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An experimental test of mate choice for red carotenoid coloration in the marine copepod *Tigriopus californicus*

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Abstract

Colorful ornaments are hypothesized to have evolved in response to sexual selection for honest signals of individual quality that provide information about potential mates. Red carotenoid coloration is common in diverse groups, and in some vertebrate taxa, red coloration is a sexually selected trait whereby mates with the reddest ornaments are preferred. Despite being widespread among invertebrates, whether red carotenoid coloration is assessed during mate choice in these taxa is unclear. The marine copepod *Tigriopus californicus* displays red coloration from the accumulation of the carotenoid astaxanthin. Previous research on copepods has shown that astaxanthin provides protection from UV radiation and xenobiotic exposure and that carotenoid production is sensitive to external stressors. Because of the condition dependency of the red coloration, we hypothesized that *Tigriopus* would use it as a criterion during mate choice. To test this hypothesis, we conducted trials in which males chose between females that were wild-type red (carotenoid-rich algae diet) or white (carotenoid-deficient yeast diet). To control for dietary differences and to isolate the effect of carotenoid coloration, we also presented males with restored-red females fed a carotenoid-supplemented yeast diet. We found that wild-type red females were weakly preferred over white females. After controlling for diet, however, we found that restored-red females were avoided. Our observations do not support the hypothesis that male copepods prefer the carotenoid coloration of females during mate choice. We hypothesize that algal-derived compounds other than carotenoids play a role in mate choice. Red coloration in copepods appears to be a condition-dependent trait that is not assessed during mating.

KEYWORDS

astaxanthin, crustacean, mate guarding, ornamentation, sexual selection, zooplankton

1 | INTRODUCTION

The indicator model of sexual selection proposes that ornamental traits function as reliable signals of the quality or condition of potential mates (Andersson & Iwasa, 1996; Weaver, Koch, & Hill, 2017). One of the best studied condition-dependent ornaments is the vibrant yellow to red coloration produced through the deposition of carotenoid pigments in body tissues. It is hypothesized that

animals in good condition tend to display more richly colored ornaments than animals in poor condition (Svensson & Wong, 2011). One possible explanation for the link between carotenoid coloration and quality is that animals cannot synthesize carotenoids *de novo* and must rely on ingested precursors for color displays (Hill, 2002). In taxa that consume photosynthetic organisms directly or that consume herbivorous prey, the majority of dietary carotenoids are yellow in color (McGraw 2006). Once ingested, carotenoids can either

be deposited to tissues unmodified or be oxidatively metabolized to produce red ketocarotenoids (Hill, 2006; Weaver, Santos, Tucker, Wilson, & Hill, 2018). The association between individual condition and red carotenoid coloration is hypothesized to arise either through internal trade-offs between the production of red color and the use of carotenoids to aid homeostasis (Alonso-Alvarez et al., 2004; Koch & Hill, 2018) or through pathways shared by pigmentation and vital cellular processes related to energy production (Hill et al., 2019).

Evidence for mate choice for carotenoid-ornamented traits has been demonstrated in a variety of vertebrate animals. For example, male two-spotted gobies prefer females with more red carotenoids (Amundsen & Forsgren, 2001), male spotted pipefish prefer females with more blue carotenoids (Berglund, Rosenqvist, & Svensson, 1986; Czezug, 1980), female guppies (Houde, 1997; Kodric-Brown, 1989) and sticklebacks (Milinski & Bakker, 1990) prefer males with more red carotenoids, female chuckwalla lizards prefer males with redder carotenoid coloration (Kwiatkowski & Sullivan, 2002), and female house finches prefer males with more red carotenoids in their feathers (Hill, 1991, 2006). In these species, mating preference for red coloration may have evolved because carotenoid coloration serves as a reliable signal of condition (Endler, 1980; Hill, 1991; Svensson & Wong, 2011) such that preference for redder mates

selects for increased reproductive fitness (Candolin, 2000; McGraw & Ardia, 2003). In these cases, individuals select for mates with a greater concentration of ornamental carotenoids. Examples of sexual selection for carotenoid-based coloration in invertebrates are rare (but see: [Baldwin & Johnsen, 2009; Detto, 2007]) despite the wide taxonomic distribution of this trait, particularly in crustaceans (Maoka, 2011).

The marine copepod *Tigriopus californicus* (Figure 1) has striking coloration that comes from the bioconversion of yellow dietary carotenoids to the red ketocarotenoid astaxanthin (Weaver, Cobine, & Hill, 2018). In the wild, *Tigriopus* copepods acquire dietary carotenoids, such as zeaxanthin, needed to make astaxanthin from a grazing diet that is rich in photosynthetic micro-algae (Powlik, 1996). In copepods, astaxanthin accumulation has been shown to protect against damage from UV radiation (Caramujo, de Carvalho, Silva, & Carman, 2012; Davenport, Healy, Casey, & Heffron, 2004; Weaver, Wang, Hill, & Cobine, 2018). In these studies, copepods with more astaxanthin had improved survival when exposed to short wavelength UV radiation. Moreover, copepods exposed to UV radiation lost between 77% and 92% of their astaxanthin coloration during treatment, in contrast to unexposed controls (Caramujo et al., 2012). Copepods, including *T. californicus*, also lose red astaxanthin

