



Natural variation of carotenoids in the eggs and gonads of the echinoid genus, *Strongylocentrotus*: implications for their role in ultraviolet radiation photoprotection

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Abstract

We examined variability in carotenoid concentration in the gonads and eggs of four sea urchin species (*Strongylocentrotus purpuratus*, *Strongylocentrotus franciscanus*, *Strongylocentrotus pallidus* and *Strongylocentrotus droebachiensis*) to explore the possible role of carotenes as photoprotectants. Carotene concentrations were measured in gonads and gametes of each species, while in eggs the ultraviolet radiation (UV-R) sensitivity and self-shading capacity by carotenes were calculated. Mean concentrations of carotenes in gonads ranged from $0.13 \pm 0.017 \text{ mg g}^{-1} \text{ dw}$ (*S. purpuratus*), $0.14 \pm 0.019 \text{ mg g}^{-1} \text{ dw}$ (*S. franciscanus*), $0.29 \pm 0.079 \text{ mg g}^{-1} \text{ dw}$ (*S. pallidus*) to $0.36 \pm 0.06 \text{ mg g}^{-1} \text{ dw}$ (*S. droebachiensis*). In eggs, concentrations ranged from 0.026 ± 0.003 to $0.09 \pm 0.034 \text{ mg g}^{-1} \text{ dw}$. UV-R sensitivity in eggs was quantified by measuring UV-R induced first-cleavage delay. Intra-specifically, cleavage delay varied significantly between individuals, and could be correlated with carotene concentration. Interspecific differences in cleavage delay and carotene concentrations were not correlated. Using the observed concentration of β , β -echinenone (which makes up between 82.4% and 94.9% of the total carotene concentration in the eggs) and a molar extinction coefficient of $\epsilon = 13.7 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 334 nm, we calculated self-shading efficiency in the eggs. Self-shading capacity (J_{334}) indicated that the eggs could only screen from 4.6% ($J_{334} = 0.046$) down to 1.5% ($J_{334} = 0.015$) of UV-R at 334 nm. While not sunscreens, we suggest that carotenes can photoprotective in echinoid eggs, probably by mitigating the effects of reactive oxygen species.

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Keywords: Carotenoid; Echinoid; Anti-oxidant; Reactive oxygen species; Photoprotection; Eggs; Ultraviolet radiation

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1. Introduction

Stratospheric ozone depletion has resulted in increased amounts of ultraviolet-B radiation (290–320 nm) reaching the sea surface (Smith et al., 1992; Hofmann, 1996), and researchers are now seeking a greater understanding of the harmful affects of ultraviolet radiation (UV-R) on marine biological systems (Hockberger, 2002). Ultraviolet-B (290–320 nm) and UV-A (320–400 nm) radiation can be deleterious to the early life-history stages of marine invertebrates. This is true for echinoid eggs (Giese, 1939; Adams and Shick, 1996), which contain a number of biochemical mechanisms to mitigate UV-R damage. These include defences against UV-radiation such as small molecule anti-oxidants (Turner et al., 1988, Dunlap and Yamamoto, 1995), sunscreens (Adams and Shick, 1996), free radical scavengers, photoreactivation (Marshak, 1949; Wells and Giese, 1950), DNA-excision repair, and reactive oxygen species scavengers.

Carotenoids are ubiquitous in marine species and are well known to act in photoprotection (against the direct and indirect effects of UV-A) through their high affinity to the triplet state of photosensitised molecules and reactive oxygen species such as singlet oxygen ($^1\text{O}_2$) (Edge et al., 1997). Recent data also indicate that they mitigate the effects of superoxide (O_2^-), hydroxyl radical ($\cdot\text{OH}$), and hydrogen peroxide (H_2O_2) (Kim et al., 2001). Reactive oxygen species damage biological systems through the oxidation of proteins, lipids and DNA (Schafer et al., 2002).

The role that carotenes might play in UV-R-mediated defences in marine invertebrates such as echinoids is still equivocal. In this paper, we investigate the variation in carotenoids of four sympatric *Strongylocentrotid* species from the Pacific Northwest (*S. droebachiensis*, *S. pallidus*, *S. franciscanus* and *S. purpuratus*) to examine if carotenoids can have a photoprotective role in their eggs. If carotenoids are important for providing photoprotection, we might expect that (1) there would be a relationship between UV-R sensitivity and carotenoid concentration both intra- and interspecifically and, (2) the eggs of those species that experience higher levels of UV-R in their natural environment (i.e. intertidal species, summer spawners) would have higher carotenoid concentrations invested in eggs.

Given these points, we examined four aspects related to this question. First, to establish the maternal investment of carotenoids in gametes, we quantified the seasonal change in carotenoid concentrations over 1 year, concentrations in male versus female gonad, and concentrations in ripe female gonad and eggs. Second, concentrations of carotenoids in the gonad and eggs of sea urchins typically show high intra-specific variation (Griffiths and Perrot, 1976; Goebel and Barker, 1998), and we used this natural variation in carotenoid concentrations to compare the relationship between UV-R sensitivity and carotenoid concentration within and among the four species examined here. We examined the absorption properties of carotenoids in the eggs, estimating the capacity of carotenoids to protect vital cellular components (the cell nucleus) within the eggs of these species. Lastly, we measured natural UV-B and UV-A irradiances in the water column over one year and discuss the possible role of carotenoids given these in situ levels of radiation.

2. Materials and methods

2.1. Sample collection

Gonads and eggs were sampled from four species of echinoids: *Strongylocentrotus droebachiensis*, *Strongylocentrotus franciscanus*, *Strongylocentrotus pallidus*, and *Strongylocentrotus purpuratus*. Collections were made from Clallum Bay and in the San Juan Islands, Washington State, USA over a 1-year period (Table 1). Samples of 10 individuals were taken at approximately monthly intervals over a year for each of the species, except for *S. purpuratus* where monthly samples were taken over a 5-month period that included the annual spawning.

For each individual, a gonad index was recorded as

$$\text{Gonad Index (\%)} = \frac{\text{total gonad weight (g)}}{\text{total drained wet weight (g)}} \times 100 \quad (1)$$

and a sample of gonad was wrapped in aluminium foil and frozen at -80°C . For each species, an additional sample of five ripe females and five ripe males were taken from field populations immediately prior to spawning. Each of these individuals was dissected and one intact gonad removed and frozen to -80°C . The remaining gonadal tissue was removed from the animal and the gametes stripped, washed, and frozen to -80°C . All frozen samples were lyophilised then returned to -80°C for storage prior to carotene extraction.

