Hydrobiologia **298**: 133–139, 1995.
D. Belk, H. J. Dumont & G. Maier (eds), Studies on Large Branchiopod Biology and Aquaculture II.

Food-dependent color patterns in *Thamnocephalus platyurus* Packard (Branchiopoda: Anostraca); a laboratory study

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Key words: β-carotene, baker's yeast, growth, survival

Abstract

Coloration of phyllopods varies from place to place and from one life stage to another. It ranges from translucent or whitish through gray, blue, green, orange, and reddish. Here, we present experimental evidence for a food-dependent color pattern in *Thamnocephalus platyurus* Packard. The presence or absence of the synthetic pigment trans-β-carotene in a baker's yeast diet was the controlling factor. All the 24 old larvae used in the experiment were whitish in color. From day 6 until the end of the experiment (day 11), 100% of the shrimps under a diet with synthetic trans-β-carotene (treatment 1) exhibited a characteristic color pattern which consisted of an orange color in the cercopods, and in all thoracopods; the rest of the body exhibited no particular color. In comparison, 100% of the shrimps under a diet without synthetic trans-β-carotene (treatment 2) were whitish throughout the body. In females from treatment 1, the ovaries and oocytes were green-bluish, while in females from treatment 2 the ovaries and oocytes were whitish. No significant differences in survival and growth were found, except that at day 9, there was a significant difference in growth, the females with the synthetic trans-β-carotene group growing faster.

Introduction

Coloration of phyllopods is highly variable, differing from place to place and from one life stage to another (Dexter, 1959; pers. obs.). Pennak (1989) pointed out that their color ranges from translucent or withish through gray, blue, green, orange, to reddish, probably governed to a large extent by the type of food ingested.

Packard (1883) quoted Dr Watson, who noted that *Streptocephalus texanus* Packard collected in Kansas in June and July had red tails and some had blue bodies, while specimens collected in October and November were pure white, but specimens of *Thamnocephalus platyurus* Packard were mostly pinkish, with the edge of the tail red, and the genitals light blue. Dexter (1943) reported much variation in the color of *Eubranchipus vernalis* (Verrill) from different ponds, although specimens from single ponds were usually uniform in color. Specimens were orange-pink, or light orange, but in two ponds they were colorless, and in eight, females were bluish-gray while males were light green. In later visits to three of the ponds which had orange-pink populations, the females were bluish-gray while the males were light-green (Dexter, 1943). Dexter & Ferguson (1943) found that metanauplii of *E. serratus* Forbes were of a brilliant salmon color, and as they grew the color became light gray, brilliant green, brown, or reddish-brown. These reports illustrate that as fairy shrimps grow, their color changes. Later, Dexter (1946) reported a population of *E. vernalis* which had an unusual light blue color. He pointed out that the pigment was found throughout the body but was particularly noticeable in the head and at the tips of the appendages, and suggested that a chemical property of the water was possibly responsible for the color of the shrimps.

Ermakow (1928, in Gilchrist & Green, 1960) described the coloration of *Artemia* by noting that females were salmon pink with green heads and thoracopods, while males had green bodies and reddish thoracopods. Bond (1933) mentioned that it was supposed, at that time, that the color of *Artemia* depended on the strength of the brine, or on food. He concluded that *Artemia* specimens fed on colorless bacteria were transparent to white, those fed on green organisms such

Table 1. Survival of *Thamnocephalus platyurus* reared at 25.5±1.0 °C in a flow-through system under treatments 1 (baker's yeast, corn oil, and synthetic trans-β-carotene) and 2 (baker's yeast, and corn oil) of four replicates each. The experiment started with 24 h-old larvae. Volume of culture medium 0.5 l per vessel.

