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ARTICLE

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

Mitchel G.E. Dender, Pauline M. Capelle, Oliver P. Love, Daniel D. Heath, John W. Heath, and Christina A.D. Semeniuk

Abstract: The selection for a single organismal trait like growth in breeding programs of farmed aquaculture species can counterintuitively lead to lowered harvestable biomass. We outbred a domesticated aquaculture stock of Chinook salmon (*Oncorhynchus tshawytscha* (Walbaum in Artedi, 1792)) with seven wild stocks from British Columbia, Canada. We then examined how functionally related traits underlying energy management – diel variation in cortisol and foraging, social, and movement behaviours — predicted stock-level variation in growth during the freshwater life history stage, which is a performance metric under aquaculture selection. Outbreeding generated significant variation in diel cortisol secretion and behaviours across stocks, and these traits co-varied, suggesting tight integration despite hybridization. The coupling of nighttime cortisol exposure with the daytime behavioural phenotype was the strongest predictor of stock-level variation in body mass. Our results suggest that selection for an integrated phenotype rather than on a single mechanistic trait 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.

Résumé : La sélection d'un seul caractère d'organismes comme la croissance dans le cadre de programmes d'élevage d'espèces aquacoles peut, de manière contre-intuitive, mener à une plus faible biomasse exploitable. Nous avons croisé un stock aquacole domestiqué de saumons quinnats (*Oncorhynchus tshawytscha* (Walbaum in Artedi, 1792)) avec sept stocks sauvages de la Colombie-Britannique (Canada). Nous avons ensuite examiné comment des caractères fonctionnellement reliés qui sous-tendent la gestion de l'énergie – les variations nycthémérales du cortisol et des comportements sociaux, d'approvisionnement et de déplacement – prédisent les variations au niveau du stock de la croissance durant l'étape du cycle biologique en eau douce, une mesure de la performance visée par la sélection aquacole. Les croisements ont produit des variations significatives de la sécrétion nycthémérale de cortisol et des comportements dans tous les stocks, et ces caractères étaient covariables, ce qui indiquerait une intégration étroite malgré l'hybridation. Le jumelage de l'exposition nocturne au cortisol avec le phénotype comportemental diurne était le meilleur prédicteur des variations de la masse corporelle à l'échelle du stock. Nos résultats donnent à penser que la sélection visant un phénotype intégré plutôt que des caractères mécanistes particuliers peut avoir le plus grand effet sur la croissance des poissons aquacoles dans des pratiques de croisement éloigné. En outre, la sélection visant ces caractères au niveau du stock pourrait accroître l'efficacité des méthodes d'élevage ayant pour but de maximiser uniformément la performance des poissons à grande échelle. [Traduit par la Rédaction]

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 and 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, namely how 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 brood stock (Bentsen and 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 brood stock 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

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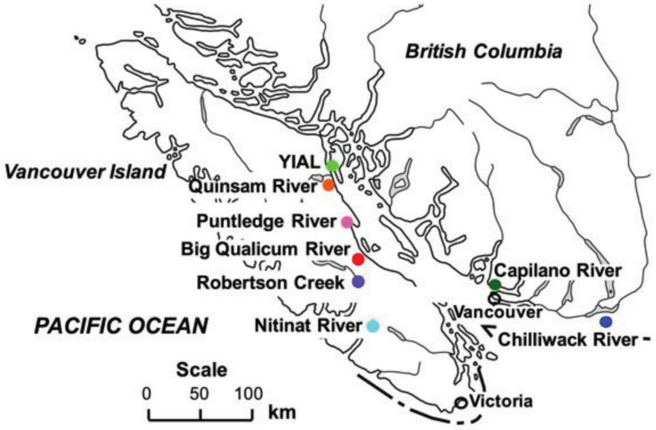
in unison. As such, when genetic recombination disrupts the evolutionary orchestration of integrated traits, individual performance and fitness can be compromised (Lancaster et al. 2010).