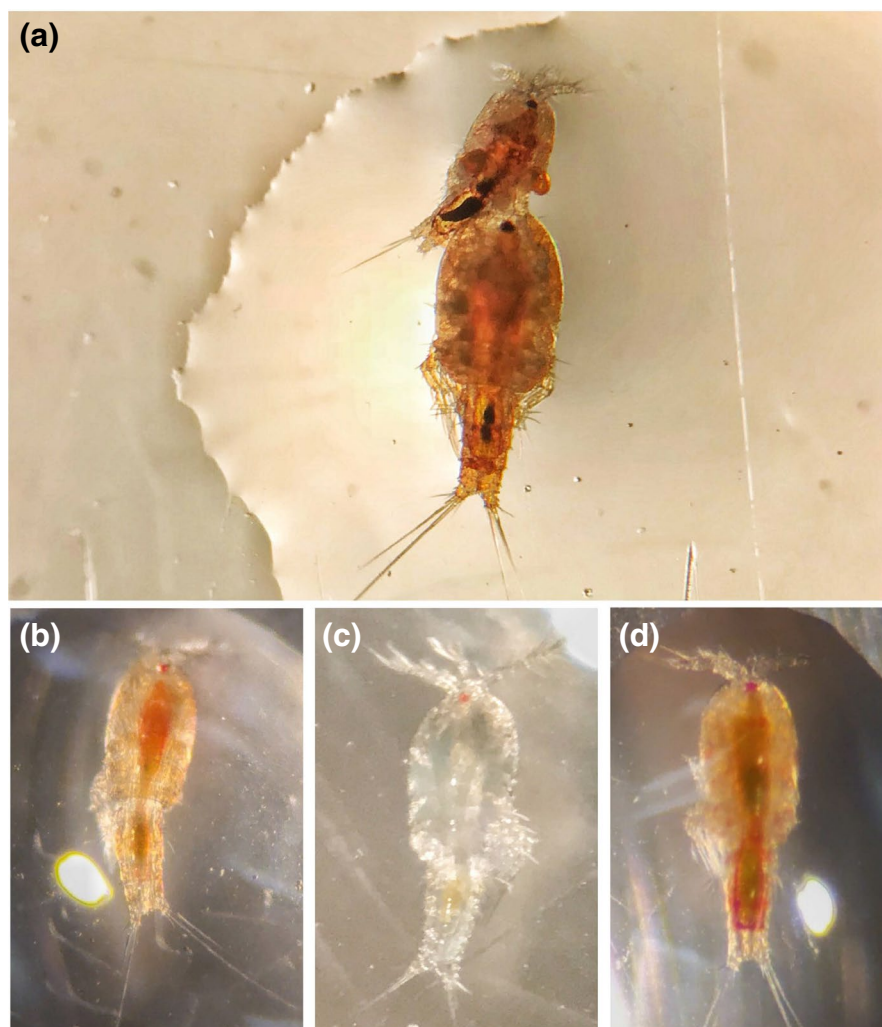


FIGURE 1 *Tigriopus californicus* under a dissecting scope. (a) Adult male *T. californicus* clasp and guarding a stage IV female copepodite, (b) wild-type red female fed a diet of mixed algae, (c) white female fed a diet of nutritional yeast deficient of carotenoid precursors, and (d) restored-red female fed a diet of yeast and supplemented with 20 µg/ml zeaxanthin [Colour figure can be viewed at wileyonlinelibrary.com]

coloration when exposed to toxic concentrations of metals, such as copper (Caramujo et al., 2012; Weaver, Hill, Kuan, & Tseng, 2016), suggesting that carotenoid accumulation and coloration is correlated with the health or condition of the animal. In vertebrates, the benefits of information about the condition of prospective mates are hypothesized to maintain mating preferences for carotenoid coloration which in turn maintain the color displays (Hill, 2006; Svensson & Wong, 2011). However, evidence that copepods assess mates based on carotenoid coloration is limited.

In the single empirical test for preference related to carotenoid coloration in *T. californicus* copepods, females were observed to have a slight but non-significant preference for red males (Palmer & Edmands, 2000). There was no control for chemical cues in these female preference trials, and the authors also suggested this result may have been complicated by differences in mate body size. Palmer and Edmands (2000) also documented female avoidance of inbreeding. However, since the completion of this study, female choice in *T. californicus* has been documented only once more: Tsuboko-Ishii and Burton (2017) observed that already-mated females attempt to escape male clasping though increased physical effort.

In many animals, where males mate multiple times and females only once, females are the more selective sex. In *T. californicus*, however, males appear to engage in more active mate choice than females because they coerce and clasp the individual that they choose. Although there is evidence that females try to escape unwanted male clasping attempts (Tsuboko-Ishii & Burton, 2017), in most cases females appear to have little choice but to mate with the male that successfully clasps them. Active choice by *Tigriopus* males makes sense because male investment in a single fertilization is large: they engage in precopulatory mate guarding by physically clasping immature females (Figure 1a) for up to 2 weeks or until females reach maturity. This period can last up to approximately 25% of the male's reproductive lifespan (Alexander, Richardson, & Anholt, 2014; Burton, 1985). Indeed, the large investment for males in a single mating event is cited as a major driver for the evolution of selective behavior in response to a variety of mating criteria (Burton, 1985; Frey, Lonsdale, & Snell, 1998; Ting & Snell, 2003). Because *Tigriopus* males exhibit a variety of non-random mating behavior and because male mate choice for astaxanthin coloration has been demonstrated in other marine taxa such as gobies (Amundsen & Forsgren, 2001), crabs (Baldwin & Johnsen, 2009) and pipefish (Berglund et al., 1986; Czczuga, 1980), we hypothesized that male *T. californicus* may also assess carotenoid content in females. To date, no test for male preference for red coloration in this species has been completed.

While there have been functional tests of vision in *Tigriopus* copepods, they have been mostly framed in the context of measuring responses to UV or polarized light (Martin, Speckmann, & Beidler, 2005; Porter, Steck, Roncalli, & Lenz, 2017). The *T. californicus* eyespot contains a layer of red pigment located between two sensory cells and next to a retroreflective layer used to concentrate low levels of light (Martin et al., 2005). The function of this pigment has not yet been determined; however, the pigmented layer in the eye of marine animals may serve to enhance discrimination of UV and

red light and may aid pooling of vitamin A for vision (Chiou, Place, Caldwell, Marshall, & Cronin, 2012; Price, Weadick, Shim, & Rodd, 2008). Despite the physiology mentioned above, whether *T. californicus* can or cannot detect differences between colors has not been tested, even though other copepod species have been documented to rely on visual cues during mating (Land, 1988). Because *T. californicus* vision may be limited compared with higher taxa, they may only be able to detect large differences in coloration between potential mates.

In this study, we tested whether carotenoid-based coloration is a criterion for mating in *T. californicus* by conducting male mate choice experiments using females fed either a wild-type algal diet, a carotenoid-deficient nutritional yeast diet, or a carotenoid-restored nutritional yeast diet. These experimental diets produced a red carotenoid-rich coloration (wild-type algal and carotenoid-restored) and a white carotenoid-deficient coloration (nutritional yeast). We used white copepods in comparison with copepods with red coloration as a coarse-level test to see whether color appeared to be a criterion in copepod mate choice. Furthermore, we fed yeast copepods a carotenoid-restored diet to isolate the effect of carotenoid color from other dietary differences in the yeast and algae diets. A similar all-or-none experimental manipulation of ornamentation was used in the initial experiments to test whether birds respond behaviorally to red feather coloration (Peek, 1972; Smith, 1972) or to ultraviolet coloration (Bennett, Cuthill, Partridge, & Maier, 1996), and these experiments formed the foundations for numerous subsequent studies. As was argued in the foundational bird studies, if *Tigriopus* copepods do not discriminate between two very different color phenotypes, then we would not predict that they would discriminate based on finer color differences as is observed in vertebrates (Svensson & Wong, 2011). We predicted that if red carotenoid coloration is used during mate choice, males would clasp red females more than white females in both wild-type red versus white and restored-red versus white mating trials.

