OSU Oregon State UNIVERSITY

Open Access Articles

Lack of Protection following Re-Exposure of Chinook Salmon to Ceratonova shasta (Myxozoa)

The Faculty of Oregon State University has made this article openly available.
Please share how this access benefits you. Your story matters.

Citation	Hurst, C. N., & Bartholomew, J. L. (2015). Lack of Protection following Re-Exposure of Chinook Salmon to Ceratonova shasta (Myxozoa). Journal of Aquatic Animal Health, 27(1), 20-24. doi:10.1080/08997659.2014.970716
DOI	10.1080/08997659.2014.970716
Publisher	Taylor & Francis
Version	Accepted Manuscript
Terms of Use	http://cdss.library.oregonstate.edu/sa-termsofuse

Oregon State UNIVERSITY OSU

1 **Re-exposure of Chinook salmon (*Oncorhynchus tshawytscha*) to *Ceratonova shasta***
2 ***(Myxozoa)***
3 **Running Head: *Ceratonova shasta* re-exposure**
4
5 C. N. Hurst and J. L. Bartholomew*
6 Department of Microbiology, College of Science, Oregon State University, Campus Way,
7 Corvallis, Oregon 97330, USA
8 *Corresponding author: bartholj@science.oregonstate.edu
9
10

<A>Abstract: The recent identification of multiple genotypes of the salmonid parasite *Ceratonova shasta* with different virulence levels in Chinook salmon (*Oncorhynchus tshawytscha*) suggests that it may be possible to immunize fish against subsequent infection and disease. We hypothesized that exposure of Chinook salmon to the less virulent parasite genotype (II) prior to the more virulent parasite genotype (I) would decrease disease and/or result in fewer mature parasites compared to fish only infected with the more virulent genotype. To test this, fish were challenged in a combination of field and laboratory exposures and we measured infection prevalence, percent morbidity, and mature parasite production. Neither mortality nor mature parasite production were reduced when fish were exposed to genotype II prior to genotype I as compared with fish exposed only to genotype I, suggesting that protection using a less virulent parasite genotype does not occur.

<A>Introduction: In aquaculture, there are opportunities for the control of diseases through vaccination and/or treatment. Vaccines have been developed for a variety of bacterial and viral pathogens, but no commercial parasite vaccines exist in aquaculture (Sommerset et al. 2005). However, there is evidence that fish can acquire resistance after a natural exposure to a parasite. In salmonids, resistance to parasite reinfection has been demonstrated for the microsporidians *Kabatana takedai* (Awakura 1974) and *Loma salmonae* (Speare et al. 1998; Kent et al. 1999), the monogenean *Discocotyle sagittata* (Rubio-Godoy and Tinsley 2004) and the myxozoan *Tetracapsuloides bryosalmonae* (Foott and Hedrick 1987). However, one drawback with using a re-exposure strategy based on exposure only to a virulent parasite is the inability to achieve high infection prevalence without causing a high incidence of disease and/or mortality after the initial

33 exposure. For example, Foott and Hedrick (1987) demonstrated that when infection prevalence
34 with *T. bryosalmonae* was high (82%), most fish exhibited clinical disease signs.

35 Alternatively, prior exposure to attenuated parasites or naturally occurring less virulent
36 parasite species/strains may also provide some protection from disease or reduce disease severity
37 (Smith et al. 1999; Read and Taylor 2001). Attenuation decreases parasite virulence while
38 simultaneously eliciting a protective immune response against future infections with an unaltered
39 parasite. For example, prior exposure to an attenuated strain of the hemoflagellate *Cryptobia*
40 *salmositica* protected rainbow trout (*Oncorhynchus mykiss*) against disease development (Woo
41 and Li 1990). Protection has also been achieved for the myxozoan parasite, *Myxobolus*
42 *cerebralis,* where researchers exposed fish to infective actinospores treated with UV irradiation
43 63 days prior to their exposure to fully infective parasites. Prior exposure resulted in decreased
44 infection, parasite survival within the host and mature parasite production after fish were
45 exposed for a second time (Hedrick et al. 2012). Immunization of rainbow trout to the ciliate
46 *Tetrahymena thermophila* (a less virulent parasite species) prior to another ciliate *Ichthyopthirius*
47 *multifilus* 6-10 weeks later increased host survival by approximately 50% (Wolf and Markiw
48 1982). Similarly, Sánchez et al. (2001) demonstrated that rainbow trout exposed to a less virulent
49 strain of *L. salmonae* 15 weeks prior to a more virulent strain resulted in a reduction in xenoma
50 intensity in the gills.

