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### Phenotypic integration of behavioural and physiological traits is related to variation in growth among stocks of Chinook salmon

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**Phenotypic integration of behavioural and physiological traits is related to variation in growth among stocks of Chinook salmon**

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1 **Phenotypic integration of behavioural and physiological traits is related to**  
2 **variation in growth among stocks of Chinook salmon**

3  
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16  
17 **Running headline:** Phenotypic integration benefits salmon growth

## 18 **Abstract**

19 The selection for a single organismal trait like growth in breeding programs of farmed  
20 aquaculture species can counter-intuitively lead to lowered harvestable biomass. We outbred a  
21 domesticated aquaculture stock of Chinook salmon (*Oncorhynchus tshawytscha*) with 7 wild  
22 stocks from British Columbia, Canada. We then examined how functionally related traits  
23 underlying energy management - diel variation in cortisol; foraging, social, and movement  
24 behaviours - predicted stock-level variation in growth during the freshwater life history stage, a  
25 performance metric under aquaculture selection. Outbreeding generated significant variation in  
26 diel cortisol secretion and behaviours across stocks, and these traits covaried, suggesting tight  
27 integration despite hybridization. The coupling of nighttime cortisol exposure with daytime  
28 behavioural phenotype was the strongest predictor of stock-level variation in body mass. Our  
29 results suggest selecting for an integrated phenotype rather than on single mechanistic traits  
30 alone can generate the greatest effect on aquaculture fish growth under outbreeding practices.  
31 Furthermore, selecting for these traits at the stock level may increase efficiency of farming  
32 methods designed to consistently maximize fish performance on a large scale.

33

34 **Key words:** Aquaculture, Behaviour, Diel cortisol, Phenotypic integration, Functional traits,  
35 Growth, Chinook salmon,

## 36 **Introduction**

37           For the first time on record, people are consuming more farm-raised than wild-caught fish  
38 (FAO 2016), and urbanization is simultaneously creating a greater demand for both improved  
39 quantity and quality protein due to its recognition in healthy diets (Clapp & Cohen 2009;  
40 Henschion et al. 2017). Aquaculture production has been relieving the pressure on wild fish  
41 populations by attempting to provide a more sustainable and economic source of protein (FAO  
42 2016). However, aquaculture faces the same challenges as other farming practices: to harvest the  
43 greatest amount of product at the minimum operator cost. As with any domestication process,  
44 fish stocks have the tendency to inadvertently become inbred when only selecting the largest,  
45 fastest growing individuals as broodstock (Bentsen & Olesen 2002), thereby becoming  
46 susceptible to disease (Arkush et al. 2002), experiencing slower growth (Kincaid 1983), or  
47 having low fecundity (Su et al. 1996). (Re)introducing alleles at fertilization is the classic  
48 method of lessening the effects of inbreeding depression via the outbreeding of domestic  
49 broodstock with wild individuals (Lehnert et al. 2014). First generation (F1) hybrid offspring of  
50 wild-caught and domesticated parents may experience benefits associated with outbreeding such  
51 as increased size (i.e., hybrid vigour: Gharrett et al. 1999; Whitlock et al. 2000). However, stocks  
52 may also experience outbreeding depression (Allendorf et al. 2001; Neff et al. 2011), whereby a  
53 shift in mean phenotype occurs and causes a reduction in growth (Tymchuk et al. 2006) and  
54 survival (Gharrett et al. 1999; Tallmon et al. 2004; Edmands 2007). These discrepancies can be  
55 explained through the evolutionary concept of an integrated phenotype (Murren 2012), which  
56 recognizes that optimal functioning of the organism requires multiple traits to work in unison. As  
57 such, when genetic recombination disrupts the evolutionary orchestration of integrated traits,  
58 individual performance and fitness can be compromised (Lancaster et al. 2010).

59 To enhance the performance of captive stocks, aquaculture producers should ideally be  
60 able to assess whether the genotypes selected for outcrossing will maintain the expression of  
61 maximal performance metrics such as growth through the continued integration of key  
62 phenotypic traits (e.g., traits related to energetic management) that ultimately increase harvest  
63 biomass (Tallmon et al. 2004). One of these metrics is the ability to manage energetic  
64 homeostasis, both physiologically and behaviourally, since energetic management is widely  
65 recognized as critical for maximizing growth in aquaculture (Boisclair and Sirois 1993;  
66 Silverstein et al. 1999). In particular, diel management of baseline levels of glucocorticoids is  
67 important for overall energetic balance via the optimal timing of fuel mobilization (e.g., glucose,  
68 lipids, and fatty acids; Gregory and Wood 1999). Likewise, behavioural traits related to  
69 energetics such as aggression, schooling, neophilia, and coping style all directly affect the ability  
70 of an individual to acquire, consume, and efficiently convert food to tissue (Gregory and Wood  
71 1999; Almazán-Rueda et al. 2005; Martins et al. 2006). Collectively, physiological and  
72 behavioural traits related to energy management are expected to impact fitness (Gilmour et al.  
73 2005), yet the linkages among these traits in novel environments remain largely unexplored.

