APPENDIX C
Photographs of example deformities and Summary of the Deformity Rankings for Each Sample-CSU

Deformity Assessment

The general scoring criteria were adopted from Holm et al. (2003) and included assessments of craniofacial deformities, mostly of the head, eyes, and jaw, vertebral deformities, fin deformities, and edema. The original publication showed pictures of some deformities but others, particularly the intermediate categories were not illustrated or were poorly described. More specific definitions for each of the assessment categories were developed to give better repeatability and consistency across studies, and to aid others in learning the range of deformities possible.

Deformities in each of the categories described above were given a score from 0-3, with 0 being a normal condition and 3 being the most deformed. Some range finding was conducted over the first several samples to find background and severe levels of deformities in each category. Initial samples were rescored as necessary to bring them into compliance with the standards that were used throughout the assessment. In the second batch of fish analyzed (~100 from 5 LSV2C sites), it was not always possible to score each fish for each category due to the condition of the organism. Therefore, in several cases no scoring was possible.

The protocol for assessing damage was to place several fish, head to the left, in a Petri dish and examine them under a dissecting microscope and 10X magnification. The lateral side was examined for spinal deformities (lordosis, kyphosis), appearance of the eye, head and snout shape, edema, and fin deformities. The fish was turned ventrally to look for mouth deformities and further spinal deformities (scoliosis), turned laterally again for the same criteria as the other side, and then dorsally for issues associated with eyes, head size, spinal deformities.

Craniofacial deformities included shortening of the jaw, snout, and missing or poorly developed eye or eyes, and head shape abnormalities. A slightly shortened lower jaw (<= 1 lip width) received a 1, a shortened jaw = 2 lip widths or a slightly shortened and slightly disfigured jaw = 2, and a flat lower jaw or much disfigured (non-functional) jaw = 3. An assessment of fish independent of this study revealed that other brown trout of the same size and developmental state did not have the slight deformity that was assessed as CF =1 for the jaw (J). Thus, the CF = 1 score where the J was concerned were deemed real. A slightly blunted snout (about 50% eye diameter, usually is > than that) = 1, very blunt or flat = 2, deformed or bulbous = 3. Eye deformities were scored as one eye blind or poorly pigmented or poorly developed =1, both poorly developed = 2, both blind = 3. Skulls that were slightly bulbous (1/3 > normal) = 1, moderately bulbous (2/3 > normal) = 2, and bulbous (1x or > than normal) = 3. Usually factors occurred together so a combination of two “1” conditions = 2, three “1” conditions = 3, or a 1 and a 2 = 3, and so on. For example, a deformed jaw and a blind eye = 2, two blind eyes = 2, but a badly deformed jaw (= 2 alone) plus a blind eye (= 1 alone), = 3.

Skeletal deformities included any deformity of the vertebrae or spines. A slight bend of less than 45 degrees (but > than body width off of straight) or a minor body constriction (e.g. a tight rubber band about the body effect) was given a score of 1, 2 slight bends or constrictions anywhere, or bend of > 45-90 degrees was scored a 2, and multi-directional

bends > 90 degrees were given a 3. Bends caused by skeletal deformities were usually detectable from normal bending of the body during preservation (these fish were usually well preserved, very straight) by presence of a slight or greater bump below the surface of the epidermis on the outside of the bend. However, some fish with SD = 1 had just a very slight bend in the range the deformity described but could be due to preservation or the poor condition of the fish. This was sometimes especially true in larger fish, which may be more muscular and undergo stronger contraction during preservation and thus, bend slightly. A score "CF = 1" was a slight deformity, if at all. The scores of SD = 1 involving kyphosis or lordosis were deemed real because that is an unusual preservation deformity. Also, samples BKD 015 SU (i.e., extra fry from CC-150-015 at swim-up), LOW 008 SU (i.e., extra fry from CC-350-008 at swim-up), and SC 003 SU (i.e., extra fry from SC-003 at swim-up) were re-examined; most fish were very straight so some samples with higher SD scores (e.g., PSU samples) were determined accurate. Thin fish difficult to score, and often looked like they were underfed or starving.

Fin deformities included variation in fin or finfold morphology and a slightly smaller or missing fin (in thin fish, the adipose fin was often absent, indicating fat absorption, not uncommon and scored 1) or one with a bend or incomplete ray development (in older fish) was given a 1, 2 fins damaged or malformed = 2, and > 2 fins malformed or if fins were missing (except adipose) was = 3. Often fins were malformed associated with vertebral deformities that did not permit proper development. Folded finfolds as a result of preservation were not counted.

Edema was not originally scheduled for assessment because it was thought sometimes not a teratogenic effect and may be transitory as fish develop. However, it was assessed because it was common in one early sample and not others, and because it was considered a condition that could affect emergence, mobility, and other factors that may limit survival of fish in the wild. Edema was detected by an obvious swelling and fluid buildup, usually abdominally, and ventrally, which often displaced the gut, and was usually clear fluid that was slightly soft when touched with a blunt probe. The yolk, which was present in some quantity in some study specimens, also created some swelling but was typically yellowish, opaque, and small, and hard to the touch in preservation. Slight edema = 1 was for a fish with up to 1X swelling of the normal body width or depth, up to 2x = 2, and > 2x = 3.

