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Retention of photosynthetic chloroplasts in five sacoglossans (Mollusca: Opisthobranchia) from the Houtman Abrolhos Islands

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Abstract – Retention abilities of photosynthetic chloroplasts are presented for five sacoglossans from Western Australia. The photosynthetic activity was measured as maximum quantum yield of chlorophyll a fluorescence (Φ_{lie}) in *Elysia* sp., *Elysia thompsoni, Elysiella pusilla, Thuridilla undula* and *Thuridilla moebii*. None of these species have previously been recorded as photosynthetic, and they are compared to known definitions kleptoplasty.

Key words: Photosynthesis, Sacoglossa, PAM

INTRODUCTION

During a field trip to the field station of the Department of Fisheries, the Saville-Kent Centre, at the Houtman Abrolhos Islands, 19-25 July 2004, five species of sacoglossa, Elysia sp., Elysia thompsoni Jensen, 1993, Elysiella pusilla Bergh, 1872, Thuridilla undula Gosliner, 1995 and Thuridilla moebii (Bergh, 1888) were collected. Two of the species are recently described, and all have few records from the area. The sacoglossans were investigated for photosynthetic activity measured as maximum quantum yield of chlorophyll (chl) a fluorescence (Φ_{ue}), using a Pulse Amplitude Modulated Fluorometer (PAM). Kleptoplasty (keeping photosynhtetically active chloroplasts in the digestive system for varying time periods) is a widespread feature within the Sacoglossa (Clark et al. 1990), but very few taxa have actually been investigated (Wägele and Johnsen 2001). In this study the retention ability of photosynthetic chloroplasts in all five species is presented.

MATERIAL AND METHODS

All sacoglossans were collected around Rat Island, the Wallaby Group of the Houtman Abrolhos Islands, both by snorkelling and SCUBAdiving in shallow waters from 0.5 to 3 meters depth. The sacoglossans were identified with the aid of Jensen (1993 and 1997) and Wells and Bryce (2000).

Animals were kept in plastic containers at indoor conditions during experiments. Number of individuals measured for each species is given in Table 1. All measurements of Φ_{IIe} were done in darkness. A thorough description of measuring photosynthetic activity in opisthobranchs is presented in Wägele and Johnsen (2001).

A PAM (Walz, Germany) was used to measure the fluorescence originating in photosystem II in the chloroplasts. About 1% of the light absorbed by functional chloroplasts will appear as chl a fluorescence (chl a is the final light acceptor molecule during the light harvesting process) (Govindjee 1995). The PAM consists of a main instrument, a cosinus-corrected light collector, and an optic fibre. The fibre detects the fluorescence of the chloroplasts in the sacoglossan by probing the ground fluorescence (defined as F₀ measured in dark acclimated chloroplasts) using a very low light source, sending light pulses at a frequency of 0.6 kHz and giving an irradiance of approximately 0.15 µmol quanta m⁻² s⁻¹. In dark acclimated chloroplasts (irradiance (E) is zero), all reaction centres are open. A flash of approximately 10 000 µmol quanta m⁻² s⁻¹ for 0.8 seconds is applied via the fibre to obtain a maximum fluorescence (defined as F_m in dark acclimated sacoglossans, closing all reaction centres), giving a maximum quantum yield of chl a fluorescence $(\Phi_{IIe'}$ defined as $(F_m - F_0)/F_m$ when dark acclimated for 15 minutes). The settings on the PAM were set to: measuring frequency = low, saturation width 0.8 seconds, saturation intensity = 8, and measuring intensity 8, and were kept constant during the experiments. Battery voltage not allowed below 12.1 V.

The Φ_{IIe} values are plotted as a function of time, and a regression line using the least square method is fitted to the plot, where y = ax + b gives y as the measured Φ_{IIe} , x as the time in days, and a as the slope of the curve (Figure 1). From this, a can be used as a decrease rate of Φ_{IIe} per day, and by dividing the Φ_{IIe} measured at the day of collection (day 0) with a, the Retention ability of Photosynthetic Chloroplasts (RPC) is given as a number of days (Table 1).



Figure 1 Φ_{Ile} values of the five sacoglossans investigated for retention ability of photosynthetic chloroplasts (RPC). Fitted regression lines indicate decrease of Φ_{Ile} per day. Depending on the Φ_{Ile} value measured on the same day as the animal is collected (day 0), and on the calculated slope (*a* in Table 1), the RPC denotes how many days the sacoglossans is able to stay photosynthetic.

RESULTS AND DISCUSSION

The plotted Φ_{IIe} values for each species and the fitted regression line is given in Figure 1. The measured Φ_{IIe} values and calculated values of *a*, RPC and R² are presented in Table 1. From this we can see that two of the species, *Elysia* sp. and *Thuridilla moebii* have the longest retention times of chloroplasts, with RPC values of 25 and 26 days. *Elysia thompsoni* and *Thuridilla undula* have twice as high decrease rates of Φ_{IIe} , and have thus only RPC values of 11 and 12 days. *Elysiella pusilla* has a very rapid decrease in Φ_{IIe} and has a low RPC of only two days.

Different levels of chloroplast retention have been described by several authors (Hinde and Smith 1974; Trench 1975; Marin and Ros 1988) culminating in the six levels of kleptoplasty suggested by Clark *et al.* (1990). The first three levels include direct digestion to retention of non functional chloroplasts (no photosynthesis). Level four a short term functional retention with photosynthetic activity lasting less than 24 hours. Level 5 a medium term functional retention with photosynthetic activity lasting for more than 24 hours. And Level six with a long term functional retention with photosynthetic activity lasting more

Table 1 Mean Φ_{IIe} values for the investigated sacoglossans (where n individuals is > 1, else it is the directly measured value), the calculated decrease rate of Φ_{IIe} (*a*), the calculated retention ability of chloroplasts (RPC, in days), the R² of the fitted line, and the number of individuals per species measured (n).

day	Elysia sp.	Thuridilla moebii	Elysia thompsoni	Thuridilla undula	Elysia pusilla	
0	0.722	0.680	0.772	0.688	0.558	*****
1	0.655	0.585	0.733	0.570	0.210	
2	0.664	0.627	0.635	0.569		
slope (a)	-0.029	-0.026	-0.068	-0.060	-0.348	
rpc	25	26	11	12	2	
rsqr	0.64	0.31	0.94	0.76		
n	3	2	3	1	1	

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than one week. Compared with the RPC values presented for the five species in this study, we find that *E. pusilla* fits the Level 5 definition, and that the rest of the species fit the Level 6 definition. The retention abilities vary greatly within the sacoglossa (Wägele and Johnsen 2001) and depend on the food source (Clark and Busacca 1978; Williams and Walker 1999). The designation of sacoglossans species into levels of kleptoplasty is therefore a delicate pursuit, but a useful tool in assembling the diversity of retention abilities of photosynthetic chloroplasts within the Sacoglossa. Using the PAM is an easy way to assess the presence of chloroplasts and their activity. Combining PAM measurements with pigment characterisation of potential food sources and the investigated sacoglossans, will eliminate food source uncertainty, and must be a future goal in chloroplast retention research. The presence of photosynthetic activity and retention abilities are presented for the first time for the species in this study. All species have earlier been recorded from the Abrolhos islands (Jensen 1993 and 1997; Wells and Bryce 2000).

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