

IDENTIFICATION OF MANUBRIUM PIGMENTS IN TWO SPECIES OF *Turritopsis* MEDUSAE (CNIDARIA, HYDROZOA) IN JAPAN

By

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Abstract

In *Turritopsis rubra* and *T. sp.*, which are c. 10 mm and several mm in diameter and are distributed in northern and southern Japan, respectively, the same volume of their manubriums, c. 0.1 g in the total wet weight, were cut out under a stereomicroscope and major pigments were extracted with acetonitrile, then analyzed at 480 nm with high performance liquid chromatography (HPLC). As a result, two sharp peaks were found in *T. rubra*, of which manubrium is scarlet color, and these two kinds of pigment substances were comparatively analyzed with standard astaxanthin and canthaxanthin by HPLC, and proved to be these two chemical compounds. In contrast, in *T. sp.*, of which manubrium is pale yellow, only astaxanthin was found. In relation to function of pigments and ecology of medusae, biological meaning of their differential coloration is discussed, and tentative suppositions, prevention of leak of bioluminescent baits within the manubrium and sun-screening effects, are proposed.

Introduction

Jellyfish color is sometimes constant and treated as one of the specific characters like an immortal jellyfish *Turritopsis* spp. in Japan (Kubota 2005; Kitada & Kubota 2015; Hasegawa *et al.* 2016). There are few studies on pigments of jellyfish (Cheesman, *et al.* 1967; Wade *et al.* 2009) and it has been known that *Periphylla periphylla* contains porphyrin (Herring 1972; Bonnett *et al.* 1979), *Physalia physalis* contains biliprotein, *Veleva* and *Porpita* contain carotenoprotein (Cheesman *et al.* 1967; Herring 1971a, b). Two of these kinds of pigments are originated from baits, i. e. biliprotein is from fish and carotenoprotein is from planktonic crustaceans. As a member of neuston, body surfaces of *Physalia*, *Veleva*, and *Porpita* are covered with pigments that absorb ultraviolet rays as a sun-screening effect, whereas pigments

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contained in manubriums of a deep-water jellyfish *Periphylla* (Jarms *et al.* 2002) are effective to conceal bioluminescent light of baits within the stomach.

In Japanese waters, three species of *Turritopsis* were inhabited and the largest one is distributed in northern Japan and its manubrium is scarlet colored identifiable as *T. rubra* (Fig. 1a). On the other hand, in southern Japan two species (*T. sp.* and *T. dohrnii*) are distributed. They are small and their manubriums (Fig. 1b) are not scarlet colored (Kubota 2005; Miglietta *et al.* 2007; Kubota & Nagai 2018). Major pigments contained in the manubrium of *T. rubra* and *T. sp.* were identified in the present study, using liquid chromatography. The biological meaning of difference of their colors between the present two species is still in question, but discussed briefly, comparing with neuston blue-colored medusae and deep-water black ones, etc.

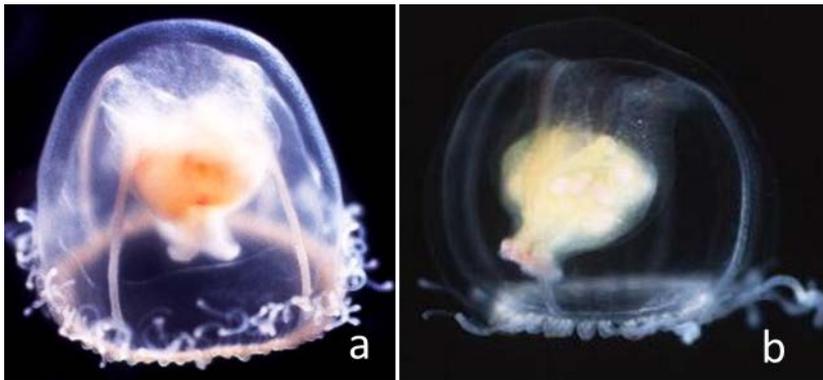


Fig. 1. Two *Turritopsis* species in Japan, used for the present pigment analysis, a: *T. rubra* (c. 10 mm in diameter); b: *T. sp.* (c. several mm in diameter).

