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Food type and concentration affect chlorophyll and carotenoid destruction during copepod feeding

Abstract—To evaluate the use of pigments as tracers for determining copepod grazing rates and selectivity, we examined the stability of several biomarker pigments during copepod feeding incubations. During these incubations, we measured changes in phytoplankton-derived chlorophylls and carotenoids in the particulate and dissolved pools. Budgets were calculated to determine changes in pigment concentrations in the food, copepod fecal pellets, copepod guts, and dissolved/colloidal fraction. For each of six algal diets, adult female *Acartia tonsa* copepods were fed limiting and saturating food concentrations, approximately 100 and 500 μg C L^{-1}, respectively. *Thalassiosira weissflogii, Rhodomonas lens, Chroomonas salina, Dunaliella tertiolecta,* and two *Tetraselmis* strains were used in the feeding experiments to investigate the fate of fucoxanthin, alloxanthin, lutein, chlorophyll (Chl) *b,* and Chl *a.* In all experiments using saturating food concentrations, the dissolved/colloidal pool contained no more than 3% (usually less than 1%) of any pigment, whereas in experiments using limiting food conditions, pigments were undetectable in the dissolved/colloidal pool. Pheopigments were present in fecal pellets and copepod guts in most of the experiments. Chl *a* destruction (conversion to colorless products) was variable among the different experiments, depending on algal species and food concentration and, in most cases, Chl *a* was destroyed to a greater extent than the carotenoids. In all cases, pigment destruction was higher when copepods were fed limiting rather than saturating food concentrations. These data attribute the variability in pigment destruction to algal species and concentration, and suggest caution when pigments are used as tracers of herbivory. In such studies, assumptions about conservative behavior, even for the carotenoids, would need to be verified for each set of experimental conditions and grazers.

Phytoplankton pigments are commonly used as taxonomic markers in aquatic studies (e.g., Carpenter et al. 1986; Levinton and McCartney 1991; Barlow et al. 1995; Bianchi et al. 1996). Chlorophyll (Chl) *a,* found in all phytoplankton, is used as an index of total biomass, while other pigments (e.g., Chl *b* and certain carotenoids) can be used as markers for phytoplankton community composition because some pigments are taxon specific (*see reviews by* Goodwin 1971; Jeffrey 1980; Millie et al. 1993; Jeffrey et al. 1997). Analysis of pigments in the guts of grazers has become a popular tool for measuring the feeding activity of planktonic herbivores (Mackas and Bohrer 1976; Baars and Oosterhuis 1984; Dagg and Walser 1987; Kleppel et al. 1988; Dam and Peterson 1991; Quiblier et al. 1994, 1996*a,b*). Although most such studies have used only Chl *a* to estimate grazing rates, both chlorophylls and carotenoids can be measured using high-performance liquid chromatography (HPLC). This information makes it possible to evaluate selectivity of grazers for different phytoplankton. However, in order to use these pigments as quantitative tracers for zooplankton grazing, the extent and variability of pigment destruction during feeding and digestion must be quantified.

Throughout the present text, the term degradation refers to the breakdown of pigments into detectable derivatives (e.g., pheopigments), whereas the term destruction refers to the conversion of pigment into colorless forms, undetectable by light absorption. It is well established that Chl *a* degradation to pheopigments occurs during grazing, although there is no consensus as to the extent to which destruction into colorless products occurs; the range of values for conversion by copepods of Chl *a* and pheopigments into colorless products has been reported from 0 to 100% (*see review by* Dam and Peterson 1988). Most studies have found chlorophyll breakdown products (i.e., pheopigments) in copepod guts and fecal pellets (Downs 1989; Head and Harris 1992, 1994); however, not all of the chlorophyll is always recovered as pheopigments. Downs (1989) found that the copepod *Calanus pacificus* converted on average 63% of ingested Chl *a* to pheophorbide and pheophytin, while the rest was destroyed. Although various estimates of chlorophyll conversions have been reported, an average of ~30% pigment loss (conversion to undetectable, colorless products) is assumed in many gut fluorescence studies (e.g., Dam and Peterson 1988).

