

Different UV protective compounds in copepods occurring in desert sinkholes in response to UVR and fish predation

Wiebke J. Boeing¹ and Samuel Hylander²

¹Department of Fish, Wildlife and Conservation Ecology, New Mexico State University, Las Cruces, NM, 88003

²Institute of Ecology/Limnology, Lund University, Lund Sweden

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Abstract

Zooplankton accumulate several groups of photoprotective compounds to shield against damaging ultraviolet (UV) radiation. One of these groups, the carotenoids, however make the animals more conspicuous to visually hunting predators like fish, whereas others, such as the mycosporine-like amino acids (MAAs) do not. The blend of photoprotective compounds is therefore important for the UV defense but also for the ability to escape predation through crypsis. Here we use field data from twelve sinkholes in New Mexico to examine how UV, predation threat and pigment availability (in the food) affects the mixture of UV protective pigments in copepods. Overall the results show that copepods have more MAAs than carotenoids and that there is a positive relationship between carotenoid and MAA concentration. The blend of MAAs and carotenoids in copepods cannot be explained by the availability of MAAs in the food or the presence of visually hunting predators but mainly by the UV threat. Copepods up-regulate their MAA content (and carotenoid content) when UV threat is increasing.

Introduction

Pigmentation is a widespread phenomenon in nature with several important functions. One of the most obvious examples is chlorophyll that harvests the solar energy during photosynthesis. Other functions of pigments include vivid coloration in plants to attract pollinators or as deposits used as sexual ornamentation. For example, birds and fish deposit brown melanins and red carotenoids in their bodies to indicate high fitness (Niecke et al. 2003; Pike et al. 2007). Deficiency of pigments can however be detrimental, illustrated by the common salmon disease M-74 that may arise due to a deficiency of thiamine and carotenoids (Pettersson and Lignell 1999).

Aquatic zooplankton also display a wide range of pigmentation. In arctic and high altitude areas, for example, the common cladoceran *Daphnia*, sometimes has a melanized carapace suggested to work as a UV (ultraviolet) sunscreen (Hebert and Emery 1990; Hessen 1994; Hansson et al. 2007). Calanoid copepods on the other hand, typically have a red carotenoid pigmentation (Hairston 1979). These deeply colored populations are usually found at high latitude or altitude areas but pigmentation is also observed in temperate regions (Byron 1982; Hansson 2000; Hansson 2004). In a series of studies Hairston (1979) concluded that carotenoids mainly function as photoprotectants in high light environments, since carotenoids are antioxidants scavenging photoproducted radicals (Goodwin 1986). Zooplankton with lower levels of protective pigmentation indeed suffer from greater mortality when exposed to UV radiation (Ringelberg et al. 1984; Hessen 1996). It was also early established that large size and non-cryptic coloration is disadvantageous to zooplankton that are exposed to visually selective fish predators (Hrbáček 1961; Brooks and Dodson 1965; Hairston 1979). Recently the predation and photoprotection results have been integrated demonstrating that copepods are plastic in their pigmentation making a trade-off between high and low pigmentation in relation to the prevalent fish:UV threat ratio (Hansson 2004; Hylander et al. 2008).

Also other substances with UV protection abilities, the mycosporine-like amino acids (MAAs), have been observed in copepods (Sommaruga and Garcia-Pichel 1999; Hansson et al. 2007). These substances have an absorption maximum between 310 and 360 nm (Sinha et al 2007) and are thus invisible in visible light. Accumulation of MAAs can therefore be hypothesized to function as an alternative strategy, compared to the carotenoids, giving UV protection but not making the animals more conspicuous to visual hunting predators. The seasonal variation in the blend of MAAs and carotenoids adds to this hypothesis with high carotenoid concentrations in spring corresponding to a relatively high UV threat and low predation threat (Hansson 2004). But during summer, high MAA and low carotenoid concentrations have been observed which coincides with the highest fish predation threat during a season (Hansson 2004; Moeller et al. 2005; Persaud et al. 2007). MAAs have been detected in several zooplankton species including rotifers and copepods (Tartarotti et al. 2001) and it is well established that they function as sunscreens protecting against damage from harmful levels of UV radiation (Shick and Dunlap 2002). Like the carotenoids the MAAs have to be accumulated from the algal food since these substances are not produced de novo by animals (Goodwin 1986; Bandaranayake 1998).