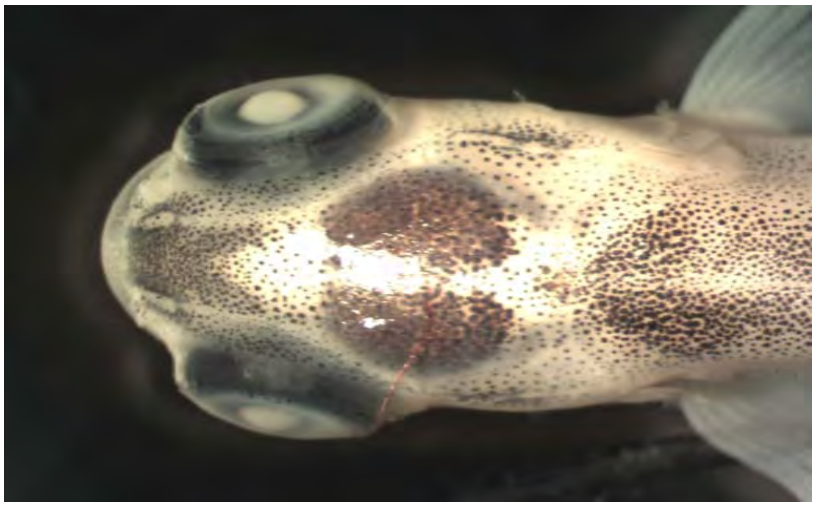
A sample of 50 fish and a sample of 30 fish were scored twice, the same fish for each batch but not necessarily the same order. This sample was characterized by a low incidence of fin deformities (slow development) and a high incidence of jaw deformities and blindness (SC 003 SU). Those cranio-facial traits are difficult to score because they are additive, and subjective as to severity. Thus, the results may be a conservative view of what score replicability should be like for other traits in other samples that are easier to score.

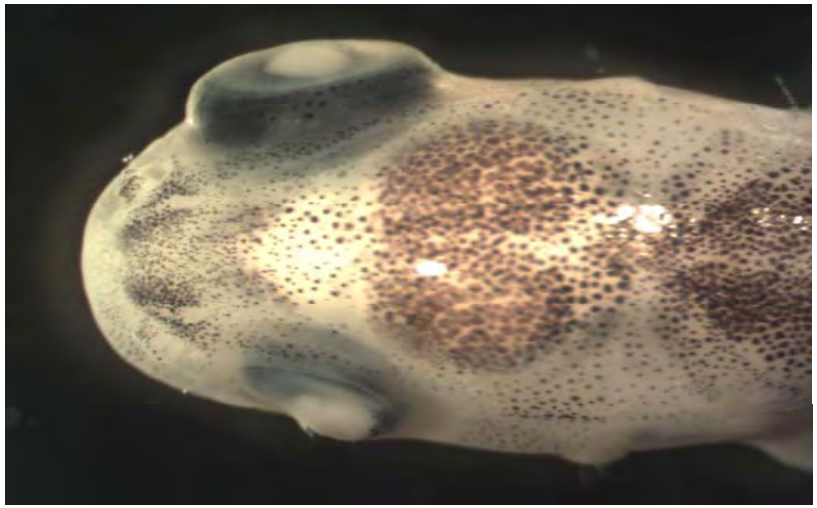
Replicability of frequency of cranio-facial abnormalities was high among assessments at 50 and 52% in the first sample of 50 fish, and identical frequencies of 46.7 % in each assessment for the sample of 30 fish. The cumulative sums of the scores were also quite

close, but reflecting variability in scoring for all three categories of severity in each sample. Replicability of fin ray development assessments for both frequency and the sum of the scores was identical in both samples.

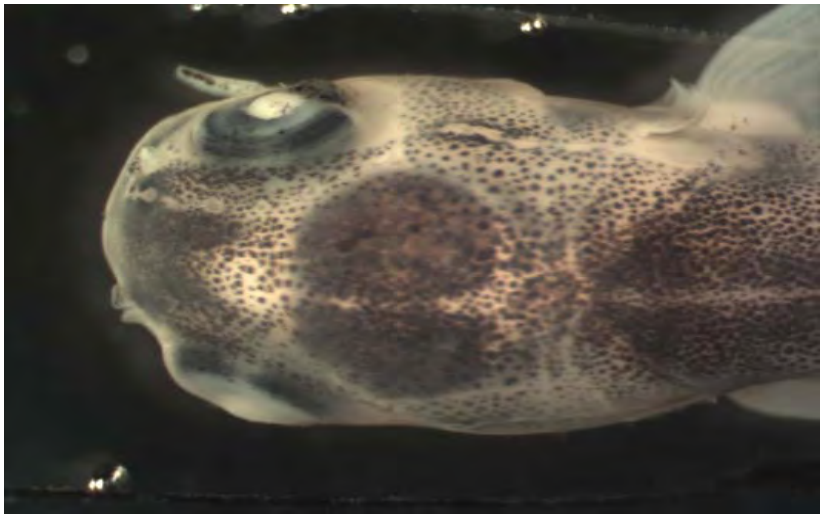
Below we have included photographs of each of the deformities assessed described above, demonstrating scoring values of 0 – 3 for each of the deformities.