74 Here we apply the framework of phenotypic integration (Ketterson et al. 2009; Lancaster  
75 et al. 2010; Murren 2012) to assess the impacts of outbreeding on performance at an important  
76 early life-history stage within an emerging model of Pacific salmonid aquaculture (Chinook  
77 salmon; *Oncorhynchus tshawytscha*). Specifically, our objectives are to determine whether: 1)  
78 outcrossing a domesticated stock with multiple wild populations generates variability in traits  
79 expected to impact juvenile growth (e.g., diel cortisol patterns, exposure to cortisol during  
80 nighttime and daytime, and behavioural phenotype); 2) physiological and behavioural traits are  
81 tightly coupled; and 3) taking an integrative, compared to a traditional, single-trait approach,

82 better predicts variation in growth in an aquaculture setting. We examine these questions using a  
83 domesticated stock outbred with wild genotypes from 7 regional populations in coastal British  
84 Columbia, Canada. We focused these questions on an early-life stage given the recently  
85 highlighted importance of identifying mechanisms controlling early development since these are  
86 considered significant for the determination of developmental windows that most impact growth  
87 variation and survival (Valente et al. 2013). Given the high degree of local adaptation of  
88 semelparous Pacific salmon (Taylor 1991), reflecting the environment-specific functional  
89 properties of specific loci (Carlson & Seamons 2008), our chosen system also provides the  
90 unique opportunity to examine whether physiology and behaviours associated with energy  
91 management become decoupled in a hybrid F1 generation, and whether the expression of energy-  
92 management phenotypes is optimally related to growth in a novel, captive environment. Given  
93 that adaptive covariation between physiology and behaviour should be ultimately linked to  
94 performance (McGlothlin et al. 2007), selection for suitable captive phenotypes expressing  
95 optimal cortisol-behavioural trait combinations should be critical for producing the ideal farmed  
96 fish. As such, we expected hybridized stocks expressing an optimal combination of low daytime  
97 baseline cortisol (representing low energetic demand), intermediate nighttime baseline cortisol  
98 (representing the ability to mobilize energy without entering stress-induced levels; Mommsen et  
99 al. 1999), and favorable energy management behaviours (e.g., high foraging rates, high sociality,  
100 and lower movement) to exhibit the largest mean body mass.

101

102

103

104



## 105 **Methods**

### 106 **Animal husbandry and growth**

107 Research was conducted at Yellow Island Aquaculture Ltd. (YIAL) on Quadra Island, British  
108 Columbia (latitude: N 50° 7' 59.124"; longitude: W 125° 19' 51.834"). The YIAL Chinook  
109 salmon population originated from crosses made in 1985 from two nearby hatcheries: Robertson  
110 Creek (latitude: N 49° 18' 37.8792"; longitude: W 124° 57' 36.4392") and Big Qualicum River  
111 (latitude: N 49° 21' 56.3616"; longitude: W 124° 39' 6.2964"). The domesticated YIAL stock has  
112 been maintained in captivity for seven generations. In 2013, sires from 7 wild stocks originating  
113 from tributaries on Vancouver Island and the lower mainland of British Columbia (Figure 1)  
114 were selected for generating outbred crossed stocks with YIAL dams, with an additional YIAL x  
115 YIAL stock serving as a control (Semeniuk et al. submitted). Gamete collection and fertilization  
116 occurred during October and November of 2013, with milt of 10 males from each stock  
117 fertilizing mixed eggs from 17 YIAL dams, who were the offspring of self-fertilization in one  
118 functional hermaphrodite, to control for known maternal effects on growth (Heath et al. 1999).  
119 After incubation in vertical-stack trays, fry were transferred on March 14, 2014 to 200 L barrels  
120 (mean:  $122 \pm 5$  fish per barrel; range: 120-136) split by stock and duplicated to account for barrel  
121 effects ( $n=16$  barrels), with a flow through water system maintained between 10–12 °C and water  
122 turnover at 1 L/min. All barrels were cleaned approximately every 7 days and dissolved oxygen  
123 was monitored once a week and maintained above 80% saturation. Fish were fed to satiation 8  
124 times daily between 8:00am to 5:00pm, and mortalities removed every two days (mean mortality  
125 rate (March 26-June 4, 2014):  $2.52 \pm 3.9\%$  per barrel; range: 0-16 fish). For this study, barrels for  
126 each stock consisted of a mixture of all 10 families. On June 6, 2014, a random subset of 10–13  
127 individual fish were removed from every barrel, individually weighed to the nearest 0.01g, and

128 fish were returned to their original barrel. Body mass at this stage was used as our performance  
129 metric to represent early growth from the fry stage, a critical freshwater life-history stage under  
130 strong selection in aquaculture (Piper et al. 1982).

