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Plasticity in coloration as an antipredator strategy among zooplankton

Abstract—We show that marine zooplankton change their level of coloration both with depth and time of the day. The carnivorous copepod *Pareuchaeta norvegica* caught near the bottom in 200–400-m deep-water columns were darker than specimens caught higher in the water column. A diel rhythm in coloration occurred even at several hundred meters' depth, with individuals caught during night time being more pigmented than the ones caught during the day. We hypothesize that individuals actively adjust their degree of coloration to achieve optimal camouflage at the prevailing light regime.

The pelagic realm is without hiding places, and its inhabitants have adopted various mechanisms to become inconspicuous to visual predators. This includes transparency and crypsis (Johnsen 2001), as well as behavioral adaptations, like hiding in dark, deep water during daytime (Gliwicz 1986).

Light regimes in water columns are not homogeneous. Optical conditions change with depth and time, turbidity, and viewing angle. Crypsis will therefore be depth and time dependent (Johnsen 2002). In well-illuminated epipelagic layers, transparency is the only form of camouflage that is successful from all viewpoints (Johnsen 2001), and epipelagic zooplankton species are generally transparent (Herring and Roe 1988). Mesopelagic species, on the other hand, tend to be half-red (red coloration of guts and opaque tissue), while bathypelagic species are uniformly red, purple, or black (Herring and Roe 1988). In these deeper layers, any downwelling radiation consists of only a tiny amount of weak, parallelized blue light (Jerlov 1968). The dominating light source here is bioluminescent organisms. Approximately 90% of all the animals in the mesopelagic zone (200–1,000 m) are bioluminescent, primarily emitting blue light (400–479 nm) (Herring 1983). In this light regime, red, purple, or black coloration may be more cryptic than transparency, because even a perfectly transparent object causes surface reflection (Johnsen 2001).

Both nonmigrating and dielly migrating zooplankton will experience a range of light conditions (Jerlov 1968). If an organism can slightly alter its coloration under changing optical conditions, this might result in nearly perfect camouflage (Johnsen and Sosik 2003). It has been noted that mesopelagic shrimps with large chromatophores would have the potential of adjusting their color in response to changes in the ambient light conditions, including on a daily basis (Foxton 1970; Herring 1973; Herring and Roe 1988), but to our knowledge, no data have been provided.

Pareuchaeta norvegica is a large, carnivorous copepod with prosome length of adult females of nearly 6 mm (Vestheim et al. 2005). Like its congeners, it may perform diel vertical migrations with amplitudes of hundreds of meters (Longhurst and Williams 1979; Mauchline 1992; Zmijewska and Yen 1993), but several studies also report quite similar nighttime and daytime distributions (Skarra and Kaartvedt 2003; Vestheim et al. 2005). In a previous study, we noted that the most shallow-living specimens were more transparent than their deeper-living conspecifics, although no quantifications were made (Vestheim et al. 2005). We further reported that transparent specimens became considerably redder after 24 h in darkness. In the current study, we aimed to assess and quantify the degree of coloration of individuals from different depths and under different light regimes (day vs. night) in situ. We hypothesize the presence of adjustable coloration explained by the light conditions that will be expressed both related to depth and time.

Material and methods—We chose to assess level of coloration by digital photography rather than extraction of pigments (a common approach). Color changes may be mediated by the presence of chromatophores and different distribution patterns of pigments rather than, or as well as, by changes in total pigment level (Ghidalia 1985). In fact, previous studies of extracted pigment concentrations in oceanic crustaceans have shown no consistent depth-related trends (Fisher et al. 1952; Herring 1973), while subjective assessments of their color indicate that deep-living species are darker (Foxton 1970).

Digital image analysis is simple, nonpolluting, and economical. Unlike chemical methods such as high-performance liquid chromatography, a sample can consist of a single individual or even a single body part of very tiny individuals. The method is, however, strongly dependent on ambient light and aperture settings, making quantitative comparisons difficult. Nevertheless, by standardizing photographing procedures, quantitative comparisons between different sampling series are achievable (Villafuerte and Negro 1998).

We conducted a preliminary study in the 430-m-deep Lurefjorden, Norway, on 06–07 November 2004. A major objective for that effort was to assess the feasibility of using digital photography for quantifying coloration. Copepods were sampled in five depth intervals during day and night with a Hydrobios Multinet. The digital photos suggested diel patterns in coloration even in the deepest interval (300–400 m) (Fig. 1), but quantification turned out to be impossible as a result of fluctuating (nonstandardized) background light (cf. Fig. 1). To assure standard light conditions during subsequent trials, we built a completely dark box equipped with two cold light sources.