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

Here we apply the framework of phenotypic integration (Ketterson et al. 2009; Lancaster et al. 2010; Murren 2012) to assess the impacts of outbreeding on performance at an important early life-history stage within an emerging model of Pacific salmonid aquaculture (Chinook salmon; *Oncorhynchus tshawytscha* (Walbaum in Artedi, 1792)). Specifically, our objectives are to determine whether: (*i*) outcrossing a domesticated stock with multiple wild populations generates variability in traits expected to impact juvenile growth (e.g., diel cortisol patterns, exposure to cortisol during nighttime and daytime, and behavioural phenotype); (*ii*) physiological and behavioural traits are tightly coupled; and (*iii*) taking an integrative approach, instead of a traditional, single-trait approach, better predicts variation in growth in an aquaculture setting. We examine these questions using a domesticated stock outbred with wild genotypes from seven regional populations in coastal British Columbia, Canada. We focused these questions on an early-life stage given the recently highlighted importance of identifying mechanisms controlling early development as these are considered significant for the determination of developmental windows that most impact growth variation and survival (Valente et al. 2013). Given the high degree of local adaptation of semelparous Pacific salmon (Taylor 1991), reflecting the environment-specific functional properties of specific loci (Carlson and Seamons 2008), our chosen system also provides the unique opportunity to examine whether physiology and behaviours associated with energy management become decoupled in a hybrid F1 generation and whether the expression of energy-management phenotypes is optimally related to growth in a novel, captive environment. Given that adaptive covariation between physiology and behaviour should be ultimately linked to performance (McGlothlin et al. 2007), selection for suitable captive phenotypes expressing optimal cortisol – behavioural trait combinations should be critical for producing the ideal farmed fish. As such, we expected hybridized stocks expressing an optimal combination of low daytime baseline cortisol (representing low energetic demand), intermediate nighttime baseline cortisol (representing the ability to mobilize energy without entering stress-induced levels; Mommsen et al. 1999), and favorable energy management behaviours (e.g., high foraging rates, high sociality, and lower movement) to exhibit the largest mean body mass.

Fig. 1. Map of British Columbia, Canada, showing the source of wild Chinook salmon stocks used for outbreeding in the study. A captive, inbred stock was used as the basis for all crosses and is held at Yellow Island Aquaculture Ltd. Colours: bright green, YIAL; purple, Robertson Creek; orange, Quinsam River; pink, Puntledge River; light blue, Nitinat River; dark blue, Chilliwack River; dark green, Capilano River; red, Big Qualicum River. [Colour online.]



