

Physiological and morphological colour change in Antarctic krill, *Euphausia superba*: a field study in the Lazarev Sea

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SUMMARY

Antarctic krill, *Euphausia superba*, is very susceptible to harmful solar radiation because of its unique genetic setup. Exposure occurs in spring to autumn during vertical diel migration and during occasional daytime surface-swarming. We have investigated colour change in Antarctic krill, *Euphausia superba*, during summer and winter in the Lazarev Sea in response to ultraviolet radiation (UVR) and photosynthetically active radiation (PAR). Short-term physiological colour change and long-term (seasonal) morphological colour change are present. Both are facilitated by a single type of monochromatic red chromatophore, i.e. erythrophones, of 20–450 µm diameter. Superficial erythrophones cover large dorsal areas, especially above vital organs (brain, sinus glands), additional ‘profound’ erythrophones cover internal organs (heart, gut, nerve cords). Short-term change in light regime causes rapid physiological colour change along dense bundles of microtubules: pigment disperses into chromorhizae upon exposure to PAR and UVA and to a lesser extent to UVB. Darkness leads to aggregation of pigment in the centre and hence blanching. There is no circadian rhythm in the dispersal state of erythrophones present in winter. Physiological colour change in adult krill is two to three times more rapid in summer than in winter. Furthermore, seasonal changes in light regime also result in a profound morphological colour change: in summer animals, abdominal astaxanthin concentration is 450% and erythrophone count is 250–480% higher than in winter krill. We conclude from our results, that pigmentation of *E. superba* serves in the protection from harmful solar radiation and is adapted to the varying diel and seasonal light conditions.

Key words: colour change, chromatophore index, UV protection, pigmentation, astaxanthin, erythrophone.

INTRODUCTION

Antarctic krill (*Euphausia superba*) – a key organism in the trophic web of the Southern Ocean – leads a life in twilight. This culminates in almost complete darkness in Antarctic winter, when the sun reaches a low zenith or is absent, the days are very short and sea ice covers most of the krill’s habitat. During the transitional seasons and in summer, juveniles, sub-adults and adults perform more or less regular diel vertical migrations, spending the day in depths of 50 m or below (i.e. in relative darkness) and the night close to the surface layer (Nicol, 2006). Such migration pattern is thought to be a result of environmental factors such as avoidance of predation and food availability but also to avoid potentially harmful radiation. Krill is particularly susceptible to ultraviolet radiation (UVR; 280–400 nm) as it results in DNA damage (Jarman et al., 1999) and increases mortality (Newman et al., 1999). Ultraviolet radiation B (UVB; 280–320 nm) is the more harmful than UVA (320–400 nm) and photosynthetically active radiation (PAR; 400–700 nm) in this regard. Recent research provided evidence that *E. superba* responds to solar radiation by moving away from the light source (Newman et al., 2003), ensuring a safe distance from UVR.

Owing to the high attenuation of UVR by the seawater column (Jerlov, 1976), the vertical migration pattern is a very effective avoidance of damaging radiation. The migration is not completely regular, however, and large surface swarms of krill have been observed occasionally during day light (e.g. Marr, 1962; Ozawa et

al., 1968; Kubotka, 1981). It is during such periods, and during ascent and/or descent, when krill is exposed to a significant portion of UVR, which can penetrate clear Antarctic waters to at least 20 m (Jerlov, 1976; Karentz and Lutze, 1990; Ban et al., 2007). Owing to the loss of stratospheric ozone, the amount of UVR reaching the surface of the oceans, especially UVB, has increased in the recent decades (Solomon, 1990; Smith et al., 1992) and so should have exposure of krill to harmful UVR.

In addition to behavioural changes, crustaceans can respond to varying light conditions with two types of colour change: (1) physiological colour change (chromomotor change) and (2) morphological colour change (chromogenic change). Physiological colour change is a relatively fast movement (seconds to minutes) of pigments in specialised cells. These chromatophores of vertebrates and invertebrates enable fast pigment movement within their limits. Many crustaceans contain clusters of differently coloured chromatophores (erythrophones, leucophores, melanophores and xanthophores, which are red, white, black and yellow, respectively). These clusters are called chromatosomes, which facilitate, together with diffuse pigmentation around them, adaptation to a wide range of background colours for camouflage (Noël and Chassard-Bouchard, 2004). The enclosure of pigments, such as astaxanthin, in chromatophores guarantees their fast movement over a defined area of the animal’s surface and enables adaptation to background colours and protection from solar radiation. Pigment granules can

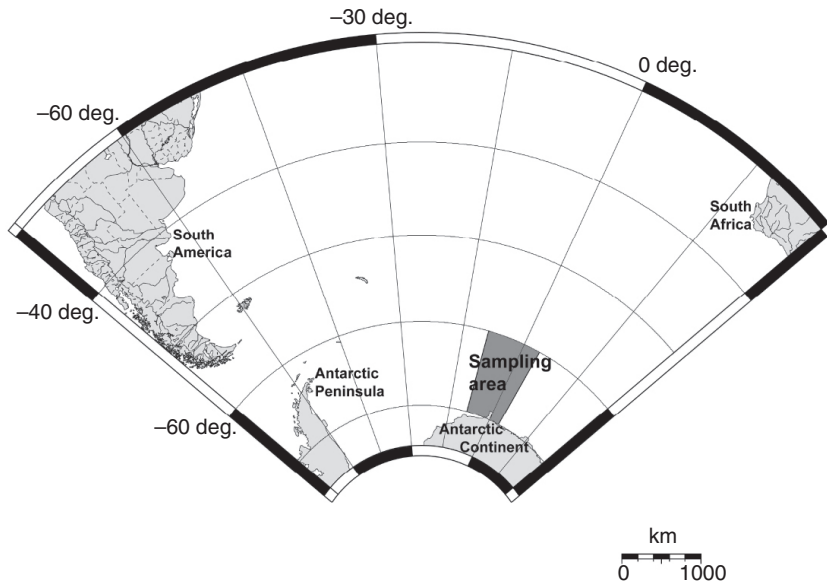


Fig. 1. Study area in the Lazarev Sea (dark grey).

