

Solar Powered Seaslug – Mutualistic Symbiosis of Aeolid Nudibranchia (Mollusca, Gastropoda, Opisthobranchia) with *Symbiodinium*

INGO BURGHARDT^{1*}, JUSSI EVERTSEN², GEIR JOHNSEN²,
and HEIKE WÄGELE¹

¹Lehrstuhl für Spezielle Zoologie, Ruhr-University Bochum,
D-44780 Bochum, Germany, Tel. +49-234-3228857, Fax. +49-234-14114,
Emails. Ingo.Burghardt@rub.de and Heike.Waegle@rub.de;

²Department of Biology, Trondhjem Biological Station,
Norwegian University of Science and Technology, N-7491 Trondheim,
Norway, Tel. +47-73591587, Fax. +47-73591597,
Emails. jussi.evertsen@bio.ntnu.no and Geir.Johnsen@bio.ntnu.no

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Abstract

In this study, we investigate the mutualistic symbiosis of two aeolid nudibranchs, *Phyllodesmium briareum* Ehrenberg, 1831 and *Pteraeolidia ianthina* (Angas 1864), with zooxanthellae (Dinophyceae) of the genus *Symbiodinium* Freudenthal, 1962. These are the first long-term experiments with a Diving-PAM (submersible Pulse Amplitude Modulated Fluorometer) on a system, where an organism gets its symbiotic partners from a primary host, namely cnidarians. Long-term experiments with the nudibranchs kept under different environmental conditions indicate a highly evolved symbiotic relationship with zooxanthellae for both species, although the efficiency in photosynthesis is higher in *Pteraeolidia ianthina*. The relationship with *Symbiodinium* helps the adult nudibranch to overcome a period of food shortage by getting photosynthetic products. Juveniles of *Phyllodesmium briareum* are not able to survive without getting their primary food source, the soft coral *Briareum violacea* (Roule, 1908). In dark the number of photo-

*The author to whom correspondence should be sent.

synthetically active zooxanthellae decreases in cultivated slugs. According to measured operational quantum yield of Chl a fluorescence (photosynthetic electron transfer rate), *Phyllodesmium briareum* and *Pteraeolidia ianthina* seem to house different types or even clades of *Symbiodinium*.

Keywords: Nudibranchia, symbiosis, mutualism, *Phyllodesmium briareum*, *Pteraeolidia ianthina*, *Symbiodinium*, zooxanthellae, photosynthesis, Pulse Amplitude Modulated fluorometry, irradiance

1. Introduction

A mutualistic symbiosis with unicellular dinoflagellates of the genus *Symbiodinium* Freudenthal, 1962, called zooxanthellae, is known from different marine invertebrates. Reef building hard corals (Hexacorallia, Madreporaria) are the most famous ones, using the metabolites of the zooxanthellae for their own nutrition. Some taxa of the Nudibranchia (Mollusca, Gastropoda, Opisthobranchia) are also known to house zooxanthellae inside of their body (namely inside the epithelial cells of the digestive gland). This was already described by Rousseau (1934, 1935). The nudibranchs get the dinoflagellates through their prey, mainly from cnidarians. Within the Nudibranchia, zooxanthellae have been found only in members of the taxon Cladobranchia but not in the Anthobranchia (Wägele and Johnsen, 2001). The importance of the relationship between intracellular symbiotic dinoflagellates and slugs is unknown in most cases. Zooxanthellae inside the nudibranch's digestive glandular cells were often detected only by histological and ultrastructural investigations (Kempf, 1984; Rudman, 1981a,b; 1982a,b; 1991; Marin and Ros, 1991; Wägele and Johnsen, 2001). These observations do not necessarily prove mutualism. In the investigated species, digestion of the zooxanthellae apart from their primary food (corals) cannot be excluded.

It has been assumed that camouflage offers a selective advantage which favours the retention of zooxanthellae in the digestive system of nudibranchs (Rudman, 1987). Additionally the zooxanthellae may enhance the ability of the seaslugs to survive periods of food shortage and allow them to search and test other food sources, e.g., other coral species. Finally in a mutualistic relationship the nudibranch would profit from the metabolites produced by the endosymbiotic zooxanthellae. An exchange of metabolites between host and endosymbiont has not been proved for nudibranchs' zooxanthellae yet, although studies of Hoegh-Guldberg and Hinde (1986) and Hoegh-Guldberg et al. (1986) suggest a transfer of organic carbon from *Symbiodinium* sp. to *Pteraeolidia ianthina* (Angas, 1864).