The inconsistent results among studies of Chl *a* loss during grazing may be the result of the physiology and trophic history of the animals (Mayzaud and Razouls 1992; Tirelli and Mayzaud 1998). Penry and Frost (1991) showed that copepods that had been acclimated for long periods of time (several days) to high algal food concentrations destroyed more pigment than those acclimated to low food. Gut residence time that is negatively correlated with food concentration (Dagg and Walser 1987) has also been hypothesized to affect the extent of pigment alteration (Penry and Frost 1991), presumably because longer gut residence time allows for more breakdown, although elsewhere it has been suggested that destruction may actually occur prior to entry of food into the guts (Head and Harris 1996). Some studies (e.g., Penry and Frost 1991; Head and Harris 1996) have demonstrated that the extent of Chl *a* destruction varies with the ingestion rates of the copepods. Therefore, animal physiology, trophic history, and potentially other variables (e.g., phytoplankton physiology, species, light environment, etc.) may all play a role in the extent of pigment destruction that occurs during grazing.

With the availability of HPLC methods, pigments other than Chl *a,* particularly carotenoids, have been used to determine which phytoplankton groups are being grazed. However, little is known about carotenoid destruction during copepod feeding. If carotenoids are destroyed during feeding, then grazing rates calculated from gut contents will be underestimated (given the conventional assumption of no ca-

rotenoid destruction; e.g., Quiblier et al. 1994, 1996*b*). If they are not destroyed, on the other hand, grazing experiments that rely on pigment disappearance from the medium to estimate ingestion will also give underestimates. Nelson (1989) and Quiblier et al. (1994, 1996*b*) found that carotenoids present in the phytoplankton food were also present in the fecal pellets and zooplankton guts, respectively. These authors inferred that carotenoids were conserved during copepod feeding, although budgets of pigments were not calculated. Even if carotenoid breakdown products are not observed in guts or fecal pellets, it is inappropriate to assume that the pigments are conserved without a mass balance or budget, because pigments may be converted into undetectable (colorless) chemical forms or assimilated by the copepods. On two occasions Head and Harris (1992, 1994) quantitatively examined the effect of copepod feeding on carotenoids and reported almost 100% destruction of the carotenoid fucoxanthin and 38–95% destruction of diadinoxanthin. In addition, a few additional reports have been published on the variability of pigment destruction (Kleppel 1998; Strom et al. 1998; Tirelli and Mayzaud 1998; Descy et al. 1999); however, pigments continue to be used as tracers of copepod grazing, often with no or minimal efforts to correct for pigment destruction (e.g., Quiblier et al. 1996; Hwang et al. 1998; Gowen et al. 1999).

The goal of the present study was to reassess the reliability of methods using pigments as tracers of herbivory. We evaluated the effect of copepod feeding on phytoplankton pigments to determine whether, and to what extent, they are destroyed after being ingested by copepods and what factors contribute to variability in destruction. Pigment budgets were calculated for six unialgal diets fed to the calanoid copepod *Acartia tonsa* to investigate the fate of three carotenoids and of Chls *a* and *b*.

On six occasions between July and December 1996 zooplankton were collected in Fishers Island Sound (41°17′N, 72°01′W). Five-minute surface tows were made using a 0.75-m-diameter plankton net equipped with a 202-μm mesh and a closed cod end. The catch was diluted immediately with surface seawater into a 10-liter carboy and transported back to the laboratory within 2 h of collection. In the laboratory, adult female *A. tonsa* copepods were sorted under a dissecting microscope for experiments. Animals were acclimated to the incubation temperature in ambient unfiltered seawater for 2–3 d before being used in the experiments.