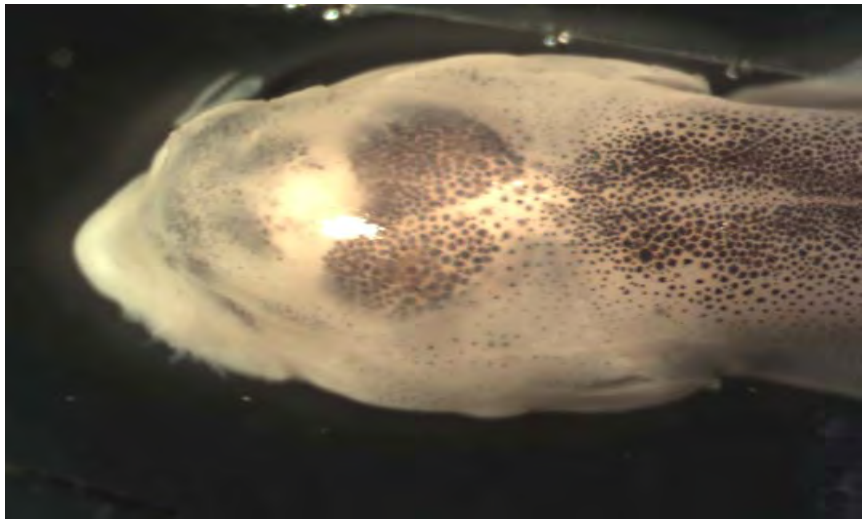
Photos 1 and 2: Example of normal brown trout eyes (left) and an example of a cranio-facial eye deformity with a score of 1 (right).



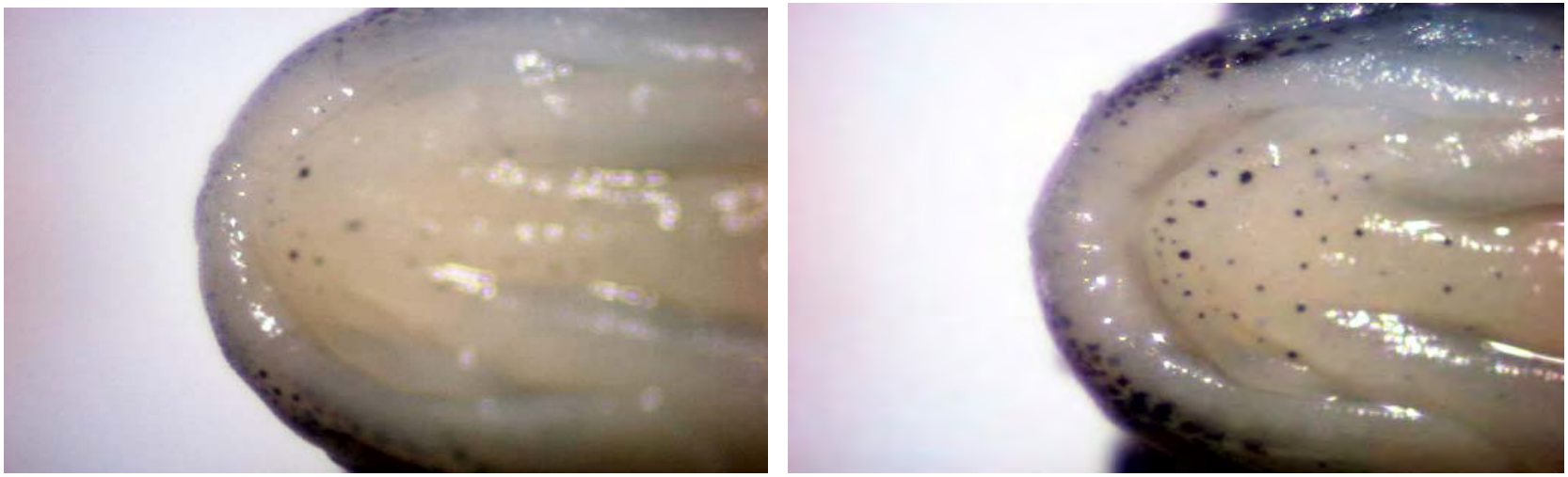


Photos 3 and 4: Examples of cranio-facial eye deformities with a score of 3 (both).

