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Hansson, Lars-Anders

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PO Box 117
221 00 Lund
+46 46-222 00 00

Induced pigmentation in zooplankton: a trade-off between threats from predation and ultraviolet radiation

Lars-Anders Hansson

Institute of Ecology/Limnology, Ecology Building, Lund University, SE-223 62 Lund, Sweden (lars-anders.hansson@limnol.lu.se)

Ultraviolet (UV) radiation is harmful to all life, and the ongoing depletion of the ozone layer is likely to affect interactions among both terrestrial and aquatic organisms. Some organisms have evolved adaptations to reduce radiation damage, such as the various types of protective pigmentation of freshwater zooplankton. However, strong pigmentation also increases vulnerability to visually hunting predators. Hence, where both UV radiation and predation are intense, zooplankton may be sandwiched between conflicting selective pressures: to be pigmented and to be transparent at the same time. Here, I show that the level of pigmentation in copepods is up to ten times higher in lakes without predatory fishes than where fishes are present. Moreover, animals from the same population exposed to either UV light or predator scent showed a 10% difference in pigmentation after only four days, suggesting that pigmentation is an inducible trait. Hence, individual copepods are not passive victims of selective predation or radiation damage, but adjust the level of pigmentation according to the prevailing threat. The ability to adjust pigmentation level rapidly may be especially useful in situations where risk assessment is difficult due to strong seasonal and spatial variation in risk variables, such as in Arctic regions. With progressive thinning of the ozone layer, the ability of some but not other organisms to adjust protection against UV radiation may lead to counter-intuitive, large-scale alterations in freshwater food webs.

Keywords: pigmentation; copepod; zooplankton; ultraviolet radiation; predation

1. INTRODUCTION

Pigmentation in animals generally constitutes sexual ornamentation to attract partners or camouflage to reduce the risk of predation, but in some planktonic crustaceans pigmentation is used as a protection against harmful ultraviolet (UV) radiation. The pigment melanin, which functions as a sunscreen, is common in *Daphnia* spp. for example (Hebert & Emery 1990; Hill 1992), whereas copepods, crustacean zooplankters common in almost all freshwater and marine environments, use the red carotenoid astaxanthin as an antioxidant, which neutralizes free radicals formed when the animal is exposed to UV radiation (Hairston 1979; Siebeck *et al.* 1994). Such protective pigments may prove especially useful in lakes at high latitude or altitude where UV radiation is intense, and many zooplankters certainly tend to have high pigment concentrations in such lakes (Luecke & O'Brien 1981; Jarvis 1988). However, being intensely coloured increases the risk of mortality through predation from visually hunting predators such as fishes, as shown for example in pigmented versus transparent copepods (Hairston 1979; Luecke & O'Brien 1981; Utne-Palm 1999). The explanation generally presented as to why copepods are less pigmented in the presence of predators than in their absence is that selective predation occurs on pigmented morphs. Hence, in Antarctic and sub-Antarctic lakes, where UV radiation is intense but fish predation is absent due to biogeographical isolation (Jarvis 1988), copepods are bright red. On the other hand, in temperate lakes fish predation is generally high and UV radiation moderate and, accordingly, copepods tend to have low concentrations of

pigments. In Arctic lakes the rate of ozone depletion and, consequently, the UV radiation reaching the lake surface is as high as in Antarctica (Müller *et al.* 1997; Björn *et al.* 1998). Here, where predatory fishes are present in many but not all lakes, there should exist a trade-off between either being protected against predation or being protected against harmful radiation. This offers an ideal and unique situation in which to test the potential trade-offs in those factors influencing pigmentation.

Recently, it has been suggested that not only are direct consumer-prey interactions important in shaping aquatic food webs, but also that chemicals released by a consumer (kairomones) are used as information by prey, inducing behavioural or morphological defences (Tollrian & Harvell 1999). An inducible defence, in contrast to a constitutive defence, is only activated when needed; that is, when the organism receives cues from the environment indicating that the defence should be activated. Well-known and striking morphological defences in zooplankters induced by predators are helmet or neck-teeth formation in cladocerans (Tollrian 1995) and spine formation in some rotifer species (Stemberger & Gilbert 1987). Pigmentation is a functional trait in situations where UV radiation is intense, but this response should not occur in the presence of predators. Hence, a plausible hypothesis would be that not only selective predation on pigmented individuals, but also individual adjustment in the level of pigmentation are mechanisms behind the considerable variation in pigmentation among copepods. This hypothesis was tested in extensive field studies in Arctic Canada and Nunavut, and in temperate Swedish lakes, complemented with a laboratory experiment.

