

University of Windsor

Scholarship at UWindsor

Biological Sciences Publications

Department of Biological Sciences

2008

Why are salmon eggs red? Egg carotenoids and early life survival of Chinook salmon (*Oncorhynchus tshawytscha*)

Selene T. Tyndale
University of Windsor

Robert J. Letcher
University of Windsor

John W. Heath

Daniel D. Heath
University of Windsor

Follow this and additional works at: <https://scholar.uwindsor.ca/biologypub>



Part of the [Biology Commons](#)

Recommended Citation

Tyndale, Selene T.; Letcher, Robert J.; Heath, John W.; and Heath, Daniel D., "Why are salmon eggs red? Egg carotenoids and early life survival of Chinook salmon (*Oncorhynchus tshawytscha*)" (2008). *Evolutionary Ecology Research*, 10, 8, 1187-1199.
<https://scholar.uwindsor.ca/biologypub/1167>

This Article is brought to you for free and open access by the Department of Biological Sciences at Scholarship at UWindsor. It has been accepted for inclusion in Biological Sciences Publications by an authorized administrator of Scholarship at UWindsor. For more information, please contact scholarship@uwindsor.ca.

Why are salmon eggs red? Egg carotenoids and early life survival of Chinook salmon (*Oncorhynchus tshawytscha*)

Selene T. Tyndale^{1*}, Robert J. Letcher^{1#}, John W. Heath² and Daniel D. Heath¹

¹Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario N9B 3P4 and ²Yellow Island Aquaculture Ltd., Campbell River, British Columbia V9W 6K9, Canada

ABSTRACT

Background: The characteristic red colour of salmon eggs is due to provisioning of carotenoids at the cost of the mother.

Hypothesis: Carotenoid-based pigmentation of salmon eggs provides increased offspring survival during incubation and elevated disease resistance upon seawater entry.

Organism: Chinook salmon (*Oncorhynchus tshawytscha*, Walbaum); Quinsam River, Chehalis River, and domestic stocks.

Methods: We tested for correlations of egg carotenoid levels measured in Chinook salmon eggs reared as maternal families with (1) incubation survival and (2) specific disease (vibriosis) challenge resistance.

Conclusions: Egg carotenoids were primarily astaxanthin and declined throughout larval development. Incubation survival and disease resistance were significantly and positively correlated with mean family carotenoid concentration measured in eggs. A 1.0 $\mu\text{g} \cdot \text{g}^{-1}$ increase in egg carotenoid concentration confers an approximately 10% survival benefit. However, the incubation–survival relationship was asymptotic, indicating a threshold effect of carotenoids on incubation survival. Egg carotenoid concentration was also positively correlated with specific disease resistance 7 months later in life.

Keywords: carotenoid, Chinook salmon, disease resistance, eggs, fitness, immune function, incubation survival, trade-off.

INTRODUCTION

The eggs of salmonids are coloured by carotenoids, as are the egg yolks of many bird and reptile species (Blount *et al.*, 2000). Carotenoids are antioxidant compounds synthesized in plants

* Correspondence: D. Heath, Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B 3P4, Canada. e-mail: dheath@uwindsor.ca

* Present address: Oregon Department of Human Services, Public Health Laboratory, Newborn Screening Section, Hillsboro, OR 97124, USA.

Present address: Wildlife and Landscape Science Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Consult the copyright statement on the inside front cover for non-commercial copying policies.

and obtained by animals through their diet (Goodwin, 1986; Lubzens *et al.*, 2003). In salmon, the primary egg carotenoid is astaxanthin (Lubzens *et al.*, 2003), a red pigment that is highly visible to potential predators, especially in contrast to the streambed aggregate (S. Tyndale, personal observation). Although high visibility generally increases predation risk, and egg carotenoid pigmentation has been speculated to incur significant predation risk (Lubzens *et al.*, 2003; Palace and Werner, 2006), there have been no direct tests of the effect of carotenoid pigmentation on predation of salmonid eggs. Additionally, the extent to which animals absorb, metabolize, allocate, and retain carotenoids from the diet incurs metabolic costs (Goodwin, 1986; Lubzens *et al.*, 2003), and is heritable (e.g. Craig and Foote, 2001; Rajasingh *et al.*, 2008). Therefore, salmonid offspring must derive sufficient benefit from carotenoid pigments to offset the costs of maternal accumulation and allocation, as well as potentially increased egg predation. Comparative studies have proposed that salmon eggs carry especially high carotenoid and antioxidant loads because of their large size and the long developmental times of the eggs, coupled with their low oxygen incubation environment, which can lead to high oxidative stress levels (Palace and Werner, 2006). Alternatively, the red coloration in sexually mature salmonids is known to have a role in sexual selection (Craig and Foote, 2001), hence egg carotenoid allocation may be a by-product of sexual selection (although sexually mature Chinook salmon generally do not develop red coloration in their skin). However, to date, there has been no empirically supported, adaptive explanation as to why salmon allocate such high levels of carotenoids to their eggs (Palace and Werner, 2006), which begs the question, why are salmon eggs red?