131

### 132 **Blood sampling and cortisol assays**

133 We sampled fish for diel cortisol patterns at 8 months post fertilization, over a three-day period  
134 beginning on June 25, 2014. Specifically, six fish from each stock were sampled (alternating  
135 between barrel replicates) at 6 time periods across the entire diel cycle for a total sample size of  
136 36 fish per stock (Figure 2A). These specific sampling times were chosen to represent both key  
137 periods over the 24-hour period where fish have been shown to be active behaviourally and  
138 physiologically (Thorpe et al. 1987; Gries et al. 1997; Pavlidis et al. 1999; Bremset 2000), to  
139 capture as much finer-scale variation with day- and night-time periods as possible. Fish were  
140 captured via dip net, immediately euthanized in a clove oil solution, and blood was collected via  
141 caudal severance using heparinized hematocrit tubes. All sampling was completed within 5  
142 minutes of the initial disturbance to account for increases in cortisol due to capture and handling,  
143 and a given barrel was only sampled once every 24 hours to ensure that individuals had not been  
144 disturbed from a previous capture session. Packed red blood cells were then separated from  
145 plasma via centrifugation at 14,000 rpm (13,331 g) and plasma was stored at -80°C until further  
146 analyses. Baseline plasma cortisol was determined using a cortisol enzyme-linked  
147 immunosorbent assay (Cayman Chemical, MI, USA) optimized in-house for use in juvenile  
148 Chinook salmon (Capelle et al. 2016). Samples were run in triplicate across fourteen assay plates  
149 at 1:20 dilution. Individual assay plates included standard curves and common control samples,

150 and were read at a wavelength of 412nm using a spectrophotometer (Biotek Inc). Inter- and  
151 intra-assay coefficients of variation were 11.0% and 4.6%, respectively.

152

### 153 **Behavioural analysis**

154 All barrels (2 barrels per stock) were each filmed once over a span of seven days (June 3–10,  
155 2014). GoPro cameras (Woodman Labs, USA) were placed on the bottom center of the barrels at  
156 ~6:00am and behaviours were recorded for 1.5 hours. At 1 hour post camera deployment, fish in  
157 the barrels were fed ~4.2g of feed. Feed amount was calculated using Taplow Feeding charts  
158 (Chilliwack, BC, Canada), with daily amounts representing ~2% of the biomass in a barrel to  
159 match a satiated growth promotion diet. Behavioural analyses consisted of: assigning a score to  
160 the degree of group cohesion, calculating individual swimming velocity, and scoring uniformity  
161 of gregarious swimming direction fifteen minutes prior and post the addition of food, at one-  
162 minute intervals. During food presentation, position and movement of a subset of fish (~60) were  
163 noted, as well as latency (in seconds) to resume pre-feeding behaviours (see supplementary  
164 materials, Tables S1 and S2). Behavioural videos of the Capilano-River stock were highly  
165 distorted due to unforeseen technical issues and thus this population was excluded from all  
166 behavioural analysis.

167

### 168 **Statistical analysis**

169 All analyses were conducted using JMP version 12 (SAS Institute Inc.), except where indicated.  
170 Prior to analysis, model assumptions of normality and homogeneity of variances were tested by  
171 visual inspection of residual versus predicted plots, and normality was confirmed using the

172 Shapiro–Wilk test. Non-normally distributed data were  $\log^{10}$  transformed where indicated. In all  
173 cases, results were evaluated for significance at  $\alpha = 0.05$ .

174 To describe diel cortisol variation across all stocks, a general linear model with stock,  
175 time of day, and their interaction included as fixed effects was used. Cortisol data were  $\log^{10}$   
176 transformed to achieve normality. Next, the area under the diel cortisol curve (AUC) was  
177 calculated for daytime (6am–6pm) and nighttime (6pm–6am) cortisol (see Figure 2A), following  
178 Pruessner et al. (2003) as an estimate of the amount of hormone fish were exposed to  
179 (aggregated at the stock level), and as a means to capture the potential different roles of cortisol  
180 across the 24-hour cycle. Daytime and nighttime cortisol exposures were pooled separately for  
181 each stock but differences were examined using a paired t-test.

182  
183 Behavioural variables from video recordings were grouped *a priori* into three categories with  
184 known implications for fish growth and consisting of distinct variables: Foraging, Sociality, and  
185 Movement (Fernö et al., 1988; Fernö et al., 1995; Oppedal et al., 2011). A principal component  
186 analysis (PCA) with varimax rotation was used for initial exploration of each behavioural  
187 category independently to reduce redundancies in the data and to create more homogenous  
188 groupings. Each ‘factor’ extracted explained a minimum of 35% of the variance in behaviour  
189 based on the Kaiser criterion (eigenvalue  $>1$ ) and visual inspection of variance plots (Table S1),  
190 and were retained for the second stage of PCA. In the second stage, the rotated factors resulting  
191 from each behavioural category were loaded into a PCA to produce an overall behavioural  
192 phenotype. This PCA produced only a single component with an eigenvalue score that was  $>1$   
193 (eigenvalue = 2.3), explained 38.3% of the overall variance, and was used in subsequent models.  
194 High positive scores were associated with fish that spent longer time feeding, retained group

195 cohesiveness, and collectively exhibited consistent swimming directions, suggestive of more  
196 efficient energetic management.