2. MATERIAL AND METHODS

The Arctic field study was performed during the Swedish Tundra Northwest expedition in August 1999, on board the Canadian Coast Guard icebreaker *Louis S St. Laurent*. Zooplankton samples were taken from 16 lakes (six with fishes and ten without fishes), ranging in latitude from 69° N to 78° N (Canadian mainland to the Magnetic North Pole) and in longitude from 65° W to 139° W (Baffin Island to Ivvavik). Samples were taken in surface water (depth 0–0.5 m) close to the shore using a zooplankton net (diameter 300 µm, length 500 mm, mesh size 300 µm) mounted on an expandable handle (2 m). The net was swept back and forth while walking along the shore for about 5 min, which theoretically, assuming no net resistance, would have sampled about 13 000 l, ensuring that a sufficient subsample of surface water was taken. Samples were generally taken between 09.00 and 16.00. As a comparison, copepods were also sampled, using the same method, in temperate Swedish lakes; eight with fishes and four without fishes. In the Arctic study, almost all copepods were calanoids and the majority of these belonged to the genus *Diaptomus*, whereas the animals in the Swedish study were a mixture of calanoid and cyclopoid copepods. In both studies, the animals were transported live to the laboratory where a subsample of about 100 individuals was put into a plastic scintillation vial (25 ml) containing 8 ml of ethanol (96%) as an extraction solvent. The zooplankters were then immediately disintegrated with a Heidolph Diax 900 disintegrator (Heidolph, Kahlheim, Germany) and extracted for 12 h at room temperature. Samples were then centrifuged for 5 min at 3000 rpm. Quantification of the extracted red pigment in the supernatant was performed using a Cadas 100 spectrophotometer (Dr Lange, Berlin, Germany) at 474 nm, which is the absorbance peak for the active pigment (Hairston 1979). Since variances in copepod pigment content were not similar between lakes with and without fishes, differences were tested using the non-parametric Mann–Whitney *U*-test.

In the laboratory experiment, I used a 2 × 2 factorial design with high and low light stress crossed with addition, or no addition, of fish exudates (predator cue). Each of the four treatments was replicated eight times giving 32 experimental aquaria (volume 5 l, diameter 22 cm). At the start of the experiment, I added about 200 *Diaptomus* sp. from a population sampled near Pond Inlet (Baffin Island, Nunavut) to each aquarium. Prior to the addition, animals were mixed in a large bucket and subsamples were taken out and added randomly to each aquarium, ensuring that there were no differences in pigmentation between treatments at the start of the experiment. The experiment was conducted in an outdoor-container laboratory on board the ship, allowing temperature to fluctuate naturally (2–7 °C). Fish exudates (mimicking the presence of predatory fishes) were supplied from an aquarium containing five nine-spined sticklebacks (*Pungitius pungitius* L.) caught in the area (Banks Island). The fishes were fed zooplankters a few hours before the exudates were taken out. Once a day, 25 ml of fish exudates were taken out, filtered through a 10 µm net and added to each fish-treatment aquarium. Since zooplankton pigments are derived from algal food (Ringelberg 1980), animals were fed a culture of small, easily accessible algae (*Rhodomonas* spp. and unidentified green algae), ensuring that food shortage did not occur during the experiment. The similar nutritional status between treatments excludes food as a factor affecting the results of the experiment. Two levels of light stress were used: low-light-stress treatment

Table 1. *Experimental light intensities and treatment times in treatments with high (UV radiation) and low (visible light) light stress*

(Times are expressed as percentages of total experimental time. PAR, photosynthetically available radiation, µmol m⁻² s⁻¹; UVA, 320–400 nm, W m⁻²; UVB, 280–320 nm, W m⁻². For comparison, natural light intensities measured under a clear sky are also given for temperate Swedish (Granéli et al. 1998) and Arctic conditions.)