2.2. Carotenoid extraction

Samples of freeze dried tissue (100 mg) were homogenised in 10 ml of 100% acetone with an OCI Instruments Omnimixer 17105 homogeniser. The homogenate was centrifuged (5000 rpm, 5 min) and the supernatant transferred to a 20-ml scintillation vial. The pellet under went a second extraction in 10 ml of 100% acetone and the two supernatants were combined. The sample was evaporated to dryness under reduced pressure using a SAVANT SpeedVac SVC100. The residue was reconstituted in 2 ml ether and 2 ml 10% methanolic KOH, flushed with N_2 , sealed and left to stand at room temperature for 2 h. At this time, 4 ml of 5% NaCl was added, the vials resealed and shaken vigorously, and mixture allowed to separate into two phases. The upper organic phase was pipetted off and

Table 1
Details of sampling of *Strongylocentrotus*

Species	Site	Depth	No. sampled	Sampling dates	Spawning period	Egg Size (μm)
<i>S. droebachiensis</i>	San Juan Is.	120 m	10	28/01 to 27/12	Mar–Apr	148.2
<i>S. franciscanus</i>	San Juan Is	15 m	10	02/02 to 27/12	May–Jun	132.3
<i>S. pallidus</i>	San Juan Is	120 m	10	28/01 to 27/12	Feb–Mar	151.5
<i>S. purpuratus</i>	Clallum Bay	Intertidal	10	22/01 to 20/05	Jan–Mar	83.3

Information on spawning period, and mean egg diameter (μm) are given.

the remaining aqueous phase was re-extracted a further two times to recover all the carotenoids. The extracts were evaporated to dryness, and reconstituted in 2 ml of 100 % methanol prior to chromatographic analysis. To minimise photooxidation of samples, all extractions were carried out under yellow light.

2.3. High-performance liquid chromatography (HPLC)

The HPLC system consisted of an ISCO Gradient Programmer (Model 2360), an ISCO HPLC pump (Model 2350) and an ISCO V⁴® Variable Wavelength Absorbance Detector (ISCO, Lincoln, NE). Operation of the system and data acquisition was made by computer, running the software JCL 6000 Chromatography Data System Version 2 (Jones Chromatography USA, Lakewood, CO). Samples were run through a *Phenosphere Next* 5 µm C18 250 × 4.60-mm column of 5-µm silicon (Phenomenex, Torrance USA) fitted with a C18 (ODS, Octadecyl) guard cartridge. Separation of extracts was made isocratically in a mobile phase consisting of Acetonitrile/Dichloromethane/Methanol, 70:20:10 by volume, at a flow rate of 1 ml min⁻¹ (pressure ≈ 590 psi). For each sample, 10 µl was injected and the absorbance of the extracts monitored at 445 nm.

2.4. Reference samples

Four carotenoids were quantified using reference samples. Echinenone (β,β-carotene-4-one) standards were obtained from VKI (Denmark). Astaxanthin ((3*S*,3' *S*)-3,3' -dihydroxy-β,β-carotene-4,4' -dione), β-carotene (β,β-carotene) and β -carotene ((6' *R*)-β,ε-carotene) were obtained from Sigma (St. Louis, MO). Stock solutions were prepared for each compound, and quantification made from the standard curves of peak-height ratios versus concentration. Quantification of unknown samples was made from the standard curves using JCL 6000 software.

2.5. Self-shading coefficients

Ultraviolet radiation self-shading of the nucleus by echinenone within the echinoid eggs was calculated for 334-nm wavelength. The self-shading coefficient (J) is an estimate of the proportion of UV-R light absorbed in the egg by the absorbing compound at a particular wavelength and is calculated using the equation:

$$J = 1 - \left(\frac{1}{aR} \right) - \frac{\exp(-a2R) - 1}{2(aR)^2} \quad (2)$$

where R = egg radius and the absorption coefficient $a = (2.3\epsilon)c$, with c = molar concentration of the absorbing compound, and ϵ = molar extinction coefficient of the compound (Garcia-Pichel, 1994). The molar extinction coefficient for echinenone at 334 nm was calculated from the absorption through a 1-cm pathway of a 2.276-µmol solution of echinenone in 100% EtOH.

2.6. Ultraviolet radiation sensitivity

Previous researchers have shown that UV-R will delay cell division in sea urchin eggs (see Giese, 1939; Adams and Shick, 1996), and the degree of the delay can be used as an estimate of UV-R sensitivity. Therefore, eggs from four females of each species were exposed to UV-R (290 to 400 nm), and differences in UV-induced delay of the first cell division used to estimate relative UV-R sensitivity. Eggs were collected from females by inducing spawning with an inter-coelomic injection of 1–2 ml of 0.5 M KCl. These eggs were exposed to UV-R for a total of 1 h (previously shown to significantly delay cell division in these eggs) in six-well dishes covered with an acetate filter (Laird Plastics), giving the eggs a total UV-A and UV-B radiation dose (measured using a LiCor Li-1800UW spectroradiometer) of 1810 and 1472 J m⁻², respectively. UV-R exposures were performed using four Optronics UVA-340 bulbs (see Beasley et al., 1996 for emission spectrum). All bulbs had been pre-burned for at least 100 h to ensure a stable output of UV-A (320 to 400 nm) and UV-B (300 to 320 nm) of 0.502 and 0.408 W m⁻² s⁻¹, respectively. For each set of eggs, a control treatment was established by covering identical six-well dishes containing eggs with a lexan filter (Laird Plastics) that blocks all UV-wavelengths but transmits visible light. Transmission spectra of these plastic filters are reported in Karentz and Lutze (1990).

For each female, three replicates of the treatment and control were used, with temperatures in the wells maintained at 11.5 ± 0.1 °C. Following exposure, eggs were fertilised and the time that 50% of the embryos had completed first-cell division recorded, and UV-R induced cleavage delay calculated as

$$\% \text{Delay} = \frac{T_{\text{UV}} - T}{T} \times 100 \quad (3)$$

where T_{UV} = time to 50% first-cell division in UV-R exposed embryos and T = time to 50% first-cell division in control embryos. To avoid photoreactivation of UV damage in the embryos, all experiment were carried out in a room illuminated by yellow light. Carotenoid concentrations of eggs from each female were quantified using HPLC as described above.

2.7. Field measurements of ultraviolet radiation

Midday measurements of in situ UV-B and UV-A irradiances were made as often as possible for a 1-year period (1 January 1999 to 3 December 1999). In total, 194 days were sampled. On each day, a LiCor Li-1800UW spectroradiometer recorded UV-B (300 to 320 nm) and UV-A (320 to 400 nm) at the surface and at depths of 0.2, 0.5, 1.0, 5.0 and 10.0 m. Each reading was an average of two casts scanning at 2-nm intervals.

