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Original Article

Redder isn't always better: cost of carotenoids in Chinook salmon eggs

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Carotenoids provide animals with many fitness benefits through increased mating success, immune function, gamete quality, and anti-oxidant capacity. Despite these benefits, carotenoids are not utilized equally by all animals, implying trade-offs associated with the pigments; although, few studies have quantified fitness costs of carotenoid pigmentation. Salmon are known for their conspicuous red coloration; however, amongst Chinook salmon (*Oncorhynchus tshawytscha*), a natural genetic color polymorphism exists (red and white morphs) which results in carotenoid-based color differences in eggs and other tissues. Although the fitness benefit of egg carotenoid content on egg incubation survival has been demonstrated, carotenoid pigmentation also results in highly visible eggs vulnerable to predation. Therefore, although white Chinook salmon eggs experience costs in terms of viability, a potential benefit in terms of reduced predation could help explain the maintenance of the polymorphism. Here, using red and white eggs from wild Chinook salmon, we show that increased carotenoid content of salmon eggs leads to greater predation risk. We found that 2 populations of wild-type rainbow trout (*O. mykiss*; an ecologically relevant predator) showed a significant bias for red eggs over white eggs under choice experiments, where red eggs were consumed first twice as often and significantly faster than white eggs. Our study suggests that trade-offs between red and white Chinook salmon during the egg stage provide an evolutionary mechanism promoting the maintenance of the unique Chinook salmon color polymorphism in nature, while also, for the first time, demonstrating a direct fitness cost of carotenoids in salmon.

Key words: **carotenoids, color polymorphism, Chinook salmon, egg predation, rainbow trout, trade-offs.**

INTRODUCTION

Variation in carotenoid pigmentation has been widely studied in the context of both natural and sexual selection (reviewed in Svensson and Wong 2011). Higher carotenoid content has been correlated with enhanced immune function (McGraw and Ardia 2003; Amar et al. 2004), increased mating success (Blount et al. 2003; Craig and Foote 2001; Yang et al. 2013), improved anti-oxidant status (Pike et al. 2007; Hõrak et al. 2007), and higher offspring quality (Tyndale et al. 2008; Bazyar Lakeh et al. 2010). Indeed, appropriate levels of carotenoids appear to be important for fitness since high levels of carotenoids can also be detrimental in some cases (see Kolluru et al. 2006; Brown et al. 2016). Initial research on carotenoid signals was focused on the benefits for mate attraction and the potential cost of predation risk (Endler 1980). However, the understanding that carotenoids contributed to many physiological functions within the organism (Lozano 1994) resulted in a shift in research focus from predation to the potential trade-offs in carotenoid utilization within the animal (i.e., immune function vs. secondary sexual traits; Blount et al. 2003; Pike et al. 2007; Baeta et al. 2008). Although it is widely speculated that carotenoid pigmentation increases predation risk, aside from studies on a few species, there have been few direct tests of this hypothesis (Kotiaho 2001; Svensson and Wong 2011). For example, in guppies (*Poecilia reticulata*) and sticklebacks (*Gasterosteus aculeatus*), brightly colored males are favored by females, but experience greater predation risk relative to conspecific drab males (Moodie 1972; Godin and McDonough 2003). Similarly, in the copepod *Eurytemora affinis*, increased carotenoid pigmentation results in higher growth rates at the cost of increased predation risk (Gorokhova et al. 2013). Higher predation risk on carotenoid pigmentation found in these species may be driven by an innate preference for red or orange coloration (i.e., sensory bias) in the predator, which has been demonstrated

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across taxa (Rodd et al. 2002; Smith et al. 2004; Grether et al. 2005; Spence and Smith 2008). Although some species can respond to predation cues by reducing carotenoid coloration (van Der Veen 2005; Anderson et al. 2015), this is not an option for all species as carotenoid signals are not always phenotypically plastic traits.