The present field study was therefore aimed at providing initial information on aspects of colour change in Antarctic krill. They were accumulated during observations and experiments onboard a summer and a winter cruise in the Lazarev Sea and are discussed in the context of exposure of krill to environmental conditions.

MATERIALS AND METHODS

Animals

Adult and sub-adult male *Euphausia superba* Dana were caught during the expeditions of R.V. Polarstern in the Lazarev Sea in austral summer 2005–2006 (ANT-XXIII/2; 15.11.2005–12.01.2006) and winter 2006 (ANT XXIII/6; 11.06.2006–27.08.2006). The sampling area (Fig. 1) was in the region of 60–69°S and 3°E–6°W (summer) and 60–69°S and 3°E–3°W (winter). In summer, krill was sampled by rectangular midwater trawl (RMT) from depths of between

200 m and the surface whereas in winter, in addition, a submersible under-ice trawl (SUIT) was employed to catch krill from directly under the sea ice. Physiologically viable specimens were selected from the catch and immediately transferred into plastic containers containing 60 litres filtered (0.45 µm) seawater and stored at $-1 \pm 1^\circ\text{C}$. Prior to experimentation, individuals were transferred into 50 ml containers with the same water conditions and were kept without food in complete darkness for at least 24 h prior to experimentation. Freshly caught krill was immediately frozen in liquid N_2 and stored at -80°C for analysis of astaxanthin concentration and chromatophore count later.

Physiological colour change

Experiments were generally executed at a water temperature of $0\text{--}1^\circ\text{C}$ with no light except the experimental illumination and a dim microscope light present. The same group of lateral chromatophores on the 5th abdominal segment of each individual krill was observed in all experiments.

Experimental illumination

Light quality and quantity was determined using a Li-250 light meter (for PAR; LiCor Biosciences, Lincoln, NE, USA) and a UVA/B radiometer PMA 2100 V 1.19 (Solar Light Company, Glenside, PA, USA). Experimental illumination was provided by 8 W fluorescent tubes. At a distance of 20 cm, the PAR lamp (Snode CW) emitted 0.11 mW cm^{-2} of visible light, 0.005 mW cm^{-2} of UVA radiation and no UVB radiation. The UVA tube (Silvania BL350) produced 0.01 mW cm^{-2} PAR, 0.1 mW cm^{-2} UVA and 0.003 mW cm^{-2} UVB. The UVB tube (Vilber Lourmat VL-8 M) produced 0.001 mW cm^{-2} PAR, 0.005 mW cm^{-2} UVA and 0.211 mW cm^{-2} UVB. Grafix™ cellulose acetate film (Grafix, Cleveland, OH, USA) was used to filter out UV radiation of wavelengths lower than 293 nm when the UVA and UVB tubes were used. The comparison of physiological colour change in winter and summer was conducted at 0.05 mW cm^{-2} PAR, 0.001 mW cm^{-2} UVA and no UVB. Similar size adults [carapax length (CL), summer: $13.4 \pm 1.0 \text{ mm}$; winter: $13.6 \pm 1.9 \text{ mm}$] were used.

Staging of chromatophores

For the investigation of a possible physiological colour change, a standard for krill was established against which dispersal or

be transported towards the centre, so concentrating them and producing 'blanching' or can be spread out into extensions (chromorhizae) of the cell, dispersing them and producing colouration. The granules move along microtubules of the cytoskeleton with the help of kinesin and myosin protein motors (Boyle and McNamara, 2005). Pigment movement inside crustacean chromatophores is under endocrine control. Upon reception of an environmental trigger (e.g. light), this is facilitated by the release of antagonistic chromatophorotropic hormones from the sinus gland in the eyestalk: the octapeptide RPCH (red pigment-concentrating hormone) triggers pigment aggregation whereas the octadecapeptide(s) PDH (pigment-dispersing hormone) leads to the dispersion of pigments in the chromatophores (see Rao, 2001; Kwok et al., 2006).

Morphological colour change is defined as a protracted (e.g. seasonal) increase and/or decrease in (1) pigment concentration, (2) chromatophore count or (3) a combination of both (Green, 1964). Physiological and morphological colour changes are interlinked: When the pigment remains dispersed or concentrated for an extended period (days, months) due to prolonged stable light or background conditions, the concentration of pigment and/or the number of chromatophores increases or decreases. This relationship was postulated by Babak (Babak, 1913) and has therefore become known as Babak's law.

Colouration of krill has been used in the fishing industry as an indicator for quality (Kawaguchi and Nicol, 2007): 'Red' krill (as compared with 'white' and 'pink' krill) is caught during summer and from close to the surface and is red because of its overall pigmentation. It is considered lower quality than white krill caught in great depth and white 'winter krill'. The 'reddishness' is obvious when looking at pictures of surface-swarming krill as well of freshly caught krill [e.g. fig. 4b in Kawaguchi and Nicol (Kawaguchi and Nicol, 2007)]. The red colour is caused by the carotenoid astaxanthin, the dominant pigment in *E. superba* (Grynbaum et al., 2005) and *E. pacifica* (Funk and Hobson, 1991), and its esters. Astaxanthin is thought to be responsible for photo-protection in crustaceans (Green, 1966; Gilchrist and Lee, 1972; Hairston, 1979).

Phenomena of colour change have only been studied in a few of crustaceans (Noël and Chassard-Bouchard, 2004), most of them decapods. Data are scarce for Antarctic krill and other euphausiids.

