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16

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

Abstract

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

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

Introduction

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

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Methods

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 ± 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 $^{\circ}$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

Behavioural analysis

153

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

Statistical analysis

168

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   (eignevalue = 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_{night} or AUC_{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_{night}) or daytime

205 cortisol exposure (AUC_{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 (AICc) 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*(AUC$_{night}$ −

234 75.31) $^{\wedge}$ 2); (behaviour: $R^2 = 0.95$, $p = 0.003$, AICc = 5.8, quadratic regression: body mass =

235 (4.03 − (0.12 * behaviour $^{\wedge}$ 2)). AUC$_{day}$ 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

11

241   of daytime cortisol exposure with behavioural phenotype on body mass. All models tested are

242   included in Table S3.

243

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

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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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.

595

29
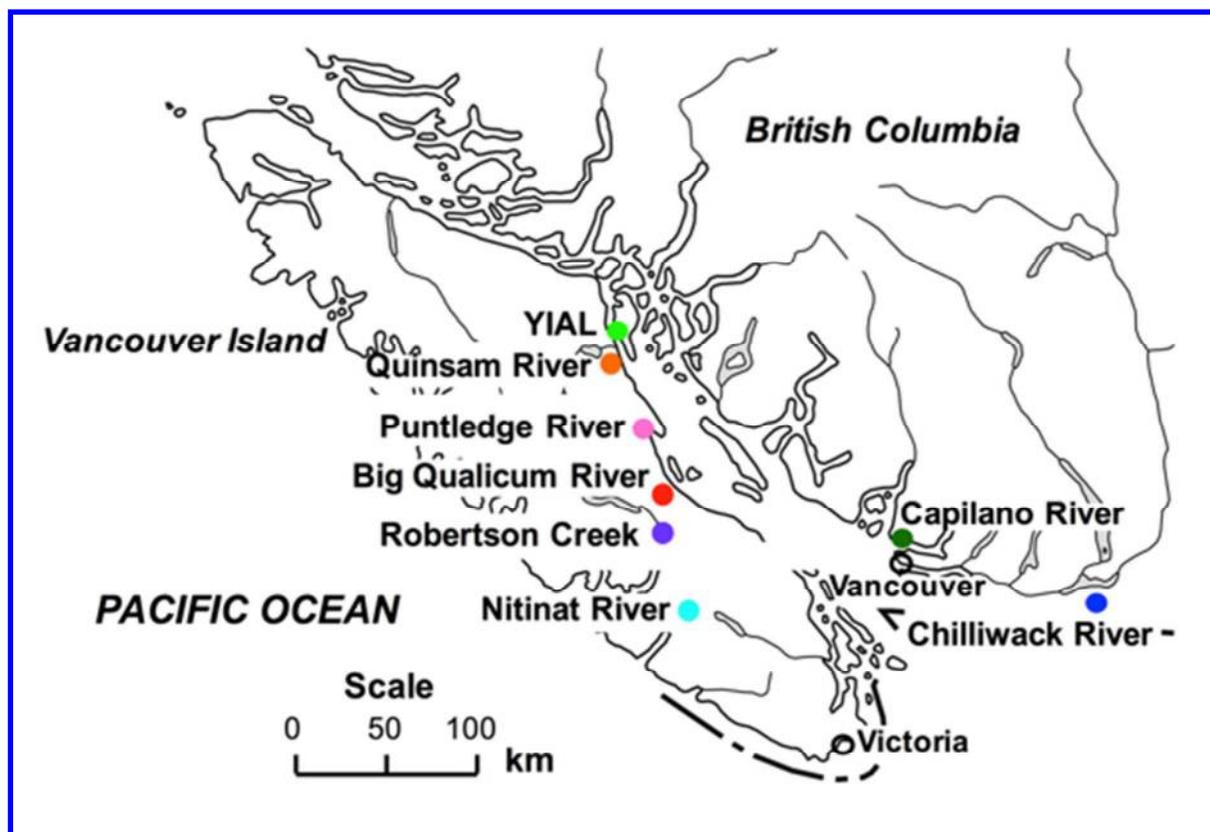
596 **Figure 2.** Inter-population variation in (A) diel patterns of baseline cortisol secretion, (B)