197 To investigate the effects of cortisol and behaviour on growth, analyses were conducted  
198 using daytime and nighttime cortisol exposure separately. To first confirm the coupling of  
199 cortisol during daytime and nighttime directly with behaviour, we used a linear regression with  
200 the behavioural phenotype score regressed against cortisol exposure ( $AUC_{\text{night}}$  or  $AUC_{\text{day}}$ ).  
201 Next, we assessed whether each trait in isolation or in combination influenced size using linear  
202 regressions with mean body mass per population as the response variable (from the sub-sample  
203 of individuals weighed prior to the experiment and averaged across replicate barrels), and  
204 population-level behavioural phenotype with nighttime cortisol exposure ( $AUC_{\text{night}}$ ) or daytime  
205 cortisol exposure ( $AUC_{\text{day}}$ ) as the explanatory variables. Both linear and quadratic terms for  
206 cortisol and behaviour were used in a subset of models to account for any hormetic relationships  
207 (Schreck 2010). For model selection, we used a combination of fit, significance, and AIC  
208 modified for small sample size ( $AIC_c$ ) using the package “AICcmodavg” in R (Mazerolle and  
209 Mazerolle 2017).

210  
211 All analyses were aggregated to the level of the population since screening at the individual level  
212 is not necessarily a feasible practice within aquaculture (Castanheira et al. 2013; Pigliucci, 2003).  
213 Instead, determining whether integrated phenotypes exist at the population level is not only  
214 practical, but since intraspecific variability influences fundamental evolutionary processes of  
215 adaptation (Bennett et al. 2016), it also increases the potential that the desired phenotypes will  
216 remain robust under the stresses of captivity in subsequent generations.

217

## 218 **Results**

### 219 **Variation in diel cortisol and behaviour among stocks**

220 Stock and time of day interacted to influence baseline cortisol levels ( $F_{47,279} = 14.01$ ,  $n = 280$ ,  $p <$   
221  $0.001$ ). While each stock showed similar diel rhythms in their baseline cortisol (Figure 2A), they  
222 differed in overall exposure to cortisol across the full 24-hour cycle (Figure 2B). In addition,  
223 daytime cortisol exposure was significantly lower than nighttime exposure (*paired t*(6) = 5.59,  $p$   
224  $< 0.001$ ). The behavioural phenotype scores for different stocks ranged from  $-1.88$  (Nitinat) to  
225  $2.23$  (Chilliwack), and were additionally composed of differences in the contributions of each  
226 independent behavioural trait (Figure 2C).

227

### 228 **Integrated effects of cortisol and behaviour on growth**

229 Daytime cortisol exposure had a negative linear relationship with behavioural phenotype ( $R^2 =$   
230  $0.64$ ,  $p = 0.031$ ; Figure 3), while nighttime cortisol exposure showed no such relationship ( $R^2 =$   
231  $0.12$ ,  $p = 0.44$ ). As univariate predictors of performance, nighttime cortisol exposure and  
232 behavioural phenotype each showed a non-linear, hormetic relationship with body mass (cortisol:  
233  $R^2 = 0.94$ ,  $p = 0.003$ , AICc = 6.1; quadratic regression: body mass =  $(4.44 - 0.002 * (\text{AUC}_{\text{night}} -$   
234  $75.31)^2$ ); (behaviour:  $R^2 = 0.95$ ,  $p = 0.003$ , AICc = 5.8, quadratic regression: body mass =  
235  $(4.03 - (0.12 * \text{behaviour}^2))$ ). AUC<sub>day</sub> had no effect on body mass (linear or quadratic  
236 relationships;  $R^2 < 0.4$ ,  $p$ -values  $> 0.36$ , AICc's  $> 11.0$ ). However, the inclusion of both  
237 nighttime cortisol exposure and behaviour increased the model's predictive ability and its  
238 weighting to explain variation in body mass, with stocks exhibiting mid-range in nighttime  
239 cortisol exposure and high energy-management behaviours having the largest body masses ( $R^2 =$   
240  $0.998$ ,  $p = 0.07$ , AICc =  $-143.3$ ; Figure 4A). There was no meaningful or significant integration

241 of daytime cortisol exposure with behavioural phenotype on body mass. All models tested are  
242 included in Table S3.

243

## 244 **Discussion**

245 Rapid human population growth and increases in anthropogenic stressors on wild ecosystems  
246 require improved efficiency in alternative food-production systems such as aquaculture to keep  
247 pace with world demand for protein (Green et al. 2005). However, aquaculture practices such as  
248 selecting for fast growing individuals can inadvertently decrease stock genetic diversity and/or  
249 co-select for undesirable traits that will ultimately impact production (Castanheira et al. 2017).  
250 We sought to determine whether outbreeding a domesticated Chinook salmon stock with  
251 multiple wild populations resulted in the optimal integration of physiological and behavioural  
252 traits at the nexus of energetic management, and if this integration lead to maximized  
253 performance at an early-life freshwater stage. Outbreeding generated significant variability in  
254 physiological and behavioural traits that are associated with energetic management and known to  
255 impact growth. As predicted by the theory of phenotypic integration (Murren 2012), growth was  
256 influenced by a coupling of nighttime cortisol exposure and behavioural phenotype, specifically  
257 with intermediate cortisol values and high energy-management behaviours combining to  
258 generate an optimized hormetic relationship to maximize body size. Our results also suggest that  
259 examining the effects of the co-variation of multiple functionally related traits on body mass is a  
260 stronger approach than examining these traits in isolation. Moreover, relationships that exist  
261 between phenotypic traits such as daytime cortisol exposure and behaviour do not imply  
262 integration (and by extension maximized performance), highlighting that only within the context  
263 of a performance measure can phenotypic integration be revealed. While phenotypic correlations