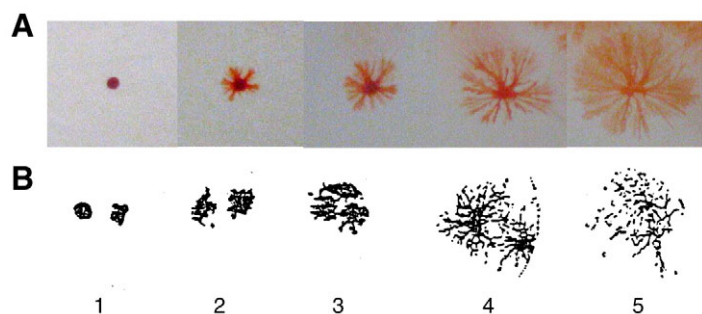


Fig. 2. Chromatophore index (CI) of (A) an erythrochore from the fifth abdominal segment of a sub-adult krill and, for comparison, (B) of the frog, *Xenopus laevis*, taken from Hogben and Slome (Hogben and Slome, 1931). The CI ranges from 1 (pigment completely aggregated) to CI 5 (pigment fully dispersed).

concentration of pigment inside the chromatophores was estimated (Fig. 2A). The resulting chromatophore index (CI) is based on that suggested previously (Fig. 2B) by Hogben and Slome (Hogben and Slome, 1931) for a frog. The index uses stage 1 (pigment fully concentrated in centre of cell) to stage 5 (pigment fully dispersed into chromorhizae) with three stages spaced in-between. For better resolution, half stages were used during observations (not depicted).

For photographs of physiological colour change, individual krill was placed in a dissecting dish (water temperature $\sim 1^{\circ}\text{C}$) and held in position by insect pins without harming the animals. Pictures were taken using a Stemi DV 4A DR microscope (Carl Zeiss) equipped with a Canon PowerShot G5 digital camera. Single chromatophores were photographed using an Axiovert 135 microscope equipped with an Axiocam MRc 5 digital camera (Carl Zeiss).

Morphological colour change

Chromatophore count

Chromatophores on the fourth, fifth and sixth abdominal segment of similar-size male adults (CL: 13.9 ± 1.2 mm winter; 14.1 ± 1.5 mm summer) and sub-adults (CL: 10.3 ± 1.2 mm winter; 10.6 ± 1.2 mm summer) were counted under a dissecting microscope (Zeiss Stemi DV 4A DR) after the animals had been exposed to light (to improve visibility of chromatophores).

Astaxanthin content

Standardisation of astaxanthin quantification was necessary: its concentration in krill is gender and age specific (Jackowska et al., 1980; Funk and Hobson, 1991). Moreover, eyestalks contain large amounts of astaxanthin (Maoka et al., 1985; Funk and Hobson, 1991). In addition, the stomach of (feeding) summer krill most probably contains more ingested pigments than that of non-feeding winter krill. To keep the influence of such variations low, only abdomens of male sub-adults of similar size (see above) were chosen



Fig. 3. Light-exposed (top) and dark-adapted (bottom) sub-adult male krill. Scale bar, 5 mm. Arrows indicate the 5th abdominal segment that was used in most experiments.

and analysed separately. They were ground to a fine powder under liquid N_2 and astaxanthin was subsequently extracted twice in 100% methanol. The supernatants were combined and analysed by HPLC according to the method of Auerswald and Gäde (Auerswald and Gäde, 2005) using synthetic astaxanthin (Sigma, St Louis, MO, USA) as standard.

RESULTS

Physiological colour change

Exposure of adult and sub-adult *E. superba* to PAR causes a reddening of the animal whereas dark-adaptation causes blanching (Fig. 3). Microscopic investigation of *E. superba* colouration (Fig. 4) revealed that the pigment is contained exclusively in bright red chromatophores: i.e. erythrochores. No halo of diffuse pigment was found around the erythrochores. Erythrochores are present in various body regions. The highest concentration is found, however, in the integument of the dorsal part of the animals whereas the ventral part is almost free of them. In addition, some erythrochores are present internally and known as 'profound' (deep-lying) chromatophores. The size of erythrochores ranges from

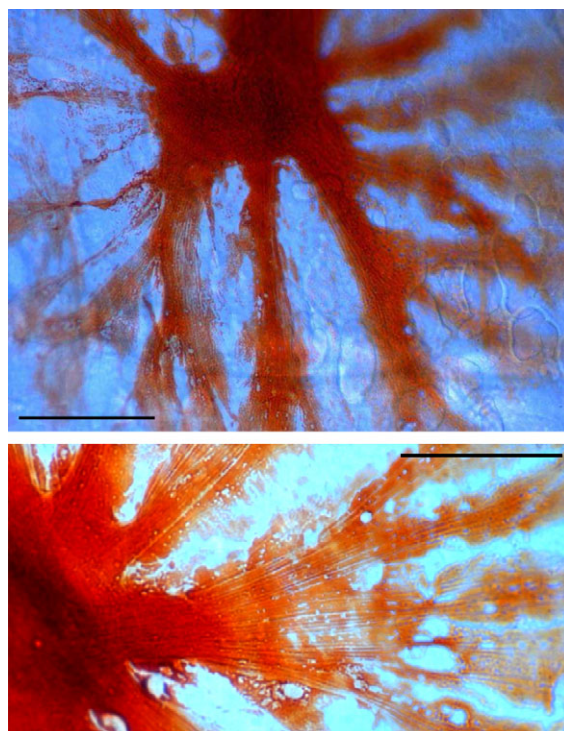


Fig. 4. Single chromatophores from the 5th abdominal segment of a sub-adult male krill (cuticle removed from abdomen) at CI 4–5. Scale bar, $50 \mu\text{m}$.

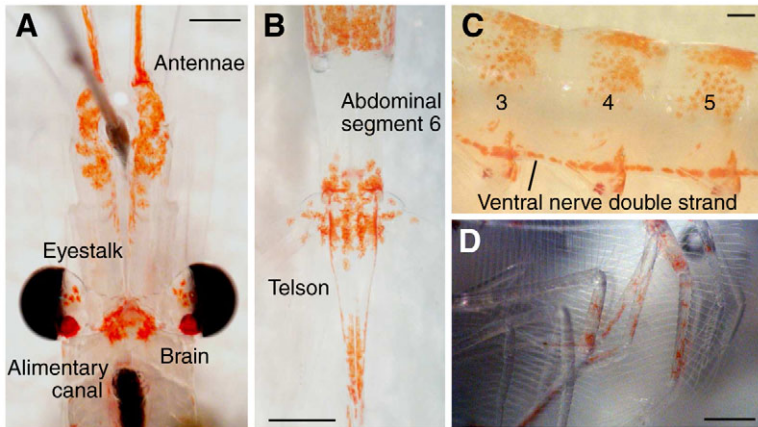


Fig. 5. Distribution of chromatophores at a sub-adult *E. superba*. (A) Dorsal view of the anterior region and (B) last two abdominal segments and telson; (C) lateral view of the third to fifth abdominal segments (numbered from the thorax) and (D) of thoracopods of the filtering basket. Scale bars, 2 mm.

approximately 20 to 450 μm diameter and they are characterised by a high density of microtubules which are visible when pigment is dispersed (Fig. 4, lower panel). Erythrophares are often arranged into groups in specific regions on the surface such as the base of the antennae, the eyestalks (Fig. 5A), the carapace, the telson (Fig. 5B) and the abdominal segments (Fig. 5C). Profound erythrophares cover internal organs such as the brain, alimentary canal (Fig. 5A) and heart. In addition, the ventral nerve cord is wrapped in such deep-lying erythrophares too (Fig. 5C). Some appendages, such as the thoracic filter legs (Fig. 5D), also carry erythrophares.

