

NOTE

Translocation of algal pigments to oocytes in *Capitella* sp. I (Annelida: Polychaeta)

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ABSTRACT: Juvenile *Capitella* sp. I were raised on 3 diets: a processed mixed cereal; benthic diatoms; and a green macroalgae. Accessory pigments from algal diets were translocated to developing oocytes. Worms fed mixed cereal produced eggs with no pigments; worms fed diatoms produced eggs with fucoxanthin, diadinoxanthin, diatoxanthin and β -carotene; worms fed macroalgae produced eggs with lutein, lutein cis-isomers and β -carotene. In addition to accessory pigments, chlorophylls and their breakdown products (pheophytin and pheophorbide) and β -carotene anabolic products (canthaxanthin and echinenone) were also translocated to oocytes. The presence of intact chlorophyll *a* in eggs is evidence that *Capitella* sp. I can rapidly assimilate and allocate materials to reproduction. Given the importance of carotenoids in aquaculture diets, differences in oocyte carotenoid compositions may significantly alter growth and development rates of eggs produced on different food resources in field populations. In future studies, it may be possible to identify the food resources of opportunistic deposit-feeders from the biochemical composition (fatty acids, sterols, and pigments) of their oocytes.

In temperate estuaries, opportunistic deposit feeders generally exhibit high fecundities, rapid oogenesis, and continuous reproduction, producing large seasonal fluctuations in a population's reproductive output (Grémare et al. 1989). Seasonal variations in reproduction have been attributed to changes in food availability (Levin 1986, Zajac 1986), especially when phytodetritus sedimentation increases after phytoplankton blooms (Davies & Payne 1984, Christensen & Kenneworff 1985). Because phytoplankton blooms are seasonally ephemeral, a population of opportunists must immediately allocate assimilated food to reproduction with little time to catabolize those resources. Thus, in opportunists with high yolk requirements, nutrition of

developing gametes is a problem when yolk-precursors must be translocated rapidly to as many oocytes as a body coelom can accommodate in the shortest amount of time as necessary for adequate yolk formation (Eckleberger 1986).

The opportunistic polychaete *Capitella* sp. I has been shown to translocate fatty acids and sterols directly from its diet to developing oocytes (Marsh et al. 1990). In addition, Marsh et al. (1990) reported a large phytol peak (the C29 isoprenoid side-chain of chlorophyll) in *Capitella* sp. I eggs produced by females raised on algal diets. The presence of phytol in these eggs suggests that algal pigments or their breakdown products could be translocated to developing oocytes. In crustaceans, dietary carotenoids may function as growth hormones, lipoprotein crosslinks to control membrane permeability, free-radical scavengers, antiphototoxidants and UV light absorbers (Castillo 1981). Thus, the presence of carotenoids in *Capitella* sp. I oocytes is not a trivial observation. At the very least, carotenoids could potentially serve as chemical markers for identifying the temporal food resources of deposit feeders. This study compares the pigment profiles of eggs produced by *Capitella* sp. I fed either a mixture of diatoms (*Nitzschia* and *Navicula* spp.) or a macroalgae (*Ulva* sp.).

Materials and methods. Stock cultures of *Capitella* sp. I, started from individuals collected at Woods Hole, Massachusetts (USA), were maintained at 20 °C and 25 ‰S (Chesney & Tenore 1985). Fecundity experiments were carried out by Grémare et al. (1988) to examine the effect of diet on egg production in *Capitella* sp. I. Immature juveniles (80 μ g dry wt) were sorted from stock cultures and placed in replicate finger bowls with different rations of either: (1) Gerber's Pablum, a mixed-grain cereal commercially available for babies (Gerber), (2) benthic diatoms, a mixture

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a freeze-dried *Nitzschia* sp. and *Navicula* sp. (diatoms), or (3) *Ulva* sp., a green macroalgae collected from Chesapeake Bay and freeze-dried (*Ulva*). The levels of carbon, nitrogen, fatty acids, and amino acids in these foods is described elsewhere (Marsh et al. 1989). After 15 d mature eggs were dissected from gravid females and frozen in groups of 1000 to 2000.