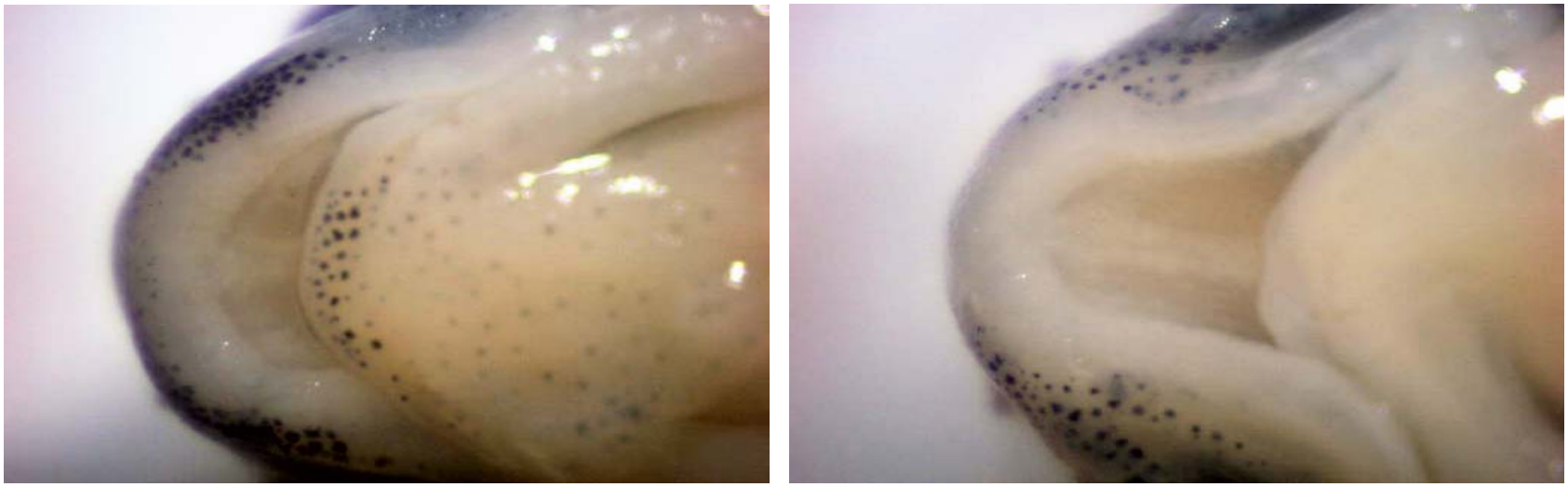




Photos 1 and 2: Example of a normal brown trout jaw (left) and an example of a cranio-facial jaw deformity with a score of 1 (right).



Photos 3 and 4: Example of a cranio-facial jaw deformity with a score of 2 (left) and 3 (right).



Photos 1 and 2: Example of a healthy brown trout fish (left) and an example of the spinal deformity constriction with a score of 1 (right).

Photo 3: Example of the spinal deformity constriction with a score of 1.

Photos 1 and 2: Example of a healthy brown trout fish (left) and an example of the skeletal deformity kyphosis with a score of 1 (right).

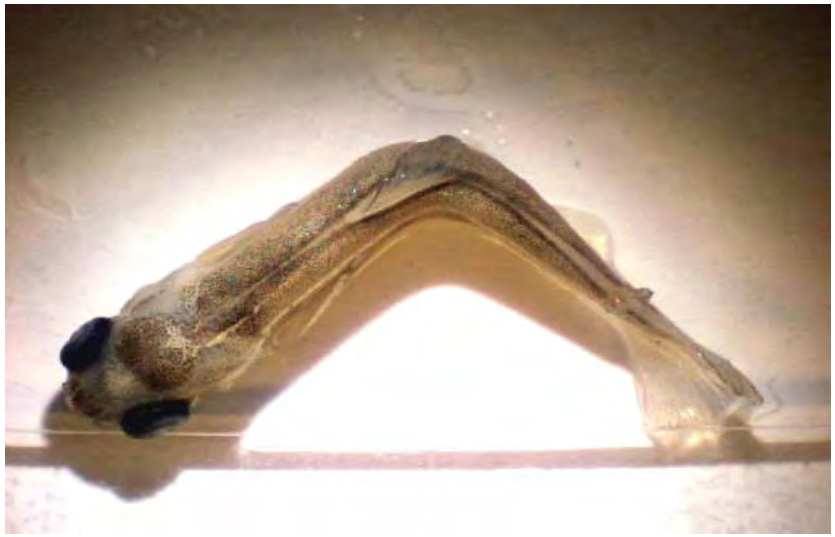
Photos 3 and 4: Example of the skeletal deformity kyphosis with a score of 2 (left) and 3 (right).

Photos 1 and 2: Example of a healthy brown trout fish (left) and an example of the skeletal deformity lordosis with a score of 1 (right).

Photos 3 and 4: Example of the skeletal deformity lordosis with a score of 2 (left) and 3 (right).

Photos 1 and 2: Example of a healthy brown trout fish (left) and an example of the spinal deformity scoliosis with a score of 1 (right).