The best studied carotenoid-based cost–benefit relationships are in birds (Hamilton and Zuk, 1982; Gray, 1996; Møller *et al.*, 2000), where correlations between parental and egg carotenoid levels and offspring performance have been shown (Blount *et al.*, 2000; McGraw *et al.*, 2005). Recent studies have suggested several mechanisms for carotenoid–fitness correlations. A direct performance benefit would result from yolk carotenoids reducing peroxidative damage, thus preserving the integrity of the lipid-rich embryonic tissues, organs, and other maternally allocated embryonic resources (Haq *et al.*, 1996; Sujak *et al.*, 1999; Palace and Werner, 2006). In addition, the availability of carotenoids during early development, from endogenous (yolk absorption) and exogenous (feeding) sources, may increase offspring capacity for carotenoid absorption and use into adult life, as shown in birds (Blount *et al.*, 2003; Koutsos *et al.*, 2003; McGraw *et al.*, 2005). In effect, early carotenoid availability may ‘prime’ an individual’s metabolic capacity for more efficient carotenoid use throughout life (McGraw *et al.*, 2005).

Despite the expectation that the red hue, indicative of carotenoid content of the eggs, reflects ‘egg quality’ or offspring performance (Hubbs and Stavenhagen, 1958; Czezugza, 1975; Palace and Werner, 2006), studies of hatchery-reared salmon from both wild and domestic egg stocks generally do not support such a relationship (Craik, 1985; Tveranger, 1986; Christiansen and Torrissen, 1997; Palace and Werner, 2006). Carotenoids were also found to have little or no effect on egg quality or viability in other teleosts (Kolluru *et al.*, 2006; Svensson *et al.*, 2006). However, under high oxidative stress, maternal egg batches that were pale in colour were both low in carotenoids and incurred the highest rates of early stage offspring mortality (Palace *et al.*, 1998; Pettersson and Lignell, 1999). Most studies of egg carotenoid and offspring performance in salmon have incorporated experimentally manipulated carotenoid levels in the maternal diets (Harris, 1984; Tveranger, 1986; Christiansen and Torrissen, 1997), or have compared records of egg carotenoid pigmentation and offspring survival from fish reared in different hatcheries or from different spawning seasons (Craik, 1985). Such approaches may limit the likelihood of detecting egg carotenoid–offspring survival relationships. Finally, Craik (1985) proposed a carotenoid threshold, above which varying levels of carotenoid would have no impact on egg or larval survival. Thus a

survival–carotenoid relationship was suggested to be expected at low levels ($< 1\text{--}3 \mu\text{g} \cdot \text{g}^{-1}$) of carotenoids only (Craik, 1985).

Chinook salmon (*Oncorhynchus tshawytscha*) carotenoid phenotypes represent the most diverse range of naturally occurring flesh and egg carotenoid levels of any salmon species (Ando *et al.*, 1994). A genetic polymorphism in Chinook salmon that has a strong effect on carotenoid accumulation and retention capacity (Withler, 1986; Rajasingh *et al.*, 2008) provides a very large natural range of carotenoid flesh and egg colour phenotypes and thus allows the elimination of potential biases due to species differences, maternal diet manipulations, and rearing conditions. In this study, maternal egg clutches of Chinook salmon were selected to maximize the natural range of carotenoid levels. We measured incubation survival and resistance to a specific disease challenge in later-stage smolt offspring, and tested for correlations with development stage levels of egg carotenoids. Our results provide a quantitative estimate of the early life survival benefits resulting from maternally derived egg carotenoid pigments in Chinook salmon, demonstrating potential direct fitness benefits to offset the costs of carotenoid accumulation and allocation in female salmon and their offspring.

MATERIALS AND METHODS

Spawning and rearing

In the fall of 2002, egg clutches from 31 mature female Chinook salmon were selected from three spawning populations. Eggs of ocean-returning Chinook salmon were obtained from the Quinsam River, Vancouver Island ($N = 10$) and the Chehalis River, a tributary of the Harrison River ($N = 10$). Chinook salmon in the Chehalis River display very high variation in carotenoid pigmentation due to a genetic polymorphism (Withler, 1986; Rajasingh *et al.*, 2008), resulting in ‘white-fleshed’ individuals (Hard *et al.*, 1989). Eggs from domestic Chinook salmon were also included; females were selected from broodstock at Yellow Island Aquaculture Ltd. (YIAL, Quadra Island, British Columbia; $N = 11$), where all crosses, rearing, and experiments took place. The YIAL fish were fed an artificial diet supplemented with both astaxanthin and canthaxanthin, although astaxanthin was the predominant carotenoid in the feed. Our use of maternal stock from three populations known to differ in carotenoid accumulation and exposure profiles provides a large range of carotenoid concentration, but may also generate a source-population effect; we thus performed within-source population analyses (see below).

Eggs were taken from 16 females on 1 November 2002 (10 Quinsam and 6 YIAL) and from 15 females on 13 November 2002 (10 Chehalis and 5 YIAL). Subsets of 30 unfertilized eggs per female were frozen (-20°C) and later stored at -80°C until carotenoid extraction (‘green eggs’). The remaining eggs ($N \pm \text{s.d.} = 359 \pm 25$ per female) were fertilized with sperm from a common domestic male (one per spawn date), creating two sets of half-sib families. Fertilized eggs from each family were held in separate compartments of a vertical incubation stack and reared under standard hatchery conditions in fresh water at 8.8°C . Since the incubation water was supplied from artesian wells, the temperature varied very little over the incubation period (range = $8.3\text{--}9.2^{\circ}\text{C}$). As the eye spots of developing embryos became visible at the ‘eyed-egg’ stage (accumulated thermal units (ATU) $\pm \text{s.d.} = 341 \pm 9.0$), eggs were subjected to a mechanical shock, and all non-viable eggs were removed and counted. The application of a mechanical shock at the eyed-egg