Methods

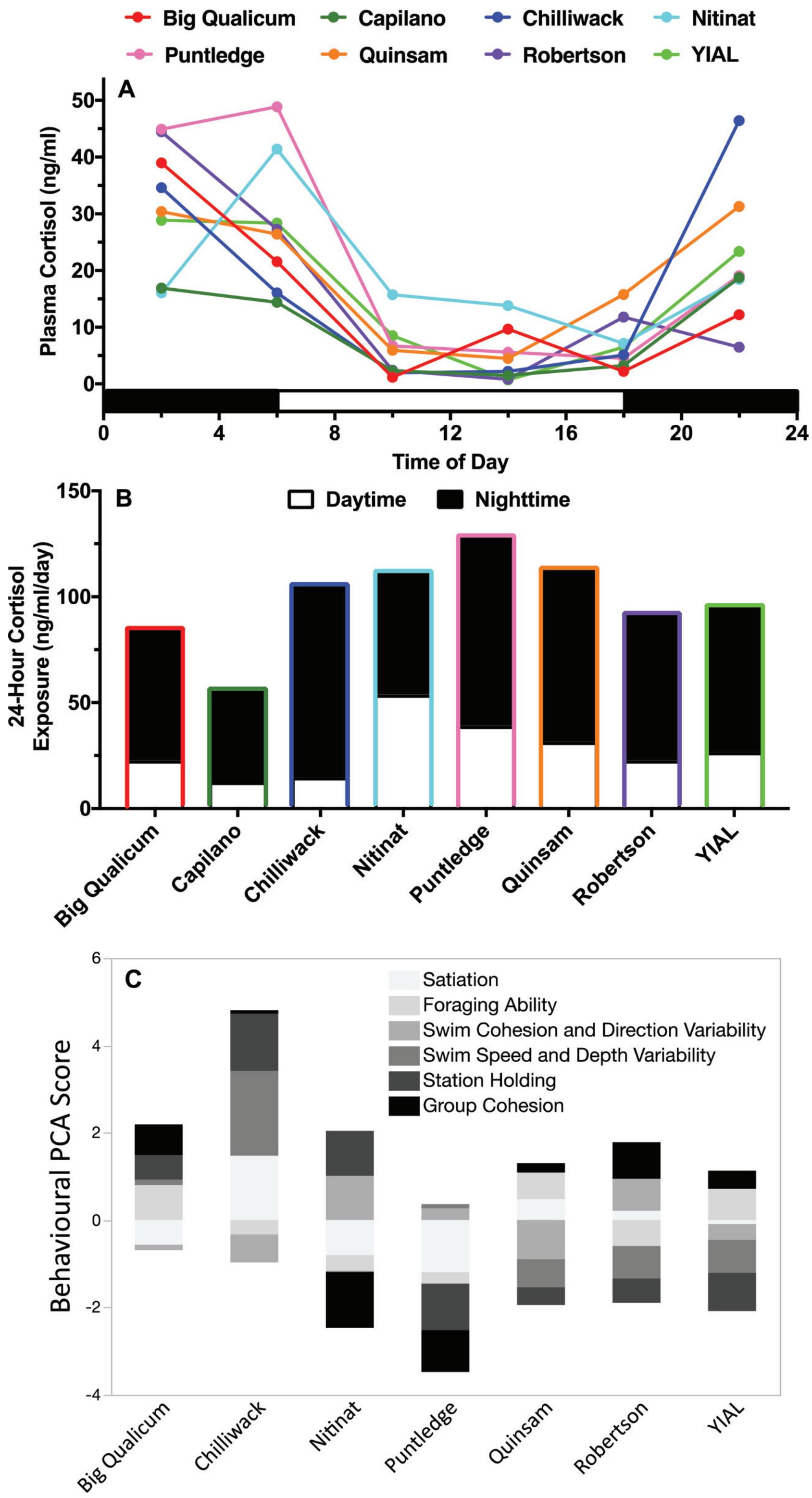
Animal husbandry and growth

Research was conducted at Yellow Island Aquaculture Ltd. (YIAL) on Quadra Island, British Columbia (latitude: N 50°7′59.124″; longitude: W 125°19′51.834″). The YIAL Chinook salmon population originated from crosses made in 1985 from two nearby hatcheries: Robertson Creek (latitude: N 49°18′37.8792″; longitude: W 124°57′36.4392″) and Big Qualicum River (latitude: N 49°21′56.3616″; longitude: W 124°39′6.2964″). The domesticated YIAL stock has been maintained in captivity for seven generations. In 2013, sires from seven wild stocks originating from tributaries on Vancouver Island and the lower mainland of British Columbia (Fig. 1) were selected for generating outbred crossed stocks with YIAL dams, with an additional YIAL × YIAL stock serving as a control (Semeniuk et al. submitted). Gamete collection and fertilization occurred during October and November of 2013, with milt of 10 males from each stock fertilizing mixed eggs from 17 YIAL dams, who were the offspring of self-fertilization in one functional hermaphrodite, to control for known maternal effects on growth (Heath et al. 1999). After incubation in vertical-stack trays, fry were transferred on 14 March 2014 to 200 L barrels (mean: 122 ± 5 fish per barrel; range: 120–136) split by stock and duplicated to account for barrel effects (n = 16 barrels), with a flow through water system maintained between 10 and 12 °C and water turnover at 1 L/min. All barrels were cleaned approximately every 7 days, and dissolved oxygen was monitored once a week and maintained above 80% saturation. Fish were fed to satiation eight times daily between 8:00 am and 5:00 pm, and mortalities removed every 2 days (mean mortality rate (26 March – 4 June 2014): 2.52% ± 3.9% per barrel; range: 0–16 fish). For this study, barrels for each stock consisted of a mixture of all 10 families. On 6 June 2014, a random subset of 10–13 individual fish were removed from every barrel, individually weighed to the nearest 0.01 g, and returned to their original barrel. Body mass at this stage was used as our performance metric to represent early growth from the fry stage, a critical freshwater life-history stage under strong selection in aquaculture (Piper et al. 1982).

