ARTICLE ADDENDUM

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Switching off photosynthesis

The dark side of sacoglossan slugs

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Sometimes the elementary experiment can lead to the most surprising result. This was recently the case when we had to learn that so-called "photosynthetic slugs" survive just fine in the dark and with chemically inhibited photosynthesis. Sacoglossan sea slugs feed on large siphonaceous, often single-celled algae by ingesting their cytosolic content including the organelles. A few species of the sacoglossan clade fascinate researcher from many disciplines, as they can survive starvation periods of many months through the plastids they sequestered, but not immediately digested – a process known as kleptoplasty. Ever since the term "leaves that crawl" was coined in the 1970s, the course was set in regard to how the subject was studied, but the topics of how slugs survive starvation and what for instance mediates kleptoplast longevity have often been conflated. It was generally assumed that slugs become photoautotrophic upon plastid sequestration, but most recent results challenge that view and the predominant role of the kleptoplasts in sacoglossan sea slugs.

Our results[1] on the 2 slugs *Elysia timida* and *Plakobranchus ocellatus* came as a surprise to many and as such have generated quite some media attention: the article was featured in Nature, Scientific American, on a blog by Ed Yong at National Geographic, and many other sites such as science.org and phys.org. First of all, we need to stress that our findings do neither "…*disprove the idea that the slugs somehow derive energy from the photosynthesizing cells*..." nor that they are not "*solar-powered*" at all as some reports claim (phys.org and Ed Yong, respectively). Our results do, however, question the importance of photosynthesis for the so-called "crawling leaves"[2] and their survival during starvation. A critical examination of the topic generally highlights the importance to again think about statements such as "…, *after which plastids are able to support continued growth of the animal*" or "...*i.e. an animal (*E. chlorotica*) is able to sustain itself solely by photoautotrophic CO_2 fixation, as a plant.*" and "…*enabling their animal host to survive photo-autotrophically.*"[3-5, respectively] Juvenile slugs need to feed after hatching to establish stable kleptoplasty and adults are also observed to continuously feed as long as algae are available in the wild, demonstrating that, if anything these slugs are photoheterotrophic. Furthermore, we would argue that currently no single line of evidence for a single slug species exists that demonstrates the animals are phototrophic to a degree that allows the slugs to maintain, let alone gain, body mass through on-going photosynthesis and CO_2 fixation during prolonged starvation.

Costasiella ocellifera, Elysia viridis, *E. chlorotica*, *E. timida* and *P. ocellatus* have all been demonstrated to fix CO_2 in a light dependent manner.[1,6-8] But for how long during starvation, and is the amount of fixed CO_2 sufficient for them to really grow? While we currently have good evidence, based on pulse amplitude modulation (PAM) measurements, that the "light-dependent" reactions of the photosystem II continue to work inside the slugs[9-13] – in some cases for months – we lack the same kind of evidence for the "light-independent" reactions of the Calvin-Benson-Bassham (CBB-) cycle. In fact, the sparse

Figure 1. Sacoglossan slugs shrink during starvation. The image shows 2 individual *Plakobranchus ocellatus* specimens. The slug on the right was approximately of equal size, before it was starved for 6 months.

amount of data available suggests that the activity of the CBB-cycle declines rather rapidly. Experiments on *P. ocellatus*, *C. ocellifera*, *E. viridis* and *E. timida* show the CO_2 fixation rate decreases during the first 5–20 d to a rate comparable to that observed for slugs kept in the dark.[7,14-16] While for *P. ocellatus* the experiment was terminated after 27 d, *C. ocellifera* and *E. viridis* were kept alive for another 50 or 80 d, respectively, all while apparently not fixing CO_2. In *E. timida* the CO_2 fixation rate after 5–10 d of starvation had declined to the level measured for *Thuridilla hopei* for comparison, and whose kleptoplasts loose their photosynthetic ability over the first few days of starvation.[16] Should this be confirmed, through experiments dedicated to investigating CO_2 fixation rates over time, we will need to find an explanation of what happens with the ATP and NADPH$^+$ generated by the light-dependent reaction. During the early phases of starvation CO_2 fixation is probably advantageous for the slugs, but suggesting these animals to survive starvation because they are photoautotrophic is currently not supported by the data available. This would, at the very minimum, require evidence for on-going CO_2 fixation and the perpetuation of body mass during starvation.

Our results demonstrate that starving *E. timida* and *P. ocellatus* shrink and loose weight in the absence of fresh food (see also **Fig. 1**), whether being able to photosynthesize or not.[1] This was also observed by West,[17] who, for *E. chlorotica*, concluded that "*Statistical analysis of the growth experiment demonstrated that light intensity is not important to the size*." Klochkova and colleagues[18] questioned the importance of the kleptoplasts' remaining photosynthesis in *E. nigrocapitata*, since under natural light conditions they "*may not properly function in the natural habitat for a long time without recruit of new chloroplasts*". This not only contradicts the 'photoautotrophic concept', but also raises the question of the kleptoplasts true purpose.

Our current working hypothesis is that in adult slugs kleptoplasts are primarily stored as a rich nutritional source, but it is further noteworthy to mention that viewing plastids as organelles that fix only CO_2 to transform it into 'sugar cubes', whose energy is then available to the host, oversimplifies the biochemistry of the organelle. Their biochemical properties further include, but are not limited to, fatty acid, iron-sulfur cluster and amino acid synthesis.[19] Hence, the interaction of kleptoplasts and slug is more complex than it appears at first sight. Photosynthesis might still be beneficial for the animals, but exactly how and when remains to be determined. To distinguish the importance of light for the animals development from photosynthesis presents a further challenge, as the slugs likely require light, like most animals including us humans do, for their normal development and living.

The last few years have reminded us not to judge a book by its cover, nor a slug just by its color. New data forces us to become more critical about what we think we know, and reconsider the prime role of kleptoplasts in greenish sacoglossan slugs. In any case, we should not refer to these slugs as photoautotrophic animals, but determine for how long and to what degree they are phototrophic during starvation. Notwithstanding, the most previous results do not belittle the phenomenon of kleptoplast longevity and recent data suggests that the genomes of the plastids being sequestered might hold the key after all.[20] Their higher genetic autonomy in comparison to land plant plastids might translate into a better servicing of damaged photosystem II, hence less leakage of reactive oxygen species. In turn this might allow the slugs to store their kleptoplasts for longer periods of time, as their degradation through ROS-induced autophagy[21] is postponed. Sacoglossan slugs and their robust plastids remain a fascinating and rich field to study, presenting ever more avenues for future research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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