	PAR	UVA	UVB	darkness
high light stress				
intensity	15.7	2.9	0.4	—
time	9	91	91	0
low light stress				
intensity	15.7	0.0	0.0	—
time	69	0	0	31
natural conditions				
Sweden	400	30	0.2	
Arctic	900	15	1.0	

with warm white lamps in combination with plexiglass lids covering the experimental containers, allowing only visible light to reach the animals; that is, photosynthetically available radiation (Röhm GS 233 Plexiglass, Germany; table 1). Moreover, for 31% of the experimental time, the light was turned off to reduce the light stress further. In the high-light-stress treatment, experimental containers were covered with UV-transparent Plexiglass (Röhm GS 2458), allowing all wavelengths to penetrate. This exposed the animals to both UV (91% of the experimental time) and visible light (table 1). Intensities of UV light throughout the experiment were similar to, or lower than, in natural conditions (table 1). Light measurements were made using an IL 1400A radiometer (International Light, Newburyport, MA, USA; table 1). At the end of the experiment, animals were collected using a 300 µm net, counted and pigments were extracted in the same way as in the field study. Differences between treatments were tested using ANOVA and Fisher's PLSD contrast analysis.

3. RESULTS AND DISCUSSION

In the 16 Arctic lakes included in this study, the mean pigmentation was more than seven times higher in lakes without fishes compared to those with fishes ($Z = 3.254$, $p < 0.002$, $n_{\text{fishes}} = 6$, $n_{\text{no fishes}} = 10$, Mann–Whitney *U*-test; figure 1). A similar comparison between temperate Swedish lakes with and without fishes showed that copepods were only three times more pigmented in the absence than in the presence of fishes ($Z = 2.717$, $p < 0.007$, $n_{\text{fishes}} = 8$, $n_{\text{no fishes}} = 4$, Mann–Whitney *U*-test; figure 1). These comparative field data show that in the presence of fish predators, copepod pigmentation is low and similar in temperate and Arctic lakes, whereas in fish-free lakes, copepods are less pigmented in temperate than in Arctic areas (figure 1). This is most likely to be a result of higher UV intensity in the Arctic. Although seldom explicitly stated or tested, the traditional explanation for low pigmentation levels in the presence of fishes is selective predation on pigmented individuals (Hairston 1979; Luecke & O'Brien 1981). However, the observations from

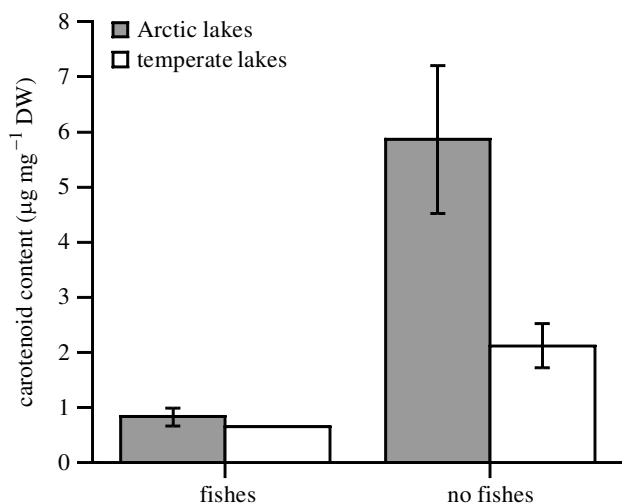


Figure 1. Copepod pigmentation in natural systems. Carotenoid concentration ($\mu\text{g mg}^{-1}$ dry weight) in temperate lakes (white bars; $n_{\text{fishes}} = 8$, $n_{\text{no fishes}} = 4$) and Arctic lakes (grey bars; $n_{\text{fishes}} = 6$, $n_{\text{no fishes}} = 10$). Mean values \pm s.e.m. are given for lakes with and without fishes. Note that the s.e.m. for temperate lakes with fishes is lower than can be seen on the graph.

the field survey suggest the intriguing hypothesis that the level of pigmentation in a specific population of copepods is not only a result of selective predation but may also be an inducible defence adjusted according to the trade-off between the risks of predation and harmful radiation.