In natural systems there is a strong positive correlation between altitude and zooplankton MAA as well as carotenoid concentrations (Byron 1982; Tartarotti et al. 2001). The increase in photoprotective compounds with altitude is interpreted as protection against increasing UV exposure. This is confirmed by a positive correlation between MAAs in zooplankton and the ratio between the 1% attenuation depth of UV and the maximum lake depth (Tartarotti et al. 2001; Tartarotti et al. 2004). The levels of carotenoids are usually highest in lakes without fish compared to lakes with fish predators (Hansson 2000). The levels of carotenoids, furthermore, fluctuate over the year with peaks in spring and autumn and these fluctuations are correlated with the prevalent UV to predation threat ratio (Hansson 2004).

Thus, the blend of carotenoids and MAAs in copepods is important for the UV defense but also the ability to escape predation through crypsis. The levels of both carotenoids and MAAs have in laboratory experiments been shown to be inducible and increase with increasing UV exposure (Moeller et al. 2005; Hansson et al. 2007). Upon exposure to fish cues the amounts of carotenoids however decrease (Hansson 2004; Hylander et al. 2008). In natural systems the blend of carotenoids and MAAs is not well described and only few studies have measured carotenoids and MAAs simultaneously and then only in a few lakes at a time (Moeller et al. 2005; Hansson et al. 2007; Persaud et al. 2007).

Here we focus on which factors regulate the blend of carotenoids and MAAs in calanoid copepods. Since the ability to show a plastic response to environmental change is likely to infer costs on fitness variables (DeWitt et al. 1998), we hypothesize that there would be an inverse relationship between the substances since it would be too costly to accumulate both substances simultaneously. We test whether carotenoids and MAAs are complementary substances; i.e., if one is high when the other is low.

Methods

A total of 12 sinkholes in New Mexico USA (33° N, 104°) were sampled. For chemical and physical characteristics of the sinkholes see table 1. Salinity levels of the sinkholes range from 4-29 ppt and DOC levels in the studied dry-temperate lakes ranged from 1.2 to 17.0 mg L⁻¹. The fish community is dominated by Pecos pupfish (*Cyprinodon pecosensis*), Pecos gambusia (*Gambusia nobilis*) and plains killifish (*Fundulus zebrinus*).

Sampling was performed from 28 July to 6 August, 2007. In each sinkhole calanoid copepods were collected for pigment analysis (carotenoids and MAAs) with net hauls (mesh size <300 μm) from a boat. Copepods were transported to the laboratory in filtered water for gut evacuation (Whatman GF/C) in a dark and cool bag. At the sinkhole,

surface water was pre-filtered through 50 μm (to remove zooplankton) and then 0.1–1.5 L was filtered through a GF/F filter (Whatman GF/F) for MAA and chlorophyll analysis in seston. From the same water a subsample for nutrients was taken. Finally, a sample for DOC was taken by filtering water through 0.2 μm in the subarctic and dry-temperate samplings (25 mm Membrane filters, Whatman) and through GF/F filters in the temperate sampling (Glass microfibre filters, Whatman). Samples for DOC, nutrients and chlorophyll were stored in -20°C and analyzed according to standard methods (Schimadzu 6500 TOC-analyzer; ICP MS ELAN-6000; Beckman DU 800).

In the laboratory live copepods were stranded on a filter (mesh size 5–10 μm) and under a stereo microscope two samples (for carotenoids and MAAs respectively) of 25–100 individuals each were collected from all lakes. The copepods were all calanoid copepods (*Eudiaptomus* sp.) with similar morphological features, i.e., evenly distributed pigment deposits and about 1 mm long (only adults and later stage copepodites, egg bearing females always excluded). Copepod and seston samples were then stored in -80°C until analyzed within seven months.

Carotenoid and MAA quantification followed standard methods (Tartarotti and Sommaruga 2002) and are described in detail in Hansson et al. (2007) and Hylander et al. (2008). In short, carotenoid samples were extracted in ethanol (95%) and quantified with a Beckman DU 800 spectrophotometer at 474 nm, the absorption peak for common carotenoids in copepods, i.e., astaxanthin and its esters (Hairston 1979; Hansson 2000, 2004). MAAs were extracted in 25% MeOH in water and were analyzed according to conventional methods in high-performance liquid chromatography (HPLC; Tartarotti and Sommaruga 2002; Tartarotti et al. 2004; and Hansson et al. 2007). The concentration of photoprotective compounds was normalized to dry weight, calculated from published relationships between length and dry weight for calanoid copepods (Persson and Ekbohm 1980). To assure that algal pigments

would not influence the results, the copepods were kept in tap water for at least 1 hour before sampling. No peaks were observed at the absorption maximum of chlorophyll (665 nm), indicating that gut evacuation had been effective and that carotenoids and MAAs from algae in the gut were not included in the analysis.