Photos 3 and 4: Example of the spinal deformity scoliosis with a score of 2 (left) and 3 (right).



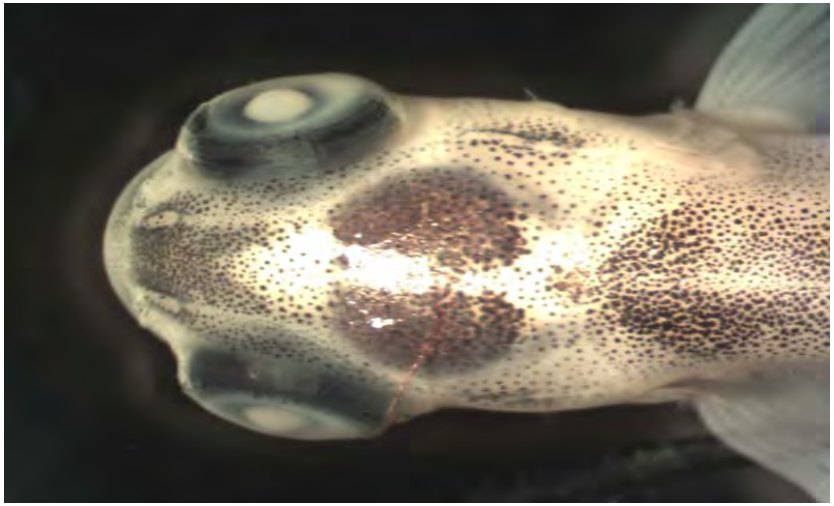
Photos 1 and 2: Example of a healthy brown trout fish (left) and an example of a fin deformity with a score of 1 (right).

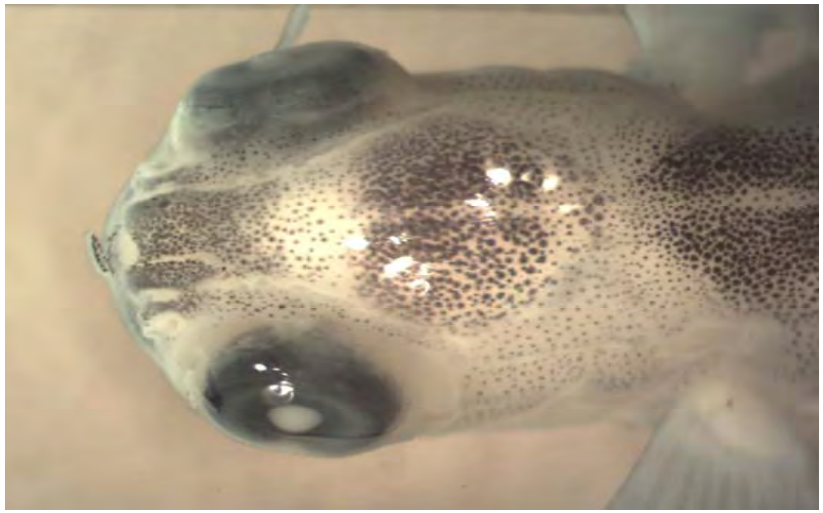
Photos 3 and 4: Example of a fin deformity with a score of 2 (left) and 3 (right).

Photos 1 and 2: Example of a healthy brown trout fish (left) and an example of abdominal edema with a score of 1 (right).

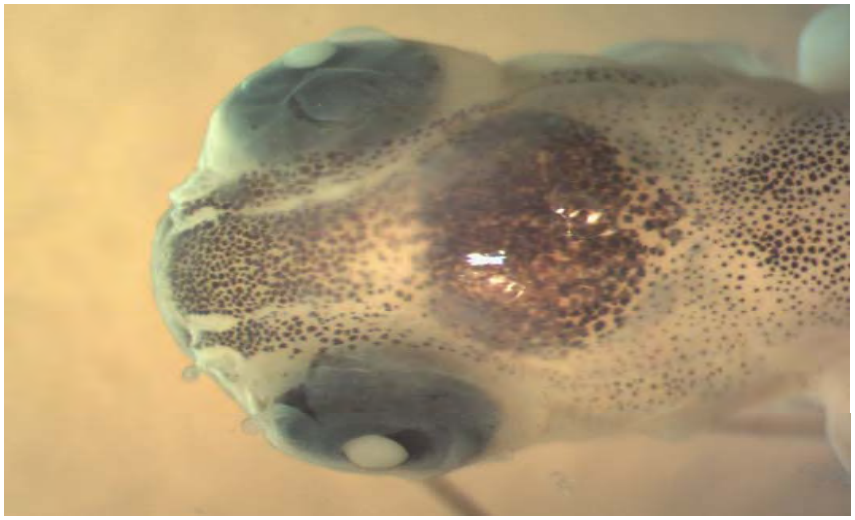
Photos 3 and 4: Examples of abdominal edema with a score of 2 (left) and 3 (right).