During winter and early spring, when *A. tonsa* was not abundant in Fishers Island Sound (for experiments using *Tetraselmis* strain PLY 429 and *Dunaliella tertiolecta* as food), laboratory-cultured copepods were used. The animals in culture were kept in an environmental chamber at 20°C on a 12-h light : 12-h dark (12L : 12D) cycle. They were fed a mixed algal diet at ~500 μg C L^{-1}.

Copepods were fed six unialgal diets to examine the effect of feeding on three carotenoids and Chls *a* and *b*. The diatom *Thalassiosira weissflogii* (fucoxanthin), the cryptophytes *Rhodomonas lens* and *Chroomonas salina* (alloxanthin), two prasinophyte strains, *Tetraselmis* sp. and *Tetraselmis* strain PLY 429 (lutein and Chl *b*), and the chlorophyte *D. tertiolecta* (lutein and Chl *b*) were used to evaluate differences in pigment destruction. Algae were grown in F/2 medium (Guillard 1975) in an environmental chamber at 20°C (±1°C) on a 12L : 12D illumination cycle. The cultures were diluted every 3 d to ensure that cells would be in exponential growth at the start of each feeding experiment.

For each grazing experiment, copepods were first acclimated to food conditions for 24 h, then collected gently on a 63-μm screen, and sorted into experimental containers. Forty copepods were added to each of six 2-liter bottles, three replicates with limiting (~100 μg C L^{-1}) and three with saturating (~500 μg C L^{-1}) food concentrations (Houde and Roman 1987; Berggreen et al. 1988). In some cases, only two replicates for each food concentration were possible because of lack of animals. Triplicate controls without copepods were used to evaluate changes in phytoplankton pigments due to growth and/or physiological adaptation. All bottles were placed on a plankton wheel (2 rpm) to ensure that food was kept in suspension and were incubated in an environmental chamber (20°C; 12L : 12D) for 24 h. The light intensity during experiments was reduced to approximately 1 μmol m^{-2} s^{-1} to prevent photodegradation of pigments (Nelson 1993) and minimize growth of the phytoplankton.

Samples for initial pigment concentrations were taken before the copepods were added. At the beginning and every 3 h during the 24-h experiments, aliquots of 20 ml were collected and preserved with acid Lugol's solution for cell counts. At the end of the experiments, samples of 100 ml were filtered onto Whatman GF/C filters to measure total pigment (that could be present in the phytoplankton, copepod guts, and/or fecal pellets), and samples of 20 ml were collected for cell counts. The concentration of total pigment from the 100-ml sample was used in calculations for the mass balance with any pigment recovered in the dissolved/colloidal pool (see below) added to this total (the total is referred to as P_{obs} in equations below). After the 100-ml sample was collected, the remaining contents in the bottles were size fractionated to measure pigment concentrations present in specific pools at the end of the experiments as follows: the copepods and fecal pellets were separated and collected by sequential passage through 63- and 30-μm sieves, respectively, and then examined under a dissecting microscope to ensure that the samples contained the desired pool and were free of algae. The collected fecal pellets were not counted as it would have resulted in longer exposure to light and thus possible photodegradation. However, this visual evidence supported the idea that coprophagy was not a problem during the experiments, as no broken-apart fecal pellets were observed in this size fraction under the microscope. The copepods and fecal pellets were transferred to GF/C filters after they were separated. The remaining contents (<30 μm, presumably phytoplankton and possibly smaller broken fecal pellets) of each bottle were filtered onto GF/C filters. To measure the concentrations of pigments in the dissolved/colloidal fraction, a subsample of 100 ml of this filtrate was passed through a solid-phase extraction cartridge (activated by successive methanol and deionized water rinses) (Alltech, C18, 300 mg), and adsorbed pigments were eluted in acetone. Cell abundance in the Lugol's-preserved samples was measured on an Elzone 280 particle counter. The particle size distributions obtained by the Elzone served as another indicator that coprophagy was not likely because only phy-

toplankton size particles were observed. All filter pads were extracted in 90% acetone for 24 h at −20°C, and after extraction they were stored at −70°C until they were analyzed for pigments by HPLC (approximately 1–2 weeks later) (Mantoura et al. 1997).