Krill was sometimes bright red (i.e. pigment dispersed) when taken from darkness. As this was unexpected, the first experiments were conducted to exclude factors other than light that could influence pigment movement. Two such factors taken into consideration are, a potential natural circadian variation and stress or disturbance.

Circadian rhythm

Circadian variation of pigment dispersal has been reported for some crustacean species (see Noël and Chassard-Bouchard, 2004) and this was hence tested in krill. Individual sub-adult krill were set up in 50 ml containers in the dark and, starting after an adaptation period of 12 h, the chromatophore index (CI) of six individuals was observed at intervals of 2–3 h. Individuals were taken out one by one and great care was taken to avoid disturbance of other krill set

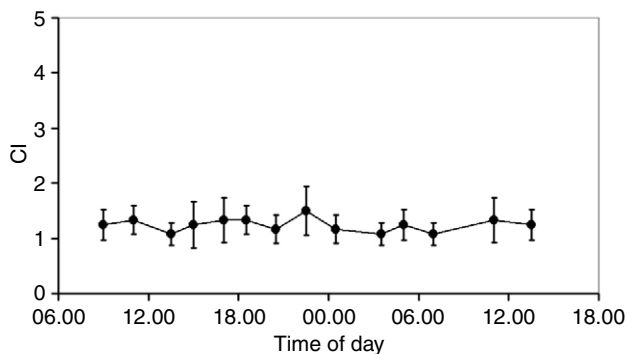


Fig. 6. Course of the chromatophore index (CI) of lateral chromatophores from the fifth abdominal segment of a sub-adult male krill during a period of complete darkness. No significant differences were found (ANOVA, *post-hoc* Tukey test, $P < 0.05$). Values are means \pm s.d. ($N=6$).

up for later observation. Although there was some variation in the CI between a minimum of 1.1 ± 0.2 and a maximum of 1.5 ± 0.4 , there was no indication of a circadian rhythm during the experimental period of 28.5 h (Fig. 6). The lowest individual CI recorded was 1 whereas the highest was 2.

Stress

To determine the effect of stress, two groups of individually kept krill were adapted to the dark. The possible influence of stress (caused by disturbance) was investigated by agitating individuals of one group in the dark by consistent gentle stirring (with a glass rod) for 10 min after which the CI was recorded. The CI of the undisturbed dark-adapted control group was recorded for comparison. The CI of stressed krill (3.1 ± 0.6) was significantly higher than in undisturbed animals (1.3 ± 0.4), confirming that stress causes pigment dispersal (Fig. 7). It is noteworthy in this regard that dying krill has a CI of 5 and that eyestalk ablation leads to irreversible dispersal to stage 5.

Time course

Sub-adult male krill was exposed to a relatively low PAR density of 0.007 mW cm^{-2} (to avoid too much overlap with dose response; see below) to study the time course of pigment dispersal. Animals were placed under the microscope and the CI was observed during 30 min of light exposure at intervals, as shown in Fig. 8. The maximum CI

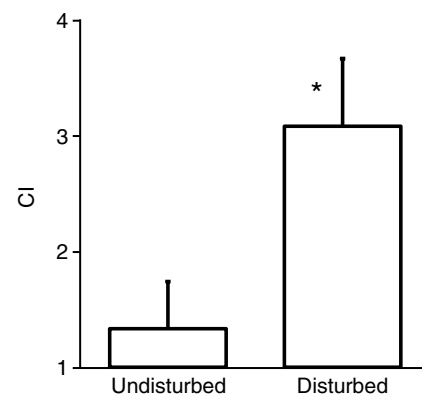


Fig. 7. Chromatophore index (CI) of lateral chromatophores from the fifth abdominal segment of disturbed and undisturbed dark-adapted sub-adult male krill. *Significantly different to undisturbed krill ($P < 0.05$, Student's *t*-test). Values are means \pm s.d. ($N=6$).

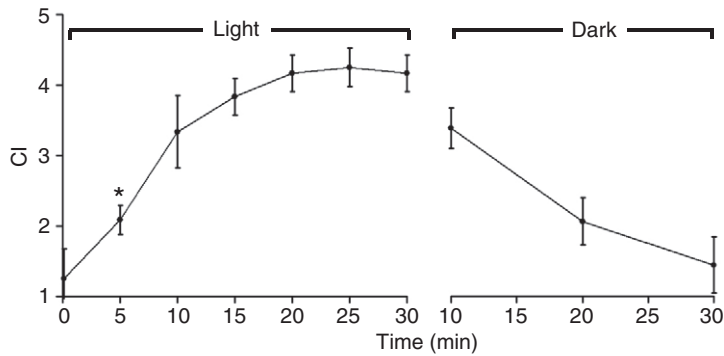


Fig. 8. Time course of chromatophore index (CI) in lateral chromatophores from the fifth abdominal segment of sub-adult male krill during exposure to photosynthetically active radiation (PAR) and after subsequent return to complete darkness. *Significantly different to starting value ($P < 0.05$, paired t -test). Values are means \pm s.d. ($N=6$).

of 4.2 ± 0.3 was reached after approximately 20 min, when pigment dispersal reached a plateau (Fig. 8). Subsequent darkness after 30 min of light exposure caused relatively fast pigment aggregation: the CI returned to baseline levels of 1.4 ± 0.4 within 30 min in darkness, which was not significantly different (paired t -test) from the value at the start of light exposure (CI 1.3 ± 0.4). It was, however, significantly lower than the CI after 5 min of exposure ($P < 0.05$). It is noteworthy that, under the described experimental conditions, the maximal recorded CI was 4.5 and that no animal reached a CI of 5. The time of half maximal dispersal (ET_{50}) was approximately 7 min and maximal dispersal was reached after approximately 20 min.