Blood sampling and cortisol assays

We sampled fish for diel cortisol patterns at 8 months post fertilization, over a 3 day period beginning on 25 June 2014. Specifically, six fish from each stock were sampled (alternating be-

Fig. 2. Interpopulation variation in (A) diel patterns of baseline cortisol secretion, (B) daytime and nighttime exposure to baseline cortisol, and (C) variation in behavioural traits of Chinook salmon parr. [Colour online.]



tween barrel replicates) at six time periods across the entire diel cycle for a total sample size of 36 fish per stock (Fig. 2A). These specific sampling times were chosen to represent both key periods over the 24 h period where fish have been shown to be active behaviourally and physiologically (Thorpe et al. 1987; Gries et al. 1997; Pavlidis et al. 1999; Bremset 2000) to capture as much finer scale variation with day- and night-time periods as possible. Fish were captured via dip net, immediately euthanized in a clove oil solution, and blood was collected via caudal severance using heparinized hematocrit tubes. All sampling was completed within 5 min of the initial disturbance to account for increases in cortisol due to capture and handling, and a given barrel was only sampled once every 24 h to ensure that individuals had not been disturbed from a previous capture session. Packed red blood cells were then separated from plasma via centrifugation at 14 000 r/m (13 331g), and plasma was stored at –80 °C until further analyses. Baseline plasma cortisol was determined using a cortisol enzyme-linked immunosorbent assay (Cayman Chemical, MI, USA) optimized in-house for use in juvenile Chinook salmon (Capelle et al. 2016). Samples were run in triplicate across 14 assay plates at 1:20 dilution. Individual assay plates included standard curves and common control samples and were read at a wavelength of 412 nmol/L using a spectrophotometer (Biotek Inc.). Inter- and intra-assay coefficients of variation were 11.0% and 4.6%, respectively.

Behavioural analysis

All barrels (2 barrels per stock) were each filmed once over a span of 7 days (3–10 June 2014). GoPro cameras (Woodman Labs, USA) were placed on the bottom center of the barrels at ~6:00 am and behaviours were recorded for 1.5 h. At 1 h post camera deployment, fish in the barrels were fed ~4.2 g of feed. Feed amount was calculated using Taplow Feeding charts (Chilliwack, BC, Canada), with daily amounts representing ~2% of the biomass in a barrel to match a satiated growth promotion diet. Behavioural analyses consisted of: assigning a score to the degree of group cohesion, calculating individual swimming velocity, and scoring uniformity of gregarious swimming direction 15 min before and after the addition of food, at 1 min intervals. During food presentation, position and movement of a subset of fish (~60) were noted, as well as latency (in seconds) to resume pre-feeding behaviours (Supplementary Tables S1 and S2[1]). Behavioural videos of the Capilano River stock were highly distorted due to unforeseen technical issues, and thus, this population was excluded from all behavioural analysis.

Statistical analysis

All analyses were conducted using JMP version 12 (SAS Institute Inc.), except where indicated. Prior to analysis, model assumptions of normality and homogeneity of variances were tested by visual inspection of residual versus predicted plots, and normality was confirmed using the Shapiro–Wilk test. Non-normally distributed data were log10 transformed where indicated. In all cases, results were evaluated for significance at $\alpha = 0.05$.

To describe diel cortisol variation across all stocks, a general linear model with stock, time of day, and their interaction included as fixed effects was used. Cortisol data were log10 transformed to achieve normality. Next, the area under the diel cortisol curve (AUC) was calculated for daytime (6:00 am – 6:00 pm) and nighttime (6:00 pm – 6:00 am) cortisol (Fig. 2A), following Pruessner et al. (2003) as an estimate of the amount of hormone fish were exposed to (aggregated at the stock level) and as a means to capture the potential different roles of cortisol across the 24 h cycle. Daytime and nighttime cortisol exposures were pooled separately for each stock, and differences were examined using a paired *t* test.