Pigments were separated by reverse-phase chromatography with a Waters 600 HPLC connected to Waters absorbance (model 440) and fluorescence (model 420-C) detectors. An ODS-hypersil C18 column (Keystone Scientific) with a 5-μm particle size and 120-Å pore size was used. Chromatograms were analyzed using PE Nelson chromatography software (Turbochrom 4). The separation procedure was a modification of the methods of Mantoura and Llewellyn (1983) and Van Heukelem et al. (1992) using two elution solvents (80% methanol : 20% 0.5 M aqueous ammonium acetate and 80% methanol : 20% acetone). The instrument was calibrated using pure pigment standards (Chls *a* and *b* from Sigma and carotenoids from the U.S. Environmental Protection Agency). Pheopigment standards were produced by acidification of chlorophylls. Pigments were quantified by absorbance at 430 nm using the area under chromatogram peaks and conversion factors (ng area^{-1}) that were calculated for each pigment standard and scaled to that of Chl *a*:

$$P = (\text{Chl } a_{\text{std}})(A_{\text{p}} \div A_{\text{Chl}})(F_{\text{p}} \div F_{\text{Chl}})(E \div V) \quad (1)$$

where P represents pigment concentration (μg L^{-1}), Chl a_{std} is the concentration of a daily chlorophyll standard (μg L^{-1}), A_{p} and A_{Chl} are the measured areas of pigment (area count) in that sample and in the chlorophyll standard, respectively; F_{p} and F_{Chl} are the conversion factors (ng area^{-1}) for the pigment standard of interest and for the chlorophyll standard, respectively; E is the extract volume (ml) and V is the volume of sample filtered (ml). A daily injection of Chl *a* standard thus provided a calibration for all other pigments, assuming that variations in instrument-related factors (e.g., lamp strength) affected all pigments equally. Extracts from known algal cultures were processed every 2–3 d to monitor pigment retention times. Chromatograms were also compared with published data to identify unknown peaks (Wright et al. 1991). Fluorescence chromatograms served as an aid in identifying chlorophylls and their derivatives.

Ingestion and clearance rates were calculated according to the equations of Frost (1972). To determine if pigments were destroyed, the total amount of pigment observed at the end of the experiment was compared to the predicted pigment (μgL^{-1}) that was calculated as follows:

$$P_{\text{pred}} = P_0 e^{(k-g)(t_2-t_1)} \quad (2)$$

where P_0 is the initial pigment concentration (μg L^{-1}), k is the cell growth coefficient (h^{-1}; obtained from changes in cell abundance in the controls), g is the grazing coefficient (h^{-1}; obtained from the experimental bottles), and t is time (h). Our hypothesis was that as the cells disappear, the pigment disappears as well. If the amount of predicted pigment equals what is observed at the end of the experiment, then 100% of ingested pigment was degraded or destroyed. If the observed pigment is greater than what was predicted, some of the pigment that was present in the grazed cells survived ingestion and digestion. To determine how much of a given pigment survived ingestion, the amount ingested was compared to that observed in the detrital pools (i.e., copepod guts, fecal pellets, and dissolved fraction) at the end of the experiment:

$$P_{\text{det}} = P_{\text{obs}} - P_{\text{cell}} \quad (3)$$

where P_{obs} was the total pigment concentration in the grazer-containing bottles at the end of the experiment, and P_{cell} was the amount of pigment contained in just the phytoplankton cells at the end of the experiment (determined from cell abundance at the end of the experiments times pigment per cell, which did not vary in the controls during the experiments). Note that for chlorophylls, P_{det} includes undegraded chlorophyll plus pheopigments (where pheopigments were converted by weight to chlorophyll equivalents), whereas for carotenoids, it includes undegraded carotenoids only. The detrital pigment was calculated in this way instead of adding up the recovered detrital pools because it was impossible to separate detrital pigment in the <30-μm size fraction (i.e., small pieces of broken fecal pellets) from uningested phytoplankton. However, subsequent comparison of the product of pigment content per cell and the cell concentration at t_{24} with the measured pigment concentration in the <30-μm fraction showed that little detrital pigment was present in that size range in any of the experiments. This is further evidence that coprophagy was not occurring significantly during the experiments.