2 | MATERIALS AND METHODS

2.1 | Animal husbandry and generation of color groups

Our laboratory has maintained large panmictic populations of *Tigriopus californicus* since 2014 under the following conditions: 35 psu artificial seawater at approximately 23°C on a 12:12-hr light:dark cycle and fed live micro-algae (*Isochrysis galbana* and *Tetraselmis chuii*). We refer to copepods raised under these conditions as "wild-type red" (Figure 1b).

Because carotenoids must be acquired through the diet, feeding *Tigriopus* copepods a carotenoid-free diet of nutritional yeast (Bragg) results in copepods that are colorless and contain only minute amounts of astaxanthin in their bodies (Figure 1c) (Weaver, Cobine, et al., 2018). To produce white copepods, we switched a subset of the wild-type red population to a nutritional yeast-only diet for several generations prior to the experiments to ensure cultures were clear of

carotenoid precursors (Weaver, Wang, et al., 2018). Nutritional yeast lacks the precursors necessary to produce astaxanthin. We refer to these copepods as “white” copepods (Figure 1c). To isolate the effect of carotenoid coloration and control for dietary differences between algae and yeast-fed copepods, we fed a subset of copepods a nutritional yeast diet supplemented with zeaxanthin—an astaxanthin precursor (Weaver, Cobine, et al., 2018), at a concentration of 20 $\mu\text{g}/\text{ml}$. We refer to these copepods as “restored-red copepods” (Figure 1d). In summary, our experimental design generated three phenotypes: wild-type red, white, and restored-red (Figure 1).

2.2 | Obtaining virgin females

Adult male *Tigriopus* perform precopulatory mate guarding by clasping immature females with their first antennae, forming a “clasp pair” until she molts into the adult stage. To obtain virgin females, we first isolated clasped pairs from the populations and then carefully separated the virgin female from the male using fine needles under a dissecting scope. We kept separated females in isolation for 1 day before trials to allow for recovery, and only free-swimming males were used in trials. We took pictures of each female under a dissecting scope on a calibrated slide (Figure S1) and measured body length and width from the images using Fiji (Schindelin et al., 2012). We used these measurements to size-match red and white females to control for preferences due to developmental stage.

2.3 | Mitigation of chemosensory signals via trypsin treatment

Previous work has shown that male *Tigriopus* copepods clasp the largest, most developmentally advanced virgin females over smaller, less developed females (Burton, 1985). Male copepods detect differences in female development through chemical cues that are likely amino acid-based pheromones (Kelly, Snell, & Lonsdale, 1998) or surface-bound glycoproteins (Ting, Kelly, & Snell, 2000; Ting & Snell, 2003). Treatment with the protease trypsin to remove these glycoproteins results in highly reduced male discrimination for developmental stage (Ting et al., 2000; Ting & Snell, 2003). To control for the known preference for developmental stage/size, we treated subsets of female copepods in our red versus white choice trials with trypsin (625 U in artificial seawater) (Figure 2). After 2 hr of trypsin exposure, we rinsed individuals three times with artificial seawater and conducted the mate choice trials.

To confirm that trypsin could remove chemical signals responsible for male preference for developmentally advanced females, we conducted separate choice trials ($n = 80$) in which a male was presented with a small, early development wild-type red virgin female or a large, late-development wild-type red virgin female (Figure 2). In approximately half of the trials ($n = 41$), both females were treated with trypsin, and in the remainder of the trials ($n = 39$), both females were untreated.

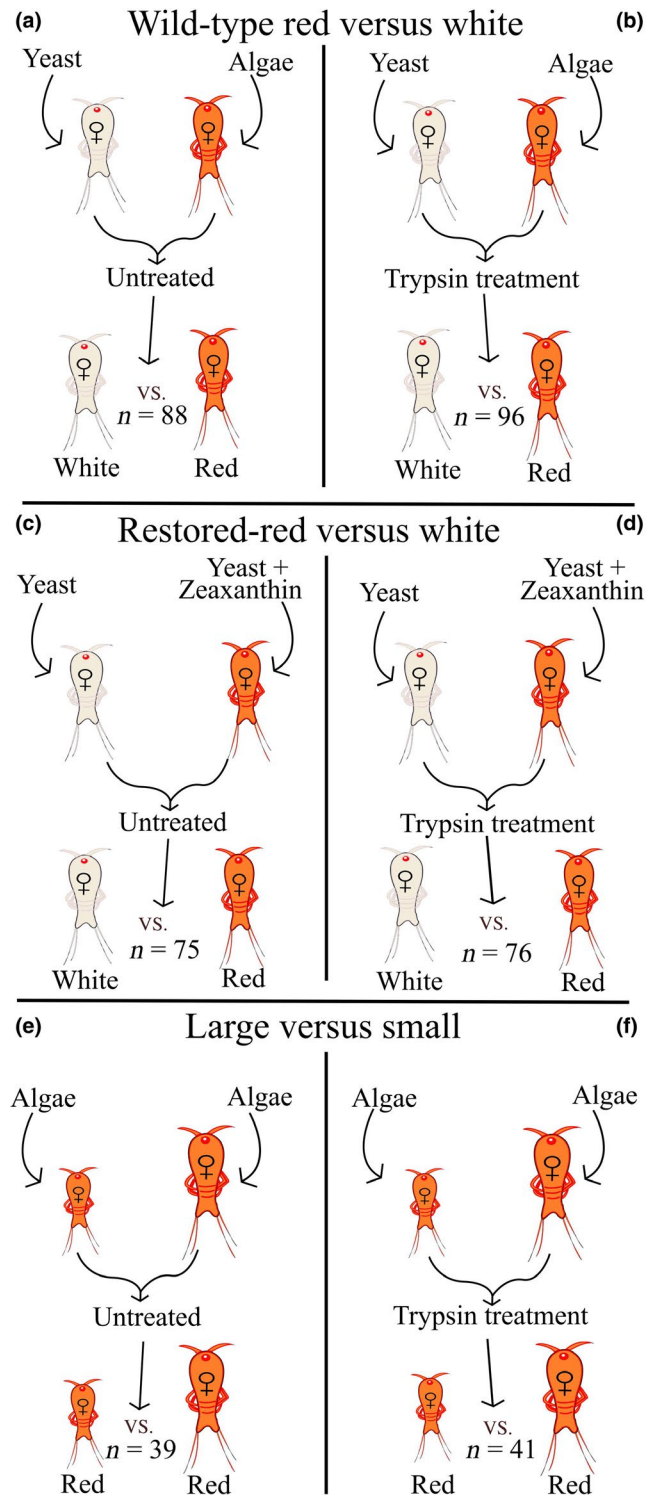


FIGURE 2 A summary of the experimental manipulations, feeding regimes, and comparisons made in this study. (a) Wild-type red versus white females untreated with trypsin, (b) wild-type red versus white females treated with trypsin, (c) restored-red versus white females untreated with trypsin, (d) restored-red versus white females treated with trypsin, (e) large versus small females untreated with trypsin, and (f) large versus small females treated with trypsin. In e and f, both large and small females were colored red from a diet of natural mixed algae [Colour figure can be viewed at wileyonlinelibrary.com]

