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FIFTY SHADES OF ORANGE IN AMPHIBIANS AND CRUSTACEANS: TWO SIDES OF THE SAME MEDAL

SUMMARY

This research was firstly performed to evaluate the presence of molecules that could have antioxidant power and therefore a possible cosmetic, nutraceutical and biotechnological application of the marine crustacean *Aristeus antennatus*, a species rich in carotenoids. Then, we applied this model to the analysis of *Salamandrina perspicillata* skin, because this species is characterized by a high amount of bright red-orange colouration on the legs and tail skin.

Key words: *Aristeus antennatus, Salamandrina perspicillata,* carotenoid, astaxanthin, visible spectroscopy, antioxidant power.

RIASSUNTO

Cinquanta sfumature di arancione in anfibi e crostacei: due facce della stessa medaglia. Al fine di valutare la presenza di molecole con potenziale capacità antiossidante e quindi con una possibile applicazione cosmetica, nutraceutica e biotecnologica, abbiamo preso come modello il gambero marino *Aristeus antennatus*, specie ricca di carotenoidi. Scelto il modello di partenza, abbiamo deciso di ampliare il lavoro ad un anfibio, in particolare alla *Salamandrina perspicillata*, la cui caratteristica è possedere una colorazione rosso-arancio sulla pelle delle zampe e della coda.

Parole chiave: *Aristeus antennatus, Salamandrina perspicillata,* carotenoidi, astaxantina, spettroscopia visibile, potere antiossidante.

Introduction

Carotenoids are red, lipid-soluble plant pigments. In nature there are over 600 types of carotenoids that show provitamin A or antioxidant activities. Astaxanthin is the most efficient carotenoid pigment displaying antioxidant activity on lipids, proteins, DNA and membrane lipoproteins of living cells and tissues (GOTO *et al.*, 2001; RANGA RAO *et al.*, 2013, 2014).

Astaxanthin is found in various microorganisms and in many species of marine animals, including crustaceans such as the shrimp *Aristeus antennatus*. As widely known, the orange colour of astaxanthin is given by the spectral absorbance of this pigment around 470 nm. Astaxanthin has a more efficient biological activity than other antioxidants since it seems to bind to the cell membrane protecting it from pro-oxidant agents. It is a promising photoprotective molecule for the treatment of ocular inflammation but also has a preventive role for UV-induced skin damage (OHGAMI *et al.*, 2003; RANGA RAO *et al.*, 2013, 2014). Furthermore, it has been shown to protect from damage caused by high glucose levels and this could reduce oxidative stress in the diabetic (UCHIYAMA *et al.*, 2002).

In this context, *Aristeus antennatus* processing waste can represent an important resource, finding use in various fields, including the industrial, cosmetic and pharmaceutical sectors.

Considering that in nature the red pigment is generally due to the presence of carotenoids, we decided to expand the well characterised model to amphibians, in particular to *Salamandrina perspicillata*, because of its red leg and tail skin, likely due to carotenoid presence. This animal displays its bright belly colours by arching its back, a defensive behaviour against predators, called *Unkenreflex* (ANGELINI *et al.*, 2007; Fig. 1).

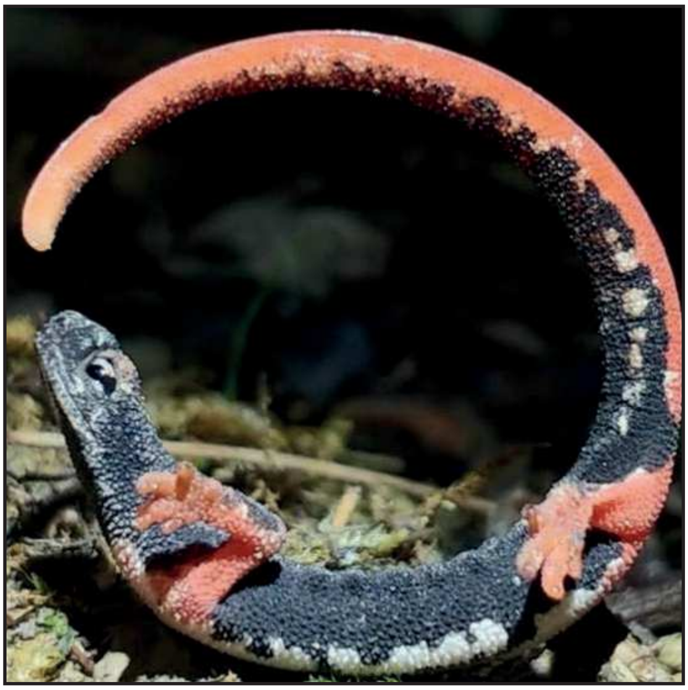

Fig. 1 — Salamandrina perspicillata defensive behaviour against predators, called *Unkenreflex*.