Using the spectroradiometer measurements, the attenuation of UV-B and UV-A was modelled for each day using the Lambert–Beer Law:

$$I_D = I_0 \exp^{-kd} \quad (4)$$

where I_D = UV-R irradiance at depth (d), I_0 = UV-R irradiance at the surface, k = UV-R extinction coefficient, and d = depth (m). We rearranged this equation to calculate the

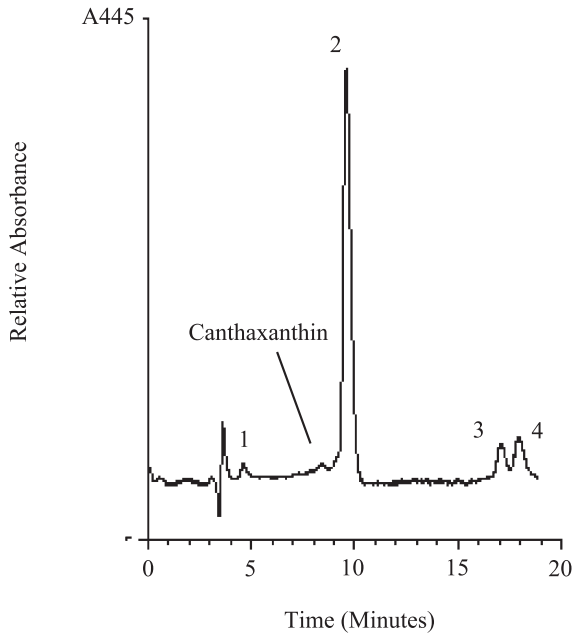


Fig. 1. HPLC chromatograph of a *S. franciscanus* gonad extract. Carotenoid pigments are (1) astaxanthin, (2) echinenone, (3) β -carotene, (4) δ -carotene. Canthaxanthin is tentatively identified eluting at ≈ 9.5 min.

maximum depth that laboratory equivalent UV-B and UV-A exposures occurred on each day as:

$$d = \frac{\ln\left(\frac{I_D}{I_0}\right)}{-k} \quad (5)$$

where I_D is the irradiance of the laboratory exposures (0.408 and $0.502 \text{ m}^{-2} \text{ s}^{-1}$ for UV-B and UV-A, respectively).

Table 2

Annual mean (\pm S.E.), maxima (\pm S.E.) and minima (\pm S.E.) total gonadal carotenoid concentration (mg g^{-1} dw) for each *Strongylocentrotus* species

	Annual mean \pm S.E. (<i>n</i>)	Annual maximum (month, <i>n</i>)	Annual minimum (month, <i>n</i>)
<i>S. droebachiensis</i>	0.36 ± 0.06 (59)	0.67 ± 0.024 (Apr, 6)	0.08 ± 0.01 (Dec, 7)
<i>S. pallidus</i>	0.29 ± 0.07 (66)	1.87 ± 0.60 (Jun, 4)	0.07 ± 0.01 (Mar, 4)
<i>S. franciscanus</i>	0.14 ± 0.01 (57)	0.32 ± 0.14 (Feb, 4)	0.009 ± 0.003 (Nov, 9)
<i>S. purpuratus</i>	0.13 ± 0.01 (26)	0.16 ± 0.04 (Apr, 6)	0.11 ± 0.01 (Feb, 10)

The month when maximum and minimum concentrations were recorded are given.

n = sample size.

2.8. Statistical analysis

Tests for normality of data, heteroscedasticity, and homogeneity of variances were made on data prior to statistical analysis. Those data that do not meet these assumptions were transformed either by $\ln(x+1)$ for non-percentages, or by $\arcsin\sqrt{(x/100)}$ for percentages. All analyses were made using the SYSTAT v.5.2.1 statistical package (Wilkinson, 1989).

3. Results

3.1. HPLC chromatographs

Four carotenoids were separated using HPLC analysis (Fig. 1) with their identity confirmed by comparison with retention times of pure carotenoid samples. Canthaxanthin

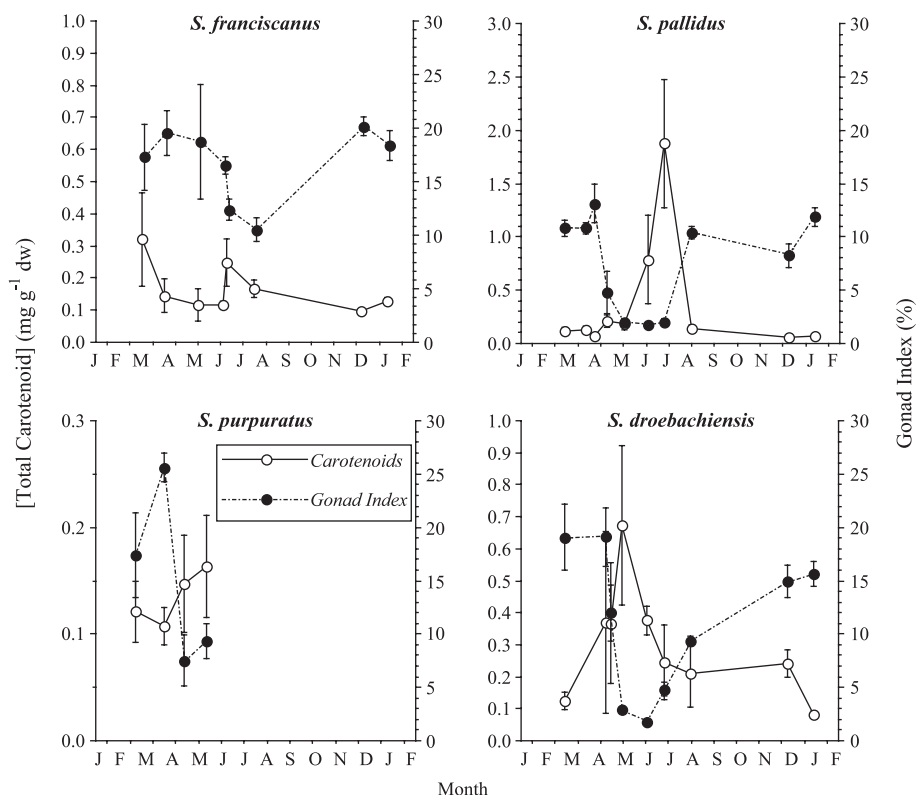


Fig. 2. Changes in total gonad carotenoid concentration ($\text{mg g}^{-1} \text{ dw}$) and gonad index (%) over time for four *Strongylocentrotus* species. Standard errors are indicated. Note that the Y-axis scales differ among some figures.

is known to be present in *Strongylocentrotus* gonads (Griffiths, 1966) but was not quantified in this current study. We often observed a peak in the HPLC chromatographs eluting at 9.5 min, which was probably canthaxanthin.