Morphological and behavioural adaptations in zooxanthellae-bearing nudibranchs were described by Rudman (1981b, 1982a, 1991) and Burghardt and Wägele (2004). Histological and ultrastructural investigations (Rudman, 1981a,b, 1982a,b, 1991; Wägele and Johnsen, 2001) show a correlation between morphological adaptations, e.g., branching structures of the digestive gland, and the housing of zooxanthellae. The branching of the digestive glandular system is much more extensive in Cladobranchia, which house zooxanthellae, than in those with no zooxanthellae. More extensive branching of the digestive gland enlarges the surface area for storage of zooxanthellae and allows higher photosynthetic light absorption and utilization. Rudman (1991) assumed a correlation between the branching patterns of the digestive gland, the evolution of the genus *Phyllodesmium* Ehrenberg, 1831 and the efficiency of photosynthetic performance inside the seaslug.

The aeolid genus *Phyllodesmium* known from the Indopacific with 15 described and approximately 11 undescribed species show a high diversity compared to other aeolid genera (Wägele, 2004). All known *Phyllodesmium* species exclusively feed on soft corals (Octocorallia). Some of these slug species are described to house zooxanthellae, but only by histological investigations (Rudman, 1991).

Here we investigate the symbiotic relationship of two nudibranch species for the first time with new methods in long-term experiments: *Phyllodesmium briareum* Ehrenberg, 1831 and *Pteraeolidia ianthina* (Angas, 1864). Additionally we compare the data of these nudibranchs with the primary food source of *P. briareum*, the octocoral *Briareum violacea* (Roule, 1908).

2. Material and Methods

Ten specimens (9 juveniles and 1 adult) of *Phyllodesmium briareum* and two adults of *Pteraeolidia ianthina* were collected in different sites around Lizard Island and investigated at Lizard Island Research Station (LIRS; Great Barrier Reef, Australia) between July and September 2002. For details of locality and the specimens see Table 1. Samples of the food of *P. briareum*, namely *Briareum violacea*, were also collected and studied. All investigated specimens were kept in aquaria with a flow-through water system at LIRS under moderate light conditions (up to a maximum of $\sim 350 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at solar noon). The water temperature was approximately 23–25°C. A Pulse Amplitude Modulated Fluorometer (Diving-PAM, Walz, Germany) was used to detect *in vivo* photosynthetic activity of zooxanthellae in *Phyllodesmium briareum*, *Briareum violacea* and *Pteraeolidia ianthina* by measuring the fluorescence emitted by Photosystem II (PSII) of chlorophyll a, the oxygen evolving side. This in turn allows distinguishing between active photosynthetic

Table 1. Synopsis of the investigated specimens of *Phylloidesmium briareum* and *Pteraeolidia ianthina* and the environmental conditions they were kept during experiments.

Species	Cultivation conditions	No.	Status	Collection date/ location	Cultivation (days)		Survival period/death
					In light	In darkness	
<i>Phylloidesmium briareum</i>	Without soft corals as food source (in light)	Pb1	Adult	12.07.2002 Cobia Hole (~16 m, on rocks)	70	-	Artificial death after 70 days (20.09.2002)
		Pb7	Juvenile	23.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	32	-	Natural death after 32 days (24.08.2002)
	On <i>Briareum violacea</i> (food source) in light	Pb8	Juvenile	24.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	58	-	Artificial death after 58 days (20.09.2002)
		Pb10	Juvenile	25.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	58	-	Artificial death after 58 days (21.09.2002)
	Without soft corals as food source (in dark)	Pb2	Juvenile	22.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	3	21	Natural death after 24 days (15.08.2002)
		Pb6	Juvenile	23.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	3	28	Natural death after 31 days (23.08.2002)
	Without food in darkness/after 33 days of starvation offer of soft corals	Pb5	Juvenile	22.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	2	31/3 starv./soft corals	Natural death after 36 days (27.08.2002)

Table 1. Continued.

Species	Cultivation conditions	No.	Status	Collection date/ location	Cultivation (days)		Survival period/ death
					In light	In darkness	
<i>Phylloidesmium briareum</i>							
	Without food in darkness/ after 34 days on <i>Briareum</i> in darkness	Pb4	Juvenile	22.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	2	32/2 starv./ <i>Briareum</i>	Natural death after 36 days (27.08.2002)
	Without food in darkness; back in light on <i>Briareum</i>	Pb3	Juvenile	22.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	2	30 5 (on <i>Briareum</i>)	Natural death after 37 days (29.08.2002)
	Without food in darkness; without food in light	Pb9	Juvenile	24.07.2002 North Point (~18 m, on <i>Briareum</i> -colony)	1	16 16	Natural death after 33 days (25.08.2002)
<i>Pteraeolidia ianthina</i>							
	Without food in light	Pte1	Adult	10.07.2002 Coconut Beach (intertidal, on coral rubble)	71	-	Artificial death after 71 days (19.09.2002)
	Without food in light; without food in darkness	Pte2	Adult	01.09.2002 Blue Lagoon between Lizard Is. and South Is.	9	11	Artificial death after 20 days (20.09.2002)

zooxanthellae inside the nudibranch and digested ones (Wägele and Johnsen, 2001).