Wavelength

Chromatophores react to the UVA and UVB component of PAR. After recording the initial CI, krill were exposed to similar doses of PAR (33 mJ cm^{-2}), UVA (30 mJ cm^{-2}) and UVB (63 mJ cm^{-2}) and, after 5 min, the CI was recorded again (Fig. 9). PAR and UVA caused similar changes in the CI, of 2.2 ± 0.3 and 2.3 ± 0.5 , respectively, whereas the change in the CI was less (1.4 ± 0.4) after exposure to UVB. All changes were higher than in a dark-adapted control group (0.4 ± 0.6). Within all experimental groups, except control, CI changes were significant ($P < 0.02$, paired t -test).

Dose dependence

After recording the initial CI, groups of individually kept krill were exposed to various doses of PAR over a 5 min period, after which the CI was recorded again and the difference, i.e. the change in CI,

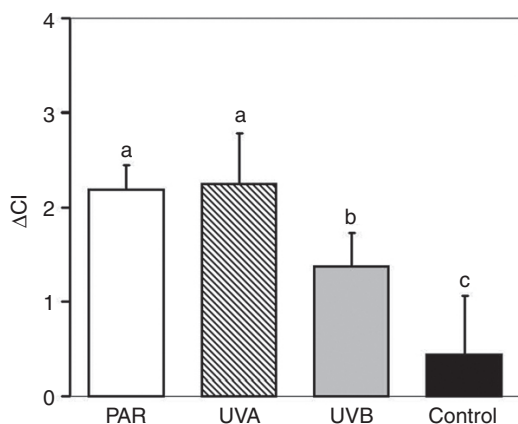


Fig. 9. Change in chromatophore index (CI) following exposure to photosynthetically active radiation (PAR), UVA and UVB radiation in comparison with a dark-adapted control. Different lower-case letters indicate significant difference (ANOVA, *post-hoc* Tukey test, $P < 0.05$). Values are means \pm s.d. ($N=8$).

was plotted (Fig. 10). A maximum change in the CI, of 2.7 ± 0.4 , was reached after exposure to 375 mJ cm^{-2} , similar to CI changes after exposure to doses of 90 and 187 mJ cm^{-2} PAR, respectively. The lowest dose investigated (0.6 mJ cm^{-2}) changed the CI by 1.1 ± 0.4 . All doses investigated caused a significant change of CI ($P < 0.005$; paired t -test). The calculated best-fit curve revealed an EC_{50} of 5.13 mJ cm^{-2} . When pigment is fully dispersed, large areas of the abdominal segments are covered (Fig. 11).

Comparison of summer vs winter responses

Physiological colour change in adult male krill was significantly faster in summer than in winter: the time to reach maximum dispersion (ET_{MAX}) was approximately three times longer in winter than in summer, and ET_{50} was more than double (Fig. 12). ET_{50} in summer of $2:35 \pm 0:21$ min was significantly different from the ET_{50} in winter of $5:37 \pm 0:55$ min (Student's t -test, $P < 0.05$).

Morphological colour change

Astaxanthin concentration

The astaxanthin concentration in the abdomens of sub-adult male krill caught in summer was approximately 450% higher than in those sampled in winter (Table 1).

Chromatophore count

The number of chromatophores is generally higher in krill caught in the summer than in winter. This was quantified in a clearly definable part of the body: the fourth to sixth abdominal segments. There were

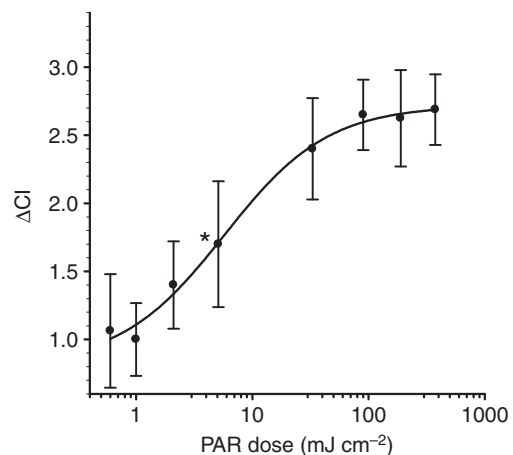


Fig. 10. Dose-dependent change in chromatophore index (CI) following exposure to photosynthetically active radiation (PAR), $r^2=0.9936$. *First change of CI that is significantly different from that of the lowest dose (ANOVA, *post-hoc* Dunnett test). Values are means \pm s.d. ($N=8$).

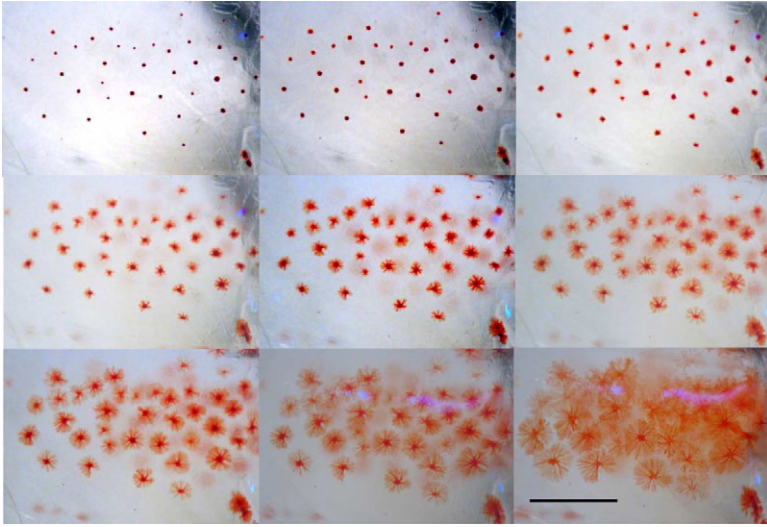


Fig. 11. A group of chromatophores from the fifth abdominal segment of a sub-adult krill (lateral view) during dispersion of pigment (from top left to bottom right). Scale bar, 1 mm.

significantly more chromatophores on all these segments of adult and sub-adult krill; however, this difference was most pronounced in the sixth segment, with 250% and 483% more chromatophores in sub-adult and adult summer krill, respectively (Table 1). The difference was most obvious in the sixth abdominal segment: it is almost free of chromatophores in winter (sub-adults depicted in Fig. 13).