3.2. Total carotenoid concentrations

Gonadal concentrations of carotenoids differed among species, being highest in *S. droebachiensis* over the year (Table 2) and lowest in *S. purpuratus*. Total carotenoid concentrations showed distinct seasonal variation in all four species (Fig. 2). Seasonal variation was greatest in *S. pallidus* with monthly concentrations ranging from 0.07 to 1.87 mg g⁻¹ dw (Table 2), and lowest in *S. purpuratus* with an annual variation of 0.11 to 0.16 mg g⁻¹ dw (although we only sampled this species during the spawning season). Seasonal variation in total carotenoid concentrations is related to annual reproductive cycles in each species. Carotenoid concentrations increased during periods of decreasing

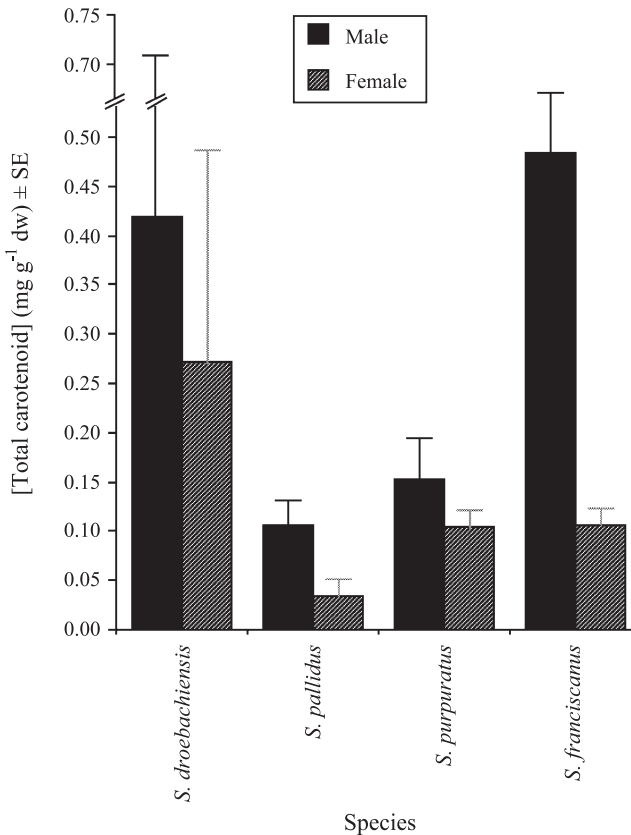


Fig. 3. Mean total carotenoid concentrations (mg g⁻¹ dw) in male and female gonadal tissue for four *Strongylocentrotus* species. Gonad samples were taken just prior to spawning. $n = 5$ for each column and standard errors are indicated.

gonad index when gametes were being shed (Fig. 2), while periods of increasing gonad indices corresponded with decreasing concentration of carotenoids. Concentrations tended to be lowest when gonad indices are highest. Concentrations of carotenoids were consistently higher in males than in females for all four species (Fig. 3), with differences between sexes ranging from 4.5-fold in *S. franciscanus*, down to 1.4-fold in *S. purpuratus*.

One-way ANOVA indicated that concentrations of carotenoids in the eggs (Fig. 4) differed significantly among species ($F_{(3,39)} = 3.856$, $P = 0.016$), with mean concentrations

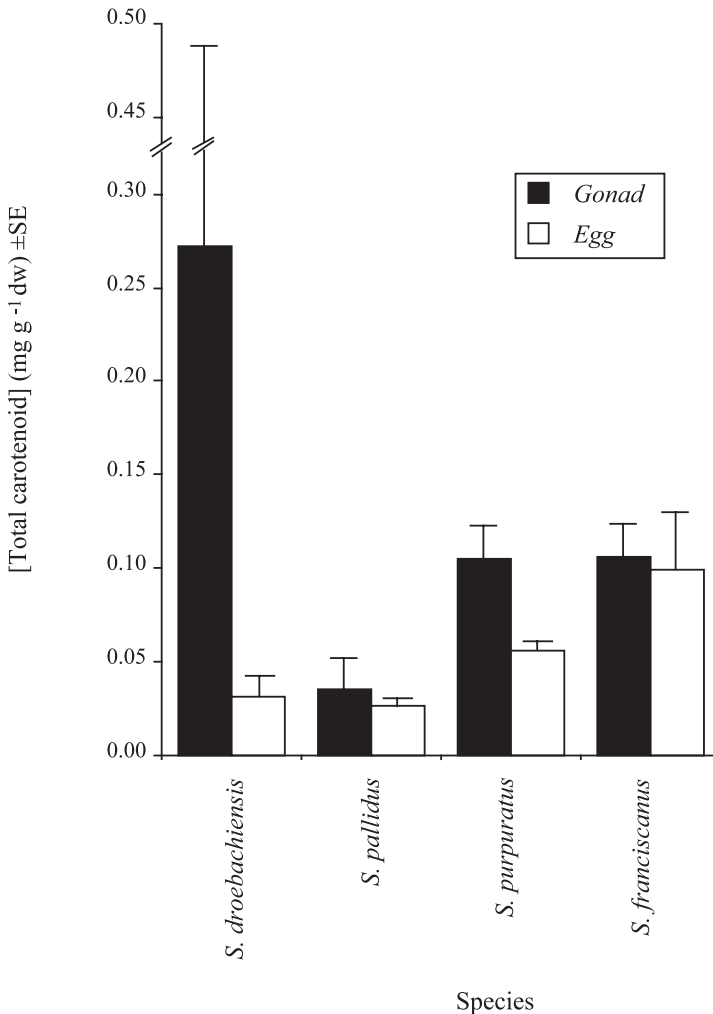


Fig. 4. Mean total carotenoid concentrations ($\text{mg g}^{-1} \text{dw}$) in female gonadal tissue and spawned eggs for four *Strongylocentrotus* species. Gonad and egg samples were taken from the same female just prior to spawning. $n = 5$ for each column and standard errors are indicated.