Sampling was thereafter carried out from the RV *Trygve Braarud* at a 200-m-deep location (59°38′N, 10°38′S) in the Oslofjord, Norway, on 15–16 February 2005. *Pareuchaeta norvegica* was sampled with a modified Nansen net, mesh size 500 μm, equipped with a nonfiltering cod end, in series

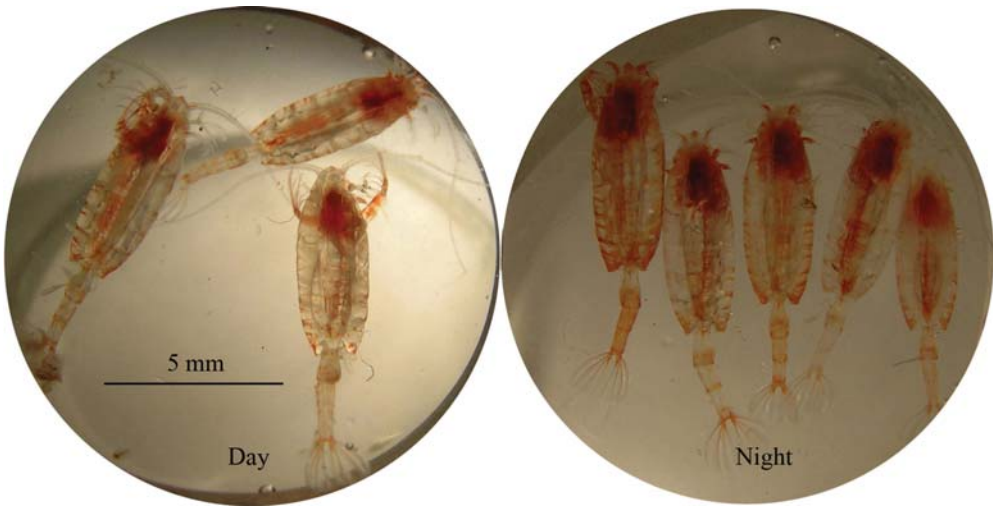

Fig. 1. Photos of adult females of *Pareuchaeta norvegica* sampled at 300–400 m in the daytime and nighttime in Lurefjorden, 08 November 2004. Note the fluctuating background light level, which precludes quantification of an apparent stronger pigmentation in nighttime samples.

comprising 4 × 50–m depth intervals covering the whole water column. The coarseness of the sampling intervals may have obscured finer-scale aspects of vertical structure in plankton distributions. Vertical distributions were established by sampling one depth interval at the time in nine full vertical series (four at day and five at night). Photographing was slightly more time-consuming than sampling, and to assure that only freshly collected individuals were included to assess pigmentation, some series were excluded in this analysis before photographing. Coloration was assessed based on four series taken during the day and three at night—28 net tows in total. Only mature females without visible gonads were assessed in the analysis.

To avoid flexion of the body during photographing, the animals were gently narcotized by CO_2 excess produced by adding a small amount of Nyco fruktsalt ($NaHCO_3$, $C_4H_6O_6$, $C_4H_4Na2O_6{\cdot}2H_2O$, $C_7H_5NO_3S$) to the water. This treatment did not affect the coloration and did not kill the animals. Thereafter the animals were immediately photographed with Nikon Coolpix 5400 (5.0 megapixels). The camera was set to RGB (red–green–blue) mode, and illumination, aperture, exposure, and ISO speed ratings were kept constant. No filters were applied. The camera was mounted on the dark box equipped with two cold light sources. One by one, the animals were placed inside the box in a transparent, colorless dish filled with filtered seawater. The dish was positioned on a white background and kept in a constant distance from the camera. Every individual was photographed within 15 min after sampling.

The intensity of coloration of all animals was measured as the mean gray values of the pixels of an elliptical selection fitted to the prosome part of the individual (Tollrian and Heibl 2004). Because small differences in mean gray values were detected in the area surrounding the animals, the mean gray values were also measured in two circles on each side of the animal. The average of the mean gray values of these two circles was defined as white (gray value = 255), and each histogram of the prosome part was shifted by multiplying their mean value with a calibrating factor, CF, where

$$\mathrm{CF} = 255/(\Sigma\text{Mean gray value on each side of the animal}/2)$$

Image analyses were performed in Adobe Photoshop 7.0 on raw (TIFF) images. Adobe Photoshop 7.0 uses weighted grayscale converting of RGB images (gray = 0.299 red + 0.587 green + 0.114 blue).

Statistical analyses were performed in S-plus R 6.1 for Windows (Venables and Ripley 2002). Data were normally distributed and had equal variance; we calculated one-way analysis of variance for each depth interval and time.

Results—Most individuals were found at 100–200 m, and there was no major difference in vertical distributions between day and night (Fig. 2).

The individuals found in the shallowest water had significantly higher mean gray values and had thus less coloration than individuals caught in deeper water both during the day and night (Fig. 3; $F_{3,183} = 6.68$, $p < 0.001$). Also, individuals had a significantly higher mean gray value (and had less coloration) during day than night (Fig. 3; $F_{1,183} = 14.3$, $p < 0.001$). No interactions between time and depths were found.