The specimens of *Phyllodesmium briareum* were either kept in natural light conditions under starving conditions without any additional food (e.g., soft corals) or in natural light conditions with the primary food source (*Briareum violacea*). Natural light conditions means natural light climate conditions in which irradiance, spectral irradiance and photoperiod are driven by the suns position (sun angle), clouds, the extinction coefficient of water and depth. Another group of specimens of *P. briareum* (juvenile animals) was kept under starving conditions, but after a few days in light they were put in total darkness in order to investigate behavioural and physiological changes of the slugs and changes of the photosynthetic performance. To some of these specimens different soft corals were offered as food source to investigate feeding preferences. Other specimens kept in darkness were brought back to the light after starvation in the darkness (Table 1). One specimen of *Pteraeolidia ianthina* (Pte1) was kept under natural sunlight conditions without any food in the aquarium for 71 days. The second specimen of *Pteraeolidia ianthina* (Pte2) was kept under the same conditions as Pte1 for the first 9 days, but afterwards brought in total darkness under continued starving conditions for 11 days (Table 1). For both nudibranch species the state of health was documented by taking digital photos of their external appearance.

The soft coral *Briareum violacea* was kept under natural light conditions, similar as specimens of *Phyllodesmium briareum*. Long-term experiments in the darkness were not performed because the polyps of the coral are retracted during night time. The zooxanthellae are not located in the coenenchyme, therefore no reliable measurements of the maximum quantum yield ($\Phi_{IIe-max}$) were possible.

All animals were exposed to different irradiances of natural sunlight between 0 and 1,500 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for photosynthesis versus irradiance curves (P-E curves) with the Diving-PAM. Measurements were taken in the "open-air-lab" under a transparent roof at LIRS. For the attenuation of different irradiances spectrally neutral white cotton tissue and paper were used for shading. The fiber optics of the PAM was placed 0.5 cm from the part of the nudibranch with the highest concentration of zooxanthellae as detected by highest *in vivo* fluorescence – in aeolids mainly the cerata. Between the measurements breaks of at least 10 minutes were taken to allow the reaction centres of PS II to recover after light saturation.

There are sources of error when using PAM measurements. These need to be considered in order to interpret the results correctly. Usually the distance between the fiberoptics of the PAM and the measured animal was 0.5 cm, but this distance influences the F_0 values. The breaks between the measurements were generally 10 minutes, but after measurements in the full sunlight it was

often necessary to take longer breaks to allow the reaction centres of PS II to recover. This was the case for species with lowlight-adapted zooxanthellae (e.g. *Phyllodesmium briareum*). Another important factor was the "measuring spot" on the animal. We always tried to find the part of the slug with the highest F_0 signal, but because of the movements of the animals and the size of the fiberoptics it was not always possible to be absolutely accurate. Measurements, when not taken during darkness, were all taken in natural sunlight where clouds and changing humidity also influenced values to a certain degree. To reduce the effects of these errors, the number of measurements was high (e.g. 444 measurements for *Pteraeolidia ianthina*, Pte1).

Measurements of the maximum fluorescence yield ($\Phi_{IIe-max}$) were taken in darkness (during night time about 3 hours after sunset). $\Phi_{IIe-max}$ was plotted versus time in diagrams (Figs. 2 and 3) in order to show how long the zooxanthellae are able to stay photosynthetically active inside the nudibranch. For methodological details see Wägele and Johnsen (2001).

The photosynthesis versus irradiance curves (P-E-curves) and the resulting values (P_{max} , E_k , α) were analysed by the statistics software Kaleidagraph 3.6. For the calculations the following equation was used (for further details see Wägele and Johnsen, 2001):

$$P = P_{max} * (1 - \exp(-\alpha * E / P_{max}))$$

$$P = \text{Photosynthetic rate at a given actinic irradiance, } P = \Phi_{IIe} \cdot E$$

where:

$$P = \Phi_{IIe} (\text{mol charge separation} \cdot \text{mol quanta absorbed}^{-1}) \cdot E (\mu\text{mol quanta m}^{-2} \text{s}^{-1})$$

$$P_{max} = \text{Maximum photosynthetic rate (same units as P)}$$

$$\alpha = \text{Maximum light utilization coefficient } (\alpha = (\Phi_{IIe} \cdot E) \cdot E^{-1})$$

$$E = \text{Irradiance (PAR, 400-700 nm; } \mu\text{mol quanta m}^{-2} \text{s}^{-1})$$

$$E_k = P_{max} / \alpha = \text{light saturation index in } \mu\text{mol quanta m}^{-2} \text{s}^{-1}$$

After the long-term experiments all specimens were preserved in formalin/seawater for later histological investigations.