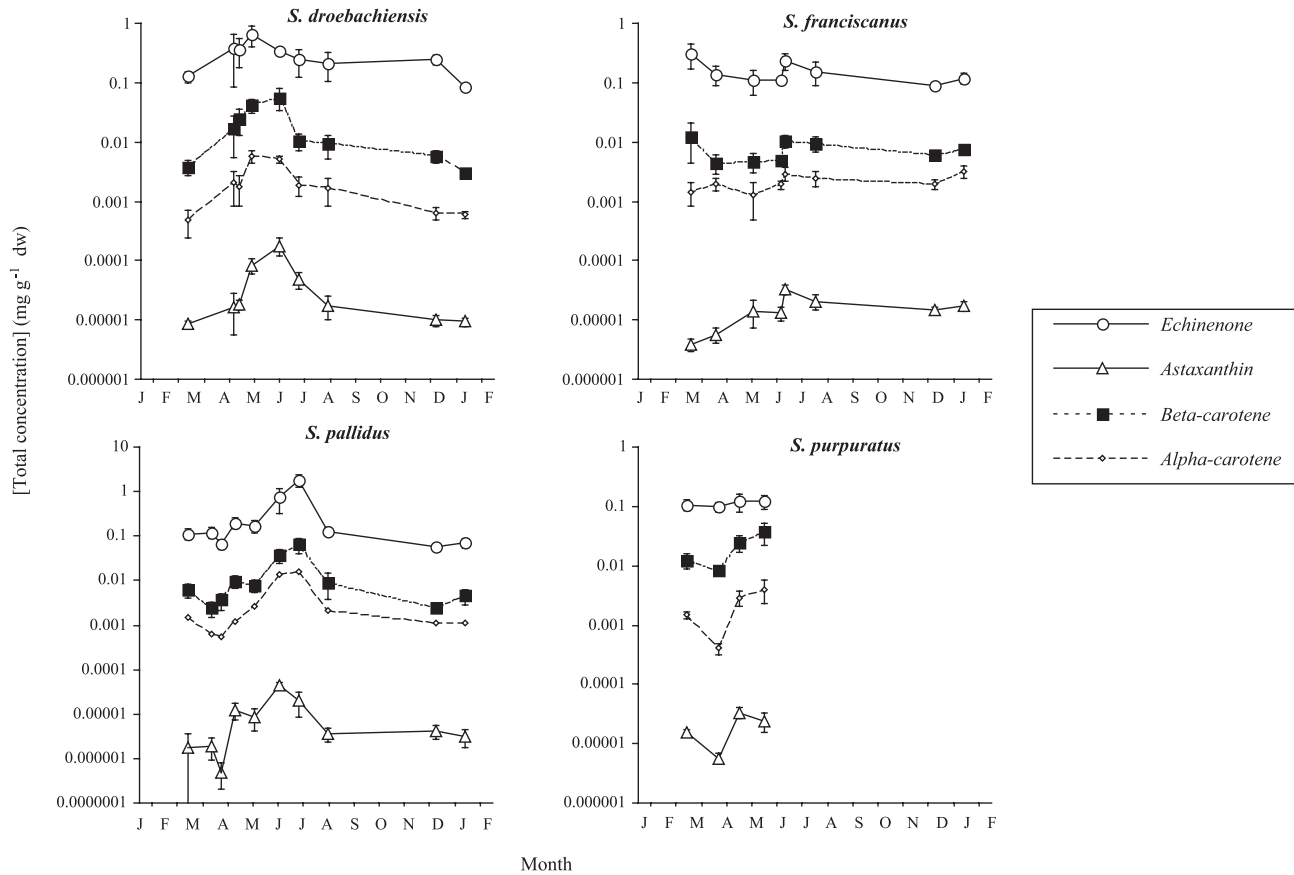


Fig. 5. Change in the gonadal concentration ($\text{mg g}^{-1} \text{ dw}$) of Echinenone, Astaxanthin, β -carotene, and α -carotene over time for four *Strongylocentrotus* species. Note that the Y-axis scales differ among some figures. Standard errors are indicated.

Table 3
Estimates of self-shading coefficients by echinenone in eggs of four *Strongylocentrotus* species

Species	Egg diameter (μm) \pm S.D.	Molar concentration ($\mu\text{mol g}^{-1}$ dw)	S.E.	$J_{334 \text{ nm}}$
<i>S. franciscanus</i>	132.3 \pm 3.9	3.49	0.059	0.046
<i>S. pallidus</i>	151.5 \pm 8.4	0.96	0.007	0.015
<i>S. droebachiensis</i>	148.2 \pm 6.1	2.70	0.0015	0.017
<i>S. purpuratus</i>	83.3 \pm 3.7	3.08	0.011	0.017

Egg molar concentrations of extracts were calculated assuming that the egg is 90% water. Mean egg radius was calculated from 20 unfertilised eggs. Molar extinction coefficient of echinenone at 334 nm used in the calculations was calculated as $\epsilon = 13.7 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$.

(\pm S.E.) lowest in *S. pallidus* ($0.026 \pm 0.003 \text{ mg g}^{-1}$ dw) and highest in *S. franciscanus* ($0.099 \pm 0.034 \text{ mg g}^{-1}$ dw). Carotenoid concentration was consistently lower in the eggs than in gonads, with the differences ranging from 8.6-fold in *S. droebachiensis* down to 1.1-fold in *S. franciscanus*.

3.3. Proportions of each carotenoid

All four carotenoids were found in gonad tissue of each species of echinoid examined (Fig. 5). Echinenone was the most abundant compound, making up between 83.1% and

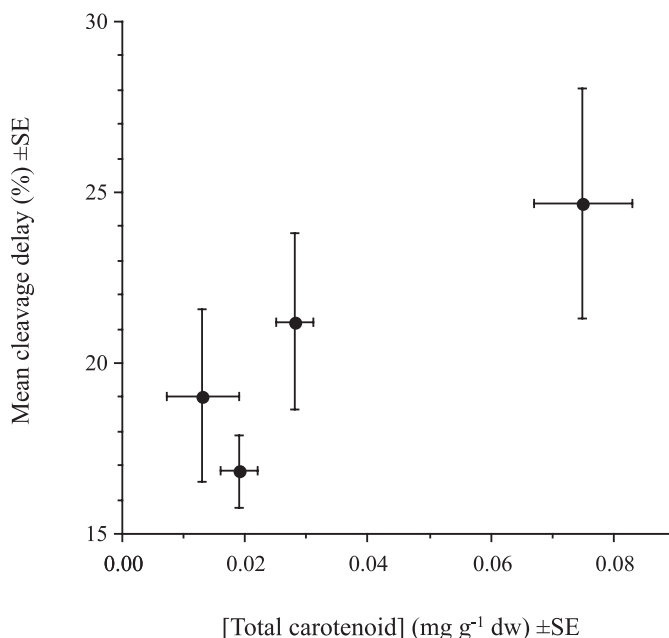


Fig. 6. The relationship between mean total carotenoid concentration in eggs (mg g^{-1} dw) and mean ultraviolet radiation induced delay to first cleavage of eggs from four *Strongylocentrotus*. $n=4$ for each error bar.