Behavioural variables from video recordings were grouped a priori into three categories with known implications for fish growth and consisting of distinct variables: foraging, sociality, and movement (Fernö et al. 1988, 1995; Oppedal et al. 2011). A principal component analysis (PCA) with varimax rotation was used for initial exploration of each behavioural category independently to reduce redundancies in the data and to create more homogenous groupings. Each "factor" extracted explained a minimum of 35% of the variance in behaviour based on the Kaiser criterion (eigenvalue >1) and visual inspection of variance plots (Supplementary Table S1) and were retained for the second stage of PCA. In the second stage, the rotated factors resulting from each behavioural category were loaded into a PCA to produce an overall behavioural phenotype. This PCA produced only a single component with an eigenvalue score that was >1 (eignevalue = 2.3), explained 38.3% of the overall variance, and was used in subsequent models. High positive scores were associated with fish that spent longer time feeding, retained group cohesiveness, and collectively exhibited consistent swimming directions, suggestive of more efficient energetic management.

To investigate the effects of cortisol and behaviour on growth, analyses were conducted using daytime and nighttime cortisol exposure separately. To first confirm the coupling of cortisol during daytime and nighttime directly with behaviour, we used a linear regression with the behavioural phenotype score regressed against cortisol exposure ($\text{AUC}_{\text{night}}$ or AUC_{day}).

Next, we assessed whether each trait in isolation or in combination influenced size using linear regressions with mean body mass per population as the response variable (from the subsample of individuals weighed prior to the experiment and averaged across replicate barrels) and population-level behavioural phenotype with nighttime cortisol exposure ($\text{AUC}_{\text{night}}$) or daytime cortisol exposure (AUC_{day}) as the explanatory variables. Both linear and quadratic terms for cortisol and behaviour were used in a subset of models to account for any hormetic relationships (Schreck 2010). For model selection, we used a combination of fit, significance, and AIC modified for small sample size (AIC_{c}) using the package "AICcmodavg" in R (Mazerolle and Mazerolle 2017).

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

Results

Variation in diel cortisol and behaviour among stocks

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

Integrated effects of cortisol and behaviour on growth

Daytime cortisol exposure had a negative linear relationship with behavioural phenotype ($R^2 = 0.64$, $p = 0.031$; Fig. 3), whereas nighttime cortisol exposure showed no such relationship ($R^2 = 0.12$, $p = 0.44$). As univariate predictors of performance, nighttime cortisol exposure and behavioural phenotype each showed a nonlinear, hormetic relationship with body mass (cortisol: $R^2 = 0.94$, $p = 0.003$, $\text{AIC}_{\text{c}} = 6.1$; quadratic regression: body mass = $4.44 - 0.002\,(\text{AUC}_{\text{night}} - 75.31)^2$; behaviour: $R^2 = 0.95$, $p = 0.003$, $\text{AIC}_{\text{c}} = 5.8$, quadratic regression: body mass = $4.03 - (0.12\,(\text{behaviour})^2)$. AUC_{day} had no effect on body mass (linear or quadratic relationships; $R^2 < 0.4$, p values >0.36, AIC_{c} values >11.0). However, the inclusion of both nighttime cortisol exposure and behaviour increased the model's predictive ability and its weighting to explain variation in body mass, with stocks exhibiting mid-range in nighttime cortisol