264 among traits have been extensively studied, fewer have been examined in light of performance-  
265 or fitness outcomes, and fewer still across multiple populations. This is the first study to show  
266 how among-population coupling of physiological and behavioural traits can influence early-life  
267 metrics such as growth rate (here measured as body mass), considered important in assessing  
268 long-term success in aquaculture (Valente et al. 2013). This work therefore suggests that  
269 researchers and producers may benefit from determining how phenotypic integration impacts  
270 early-life performance when employing methods such as outbreeding to enhance or rescue  
271 breeding programs.

272

### 273 **Impacts of outcrossing on variability in performance-mediated traits**

274 Optimal management of energy budgets over both short- and long-term periods within the  
275 constraints of local environmental variation is expected to maximize fitness (Fong 1975; Taylor  
276 1991; Sanford & Kelly 2011), a critical consideration for production output in aquaculture  
277 (Akvaforsk 2005). Outbreeding in our study produced substantial variation in diel physiological  
278 and behavioural traits central to daily energetic management. We focused on diel variation in  
279 baseline cortisol because it can differentially affect growth by regulating metabolic processes and  
280 promoting homeostasis in fish (Mommsen et al. 1999). In diurnal vertebrates, an optimal diel  
281 cortisol cycle consists of high levels early in the morning to provide the energy via  
282 gluconeogenesis following night fasting (Dallman et al. 1993), to initiate foraging and other  
283 activities (Astheimer et al. 1992; Breuner et al. 1999; Breuner and Hahn 2003). Levels then tend  
284 to decline for the remainder of the day to avoid the “high cost of living” associated with  
285 maintaining high baseline circulating glucocorticoid levels (Sapolsky et al. 2000; Bernier et al.  
286 2004). Although our data confirm that all populations showed strong diel rhythms in baseline



287 cortisol, populations nonetheless differed significantly in patterns of cortisol exposure during  
288 night and day. Outbreeding also generated a range in behavioural traits associated with energetic  
289 management, and when combined with differences in cortisol exposure, revealed certain stocks  
290 to be less ideally suited to captive environments as evidenced by the resultant low body size.

291         The differences observed among stocks are presumably driven by large sire effects that  
292 result from genetic differences among the source stocks that have arisen through local adaptation  
293 and potentially genetic drift (Fraser et al. 2011). The sire effect would have dominated possible  
294 dam effects among stocks given that we used a common inbred line of domestic dams to both  
295 minimize maternal effects and equalize any influences across all stocks (as eggs were also  
296 pooled). Within our study, not all hybrid stocks showed high performance. While there are many  
297 possible causes of this pattern of reduced performance, one may be the poor performance of the  
298 F1 generation is due to the intermediate phenotypes generated by hybridization which are  
299 unsuited to the domestic parental environments (Frankham et al. 2002; McClelland & Naish  
300 2007). Another mechanistic cause of lowered performance in hybrid stocks may be intrinsic  
301 outbreeding depression due to the disruption of epistatic interactions in large co-adapted gene  
302 complexes (Edmands 1999); however, we do not believe this to be the case since previous  
303 studies have shown Chinook salmon F1 hybrids of genetically different parental lines  
304 demonstrate no evidence of hybrid breakdown (Lehnert et al. 2014). Instead, suboptimal  
305 performance of juvenile Chinook salmon of certain stocks most likely reflects local adaptation of  
306 performance traits within freshwater systems that are very important for the success of early life-  
307 history stages of anadromous, semelparous salmonids (Waples 1991). When different genotypes  
308 are raised in common environments, the impacts of these locally adapted phenotypes persist and  
309 are known to generate significant variation in performance outcomes (McClelland & Naish

310 2007), and so deliberate selection of these tightly integrated traits will be robust past the F1  
311 generation and persist through multiple generations. However, the potential for non-additive  
312 genetic effects coupled with residual epigenetic effects may interfere with the predictable  
313 inheritance of some of the traits measured.

314

315 **Effects of outcrossing on the outcome of phenotypic integration under novel captive**  
316 **conditions**

317 Traditionally, researchers have focussed on single traits rather than multi-trait combinations to  
318 predict fitness or performance of wild populations (Gilmour et al. 2005; Killen et al. 2013).  
319 However, since traits that depend on common mechanisms for their expression may evolve as a  
320 unit (McGlothlin & Ketterson 2008), ignoring the potential for synergistic effects of integrated  
321 phenotypes on performance can limit our appreciation of their adaptive value (Pigliucci 2003;  
322 Ketterson et al. 2009; Laughlin & Messier 2015). In our study, examining interactions between  
323 physiology and behaviour suggested that daytime cortisol exposure predicted behavioural  
324 phenotype in a simple linear fashion, setting up the expectation that both would interact to  
325 impact growth. However, an integrated phenotypic approach revealed it was the combination of  
326 exposure to nighttime cortisol and behaviour that was the best predictor of maximized growth  
327 across the 7 stocks, with stocks having mid-range nighttime cortisol exposure and maximal  
328 energetics behaviour exhibiting the highest body mass (Figure 4A).