Additionally, some data (irradiance and yield measurements) were taken *in situ* under water by Scuba Diving. The irradiance in the intertidal zone (0-1 m depth) and at 15-20 m was typically ~1,500 and 50-100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ respectively at solar noon.

3. Results

P-E (photosynthesis versus irradiance curves)

The E_k , α and P_{max} values of starved and fed specimens of *Phyllodesmium briareum*, *Briareum violacea* and *Pteraeolidia ianthina* are listed in Table 2.

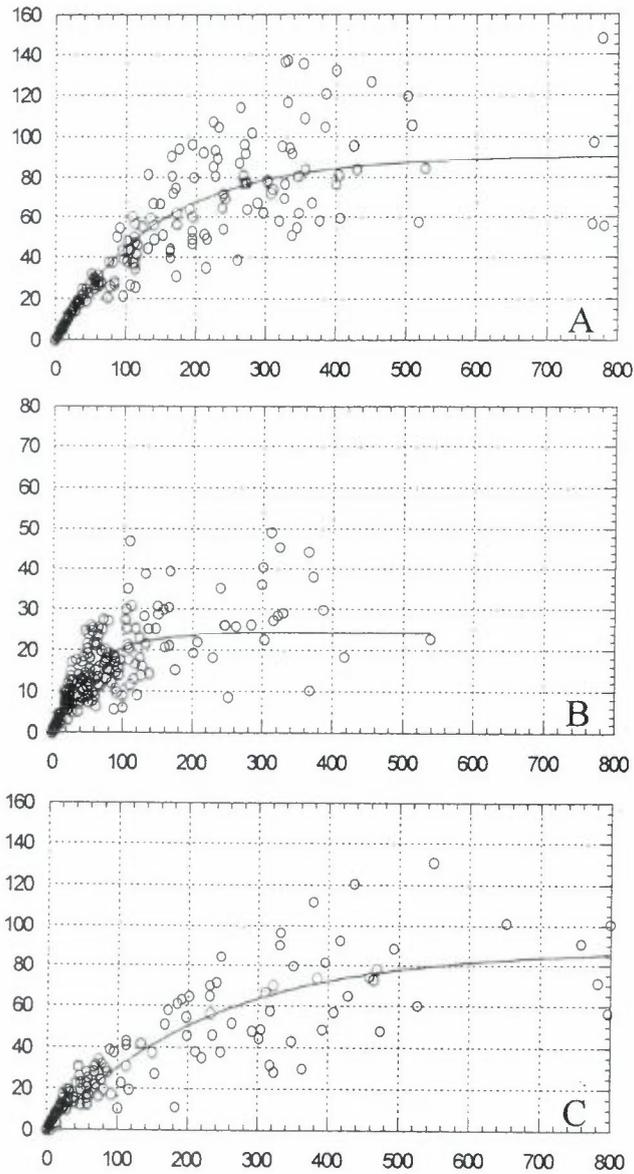


Figure 1. Photosynthetic rate ($P = \Phi_{IIe} * E$) versus irradiance (E ; [$\mu\text{mol quanta m}^{-2} \text{s}^{-1}$]) of *Briareum violacea*, *Phylloidesmium briareum* and *Pteraeolidia ianthina*. A: *Briareum violacea*. B: *Phylloidesmium briareum* Pb1. C: *P. briareum* Pb8.