To test whether this is the case, I performed a complementary laboratory experiment where copepods from one population of the calanoid *Diaptomus* sp. were exposed to high light stress (UV + visible light) or low light stress (visible light + darkness) crossed with the presence or absence of cues from a predator. To test whether any response in terms of pigmentation occurs within an individual's lifetime, I chose a short experimental period (95 h). At the end of the experiment, copepods differed significantly in pigmentation between treatments ($F_{3,28} = 8.038$, $p < 0.001$, ANOVA; figure 2). In the absence of predators, pigmentation in animals suffering from high light stress ('UV') was almost 7% higher than in animals exposed to low light stress ('v') ($p < 0.004$, Fisher's PLSD contrast; figure 2). Furthermore, in the treatment with high light stress crossed with increased predator risk ('UV-F'), animals reduced their pigmentation ($p < 0.007$, Fisher's PLSD contrast) to levels similar to those at low light stress ($p > 0.80$, Fisher's PLSD contrast; figure 2). The mean difference in pigmentation between the two extreme treatments, where copepods received either a clear indication of high light stress and no indication of predator presence ('UV') or a clear indication of predator presence and no light stress ('v-F'), was as high as 10% ($p < 0.0001$, Fisher's PLSD contrast). Given the short duration of the experiment, these data clearly illustrate that pigmentation is rapidly adjusted to the present risk within the lifetime of an individual and that these animals are receptive to light cues as well as to cues from predator exudates.

UV radiation may be less of a problem in deep than in shallow lakes since zooplankters are able to migrate on a daily basis, thereby escaping from the high UV intensities

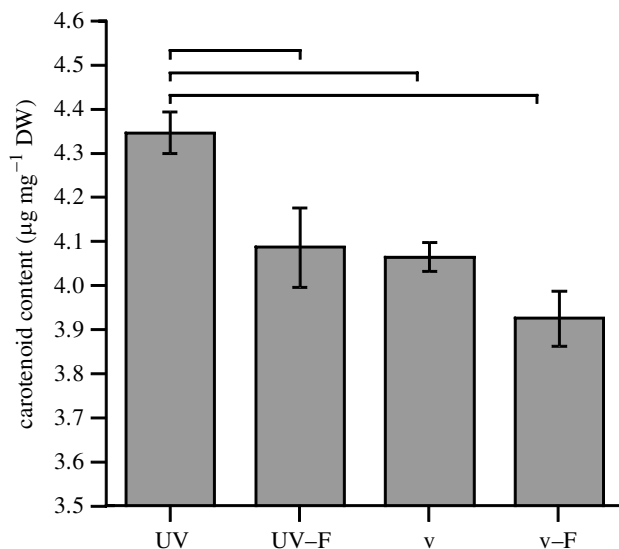


Figure 2. Experimentally manipulated copepod pigmentation. Bars show mean carotenoid content \pm s.e.m. of the copepod *Diaptomus* sp. at the end of the experiment after the animals had been subjected to different light and predator cues. Treatments are UV, ultraviolet radiation (high light stress); F, fish exudates added; v, visible light and darkness (low light stress). Brackets indicate significant differences between treatments (Fisher's PLSD, $p < 0.01$). DW, dry weight.

in surface waters (Hairston 1980; Byron 1982). This notion suggests that pigmentation should be less necessary in deep than in shallow lakes and, consequently, that there should be a negative relationship between maximum depth and pigmentation. This was found to be the case in the lakes investigated in this study ($r = -0.74$, $t_{26} = 5.719$, $p < 0.001$; figure 3). However, separating lakes with and without fishes removes the relationship ($r < 0.26$, $t < 0.97$, $p > 0.20$), suggesting that depth as such does not determine the level of pigmentation. To test whether the level of pigmentation is only a result of maximum lake depth (refugium from UV radiation), or whether predation risk is also involved, it would have been necessary to include deep lakes without fish predators. Unfortunately, such lakes are rare in the Arctic, since they never freeze to the bottom, allowing fishes to survive the winter, and none were found during the present expedition. However, 12 (43%) out of the 28 investigated lakes overlap in maximum depth (shaded area in figure 3) covering a depth interval of 2–9 m. If depth (refugium) had been the only explanation for the level of pigmentation in copepods, we would have expected a random distribution among lakes with and without fishes within this overlapping depth interval. This was not the case: copepods in lakes with fishes had a significantly lower pigment level than those in lakes without fishes ($Z = 2.928$, $p < 0.004$, Mann-Whitney U -test, $n_{\text{fishes}} = 8$, $n_{\text{no fishes}} = 4$; figure 3). Hence, depth may function as a refugium from photodamage where animals can escape the intense UV radiation in surface waters (Hairston 1980; Luecke & O'Brien 1981), allowing them to reduce investment in pigments. However, the field data also show that if fishes are absent copepods have a higher level of pigmentation than if fishes are present, irrespective of lake depth.