329 Chronically high cortisol levels are known to negatively impact behavioural traits such as  
330 foraging and competitive ability due to a reduction in appetite (Gregory & Wood 1999) and can  
331 inhibit aggression and locomotion in salmonids (Øverli et al. 2002). As such, prolonged exposure  
332 can lead to tertiary responses such as slow growth, reduced immune function, and compromised

333 survival (Pickering & Pottinger 1983; Barton 2002). In our study, stocks with individuals that  
334 expressed elevated daytime cortisol exposure may have incurred these behavioural costs that led  
335 to reduced growth. During nighttime, when fish were not fed and feeding activity was much  
336 reduced (Fraser & Metcalfe 1997), cortisol plays a more direct role in growth: elevated baseline  
337 levels maintain energetic homeostasis, allowing stored glycogen in the liver (generated from  
338 food consumed and digested during the day) to be mobilized as glucose to fuel the maintenance  
339 and growth of tissues (see Dallman et al. 1993). Behaviours that promote energy gain while  
340 reducing energetic costs of locomotion (including sociality) are generally assumed to lead to the  
341 highest growth (e.g., foraging efficiency: Eklöv, 1992; high cohesiveness: Johnsson 2003).  
342 Interestingly, as a sole (univariate) measure, these maximized behaviours seemingly had a  
343 counter-intuitive, negative effect on growth (i.e., significant hormetic relationship), and yet as an  
344 integrated unit with physiology, these same behaviours conferred the greatest performance. We  
345 therefore argue that an integrated approach situated within a performance context is needed to  
346 understand how underlying traits will ultimately impact performance in aquaculture. We also  
347 demonstrate that with this integrated relationship - visualized as a contour plot (Figure 4B), there  
348 exists the potential for further selection of integrated traits to maximize production. For instance,  
349 *actively* selecting for specific behaviours (i.e., greater locomotory-, foraging efficiency, and  
350 sociality) - should these traits remain co-varied within a physiological range, can result in even  
351 greater mass gains than those observed. This integrated selection approach at the stock level can  
352 be of value for other performance traits (such as disease resistance) and in other breeding  
353 programs, outside of salmon culture: it is not only feasible for farming methods that must  
354 routinely document production data of functional traits and performance on a large scale, but can  
355 be used to possibly increase efficiency as well.

**356 Conclusions**

357 While phenotypic integration can increase organismal performance by optimizing the adaptive  
358 phenotype over evolutionary time, it may decrease flexibility in a changing environment  
359 (Schlichting 1989). This apparent trade-off is an important realization for evolutionary biologists  
360 and applied producers since both robust and flexible phenotypes carry costs due to (1) the  
361 potential for a “mismatched” phenotype to a given environment (Hendry 2004; Nosil et al. 2005)  
362 and (2) the associated energetic costs of flexibility (DeWitt et al. 1998; Snell-Rood 2013),  
363 respectively. In fact, with regards to food production, abrupt changes in environment (e.g., from  
364 the wild to captivity, or fresh water to salt water) may better suit a less flexible and instead a  
365 more integrated phenotype, as the temporal lag in endocrine activity and its associated  
366 behavioural response may be too slow (and take several generations) to adaptively respond to  
367 changing conditions (Taff & Vitousek 2016). Since the management of cortisol is intimately  
368 involved in the osmoregulatory transition of fish from fresh to salt water (rev. in McCormick  
369 2012), we would predict that (above and beyond energetic management) a tighter integration  
370 between cortisol and behaviour could also result in a less costly transition. Taken together,  
371 appreciating population-level effects associated with integrated energetic management has the  
372 ability to better inform researchers and aquaculture producers about early-life stage mechanisms  
373 that influence variation in growth and survival (Valente et al. 2013), and that can also undergo  
374 intentional selection for increased food production long-term.