94.2% of the total carotenoids, while astaxanthin contributed 0.005% to 0.13% of the total carotenoids. Within eggs, echinenone was also the dominant carotenoid making up all of the detectable carotenoids in *S. droebachiensis* and *S. pallidus*, and 82.4 and 94.9% of the carotenoids in *S. franciscanus* and *S. purpuratus*, respectively. Two-way ANOVAs of carotenoid concentration within each species (dependent variable = carotenoid concentration, factors = month, carotenoid type) were undertaken to identify statistically significant differences in the proportions of carotenoids over time. The analyses indicated that there were no seasonal differences in the relative concentrations of each carotenoid type for any of the *Strongylocentrotids* (Fig. 5). Analyses indicated that changes in carotenoid concentrations were synchronous over the season (i.e. the

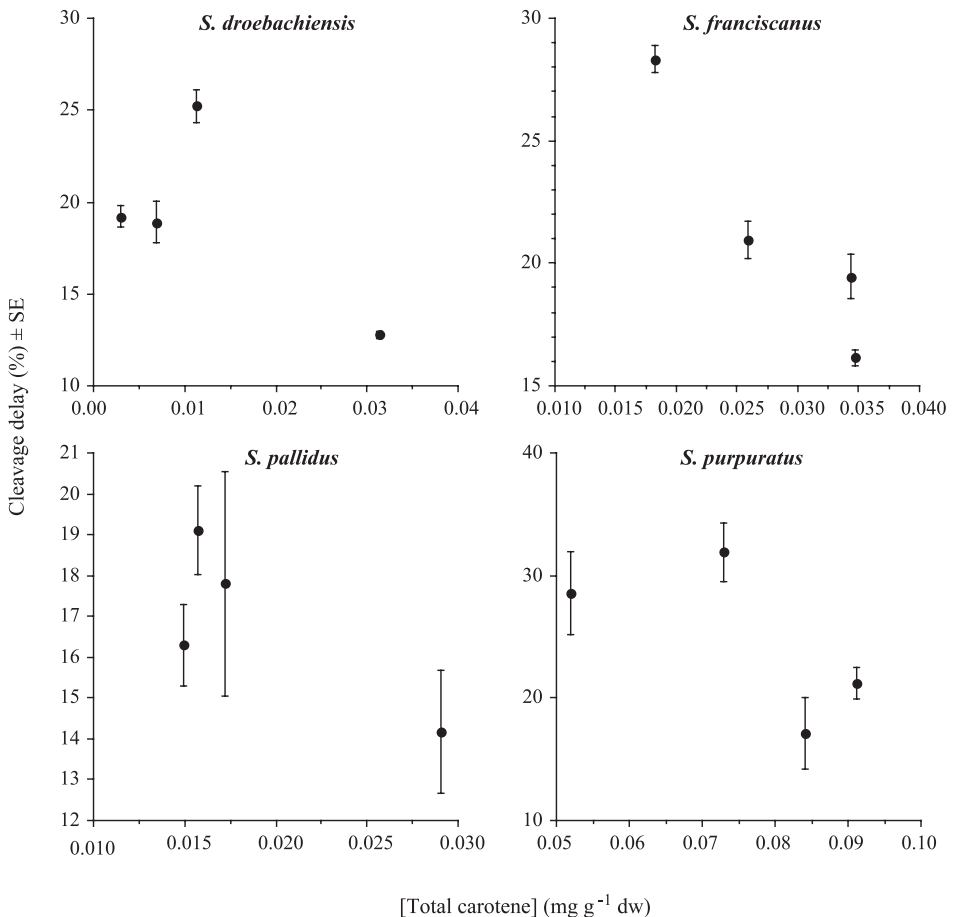


Fig. 7. The relationship between total carotenoid concentration (mg g⁻¹ dw) and ultraviolet radiation induced delay to first cleavage of eggs for four species of *Strongylocentrotus*. Note that both the X-axis and Y-axis scales differ among some figures. $n=4$ for each error bar.

interaction term for month*carotenoid was $P \geq 0.191$ for each carotenoid in all four species).

3.4. Self-shading coefficients

Estimates of the self-shading coefficient (J) of echinenone at 334 nm was highest in *S. franciscanus* ($J_{334 \text{ nm}}=0.045$), with the remaining three species having very similar self-shading ($J_{334 \text{ nm}} \approx 0.015$ to 0.017). These calculations suggest that *Strongylocentrotus* eggs are shading the nucleus by echinenone of between 1.5% and 4.5% light at 334-nm wavelength (Table 3).

3.5. Cleavage delay and carotenoid concentration

One-way ANOVA indicated that ultraviolet radiation-induced cleavage delay (Fig. 6) varied significantly among species ($F_{(3,30)}=11.154$, $P=0.001$), ranging from $16.8 \pm 0.01\%$ in *S. pallidus* up to $24.6 \pm 0.07\%$ in *S. purpuratus*. Among the species there was a trend of increasing total carotenoid concentration associated with increasing cleavage delay (Fig. 6) although linear regression analysis indicated that the linear relationship between the two variables was not significant ($F_{(1,2)}=7.213$, $P=0.115$). Within each species, cleavage delay tended to decrease with increasing carotenoid concentration (Fig. 7), and linear regression analysis of the two variables indicated that a significant relationship existed between the two for *S. franciscanus* and *S. droebachiensis* (Table 4). For these two species, carotenoid concentration could explain 82.2% and 44.7% of the variation in cleavage delay, respectively. The relationship was not significant for the remaining two species.

3.6. Field measurements of ultraviolet radiation

The depth of UV-B and UV-A penetration varied seasonally (Fig. 8), with the maximum depth of laboratory equivalent irradiance (0.408 and $0.502 \text{ W m}^{-2} \text{ s}^{-1}$ for UV-B and UV-A, respectively) occurring in May and August (0.93 and 5.4 m, respectively). The depth of penetration decreased during the mid-summer due to the higher standing crop of phytoplankton (Chl-*a* ranged from 1.1 to 5.14 mg m^{-3} during June 1999 and July 1999 compared with 0.13 to 1.9 mg m^{-3} between January 1999 and May 1999).

Table 4

Regression analysis of cleavage delay (%) versus carotenoid concentration ($\text{mg g}^{-1} \text{ dw}$) in eggs of four *Strongylocentrotus* species

Species	<i>n</i>	Regression equation	S.E. of slope	ANOVA (<i>df</i> , <i>F</i> , <i>p</i>)	R^2
<i>S. droebachiensis</i>	12	$y=0.49 - 3.65x$	1.28	11, 8.074, 0.0175	0.447
<i>S. pallidus</i>	12	$y=0.49 - 3.59x$	1.98	11, 3.299, 0.099	0.248
<i>S. franciscanus</i>	11	$y=0.68 - 7.34x$	1.41	10, 41.429, 0.001	0.822
<i>S. purpuratus</i>	12	$y=0.67 - 1.89x$	1.70	11, 1.227, 0.294	0.109

Cleavage delay data are arcsine transformed and carotene concentrations are $\ln(x+1)$ transformed.