To get an estimate of the UV threat in each lake, defined here as the daily UV radiation at a depth of 0.2 m ($I_{0.1}$), we first calculated the intensities of UVA reaching the lakes during 24 hr using the TUV model (available online, http://cprm.acd.ucar.edu/Models/TUV/Interactive_TUV/) after corrections for the atmospheric ozone thickness using ‘Total Ozone Mapping Spectrometer’ (available online, http://toms.gsfc.nasa.gov/teacher/ozone_overhead_v8.html). To be able to account for previous UV exposure a mean radiation value was then calculated by taking the mean UVA radiation of the sampling date and of four prior days to the sampling date. For each simulation, sampling date, altitude, latitude and longitude and over head ozone column were varied to obtain a compound variable for the UV threat. Surface albedo was set to 0.1 in all calculations. Since UV radiation is attenuated to different extents in natural waters the daily UVA threat averaged over five days at a depth of 0.2 m was then finally calculated from $I_{0.1} = I_0 e^{(-K_{320} \cdot 0.1)}$ (Hansson 2004), where I_0 was the averaged daily UVA radiation (above the surface) estimated by the TUV model. The diffuse attenuation coefficient at 320 nm (K_{320}) and the 1% attenuation depth were calculated using the relationship between the absorption coefficient (A_{320}) and the diffuse attenuation coefficient (Kirk 1994; Morris et al. 1995). To obtain the absorption coefficient (A_{320}), water samples were analyzed for absorbance at 320 nm (Beckman DU 800 spectrophotometer).

As a proxy of fish predation pressure total fish density was calculated using mark-recapture estimates (Swaim 2008).

Statistical tests and calculations

Statistical differences in MAA and carotenoid content in copepods, as well as physical parameters were tested in a MANOVA analysis. Relationships were analyzed with linear regressions and correlations. In one case (Sinkhole 38) MAA analysis failed and therefore we only have a carotenoid reading from this lake. All analyses were performed in SPSS 15.0 for Windows.

Results

Field survey

MAA levels were high ($25.3 \mu\text{g mg dry wt}^{-1}$) and carotenoid concentrations low (mean of $0.8 \mu\text{g mg dry wt}^{-1}$) (Fig. 1). Copepod carotenoid levels ranged from 0.2 to $1.3 \mu\text{g mg dry wt}^{-1}$.

The relationship between MAA and carotenoid content in copepods was positive, i.e., when MAA concentrations were high the carotenoid concentrations also increased (Pearson Correlation, $r = 0.44$, $p = 0.04$, $n = 12$; Fig. 2).

Seston MAA concentrations ranged from not detectable to $24 \mu\text{g L}^{-1}$ and there was no significant relationship between MAA content in copepods and seston (Fig. 3).

When plotting MAAs over different UV threats (i.e., $I_{0.1}$) there was a positive relationship between MAAs and UV threat (Fig. 4; $r^2 = 0.430$, $F_{1,9} = 6.8$, $p = 0.03$).

For carotenoids there was no significant relationship between pigment and UV threat ($r^2 = 0.012$, $F_{1,10} = 0.1$, $p = 0.74$).

Mean lengths of copepods were 1.0 ± 0.1 mm (mean \pm 1 SD). There were also no significant relationships between dry weight specific content of carotenoids or MAAs and length ($r^2 < 0.001$, $F_{1,35} = 0.001$, $p = 0.98$; $r^2 = 0.069$, $F_{1,34} = 0.16$, $p = 0.69$ for carotenoids and MAAs over length, respectively).

Fish density for each sinkhole calculated from mark-recapture estimates ranged from 0 to 4.9 g fish m⁻³.