Materials and methods

Turritopsis rubra (c. 10 mm in diameter: Fig. 1a) was collected from sea surface in October, 2013 at the Onahama fishing port, Iwaki City, Fukushima Prefecture, Japan, and *T. sp.* (c. several mm in diameter: Fig. 1b) was collected in the sea surface by towing plankton net in August, 2013 in the Tanabe Bay, Wakayama Prefecture, Japan by Shin Kubota. They were kept alive without foods for three days in order to avoid contamination of ingested food remained in their stomach. Then, their manubriums were cut out from bodies under a stereomicroscope, and their pigments were analyzed by similar methods carried out by Kuhn and Sorensen (1938) and Haxo (1959). Five individuals were used in *T. rubra*, while 20 ones in *T. sp.* The manubrium volume of *T. sp.* is about 1/8 of that of *T. rubra*, therefore much more individuals were required for *T. sp.* in order to analyze the same volume of manubrium (c. 0.1 g in the total wet weight).

The manubriums were put in a ceramic bowl (outer diameter 9 cm), mixed with 3 ml of acetonitrile, 2 ml of n-hexane saturated with acetonitrile, 1 g of anhydrous sodium sulfate and 0.2 g of quartz sand, and ground fully with a ceramic bar. After centrifugation at 3000 rpm for 10 min, acetonitrile layer was collected (extracted liquid 1). Furthermore, 2 ml of acetonitrile was added to residual hexane layer and they were mixed well. After centrifugation at 3000 rpm for 10 min, acetonitrile layer was collected (extracted liquid 2). At last the extracted liquid 1 was mixed with the extracted liquid 2, and 1 ml of n-propanol was added. The mixture was decompressed and concentrated to 0.5 ml under vacuum below 40°C. Comparative HPLC analysis was performed using HITACHI HPLC, L-7000 series with the measuring condition below. The apparatus; the HPLC apparatus consisted of a Hitachi (Tokyo Japan) L-7100 pump, an L-7400 detector and a D-7500 integrator. Measuring conditions, absorbance at 480nm, separation using a Nucleosil 100-5C18 column (GL Sciences, Tokyo, Japan) at room temperature, mobile phase; methanol, flow rate; 1.0 ml/min and injection loop; 20 µl. Standard samples for astaxanthin and canthaxanthin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Results

In *Turritopsis rubra*, two major sharp peaks were detected after analyzation by HPLC (Fig. 2a). The retention time of each peak was 4.91 min and 9.22 min, and pigments are mainly composed of these two kinds of substance. No major peaks were observed except for those shown in Fig. 2a. In order to identify these two substances, the manubrium samples were comparatively analyzed with astaxanthin or canthaxanthin. First, a standard sample of astaxanthin was dissolved in acetonitrile and comparatively analyzed by HPLC. The retention

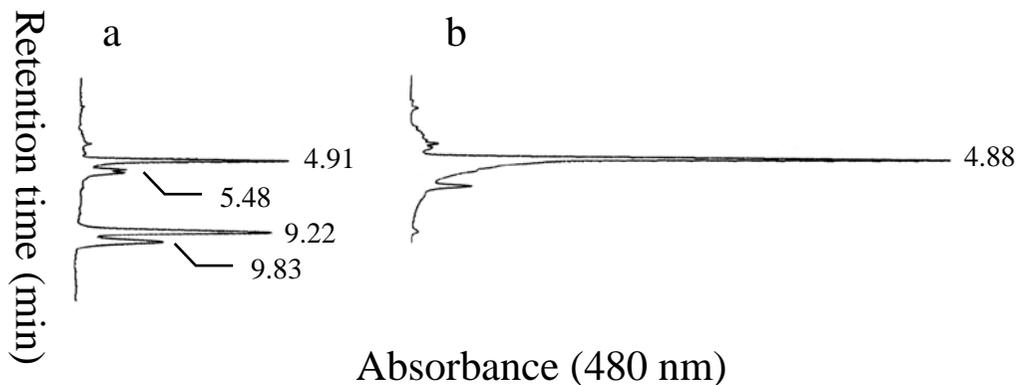


Fig. 2. Pigment analysis of manubrium of *Turritopsis rubra* by HPLC (a) and astaxanthin standard test (b).