Crustaceans constitute a model for the high and all well documented presence of astaxanthin. In amphibians, the pigments of the epidermis are far from being well characterized. With this preliminary work we wanted to understand differences/similarities bet ween these two groups so distant from an evolutionary point of view.

Materials and methods

Aristeus antennatus were captured in the Ligurian Sea (Fig. 2). Each shrimp was separated into 4 parts: muscle (M), abdominal exoskeleton (EA), viscera (V), shell (C) and each of these components was placed in a test tube with ethanol at 4 ° C for one week in order to dissolve reddish pigments, then after centrifugation (1000 rpm, 10 minute, 4 °C) debris were separated and clear solution was analyzed.

The antioxidant power was measured by means of DPPH test using Trolox as standard. In order to correlate antioxidant power with astaxanthin content the same sample was also analysed by absorbance and light spectroscopy.

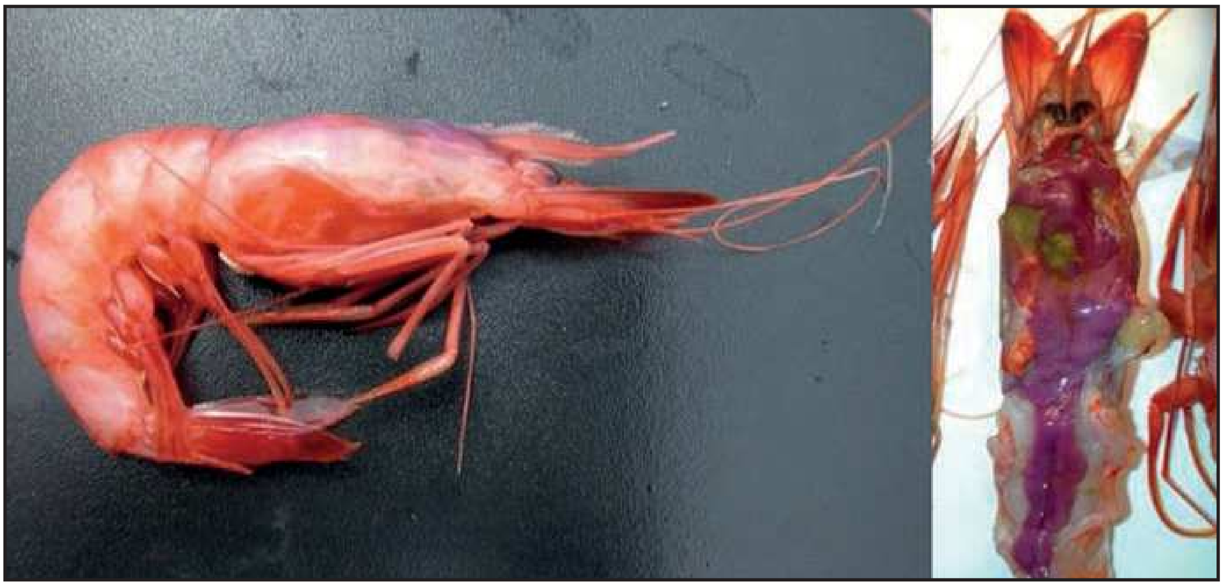

Fig. 2 — Aristeus antennatus: entire organism and viscera of a mature individual.

Salamandrina perspicillata individuals were captured in Val Borbera (Piedmont, NW Italy; Fig. 3). A small part of the tail was taken from each salamander, and divided in two parts: blackish dorsal and reddish ventral which were then placed separately in a test tube with ethanol at 4 °C for a week, to dissolve pigments.

Fluorescence and absorbance spectra were recorded to identify the nature of the red pigment. Subsequently, we measured the fluorescence by exciting the samples through a green laser source (532 nm) and the absorbance was measured at extended spectral ranges. Preliminary histological analyses were also performed.