Chinook salmon (*Oncorhynchus tshawytscha*) represent an ideal system to study fitness consequences of carotenoids because a genetic color polymorphism results in red and white morphs that differ in their ability to deposit carotenoids (primarily astaxanthin; see Tyndale et al. 2008; Garner et al. 2010) into their eggs (Figure 1a, b), flesh, and skin (see Lehnert et al. 2016a), therefore resulting in coloration differences between morphs during multiple life stages. Although individuals can be categorized discretely as red (pigmented) and white (unpigmented), variation in the degree of pigmentation may exist across red individuals. Chinook salmon are the only salmonid species that exhibit this color polymorphism, and it is suggested that the white color phenotype may have first appeared during the last glaciation (Hard et al. 1989). In natural populations, morphs vary in frequency, where white individuals can represent 0–100% of the population. However, the evolutionary mechanisms responsible for the maintenance of the polymorphism remain unknown. Yet, the persistence of the polymorphism underlies the mystery of why some genera within the family Salmonidae alone among teleosts have evolved such dramatic red coloration. One plausible explanation is that natural selection operates differentially on red and white Chinook salmon across life stages resulting in potential fitness trade-offs between color morphs that balance lifetime fitness in the 2 morphs. In this case, white Chinook salmon may experience different fitness benefits than red Chinook salmon if the lack of carotenoids can provide advantages at certain life stages or if white Chinook salmon have evolved compensatory mechanisms to counteract the expected handicap (see Lehnert et al. 2016a). For example, in sockeye salmon (*O. nerka*), incipient speciation has led to a nonanadromous morph (known as kokanee) that have limited access to environmental carotenoids (Craig and Foote 2001). Kokanee have evolved mechanisms to counteract a low carotenoid diet by increasing the efficiency of carotenoid sequestration thus allowing both morphs to display similar red spawning coloration despite strong differences in carotenoid availability

(Craig and Foote 2001). Similarly, white Chinook salmon may have evolved other means to counteract the absence of carotenoids, such as through functional genetic mechanisms where selection may operate differentially on immune genes between morphs (see Lehnert et al. 2016a) or white Chinook salmon may replace carotenoids with other antioxidants as suggested in Gobiidae species with striking differences in egg carotenoid content (Svensson et al. 2009).

The cost of carotenoids has been overlooked throughout all life stages in salmon, and in particular, the egg stage represents an important developmental stage where strong selection pressures can operate (Heath et al. 2003). For example, maternal allocation of carotenoids can increase salmon egg survival, as Tyndale et al. (2008) demonstrated that eggs from red Chinook salmon females experienced greater incubation survival relative to eggs from white females. However carotenoid pigments (primarily astaxanthin as it represents >95% of the total carotenoids found in the eggs; see Tyndale et al. 2008), are clearly visible in salmon eggs thus making them highly conspicuous to predators. In nature, many fish prey on salmon eggs such as sculpins (Foote and Brown 1998) and other salmonids including rainbow trout (*O. mykiss*), coho salmon (*O. kisutch*), and Dolly Varden (*Salvelinus malma*) (Kline et al. 1990; Willson and Halupka 1995). These predators can consume salmon eggs either during spawning, or when eggs are mobilized from redds (nests) by digging females or hydraulic actions of rivers. If predators have a bias for red Chinook salmon eggs, this could provide one mechanism by which white Chinook salmon may gain a relative fitness advantage from the absence of carotenoids (and may help explain why not all fish store carotenoids as salmonids do). To test this hypothesis, we used rainbow trout, an ecologically relevant predator (Kline et al. 1990) capable of color discrimination (Ginetz and Larkin 1973), in choice experiments with red and white Chinook salmon eggs.

METHODS
Predator: rainbow trout

In August 2015, rainbow trout derived from hatchery stock populations of 2 wild strains were transported from the Fraser Valley Trout Hatchery in Abbotsford, British Columbia, Canada to the Center for Aquaculture and Environmental Research (CAER) in
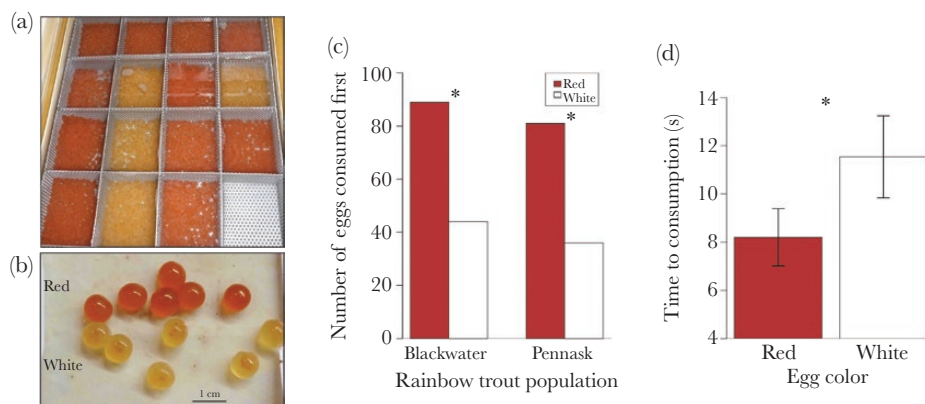
Figure 1
Typical red and white eggs from red and white Chinook salmon (*Oncorhynchus tshawytscha*) females from the Quesnel River, British Columbia, Canada, where (a) shows red and white fertilized eggs in an incubation tray and (b) shows unfertilized red and white eggs with scale (1 cm). (c) Number of red and white Chinook salmon eggs consumed first by rainbow trout (*O. mykiss*) from 2 trout populations (Blackwater and Pennask) during egg predation experiments, where significant differences between egg colors based on chi-square tests are indicated by asterisks (*). (d) Mean (± standard error) time required by rainbow trout from both populations (Blackwater and Pennask) to consume (or attempt to consume) red and white Chinook salmon eggs over all trials (*n* = 447 eggs), where asterisk (*) indicates significant difference between egg colors based on linear mixed models (see Table 1 for full model results).