time of astaxanthin peak was 4.88 min, almost the same as the first peak of *T. rubra* (Fig. 2b). It is known that astaxanthin, which is a kind of carotenoid, is present in most red-colored aquatic organisms such as salmon and shrimps.

Next, the manubrium samples from *T. rubra* were compared with canthaxanthin by HPLC and the second peak of manubrium samples appeared at the retention time of 10.69 min, almost coincidentally with the peak of canthaxanthin (the retention time: 10.68 min) as shown in Fig. 3. Therefore, it is clear that major pigments of the manubrium of *T. rubra* (Fig. 1a) are derived from astaxanthin and canthaxanthin.

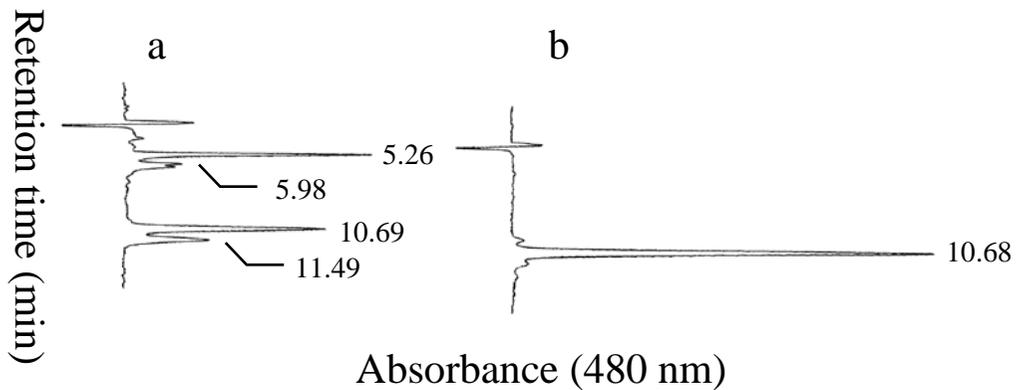


Fig. 3. Pigment analysis of manubrium of *Turritopsis rubra* by HPLC (a) and canthaxanthin standard test (b).

On the other hand, the HPLC analysis of manubrium in *T. sp.* is shown in Fig. 4. Several small peaks appeared, and among them the peak of the retention time of 5.02 min corresponded to the peak of astaxanthin (the retention time: 4.96 min). The peak of canthaxanthin was not detected and the major peak (the retention time: 2.84 min) was not coincident with canthaxanthin. Therefore, the pigments of manubrium of this species are at least derived from astaxanthin, but not from canthaxanthin. Several small peaks besides the peak of astaxanthin appeared in Fig. 4a. Since *T. sp.* is too small to cut out manubrium exclusively, some contamination of other tissues might be contained. High concentration of astaxanthin is scarlet color in acetonitrile, as is observed in crustaceans and fish (carp and seabream, etc.), while low concentration of it is pale yellow. Actually, the manubrium of *T. sp.* is pale yellow (Fig. 1b), and the concentration of astaxanthin may be lower in this species.