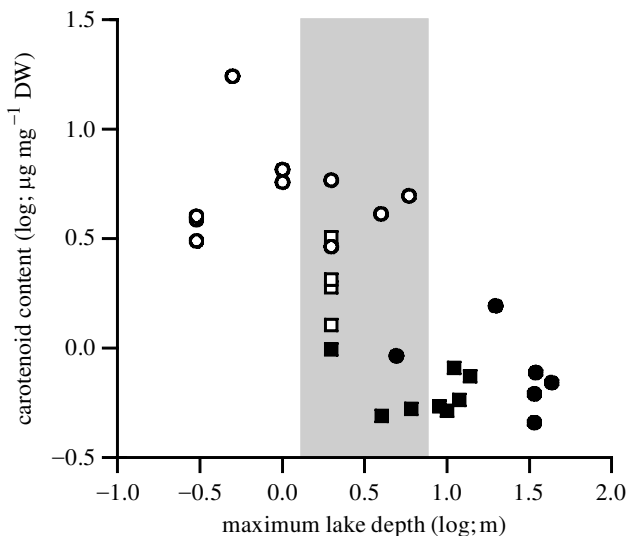


Figure 3. Copepod pigment levels in natural systems of different depths. Maximum depth (log; m) versus copepod carotenoid concentration (log; $\mu\text{g mg}^{-1}$ DW) in lakes with fishes (closed symbols) and lakes without fishes (open symbols). Squares indicate Swedish lakes and circles indicate Arctic lakes. DW, dry weight.

Olfactory reception of chemical cues exuded by a consumer (kairomones) resulting in induction of morphological or behavioural defences has recently received considerable attention, especially with respect to predator-prey relationships (Harvell 1990; Brönmark & Miner 1992; Krieger & Breer 1999; Tollrian & Harvell 1999; Brönmark & Hansson 2000), as well as in herbivore-plant interactions (Agrawal 1998; Agrawal *et al.* 1999; Hansson 2000). The existence and perception of a 'chemical network' of information about hazards in the environment and the proper response by prey organisms to such information opens up new perspectives for food-web theory and modelling of population dynamics (Hansson 1996, 2000), which, at present, are almost entirely based on direct encounters between consumers and prey.

All organisms on earth are continuously faced with multiple sources of information. Despite this, most studies deal only with a single stimulus, and may therefore not tell the whole story about the cause of a response. When exposed to multiple sources of conflicting information, an organism may respond differently from when it is exposed to one stimulus at a time. One such example is the response of a crayfish species (*Orconectes virilis*) exposed to both predator and food cues, which shows that the crayfish adjusts its behaviour to the relative strength of the two cues (Hazlett 1999). My results show that in both temperate and Arctic regions, copepods adjust the level of pigmentation to meet the threats from two lethal factors: predation and UV radiation. Hence, the level of pigmentation in copepod populations is influenced not only by direct predation, but also by more subtle, indirect processes such as assessment of current levels of harmful radiation and predation risk. From an evolutionary perspective, adaptations arising from the use of multiple sources of information, be they predation risk, food cues or risk of UV-radiation damage, are likely to be crucial fitness variables.

The observation that some organisms, such as copepods, are able to adjust their protection against harmful UV radiation is of considerable evolutionary interest but may also have large-scale implications related to the thinning of the ozone layer (Björn *et al.* 1998). Particularly in clear-water lakes, which are common in polar regions, UV penetration is considerable (Schindler *et al.* 1996) and an accelerating ozone depletion is therefore likely to strongly affect all aquatic organisms. However, organisms with inducible defences against UV radiation, such as crustacean copepods, will not be as severely affected as their competitors or predators, which may result in counterintuitive alterations in dominance patterns and food-web interactions (Bothwell *et al.* 1994; Vinebrooke & Leavitt 1999). Hence, we may begin to disentangle mechanisms suggesting which organisms are likely to become dominant in future ecosystems assuming that the anthropogenic effects on natural systems are not reduced.

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