West Vancouver, BC. All rainbow trout used in our study were one generation removed from the wild and had no prior experience with salmon eggs (i.e., naïve to salmon eggs as a food source). The wild sources of the rainbow trout were Pennask Lake and Blackwater River both of which are riverine spawners. Pennask Lake (GPS coordinates 49°59'24"N and 120°05'57"W) is part of the Thompson River drainage and is located southeast of Merritt, BC, whereas Blackwater River (GPS coordinates 53°18'38"N and 122°52'32"W) is part of the Fraser River drainage and is located northwest of the mouth of the Quesnel River, BC. The 2 trout populations differ in their overlap with spawning Chinook salmon populations, as Blackwater River trout will have contact with spawning Chinook salmon as the river is located near the Quesnel River where both red and white morphs occur at equal frequency, whereas Pennask Lake does not support a spawning Chinook salmon population. Trout were held in 200 L tanks supplied with aerated fresh water at CAER and were fed a commercial salmon pellet diet (Skretting Canada Ltd.) until the experiment was initiated (approximately 2 months).

Eggs: Chinook salmon gamete collection

Eggs used in the study were collected from adults captured from a wild population of red and white Chinook salmon in the Quesnel River, Likely, BC (GPS coordinates 52°36'23"N and 121°32'57"W; see Lehnert et al. 2016a). Eggs from red and white Chinook salmon females were fertilized on September 21, 2015 at the Quesnel River Research Center, Likely, BC. Eggs of 3 red and 4 white females were fertilized with mixed milt from 2 males to ensure high fertilization success. The color of the male is not expected to influence egg color, however all eggs were fertilized with sperm from paired red and white males, with the exception of the eggs of 1 red female which were fertilized by 2 white males. Egg weight of red females ranged from 0.247–0.296 g (mean = 0.273 g) and egg weight of white females ranged from 0.209–0.296 g (mean = 0.268 g). Egg weight did not differ significantly between red and white females in our study ($t = 0.25$, df = 4.88, $P = 0.81$). Additionally, to assess egg size differences between the morphs in the population overall, we compared egg weight for red and white females ($n = 19$ red; 18 white) collected from the Quesnel River population over 3 spawning seasons (2013–2015). We found no significant difference in egg weight between red and white females ($t = 0.15$, df = 34.56, $P = 0.88$), where mean egg weight was 0.281 and 0.279 grams for red and white females, respectively. Following fertilization, eggs were treated for 10 min with 100 ppm free iodine disinfectant solution (Ovadine; DynamicAqua Supply, Canada). This treatment does not affect egg coloration, however disinfection is necessary when eggs will be transported to other locations (i.e., to reduce spread of disease). Eggs were then incubated in vertical stack trays with eggs separated by color. At 15 days postfertilization (approximately 150 accumulated thermal units), eggs were transported to the CAER and placed in vertical stack incubation trays until the experiment. Any dead eggs were removed and not used in the experiment.