Discussion

Here we suggest that MAA concentration is high in our samples, while carotenoid concentration is negligible. Therefore, copepods are already adapted to overall high fish predation pressure, which explains the lack of relationship between carotenoids and fish density. MAAs are accumulated in copepods from food, however, we did not find a positive relationship between seston MAAs and copepod MAAs. This might suggest that MAA concentration in the food is not limiting copepod MAA accumulation. In our observed sinkholes, there was a positive relationship between copepod MAA concentration and UV threat, identifying UV radiation as the main environmental factor driving pigment accumulation in copepods.

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Table 1. Physical and biological parameters from the sampled sinkholes. All parameters are tested together with copepod MAA and carotenoid content in MANOVA analysis. *p*-values for multiple comparisons (Tukey's test) in the MANOVA are given.

Sinkhole	Altitude (m asl)	Z _{max} (m)	TN ($\mu\text{g L}^{-1}$)	TP ($\mu\text{g L}^{-1}$)	Chloro- phyll ($\mu\text{g L}^{-1}$)	DOC (mg L^{-1})*	Attenuation depth, 320 nm, 1%	Seston MAAs (ng L^{-1})
1	1070	5.5	959	10.0	2.7	9.2	0.97	9098
2	1070	5.6	667	n.a.	2.7	7.0	1.14	8426
7	1070	9.3	547	10.0	0.9	4.8	1.73	235
9	1070	6.2	838	20.0	1.6	8.4	0.75	2948
10	1070	2.9	1103	50.0	3.7	11.1	0.56	24056
11	1070	6.6	1581	40.0	1.1	11.2	0.39	12712
19	1070	3.3	2218	110.0	12.8	17.0	0.36	1589
20	1070	4.2	750	20.0	4.3	6.0	0.85	1838
32	1070	1.8	1122	20.0	1.1	11.7	0.30	17977
37	1070	14.5	497	40.0	0.5	3.0	0.90	1829
38	1070	2.1	826	40.0	2.1	1.2	8.00	1505
59	1070	4.3	222	20.0	0.5	2.5	0.97	3585
sign. (<i>p</i> -value)	n.a.	=0.002	= 0.36	= 0.05	< 0.001	= 0.22	< 0.001	< 0.001