stage is a standard salmon-rearing practice (e.g. Christiansen and Torrissen, 1997) and it serves to identify dead eggs (they become opaque). Live embryos are not affected by this gentle mechanical shock. Two families (YIAL; spawned on 13 November) were found to have extremely low fertilization success and were removed from further analyses. A sub-sample of 25 (live) eyed eggs from each family was frozen and stored at -80°C until carotenoid extraction ('eyed eggs'). Subsequent to the eyed-egg stage, dead eggs were removed three to four times each week and tabulated by family. Once offspring had completely absorbed their yolk-sacs just before the start of feeding at the 'swim-up' stage ($\text{ATU} \pm \text{s.d.} = 1045 \pm 9.0$), 20 offspring per family were frozen and stored at -80°C until carotenoid extraction ('swim-up fry'). Remaining offspring ($N \pm \text{s.d.} = 163.3 \pm 18.2$) from 25 families were transferred to individual 200-litre barrels for the remainder of freshwater rearing. Each barrel received a daily ration of approximately 250 ml of Organic Chinook Fry Grower (Taplow feeds, Victoria, BC), with no carotenoid added, until the fish reached the smolt stage (see below).

Carotenoid determination

In a previous study, Li *et al.* (2005) identified the major carotenoid in Chinook salmon eggs to be astaxanthin, with much lower levels of all-*trans*-retinol, lutein, and canthaxanthin, using a method based on high-performance liquid chromatography (HPLC) coupled to electro-spray ionization-mass spectrometry (HPLC-ESI-MS) or to UV-visible spectroscopy. In the present study, carotenoids were extracted and quantified in duplicate batches of approximately 1 g (2–4) eggs per maternal family. Using the sample work-up methodology of Li *et al.* (2005), under dimmed lights, crude egg homogenate was extracted with acetone, followed by phase separation with acetone-water and methyl-*tert*-butyl-ether (MtBE). As the majority of all carotenoids partition into the MtBE, this step was repeated three times, the MtBE combined for each replicate, then condensed, filtered, sealed under nitrogen, and stored at -80°C until analysis. The MtBE egg extracts were analysed in triplicate at three injection volumes using a Waters 2695 HPLC coupled to a Waters 487 dual-channel UV-visible detector (Li *et al.*, 2005). A gradient elution of methanol (MeOH) and MtBE over 25 min was used, and the eluting carotenoids were detected and quantified via a UV-visible absorption response at a maximum wavelength of 480 nm. Carotenoids were identified and quantified by comparison with the retention times and signal responses of known carotenoid standards. External standards included astaxanthin, lutein, zeaxanthin, canthaxanthin, and β -carotene. β -Cryptoxanthin was used as an internal calibration standard at a concentration of 50 ppm with 100 μl added per gram of sample with recovery efficiencies of $\geq 90\%$. Response peak areas were averaged with respect to injection volume, and the concentrations calculated (μg carotenoid per g original sample wet weight) were averaged by extract and family at each development stage. Astaxanthin and canthaxanthin constituted over 98% of the measured carotenoids in all samples. Canthaxanthin was found only in the domestic YIAL eggs and represented approximately 14% of the total carotenoids in those eggs. We used total carotenoid values for all analyses; however, no substantial change in statistical significance resulted when we used astaxanthin concentrations as the independent variable.

Incubation survival and disease challenge

Incubation survival was calculated for the incubation period as the percent survival from fertilization to the swim-up stage. Egg batches ($N = 2$) that displayed low fertilization

success were eliminated from subsequent analyses. Survival calculations were corrected for the eggs removed for carotenoid analyses.

At the smolt stage ($ATU \pm s.d. = 2057 \pm 11.9$), 714 fish from 23 families (those with sufficient numbers remaining) were included in an experimental challenge with the causative agent of vibriosis (*Listonella anguillarum*, formerly *Vibrio*), a common Pacific marine pathogen. A live vibrio culture (Dr. A. Osborn, Pacific Biological Station, Nanaimo, British Columbia) was grown on marine agar at 25°C to obtain a homogeneous bacterial lawn. A stock suspension was prepared in phosphate-buffered saline ('PBS'; 0.85% saline, 0.1% peptone, pH 7.04) and diluted to an approximate bacterial concentration of 10^5 cfu·ml⁻¹. Fish ($N = 31 \pm 1$ per family) were netted, anaesthetized by brief exposure to tricane methanosulphate (30–45 s, 25 mg·l⁻¹, 2:1 sodium bicarbonate buffer), and injected with 0.1 ml of either the vibrio suspension ($N = 21 \pm 1$) or a PBS blank control ($N = 10$). Subsequently, moribund fish were removed and humanely euthanized every 2–6 h, counted, and the time since the start of the challenge recorded by family. Dose concentration was verified by serial dilution: a 20 µl/dilution aliquot was smeared by sterile glass rod on a marine agar plate in triplicate and incubated at 25°C. After 16–20 h, the numbers of colonies per plate were counted, replicates averaged, and dilutions calculated to obtain a dose concentration in colony-forming units (cfu) per millilitre for each injection batch of families. The dose concentration averaged $3.12 \pm 1.7 \times 10^5$ cfu·ml⁻¹ throughout the vibrio challenge. We used the time to the second death (10% mortality) within families (LT10) as a measure of disease resistance. Differences in dose were accounted for by standardizing the LT10 values by mean injection batch, thus we calculated the mean LT10/injection batch and report family challenge performance as the ratio of family LT10 : mean LT10 of the injection batch.