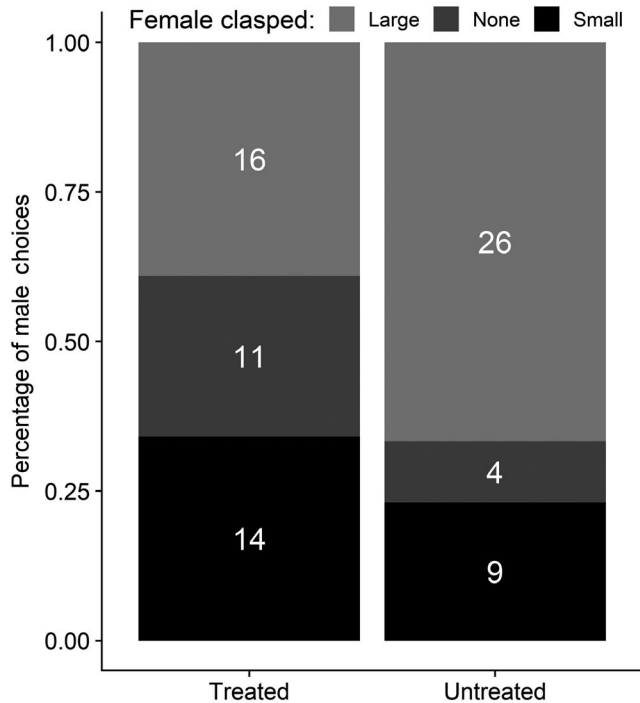


FIGURE 3 Results from size choice trials. Bars add up to 100% and individual segment sizes correspond with the percent of males that clasped each type of female. Numbers inside each bar are raw count values for the number of males that made each choice. Males significantly prefer larger, developmentally mature females over females of earlier stages in normal mating situations (untreated group). When treated with 625 U trypsin, stage-specific glycoproteins are removed from the carapace of large and small females. Therefore, males cannot distinguish between developmental stages and have no significant preference for large or small females (trypsin-treated group)

2.4 | Color choice trials procedure

In every choice trial (Figure 2), one male was placed in single 3.4-ml well of a 24-well plate in 600 μ l artificial seawater with one red female and one white female for 2 hr. Sample sizes for each experiment group are listed in Figure 2. We determined male choice by recording, at regular intervals over 2 hr, which female was clasped. We did not record full copulations. The guarding behavior associated with clasping has frequently been used as an acceptable correlate of mate choice in copepods (Snell, 2010; Titelman, Varpe, Eliassen, & Fiksen, 2007). Our trials only lasted for 2 hr, and males can clasp females for up to 2 weeks before copulation occurs (Burton, 1985) making sperm transfer a difficult measure to quantify choice in this experiment. Each 2-hr trial consisted a male choosing between one white female and either a wild-type red or a restored-red female (Figure 2). Females were kept individually in labeled wells of a 24-well plate for approximately two hours before the start of mating trials.

To begin each trial, we first placed red and white females in a single well of a clean 24-well plate filled with 600 μ l of fresh artificial seawater. Once both females were in the well, the male was added, and the trial was started. We recorded which female, if any, the male had clasped every 5 min until the 30th minute, after which we recorded

two more observations at the 1-hr and 2-hr marks. We recorded which female the male clasped first during this 2-hr time period. If a male still had not clasped a female by the end of the 2-hr observation window, we recorded this as "no choice." The majority of clasping events occurred within the first 10 min (Figure S2) as previously observed in *Tigriopus* species during mate choice experiments (Kelly et al., 1998). At the end of each mating trial, both the male and two females were placed in stock laboratory cultures not used for further mating trials.

2.5 | Statistical analyses and presentation of results

We tested for male preference for female coloration and size using a logistic regression model using the "multinom" function from the "nnet" package v7.3.12 (Venables & Ripley, 2013). This model estimates the likelihood of a male clasping a red female, a white female, or neither. We report the results as the mean probability and 95% confidence interval of a male clasping one female over another, called the relative risk (Andrade, 2015). A relative risk with confidence intervals that do not include 1 is equivalent to the p -value being $<.05$. All statistical analyses were performed in R v3.6.1 (The R Core Team, 2019) and graphed using the package "ggpubr" v0.2.1 (Kassambara, 2019) and "cowplot" (Willke, 2019).

3 | RESULTS

3.1 | The influence of trypsin on male preference for large or small females

We tested male preference for large or small females that had or had not been treated with trypsin (Figure 2). When females were untreated ($n = 39$ trials), males were 2.89 (95% CI: 1.35–6.17) times as likely to clasp large females than small females and this was statistically significant (Figure 3). When females were treated with trypsin ($n = 41$ trials), males were only 1.14 (95% CI: 0.56–2.34) times as likely to choose large over small females, but this was not statistically significant (Figure 3).

3.2 | The effect of color on male choice for wild-type red or white females

When females were untreated ($n = 88$ trials) (Figure 2a), males were 1.48 (95% CI: 0.93–2.38) times as likely to clasp wild-type red females over white females, but this result was not significant (Figure 4). When males were presented with trypsin-treated females ($n = 96$ trials) (Figure 2b), males were 1.64 (95% CI: 0.99–2.69) times as likely to clasp wild-type red over white females, but this result was also not significant (Figure 4).

3.3 | The effect of color on male choice for restored-red or white females

When females were untreated ($n = 75$ trials) (Figure 2c), males were only 0.37 (95% CI: 0.21–0.66) times as likely (63% less likely) to clasp

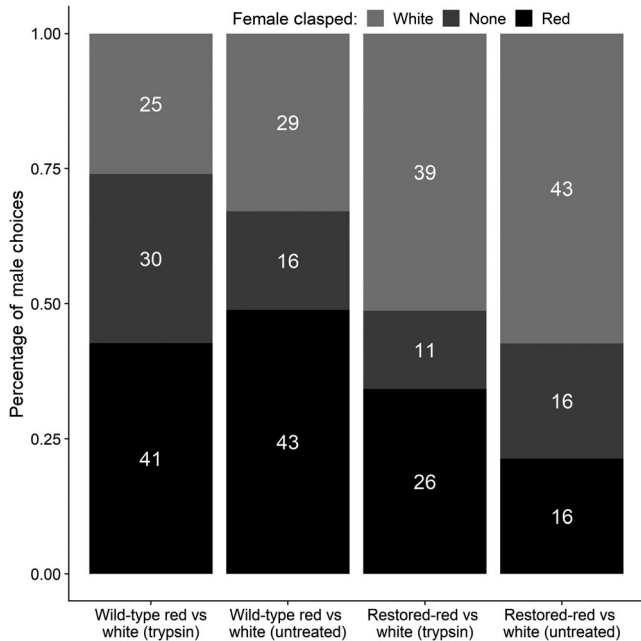


FIGURE 4 Results from mating trials testing male preference for wild-type red versus white females and male preference for restored-red females versus white females. Both experiments were completed with untreated females and females washed in trypsin to remove surface-bound glycoproteins. Bars add up to 100% and individual segment sizes correspond with the percent of males that clasped each type of female. Numbers inside each bar are raw count values for the number of males that made each choice. Significantly fewer males clasped restored-red females over white females when females were not treated with trypsin

restored-red females over white females and this result was statistically significant (Figure 4). When females were treated with trypsin ($n = 76$ trials) (Figure 2d), males were 0.67 (95% CI: 0.41–1.10) times as likely (33% less likely) to clasp restored-red females over white females, but this result was not significant (Figure 4).