* DOC from 2005

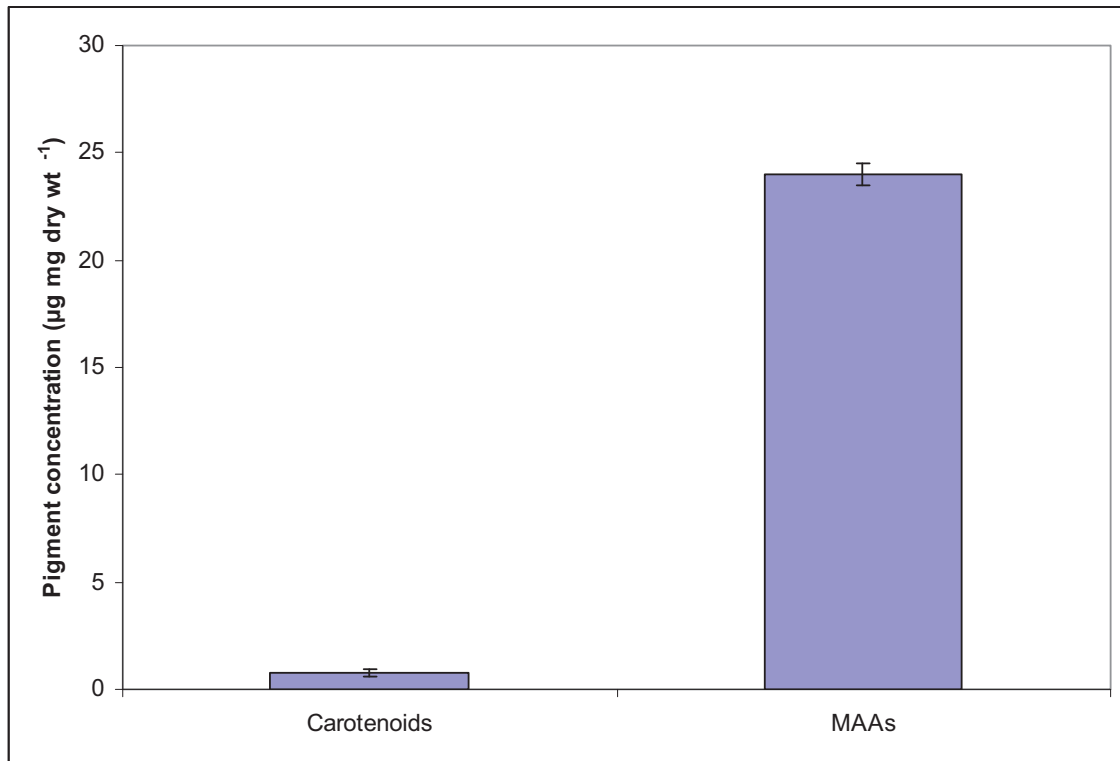


Fig. 1. Concentrations (± 1 SD) of carotenoids ($\mu\text{g mg dry wt}^{-1}$) and MAAs ($\mu\text{g mg dry wt}^{-1}$) in copepods in sinkholes in New Mexico ($n = 12$).

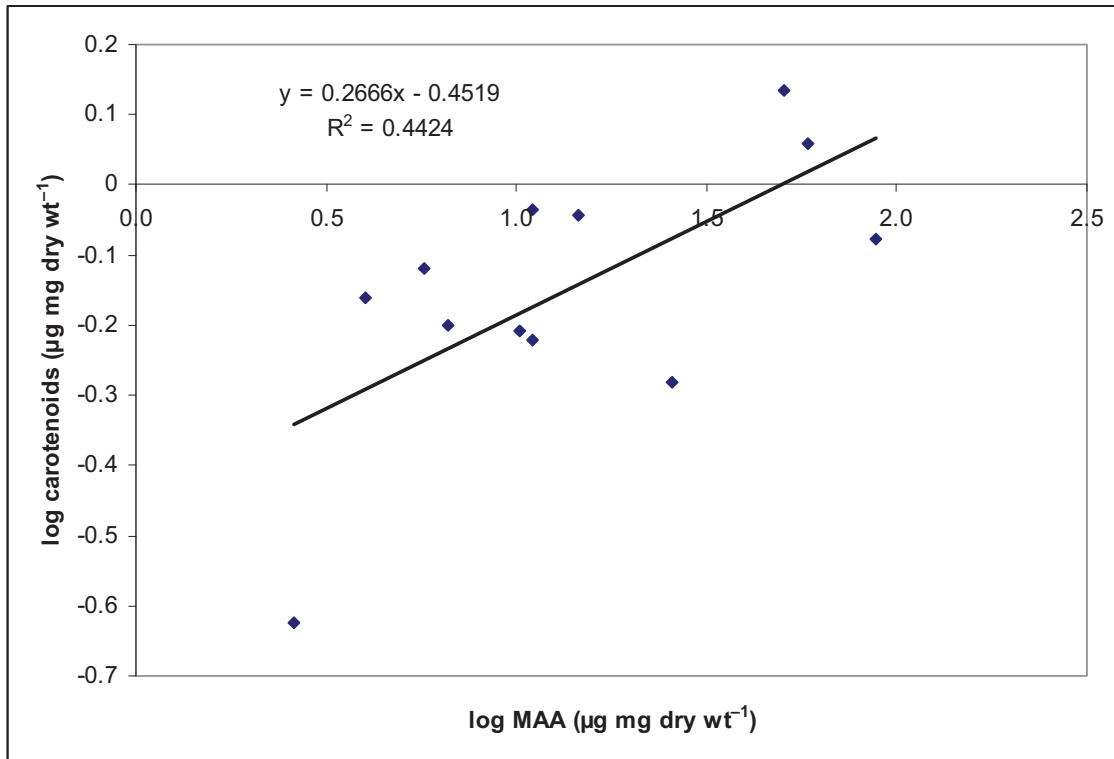


Fig. 2. Positive relation between MAA ($\mu\text{g mg dry wt}^{-1}$) and carotenoid ($\mu\text{g mg dry wt}^{-1}$) concentrations in copepods in New Mexico ($p=0.04$).