DISCUSSION

Antarctic krill employs three strategies to compensate for their highly UV-sensitive DNA composition: (1) avoidance of light (Newman et al., 2003), (2) efficient DNA repair mechanisms (Malloy et al., 1997) and (3) pigmentation (present study). The last method adapts to variations in short-term light conditions by physiological colour change, whereas long-term (seasonal) changes in the light regime result in a morphological colour change. Both are facilitated by chromatophores. Here, we describe quantitatively, for the first time, colour change for a euphausiid crustacean.

Short-term colour change

The main purpose of physiological colour change in krill seems to be protection from potentially harmful solar radiation (from above) without compromising camouflage from predators in open water against surface light (i.e. from below). To balance between the two required states, opposing and rapidly convertible appearances are required: coverage of large areas of the body by pigments for UV protection *versus* a high degree of transparency for camouflage. Complete dispersal of the pigment, as in the chromatophore index 5 (CI 5) ensures UV protection, whereas complete aggregation (CI 1) meets the requirements for camouflage. In nature, transition between the two situations is necessary during ascent and descent of the diel vertical migration. There is no scientific information available confirming a rhythmic pigmentation pattern that matches this movement. Fishery reports indicate, however, that white krill is caught close to the sea floor, whereas red krill is caught closer to the surface (Kawaguchi and Nicol, 2007). We did not find any rhythmicity of the chromatophore index in winter krill. Such a rhythm may still exist in other seasons, when periodicity of daylight is more pronounced. These periods, however, were not covered in the present study.

Fishery reports also mention that neighbouring krill aggregations often have completely different degrees of redness whereas the colouration is uniform within a single aggregation (Kawaguchi and Nicol, 2007). Assuming aggregations mentioned in those reports were exposed to similar light conditions, these observations would

be supportive of our experimental findings that stress is a trigger of pigment dispersal independent of light. In nature, such stressors could be, for example, the presence of predators or fishing operations. The uniformity of colouration would imply some sort of communication of the signal within the aggregation.

Euphausiid eyes are very sensitive to light and can detect light fields of two magnitudes below that experienced in 250 m depth during daylight (Onsrud and Kaartvedt, 1998; Zhou and Dorland, 2004). But, although this should ensure avoidance, at least of PAR, krill swarms occasionally occur, for unknown reasons, at the surface during daylight (Marr, 1962; Ozawa et al., 1968; Kubotka, 1981). Furthermore, krill remains closer to the surface during the day in early summer to take advantage of phytoplankton (Taki et al., 2005). Exposure occurs close to the sea surface but can also occur at greater depths. In the Antarctic summer, significant portions of UVR reach depths of 20–50 m (Ban et al., 2007) and are still biologically harmful at 30 m depth (Karentz and Lutze, 1990).

Krill is well equipped to respond physiologically to such exposure. Very low doses of PAR are sufficient to trigger a response of the erythrophores in *E. superba*: the lowest tested dose of 0.6 mJ cm^{-2} (5 min exposure at an irradiance level of 0.02 mW cm^{-1}), caused significant dispersal. Estimated from readings of the surface irradiance at Palmer Station at noon in early Antarctic summer (Helbing et al., 1996) and attenuation tables (Jerlov, 1976), such a dose represents

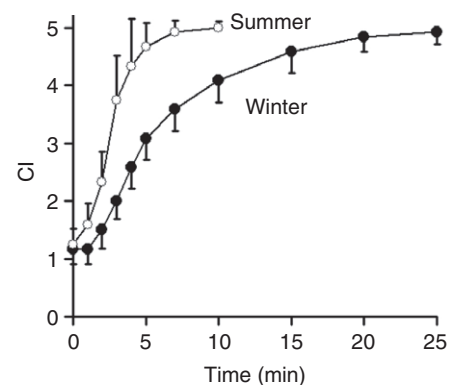


Fig. 12. Seasonal comparison of time course of chromatophore index (CI) in lateral chromatophores from the fifth abdominal segment of adult male krill during exposure to PAR. Filled circles, winter; open circles, summer krill. Values are means \pm s.d. ($N=5$).

Table 1. Seasonal differences in chromatophore count and astaxanthin concentration in the abdomen of male *E. superba*

Season	Astaxanthin concentration* ($\mu\text{g g}^{-1}\text{fw}$)	Chromatophore count					
		Adults			Sub-adults		
		Segment 4	Segment 5	Segment 6	Segment 4	Segment 5	Segment 6
Summer	45.1 \pm 12.1	172 \pm 22	182 \pm 42	70 \pm 16	151 \pm 35	168 \pm 21	63 \pm 17
Winter	8.2 \pm 1.6 [†]	89 \pm 22 [†]	102 \pm 41 [†]	12 \pm 3 [†]	67 \pm 11 [†]	94 \pm 25 [†]	18 \pm 7 [†]

Values are means \pm s.d. ($N=5$).

*Measured in sub-adults.

[†]Significantly different from respective summer value (Student's *t*-test; $P<0.05$).

0.1–0.3% of surface irradiation. Physiological colour change may therefore be starting as deep as approximately 50 m, depending on the amount of matter influencing attenuation of PAR (Jerlov, 1976). Furthermore, krill is susceptible to doses of PAR and UVR that occur in their natural environment (Newman et al., 1999) and krill DNA, because of its high content of T–A base pairs [and, therefore, high amounts of (T)_n arrays], was shown to be particularly prone to damage by UVB radiation (Jarman et al., 1999). This is countered by a very efficient photoenzymatic DNA repair mechanism (Malloy et al., 1997). In captivity, krill also responds to PAR and UVA with an avoidance strategy – which was assumed to be a mechanism to stay clear of the more harmful UVB radiation (Newman et al., 2003).