4 | DISCUSSION

Through a series of experiments in which males were given a choice of females that were either red or white, we found little to no support for the hypothesis that male *T. californicus* use redness as a criterion in mate choice. We found that male copepods clasped wild-type red females more frequently than white females, but not at a rate that differed significantly from a random pattern. The strength of preference observed in these trials was similar to that observed by Palmer and Edmands (2000) when they tested female *Tigriopus* preference for red and white males. In our study, the result was the same whether females were treated with trypsin or not, but the trend toward preference for wild-type red females was stronger in the trypsin-treated group. We may have observed this increased attraction to red females because without chemical cues, wild-type red females stood out more since they were darker. However, an increased reliance on sight would not explain why trypsin treatment

removed male preference for larger females in our size trials, since it would be predicted that males would still be able to visually distinguish between large and small mates. Based on these results, *T. californicus* eyespots may be better at detecting differences between light and dark, than resolving shape and size. This is certainly supported by the fact that *T. californicus* can distinguish between water in shade and bright light (Martin et al., 2005), and that other copepods species must use mechanical sensation to determine mate size in the absence of chemical cues (Ceballos & Kiorboe, 2010).

We isolated the effect of red coloration by supplementing carotenoid-deficient females with a single precursor carotenoid used to make the red carotenoid astaxanthin. In these red-restored trials, males not only failed to show a preference for red over white females, but also showed a significant preference for white females. This strong pattern of choice is difficult to reconcile with the hypothesis that male copepods use red coloration as a positive criterion in mate choice. Moreover, this lack of choice for red when we controlled for the gross dietary differences that were present in the yeast versus algae-fed trials suggests that the apparent preference for red in those trials was an artifact of preference for algae-related compounds. Compounds derived from an algal diet may produce a more desirable chemical signal during mate choice in *Tigriopus* copepods than carotenoid coloration.

Conversely, aqueous supplementation of powdered zeaxanthin to produce restored-red individuals may have masked or changed the chemical signal of restored-red females so that males preferred to clasp white females instead (Figure 4). It is possible for dietary carotenoids to associate with glycoproteins (Teng et al., 2016; Zsila, Molnár, Deli, & Lockwood, 2005; Zsila, Nadolski, & Lockwood, 2006). This hypothesis is speculative, and we have no data to support the idea that zeaxanthin may interrupt *T. californicus* chemical cues. It is very rare to see a white *T. californicus* copepod in the wild. In other species, such as the freshwater copepod *Acanthodiaptomus denticornis*, certain populations exposed to high predation levels have lost red astaxanthin coloration and have become white (Sereda, Debes, Wilke, & Schultheiß, 2016). In these populations, white individuals may be preferred over red if they are better able to avoid detection by predators when forming clasped pairs. This situation is unlikely to be the case in *T. californicus*, who limit their exposure to predation by inhabiting elevated tide pools that are more difficult for predators to reach (Dethier, 1980). Moreover, high UV exposure in these tide-pools would likely select against any white phenotype.

Copepods produce red integumentary coloration using the same carotenoid precursors and following the same conversion pathways as many vertebrates (McGraw, 2006; Weaver, Cobine, et al., 2018). Also, as in vertebrates, red carotenoid coloration in copepods is sensitive to environmental perturbations. Copepods lose their red coloration after exposure to UV radiation (Davenport et al., 2004; Weaver, Wang, et al., 2018), predation (Brüsin, Svensson, & Hylander, 2016), and toxic metal exposure (Caramujo et al., 2012; Weaver et al., 2016). It is widely stated and experimentally confirmed that red coloration in copepods plays an important role in protecting them from photo-oxidation (Caramujo et al., 2012; Hairston, 1976;

Moeller, Gilroy, Williamson, Grad, & Sommaruga, 2005; Weaver, Wang, et al., 2018), and this is in agreement with other marine invertebrates (Babin, Biard, & Moret, 2010; Maoka, 2011).

Unlike in vertebrates, experimental evidence of sexual selection for carotenoid color in crustaceans is rare. This could be due to a lack of empirical studies on the use of visual cues during mating events (Elofsson, 2006). The role of carotenoids in crustacean mate choice and the ability of crustaceans to perceive carotenoid-based signals has been identified as an area of research that needs more attention (Baldwin & Johnsen, 2012). Moreover, current evidence for the role of carotenoid-based visual cues in crustacean mating is inconsistent. As a primary example, Baldwin and Johnsen (2009) showed that blue crabs (*Callinectes sapidus*) prefer mates with claws displaying redder coloration; however, Bushmann (1999) showed no decrease in the tendency for blue crabs to mate when blindfolded. This could indicate that visual signals are only part of the information assessed by crustaceans and other signals, such as chemical cues, may be more important.

In contrast to the paucity of research on mate choice in crustaceans, numerous studies have investigated carotenoid pigmentation in relation to physiological functions. The dominant red carotenoid in *Tigriopus* copepods and other crustaceans, astaxanthin, has mostly been studied in the context of its roles as an antioxidant and in pigmentation, photoprotection, and production of provitamin A (Britton, Liaaen-Jensen, & Pfander, 2008; Linan-Cabello, Paniagua-Michel, & Hopkins, 2002). The association of carotenoids with improved growth, immune response, stress response, and disease resistance in crustaceans is well established (Niu et al., 2009, 2014; Paibulkichakul, Piyatiratitivorakul, Sorgeloos, & Menasveta, 2008; Supamattaya, Kiriratnikom, Boonyaratpalin, & Borowitzka, 2005). There is also evidence that astaxanthin is important during crustacean reproductive events and gonadal development (reviewed in Linan-Cabello et al., 2002).