51 The recent discovery that the myxozoan *Ceratonova shasta* comprises four genotypes (0,
52 I, II and III; Atkinson and Bartholomew 2010ab that differ in virulence presents an opportunity
53 to immunize hatchery fish prior to their release. The life cycle of *C. shasta* is complex, with a
54 waterborne actinospore stage attaching to and penetrating the gills of a salmonid. Once in the
55 host, the parasite begins proliferating and travels through the blood to the intestine (Bjork and

Bartholomew 2010). The parasite then matures into the myxospore stage and is subsequently released into the water column to infect a freshwater polychaete, *Manayunkia speciosa* (Bartholomew et al. 1997). Our study focuses on Chinook salmon (*O. tshawytscha*) which are commonly infected by both *C. shasta* genotypes I and II, with genotype I causing mortality from enteronecrosis, while infection with genotype II rarely results in myxospore production or mortality (Hurst and Bartholomew 2012a). Thus, we hypothesize that infection of Chinook salmon with *C. shasta* genotype II, followed by exposure to genotype I, will result in a decrease in disease and/or myxospore production. The association of *C. shasta* with declines in adult Chinook salmon returns (Fujiwara et al. 2011) has focused attention on strategies to reduce parasite abundance and ultimately disease. Thus, immunizing hatchery fish may improve the survival of returning adults by increasing survival of out-migrating juveniles. In addition, a decrease in myxospore production could reduce the overall number of parasites in the river system by reducing transmission to the parasite’s next host.

<A>Materials and Methods

Exposures.− Age 0 Chinook salmon (5-10 g) were obtained from Iron Gate Hatchery (Hornbrook, CA) and transported in aerated coolers to the John L. Fryer Salmon Disease Laboratory, Oregon State University, Corvallis, OR (SDL) and held until parasite exposure. A total of 360 fish were randomly placed into four treatments: no parasite (control-treatment 1), genotype II only (treatment 2), genotype I only (treatment 3) and genotype II, then genotype I (treatment 4). Two replicates were used for treatments 1 and 2 as no mortality was expected; treatments 3 and 4 had four replicates each (Figure 1). All replicates were comprised of 30 fish and exposures were conducted in cylindrical cages of 0.3x1.0 m.

79 Fish in treatments 2 and 4 were exposed to genotype II for 24 h in the Williamson River,
80 OR (N 42° 32.425, W 121° 52.787) a location where this genotype is predominant and genotype
81 I is absent (Atkinson and Bartholomew 2010a; Hurst et al. 2012b). Water temperature during
82 exposure was 16.5°C. The remaining treatments (1 and 3) were exposed to 18°C UV treated
83 Willamette River water for 24 h at the SDL.

84 Exposure to genotype I was conducted in the laboratory where a pure parasite culture
85 could be obtained. The exposure timeline was constrained by the availability of myxospores and
86 polychaetes (Hurst and Bartholomew 2012a) and the activation time required for both the innate
87 and adaptive immune responses (6-12 weeks; Sitjà-Bobadilla 2008). Thus, exposure to genotype
88 I occurred 53 days after the initial exposure to genotype II, which was supposed to allow for
89 activation of both the innate and adaptive immune response and coincided with genotype I
90 production in the laboratory. At the SDL, each treatment replicate was placed in a separate cage
91 within a flow-through 378 L tank containing either genotype I from cultured polychaete
92 populations (treatments 3 and 4) or UV treated Willamette River water (treatments 1 and 2) for
93 24 h (Figure 1). Water temperatures were 21°C at the time of the second exposure.

94 *Parasite exposure dose.*−To calculate exposure dose per fish for field and laboratory
95 challenges, 3x1 L of water were collected before and after each exposure. Water samples were
96 filtered, parasite DNA was extracted and parasite density was measured by qPCR for each
97 sample in duplicate (Hallett and Bartholomew 2006). Samples were considered positive if both
98 duplicate wells fluoresced and were re-run if a difference of more than one cycle occurred
99 between wells. One sample from the beginning and end of each exposure was tested to determine
100 if parasite DNA detection was inhibited by other components in the water sample filtrate (Hallett
101 and Bartholomew 2009). If inhibition occurred, samples were diluted 1:10 and re-run. Mean

102 parasite density for each exposure site and time was then multiplied by the velocity and exposure
103 duration and divided by the number of fish (Ray et al. 2010). Velocity was 3 L/s in the field and
104 0.0083 L/s in the laboratory.