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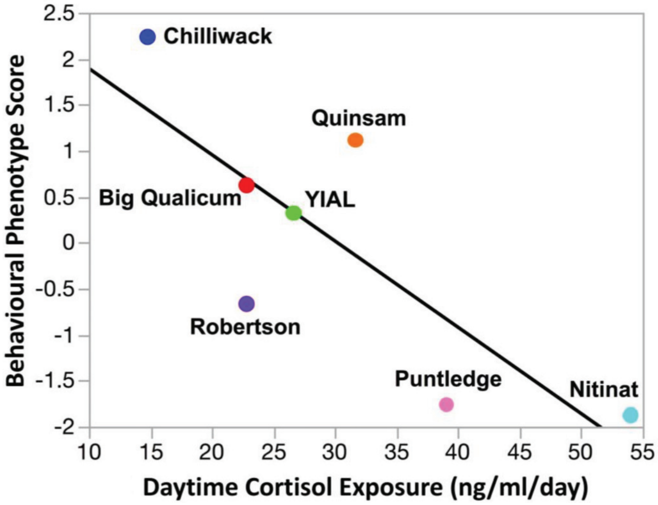
[1] Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2017-0367.

Fig. 3. Exposure to daytime cortisol predicts overall behavioural phenotype (combination of foraging, sociality, and movement behaviours) as a linear relationship across six outbred populations of Chinook salmon and the inbred control population. [Colour online.]



exposure and high energy-management behaviours having the largest body masses ($R^2 = 0.998$, $p = 0.07$, $\mathrm{AIC_c} = -143.3$; Fig. 4A). There was no meaningful or significant integration of daytime cortisol exposure with behavioural phenotype on body mass. All models tested are included in Supplementary Table S3.