Discussion

It is concluded that manubrium of two *Turritopsis* species contains two carotenoid pigments of astaxanthin and/or canthaxanthin, both chemical compounds have a strong antioxidative effect. These pigments may be derived from crustacean baits, and quantity of them

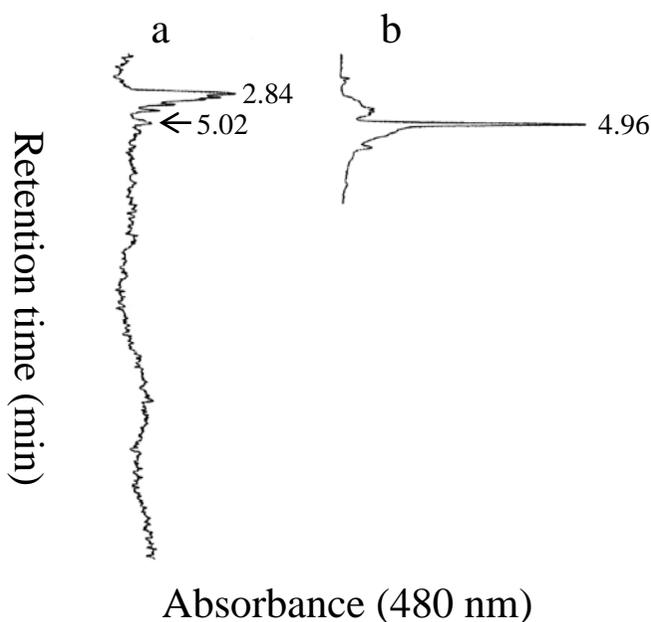


Fig. 4. Pigment analysis of manubrium of *Turritopsis* sp. by HPLC (a) and astaxanthin standard test (b). The arrow indicates the astaxanthin peak.

seem to affect the colors of the two *Turritopsis* species. The scarlet color of well-developed medusae of *T. rubra* is constant, when fed by *Artemia* nauplii (Kubota & Mizutani 2003) for two months. An immature medusa can grow to a typical, well-developed medusa with scarlet colored manubrium, when fed by *Artemia* nauplii for two months (Kubota & Nishihara 2017). In contrast, the manubrium of *T. sp.* and *T. dohrnii* does not become scarlet color when a medusa is cultured in the laboratory from an immature stage to senile one, when fed with *Artemia* nauplii for up to 77 days (Kubota 2005). The biological reason why pigments of manubrium in *T. rubra* consist of both astaxanthin and canthaxanthin, while those of *T. sp.* consist of only astaxanthin is, however, difficult to elucidate for the present. But, tentative surmise is that *T. rubra* often appears near the sea surface in the daytime in summer (Kitada & Kubota 2015) and it has two pigments in order to protect their gonads around manubrium surface from strong ultraviolet rays, like a similar role of carotenoprotein found in *Velevella* and *Porpita* (Herring 1971a, b). Another supposition is that this color, although not porphyrin pigments but carotenoids, plays a role to prevent leak of luminescent light from the surface of the manubrium after eating bioluminescent baits at night like deep-sea medusae (Bonnett *et al.* 1979). It is noteworthy here that the exumbrella of *T. rubra* is not transparent (Fig. 1a) as an exceptional case among the hydromedusae, and this fact may be related to this supposition.

On the other hand, *T. sp.* is inhabited in southern Japan (Kubota 2005), where ultraviolet rays are stronger than in northern Japan, then the medusa should be more

scarlet-colored than *T. rubra*, but the phenomenon is opposite. Further, these two *Turritopsis* species do not seem to be deep water dwellers with ecological meaningful coloration as is pointed out by Bonnett *et al.* (1979). It is, however, assumed in two ways that in *T. sp.* (1) small quantity of pigments are enough to protect their gonads from harmful effects of ultraviolet rays, (2) the medusae do not frequently appear on sea surface in the daytime, especially in summer to prevent from deleterious effects of radiation. It is reported that *Turritopsis sp.* appears mainly in summer in middle Japan (Kawamura & Kubota 2005), when ultraviolet rays is strongest in the year. Therefore, prevention of leak of bioluminescent baits within the manubrium is plausible for a role of the scarlet color of *T. rubra*, but further physiological and ecological studies are needful to demonstrate and testify the above-mentioned suppositions.