105 *Assessment of Infection.*− Immediately after each 24 h exposure, five fish from each
106 replicate were euthanized with an overdose of tricaine methanesulfonate (MS-222 Argent
107 Chemical Laboratories, Redmond, WA, USA) and the right half of the gill was excised, placed
108 on ice, and stored at -20 °C to determine infection prevalence. The remaining fish were placed
109 into 25 L tanks supplied with well water at 18°C. All fish were treated for external parasites and
110 bacterial infections, fed daily and monitored for clinical disease signs according to Ray et al.
111 (2010). Moribund fish were removed, euthanized as above and time to morbidity was recorded.
112 Myxospores were counted by removing and weighing the intestine, flushing into a
113 microcentrifuge tube using a pipette attached to one end of the intestine filled with 1 mL of tap
114 water and using a hemocytometer at 200x magnification. A 25 mg aliquot of the harvested
115 myxospores was stored at -20°C for genotype composition (see below). Fish remaining at the end
116 of the experiment at 113 days were euthanized using an overdose of MS-222 and a 25 mg piece
117 of intestine was collected and stored at -20°C to determine infection status in survivors (10 fish
118 from each treatment were assayed).

119 DNA from gills and intestines was extracted and purified as in Hurst et al. (2014). After
120 extraction, DNA from all samples was then directly tested for the presence of parasite DNA
121 using qPCR as above. To create a standard curve for estimating the parasite DNA copy number
122 in 0.1 g of host gill tissue, ten-fold serial dilutions of a synthetic parasite template were added to
123 gill tissue (Hallett and Bartholomew 2006). Harvested myxospores from the intestine of 10

124 moribund fish in each of the treatments were sequenced to determine genotype composition
125 (Hurst et al. 2014).
126 Infection prevalence and copy number were determined using gills from treatments 2 and
127 4 (genotype II; *n* = 30) and treatment 3 (genotype I; *n* = 20) at 1 day post exposure. S-PLUS
128 version 8.2 (Tibco, Palo Alto, CA) was used to compare survival between treatments 3 and 4 and
129 among treatment replicates using a Mantel-Cox test. One replicate from treatment 4 was lost
130 when water flow to the tank was stopped and therefore was not included in the analyses. A one-
131 way ANOVA with Tukey's test for highly significant differences was used to compare natural
132 log transformed (for normality) myxospore counts among treatment replicates. If no differences
133 were detected among replicates within a treatment, replicates were combined for analyses at the
134 treatment level using a student's t-test. Differences were considered significant at $P < 0.05$.
135
136 **<A>Results and Discussion**
137 In this study, previous exposure to a less virulent genotype (II) of *C. shasta* did not
138 prevent or limit mortality or myxospore production in Chinook salmon after a subsequent
139 exposure to a more virulent genotype (I). There was no significant difference in survival between
140 treatments 4 (57%) and 3 (48%; Mantel-Cox $test_1 = 2.05$, $p = 0.15$; Figure 2). There was also no
141 difference in myxospore counts between these treatments (t-test, $t_5 = 0.462$, $p = 0.663$), with
142 mean ± SE counts of 8173 ± 2108 and 7267 ± 612, respectively (Figure 3). Sequencing
143 demonstrated that myxospores obtained from fish from both treatments were only of genotype I.
144 Infection prevalence in surviving fish from treatments 3 and 4 was 50 and 40%, respectively.
145 None of the fish in treatments 1 and 2 died and parasite DNA was not detected in these fish at the
146 end of the study. These data indicate that prior exposure to genotype II does not reduce disease in

147 Chinook salmon. If genotype II was protective, we would have expected at least 74% survival
148 [the 50% infected with genotype II (45/90) in addition to 48% of the remaining naïve fish
149 exposed to genotype I (22/45)].