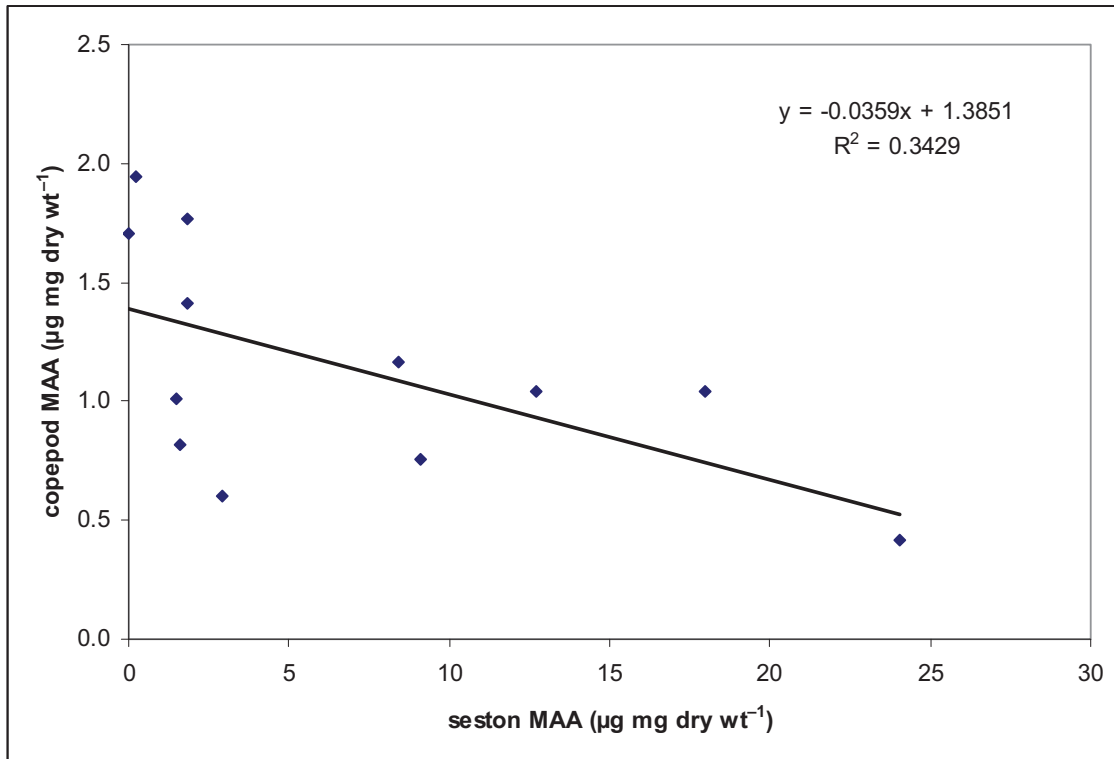


Fig. 3. Relationship between MAA concentration in copepods ($\mu\text{g mg dry wt}^{-1}$) and in seston ($\mu\text{g L}^{-1}$). There is a negative relationship between seston and copepod MAA concentrations ($p = 0.05$).