597 daytime and nighttime exposure to baseline cortisol, and (C) variation in behavioural traits of

598 Chinook salmon parr.
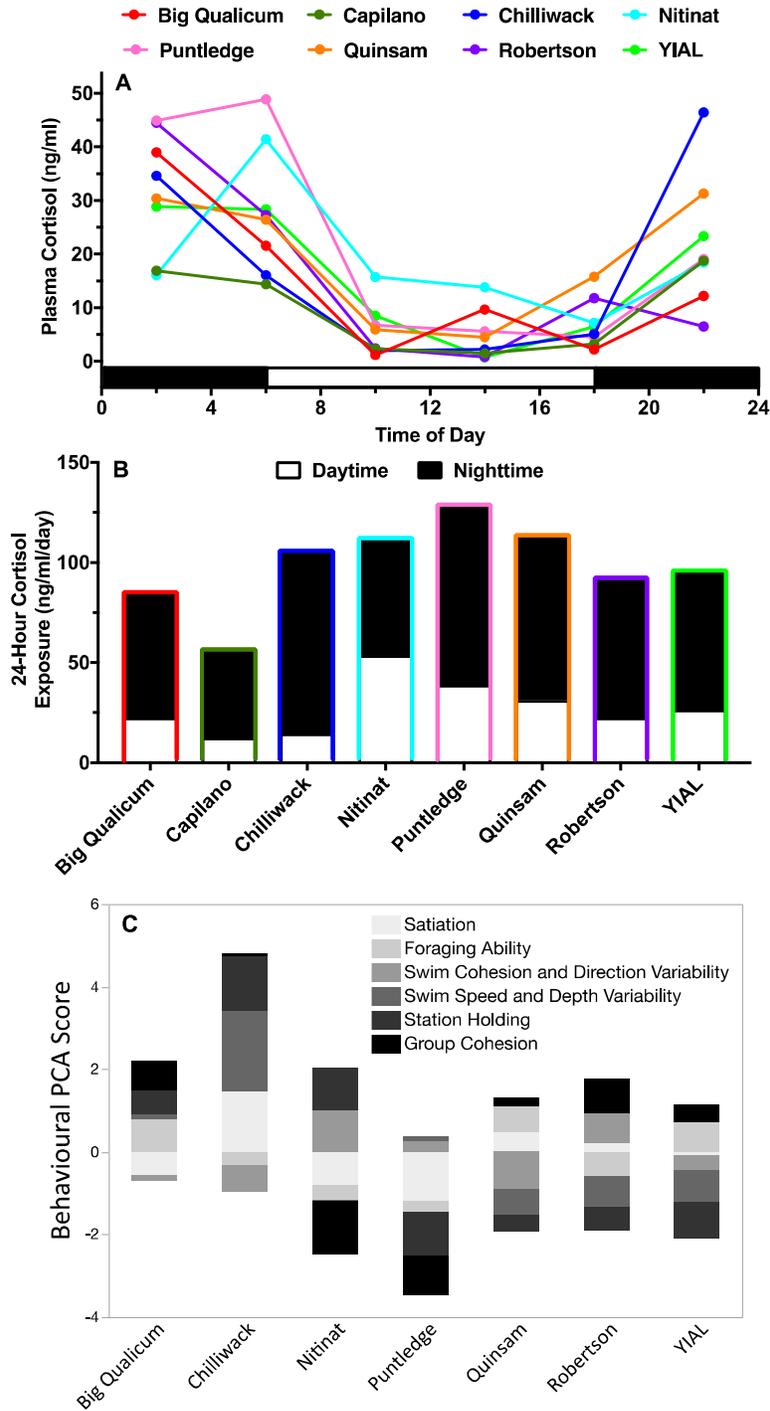
599

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.
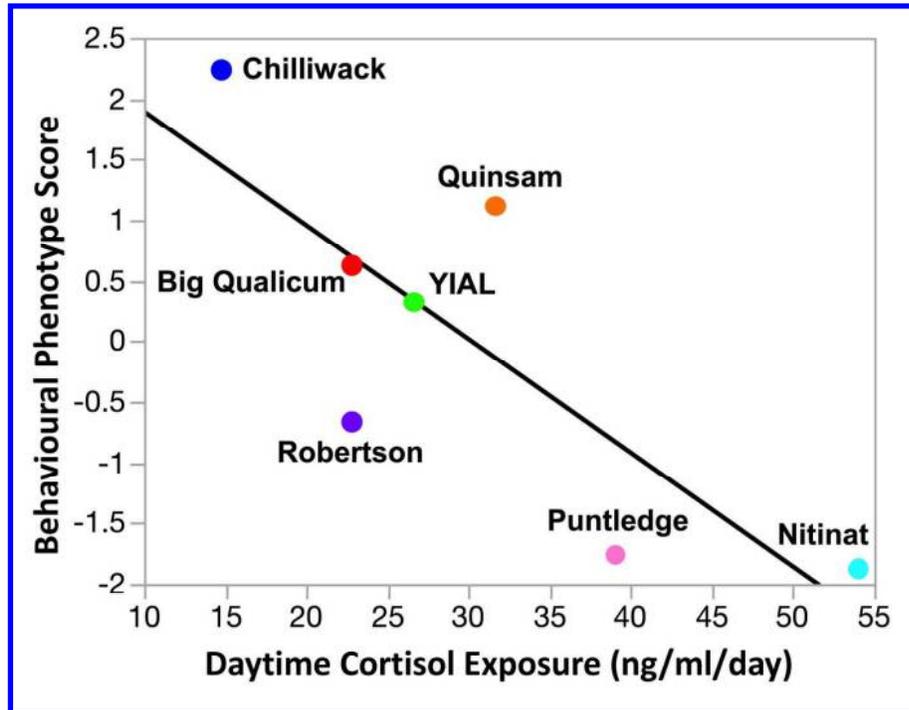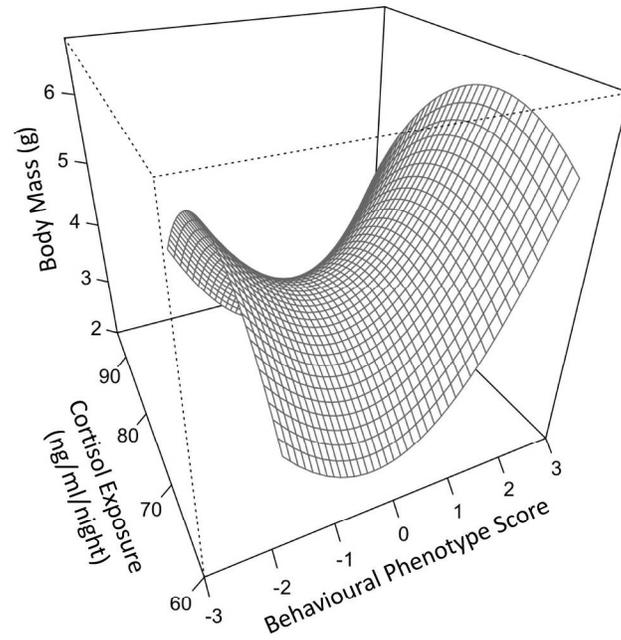

Figure 4. Body mass outcome of optimal phenotypic integration between exposure to cortisol during the night and overall behavioural phenotype (see Methods) across 6 outbred populations of Chinook salmon and the inbred control population (YIAL), represented as both (A) a three-dimensional plot and (B)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

A

B