150 Differences in exposure conditions and in genotype virulence resulted in variations in
151 exposure dose and infection prevalence. The exposure dose of parasite genotype II in the field
152 was approximately 1.1 x 10^4 actinospores/fish and 50% of the fish became infected, as
153 determined by detection of parasite DNA in fish gills. Mean parasite copy number at 24 h was
154 4.3 (SE, 1.8) using our standard curve for gill tissue ($y = -3.35x + 38.40$; $r^2 = 0.997$). Parasite
155 genotype I exposure dose in the laboratory was lower, 9.2 x 10^1 actinospores/fish, but resulted in
156 a higher infection prevalence (100%) and a higher mean parasite copy number of 6.7 x 10^2 (SE,
157 48.5). Parasite DNA was not detected in the gills of control fish. The faster replication rate of
158 genotype I (author's unpublished data) combined with more optimal flow and temperature
159 conditions for the parasite in the slower flow of the laboratory challenge tanks (Ray et al. 2013)
160 likely contributed to the higher prevalence and intensity of genotype I infections.

161 Although these results suggest that previous exposure using a low virulence genotype is
162 not effective, modifications to exposure timing may yield different results. It is unknown
163 whether the adaptive response plays a protective role in the fish host's defense against *C. shasta*;
164 however, researchers found that rainbow trout infected with a less virulent parasite genotype of
165 *C. shasta* and surviving to three months had a 700 fold increase in IgT antibody levels compared
166 to unexposed fish (Zhang et al. 2010). Work with other myxozoans indicated that specific
167 antibodies were produced from 50 to 360 days post re-exposure by turbot (*Psetta maxima*) in
168 response to *E. scophthalmi* infection (Sitja-Bobadilla et al. 2007) and as early as 35 days post
169 exposure in rainbow trout infected with *M. cerebralis* (Hedrick et al. 1998). Thus, it seems likely

170 that the adaptive immune response to *C. shasta* infection would have been mounted during our
171 experimental time frame, but a longer time between exposures may have allowed for increased
172 production of a putative protective antibody.

173 The timing of the subsequent exposure to the more virulent genotype should also
174 consider the timing of the hosts' innate immune response to the parasite, which may be elicited
175 within hours to days of infection depending on temperature and stress (Sitjà-Bobadilla 2008;
176 Gómez et al. 2014). For example, infections with *M. cerebralis* resulted in upregulation of
177 immune relevant genes as early as five minutes post exposure (Severin and El-Matbouli 2007).
178 Recently, Bjork et al. (2014) demonstrated that an inflammatory response to *C. shasta* is
179 mounted within at least two weeks of exposure and is capable of resolving infection by 90 days.
180 However, sampling was not conducted between the 25 and 90 day sample times, thus the
181 infection may have been resolved sooner. This suggests that in this study infection with genotype
182 II may have been resolved before exposure to genotype I occurred. A decrease in the interval
183 between exposures may provide some short-term protection for the fish by taking advantage of
184 the mounted inflammatory response.

185 The lack of protection after initial exposure to genotype II could be attributed to a
186 parasite dose that did not elicit a host immune response and could be rectified by exposing fish to
187 a higher dose of genotype II or lengthening the exposure time. Studies with *T. bryosalmonae* and
188 *E. scopthalmi* demonstrated resistance to parasite reinfection only after lengthy continuous
189 parasite exposures of 10 and 13 months, respectively (Foott and Hedrick 1987; Sitja-Bobadilla et
190 al. 2007). Alternatively, immunization may be parasite genotype-specific, requiring initial
191 exposure to the more virulent genotype to elicit an effective immune response. However, prior
192 exposure to the more virulent genotype increases the probability of fish developing clinical

disease. To minimize this risk, fish could be exposed to a low dose of genotype I that enables fish to resolve the infection (Bjork et al. 2014). Despite the difficulties of working with a parasite that has a complex life cycle, immunization studies with *C. shasta* are worth pursuing because they may improve our understanding of how to implement management actions such as dam removal and/or fish reintroduction in a manner that could provide fish with some level of natural protection against the parasite.

<A>Acknowledgements

We would like to thank Iron Gate Hatchery for supplying Chinook salmon. This work was funded by the Hatfield Marine Science Center William Q. Wick Marine Fisheries Award, the Flyfisher's Club of Oregon and NOAA's Graduate Sciences Program.

<A>References

Atkinson, S. D., and J. L. Bartholomew. 2010a. Spatial, temporal and host factors structure the *Ceratomyxa shasta* (Myxozoa) population in the Klamath River basin. Infection, Genetics and Evolution 10:1019-1026.