Discussion

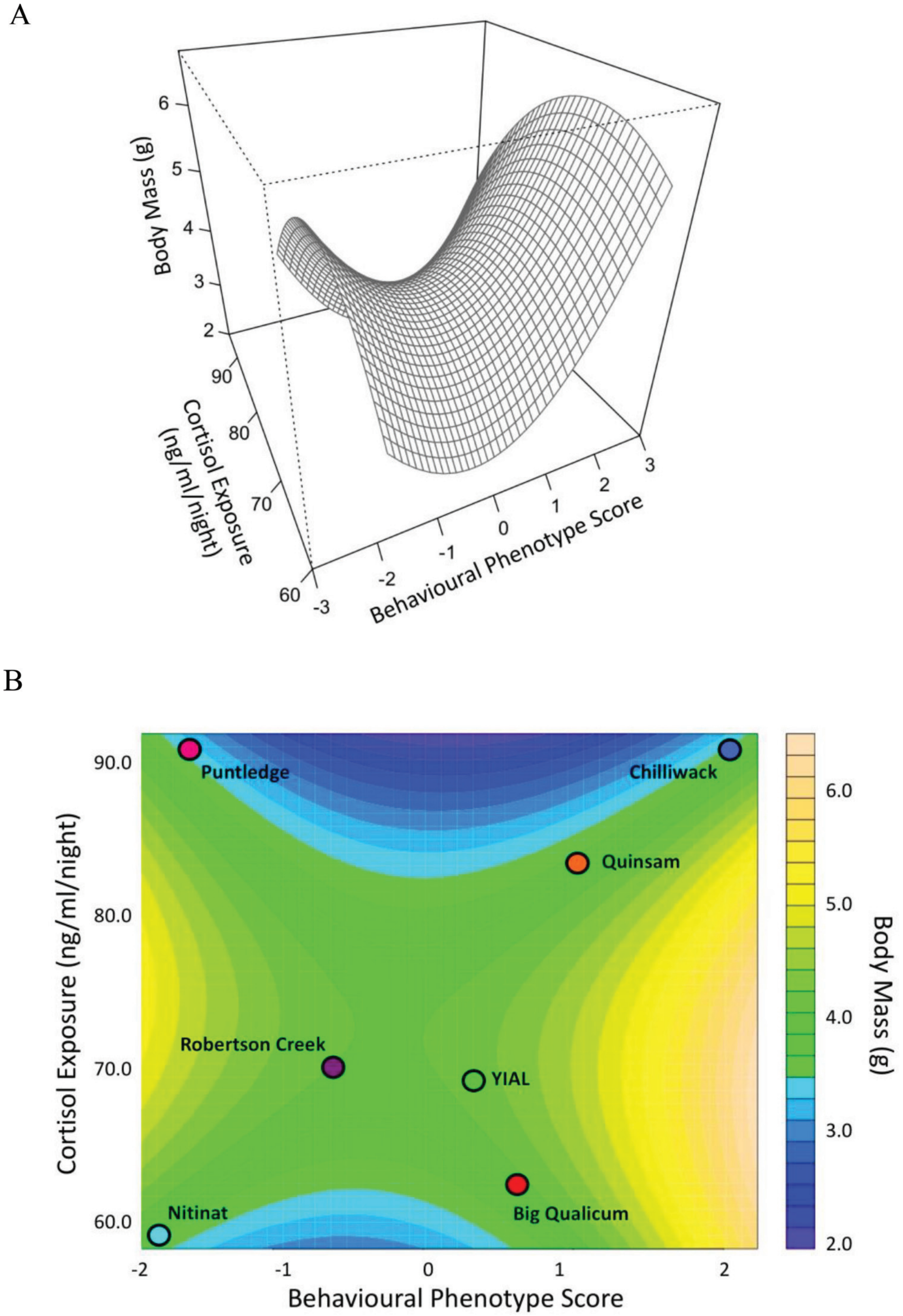
Rapid human population growth and increases in anthropogenic stressors on wild ecosystems require improved efficiency in alternative food-production systems such as aquaculture to keep pace with world demand for protein (Green et al. 2005). However, aquaculture practices such as selecting for fast-growing individuals can inadvertently decrease stock genetic diversity and (or) co-select for undesirable traits that will ultimately impact production (Castanheira et al. 2017). We sought to determine whether outbreeding a domesticated Chinook salmon stock with multiple wild populations resulted in the optimal integration of physiological and behavioural traits at the nexus of energetic management and if this integration led to maximized performance at an early-life freshwater stage. Outbreeding generated significant variability in physiological and behavioural traits that are associated with energetic management and known to impact growth. As predicted by the theory of phenotypic integration (Murren 2012), growth was influenced by a coupling of nighttime cortisol exposure and behavioural phenotype, specifically with intermediate cortisol values and high energy-management behaviours combining to generate an optimized hormetic relationship to maximize body size. Our results also suggest that examining the effects of the co-variation of multiple functionally related traits on body mass is a stronger approach than examining these traits in isolation. Moreover, relationships that exist between phenotypic traits such as daytime cortisol exposure and behaviour do not imply integration (and by extension maximized performance), highlighting that only within the context of a performance measure can phenotypic integration be revealed. Although phenotypic correlations among traits have been extensively studied, fewer have been examined in light of performance or fitness outcomes, and fewer still across multiple populations. This is the first study to show how among-population coupling of physiological and behavioural traits can influence early-life metrics such as growth rate (here measured as body mass), which are considered important in assessing long-term success in aquaculture (Valente et al. 2013). This work therefore suggests that researchers and producers may benefit from determining how phenotypic integration impacts early-life performance when employing methods such as outbreeding to enhance or rescue breeding programs.

Impacts of outcrossing on variability in performance-mediated traits

Optimal management of energy budgets over both short- and long-term periods within the constraints of local environmental variation is expected to maximize fitness (Fong 1975; Taylor 1991; Sanford and Kelly 2011), a critical consideration for production output in aquaculture (Akvaforsk 2005). Outbreeding in our study produced substantial variation in diel physiological and behavioural traits central to daily energetic management. We focused on diel variation in baseline cortisol because it can differentially affect growth by regulating metabolic processes and promoting homeostasis in fish (Mommsen et al. 1999). In diurnal vertebrates, an optimal diel cortisol cycle consists of high levels early in the morning to provide the energy via gluconeogenesis following night fasting (Dallman et al. 1993), to initiate foraging, and other activities (Astheimer et al. 1992; Breuner et al. 1999; Breuner and Hahn 2003). Levels then tend to decline for the remainder of the day to avoid the "high cost of living" associated with maintaining high baseline circulating glucocorticoid levels (Sapolsky 2000; Bernier et al. 2004). Although our data confirm that all populations showed strong diel rhythms in baseline cortisol, populations nonetheless differed significantly in patterns of cortisol exposure during night and day. Outbreeding also generated a range in behavioural traits associated with energetic management, and when combined with differences in cortisol exposure, revealed certain stocks to be less ideally suited to captive environments as evidenced by the resultant low body size.