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References

- Bonnett, R., E.J. Head and P.J. Herring. 1979. Porphyrin pigments of some deep-sea medusae. *Journal of the Marine Biological Association of the United Kingdom*, 59: 565-573.
- Cheesman, D.F., W.L. Lee and P.F. Zagalsky. 1967. Carotenoproteins in invertebrates. *Biological Review*, 42: 132-160.
- Hasegawa, Y., T. Watanabe, M. Takazawa, O. Ohara and S. Kubota. 2016. *De Novo* assembly of the transcriptome of *Turritopsis*, a jellyfish that repeatedly rejuvenates. *Zoological Science*, 33: 366-371.
- Haxo, F. 1959. Carotenoids of the mushroom *Cantharellus cinabarinus*. *Botanical Gazette*, 112(2): 228-232.
- Herring, P.J. 1971a. Biliprotein coloration of *Physalia physalis*. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 39: 739-746.
- Herring, P.J. 1971b. Stability of the blue pigment of *Velella* and *Porpita* (Coelenterata: Siphonophora). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 39: 1039-1043.
- Herring, P.J. 1972. Porphyrin pigmentation in deep-sea medusa. *Nature* 238: 276-277.
- Jarms, G., H. Tiemann and U. Båmstedt. 2002. Development and biology of *Periphylla periphylla* (Scyphozoa: Coronatae) in a Norwegian fjord. *Marine Biology* 141: 647-657.
- Kawamura, M. and S. Kubota. 2005. Seasonal change of medusa of *Turritopsis nutricula* (Hydrozoa, Anthomedusae) in Tanabe Bay, Wakayama Prefecture, Japan. *Bulletin of the Biogeographical Society of Japan*, 60: 25-30. (In Japanese with English abstract).

- Kitada, H. and S. Kubota. 2015. Violent change of seasonal appearance of three hydromedusae species (Hydrozoa, Anthomedusae) from Iwaki City, Fukushima Prefecture, Japan after the disastrous earthquake in 2011. *Bulletin of the Biogeographical Society of Japan*, 70: 267-269. (In Japanese with English abstract).
- Kubota, S. and S. Mizutani. 2003. Strange fates of degenerated medusae of *Turritopsis nutricula* (Hydrozoa, Anthomedusae, Clavidae) from northern Japan. *The Nanki Seibutu*, 45(2): 107-109. (In Japanese with English summary).
- Kubota, S. 2005. Distinction of two morphotypes of *Turritopsis nutricula* medusae (Cnidaria, Hydrozoa, Anthomedusae) in Japan, with reference to their different abilities to revert to the hydroid stage and their distinct geographical distributions. *Biogeography*, 7: 41-50.
- Kubota, S. and F. Nishihara. 2017. A large form of *Turritopsis* (Hydrozoa, Anthomedusae) collected from southernmost distributional region in this species in Japan after one century. *Bulletin of the Biogeographical Society of Japan*, 71: 199-202. (In Japanese with English abstract).
- Kubota, S. and S. Nagai. 2018. 16S mitochondrial gene sequence analysis of some *Turritopsis* (Hydrozoa, Oceanidae) from Japan and abroad. *Kuroshio Biosphere* 14: 1-6.
- Kuhn, R. and N.A. Sorensen. 1938. The coloring matters of the lobster (*Astacus gammarus* L.). *Z. Angew Chemistry*, 51: 465-466.
- Miglietta, M.P., S. Piraino, S. Kubota and P. Schuchert. 2007. Species in the genus *Turritopsis* (Cnidaria, Hydrozoa): a molecular evaluation. *Journal of Zoological Systematics and Evolutionary Research*, 45(1): 11-19.
- Wade, N.M., A. Tollenaere, M.R. Hall and B.M. Degnan. 2009. Evolution of a novel carotenoid-binding protein responsible for crustacean shell color. *Molecular Biology and Evolution*, 26(8): 1851-1864.