Statistical analyses

We corrected for differences in spawning dates by calculating the family carotenoid concentration as a function of ATU and converting to common ATU equivalents for each development stage (288 ATU for eyed-egg and 992 ATU for swim-up stages). To test for the potential effect of the two sires used on the two spawning dates, we included sire as a categorical covariate in multiple regression analyses (see below).

Changes in carotenoid concentrations within full-sibling families were visualized by plotting the family mean carotenoid values at (1) the green-egg versus eyed-egg and (2) eyed-egg versus swim-up fry stages. We used a simulation to test whether the observed decline in carotenoid concentration over the incubation period could be explained by differential mortality of the high-carotenoid eggs. Within each family, we used the observed mean and standard deviation in unfertilized egg carotenoid concentration to generate a pseudo-normal distribution; from this, 100 individuals were selected. We removed individuals to simulate the observed family mortality, targeting individuals with the highest levels of egg carotenoids, and calculated the mean carotenoid concentration for the remaining individuals.

Levene's homogeneity of variances test was used to evaluate the normality of distributions. Empirical relationships between measures of survival (incubation survival and LT10 at smolt) and egg carotenoid concentration (at various developmental stages) were examined using single and multiple regression models. Each set of dependent versus independent variables was examined using: (1) univariate regression analysis; (2) Spearman's rank-order correlation (Ramsey, 1989) to address departures from normality (where

appropriate); (3) a multiple regression model with the covariate eyed-egg weight as an estimate of maternal effects (see Heath and Blouw, 1998); (4) a multiple regression model with the covariate sire (a categorical variable) as a correction for the possible block-effect of the two sires used; and (5) three separate univariate regressions for each source population, to address the possibility of a source population effect on the correlation between incubation survival and carotenoids.

RESULTS

Carotenoid levels

The main carotenoid detected in all eggs was astaxanthin (> 95% of the total carotenoid concentration). Maternally derived egg carotenoid levels were highly variable among families, and declined somewhat from the green-egg to eyed-egg stage, but declined substantially from the eyed-egg to swim-up stage in all families (Fig. 1). In all cases, simple differential mortality could not account for the decline in carotenoid concentration during incubation, with the mean final concentration in the simulations being more than three times (mean 3.4-fold; range 1.3- to 42.2-fold) higher than the observed final carotenoid concentration. Although carotenoid concentrations were correlated between the green-egg and eyed-egg stages ($r = 0.55$; $P = 0.0012$), two obvious outliers were present (Fig. 1). When those two values are removed, the correlation between the two measures increases dramatically ($r = 0.93$; $P < 0.0001$). Although the two anomalously high green-egg carotenoid values are not far out of the observed range (Fig. 1), the large drop in carotenoid concentration from the green-egg to eyed-egg stages is unexpected, and therefore the green-egg carotenoid data were not used in subsequent analyses, as they do not provide additional information over the eyed-egg carotenoid data. Carotenoid concentrations were more weakly correlated between the eyed-egg and swim-up fry stages ($r = 0.49$; $P = 0.015$); however, no obvious outliers are present (Fig. 1).

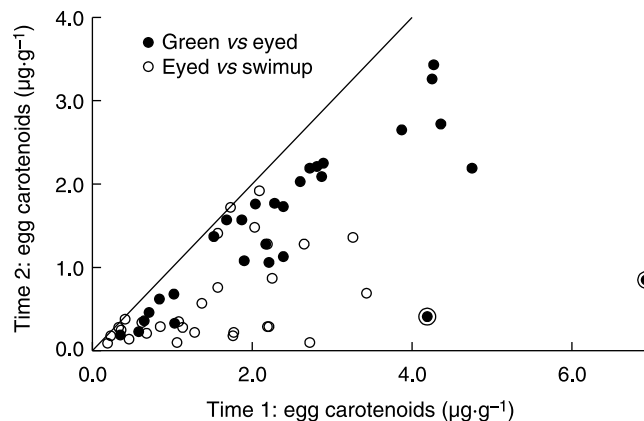


Fig. 1. Scatterplot of mean family carotenoid concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ wet weight) in Chinook salmon across developmental stages. Solid circles represent the relationship between green egg versus eyed-egg values for all maternal families, while open circles represent the relationship between eyed-egg versus swim-up fry values. The diagonal line is the 1:1 relationship. The circled (filled) points highlight anomalously high green egg carotenoid values.

Survival–carotenoid relationships

Total incubation survival varied considerably among families, and was not normally distributed (arcsine square-root transformation did not improve normality). Mean (\pm s.d.) survival across all families from fertilization to the eyed-egg stage was $83.9 \pm 10.8\%$, and to the swim-up stage $78.3 \pm 12.8\%$. Significant positive regressions were found between eyed-egg carotenoid concentrations and incubation survival (Table 1; Fig. 2), but there were no significant regressions with swim-up fry carotenoid concentrations (Table 1). Fitting a log-linear regression line to the eyed-egg carotenoid concentration versus incubation survival data substantially increased the variance explained by the regression (Fig. 2). When sire/spawning date was used as a covariate, the covariate was not statistically significant and the pattern of regression results was similar: eyed-egg carotenoid concentrations were significantly correlated with incubation survival, whereas the swim-up fry carotenoid concentration was not (Table 1).