Astaxanthin accumulation has been shown to correlate with additive genetic effects and is likely heritable, as shown by a single trait mixed model used to estimate heritability of shrimp astaxanthin coloration across a reconstructed pedigree (Nguyen et al., 2014). Genetic indicator models of sexually selected traits assume that there needs to be sufficient heritability of a trait for it become preferred (Andersson & Iwasa, 1996).

The apparent associations between astaxanthin, body condition, and improved physiological responses in crustaceans, coupled with the possibility that astaxanthin accumulation is a heritable genetic trait, begs further investigation into the role of astaxanthin coloration in crustacean behavior. Our results with *Tigriopus* copepods show that although astaxanthin accumulation is related to body condition, it has not been co-opted as a sexually selected trait as seen in birds and other vertebrates. However, our results are gathered from laboratory populations of *T. californicus* raised under optimal conditions exclusively.

Red coloration in *T. californicus* appears to be a striking, condition-dependent visual display that according to our study and results shown by Palmer and Edmands (2000) did not evolve as

a signal used in mate assessment. It is interesting to speculate that condition dependency is an inherent property of carotenoids (Weaver et al., 2017) whether or not they are assessed in mate choice and that the coevolution of condition dependency and sexual selection (Rowe & Houle, 1996) may be unnecessary in crustaceans. In order to evaluate this hypothesis, more empirical work in crustaceans testing the role of carotenoid coloration in sexual signaling is needed. Coupling knowledge on the physiological functions of carotenoids with inquiries on their potential roles in modifying behavior may prove valuable to understanding the evolution of condition dependency of carotenoid-based ornaments.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

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REFERENCES

- Alexander, H. J., Richardson, J. M. L., & Anholt, B. R. (2014). Multigenerational response to artificial selection for biased clutch sex ratios in *Tigriopus californicus* populations. *Journal of Evolutionary Biology*, 27(9), 1921–1929. <https://doi.org/10.1111/jeb.12449>
- Alonso-Alvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B., & Sorci, G. (2004). An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *The American Naturalist*, 164(5), 651–659. <https://doi.org/10.1086/424971>
- Amundsen, T., & Forsgren, E. (2001). Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences*, 98(23), 13155–13160. <https://doi.org/10.1073/pnas.211439298>
- Andersson, M., & Iwasa, Y. (1996). Sexual selection. *Trends in Ecology and Evolution*, 11(2), 53–58. [https://doi.org/10.1016/0169-5347\(96\)81042-1](https://doi.org/10.1016/0169-5347(96)81042-1)
- Andrade, C. (2015). Understanding relative risk, odds ratio, and related terms: As simple as it can get. *The Journal of Clinical Psychiatry*, 76(07), e857–e861. <https://doi.org/10.4088/JCP.15f10150>
- Babin, A., Biard, C., & Moret, Y. (2010). Dietary supplementation with carotenoids improves immunity without increasing its cost in a crustacean. *The American Naturalist*, 176(2), 234–241. <https://doi.org/10.1086/653670>
- Baldwin, J., & Johnsen, S. (2009). The importance of color in mate choice of the blue crab *Callinectes sapidus*. *Journal of Experimental Biology*, 212(22), 3762–3768. <https://doi.org/10.1242/jeb.028027>