Ingested pigment was calculated by multiplying the cellular pigment content by the number of cells ingested. The amount of pigment destroyed was then calculated as follows:

$$\%\ \text{pigment destroyed} = [(P_{\text{ing}} - P_{\text{det}})/P_{\text{ing}}][100] \quad (4)$$

where P_{ing} is the amount of pigment ingested, and P_{det} is the amount of pigment in the detrital pool at the end of the experiment.

Nonparametric statistical methods were used throughout because assumptions of analysis of variance were not met in all experiments. Kruskal–Wallis tests were performed to determine if there was a significant difference among algal species with respect to both Chl *a* and accessory pigment destruction, for both limiting and saturating food concentrations. Differences between food concentrations within each algal diet were determined using Mann–Whitney *U*-tests.

Cellular pigment content in the controls never changed by >3% between the beginning and end of the experiments; thus, any changes in pigment concentrations in the experimental bottles were the result of copepod grazing rather than physiological adaptation of the algae. Under the dim light conditions during the experiments, growth was also low. Copepod ingestion rates and clearance rates displayed the classical responses when the animals were fed different concentrations of food. Ingestion rates were lowest at limiting food concentrations and higher at high food conditions for each experiment, although the magnitude varied among algal species (Table 2).

Summaries of results for chlorophyll and carotenoid destruction in all experiments are given in Tables 1 and 2, respectively. Overall, the range of pigment destruction was 0–93% for Chl *a* and pheopigments and 0% to approximately 100% for the main accessory pigments.

Table 1. Chlorophyll *a* and *b* degradation (pheopigments) and destruction (colorless products) (±standard error) when copepods were fed each phytoplankton diet at saturating and limiting food concentrations.

Phytoplankton	Saturating Food Condition		Limiting Food Condition	
	% Degraded	% Destroyed	% Degraded	% Destroyed
a) Chl *a*				
T. weissflogii	7 (±1.4)	73 (±6.8)	2 (±0.3)	93 (±1.9)
R. lens	9 (±4.0)	60 (±3.0)	16 (±2.1)	70 (±2.5)
C. salina	14 (±2.1)	57 (±12.5)	22 (±4.3)	64 (±12.0)
Tetraselmis sp.	3 (±1.2)	0 (±0)	19 (±6.2)	52 (±1.5)
Tetraselmis PLY 429*	6 (±1.4)	13 (±1.8)	21 (±4.0)	71 (±4.4)
*D. tertiolecta**	11 (±3.1)	53 (±0.7)	29 (±3.8)	70 (±6.7)
b) Chl *b*				
Tetraselmis sp.	<1	3 (±3.0)	<1	20 (±10.5)
Tetraselmis PLY 429*	<1	43 (±7.2)	3 (±1.1)	78 (±3.5)
*D. tertiolecta**	4 (±0.9)	40 (±2.8)	7 (±2.0)	87 (±0.7)

* Experiments when cultured animals were used.

For experiments using the diatom as food, fucoxanthin did not behave conservatively, with more destruction at the limiting food concentrations. An unidentified polar breakdown product that may have originated from fucoxanthin was observed in the fecal pellets; however, assuming it to have the same extinction coefficient as fucoxanthin, it would not have accounted for all of the missing pigment given that it represented <1% of the missing fraction. Chl *a* was also converted to undetectable products more thoroughly under limiting compared to saturating food concentrations. (In all cases, the values reported as chlorophyll destruction refer to pigment loss into colorless products, not pheopigments that were converted back into chlorophyll equivalents for the mass balance.) Very little pigment was recovered in the detrital fractions, especially for fucoxanthin, and only in experiments with saturating concentrations of food were any detectable pigments found in the dissolved/colloidal fraction.