When behavioural response (avoidance) is not possible, UV protection by pigmentation, as demonstrated in our study, is yet another physiological mechanism to avoid damage and increase survival. Size and distribution of pigmentation support this interpretation: the majority of chromatophores are located dorsally so that natural radiation can be blocked. With diameters reaching 450 μm on the dorsal parts of the abdomen and thorax, krill chromatophores are large, compared with those of other crustaceans (Noël and Chassard-Bouchard, 2004) and when in CI 5, they cover large portions of light-exposed areas with a screen of astaxanthin granules (see Figs 5 and 11). Astaxanthin proved to be efficient in protecting copepod crustaceans from the harmful effects of UVR (Hairston, 1976; Hairston, 1978; Davenport et al., 2004). The highly transparent nature of krill probably necessitates additional protection of deep-lying critical structures, such as the ventral double strand of the nervous system, by ‘profound’ chromatophores.

For camouflage against surface light, all pigmentation requires to be controlled and, hence, has to be within chromatophores. The absence of visible diffuse pigmentation in *E. superba* is advantageous in this regard. In crustacean species that camouflage against a background, diffuse pigmentation is common around chromatophores or chromatosomes (see Noël and Chassard-Bouchard, 2004). Full aggregation of red pigment in the erythrophores causes blanching of the animal to an extent that it is almost completely transparent against surface light. This camouflage effect is enhanced, and the remaining silhouette further blurred, by counter-illumination by the krill's photophores, which are arranged to emit all their light downwards. To maximise efficiency of this system, the axes of both eyes and photophores are synchronously aligned towards a single-point light source [i.e. the sun (Grinnell et al., 1988)].

Many crustacean species possess a variety of differently coloured chromatophores and diffuse pigmentation for efficient camouflage (McNamara, 1981; Noël and Chassard-Bouchard, 2004). Antarctic krill, by contrast, in which UV protection is the top priority during diel vertical migration, has only one monochromatic type of chromatophores – erythrophores – and lacks diffuse pigmentation. Erythrophores are generally characterised by intense pigment movement that is facilitated by a high abundance of microtubules

(Noël and Chassard-Bouchard, 2004). They are therefore most suited for the rapid colour change required in krill.

Seasonal differences in colour change

In addition to short-term changes in pigmentation, we found evidence that there are also seasonal differences in (1) the speed of physiological colour change between summer and winter and (2) the number of chromatophores and concentration of astaxanthin (i.e. morphological colour change).

Physiological colour change was much slower in animals that were investigated in winter, when days are short and the sun is low. Since physiological colour change is energy dependent, this may be the result of the metabolic depression that postlarval krill undergoes in winter and which is characterized by low feeding and respiration rates as well as reduced activity of metabolic and digestive enzymes (Atkinson et al., 2002; Meyer et al., 2002).

Recently, it was demonstrated for the first time that the reduced metabolic activity of krill, which is already in place at the onset of winter, is triggered by the Antarctic light regime (Teschke et al.,

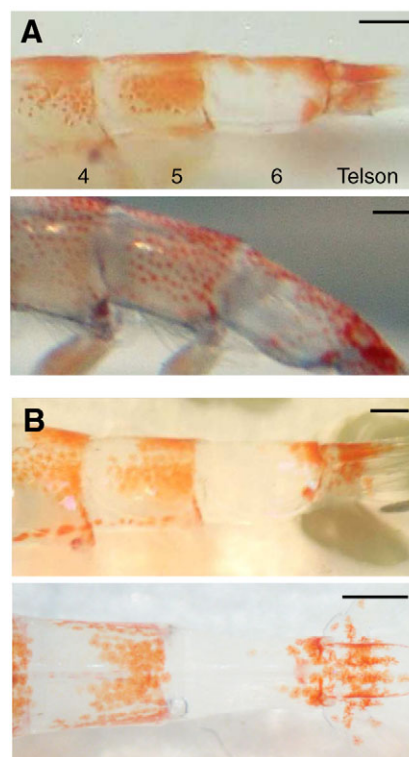


Fig. 13. Difference in chromatophore coverage of the abdominal segments (numbered from thorax, in top picture) of sub-adults of *E. superba* caught in winter (A) and summer (B). Scale bars, 2 mm.

2007). The slow colour change we found in winter might also be a result of this general slow down of metabolism.

Long-term exposure to a particular light regime or background – and therefore maintaining the chromatophores with a CI 1 or CI 5 – results in a slow change in the number of chromatophores and/or the amount of pigment, i.e. a morphological colour change, according to Babak's Law (Babak, 1913). Our results give a clear-cut indication that such a morphological colour change takes place in Antarctic krill. Both, the concentration of the major pigment astaxanthin and the number of chromatophores in krill, are substantially higher in summer – when exposure to light is maximal – than in the darkness of winter. We have confirmed quantitatively for the first time, what fisheries reports have indicated: red krill is typical for summer and white krill occurs in winter (Kawaguchi and Nicol, 2007). Our two-season sampling only provided a snapshots of the extreme 'endpoints' of a possible morphological colour change, and data are still missing for the transitional periods of spring and autumn as well as laboratory experiments that mimic a full seasonal transition.

In addition to the amount of light available, the quantity of food consumed may influence seasonal astaxanthin concentration. Astaxanthin cannot be synthesized *de novo* by crustaceans and its precursors have to be acquired *via* food intake (Goodwin, 1984; Britton, 1995). Algae form the bulk of their food and the biggest source of astaxanthin precursors for non-larval krill in summer. As an effect of light regime and sea ice coverage, phytoplankton is very much limited in the Antarctic winter: chlorophyll *a* concentrations drop by at least two orders of magnitude from $>10 \mu\text{g l}^{-1}$ seawater in summer to $<0.1 \mu\text{g l}^{-1}$ in winter (Atkinson et al., 2002). More importantly, the light regime sets in motion an internal cascade of events (Teschke et al., 2007) that leads to non-feeding in winter and metabolic depression (Quentin and Ross, 1991; Atkinson et al., 2002). So, even if phytoplanktonic (or zooplanktonic) food became available, krill would not be able to make use of it. However, experimental evidence to verify if and to what extent the lack of precursors causes a reduction in astaxanthin concentration in winter, is still unavailable.