Eggs were ultrasonicated in 100 % acetone at 4 °C after thawing. Samples were centrifuged (10 000 × *g*) and the supernatants used for pigment analyses by ion pairing, reversed-phase HPLC after Bianchi et al. (1988). A programmable pump (Constametric III, LDC Milton-Roy with MP 3000 controller) established a gradient from 100 % solvent A (80:10:10, methanol: water:5.5 % tetrabutyl ammonium acetate) to 100 % solvent B (70:30, methanol to acetone) in 30 s with a hold for 20 s. Dual channel detection was achieved with an LDC UV monitor with 440 nm filter kit for absorbance and a Perkin-Elmer 650–10S fluorescence detector, excitation 440 nm and emission 670 nm. Pigments were separated on an 250 × 4 mm C18, 5 μm Adsorbosphere column. Standard chlorophyll and degradation products were prepared after Mantoura & Llewellyn (1983). Pigments were identified by diode array spectroscopy (Waters model 990) and comparisons of retention times to isolated standards. The instruments at the Chesapeake Biological Laboratory

have been calibrated with standards and reference algal cultures made available through the Joint Global Ocean Flux Studies-Plant pigment intercalibration program (Drs Bidigare and Kennicutt, Texas A & M University, Austin).

Results. The pigment profiles of the experimental diets differed in both their quantities of chlorophyll *a* and their complement of accessory pigments. Gerber, a commercially bleached, grain-cereal extract, had no pigments present. The diatom accessory pigments were dominated by chlorophyll *c*, fucoxanthin, diadinoxanthin, and diatoxanthin (Fig. 1A). A trace of β-carotene was detected as well as an unknown pigment eluting just after diatoxanthin. The *Ulva* sp. accessory pigments were dominated by fucoxanthin, neoxanthin, lutein, and chlorophyll-*b* (Fig. 1B). Traces of β-carotene, violaxanthin, antheraxanthin, and lutein *cis*-isomers were detected.

Capitella sp. I females fed *Nitzschia* sp. and *Navicula* sp. produced eggs with diatom accessory pigments (Fig. 2A). Fucoxanthin, diadinoxanthin, diatoxanthin, canthaxanthin, echinenone and β-carotene were translocated to developing oocytes. Intact chlorophyll *a* and its breakdown products (phaeophorbides and phytol) were also present in the eggs. The mixture of phaeophorbides probably included chlorophyll *a* and *c* derivatives, however, the fluorescence detector was