Period (days)	Initial N per replicate	Survival mean±SD	Survival %	Mortality %	Cumulative %
Treatment 1					
1–3	100	72.25±6.9	72.2	27.8	72.2
3–6	70	61.00±8.0	87.1	12.9	62.8
6–9	50	33.00±11.6	66.0	34.0	41.4
9–11	10	5.50±0.5	55.0	45.0	22.7
Treatment 2					
1–3	100	81.75±8.0	81.7	18.3	81.7
3–6	70	64.25±1.7	91.7	8.3	74.9
6–9	50	33.25±1.2	66.5	33.5	49.8
9–11	10	6.25±1.2	62.5	37.5	31.1

as algae were greenish, and those fed on orange colored organisms such as *Dunaliella salina* were deep red (Bond, 1933).

Gilchrist & Green (1960) pointed out that color variation is not always due to differences in food. They mentioned that besides carotenoid pigments, the reddish coloration in *Artemia* can be due to haemoglobin (Gilchrist & Green, 1960), in a concentration which varies inversely with the oxygen content of the water (Gilchrist, 1954). Gilchrist & Green (1962) mentioned that when *Chirocephalus diaphanus* Prevost was collected in the field it was usually bright orange due to carotenoids and when kept in the laboratory and fed on yeast, the animals gradually became greenish-blue. They pointed out that this color was probably due a bile pigment and suggested that it is likely derived from the breakdown of the haemoglobin in the blood. Czeczuga (1973) identified a number of carotenoids occurring in *Branchinecta paludosa* (Muller) and suggested that some of these pigment can give a specific color to the body of this species. Gilchrist & Zagalsky (1983) isolated two canthaxanthin-proteins from females of *B. packardi* Pearse, an orange lipovitellin from yolk platelets and a blue lipoprotein from connective tissue storage cells.

It is clear that descriptions of body coloration have mainy been based on (1) the gut content, *i.e.* the color of the food itself, and (2) the pigments contained in the body. Many coloration patterns in animals are, however, partially or entirely due to structural peculiarities of the tissues concerned, rather than to the presence of pigments (e.g. iridescent tissues) (Goodwin, 1960).

While standardizing a flow-through culture system using *Thamnocephalus platyurus* as a test organism (Maeda-Martínez *et al.*, 1995; this volume), we noted different coloration patterns in the animals using baker's yeast diets, with and without β-carotene. Thus, we decided to experimentally define not only the coloration patterns but also to compare the survival and growth of animals fed baker's yeast with (treatment 1) and without (treatment 2) synthetic β-carotene.

Material & methods

Cysts of *Thamnocephalus platyurus* were produced in a mass culture (Dr D. Weaver, California, USA) and supplied to our laboratory by Dr D. Belk. The (1) incubation method, (2) culture method for the first larval stages, (3) culture system (flow-through), and (4) feeding schedule are described by Maeda-Martínez *et al.* (1995; this volume).

Survival, growth, and color were determined at the end of four subsequent culture periods (culture days 1–3, 3–6, 6–9 & 9–11) (Tables 1 & 2). Each culture period started with an equal number of shrimps in all four replicates. After the first culture period, the density was adjusted by randomly selecting a fixed number of the surviving shrimps (Table 1). Growth (Table 2) was estimated by measuring standard length. Measurements

Table 2. Growth of *Thamnocephalus platyurus* reared at 25.5±1.0 °C in a flow-through system under treatments 1 (baker's yeast, corn oil, and synthetic trans-β-carotene) and 2 (baker's yeast, and corn oil) of four replicates each. The experiment started with 24 h-old larvae.