Spearman's rank-order correlation analyses yielded the same pattern of significance, with a strong correlation between eyed-egg carotenoid concentration and incubation survival (Table 1). Survival for two families from green- to eyed-egg was low, leading to low total survival (Fig. 2). Those two families were affected by a fungal infection early during

Table 1. Summary of significance of regression and rank-order correlation analyses between mean family carotenoid concentrations (at two developmental stages) versus incubation survival and disease resistance (LT10) as dependant variables in Chinook salmon

Carotenoid	Incubation survival		Disease resistance (LT10)
	Parametric	Rank-order [#]	Parametric
Eyed egg	$P = 0.008$; $r = 0.48$	$P = 0.004$; $r_s = 0.51$	$P = 0.033$; $r = 0.44$
Sire covariate	$P = 0.048$; $r = 0.42$	—	$P = 0.041$; $r = 0.49$
<i>Source population</i>			
Quinsam	$P = 0.19$; $r = 0.45$	$P = 0.042$; $r_s = 0.58$	$P = 0.21$; $r = 0.43$
Chehalis	$P = 0.13$; $r = 0.51$	$P = 0.041$; $r_s = 0.57$	$P = 0.044$; $r = 0.61$
YIAL (domestic)	$P = 0.11$; $r = -0.55$	$P = 0.18$; $r_s = -0.31$	— ^a
Egg size covariate	$P = 0.009$; $r = 0.55$	—	$P = 0.034$; $r = 0.53$
Swim-up fry	$P = 0.051$; $r = 0.33$	$P = 0.017$; $r_s = 0.41$	$P = 0.48$; $r = 0.15$
Sire covariate	$P = 0.26$; $r = 0.22$	—	$P = 0.70$; $r = 0.10$
<i>Source population</i>			
Quinsam	$P = 0.33$; $r = 0.34$	$P = 0.18$; $r_s = 0.34$	$P = 0.99$; $r = 0.00$
Chehalis	$P = 0.038$; $r = 0.61$	$P < 0.001$; $r_s = 0.93$	$P = 0.13$; $r = 0.51$
YIAL (domestic)	$P = 0.32$; $r = -0.37$	$P = 0.10$; $r_s = -0.41$	— ^a
Egg size covariate	$P = 0.048$; $r = 0.40$	—	$P = 0.41$; $r = 0.21$

Note: 'Sire covariate' and 'Egg size covariate' denote multiple regression models with sire and egg size as covariates (partial correlation coefficients and significance for the carotenoid parameters are shown). Source population analyses were performed within the three source populations ($N_{\text{Quinsam}} = 10$, $N_{\text{Chehalis}} = 10$, $N_{\text{YIAL}} = 9$).

[#] Spearman rank-order correlation results (multivariate rank-order correlations not included).

^a Regression analysis within the YIAL source population for LT10 was not performed due to low family numbers ($N = 3$).

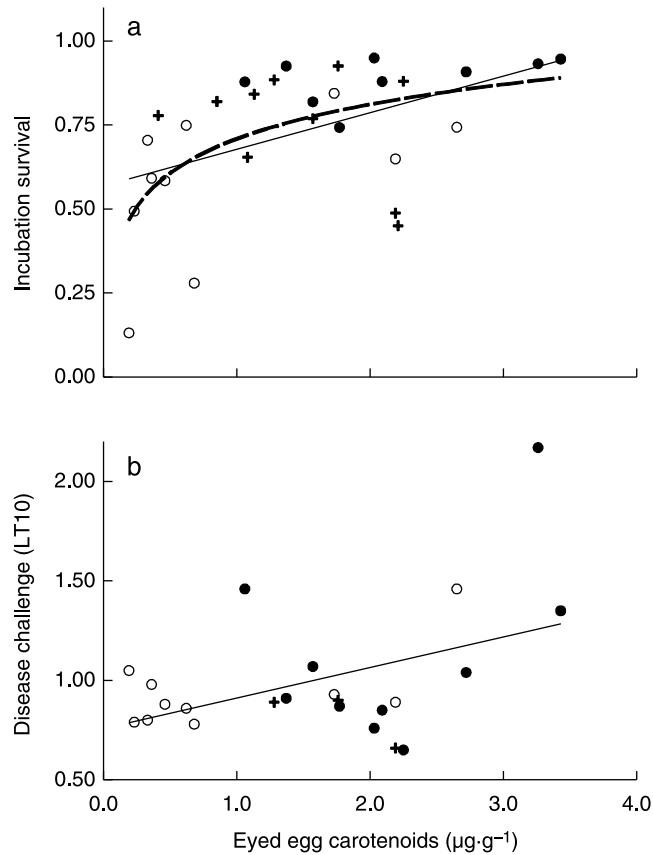


Fig. 2. Regression of early life survival and disease resistance versus mean family carotenoid concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ wet weight) measured at the eyed-egg development stage in Chinook salmon (open circles = Chehalis; solid circles = Quinsam; crosses = YIAL). (a) Linear (solid line) and log-linear (dashed line) regression for incubation survival versus eyed-egg carotenoid concentration. Two families experienced anomalously high mortality before the eyed-egg stage due to a fungus outbreak, and are evident by low incubation survival (<30%). The linear and log-linear regressions are highly significant ($R = 0.48$, $P < 0.01$ and $R = 0.56$, $P < 0.001$, respectively). (b) Linear regression of disease resistance (LT10 following vibriosis challenge) versus eyed-egg carotenoid levels. The regression was significant ($R = 0.44$, $P < 0.05$; $N = 29$).

incubation. When those two families were removed from the linear and Spearman regression analyses, the strength of the relationships declined, but the eyed-egg carotenoid relationship remained significant.