Egg predation experiment

On October 14, 2015, a total of 16 rainbow trout from each population were divided into 4 200-L tanks (8 trout/tank/population) that were light blue in color. The groups of 8 fish represented the predator populations. Artificial light (gold fluorescent light) was used during the experiment, where the spectral distribution ranged between wavelengths of approximately 500 to 750 nm, with peak

intensity occurring at 575 nm. The light conditions used here would allow discrimination between red and white eggs, as red carotenoids reflect light of longer wavelengths greater than 600 nm. Additionally, the eyes of rainbow trout have visual pigments that are sensitive to this range of wavelengths (Hawryshyn and Hárosi 1994; Sabbah et al. 2013). Fish were given approximately 22 h to acclimate to the new tank environment, and thus the experiment began on the morning of October 15. Prior to each experimental period, GoPro™ cameras were set up at the top of each tank to record trout behavior during the experiment. After 10 min of recording, a red and white Chinook salmon egg were released in air (using transfer pipettes) simultaneously less than 20 cm apart from the top of the tank near the water surface. When eggs were released and entered the water, they moved through the water column to the bottom of the tank, which contained no substrate. This design was chosen to simulate a spawning event, as eggs would be released from the female and sink towards to the gravel where during this time predators would have the opportunity to consume eggs. However, this design can also reflect the alternative situation when spawning females or river turbulence dislodge eggs from the river bottom. In this case, trout will need to move in quickly to consume eggs when the eggs are still in the water column. Eggs were simultaneously released every 2 min for a total of 5 trials during a 10-min time span. During each trial, red and white eggs were released from alternating sides (left/right) of the tank, and we note that side of tank had no effect on the time required to consume an egg ($P = 0.31$; see below), as well as no effect on which egg was consumed first during the trials ($n = 126$ left side and 124 right side; $\chi^2 = 0.016$, $P = 0.90$; see below). The trials were repeated 3 times each day, with trials occurring during morning (start time between 8:30 and 8:45), at noon (start time between 12:04 and 12:19) and during the afternoon (start time between 15:12 to 15:30). On October 19, a total of 14 sets of trials were recorded (no trials October 19 in afternoon), after which rainbow trout from all 4 tanks were sampled for weight. Mean weight (± standard error) of Pennask and Blackwater rainbow trout were 50.4 (± 4.04) g and 33.5 (± 2.54) g, respectively.

Statistical analyses

From video recordings during each trial, the amount of time required for a trout to approach and either consume or attempt to consume each egg was recorded. We note that attempts to consume eggs may not result in egg consumption; however both consumption and attempts could still lead to egg mortality, as attempts to bite an egg could result in egg damage, move an unfertilized egg away from fertilization opportunities or alert other predators to the food source. Therefore we considered time required to consume or attempt to consume an egg (hereafter referred to as consume) to represent the same predation event in our study. First, chi-square tests were used to determine whether there was a significant difference between number of times red versus white eggs were consumed first by rainbow trout within each population. Trials were excluded if both eggs were consumed at the same time ($n = 4$ out of 280 trials in total), if neither egg was consumed within 2 min of being dropped into the tank ($n = 21$ trials) or if eggs went out of frame or were not dropped simultaneously ($n = 5$ trials). Next, using linear mixed models with Gaussian error distribution in the R software (R Core Team 2014) package lme4 (Bates et al. 2009), we determined whether egg color and trout population had a significant effect on the time required to consume an egg. Thus, the model included egg color and population as fixed factors with random factors of date, trial number

(1 to 5), tank, time of day, and side of tank from which the egg was released (left/right). The interaction between egg color and population was also tested in the model, and if the interaction was not significant it was removed from the model. Eggs were removed from the analysis if they went out of the video frame within 2 min of being released ($n = 10$ eggs) or if the egg was not consumed within 2 min ($n = 97$ eggs), therefore our analysis involved a total of 447 eggs (data points). Time required to consume the egg (dependent variable) was inverse transformed (i.e., 1/time) to meet assumptions of homogeneity and improve normality of model residuals. Given that the inverse transformation results in the reverse order of data values, the inverse transformed data were reflected and reversed by multiplying the inverse by -1 then adding a constant of 2 to return data to positive values in their original order. Date was removed from the model to avoid over-parameterization, as date did not contribute to the variance observed for time required to consume an egg ($P = 0.99$). Using log-likelihood ratio tests, models were compared with and without each factor to determine their effect in the predictive capability. Finally, we also examined color bias over time, where we first used a general linear model in R with logit link function for binary data where each egg was coded as 1 if it was consumed first or 0 if it was not consumed first ($n = 500$ eggs). To test the color bias over time, we tested the interaction of color and date. If the interaction was significant signifying that egg color bias changed over time, we then used chi-square tests to compare the number of red versus white eggs consumed first on each day of the experiment.