Atkinson, S. D., and J. L. Bartholomew. 2010b. Disparate infection patterns of *Ceratomyxa shasta* (Myxozoa) in rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*) correlate with internal transcribed spacer-1 sequence variation in the parasite. International Journal of Parasitology 40:599-604.

Awakura, T. 1974. Studies on the microsporidian infection in salmonid fishes. Scientific Report of the Hokkaido Fish Hatchery 29:1-95.

Bartholomew, J. L., M. J. Whipple, D. G. Stevens, and J. L. Fryer. 1997. The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. Journal of Parasitology 83:859-868.

Bjork, S. J., and J. L. Bartholomew. 2010. Invasion of *Ceratomyxa shasta* (Myxozoa) and comparison of migration to the intestine between susceptible and resistant fish hosts. International Journal of Parasitolology 40:1087-1095.

Bjork, S. J., Y. A. Zhang, C. N. Hurst, M. E. Alonso-Naveiro, J. D. Alexander, J. O. Sunyer, and J. L. Bartholomew. 2014. Defenses of Susceptible and Resistant Chinook Salmon (*Onchorhynchus tshawytscha*) Against the Myxozoan Parasite, *Ceratomyxa shasta*. Fish and Shellfish Immunology 37:87-95.

Foott, J. S., and R. P. Hedrick. 1987. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. Journal of Fish Biology 30:477-483.

Fujiwara, M., M. S. Mohr, A. Greenberg, J. S. Foott, and J. L. Bartholomew. 2011. Effects of ceratomyxosis on population dynamics of Klamath fall-run Chinook salmon. Transactions of the American Fisheries Society 140:1380-1391.

Gómez, D., J. L. Bartholomew, and J. O. Sunyer. 2014. Biology and mucosal immunity to myxozoans. Developmental and Comparative Immunology 43:243-256.

Hallett, S. L., and J. L. Bartholomew. 2006. Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in river water samples. Diseases of Aquatic Organisms 71:109-118.

Hallett, S. L., and J. L. Bartholomew. 2009. Development and application of a duplex QPCR for river water samples to monitor the myxozoan parasite *Parvicapsula minibicornis*. Diseases of Aquatic Organisms 86:39-50.

249 Hedrick, R. P., M. A. Adkison, M. El-Matbouli, and E. MacConnell. 1998. Whirling disease:
250 re-emergence among wild trout. Immunology Reviews 166:365-376.
251
252 Hedrick, R. P., T. S. McDowell, M. A. Adkison, K. A. Myklebust, F. O. Mardones, and B. Petri.
253 2012. Invasion and initial replication of ultraviolet irradiated waterborne infective stages of
254 *Myxobolus cerebralis* results in immunity to whirling disease in rainbow trout. International
255 Journal of Parasitology 42:657-666.
256
257 Hurst, C. N., and J. L. Bartholomew. 2012a. *Ceratomyxa shasta* genotypes cause differential
258 mortality in their salmonid hosts. Journal of Fish Diseases 35:725-732.
259
260 Hurst, C. N., R. A. Holt, and J. L. Bartholomew. 2012b. Dam removal and implications for fish
261 health: *Ceratomyxa shasta* in the Williamson River, Oregon, USA. North American Journal of
262 Fisheries Management 32:14-23.
263
264 Hurst, C. N., P. Wong, R. A. Ray, S. L. Hallett, and J. L. Bartholomew. 2014. Transmission and
265 persistence of *Ceratomyxa shasta* genotypes. Journal of Parasitology Accepted.
266
267 Kent, M. L., S. C. Dawe, and D. J. Speare. 1999. Resistance to reinfection in Chinook salmon
268 *Oncorhynchus tshawytscha* to *Loma salmonae* (Microsporidia). Diseases of Aquatic
269 Organisms 37:205-208.
270
271 Ray, R. A., P. A. Rossignol, and J. L. Bartholomew. 2010. Mortality threshold for juvenile
272 Chinook salmon *Oncorhynchus tshawytscha* in an epidemiological model of *Ceratomyxa shasta.*
273 Diseases of Aquatic Organisms 93:63-67.
274
275 Ray, R. A., and J. L. Bartholomew. 2013. Estimation of transmission dynamics of the
276 *Ceratomyxa shasta* actinospore to the salmonid host. Parasitology 140:907-916.
277
278 Read, A. F., and L. H. Taylor. 2001. The ecology of genetically diverse
279 infections. Science 292:1099-1102.
280
281 Rubio-Godoy, M., and R. C. Tinsley. 2004. Immunity in rainbow trout, *Oncorhynchus mykiss*,
282 against the monogenean *Discocotyle sagittata* following primary infection. Parasitology
283 Research 92:367-374.
284
285 Sanchez, J. G., D. J. Speare, R. J. F. Markham, and S. R. M. Jones. 2001. Experimental
286 vaccination of rainbow trout against *Loma salmonae* using a live low-virulence variant of *L.*
287 *salmonae*. Journal of Fish Biology 59:442-448.
288
289 Severin, V. I., and M. El-Matbouli. 2007. Relative quantification of immune-regulatory genes in
290 two rainbow trout strains, *Oncorhynchus mykiss*, after exposure to *Myxobolus cerebralis*, the
291 causative agent of whirling disease. Parasitology Research 101:1019-1027.
292