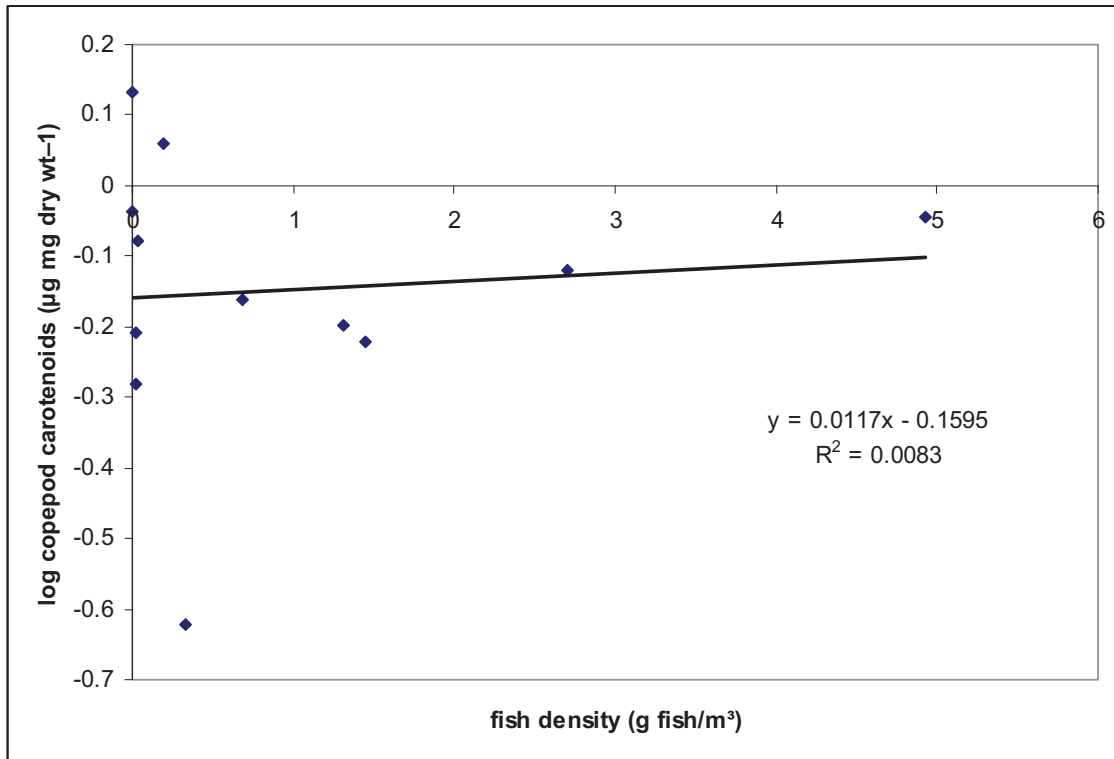


Fig. 4. Copepod carotenoid concentrations ($\mu\text{g mg dry wt}^{-1}$) at different fish threats. There is no significant relationship.

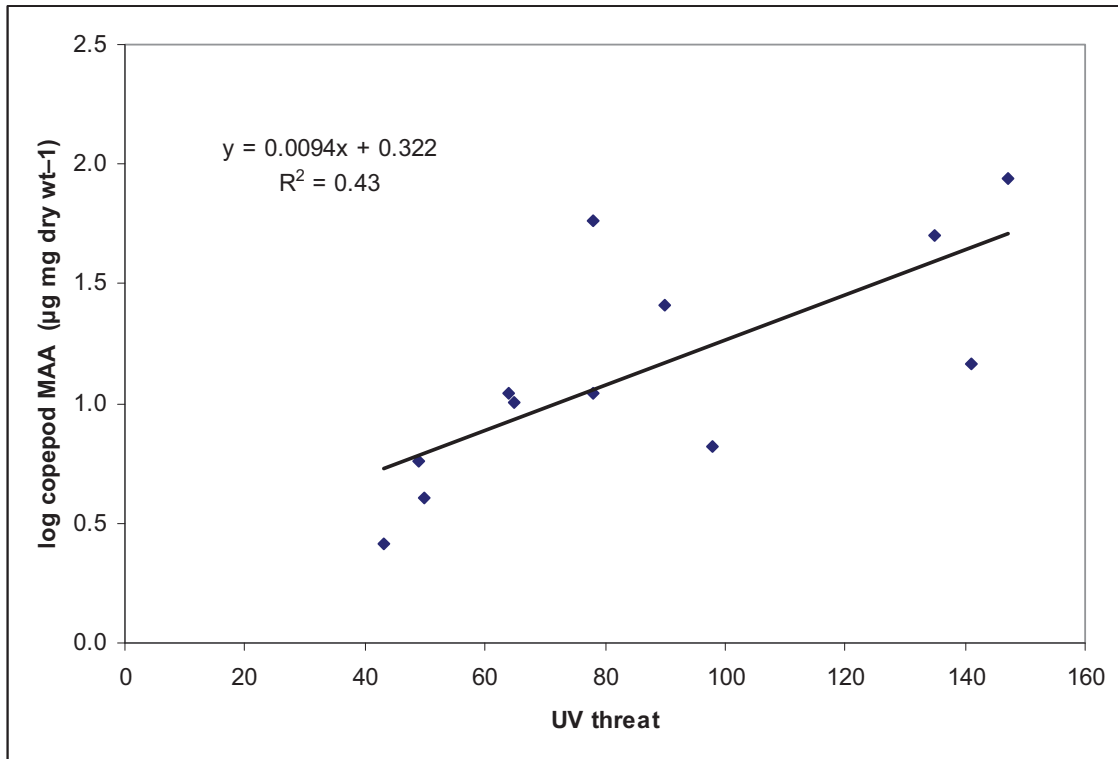


Fig. 5. Copepod MAA concentrations ($\mu\text{g mg dry wt}^{-1}$) at different UV threats. The positive relationship is significant ($p < 0.03$).