Our use of females from three different source populations introduces the possibility that the observed correlations between egg carotenoid concentration and survival may be driven by population-level covariation in carotenoids and hatchery incubation survival. However, we found significant positive correlations between carotenoid levels and incubation survival within some of the individual source populations (Table 1). Although the relationships were weaker (due to a much smaller sample size), the Spearman rank-order correlations were consistent with our full data-set regression analyses (Table 1). We thus conclude that the

relationship between egg carotenoid and incubation survival cannot be due solely to source population effects.

The mean (\pm s.d.) wet weights across all families were: 0.33 ± 0.072 g (green egg), 0.39 ± 0.10 g (eyed egg), 0.37 ± 0.082 g (swim-up fry), and 5.45 ± 0.40 g (smolt). When mean family eyed-egg weight was added as a covariate in the regression analyses, all relationships remained significant, although the significance decreased marginally (Table 1). Neither survival nor carotenoid concentration at any stage was significantly correlated with egg weight.

Disease challenge survival

Disease resistance (LT10) following the live vibriosis challenge at the smolt stage was positively correlated with egg carotenoid concentrations at the eyed-egg stage (Table 1). These results were robust to the addition of the sire categorical covariate as well as the family eyed-egg weight covariate (Table 1). No significant relationships were observed between smolt stage disease resistance and carotenoid concentration at the swim-up stage. The within-source population analyses were not possible for the YIAL families, since only three YIAL families were included in the disease challenge (Table 1); however, significant relationships remained between eyed-egg carotenoid concentration and LT10 among the Chehalis families (Table 1). Similar results were found using LT20 (i.e. time to 20% mortality); however, all relationships were non-significant when LT50 was used.

DISCUSSION

Here we report new evidence to support the expectation for a positive effect of increased egg carotenoid concentration on the survival of offspring through embryo development and beyond. Several previous studies that manipulated carotenoid concentration in various salmonid species did not find such a relationship (Torrissen, 1984; Craik, 1985; Tveranger, 1986; Christiansen and Torrissen, 1997). However, some of those studies used egg colour as a measure of carotenoid content, which can bias carotenoid estimation (Craik, 1985; Palace and Werner, 2006). Furthermore, some of those studies made comparisons across multiple populations, and our analyses showed that there are potential population-level effects on carotenoid–survival relationships. Craik (1985) noted a possible threshold effect of carotenoid concentration ($1\text{--}3 \mu\text{g} \cdot \text{g}^{-1}$) on salmon early life fitness, whereby artificially elevated concentrations would have little or no effect on survival. Our data show an asymptotic relationship between carotenoid concentration and incubation survival above $2 \mu\text{g} \cdot \text{g}^{-1}$, which is consistent with a ‘threshold effect’ as proposed by Craik (1985). Such an asymptotic relationship between egg carotenoids and offspring performance may explain the inconsistent results in other studies (e.g. Kolluru *et al.*, 2006; Svensson *et al.*, 2006). Our methodology, coupled with an exceptional range of egg carotenoid content in the Chehalis families, provides a powerful test of the role of carotenoids in Chinook salmon early performance and fitness. Furthermore, since Chinook salmon have relatively large eggs for fish, the potential fitness effects of carotenoids and antioxidants may be magnified (Palace and Werner, 2006). Thus Chinook salmon are a good model species since their large egg size enhances our ability to detect egg carotenoid–survival relationships even under the benign conditions of the hatchery environment. It is important to note that the relationship between incubation survival and egg carotenoid content may differ in the much less ‘benign’ natural river bed environment. Although the regression

explained a modest proportion of the observed variance in incubation survival, the average expected increase in survival associated with a $1.0 \mu\text{g}\cdot\text{g}^{-1}$ increase in carotenoid concentration in eyed eggs (at levels below $3.0 \mu\text{g}\cdot\text{g}^{-1}$) is approximately 10%. Such a survival increase represents a substantial fitness benefit, and likely constitutes a substantial component of the expected balancing benefit for the costs associated with producing red eggs in salmon.

Carotenoids and their metabolic retinoid derivatives are known to enhance resistance to, and recovery from, numerous diseases in humans (e.g. Wolf, 1996), while positive relationships between carotenoids and immune function have been documented in a variety of vertebrates (e.g. Christiansen *et al.*, 1995; Skarstein and Folstad, 1996; Saino *et al.*, 1999; Massimino *et al.*, 2003; Peters *et al.*, 2004). Additionally, carotenoids and their metabolic retinoid derivatives have been shown to protect valuable host resources via antioxidant mediation (Aoi *et al.*, 2003; Alonso-Alvarez *et al.*, 2004; Bhosale and Bernstein, 2005). Our results for Chinook salmon indicate that carotenoid levels during the metabolically sensitive 2 months after fertilization are correlated with disease resistance 6 months later at smoltification. This enhanced disease resistance thus comes as offspring prepare to enter the marine environment, and hence represents an important survival benefit to offspring with high maternal carotenoid allocation. Other studies have shown a positive effect of dietary carotenoids on immune function in salmonids. For example, Christiansen *et al.* (1995) reported that carotenoid-supplemented juvenile Atlantic salmon showed significantly increased resistance to an Atlantic marine bacterial pathogen, *Aeromonas salmonicida*, the causative agent of furunculosis. However, we do not know what the mechanism is that drives the early carotenoid effects on later disease resistance in salmonids. One possibility is that egg carotenoid levels may influence the metabolic capacity of offspring ('priming' the metabolism of an organism) to better absorb/incorporate carotenoids from the diet throughout ontogeny, thus leading to a better provisioned antioxidant system for the organism throughout life (e.g. Blount *et al.*, 2003; Fitze *et al.*, 2003; Koutsos *et al.*, 2003). Alternatively, salmon egg carotenoid pigmentation may be indirectly indicative of the overall resource base of the egg, thus darker pigmentation indicates better provisioned eggs, leading to fitter offspring which are then better equipped to overcome environmental stress, such as bacterial challenges (e.g. de Jong and van Noordwijk, 1992). However, we found no significant correlation between mean family carotenoid concentration and any measure of body weight or growth through the freshwater rearing period ($P > 0.05$; data not shown), suggesting that this possibility is unlikely.

