	recut 1	recut 2	recut 3	
10CRLA-1	M/mature		No		25.2	210	None	neg	None	None	None	None	None	None	
10CRLA-9	F/immature		False +		18	50	neg	Sperm	neg	neg	neg	Sperm	Sperm	Sperm	Sperm associated with piece of connective tissue/not directly in ovary
10CRLA-10	M/immature		No		16.7	45	None	None	None	None	None	None	None	None	no tissue found in cassette
10CRLA-11	F/immature		False +		17.1	58	neg	Sperm	neg	neg	neg	Sperm	Sperm	Sperm	Sperm in ovary, associated with RBCs
10CRLA-13	M/immature		No		18.7	68		neg	None	None	None	None	None	None	
10CRLA-14	F/nearly ripe		False +		32.3	400	Neg	Sperm	Neg	Neg	Neg	Sperm	Sperm	Sperm	small amount of sperm observed in every section
10CRLA-15	F/immature		No		17.4	55	neg	neg	neg	neg	neg	neg	Sperm	neg	sperm outside gonad
10CRLA-16			No		17	52	None	None	None	None	None	None	None	None	autolysis-no tissue sampled
10CRLA-17			No		17.2	48	None	None	None	None	None	None	None	None	autolysis-no tissue sampled
10CRLA-18			No		17.5	59	None	None	None	None	None	None	None	None	autolysis-no tissue sampled
10CRLA-19	M/mature		No		26.5	250	None		None	None	None	None	None	None	
10CRLA-20	F/immature		False +		16.6	44	Neg	??	neg	neg	neg	Sperm	Sperm	Sperm	a few mature sperm with RBCs deep in gonad

Block #	Sex & Maturity Determined by gross examination	Sex & Maturity determined by histology	Intersex	Total Length (cm)	Fork Length (cm)	Weight (g)	Initial Slide Females only	Initial Slide Mixed	additional slide recuts females only			additional slide recuts mixed			Comments
									recut 1	recut 2	recut 3	recut 1	recut 2	recut 3	
10CRLA-21	M/immature		No		16.6	52			None	None	None	None	None	None	
10CRLA-22			No		16.5	did not weigh fish	None	None	None	None	None	None	None	None	autolysis-no tissue sampled
10CRLA-24	M/immature		No		17.4	68	None	None	None	None	None	None	None	None	no tissue found in cassette

LAKE: CRATER

Species: Rainbow Trout

Number of Samples: 12

Block #	Sex & Maturity Determined by gross examination	Sex & Maturity determined by histology	Intersex	Total Length (cm)	Length (cm) Fork	Weight (g)	Initial Slide Females only	Initial Slide Mixed	additional slide recuts females only			additional slide recuts mixed			Comments
									recut 1	recut 2	recut 3	recut 1	recut 2	recut 3	
10CRLA-2	M/immature				30.3	330	None	neg	None	None	None	None	None	None	
10CRLA-3	F/mature		False +		34.1	519	Neg	neg	neg	neg	neg	sperm	sperm	SPERM	at edge of section, not in gonad proper
10CRLA-4	F/immature		No		30.5	342	Neg	neg	neg	neg	neg	neg	neg	neg	
10CRLA-5	M/immature		No		26.3	195	None	neg	None	None	None	None	None	None	
10CRLA-6	F/immature		No		40.5	862	neg	neg	neg	neg	neg	neg	neg	neg	
10CRLA-7	F/immature		No		20.4	100	neg	neg	neg	neg	neg	neg	neg	neg	
10CRLA-8	M/immature		No		23.3	150	None	neg	None	None	None	None	None	None	
10CRLA-12	F/immature		False +		16.2	48	neg	neg	neg	neg	neg	neg	neg	SPERM	small aggregate at edge of gonad
10CRLA-23	M/immature		No		16.3	56	None		None	None	None	None	None	None	
10CRLA-25	F/immature (male)		No		14.7	48	neg		neg	neg	neg	neg	neg	neg	Actually an immature male, no sperm present
10CRLA-26	F/immature		No		16.7	56	neg		neg	neg	neg	None	noe	noe	No gonads in recut of mix
10CRLA-27	F/immature		False +		18.1	60	neg		neg	neg	neg	None	sperm	SPERM	few mature sperm deep in gonad