- Baldwin, J., & Johnsen, S. (2012). The male blue crab, *Callinectes sapidus*, uses both chromatic and achromatic cues during mate choice. *Journal of Experimental Biology*, 215(7), 1184–1191. <https://doi.org/10.1242/jeb.067512>
- Bennett, A. T. D., Cuthill, I. C., Partridge, J. C., & Maier, E. J. (1996). Ultraviolet vision and mate choice in zebra finches. *Nature*, 380(6573), 433–435. <https://doi.org/10.1038/380433a0>
- Berglund, A., Rosenqvist, G., & Svensson, I. (1986). Mate choice, fecundity and sexual dimorphism in two pipefish species (Syngnathidae). *Behavioral Ecology and Sociobiology*, 19(4), 301–307. <https://doi.org/10.1007/BF00300646>
- Britton, G., Liaaen-Jensen, S., & Pfander, H. (2008). Functions of intact carotenoids. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids*, Vol. 4, (pp. 189–212), Natural Functions, Basel, Switzerland: Birkhauser Verlag.
- Brüsin, M., Svensson, P. A., & Hylander, S. (2016). Individual changes in zooplankton pigmentation in relation to ultraviolet radiation and predator cues. *Limnology and Oceanography*, 61(4), 1337–1344. <https://doi.org/10.1002/lno.10303>
- Burton, R. S. (1985). Mating system of the intertidal copepod *Tigriopus californicus*. *Marine Biology*, 86(3), 247–252. <https://doi.org/10.1007/BF00397511>
- Bushmann, P. J. (1999). Concurrent signals and behavioral plasticity in blue crab (*Callinectes sapidus* Rathbun) courtship. *Biological Bulletin*, 197(1), 63–71. <https://doi.org/10.2307/1542997>
- Candolin, U. (2000). Changes in expression and honesty of sexual signalling over the reproductive lifetime of sticklebacks. *Proceedings of the Royal Society B: Biological Sciences*, 267(1460), 2425–2430. <https://doi.org/10.1098/rspb.2000.1301>
- Caramujo, M., de Carvalho, C. C. C. R., Silva, S. J., & Carman, K. R. (2012). Dietary carotenoids regulate astaxanthin content of copepods and modulate their susceptibility to UV light and copper toxicity. *Marine Drugs*, 10(12), 998–1018. <https://doi.org/10.3390/md10050998>
- Ceballos, S., & Kiorboe, T. (2010). First evidences of sexual selection by mate choice in marine zooplankton. *Oecologia*, 164(3), 627–635. <https://doi.org/10.1007/s00442-010-1755-5>
- Chiou, T. H., Place, A. R., Caldwell, R. L., Marshall, N. J., & Cronin, T. W. (2012). A novel function for a carotenoid: Astaxanthin used as a polarizer for visual signalling in a mantis shrimp. *Journal of Experimental Biology*, 215(4), 584–589. <https://doi.org/10.1242/jeb.066019>
- The R Core Team (2019). *R: A language and environment for statistical computing*. Retrieved from <https://www.r-project.org/>
- Czeczuga, B. (1980). Carotenoids in fish. XXIII. Syngnathidae family. *Hydrobiologia*, 70(3), 197–199. <https://doi.org/10.1007/BF00016761>
- Davenport, J., Healy, A., Casey, N., & Heffron, J. J. A. (2004). Diet-dependent UVAR and UVBR resistance in the high shore harpacticoid copepod *Tigriopus brevicornis*. *Marine Ecology Progress Series*, 276(1), 299–303. <https://doi.org/10.3354/meps276299>
- Dethier, M. N. (1980). Tidepools as refuges: Predation and the limits of the harpacticoid copepod *Tigriopus californicus* (Baker). *Journal of Experimental Marine Biology and Ecology*, 42(2), 99–111. [https://doi.org/10.1016/0022-0981\(80\)90169-0](https://doi.org/10.1016/0022-0981(80)90169-0)
- Detto, T. (2007). The fiddler crab *Uca mjoebergi* uses colour vision in mate choice. *Proceedings of the Royal Society B: Biological Sciences*, 274(1627), 2785–2790. <https://doi.org/10.1098/rspb.2007.1059>
- Elofsson, R. (2006). The frontal eyes of crustaceans. *Arthropod Structure and Development*, 35(4), 275–291. <https://doi.org/10.1016/j.asd.2006.08.004>
- Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, 34(1), 76. <https://doi.org/10.2307/2408316>
- Frey, M. A., Lonsdale, D. J., & Snell, T. W. (1998). The influence of contact chemical signals on mate recognition in a harpacticoid copepod. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 353(1369), 745–751. <https://doi.org/10.1098/rstb.1998.0240>
- Hairston, N. C. (1976). Photoprotection by carotenoid pigments in the copepod *Diaptomus nevadensis*. *Proceedings of the National Academy of Sciences of the United States of America*, 73(3), 971–974. <https://doi.org/10.1073/pnas.73.3.971>
- Hill, G. E. (1991). Plumage coloration is a sexually selected indicator of male quality. *Nature*, 350(6316), 337–339. <https://doi.org/10.1038/350337a0>
- Hill, G. E. (2002). *A red bird in a brown bag: the function and evolution of colorful plumage in the house finch*. Oxford, UK: Oxford University Press on Demand.
- Hill, G. E. (2006). Female mate choice for ornamental coloration. In G. E. Hill, & K. J. McGraw (Eds.), *Bird Coloration*, (2nd ed., pp. 137–200). Cambridge: Harvard University Press.
- Hill, G. E., Hood, W. R., Ge, Z., Grinter, R., Greening, C., Johnson, J. D., ... Zhang, Y. (2019). Plumage redness signals mitochondrial function in the house finch. *Proceedings of the Royal Society B: Biological Sciences*, 286(1911), 20191354. <https://doi.org/10.1098/rspb.2019.1354>
- Houde, A. (1997). *Sex, color and mate choice in guppies*. Princeton, NJ: Princeton University Press.
- Kassambara, A. (2019). *ggpubr: "ggplot2" based publication ready plots*. Retrieved from <https://cran.r-project.org/package=ggpubr>
- Kelly, L. S., Snell, T. W., & Lonsdale, D. J. (1998). Chemical communication during mating of the harpacticoid *Tigriopus japonicus*. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 353(1369), 737–744. <https://doi.org/10.1098/rstb.1998.0239>
- Koch, R. E., & Hill, G. E. (2018). Do carotenoid-based ornaments entail resource trade-offs? Anevaluation of the theory and data. *Functional Ecology*, 32(8), 1908–1920. <https://doi.org/10.1111/1365-2435.13122>
- Kodric-Brown, A. (1989). Dietary carotenoids and male mating success in the guppy: An environmental component to female choice. *Behavioral Ecology and Sociobiology*, 25(6), 393–401. <https://doi.org/10.1007/BF00300185>
- Kwiatkowski, M. A., & Sullivan, B. K. (2002). Geographic variation in sexual selection among populations of an iquanid lizard, *Sauromalus obesus* (=ater). *Evolution*, 56(10), 2039–2051. <https://doi.org/10.1111/j.0014-3820.2002.tb00130.x>
- Land, M. F. (1988). The functions of eye and body movements in Labidocera and other copepods. *Journal of Experimental Biology*, 140(1), 381–391.
- Linan-Cabello, M. A., Paniagua-Michel, J., & Hopkins, P. M. (2002). Bioactive roles of carotenoids and retinoids in crustaceans. *Aquaculture Nutrition*, 8(4), 299–309. <https://doi.org/10.1046/j.1365-2095.2002.00221.x>
- Maoka, T. (2011). Carotenoids in marine animals. *Marine Drugs*, 9(2), 278–293. <https://doi.org/10.3390/md9020278>
- Martin, G. G., Speakmann, C., & Beidler, S. (2005). Photobehavior of the harpacticoid copepod *Tigriopus californicus* and the fine structure of its nauplius eye. *Invertebrate Biology*, 119(1), 110–124. <https://doi.org/10.1111/j.1744-7410.2000.tb00179.x>
- McGraw, K. J. (2006). Mechanics of carotenoid coloration. In G. E. Hill, & K. J. McGraw (Eds.), *Bird Coloration, Volume 1: Measurements and Mechanisms*. Cambridge: Harvard University Press.
- McGraw, K. J., & Ardia, D. R. (2003). Carotenoids, immunocompetence, and the information content of sexual colors: An experimental test. *The American Naturalist*, 162(6), 704–712. <https://doi.org/10.1086/378904>
- Milinski, M., & Bakker, T. C. M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, 344(6264), 330–333. <https://doi.org/10.1038/344330a0>
- Moeller, R. E., Gilroy, S., Williamson, C. E., Grad, G., & Sommaruga, R. (2005). Dietary acquisition of photoprotective compounds (mycosporine-like amino acids, carotenoids) and acclimation to ultraviolet radiation in a freshwater copepod. *Limnology and Oceanography*, 50(2), 427–439. <https://doi.org/10.4319/lo.2005.50.2.0427>
- Nguyen, N. H., Quinn, J., Powell, D., Elizur, A., Thoa, N. P., Nocillado, J., ... Knibb, W. (2014). Heritability for body colour and its genetic association with morphometric traits in Banana shrimp (*Fenneropenaeus*