RESULTS

During choice trials, we found that twice as many red eggs as white eggs were selected first by rainbow trout from both populations within trials (Figure 1c; χ^2 tests; Blackwater: $\chi^2 = 15.23$; $P < 0.001$; Pennask: $\chi^2 = 17.31$; $P < 0.001$; overall: $\chi^2 = 32.40$; $P < 0.001$). Although we found a significant color effect, we found no effect of side of tank from which the egg was released (χ^2 tests, $P > 0.78$). Given that red and white eggs were dropped from alternating sides of the tank, it is possible that fish could predict the side from which the red egg would be released next after the first trial. Therefore, we also compared the difference between the number of red and white eggs selected first during only Trial 1 for each set of predation trials. Although these trials represent only one-fifth of the data, we still found that significantly more red eggs were consumed first

overall during the first trial ($n = 35$ red and 14 white; $\chi^2 = 9.00$, $P = 0.003$), where the difference was significant in the Blackwater population ($\chi^2 = 6.00$, $P = 0.014$) and the difference approached marginal significance in the Pennask population ($\chi^2 = 3.24$, $P = 0.07$). Next, we note that not all eggs were consumed during the trials, and we found that over all trials significantly more white eggs ($n = 66$) than red egg ($n = 31$) were left unconsumed within 2 min of being released (i.e., within a trial) (χ^2 test; $\chi^2 = 12.63$, $P < 0.001$). Of the eggs that were consumed ($n = 447$ eggs), red eggs were consumed significantly faster than white eggs (Figure 1d and Table 1; Linear mixed model $\chi^2 = 8.03$, $P = 0.005$). Although we found a significant tank effect (random effect) on time to consumption ($\chi^2 = 34.5$; $P < 0.001$), there were no population (fixed effect) differences in how quickly eggs were consumed ($\chi^2 = 2.19$, $P = 0.14$) (Table 1). We also found that the amount of time required to consume an egg upon release increased significantly ($\chi^2 = 16.2$, $P < 0.001$) from Trial 1 to Trial 5 within each round of egg choice (Table 1), consistent with partial satiation. Random effects including time of day and side of tank were not significant in the model (see Table 1). Additionally, we note that there was no significant interaction between egg color and population on time to consumption ($\chi^2 = 0.29$, $P = 0.59$), therefore the interaction term was not included in the model. Next, we examined color bias over time using a logistic regression. Based on the analysis of deviance from the logistic regression, we found a significant interaction between color and date in the model ($P = 0.004$). Given the significant interaction, we used chi-square tests to compare the number of red versus white eggs consumed first on each day of the experiment. We found that the color bias was greater at the beginning of the experiment (Figure 2). On the first and second day of the experiment, we found a significant difference between the number of red and white eggs that were consumed first (Figure 2; $P < 0.001$), however the difference was no longer significant by the third and later days of the experiment ($P > 0.05$).

DISCUSSION

In our study, we found that increased redness (i.e., astaxanthin content) of salmon eggs can increase predation risk, thus demonstrating, for the first time, a cost of carotenoids in salmon. The observed behavioral bias for red egg predation demonstrated by both trout
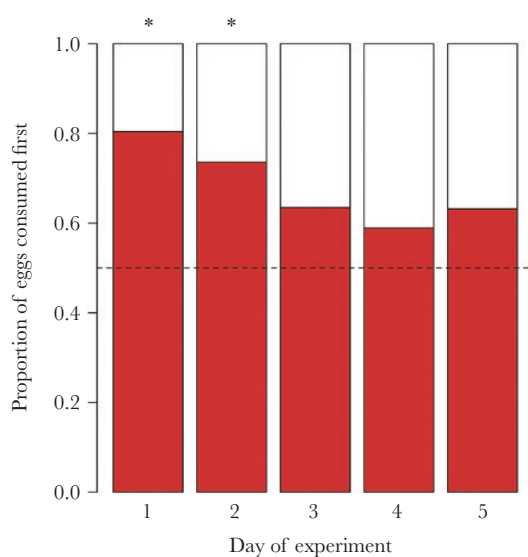
Table 1

Estimates (± standard errors) and variance components (± standard deviations) of fixed and random effects produced from linear mixed effect models with results of log-likelihood ratio tests (χ^2 and *P* value) from model comparisons