Day	N measured from each replicate	Standard length (mm) mean±SD	Growth rate mm day^{-1}
Treatment 1			
1	10	0.92±0.08	
3	10	4.63±0.53	1.85
6	pooled	9.71±0.50	1.69
	5♂♂	9.94±0.65	1.77
	5♀♀	9.48±0.59	1.61
9	pooled	12.36±0.27	0.88
	5♂♂	11.81±0.84	0.62
	5♀♀	12.92±0.59	1.14
11	pooled	15.21±0.59	0.95
	3♂♂	15.04±2.77	1.07
	2♀♀	15.30±1.93	0.79
Treatment 2			
1	10	0.89±0.09	
3	10	4.57±0.51	1.84
6	pooled	9.79±0.62	1.74
	5♂♂	9.59±0.74	1.67
	5♀♀	10.00±0.60	1.81
9	pooled	11.39±0.72	0.53
	5♂♂	11.43±0.69	0.61
	5♀♀	11.34±0.84	0.44
11	pooled	14.82±0.97	1.14
	3♂♂	14.54±1.47	1.03
	2♀♀	15.23±1.76	1.29

were carried out with an eye-piece micrometer on a stereo-microscope Wild M3 to the nearest 0.18 mm. The experiment was carried out at 25.5±1.0 °C, pH of 6.7–7.2, conductivity 580–630 μS cm^{-1}, and a light regime of 16L/8D.

The diet for treatment 1 was prepared as follows: 0.05 g of synthetic (crystalline) trans-β-carotene (Sigma-C9750) was diluted in 6 ml of corn oil (Nonkels, Belgium) at room temperature and kept at 2 °C until used. To each 20 g of baker's yeast (Koningsgist, Belgium) to be used according to the feeding schedule (see Maeda-Martínez *et al.*, 1995; this volume), 1.8 ml of corn oil with diluted synthetic trans-β-carotene was added. The diet of treatment 2 consisted of baker's yeast and corn oil without synthetic trans-β-carotene. Using absolute ethanol as a solvent, pigment extractions were analyzed under a UV-visible recording spectrophotometer (UV-160A Shimadzu). Measurements between wavelengths 300 to 600 nm gave a maximum peak of absorption at 447 nm for both the extractions from (1) synthetic trans-β-carotene, and from (2) baker's yeast with corn oil and synthetic trans-β-carotene, and at 499 nm for the extractions from (3) baker's yeast with corn oil. The absorption maximum of β-carotene in ethanol has been reported to be at 453 nm by Isler *et al.* (1956, in Goodwing, 1980), and at 449 and 475 nm by Katayama *et al.* (1972, in Davis, 1976).

Pooled data for survival, and both pooled, and sex-separated data for growth at days 3, 6, 9 & 11 were analyzed by one-way ANOVA (Tukey) (SYSTAT, Wilkinson, 1990).

Results

Survival & growth

No significant effect of the treatments on survival were found ($p>0.05$) (Table 3). Similarly, no differences in growth were found for any of the periods except at day 9 where a significant difference in growth from pooled data and females was noticed ($p<0.05$) (Table 3).

At the end of the experiment (day 11) 45.4% of the treatment 1 and 92% of the treatment 2 animals suffered from black disease.

Color patterns

Nauplii of *Thamnocephalus platyurus* exhibited a green-bluish color throughout the body except for the first and second antenae and the biramous mandibles, which were whitish. Later, all the 24 h-old larvae used in the experiment were whitish in color. At day 3, sexes could not be distinguished; about 69% of the animals in treatment 1 had an orange tail (telson and primordia of cercopods), while the rest of the body was whitish. By contrast, 100% of in animals in treatment 2 showed a whitish color throughout the body. From day 6 until the end of the experiment (day 11), sex was easily distinguished and 100% of the animals in treatment 1 exhibited a characteristic color pattern which consisted of an orange color, distributed only in the tail and all the thoracopods. The rest of the body had no particular

Table 3. Effect of diets on the growth and survival of *T. platyurus*. One-way ANOVA.