375

376

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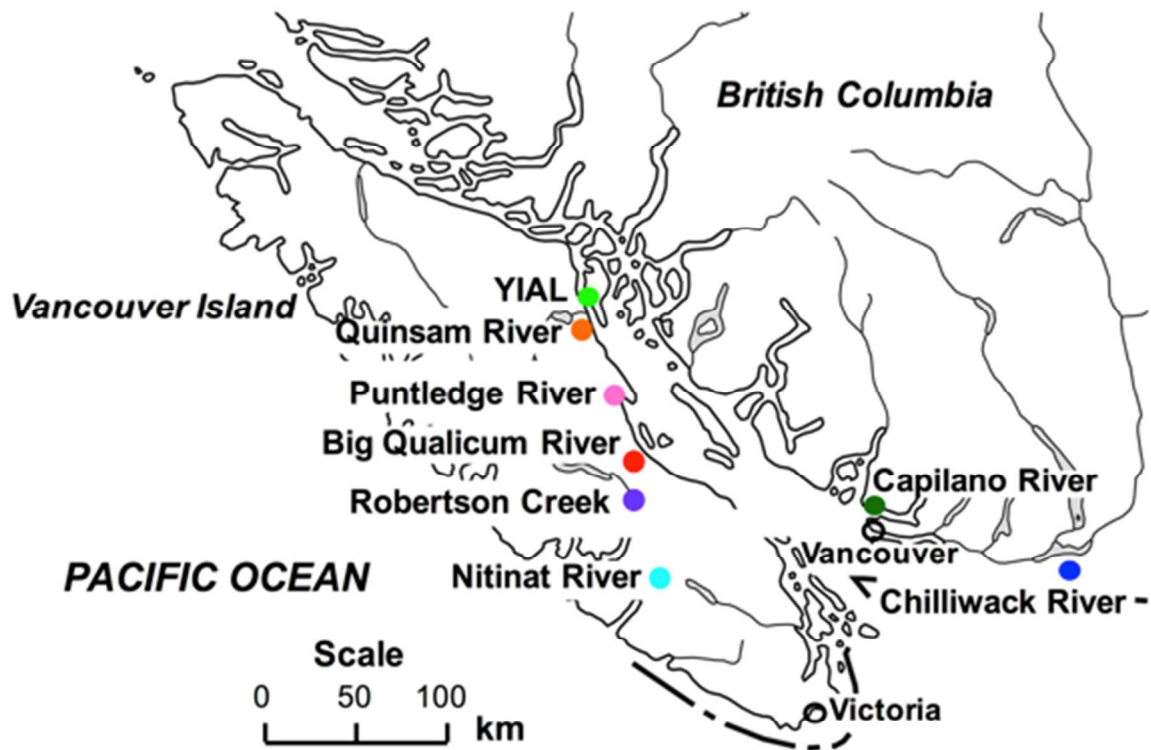
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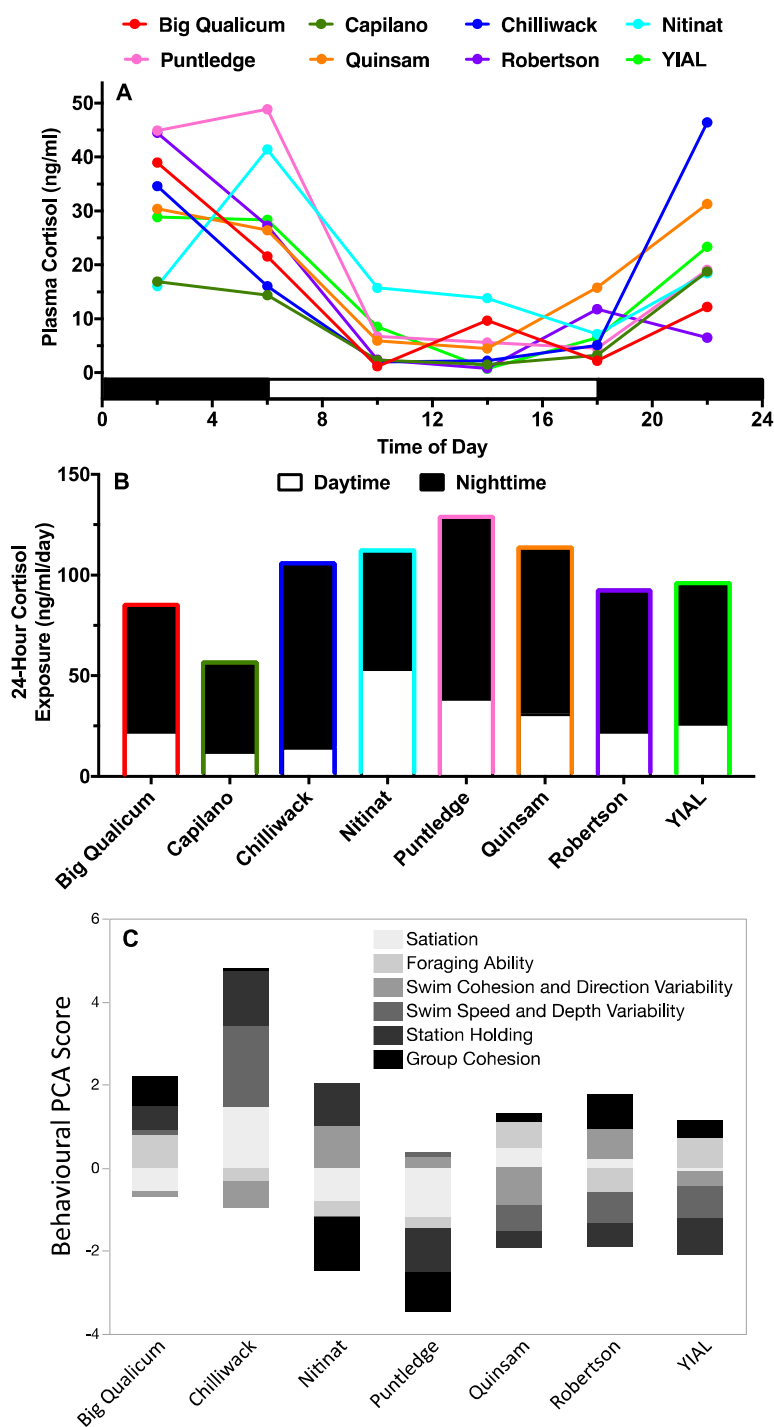
590 **Figure 1.** Map of British Columbia, Canada showing the source of wild Chinook salmon stocks  
591 used for outbreeding in the study. A captive, inbred stock was used as the basis for all crosses  
592 and is held at Yellow Island Aquaculture Ltd. Colours: Bright green – YIAL; purple – Robertson  
593 Creek; orange – Quinsam River; pink – Puntledge River; light blue –Nitinat River; dark blue –  
594 Chilliwack River; dark green – Capilano River; red –Big Qualicum River.