Factors		Estimate	± SE	Log-likelihood ratio test χ^2	*P*
Fixed effects	Intercept	1.50124	0.085		
	Egg color	0.06786	0.024	8.03	0.005*
	Population	0.13860	0.108	2.19	0.14
		Variance	± SD	χ^2	*P*
Random effects	Trial	0.00428	0.065	16.2	<0.001*
	Time of day	0.00078	0.028	1.63	0.20
	Tank	0.01100	0.105	34.5	<0.001*
	Side of tank	0.00061	0.025	1.03	0.31
	Error	0.06193	0.249		

The full model included the effects listed with the response variable of time (in seconds) required for the egg to be consumed (or attempted to be consumed). A total of 447 Chinook salmon (*Oncorhynchus tshawytscha*) eggs were consumed (or attempted to be) by rainbow trout (*O. mykiss*) during experimental predation trials. * indicates significant effect of factor in the model ($P < 0.05$).

populations in our study seems most likely to be a consequence of differences in their detection ability for the 2 egg colors, which may be a result of differences in color (red vs. white) or luminosity (dark vs. light). Rainbow trout are visual predators and a previous study found that rainbow trout food color preference was often dependent on contrast with background color (Ginetz and Larkin 1973), and in our study, conspicuous red eggs had higher contrast with tank background (light blue) compared to white eggs. Alternatively, the behavior may be driven by a pre-existing sensory bias for red coloration (as documented in fishes [Smith et al. 2004; Spence and Smith 2008], including salmon [Clarke and Sutterlin 1985]) given that rainbow trout have their own carotenoid requirements to fulfill. This hypothesis may be supported by the fact that both trout populations demonstrated a similar bias, even though only the Blackwater population (and not the Pennask population) overlaps with spawning Chinook salmon. Additionally, bias may also be motivated by preference for red eggs and/or avoidance of white egg. Although odor was not measured here, it is possible that the different chemical composition or concentration of red and white eggs could influence their odor and therefore their detection. Salmon egg predators, such as sculpin (*Cottus* sp.), have previously been demonstrated to rely on chemical cues from eggs for detection (Dittman et al. 1998). However, trout often approached eggs quite rapidly without much time to assess the odor. Therefore, the difference in visibility (due to color or luminosity) of red and white eggs is most likely responsible for the bias because we found that the color bias was greatest at the beginning of the experiment. The decrease in color bias over time may indicate that experience can alter the ability of trout to detect both egg colors and indicate that negative frequency-dependent selection may be operating to maintain the polymorphism, where the rare morph experiences a fitness advantage due to an inability of predators to recognize their unfamiliar eggs (Olendorf et al. 2006). Alternatively, changes over time

may represent a decrease in carotenoid requirements by rainbow trout if enough carotenoids have been ingested and the resource has become less valuable; however, this scenario seems unlikely given the short amount of time. Additionally, there is no evidence to suggest a detrimental effect of excess carotenoid supplementation in salmonids (Torrissen and Christiansen 1995; Amar et al. 2004; Page et al. 2005; Bazyar Lakeh et al. 2010; but see Costantini et al. 2007; Huggins et al. 2010 for evidence in other taxa) or that fishes know when they have attained their requirement.

Our documented advantage for white Chinook salmon may not only be important during the egg stage, but also during the critical life stage following hatch (alevin stage) as visible color (i.e., carotenoid) differences still exist in alevin yolk sacs. Although the percentage of salmon eggs (and alevins) that are lost due to predation is not well documented, previous studies indicate that in some rivers during spawning, salmon eggs can represent 84% of the rainbow trout diet (Idyll 1942) and >90% of the diet of other juvenile salmonids (Johnson and Ringler 1979). In sockeye salmon (*O. nerka*), it was estimated that up to 16% of spawned eggs may be consumed by sculpin predators (Foote and Brown 1998). Therefore, we estimated relative fitness for Chinook salmon during the egg stage, where we used egg incubation survival for red and white eggs from a previous study (Tyndale 2005) and we estimated predator avoidance based on our study and assuming varying levels (low to high) of egg predation (see Table 2). Using these measures, we estimate that relative fitness for white Chinook salmon eggs is 0.84 under low (5%) predation rate and 0.92 under medium (25%) predation rate (Table 2). Under a high (50%) predation rate scenario, white Chinook salmon would have a fitness advantage, where the relative fitness of red Chinook salmon eggs would be 0.95. Thus, when considering the increased predation risk due to carotenoids, the relative fitness of white eggs increases with increasing predation rate, where fitness of both morphs is equal when predation rate is approximately 41.5%. Although the advantage of reduced predation risk does not outweigh the cost of reduced incubation survival in white eggs under rates of predation that are likely ecologically relevant (low to medium risk), differences in fitness during later life stages may further minimize fitness differences between morphs. Specifically, if white Chinook salmon have evolved compensatory mechanisms to deal with their lack of carotenoids throughout life stages the relative fitness of the red and white morphs may be further modified.