293 Sitjà-Bobadilla, A., O. Palenzuela, A. Riaza, M. A. Macias, and P. Alvarez-Pellitero. 2007.
294 Protective acquired immunity to *Enteromyxum scophthalmi* (myxozoa) is related to specific
295 antibodies in *Psetta maxima* (L.)(teleostei). Scandinavian Journal of Immunology 66:26-34.
296
297 Sitja-Bobadilla, A. 2008. Fish immune response to Myxozoan parasites. Parasite 15:420-425.
298
299 Smith, T., I. Felger, M. Tanner, and H. P. Beck. 1999. Premunition in *Plasmodium falciparum*
300 infection: insights from the epidemiology of multiple infections. Transactions of the Royal
301 Society of Tropical Medicine and Hygiene 93:59-64.
302
303 Sommerset, I., B. Krossøy, E. Biering, and P. Frost. 2005. Vaccines for fish in aquaculture.
304 Expert Review of Vaccines 4:89-101.
305
306 Speare, D. J., H. J. Beaman, S. R. M. Jones, R. J. F. Markham, and G. J. Arsenault. 1998.
307 Induced resistance in rainbow trout, *Oncorhynchus mykiss* (Walbaum), to gill disease associated
308 with the microsporidian gill parasite *Loma salmonae*. Journal of Fish Diseases 21:93-100.
309
310 Wolf, K., and M. E. Markiw. 1982. Ichthyophthiriasis: immersion immunization of rainbow trout
311 (*Salmo gairdneri*) using *Tetrahymena thermophila* as a protective immunogen. Canadian Journal
312 of Fisheries and Aquatic Sciences 39:1722-1725.
313
314 Woo, P. T., and S. Li. 1990. In vitro attenuation of *Cryptobia salmositica* and its use as a live
315 vaccine against cryptobiosis in *Oncorhynchus mykiss*. The Journal of Parasitology 76:752-755.
316
317 Zhang, Y. A., I. Salinas, J. Li, D. Parra, S. Bjork, Z. Xu, S. E. LaPatra, J. L. Bartholomew, and J.
318 O. Sunyer. 2010. IgT, a primitive immunoglobulin class specialized in mucosal immunity.
319 Nature Immunology 11:827-835.

320 Figure Captions

321 Figure 1: Diagrammatic drawing of the experimental design of Chinook salmon exposure to two
322 genotypes of *Ceratonova shasta*. Both exposures were for 24 h and included four treatments;
323 control (treatment 1), genotype II only (treatment 2), genotype I only (treatment 3) and genotype
324 II followed by genotype I (treatment 4). Exposure 1 took place in both the Williamson River,
325 Oregon for treatments 2 and 4 and at the John L. Fryer Salmon Disease Laboratory at Oregon
326 State University, Corvallis, Oregon (SDL) for treatments 1 and 3. Exposure 2 took place at the
327 SDL for all four treatments. Five fish were euthanized immediately following both exposures for
328 determination of infection.

329

330 Figure 2: Percent survival of Chinook salmon after exposure to *Ceratonova shasta* genotype I
331 only (black line) or after exposure to genotype II followed by genotype I (gray line). Letters
332 indicate statistical differences using a Mantel-Cox test.

333 Figure 3: Mean number of *Ceratonova shasta* myxospores produced in 0.1 g of intestinal tissue
334 from moribund fish in treatments 3 (exposure to genotype I only) and 4 (exposure to genotype II
335 then I). Error bars indicate standard error of the mean and letters indicate statistically significant
336 differences using a student's t-test.

337