- merguiensis). *BMC Genetics*, 15(1), 1–12. <https://doi.org/10.1186/s12863-014-0132-5>
- Niu, J., Tian, L. X., Liu, Y. J., Yang, H. J., Ye, C. X., Gao, W., & Mai, K. S. (2009). Effect of dietary astaxanthin on growth, survival, and stress tolerance of postlarval shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society*, 40(6), 795–802. <https://doi.org/10.1111/j.1749-7345.2009.00300.x>
- Niu, J., Wen, H., Li, C. H., Liu, Y. J., Tian, L. X., Chen, X. U., ... Lin, H. Z. (2014). Comparison effect of dietary astaxanthin and β -carotene in the presence and absence of cholesterol supplementation on growth performance, antioxidant capacity and gene expression of *Penaeus monodon* under normoxia and hypoxia condition. *Aquaculture*, 422–423, 8–17. <https://doi.org/10.1016/j.aquaculture.2013.11.013>
- Paibulkichakul, C., Piyatiratitivorakul, S., Sorgeloos, P., & Menasveta, P. (2008). Improved maturation of pond-reared, black tiger shrimp (*Penaeus monodon*) using fish oil and astaxanthin feed supplements. *Aquaculture*, 282(1–4), 83–89. <https://doi.org/10.1016/j.aquaculture.2008.06.006>
- Palmer, C. A., & Edmands, S. (2000). Mate choice in the face of both inbreeding and outbreeding depression in the intertidal copepod *Tigriopus californicus*. *Marine Biology*, 136(4), 693–698. <https://doi.org/10.1007/s002270050729>
- Peck, F. W. (1972). An experimental study of the territorial function of vocal and visual display in the male red-winged blackbird (*Agelaius phoeniceus*). *Animal Behaviour*, 20(1), 112–118. [https://doi.org/10.1016/S0003-3472\(72\)80180-5](https://doi.org/10.1016/S0003-3472(72)80180-5)
- Porter, M. L., Steck, M., Roncalli, V., & Lenz, P. H. (2017). Molecular characterization of copepod photoreception. *Biological Bulletin*, 233(1), 96–110. <https://doi.org/10.1086/694564>
- Powlik, J. J. (1996). *Ecology of Tigriopus californicus (Copepoda, Harpacticoida) in Barkley Sound*. Vancouver, BC, Canada: University of British Columbia.
- Price, A. C., Weadick, C. J., Shim, J., & Rodd, F. H. (2008). Pigments, patterns, and fish behavior. *Zebrafish*, 5(4), 297–307. <https://doi.org/10.1089/zeb.2008.0551>
- Rowe, L., & Houle, D. (1996). The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 263(1375), 1415–1421. <https://doi.org/10.1098/rspb.1996.0207>
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. <https://doi.org/10.1038/nmeth.2019>
- Sereda, S. V. E., Debes, P. V., Wilke, T., & Schultheiß, R. (2016). Divergent mating system adaptations in microallopatric populations of *Acanthodiaptomus denticornis* (Copepoda, Calanoida). *Journal of Plankton Research*, 38(5), 1255–1268. <https://doi.org/10.1093/plankt/fbw060>
- Smith, D. G. (1972). The role of the epaulets in the red-winged blackbird, (*Agelaius phoeniceus*) social system. *Behaviour*, 41(3–4), 251–268.
- Snell, T. (2010). Contact chemoreception and its role in zooplankton mate recognition. In T. Breithaupt, & M. Thiel (Eds.), *Chemical Communication in Crustaceans* (pp. 451–466). New York, NY: Springer.
- Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M., & Borowitzka, L. (2005). Effect of a Dunaliella extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). *Aquaculture*, 248(1–4), 207–216. <https://doi.org/10.1016/j.aquaculture.2005.04.014>
- Svensson, P. A., & Wong, B. B. M. (2011). Carotenoid-based signals in behavioural ecology: A review. *Behaviour*, 148(2), 131–189. <https://doi.org/10.1163/000579510X548673>
- Teng, Y., Sheu, M., Hsieh, Y., Wang, R., Chiang, Y., & Hung, C. (2016). β -carotene reverses multidrug resistant cancer cells by selectively modulating human P-glycoprotein function. *Phytomedicine*, 23(3), 316–323. <https://doi.org/10.1016/j.phymed.2016.01.008>
- Ting, J. H., Kelly, L. S., & Snell, T. W. (2000). Identification of sex, age and species-specific proteins on the surface of the harpacticoid copepod *Tigriopus japonicus*. *Marine Biology*, 137(1), 31–37. <https://doi.org/10.1007/s002270000320>
- Ting, J. H., & Snell, T. W. (2003). Purification and sequencing of a mate-recognition protein from the copepod *Tigriopus japonicus*. *Marine Biology*, 143(1), 1–8. <https://doi.org/10.1007/s00227-003-1071-2>
- Titelman, J., Varpe, Ø., Eliassen, S., & Fiksen, Ø. (2007). Copepod mating: Chance or choice? *Journal of Plankton Research*, 29(12), 1023–1030. <https://doi.org/10.1093/plankt/fbm076>
- Tsuboko-Ishii, S., & Burton, R. S. (2017). Sex-specific rejection in mate-guarding pair formation in the intertidal copepod, *Tigriopus californicus*. *PLoS ONE*, 12(8), 1–16. <https://doi.org/10.1371/journal.pone.0183758>
- Venables, W., & Ripley, B. (2013). *Modern applied statistics with S-PLUS*. (No. 7.3.12; 3rd ed.). New York, NY: Springer Science and Business Media.
- Weaver, R. J., Cobine, P. A., & Hill, G. E. (2018). On the bioconversion of dietary carotenoids to astaxanthin in the marine copepod, *Tigriopus californicus*. *Journal of Plankton Research*, 40(2), 142–150. <https://doi.org/10.1093/plankt/fbx072>
- Weaver, R. J., Hill, G. E., Kuan, P. L., & Tseng, Y. C. (2016). Copper exposure reduces production of red carotenoids in a marine copepod. *Ecological Indicators*, 70, 393–400. <https://doi.org/10.1016/j.ecolind.2016.06.040>
- Weaver, R. J., Koch, R. E., & Hill, G. E. (2017). What maintains signal honesty in animal colour displays used in mate choice? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1724), 20160343. <https://doi.org/10.1098/rstb.2016.0343>
- Weaver, R. J., Santos, E. S. A., Tucker, A. M., Wilson, A. E., & Hill, G. E. (2018). Carotenoid metabolism strengthens the link between feather coloration and individual quality. *Nature Communications*, 9(1), 73. <https://doi.org/10.1038/s41467-017-02649-z>
- Weaver, R. J., Wang, P., Hill, G. E., & Cobine, P. A. (2018). An in vivo test of the biologically relevant roles of carotenoids as antioxidants in animals. *The Journal of Experimental Biology*, 221(15), jeb183665. <https://doi.org/10.1242/jeb.183665>
- Willke, C. O. (2019). *Cowplot: Streamlined plot theme and plot annotations for "ggplot2"*. Retrieved from <https://cran.r-project.org/package=cowplot>
- Zsila, F., Molnár, P., Deli, J., & Lockwood, S. F. (2005). Circular dichroism and absorption spectroscopic data reveal binding of the natural cis-carotenoid bixin to human α 1-acid glycoprotein. *Bioorganic Chemistry*, 33(4), 298–309. <https://doi.org/10.1016/j.bioorg.2005.03.003>
- Zsila, F., Nadolski, G., & Lockwood, S. F. (2006). Association studies of aggregated aqueous lutein diphosphate with human serum albumin and α 1-acid glycoprotein in vitro: Evidence from circular dichroism and electronic absorption spectroscopy. *Bioorganic and Medicinal Chemistry Letters*, 16(14), 3797–3801. <https://doi.org/10.1016/j.bmcl.2006.04.028>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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