We propose that the maternal allocation of carotenoid-based pigmentation in salmon eggs represents an adaptation for increased offspring survival both directly during incubation, and indirectly later in life when facing immune challenges. This is the first report of survival benefits that may serve to offset some of the costs of maternal carotenoid allocation and possible increased egg predation risk, which are costs inherent to the nearly universal red eggs of salmon. However, our results do not provide an explanation for the existence and persistence of 'white-fleshed' Chinook salmon populations (Hard *et al.*, 1989; Ando *et al.*, 1994). Presumably, those populations have evolved an alternative mechanism that replaces the cost-benefit trade-off of maternal egg carotenoids. The effect of carotenoids in enhancing individual fitness is generally accepted; however, few studies have reported a direct relationship between maternal carotenoid provisioning in eggs and offspring survival and immune function. The present study, therefore, provides a valuable model system to evaluate the mechanisms of early life carotenoid benefits.

ACKNOWLEDGEMENTS

We thank G. Cho, A. Heath, A. Newhook, and C. Bettles for their help in the field, J. Saso, H. Li, and D. Frenkel for their help in the laboratory, and B. Young and B. Dufour for their help in both the laboratory and field. This study was supported by Yellow Island Aquaculture Ltd. and the Natural Sciences and Engineering Research Council, Canada (D.D.H. and R.J.L.). An Ontario Graduate Scholarship from the Province of Ontario, Canada supported S.T.T. All analytical carotenoid standards were generously donated by Roche Vitamins Canada Inc. (Ontario, Canada). All experimental protocols were approved by the University of Windsor Animal Care and Use Committee.

REFERENCES

- Alonso-Alvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B. *et al.* 2004. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *Am. Nat.*, **164**: 651–659.
- Ando, S., Fukuda, N., Mori, Y., Sugawara, A. and Heard, Q.R. 1994. Characteristics of carotenoid distribution in various tissues from red- and white-fleshed Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture Fish. Manage.*, **25**: 112–120.
- Aoi, W., Naito, Y., Sakuma, K., Kuchide, M., Tokuda, H., Maoka, T. *et al.* 2003. Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. *Antiox. Redox. Signal.*, **5**: 139–144.
- Bhosale, P. and Bernstein, P.S. 2005. Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation. *Biochim. Biophys. Acta – Mol. Basis Dis.*, **1740**: 116–121.
- Blount, J.D., Houston, D.C. and Møller, A.P. 2000. Why egg yolk is yellow. *Trends Ecol. Evol.*, **15**: 47–49.
- Blount, J.D., Metcalfe, N.B., Arnold, K.E., Surai, P.F., Devevey, G.L. and Monaghan, P. 2003. Neonatal nutrition, adult antioxidant defenses and sexual attractiveness in the zebra finch. *Proc. R. Soc. Lond. B*, **270**: 1691–1696.
- Christiansen, R. and Torrissen, O.J. 1997. Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar L.*). *Aquaculture*, **153**: 51–62.
- Christiansen, R., Glette, J., Lie, Ø., Torrissen, O.J. and Waagbø, R. 1995. Antioxidants and immunity in Atlantic salmon, *Salmo salar L.*, fed semi-purified diets with and without astaxanthin supplementation. *J. Fish Dis.*, **18**: 317–328.
- Craig, J.K. and Foote, C.J. 2001. Countergradient variation and secondary sexual color: phenotypic convergence promotes genetic divergence in carotenoid use between sympatric anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution*, **55**: 380–391.
- Craik, J.C.A. 1985. Egg quality and egg pigment content in salmonid fishes. *Aquaculture*, **47**: 61–88.
- Czczuga, B. 1975. Carotenoids in fish. V. Salmonidae and Thymallidae from Polish waters. *Hydrobiologia*, **46**: 223–239.
- de Jong, G. and van Noordwijk, A.J. 1992. Acquisition and allocation of resources: genetic (co)variances, selection and life histories. *Am. Nat.*, **139**: 749–770.
- Fitze, P.S., Tschirren, B. and Richner, H. 2003. Carotenoid-based colour expression is determined early in nestling life. *Oecologia*, **137**: 148–152.
- Goodwin, T.W. 1986. Metabolism, nutrition and function of carotenoids. *Ann. Rev. Nutr.*, **6**: 273–297.
- Gray, D.A. 1996. Carotenoids and sexual dichromatism in North American passerine birds. *Am. Nat.*, **148**: 453–480.
- Hamilton, W.D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, **218**: 384–387.