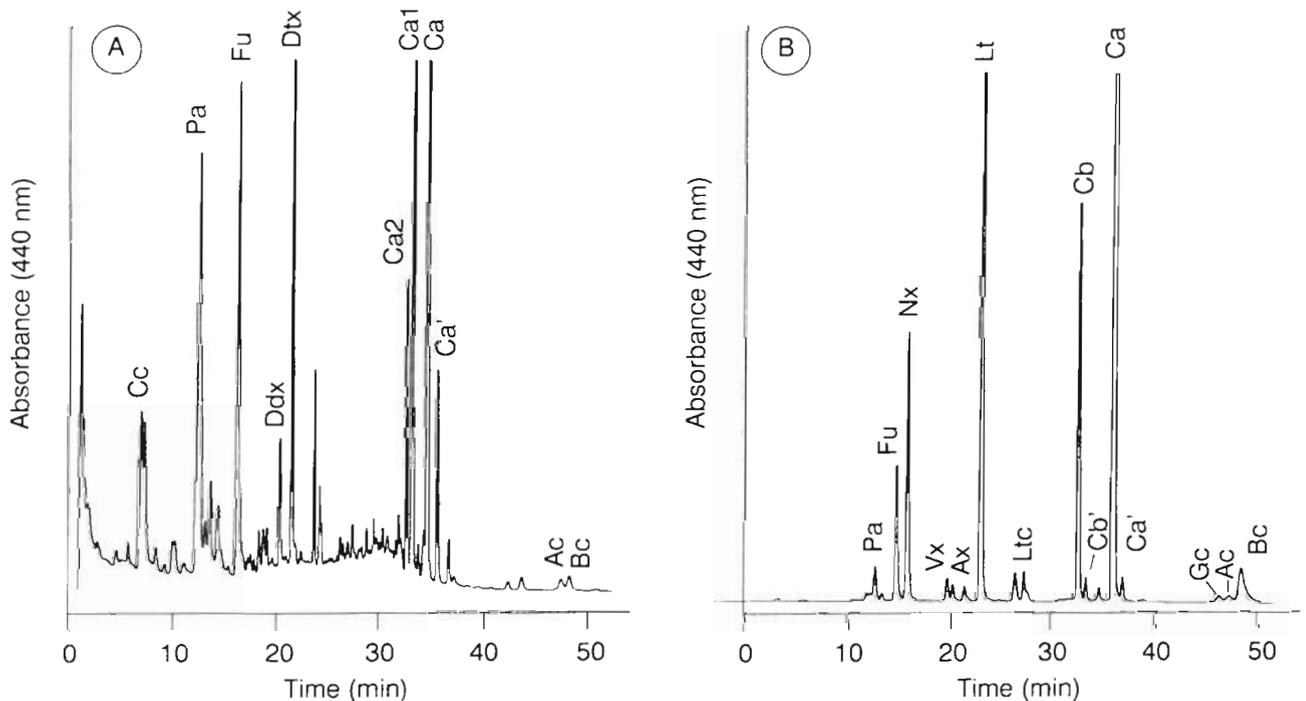


Fig. 1. Pigment profiles of the algal diets: (A) benthic diatoms, *Nitzschia* sp. and *Navicula* sp.; (B) the green macroalgae *Ulva* sp. Relative absorbance at 440 nm is followed during HPLC pigment separation. An = antheraxanthin; Ac = α-carotene; Bc = β-carotene; Ca = chlorophyll *a*; Ca' = chlorophyll *a*'; Ca1 = chlorophyll *a* allomer 1; Ca2 = chlorophyll *a* allomer 2; Cb = chlorophyll *b*; Cb' = chlorophyll *b*'; Cc = chlorophyll *c*; Ddx = diadinoxanthin; Ddt = diatoxanthin; Fu = fucoxanthin; Gc = γ-carotene; Lt = lutein; Ltc = lutein *cis*-isomers; Nx = 9'-*cis*-neoxanthin; Pa = phaeophorbide *a*; Vx = violoxanthin.

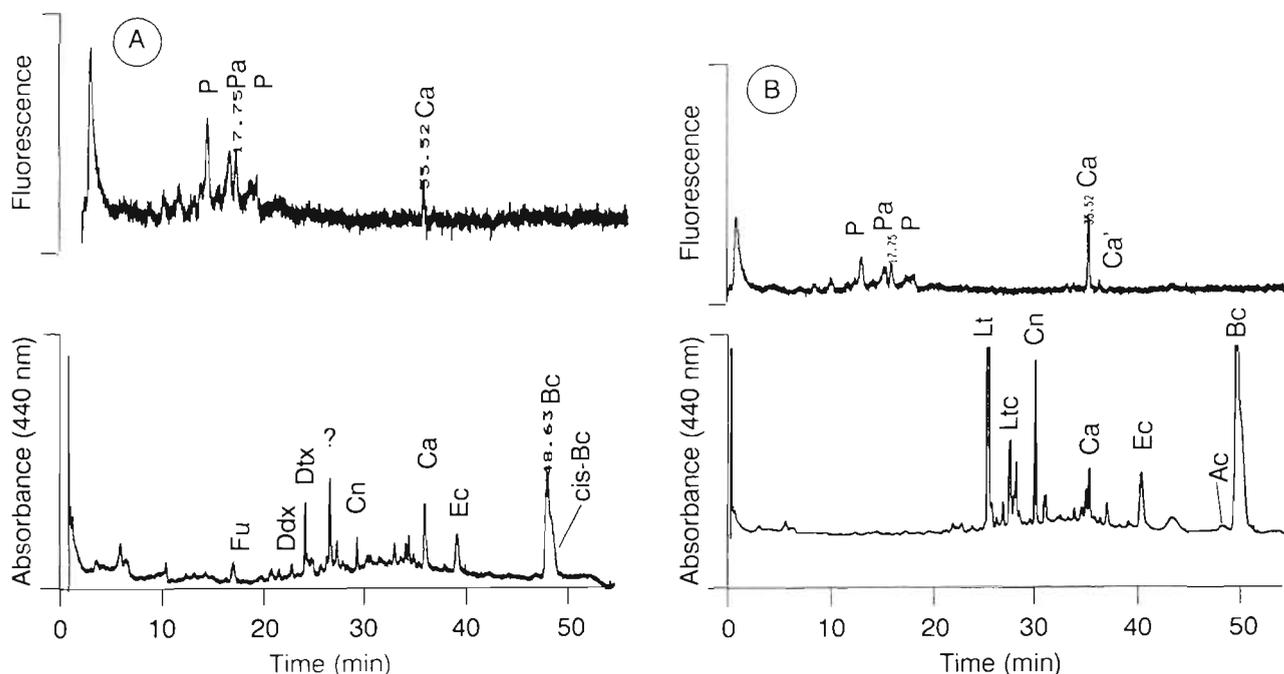


Fig. 2. *Capitella* sp. I. Pigment profiles of oocytes produced on the algal diets: (A) diatoms, (B) *Ulva* sp. Both relative absorbance and relative fluorescence are followed during HPLC pigment separation. Bc = β -carotene; Ca = chlorophyll *a*; Ca' = chlorophyll *a*'; Cn = canthaxanthin; Ddx = diadinoxanthin; Ddt = diatoxanthin; Ec = echinenone; Fu = fucoxanthin; Lt = lutein; Ltc = lutein cis-isomers; P = phaeophorbide-like compounds, probably derivatives of chlorophylls *b* and *c*; Pa = phaeophorbide *a*; ? = unknown pigment translocated from diatom diet to oocytes