Discussion—Individuals belonging to the same species adjust their degree of coloration depending both on time and depth of capture. We ascribe these findings to predator avoidance. This explanation would be in line with what is known about crypsis in weak blue light (*see* the Introduction), although we acknowledge that fish and other visually hunting predators may apply camouflage-breaking abilities like polarization and ultraviolet (UV) vision to enhance the visibility of otherwise well-camouflaged prey (*see* Losey et al. 1999 and references therein). It would also

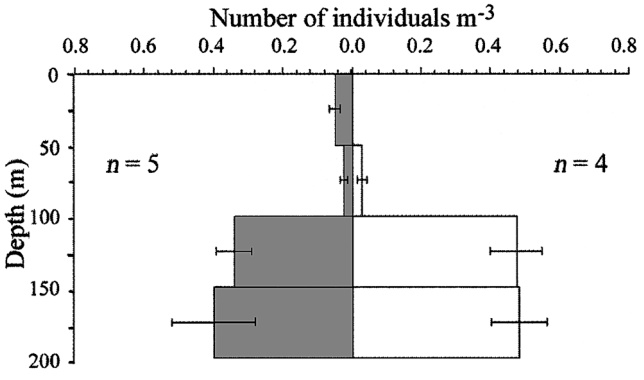

Fig. 2. Vertical distribution of adult females of *P. norvegica* night (solid bars, left) and day (open bars, right), 15–16 February 2005. Error bars denote SE of mean number of individuals m^{-3}; *n* refers to number of vertical series. Only nonovigerous females and females with attached egg mass were counted.

be in accordance with the prediction of enhanced camouflage if an organism is able to slightly alter its coloration under changing optical conditions (Johnsen and Sosik 2003).

In crustaceans with thin exoskeletons, like copepods, coloration may be caused both by pigments inside the body or in chromatophore cells. The pigments with a chromatic function are mainly carotenoids, melanins, and ommochromes, with carotenoids as the most important. Carotenoids may appear everything from blue, yellow, and red to colorless (*see*, e.g., Ghidalia 1985 for a thorough description of pigments in Crustacea). Like all animals, crustaceans do not synthesize carotenoids de novo, but ingest them from their food. Previously, a diel variation in pigmentation level correlating with nightly feeding activity has been documented for herbivore copepods both in freshwater and shallow marine environments (Ringelberg and Hallegraeff 1976; Kleppel et al. 1985). Although a diel feeding rhythm also occurs in *P. norvegica* (Olsen et al. 2000; Skarra and Kaartvedt 2003), we refute this as an explanation for the diel color shifts because nonfeeding individuals may increase their pigmentation upon exposure to darkness (Vestheim et al. 2005).

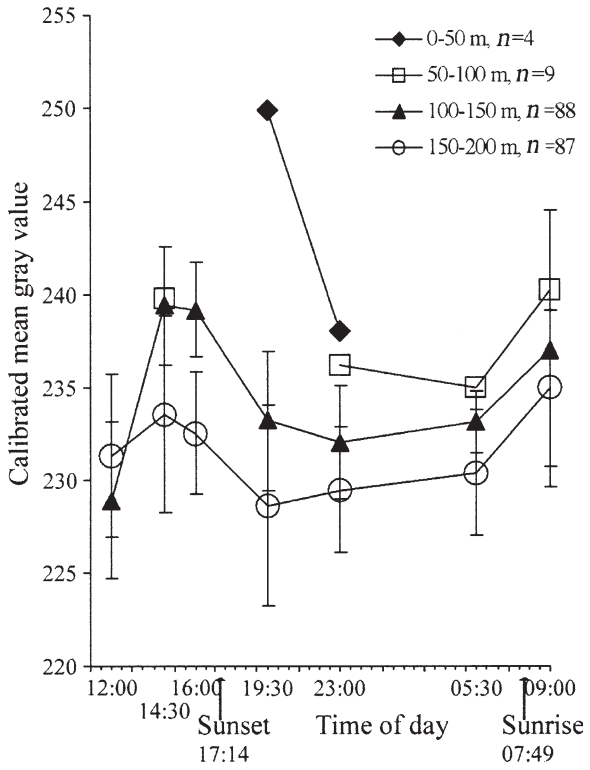

Fig. 3. Calibrated average mean gray values in relation to depth and time of capture. Error bars denote 95% confidence intervals of the mean.

In surface waters, carotenoids are important as photoprotection, and for limnic copepods, carotenoid level is shown to correlate negatively with the degree of fish predation and positively with UV intensity (e.g., Hansson 2004). However, for a deep-living species like *Pareuchaeta*, UV protection should not be of importance, and this explanation can also be refuted because inducible photoprotection would result in the opposite pattern, with enhanced coloration in brighter light. Carotenoids may also have an antioxidant function and play a role in development (e.g., Lotocka et al. 2004). We only investigated individuals of the same stage and sex, and we do not discuss these matters here.

It is not clear whether *P. norvegica* changes coloration chemically or uses chromatophores to change color (as indicated by photos of some low-pigmented individuals having small asterisk-shaped red patches spread around the body). Regardless of the physiology behind color changes, plasticity in the degree of coloration would be beneficial to become inconspicuous for visually hunting predators like fish under a range of light conditions. During the last two decades, studies on zooplankton antipredatory strategies have largely focused on diel vertical migrations (e.g., Hays et al. 1994; Yoshida et al. 2004). Here we suggest that plasticity in level of coloration is also applied in predator avoidance, both related to depth, and on a diel timescale.

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