- Haq, A., Bailey, C.A. and Chinnah, A. 1996. Effect of β -carotene, canthaxanthin, lutein, and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets. *Poultry Sci.*, **75**: 1092–1097.
- Hard, J.J., Wertheimer, A.C. and Johnson, W.F. 1989. Geographic variation in the occurrence of red- and white-fleshed Chinook salmon (*Oncorhynchus tshawytscha*) in western North America. *Can. J. Fish. Aquat. Sci.*, **46**: 1107–1113.
- Harris, L.E. 1984. Effects of a broodfish diet fortified with canthaxanthin on female fecundity and egg color. *Aquaculture*, **43**: 179–183.
- Heath, D.D. and Blouw, D.M. 1998. Are maternal effects in fish adaptive or merely physiological side-effects? In *Maternal Effects as Adaptations* (T.A. Mousseau and C. Fox, eds.), pp. 178–201. Oxford: Oxford University Press.
- Hubbs, C. and Stavenhagen, L. 1958. Effects of maternal carotenoid deficiency on the viability of darter (*Osteichthyes*) offspring. *Physiol. Zool.*, **31**: 280–283.
- Kolluru, G.R., Grether, G.F., South, S.H., Dunlop, E., Cardinali, A., Liu, L. *et al.* 2006. The effects of carotenoid and food availability on resistance to a naturally occurring parasite (*Gyrodactylus trunbulli*) in guppies (*Poecilia reticulata*). *Biol. J. Linn. Soc.*, **89**: 301–309.
- Koutsos, E.A., Clifford, A.J., Calvert, C.C. and Klasing, K.C. 2003. Maternal carotenoid status modifies the incorporation of dietary carotenoids into immune tissues of growing chickens (*Gallus gallus domesticus*). *J. Nutr.*, **133**: 1132–1138.
- Li, H.A., Tyndale, S.T., Heath, D.D. and Letcher, R.J. 2005. Carotenoids and retinoids in eggs of chinook salmon (*Oncorhynchus tshawytscha*) using high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *J. Chromatog. B: Biomed. Sci. Appl.*, **816**: 49–56.
- Lubzens, E., Lissauer, L., Levavi-Sivan, B., Avarre, J.-C. and Sammar, M. 2003. Carotenoid and retinoid transport to fish oocytes and eggs: what is the role of retinol binding protein? *Mol. Aspects Med.*, **24**: 441–457.
- Massimino, S., Kearns, R.J., Loos, K.M., Burr, J., Park, J.S., Chew, B. *et al.* 2003. Effects of age and dietary β -carotene on immunological variables in dogs. *J. Vet. Int. Med.*, **17**: 835–842.
- McGraw, K.J., Adkins-Regan, E. and Parker, R.S. 2005. Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. *Naturwissenschaften*, **92**: 375–380.
- Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N. *et al.* 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poultry Biol. Rev.*, **11**: 137–159.
- Palace, V.P. and Werner, J. 2006. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: a review. *Sci. Mar.*, **70S2**: 41–57.
- Palace, V.P., Brown, S.B., Baron, C.L., Fitzsimons, J., Woodin, B., Stegeman, J.J. *et al.* 1998. An evaluation of the relationships among oxidative stress, antioxidant vitamins and early mortality syndrome (EMS) of lake trout (*Salvelinus namaycush*) from Lake Ontario. *Aquat. Tox.*, **43**: 195–208.
- Peters, A., Denk, A.G., Delhey, K. and Kempenaers, B. 2004. Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. *J. Evol. Biol.*, **17**: 1111–1120.
- Pettersson, A. and Lignell, A. 1999. Astaxanthin deficiency in eggs and fry of Baltic salmon (*Salmo salar*) with the M74 syndrome. *Ambio*, **28**: 43–47.
- Rajasingh, H., Gjuvsland, A.B., Våge, D.I. and Omholt, S.W. 2008. When parameters in dynamic models become phenotypes: a case study on flesh pigmentation in the Chinook salmon (*Oncorhynchus tshawytscha*). *Genetics*, **179**: 1113–1118.
- Ramsey, P. 1989. Critical values for Spearman's rank order correlation. *J. Educ. Stat.*, **14**: 245–253.
- Saino, N., Stradi, R., Ninni, P., Pini, E. and Møller, A.P. 1999. Carotenoid plasma concentration,

- immune profile, and plumage ornamentation of male barn swallows (*Hirundo rusitca*). *Am. Nat.*, **154**: 441–448.
- Skarstein, F. and Folstad, I. 1996. Sexual dichromatism and the immunocompetence handicap: an observational approach using Arctic charr. *Oikos*, **76**: 359–367.
- Sujak, A., Gabrielska, J., Grudzinski, W., Borc, R., Mazurek, P. and Gruszecki, W.I. 1999. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch. Biochem. Biophys.*, **371**: 301–307.
- Svensson, P.A., Pélabon, C., Blount, J.D., Surai, P.F. and Amundsen, T. 2006. Does female nuptial coloration reflect egg carotenoids and clutch quality in the Two-Spotted Goby (*Gobiusculus flavescens*, Gobiidae)? *Funct. Ecol.*, **20**: 689–698.
- Torrissen, O.J. 1984. Pigmentation of salmonids – effects of carotenoids in eggs and start-feeding diet on survival and growth rate. *Aquaculture*, **43**: 185–193.
- Tveranger, B. 1986. Effect of pigment content in broodstock diet on subsequent fertilization rate, survival and growth rate of rainbow trout (*Salmo gairdneri*) offspring. *Aquaculture*, **53**: 85–93.
- Withler, R.E. 1986. Genetic variation in carotenoid pigment deposition in the red-fleshed and white-fleshed chinook salmon (*Oncorhynchus tshawytscha*) of Quesnel River, British Columbia. *Can. J. Genet. Cytol.*, **28**: 587–594.
- Wolf, G. 1996. A history of vitamin A and retinoids. *FASEB J.*, **10**: 1102–1107.