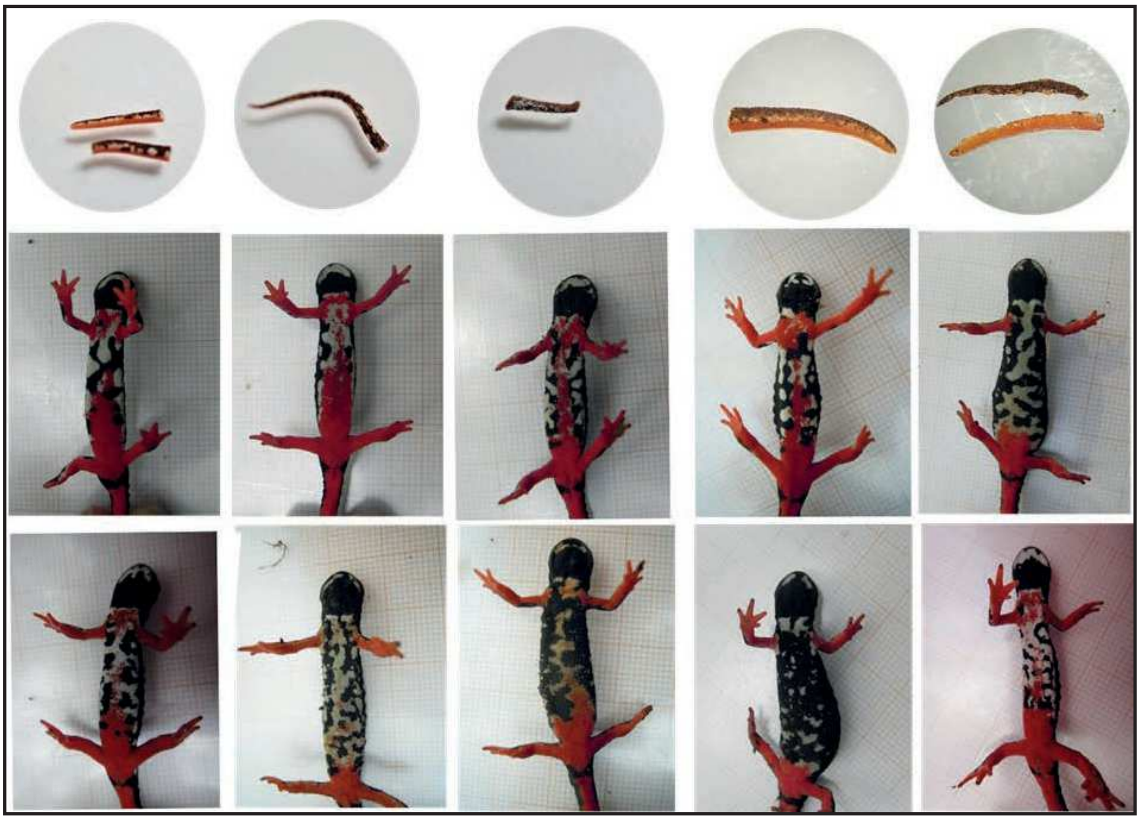

Fig. 3 — Salamandrina perspicillata individuals captured in Val Borbera.

RESULTS

Concerning *Aristeus antennatus*, a first spectroscopic analysis of the absorbance allowed to identify two peaks at 282 nm and 470 nm. The peak at 282 nm is compatible with the presence of proteins and is greater in the extracts of bowel and muscle than in the abdominal exoskeleton extracts. The absorption peak at 474 nm, on the other hand, is associated with the presence of astaxanthin and is generally more intense in the abdominal exoskeleton and in the shell (HU *et al.*, 2018).

These results were confirmed by the luminescence spectrum of the samples measured with 532 nm excitation, in which there are several peaks that identify the presence of other compounds. On the other hand, DPPH test revealed that the viscera and the muscle showed greater antioxidant power if compared to the shell and abdominal exoskeleton.

The data on *Salamandrina perspicillata*, obtained both with spectroscopic analysis of absorbance and with excitation at 532 nm, showed different peaks indicating the presence of some substances, which however, due to the complexity of the spectra, are still under study.

CONCLUSIONS

The spectroscopic measurements of absorption of the ethanolic extracts of the different components of *Aristeus antennatus* highlighted the presence of aromatic aminoacids and the presence of astaxanthin. In particular, the latter seems to be greater in the abdominal exoskeleton and in the shell, compared to the viscera and muscle, while as regards the presence of aromatic amino acids, it appears to have an opposite trend. Surprisingly, we did not find a direct correlation between the amount of astaxanthin and antioxidant power. Thus, the most orange-colored shell did not show a greater antioxidant power. On the other hand, the bowels and muscle extracts showed a greater antioxidant capability despite the faint orange color. This observation shows that the antioxidant power is probably also related to other unknown compounds found in the bowel and muscle.

The model established with this crustacean can be used to study other red or orange colorful animals such as *Salamandrina perspicillata*, in which the function of its bright ventral surface is still debated (ANGELINI *et al.*, 2007).

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