Variable	Source of variation	DF	SS	MS	F
Growth (pooled)					
Day 3	Food types				
	Growth	1	0.007	0.007	0.025^{ns}
	Error	6	1.633	0.272	
Day 6	-do-	1	0.013	0.013	0.041^{ns}
		6	1.932	0.322	
Day 9	-do-	1	1.901	1.901	6.428
		6	1.775	0.296	
Day 11	-do-	1	0.332	0.332	0.223^{ns}
		6	8.935	1.489	
Growth (sex-separated)					
Day 6	Food types				
	Growth (males)	1	0.251	0.251	0.514^{ns}
	Error	6	2.936	0.489	
Day 9	-do-	1	0.278	0.278	0.466^{ns}
	Error	6	3.569	0.595	
Day 11	-do-	1	0.485	0.485	0.098^{ns}
		6	25.587	4.931	
Day 6	Food types				
	Growth (females)	1	0.531	0.531	1.478^{ns}
	Error	6	2.518	0.360	
Day 9	-do-	1	5.024	5.024	9.438*
		6	3.194	0.532	
Day 11	-do-	1	0.011	0.011	0.003^{ns}
		6	20.496	3.416	
Survival					
Day 3	Food types				
	Survival	1	180.5	180.5	3.171^{ns}
	Error	6	341.5	56.9	
Day 6	-do-	1	21.125	21.125	0.625^{ns}
		6	202.7	33.792	
Day 9	-do-	1	0.125	0.125	0.002^{ns}
		6	414.750	69.125	
Day 11	-do-	1	1.125	1.125	1.174^{ns}
		6	5.750	0.958	

* = $p<0.05$; *ns* = $p>0.05$

color (Fig. 1B). In comparison, 100% of the animals in treatment 2 were whitish throughout the body. On day 11, at least five females in each treatment were just starting oogenesis. Their ovaries and oocytes differed in color. The females in treatment 1 had ovaries and oocytes that were green-bluish (Fig. 1D), while in females in treatment 2 they were whitish (Fig. 1C).

No differences in shell gland color could be detected among females in either treatment. The *Thamnocephalus platyurus* female has two pairs of shell glands connected to the ovisac in the brood-pouch. One pair (the dark, dorsal pair) is located in the atrium formed by the two genital segments between the gut and the base of the brood-pouch. The other pair (light, ventral pair) is located in the base of the brood-pouch. The dark dorsal pair is red-brownish to dark brown, while the light ventral pair is light-brownish to brown (Fig. 1C & D).