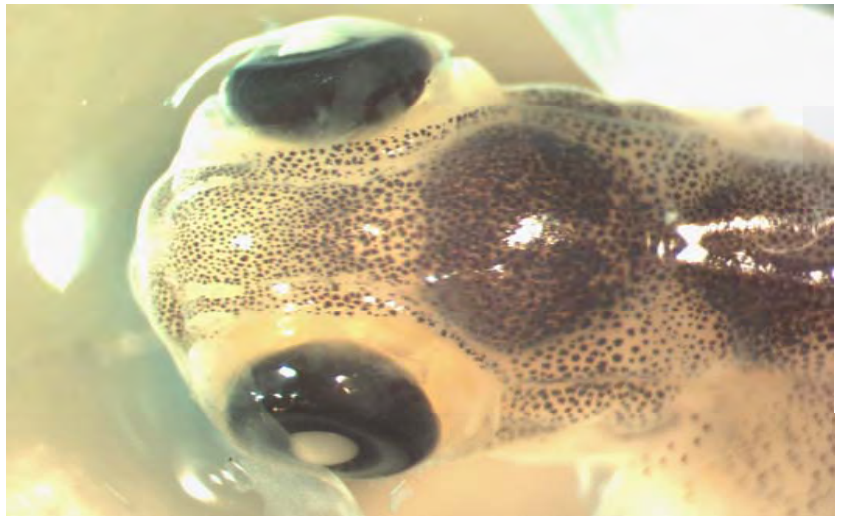
Photos 1 and 2: Example of a healthy brown trout fish (left) and an example of cranial edema with a score of 1 (right).





Photos 3 and 4: Example of cranial edema with a score of 2 (left) and 3 (right).





Photos 1 and 2: Examples of brown trout with unusual deformities (both having two heads).

Photos 3 and 4: Examples of unusual deformities.

Deformity assessment of fry preserved after death during the BT parental study.

filename: LSV2C def data.xls

0 (normal)
1 (slight/few)
2 (mod/several)
3 (severe/many)

CF = craniofacial deformities
SD = vertebral deformities
FD = fin deformities
ED = edema

Counts

CF

Location	Field Sample	0	1	2	3	Grand Total	Total assessed
LSV2C	003	0	3	83	12	98	98
	004	0	6	80	15	101	101
	005	0	14	66	9	89	89
	010	0	16	55	0	71	71
	021	0	2	53	46	101	101

CF

Location	Field Sample	0	1	2	3	Grand Total
LSV2C	003	0.0%	3.1%	84.7%	12.24%	100%
	004		5.9%	79.2%	14.85%	100%
	005		15.7%	74.2%	10.11%	100%
	010		22.5%	77.5%	0.00%	100%
	021		2.0%	52.5%	45.54%	100%

SD

Location	Field Sample	0	1	2	3	Grand Total	Total assessed
LSV2C	003	0	56	20	6	82	82
	004	0	47	13	4	64	64
	005	0	28	16	40	84	84
	010	0	30	16	7	53	53
	021	0	35	19	8	62	62

SD

Location	Field Sample	0	1	2	3	Grand Total
LSV2C	003	0.0%	68.3%	24.4%	7.32%	100%
	004		73.4%	20.3%	6.25%	100%
	005		33.3%	19.0%	47.62%	100%
	010		56.6%	30.2%	13.21%	100%
	021		56.5%	30.6%	12.90%	100%

FD

Location	Field Sample	0	1	2	3	Grand Total	Total assessed
LSV2C	003					0	0
	004		1			1	1
	005	0	13	7	35	55	55
	010					0	0
	021	0	9	5	0	14	14

FD

Location	Field Sample	0	1	2	3	Grand Total
LSV2C	003		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	004		100.0%	0.0%	0.00%	100%
	005		23.6%	12.7%	63.64%	100%
	010		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	021		64.3%	35.7%	0.00%	100%

ED

Location	Field Sample	0	1	2	3	Grand Total	Total assessed
LSV2C	003	0	47	30	7	84	84
	004	0	57	28	6	91	91
	005	0	40	13	5	58	58
	010	0	16	19	10	45	45
	021	0	62	19	1	82	82

ED

Location	Field Sample	0	1	2	3	Grand Total
LSV2C	003		56.0%	35.7%	8.33%	100%
	004		62.6%	30.8%	6.59%	100%
	005		69.0%	22.4%	8.62%	100%
	010		35.6%	42.2%	22.22%	100%
	021		75.6%	23.2%	1.22%	100%

Note: scoring criteria were not possible for all organisms due to the poor physical condtion of some samples. For these samples, no value was included.