When copepods were fed the cryptophyte *R. lens,* 65% (±1.5; standard error [SE]) and 56% (±6.5) of alloxanthin and 70% (±2.5) and 60% (±3.0) of Chl *a* were destroyed under limiting and saturating food concentrations, respectively. The distribution of pigment at the end of the experiment with *R. lens* was similar to that found with the diatom diet. Little pigment was recovered in the detrital pools, and dissolved pigment was only observed when saturating food concentrations were used. With the second cryptophyte, *C. salina,* the copepods destroyed slightly less of the alloxanthin and about the same amount of the Chl *a* as when fed *R. lens.*

Results from the experiments with *Tetraselmis* sp. showed less destruction of pigments than with any other algal species, and this was the only case in which any of the pigments behaved conservatively. When the copepods were fed saturating concentrations of this alga, none of the Chl *a* or lutein was destroyed, and only 3% (±3.0) of the Chl *b* was destroyed. Under limiting food conditions, however, all three pigments showed some destruction.

The experiments using *Tetraselmis* strain PLY 429 and *D. tertiolecta* as food were performed with copepods that were obtained from cultures. Again pheopigments were found for both Chls *a* and *b.* Some destruction occurred for each pigment and varied between algal species and with food concentrations.

For all algal species, pigments were destroyed to a greater extent when the copepods were fed limiting food concentrations (Tables 1, 2). A statistically significant difference (Mann–Whitney *U,* $P < 0.05$) was found between the two food concentrations with respect to both Chl *a* and accessory pigment destruction for the diatom and all three species of green algae. There was no significant difference found for the cryptophytes; however, the experiments using those algal diets exhibited the same general trend of more destruction

Table 2. Carotenoid destruction (% *D*) (±standard error); algal growth rates, k (h^{-1}); and ingestion rates, I (cells ingested $copepod^{-1}$ h^{-1}) for each phytoplankton diet. C_{t_0} and $C_{t_{24}}$ are initial and final food concentrations (cells ml^{-1}), respectively.

Phytoplankton	Pigment	Saturating food condition					Limiting food condition				
		% *D*	k	C_{t_0}	$C_{t_{24}}$	I	% *D*	k	C_{t_0}	$C_{t_{24}}$	I
T. weissflogii	Fucoxanthin	63 (±5.0)	0.012	3,051	2,167	4,275	99.9 (±0.1)	0.014	896	829	933
R. lens	Alloxanthin	56 (±6.5)	0.018	3,495	3,697	5,062	65 (±1.5)	0.016	2,510	2,316	2,905
C. salina	Alloxanthin	37 (±14.5)	0.009	7,211	5,601	8,049	52 (±10.5)	0.01	2,437	2,236	2,092
Tetraselmis sp.	Lutein	0 (±0)	0.01	10,843	9,681	9,707	31 (±9.0)	0.011	3,123	2,788	3,002
Tetraselmis PLY 429*	Lutein	2 (±0.4)	0.013	6,729	6,008	7,229	80 (±5.7)	0.013	4,644	4,392	4,307
*D. tertiolecta**	Lutein	35 (±2.0)	0.01	6,401	5,566	6,080	80 (±3.0)	0.01	4,073	3,711	3,496

* Experiments when cultured animals were used.

occurring under limiting food concentrations. The differences among phytoplankton species with respect to pigment destruction were significant (Kruskal–Wallis test, $P < 0.05$) for Chl *a* and for the major accessory pigment of each alga at both food concentrations.

As observed by others (Downs 1989; Head and Harris 1992, 1994), pheopigments were seen in the fecal pellets and copepod guts in most cases. Usually Chl *a* was destroyed to a greater extent than the accessory pigments. Negligible amounts of pigment were observed in the dissolved/colloidal fraction at limiting food concentrations. However, some pigment was usually recovered in this pool under saturating conditions, although it never accounted for >3% of the total pigment at the end of the experiment. Only on one occasion were pigments observed in the dissolved fraction from the controls, and this particular experiment was omitted from the study because inconsistent cell counts were obtained. This suggests that the small amount of pigment in the dissolved fraction was the result of grazing rather than leakage or autolysis of the algal cells.