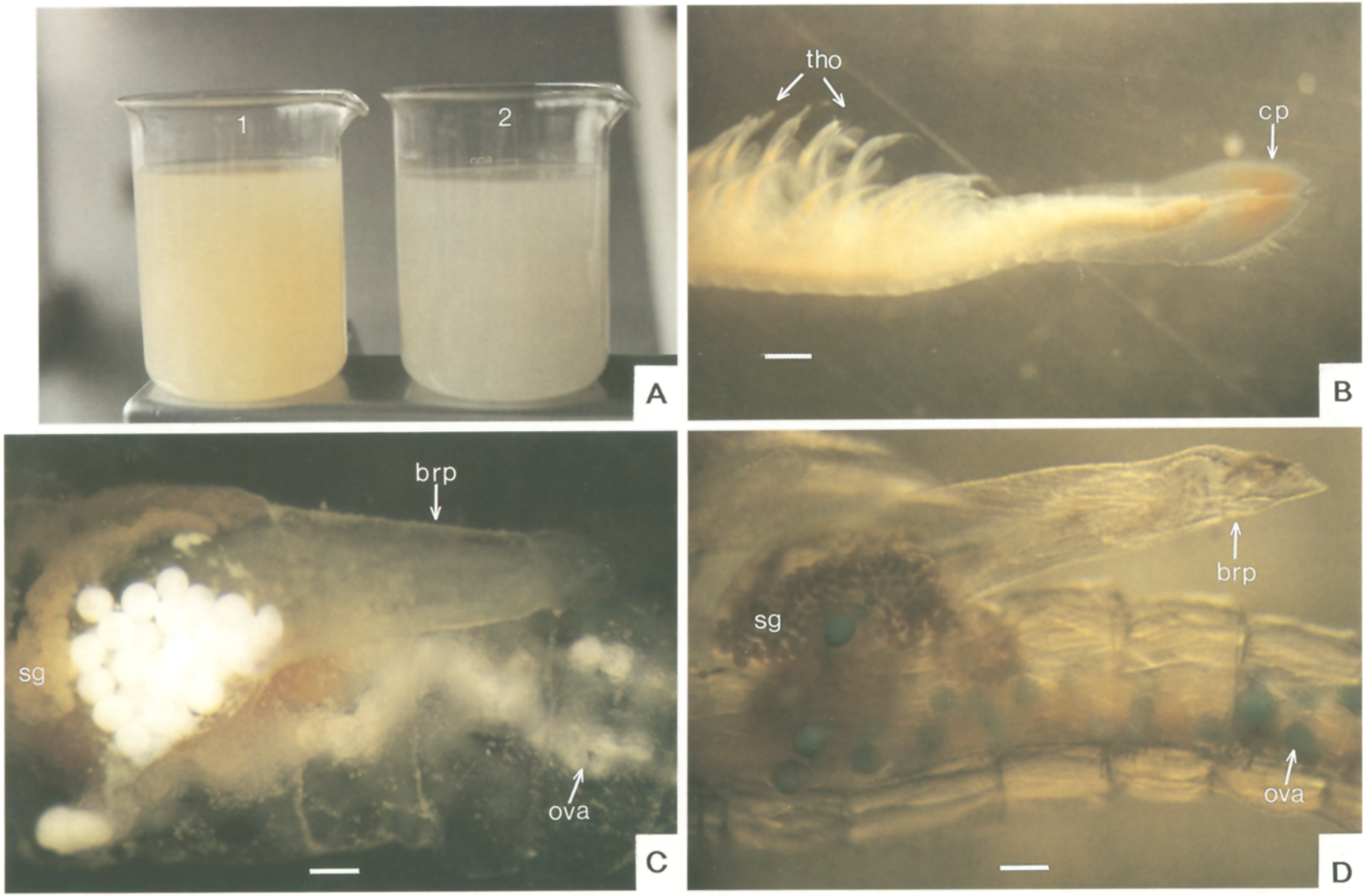

Fig. 1. A. Food suspensions of treatments 1 (baker's yeast, corn oil, and synthetic trans-β-carotene) & 2 (baker's yeast, and corn oil). B-D. *Thamnocephalus platyurus*. B. Male postlarva. Note the orange color of the cercopods and thoracopods. C. Female adult from treatment 2. Note the white color of the ovaries and oocytes. D. Female adult from treatment 1. Note the greenish-blue color of the ovaries and oocytes. brp. brood-pouch, cp. cercopods, ova. ovary, tho. thoracopods & sg. shell glands. Scale bars: B. 1.0 mm, C & D. 0.5 mm.

Discussion

In this paper, we present experimental evidence for a food-dependent color pattern in *Thamnocephalus platyurus*. The presence or absence of the synthetic pigment, trans-β-carotene in a baker's yeast diet was the controlling factor. It is known that baker's yeast contains little or no carotenes (Gilchrist & Green, 1960; Bunker, 1963; Gilchrist, 1968; Hata & Hata, 1969). Herring (1968a) declared *Daphnia magna* Straus free of carotenoids after several generations reared on a diet of yeast alone. Whereas all photosynthetic organisms and some other microorganisms can synthesize carotenoids *de novo*, it has been a dogma that animals cannot synthesize carotenoids *de novo*, but they may have the ability to structurally modify carotenoids from the diet by oxidation (Goodwin, 1984; Partali *et al.*, 1985). According to Goodwin (1984), carotenoids exist in three forms in the Crustacea: (1) as free pigments (carotenes and unesterifed xanthophylls), (2) as xanthophylls esterified to long-chain fatty acids, and (3) as xanthophylls attached to proteins to form carotenoproteins. Carotenoids in Anostraca may occur as (1) granules, (2) in solution in fat globules of phagocytic storage cells, and (3) as water-soluble carotenoproteins (Gilchrist, 1968). According to Gilchrist (1968) the carotenoid contained in the fat globules of phagocytic storage cells frequently stain them bright orange. These cells mainly occur in the labrum, thoracopods, and in the 'fat body' associated with the ovary (Gilchrist, 1968). The major carotenoid in anostracan eggs is canthaxanthin, probably derived from the maternal ovary, and there exists some evidence that Anostraca synthesize canthaxanthin from β-carotene ingested with food (Gilchrist, 1968; Hsu *et al.*, 1970).