The differences observed among stocks are presumably driven by large sire effects that result from genetic differences among the source stocks that have arisen through local adaptation and potentially genetic drift (Fraser et al. 2011). The sire effect would have dominated possible dam effects among stocks given that we used a common inbred line of domestic dams to both minimize maternal effects and equalize any influences across all stocks (as eggs were also pooled). Within our study, not all hybrid stocks showed high performance. Although there are many possible causes of this pattern of reduced performance, one may be the poor performance of the F1 generation is due to the intermediate phenotypes generated by hybridization, which are unsuited to the domestic parental environments (Frankham et al. 2002; McClelland and Naish 2007). Another mechanistic cause of lowered performance in hybrid stocks may be intrinsic outbreeding depression due to the disruption of epistatic interactions in large co-adapted gene complexes (Edmands 1999); however, we do not believe this to be the case because previous studies have shown that Chinook salmon F1 hybrids of genetically different parental lines demonstrate no evidence of hybrid breakdown (Lehnert et al. 2014). Instead, suboptimal performance of juvenile Chinook salmon of certain stocks most likely reflects local adaptation of performance traits within freshwater systems that are very important for the success of early life-history stages of anadromous, semelparous salmonids (Waples 1991). When different genotypes are raised in common environments, the impacts of these locally adapted phenotypes persist and are known to generate significant variation in performance outcomes (McClelland and Naish 2007), and so, deliberate selection of these tightly integrated traits will be robust past the F1 generation and persist through multiple generations. However, the potential for non-additive genetic effects coupled with residual epigenetic effects may interfere with the predictable inheritance of some of the traits measured.

Fig. 4. Body mass outcome of optimal phenotypic integration between exposure to cortisol during the night and overall behavioural phenotype (see Methods) across six 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. [Colour online.]



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

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

Chronically high cortisol levels are known to negatively impact behavioural traits such as foraging and competitive ability due to a reduction in appetite (Gregory and Wood 1999) and can inhibit aggression and locomotion in salmonids (Øverli et al. 2002). As such, prolonged exposure can lead to tertiary responses such as slow growth, reduced immune function, and compromised survival (Pickering and Pottinger 1983; Barton 2002). In our study, stocks with individuals that expressed elevated daytime cortisol exposure may have incurred these behavioural costs that led to reduced growth. During nighttime, when fish were not fed and feeding activity was much reduced (Fraser and Metcalfe 1997), cortisol plays a more direct role in growth, and elevated baseline levels maintain energetic homeostasis, allowing stored glycogen in the liver (generated from food consumed and digested during the day) to be mobilized as glucose to fuel the maintenance and growth of tissues (see Dallman et al. 1993). Behaviours that promote energy gain while reducing energetic costs of locomotion (including sociality) are generally assumed to lead to the highest growth (e.g., foraging efficiency: Eklöv 1992; high cohesiveness: Johnsson 2003). Interestingly, as a sole (univariate) measure, these maximized behaviours seemingly had a counterintuitive, negative effect on growth (i.e., significant hormetic relationship), and yet, as an integrated unit with physiology, these same behaviours conferred the greatest performance. We therefore argue that an integrated approach situated within a performance context is needed to understand how underlying traits will ultimately impact performance in aquaculture. We also demonstrate that with this integrated relationship — visualized as a contour plot (Fig. 4B) — there exists the potential for further selection of integrated traits to maximize production. For instance, actively selecting for specific behaviours (i.e., greater locomotion efficiency, foraging efficiency, and sociality), should these traits remain covaried within a physiological range, can result in even greater mass gains than those observed. This integrated selection approach at the stock level can be of value for other performance traits (such as disease resistance) and in other breeding programs, outside of salmon culture. It not only is feasible for farming methods that must routinely document production data of functional traits and performance on a large scale, but also can be used to possibly increase efficiency.

Conclusions

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

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