Our experiments suggest that differences among algal species may have caused the variable degrees of pigment destruction that have been observed by others (Dam and Peterson 1988). Food concentration also plays an important role in the extent of pigment destruction. Penry and Frost (1991) showed that animals acclimated to high food concentrations for long periods of time (several days) destroy more pigment than those acclimated under low food conditions. Although this is opposite of what is reported here, our experiments were performed on a time scale too short for physiological adaptation of the copepods. When given high concentrations of food for several days, acclimation results in increased production of digestive enzymes and absorption sites, enabling more efficient utilization of the amount of food present (e.g., Hassett and Landry 1990; Mayzaud et al. 1992). When animals acclimated to high food concentrations feed, they may have higher levels of digestive enzymes that may lead to more extensive destruction of pigments than do copepods acclimated to low food environments. In our study, cultured animals were acclimated over long time scales to high food concentrations and they were then separated into the high and low food experiments with only 1 d of acclimation to experimental conditions. The experiments lasted 1 d, which is not enough time for *A. tonsa* to acclimate physiologically to changes in food concentration (Hassett and Blades-Eckelbarger 1995), and thus animals in the high and low food treatments may be regarded as physiologically the same. Hence, they were only able to change their behavior in response to the treatments (i.e., ingestion rates).

The degree of pigment destruction reported here was negatively correlated with the ingestion rate. Our results are similar to those observed by Head and Harris (1996), where ingestion rates varied inversely with chlorophyll destruction. Head and Harris found that the most important factor contributing to pigment destruction in their study was ingestion rate. They suggest that degradation actually takes place before the food reaches the copepod gut, and they provide a mechanistic interpretation of chlorophyll degradation involving enzymes (chlorophyll bleaching enzymes) produced by both the algae and copepods. Head and Harris (1996) reported that at low ingestion rates, the copepod enzyme can destroy all or most of the ingested chlorophyll, while at higher ingestion rates most of the destruction is due to the algal enzymes. Our data showed that this correlation with ingestion applies to the destruction of carotenoids as well.

In summary, phytoplankton pigments are not conserved during copepod grazing, and in our experiments there was no constant value for the extent to which they were destroyed. This creates problems for using pigments as tracers in bottle incubation feeding experiments and gut pigment analyses. In both cases the amount of grazing would be underestimated because the pigments are not conserved. In incubation studies, it is assumed that pigments are destroyed during feeding so that decreases in pigment represent grazing rates (e.g., Meyer-Harms and von Bodungen 1997). However, if some of the ingested pigment is not destroyed, it would remain in the system in fecal pellets, leading to an underestimate of grazing. In gut pigment studies using carotenoid pigments, it is usually assumed that pigments are unaltered in the guts of the animals (e.g., Quiblier et al. 1996*b*; Hwang et al. 1998). If the pigments are destroyed into undetectable, colorless products, these grazing rates also would be underestimates. Although recent studies are beginning to implement caution when using pigments as tracers (e.g., Gowen et al. 1999), the precautions taken do not always suffice because there are a multitude of variables influencing the extent of destruction.

Our results underscore the many problems associated with using phytoplankton pigments as tracers of herbivory. We found variability in pigment destruction that could be attributed to algal species and concentration, as well as ingestion rates. Pigments may still serve as qualitative markers of diel or spatial differences in gut fullness; however, because it is difficult to account for all of the factors affecting the chemical behavior of pigments, studies using pigments as quantitative indices for grazing should be avoided. At best, the conservative behavior of the pigments would have to be verified for each set of experimental conditions and grazers.