Figure 2
Proportion of red (shaded bar) and white (white bar) Chinook salmon (*Oncorhynchus tshawytscha*) eggs consumed (or attempted to be consumed) first by rainbow trout (*O. mykiss*) over all tanks during each day of the predation experiment from 15 October 2015 to 19 October 2015. Asterisk (*) over bar indicates significant difference between the number of red and white eggs consumed first by rainbow trout (Days 1–2: $P < 0.006$; Days 3–5: $P \geq 0.052$). Dotted line represents equal consumption of red and white eggs.

Table 2

Relative fitness estimates of red and white Chinook salmon (*Oncorhynchus tshawytscha*) during the egg stage based on estimates of incubation survival and predation avoidance (proportions) for red and white eggs under different levels of predation

	Predation rate					
	Low (5%)		Medium (25%)		High (50%)	
Fitness trait	Red	White	Red	White	Red	White
Incubation survival[§]	0.921	0.762	0.921	0.762	0.921	0.762
Predation avoidance[†]	0.966	0.984	0.830	0.920	0.660	0.840
Overall egg survival[‡]	0.890	0.750	0.764	0.701	0.608	0.640
Relative fitness	1	0.843	1	0.917	0.950	1

[§]Based on results obtained from Tyndale (2005) from wild populations of red and white Chinook salmon. [†]Based on number of red versus white eggs consumed first over all eggs consumed and multiplied by predation rate then subtracted from 1. [‡]Incubation survival multiplied by predation avoidance.

In this case, lifetime fitness of morphs could be balanced and thus maintain the persistence of the white morph.

Salmon egg predation can occur when eggs are released by spawning females or when eggs are mobilized from the gravel by nest building activity or hydraulic actions of the river, and our study design can be argued to represent both of these situations when eggs are present in the water column. In our experiment, trout experienced no fitness costs for their choices; however, in nature, both red and white eggs will not be available from a single female at the same time. Trout may thus need to invest energetically in moving among spawning females, and thus would need to assess costs and benefits of movement decisions. If the bias in our study is driven by a preference for red eggs, trout may be willing to accept certain costs to obtain these preferred red eggs (i.e., choosiness). Under certain natural conditions, the bias for red eggs detected in our study under experimental conditions may be amplified or reduced depending on the mechanism for the bias and the environmental conditions found in the river. For example, if the mechanism driving the bias is due to color-based detection differences, then the spectrum of light entering the water column may influence the bias, where under high water clarity and light intensity, red eggs may be more detectable than white eggs thus increasing predation risk on red eggs. Whereas, under low-light conditions, predators may rely on other cues such as odor to detect eggs that may minimize predation differences between colors (unless egg colors differ in odor cues). Alternatively, if predation bias differences in our study are a result of egg luminosity, predation bias could increase under low light conditions when visual predators could more readily detect darker (red) eggs. In our study, artificial light conditions may not be representative of the conditions found in the wild, however in nature these conditions are not static, as light will change with time of day, amount of coverage, siltation, water depth, and other environmental parameters. Thus although our predation rates observed in experimental tanks may not be directly representative of predation under natural conditions, salmon bearing rivers throughout the Pacific coast can vary in environmental conditions and predator communities. Such potential sources of variation in predator bias, as well as predator density, among different rivers may explain why the white phenotype only persists in certain populations of Chinook salmon. Nevertheless, our study demonstrates a clear bias by rainbow trout egg predators for red Chinook salmon eggs; thus, a trade-off between red and white Chinook salmon in egg survival (Tyndale et al. 2008) and predation may provide an evolutionary mechanism responsible for the maintenance of this color polymorphism in nature and explain why not all fish are red.

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Data accessibility: Analyses reported in this article can be reproduced using data provided by Lehnert et al. (2016b).

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