The food-dependent colors of the ovaries and eggs exhibited by the *T. platyurus* specimens in this study were similar to those experimentally found in *Artemia* by Hata & Hata (1969). They reported that the color of yeast-fed *Artemia* eggs was white in the ovary, while the color of the pure β-carotene-fed *Artemia* ovary was blue. After chemical analysis, Hata & Hata (1969) concluded that the blue color of the eggs is due to a canthaxanthin-protein complex. The color pattern of *T. platyurus* specimens under treatment 1 is very similar to that observed in most of the specimens collected from the wild (*i.e.* cercopods and thoracopods orange, and ovaries blue) (pers. obs.). This suggests that a natural source of β-carotene was available to them, probably algae.

In the Crustacea, apart from the fact that some carotenoids are an essential primary source of vit. A, little is known of their function *per se* (Goodwin, 1984). Goodwin (1960) pointed out that the major function of carotenoid and melanin pigments is to provide the external color pattern of the animals. A number of other functions of carotenoids in crustaceans have been claimed, however (see Goodwin, 1984). Cheesman *et al.* (1967) suggested that carotenoproteins in invertebrates may participate in protection, coloration, photosensitivity, electron transport, and enzymatic activity. Nelis *et al.* (1984) found cis-canthaxanthins specifically occurring in the ovaries and eggs of *Artemia* females. They suggested that some kind of relation to reproduction and/or embryonic development exists, thus suggesting a previously unrecognized function for carotenoids in the brine shrimp and possibly in related Crustacea.

Our results show that *T. platyurus* is able to grow, apparently normally, with or without synthetic trans-β- carotene in its diet. Only at the end of the third culture period (day 9) was there a significant difference in growth between the females, with the synthetic trans-β-carotene group growing faster. Herring (1968b) reported that *Daphnia magna* is able to grow and reproduce 'normally' in the absence of carotenoids in its diet. Here, the absence of evidence for vit. A or carotenoid requirements other than for visual purposes, led to the conclusion that the natural metabolic fate and subsequent deposition of ingested carotenoid is purely fortuitous (Herring, 1968b). Partali *et al.* (1985), however, could not obtain a healthy, yet fully carotenoid depleted *D. magna*.

Summarizing, we conclude that, while carotenoids do not appear essential to animals reared under laboratory conditions, they still may have an ecological role in natural conditions (e.g. photoprotection: Hairston, 1980). Without considering the color of the food itself while in the intestinal tract, the color patterns of the phyllopod body can be explained by (1) structural peculiarities of its tissues (e.g. iridescence: Goodwin, 1960), (2) the presence of pigments produced *de novo* (e.g. haemoglobin: Gilchrist & Green, 1960), and (3) the presence of food-dependent pigments (e.g. carotenoids: Goodwin, 1984).

Acknowledgments

We thank Dr Denton Belk (Our Lady of the Lake University of San Antonio, Texas, USA) for supplying

cysts of *T. platyurus*, and J. Ali and Dr G. Murugan (Laboratory of Animal Ecology, University of Ghent, Belgium) for their assistance in statistical analyses and critical reading of the manuscript, respectively. The first author has a doctoral fellowship with Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico.

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