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The effect of calcium concentration on the calcification of *Daphnia magna*

Abstract—Soft waters are characterized by low Ca concentrations, and the distribution and relative success of Ca-demanding invertebrates could be limited by low Ca in extreme softwater localities. A further Ca depletion caused by reversed acidification could thus seriously affect freshwater crustaceans. Experimental studies on the calcification and Ca content of *Daphnia magna* clearly suggested the potential of a Ca limitation. Saturated calcification was reached at Ca concentrations >0.13 mM. Individuals reared in media with lower Ca concentrations were unable to compensate by increasing the period of postmolt Ca uptake and thus had a lower specific Ca content. Specific Ca content decreased from 4.2% to 1% of dry weight over the range 0.25–0.013 mM Ca. Even at the low Ca concentrations, only 10% of total Ca was reclaimed upon molting, the rest was lost with the old exuviae (~40%) or to the ambient medium (~50%). This incomplete calcification under low ambient Ca concentrations could represent a competitive drawback in Ca-poor waters.

Ca is an essential element to crustaceans and other groups of animals with a calcified exoskeleton. While Ca demands are easily satisfied in marine environments and hardwater lakes, Ca deficiency may limit the success of some zooplankton species in softwater lakes. These lakes may have a scattered distribution depending on local geology, or they may cover larger areas like the Canadian Shield lakes (Neary and Dillon 1988; Yan et al. 1989) and major parts of Scandinavia. In a national survey of 1,500 Norwegian lakes, the median Ca concentration was 0.025 mM Ca (Skjelkvåle et al. 1997). In another survey of 346 Norwegian lakes where acidified and high-altitude lakes were omitted, less than 25% of the lakes had Ca concentrations >0.13 mM, and more than half had Ca concentrations <0.063 mM Ca (Hessen et al. 1995*b*). In addition, reduction in the long-term inputs of anthropogenic SO_4^{2-} has led to a decrease in runoff sulfate that is matched by an equal decline in water Ca concentration (Likens et al. 1996). Because of the reduced mineral weathering rates at reduced sulfate deposition, the depletion of Ca will be long-lasting. Reduced water Ca concentrations may thus add to the prevailing Ca deficiency in softwater localities.

The major sources of Ca for crustaceans are water and food, with ionic Ca in water being the most important source of Ca for zooplankton (Cowgill et al. 1986) as for crayfish (Hessen et al. 1991). Various species have a variable Ca content, ranging from 2.8 to 7.7% of dry weight in *Daphnia* spp. (Cowgill 1976; Havas 1985; Yan et al. 1989) to as low as 0.06% in copepods (Yan et al. 1989). Most of the Ca is probably associated with carbonate and phosphate minerals in the carapace (Stevenson 1985), and thus at least some proportion of this body-Ca must be regenerated after each molt.

It has been observed that the Ca content in *D. magna* is affected by the chemical composition of the medium (Cowgill et al. 1986), and this apparent adjustment to low Ca may also imply that some of the more heavily calcified cladocerans may suffer from Ca deficiency in softwater lakes. Recent studies have shown that low Ca concentrations affect both survival and reproduction in *Daphnia* spp. (Hamza et al. 1998; D. O. Hessen et al. in press). Low Ca concentrations may hence constrain the distribution of certain branchiopods, and this will also affect community structure. The latter is supported by Tessier and Horwitz (1990), who observed a transition from large crustaceans to small rotifers with decreasing water hardness, and related this to Ca deficiency in the crustaceans. Further, Hessen et al. (1995*a*) showed a clear connection between lake-specific Ca concentration and the occurrence of *Daphnia* spp.

Many crustaceans store a proportion of the Ca withdrawn from the old exoskeleton before ecdysis. The degree of Ca storage and storage site is, however, highly variable between different taxa (Greenaway 1985). Little information is available on Ca storage in zooplankton. However, it is suggested that freshwater ostracods, due to their small size, have no Ca storage mechanisms but rather depend on rapid Ca uptake (Turpen and Angell 1971).

The aim of this study was to evaluate the role of water Ca concentration on the Ca content and calcification of *D.*