No organisms scored a "0" on any of the different assessments (i.e., CF, SD, FD, ED)

Deformity assessment results for brown trout in reproductive success study

Vaues represent the number of fish (at swimup and at test termination) in each scoring criterion (i.e., 0 - 3).
See below for a definition of scoring criteria.

filename: deformity data.xls

Count of Fish #		Craniofacial Deformities (CF)				
Location	Field Sample ID	0	1	2	3	Grand Total
CC-150	009	136	1	2	3	142
	011	114	150	2		266
	012	191	86	4	1	282
	013	183	31	28	68	310
	015	231	207	5	2	445
	016	20	2		1	23
	017	108	54	1		163
	018	288	193	2	3	486
	020	506	52			558
CC-150 Total		1777	776	44	78	2675
CC-350	006	228	122	22	14	386
	007	102	12	11	6	131
	008	315	8	5	10	338
CC-350 Total		645	142	38	30	855
LSV2C	002	531	13			544
	003		3	83	12	98
	004	63	6	80	15	164
	005	27	27	75	9	138
	008	165	24	5		194
	010		16	55		71
	012	511	39	3	1	554
	016	495	34	1		530
	017	122	16	10	2	150
	019	302	79	8	1	390
	020	257	36	3		296
	021	47	13	57	53	170
LSV2C Total		2520	306	380	93	3299
SC	001	96	14	4	1	115
	002	104	6	1	2	113
	003	174	37	55	36	302
	004	69	26	26	19	140
	005	39	3			42
	006	519	2	6	8	535
	007	119	11	6	1	137
	008	339	12	3	5	359
SC Total		1459	111	101	72	1743
SPC	001	490	75	2	1	568
	003	448	91	6		545
	005	476	82	2	1	561
	006	475	77	3	1	556
SPC Total		1889	325	13	3	2230
Grand Total		8290	1619	239	194	10342

Craniofacial deformities included shortening of the jaw, snout, and missing or poorly developed eye or eyes, and head shape abnormailities. A slightly shortened lower jaw (<= 1 lip width) received a 1, a shortened jaw = 2 lip widths or a slightly shortened and slightly disfigured jaw = 2, and a flat lower jaw or much disfigured (non-functional) jaw = 3. An assessment of fish independent of this study revealed that other brown trout of the same size and developmental state did not have the slight deformity that was assessed as CF =1 for the jaw (J). Thus, the CF = 1 score where the J was concerned were deemed real. A slightly blunted snout (about 50% eye diameter, usually is > than that) = 1, very blunt or flat = 2, deformed or bulbous = 3. Eye deformities were scored as one eye blind or poorly pigmented or poorly developed =1, both poorly developed = 2, both blind = 3. Skulls that were slightly bulbous (1/3 > normal) = 1, moderately bulbous (2/3 > normal) = 2, and bulbous (1x or > than normal) = 3. Usually factors occurred together so a combination of two “1” conditions = 2, three “1” conditions = 3, or a 1 and a 2 = 3, and so on. For example, a deformed jaw and a blind eye = 2, two blind eyes = 2, but a badly deformed jaw (= 2 alone) plus a blind eye (= 1 alone), = 3.

Deformity assessment results for brown trout in reproductive success study

Vaues represent the number of fish (at swimup and at test termination) in each scoring criterion (i.e., 0 - 3).

See below for a definition of scoring criteria.

filename: deformity data.xls

Count of Fish #		Skeletal Deformities (SD)				
Location	Field Sample ID	0	1	2	3	Grand Total
CC-150	009	109	28	3	2	142
	011	213	50	3		266
	012	237	42	3		282
	013	214	81	11	4	310
	015	402	33	8	2	445
	016	13	10			23
	017	150	11	2		163
	018	353	121	11	1	486
	020	499	44	15		558
CC-150 Total		2190	420	56	9	2675
CC-350	006	198	117	43	28	386
	007	83	22	20	6	131
	008	284	43	7	4	338
CC-350 Total		565	182	70	38	855
LSV2C	002	499	38	7		544
	003		56	20	6	82
	004	20	83	20	4	127
	005	17	44	29	43	133
	008	173	19	2		194
	010		30	16	7	53
	012	235	306	13		554
	016	486	41	3		530
	017	138	10		2	150
	019	341	46	2	1	390
	020	274	17	4	1	296
	021	20	71	32	8	131
LSV2C Total		2203	761	148	72	3184
SC	001	79	28	7	1	115
	002	75	32	3	3	113
	003	260	39	3		302
	004	99	28	6	7	140
	005	25	17			42
	006	486	42	6	1	535
	007	105	23	4	5	137
	008	291	47	8	13	359
SC Total		1420	256	37	30	1743
SPC	001	493	62	9	4	568
	003	457	64	21	3	545
	005	479	65	12	5	561
	006	488	41	22	5	556
SPC Total		1917	232	64	17	2230
Grand Total		8295	1655	291	101	10342

Skeletal deformities included any deformity of the vertebrae or spines. A slight bend of less than 45 degrees (but > than body width off of straight) or a minor body constriction (e.g. a tight rubberband about the body effect) was given a score of 1, 2 slight bends or constrictions anywhere, or bend of > 45-90 degrees was scored a 2, and multi-directional bends > 90 degrees were given a 3. Bends caused by skeletal deformities were usually detectable from normal bending of the body during preservation (these fish were usually well preserved, very straight) by presence of a slight or greater bump below the surface of the epidermis on the outside of the bend. However, some fish with SD = 1 had just a very slight bend in the range the deformity described but could be due to preservation or the poor condition of the fish. This was sometimes especially true in larger fish, which may be more muscular and undergo stronger contraction during preservation and thus, bend slightly. A score “CF = 1” was a slight deformity, if at all. The scores of SD = 1 involving kyphosis or lordosis were deemed real because that is an unusual preservation deformity. Some samples were re-examined; most fish were very straight so some samples with higher SD scores (e.g., PSU samples) were determined accurate.