Mechanisms of colour change in Antarctic krill

Quantity and quality of light are important environmental factors in triggering colour change. Detailed data on the response of pigment movement in crustacean chromatophores to different doses of radiation is very scarce, in contrast to dose dependence for their respective chromatophorotropins (see Rao, 2001). In addition, existing results are hardly comparable because of the different experimental setup, different wavelengths used and the use of chromatophores from different ontogenetic stages (i.e. eggs, larvae or adults). A recent study has investigated the relationship of pigment dispersal and UVA dose in an adult eyestalk-less decapod crustacean, and found an ED_{50} of approximately 500 mJ cm^{-2} (Gouveia et al., 2004). This is much higher than the ED_{50} of 5.13 mJ cm^{-2} for *E. superba* in the present study in response to PAR. In summer, sensitivity may be even higher than in winter. We did not establish a dose–response curve for UVA in the present study. From the change in the CI after irradiation with a dose of UVA similar to that of PAR, however, it can be assumed that such a curve would be in the same order of magnitude as that for PAR. The CI of aggregated erythrocytes of an intact shrimp species in the above-mentioned study only increased by approximately 1.5 at a dose of 2500 J cm^{-2} UVA (Gouveia et al., 2004). The eyes are the main organ for photoreception in crustaceans and the higher sensitivity in *E. superba* may be due to the fact that the eyes in our experimental animals were fully intact.

Clearly, PAR and UVA cause pigment dispersal in krill erythrocytes. Interestingly, UVB radiation triggered moderate

pigment dispersal too, although krill does not avoid UVB under laboratory conditions (Newman et al., 2003). The latter observation suggested that it cannot be detected. The result of our study may be an artefact resulting from pollution of UVB with other wavelengths and a higher dose of UVB (relative to the UVA and PAR used to irradiate the other experimental groups) or a possible direct reception of UVB by krill chromatophores. Such direct reception of radiation by chromatophores is known from experiments with decapod crustaceans (Coochill et al., 1970; Gouveia et al., 2004).

Pigment dispersal in response to light exposure in krill is very swift. This is facilitated by the exclusive presence of erythrocytes (see above) and provides rapid UV protection when necessary. Dense bundles of microtubules, a trademark of erythrocytes, are visible in krill erythrocytes (Fig. 4); similar to those in the glass shrimp *Palaemonetes vulgaris* (Robison and Charlton, 1973). Although exact details of pigment movement inside chromatophores are yet to be established, there is evidence that pigment granules move along the cytoskeleton (particularly the microtubules) in association with kinesin and myosin protein motors (Boyle and McNamara, 2005). In summer krill, a period of only 5–7 min is necessary to change from complete transparency to full colouration. Based on a diameter of a large abdominal erythrocyte of about $450 \mu\text{m}$ (see above), this is a speed (in summer) of approximately $40 \mu\text{m min}^{-1}$.

As previously noted, we did not find an endogenous circadian rhythm of pigmentation in winter krill. Antarctic krill performs more or less regular vertical migrations that are most pronounced in summer (Taki et al., 2005). Although summer krill migrates from close to the surface at night to deeper water, swarms, or parts thereof, remain in light-exposed depths above 50 m during daytime (Godlewska and Klusek, 1987; Godlewska, 1993; Taki et al., 2005). This is sufficient to trigger colour change (see above), creating the possibility of some degree of rhythmicity in light exposure. The result could well be a pigmentation rhythm in summer. Circadian variation in pigmentation in crustaceans is mediated by their antagonistic chromatophorotropins: rhythmic synthesis and release of PDH was suggested to be behind pigment dispersal in crab melanophores (Granato et al., 2004) whereas rhythmic expression of the RPCH gene was found in a crayfish (Martinez-Perez et al., 2005). Adversely, eyestalk-ablated fiddler crabs maintained their pigmentation rhythm in another study (Webb et al., 1954). An attempt to use eyestalk-ablated krill in the present study failed, because animals that were eyestalk-ablated at CI 1 subsequently displayed maximal and irreversible pigment dispersal. Future research should therefore cover summer and should also tackle the involvement of chromatophorotropic hormones in *E. superba*. This will reveal how colour change is triggered and this signal is mediated.

Adaptation of pigmentation to either UV protection (of animals living close to the surface) OR camouflage (of animals from deeper water) within one species has been shown, for two morphs of a copepod species (Luecke and O'Brien, 1981). This is a more permanent adaptation (i.e. morphological colour change) that is not suitable for a species such as krill that encounters both situations in a short period of time, with different requirements for each of them.

During morphological colour change, destruction of chromatophores can be fast and the various types of chromatophores are destroyed and produced at different rates. In *Palaemonetes vulgaris*, for example, red and black chromatophores disappeared at the highest rate (Brown, 1934). In the Hawaiian ghost crab, *Ocypode ceratophthalma*, the rate of black chromatophore destruction was actually measured and happens at a rate of $0.76 \text{ chromatophores mm}^{-2} \text{ day}^{-1}$ during 7 days after transfer from a

black to a white background (Green, 1964). In krill, the change is not as drastic as in the *Ocyropsis* experiment though. Moreover, crabs from a black background had 12 times more black chromatophores than those from a white background whereas the difference in erythrochrome count in krill from different seasons was only a factor of less than two as estimated from the surface area ($\sim 30\text{ mm}^2$) of the fifth abdominal segment (lateral and dorsal) and the difference in chromatophore count (80; see Table 1), the rate of destruction in adult krill is much lower at approximately 0.01 erythrochromes $\text{mm}^{-2}\text{ day}^{-1}$ during a period of 240 days. Future research will have to reveal the fate of astaxanthin and erythrochromes on the transition from summer to winter.

Our present study has revealed some basic information on physiological and morphological colour change in Antarctic krill. More systematic field research and laboratory studies – *in vivo* and *in vitro* – are necessary in the future to verify results presented here and to provide a comprehensive model of colour change in Antarctic krill.

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