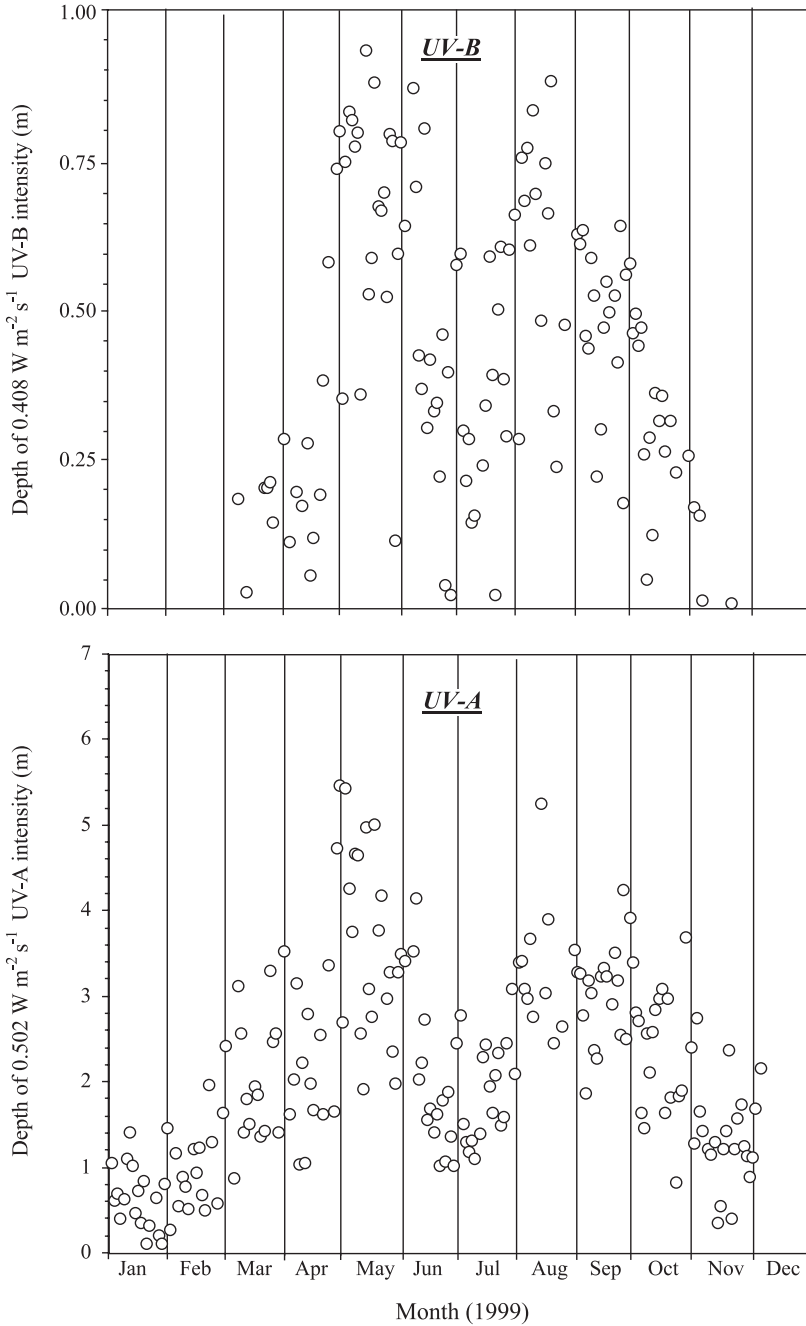


Fig. 8. Depth to which the penetration of UV-B at $0.408 \text{ W m}^{-2} \text{ s}^{-1}$ and UV-A at $0.502 \text{ W m}^{-2} \text{ s}^{-1}$ occurred in San Juan Channel, San Juan Islands. All measurements were made at mid-day using a LiCor Li-1800UW spectroradiometer, scanning at 2-nm intervals from 300 to 750 nm.

4. Discussion

Carotenoid concentrations recorded in the current study compare well with those previously made for the *Strongylocentrotus* genus. Griffiths and Perrot (1976) observed total carotenoid concentrations in the ovaries ranging from ≈ 0.8 down to $\approx 0.5 \text{ mg g}^{-1}$ dw over 1 year. Echinenone and β -carotene were found at 0.623 and 0.121 mg g^{-1} dw, making up 76.9% and 14.9% of total carotenes, respectively. In the same study, the authors reported concentrations in the eggs of 0.088 to 0.247 mg g^{-1} dw (mean = 0.137 mg g^{-1} dw). Griffiths (1966) reported total carotene concentrations of between 0.19 and 0.38 mg g^{-1} (egg protein) in *S. purpuratus*, with echinenone and β -carotene making up 67% and 23% of the total, respectively. Tsushima and Matsuno (1990) examined total gonadal concentration in six regular echinoids, finding concentrations ranging from 0.005 to 0.038 mg g^{-1} dw. In these species, echinenone was the most abundant carotene in the gonad, making up between 31.4% and 69.1% of the carotenes.

In our study, gonadal carotene concentrations varied annually in each species, with mean concentrations highest in *S. droebachiensis* (0.36 mg g^{-1} dw) and lowest in *S. purpuratus* (0.13 mg g^{-1} dw). In contrast, maternal investment in eggs was highest in *S. franciscanus* (0.09 mg g^{-1} dw) and lowest in *S. pallidus* (0.026 mg g^{-1} dw). The increase in gonadal carotene concentrations during spawning, and the lower concentration of carotenes in the eggs compared with the intact gonad, indicate that most of the carotenes were retained in the gonad (possibly in the nutritive phagocytes) and not invested into the eggs.

Carotenoids are believed to effect reproduction in echinoids, influencing the egg energy content (De Jong-Westman et al., 1995), and in some cases fecundity (Tsushima et al., 1997; Kawakami et al., 1998). Inter-specific differences in egg carotene concentrations might also be related to differences in embryonic and larval development. A good example of this is shown in the Australian echinoid genera, *Heliocidaris* (Tsushima et al., 1995). These two sympatric species differ in their larval development mode and have large differences in the carotene concentrations within the egg (i.e. *H. eurythrogramma*; indirect developer; high carotene concentration compared with *H. tuberculata*; direct developer; low carotene concentration). Other examples are given for the effects of carotene concentration on larval survival (Torrissen, 1984; George et al., 2001), larval development (De Jong-Westman et al., 1995; George et al., 2001).