Deformity assessment results for brown trout in reproductive success study

Vaues represent the number of fish (at swimup and at test termination) in each scoring criterion (i.e., 0 - 3).
See below for a definition of scoring criteria.

filename: deformity data.xls

Count of Fish #		Fin Deformities (FD)				
Location	Field Sample ID	0	1	2	3	Grand Total
CC-150	009	137	2	1	2	142
	011	266				266
	012	279	1		2	282
	013	287	17	4	2	310
	015	437	3	4	1	445
	016	23				23
	017	162	1			163
	018	483	3			486
	020	549	9			558
CC-150 Total		2623	36	9	7	2675
CC-350	006	325	16	16	29	386
	007	95	10	18	8	131
	008	303	25	7	3	338
CC-350 Total		723	51	41	40	855
LSV2C	002	528	15	1		544
	003					0
	004	48	15	1		64
	005	39	17	11	37	104
	008	194				194
	010					0
	012	544	9	1		554
	016	485	45			530
	017	144	4		2	150
	019	390				390
	020	292		1	3	296
	021	27	51	5		83
LSV2C Total		2691	156	20	42	2909
SC	001	102	7	5	1	115
	002	103	6	4		113
	003	280	21		1	302
	004	113	10	13	4	140
	005	42				42
	006	501	21	7	6	535
	007	114	11	7	5	137
	008	343	4	3	9	359
SC Total		1598	80	39	26	1743
SPC	001	542	11	10	5	568
	003	524	8	7	6	545
	005	533	16	4	8	561
	006	529	11	7	9	556
SPC Total		2128	46	28	28	2230
Grand Total		9763	346	125	108	10342

Fin deformities included variation in fin or finfold morphology and a slightly smaller or missing fin (in thin fish, the adipose fin was often absent, indicating fat absorption, not uncommon and scored 1) or one with a bend or incomplete ray development (in older fish) was given a 1, 2 fins damaged or malformed = 2, and > 2 fins malformed or if fins were missing (except adipose) was = 3. Often fins were malformed associated with vertebral deformities that did not permit proper development. Folded finfolds as a result of preservation were not counted.

Deformity assessment results for brown trout in reproductive success study

Vaues represent the number of fish (at swimup and at test termination) in each scoring criterion (i.e., 0 - 3).
See below for a definition of scoring criteria.

filename: deformity data.xls

Count of Fish #		Edema Deformities (ED)				
Location	Field Sample ID	0	1	2	3	Grand Total
CC-150	009	141	1			142
	011	266				266
	012	282				282
	013	308	2			310
	015	445				445
	016	23				23
	017	163				163
	018	485		1		486
	020	558				558
CC-150 Total		2671	3	1		2675
CC-350	006	382	3	1		386
	007	126	3	2		131
	008	337	1			338
CC-350 Total		845	7	3		855
LSV2C	002	541	3			544
	003		47	30	7	84
	004	63	57	28	6	154
	005	42	46	14	5	107
	008	180	6	8		194
	010		16	19	10	45
	012	554				554
	016	530				530
	017	135	9	5	1	150
	019	381	8	1		390
	020	296				296
	021	69	62	19	1	151
LSV2C Total		2791	254	124	30	3199
SC	001	114	1			115
	002	113				113
	003	302				302
	004	139	1			140
	005	42				42
	006	534	1			535
	007	137				137
	008	359				359
SC Total		1740	3			1743
SPC	001	565	3			568
	003	539	4	2		545
	005	558	3			561
	006	553	1	1	1	556
SPC Total		2215	11	3	1	2230
Grand Total		10262	56	22	2	10342

Edema was not originally scheduled for assessment because it was thought sometimes not a teratogenic effect and may be transitory as fish develop. However, it was assessed because it was common in one early sample and not others, and because it was thought a condition that could affect emergence, mobility, and other factors that may limit survival of fish in the wild. Edema was detected by an obvious swelling and fluid buildup, usually abdominally, and ventrally, which often displaced the gut, and was usually clear fluid that was slightly soft when touched with a blunt probe. The yolk, which was present in some quantity in some study specimens, also created some swelling but was typically yellowish, opaque, and small, and hard to the touch in preservation. Slight edema = 1 was for a fish with up to 1X swelling of the normal body width or depth, up to 2x = 2, and > 2x = 3.