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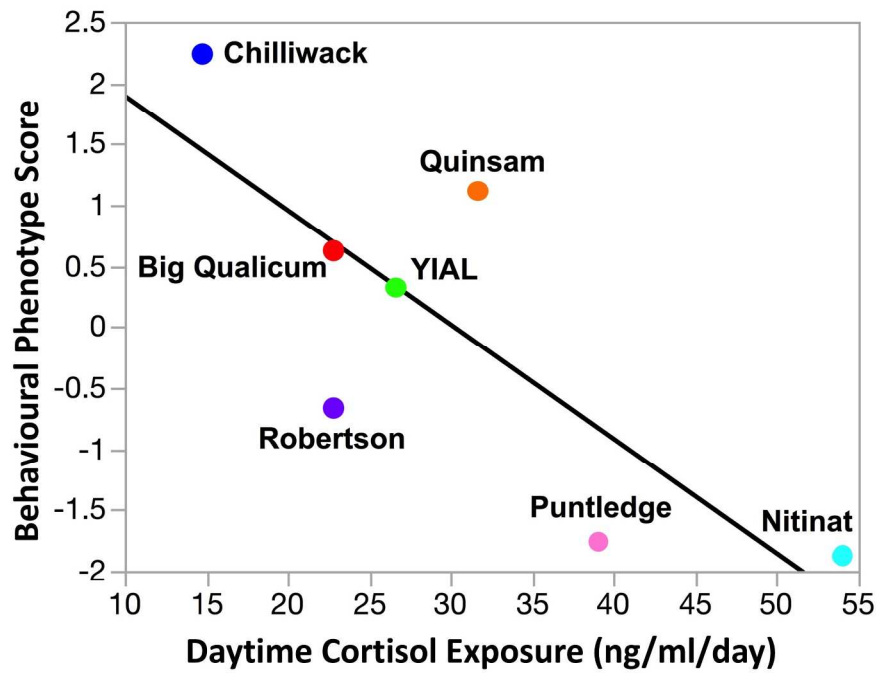


596 **Figure 2.** Inter-population variation in (A) diel patterns of baseline cortisol secretion, (B)  
 597 daytime and nighttime exposure to baseline cortisol, and (C) variation in behavioural traits of  
 598 Chinook salmon parr.



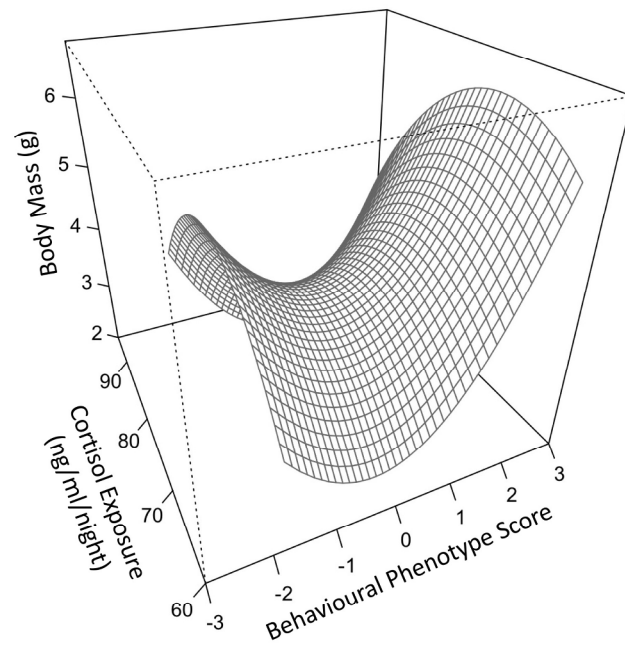
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**Figure 3.** Exposure to daytime cortisol predicts overall behavioural phenotype (combination of foraging, sociality, and movement behaviours) as a linear relationship across 6 outbred populations of Chinook salmon and the inbred control population.



**Figure 4.** Body mass outcome of optimal phenotypic integration between exposure to cortisol during the night and overall behavioural phenotype (see Methods) across 6 outbred populations of Chinook salmon and the inbred control population (YIAL), represented as both (A) a three-dimensional plot and (B) as a contour map.

A



B