Our research examined the role that carotenoids may play as photoprotectants against the damaging effects of UV-radiation. We found that within a species, an increase in total carotenoid concentration correlated with a decrease in UV-R sensitivity (as measured by egg cleavage delay). For two of the species we examined (*S. droebachiensis* and *S. franciscanus*), there was a significant negative relationship between carotenoid concentration and UV-R sensitivity, with carotenoid concentration explaining 44.7% and 82.2% of the variation, respectively. In the remaining two species, the relationship was not statistically significant, although there was a trend for higher UV-R sensitivity in those individuals with lower carotenoid concentrations.

These observations are consistent with the suggestion that carotenoids can have a photoprotective role in echinoid eggs, but could be explained by three potential mechanisms: (1) carotenoids may be acting as UV-R absorbing sunscreens; (2) carotenoids

are protecting cellular components (proteins, lipids, DNA) against reactive oxygen species or; (3) carotenoid concentrations co-vary with, but are not functionally related to photoprotective processes and UV-R sensitivity in these eggs. We consider all three possibilities.

Carotenoids are not effective sunscreens in the *Strongylocentrotus* eggs. This is in contrast to other invertebrate species (such as copepods), where carotenoids in the tissues are in sufficient concentration to be effective suncreening compounds (Hairston, 1976; Mathew-Roth, 1997; Hessen et al., 1999). In our study, the observed carotenoid concentrations and egg sizes for each species were used to calculate self-shading capacity in the UV region (334 nm), which ranged from $J=0.015$ to $J=0.046$. These represent a minor reduction in UV_{334} reaching the egg nucleus of 1.7% to 4.6%. Sunscreening in echinoids does occur in eggs, but is affected by UV-absorbing mycosporine-like amino acids (MAAs). Mean concentrations of these compounds in *S. droebachiensis* eggs range from 1.46 to 33.21 nmol mg^{-1} dw depending on algal diet Adams and Shick (1996, 2001). At these MAA concentrations, self-shading coefficients were estimated to be between $J=0.128$ and $J=0.862$ (i.e. blocking 12.8% to 86.2% of the UV-B reaching the egg nucleus).

A high suncreening capacity does not appear to be universal in *Strongylocentrotus* eggs however (Lamare, unpublished data). Mean concentrations of MAAs were measured in ripe and spent gonads (from the Puget Sound area) of *S. droebachensis* (0.525, 0.169 nmol mg^{-1} dw), *S. franciscanus* (0.005, 0.001 nmol mg^{-1} dw), *S. purpuratus* (0.004, 0.022 nmol mg^{-1} dw), and *S. pallidus* (0.0067, 0.0052 nmol mg^{-1} dw), respectively, which would correspond to low self-shading capacities in the eggs of these three later species. Furthermore, spectrophotometric examination of egg extracts (80% MeOH) could not detect any other UV-R absorbing compound, indicating that a suncreening capacity is minimal in populations of three of the four species.

The negative correlation between UV-sensitivity and carotenoid concentration is consistent with the suggestion that carotenoids are photoprotective against the effects of UV-R. The most probable mechanism is by reducing the effects of reactive oxygen species on lipid peroxidation. Carotenoids are well-known preventers of lipid peroxidation caused by singlet oxygen (1O_2), and possibly other species of reactive oxygen species (Edge et al., 1997).

The degree to which UV-R mediated effects on cells and subsequent mitigation of reactive oxygen species may be occurring in situ will depend on ambient UV-R levels in the water column. The eggs in our laboratory experiments were exposed to 1810 $J m^{-2}$ UV-A and 1472 $J m^{-2}$ UV-B, which is equivalent to instantaneous UV intensities of 0.502 $W m^{-2} s^{-1}$ UV-A, and 0.408 $W m^{-2} s^{-1}$ UV-B. Midday measurements of UV-A and UV-B through the water column indicated that UV-B intensities are typically low, and that eggs would have to be present in water less than ≈ 1 m between March and October to experience laboratory-equivalent UV-B doses. UV-A penetrates deeper, and laboratory equivalent irradiances were recorded as deep as 5.5 m. For most of the year (March to October), eggs and embryos occurring between 1 and 4 m will experience UV-A intensities equivalent to the laboratory exposures (0.502 $W m^{-2} s^{-1}$). For species that spawn during this period (*S. droebachiensis*, *S. franciscanus*, *S. pallidus*), UV-A at these irradiances, could result in damage via the production of reactive oxygen species. Under these conditions, carotenoids may provide a measurable degree of photoprotection against UV-R.

The final possibility is that carotenoid concentrations and UV-R sensitivity co-vary but are not functionally related. This would occur if carotenoid concentration co-varied with the functionality of an unrelated UV-mitigating strategy. Echinoid eggs possess a number of mechanisms to mitigate the effects UV-R, including dark-excision repair (Karentz, 1994), and photoreactivation of damaged DNA (Karentz, 1994; Lamare and Hoffmann, unpublished information), sunscreens compounds (Adams and Shick, 1996), and a number of free radical scavengers (Dunlap et al., 2000) that will all influence UV-R sensitivity. Indeed, interspecific comparisons (where the species with highest carotenoid concentrations had the highest sensitivity to UV-R) indicates that factor(s) other than carotenoid concentration determine UV-R sensitivity.

If carotenoids are important photoprotectants, then we might expect a correlation between ambient UV-R exposure of a species and their photoprotectant capacity (as has been shown for MAA concentrations within tropical corals (see Dunlap et al., 1986) and the Antarctic sea urchin *Sterechinus neumayeri* (Karentz et al., 1997)). No such relationship exists between the depth of collection and UV-R sensitivity, although the concentrations of carotenoids tend to decrease with increasing depth of collection. Carotenoid concentration in the gonad and eggs of echinoids is influenced by diet, and will be affected by food availability and concentration of carotenoids in the algae (Plank et al., 2001). Those deeper species have lower concentrations of carotenoids (*S. pallidus*, *S. droebachiensis*) which likely reflect the lower amounts of algal material available at these depths. In contrast, the two species that occur in shallower waters tend to have higher carotenoid concentrations, likely reflecting a greater availability of macroalgae and dietary accumulation of carotenoids in the gonad.

5. Conclusions

The current study indicates that gonadal carotenoid concentrations vary significantly among the four echinoid species that we examined, and that there is significant variation of carotenoid concentrations within a species. Within a species, these differences could be negatively correlated with differences in UV-R sensitivity, suggesting that carotenoids can act in a photoprotectant manner, (probably by reducing the effects of reactive oxygen species). These results would support suggests by Miki et al. (1994) that scavenging of free radicals by carotenoids protect marine species from the harmful effects of bright sunlight. To directly examine the relative capacities of eggs to mitigate the effects of radical oxygen species further, chemical trap assays to quantify the removal efficiency of $^1\text{O}_2$ (Kim et al., 2001), or reduction of nitro-blue tetrazolium to quantify superoxide scavenging (Kim et al., 2001) could be employed on egg extracts.

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