operating below its optimal response level and the identification of phaeophorbide *c* is tenuous. *Capitella* sp. I females fed *Ulva* sp. produced eggs with green algal accessory pigments (Fig. 2B). Lutein, lutein cis-isomers, canthaxanthin, echinenone, and β -carotene were translocated to developing oocytes. Again intact chlorophyll *a* and phaeophorbide products (possibly including derivatives of chlorophyll *b*) were also present and in such large quantities that these eggs had a visible yellow-green tint. *Capitella* sp. I females fed Gerber produced eggs with no pigments present.

Phaeophytins were not detected in the oocytes and supports the suggestion that *Capitella* sp. I can remove the phytol side chain of parent chlorophylls (Marsh et al. 1990). The presence of phaeophorbide and phytol in *Capitella* sp. I eggs indicates that during digestion, assimilation, and translocation, chlorophylls are degraded by removal of the magnesium ion and phytol side chain from the tetrapyrrole ring to form phaeophorbides. The degree of chlorophyll degradation is probably dependent on the rate at which digestion, assimilation and translocation proceed; the presence of intact chlorophyll molecules in the eggs indicates that these processes can occur rapidly in *Capitella* sp. I.

Discussion. Oogenesis in *Capitella* species is unique among polychaetes. The dorsal blood vessel passes

between the ovaries in each segment, allowing follicle cells to immediately assimilate macromolecules from the circulatory system by endocytosis (Ecklebarger & Grassle 1982). This characteristic provides *Capitella* species with the ability to translocate rapidly materials to developing oocytes. As a consequence, an adult female has little opportunity to metabolize or structurally rearrange materials assimilated from the gut before they are sequestered in oocytes. This may be true for other opportunists that have high fecundities, rapid oogenesis, and continuous breeding when resources are available. *Capitella* sp. I eggs produced by adults fed different diets can be discriminated in a principal component analysis of egg fatty acid and sterol composition (Marsh et al. 1990). The ability of *Capitella* sp. I to translocate chlorophyll *a* intact is evidence of the fast rates at which materials can be moved to oocytes.

Carotenoids appear to have a variety of physiological functions and their importance in aquaculture feeds has been well established (Simpson et al. 1981). Work on crustacean development with canthaxanthin (a β -carotene derivative) has shown that sequestering this carotenoid in oocytes provides a vitamin-A-like compound during development that is analogous to crustacean juvenile growth hormone (Ahl 1989). Work on fish has shown that echinenone (another β -carotene deriva-

tive) can be converted to a provitamin A metabolite that increases bacterial and fungal disease resistance (Tacon 1981). These vitamin-A-like compounds have pronounced effects on reproductive physiology and can increase gonadal development, egg maturation and viability, and larval growth and survival (Tveranger 1986).

In polychaetes, β -carotene, canthaxanthin and echinenone are common tissue constituents (Dales 1962). β -carotene is obtained in the diet and converted to its derivatives. The influence of these carotenoids on growth, reproduction and development in field populations of opportunists is unknown. Given the importance of phytodetrital sedimentation in determining opportunistic population dynamics, carotenoids may be an important dietary micronutrient, such as essential fatty and amino acids, vitamins and cofactors. Although some dietary carotenoids may not be 'essential' for survival, their presence could potentially enhance reproductive and developmental rates, and thus significantly influence the potential for rapid population increases.

A secondary importance of dietary pigments in the eggs of deposit feeders lies in the potential for identifying their food resources. *Capitella* sp. I fed *Ulva* sp. transmits a green-algal signal to its oocytes, namely lutein and probably phaeophorbide *b* compounds (at present only tentatively identified). *Capitella* sp. I fed *Nitzschia* sp. and *Navicula* sp. transmits a diatom signal to its oocytes, namely fucoxanthin, diadinoxanthin, diatoxanthin and probably phaeophorbide *c* compounds. We speculate that *Capitella* sp. I fed dinoflagellates would produce eggs with the pigment peridinin and the sterol dinosterol; *Capitella* sp. I fed cryptomonads would produce eggs with alloxanthin. All of these pigments are unmistakable markers of feeding history. Fatty acid and sterol composition of oocytes in *Capitella* sp. I have been used to identify experimental diets on which females were raised. Carotenoid composition in conjunction with fatty acid and sterol profiles of oocytes may collectively provide a sensitive discriminant function to identify the food resources of opportunistic deposit-feeders in the field.

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