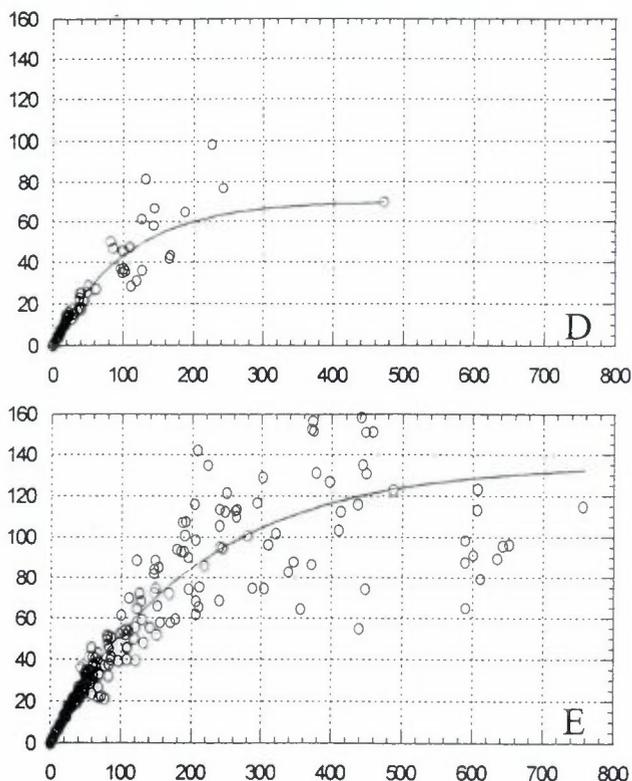


Figure 1. Photosynthetic rate ($P = \Phi_{IIe} * E$) versus irradiance (E ; [$\mu\text{mol quanta m}^{-2} \text{s}^{-1}$]) of *Briareum violacea*, *Phyllodesmium briareum* and *Pteraeolidia ianthina*. D: *P. briareum* Pb10. E: *Pteraeolidia ianthina* Pte1. x-axis: P, y-axis: E. Please note the different scales for P.

The E_k values of the starving specimens of *Phyllodesmium briareum* (here shown for Pb1) are generally lower than the ones of the fed specimens (Pb8 + Pb10). Similar results are observed for the P_{max} values (maximum photosynthetic rate) of the starving specimens in light: The mean values of these individuals are generally lower than the ones of the fed animals (Figs. 1C and 1D, Table 2). Compared to *Phyllodesmium briareum*, the P_{max} and α values of *Briareum violacea* are higher (Table 2). The E_k values are generally higher in *B. violacea* than in *P. briareum*. The P_{max} - and the E_k values of *Pteraeolidia ianthina* are significantly higher than the ones of *Phyllodesmium briareum* and *Briareum violacea* (Table 2).

Table 2. Photosynthetic characteristics of *Phyllodesmium briareum*, *Briareum violacea* and *Pteraeolidia ianthina* (calculated by Kaleidagraph 3.6, based on measurements with a Diving-PAM (Walz, Germany).

Species	Conditions	No.	State	α	P _{max}	E _k [$\mu\text{mol quanta}/\text{m}^{-2} \text{s}^{-2}$]
<i>Phyllodesmium briareum</i>						
	Without food in light	Pb1	Adult	0.46	24 (70 d)	52 (70 d)
		Pb7	Juvenile	0.57	21 (30 d)	37 (32 d)
	On food (<i>Briareum</i>) in light	Pb8	Juvenile	0.38	89 (58 d)	96 (58 d)
		Pb10	Juvenile	0.67	70 (58 d)	104 (58 d)
<i>Briareum violacea</i>						
	In light		Colony	0.63	90 (58 d)	144 (58 d)
<i>Pteraeolidia ianthina</i>						
	Without food in light	Pte1	Adult	0.67	136 (69 d)	203 (69 d)

$\Phi_{\text{IIe}} - T$ (PSII quantum yield vs. time-curves) and $F_0 - T$ (ground fluorescence vs. time-curves)

Comparisons of Φ_{IIe} and F_0 of different specimens of *Phyllodesmium briareum* indicate intraspecific differences (Fig. 2A). The starving individual kept in light (Pb1) obtained a gradual decrease in Φ_{IIe} versus time (from an average of 0.6 in the beginning to approximately 0.35 after 70 days). The curve of Pb7, the other individual kept under the same conditions as Pb1, is nearly identically, but this animal died much earlier. The curve is not shown here. By contrast, the Φ_{IIe} of the two specimens that were kept in light and fed with *Briareum violacea* (Pb8 + Pb10) stayed stable and decreased from 0.7 (start) to an average of 0.6 after 60 days in captivity (Fig. 2A).

There are also interspecific differences in the Φ_{IIe} versus time-curves between starving *Phyllodesmium briareum* (Pb1) and *Pteraeolidia ianthina* (Pte1; Fig. 2B). The Φ_{IIe} of Pte1 is close to the maximum (~0.6–0.7) during the experimental period.

Intraspecific differences between individuals of *Pteraeolidia ianthina* kept under different conditions are shown in Fig. 2C. Pte1 was kept under starvation without the coral in light for 71 days. Pte2, also kept without food, stayed in light for 8 days and was then brought into darkness for 11 days. In contrast to Pte1, the Φ_{IIe} of Pte2 decreased from 0.65 to 0.4 after being in darkness for 4 days (day 12 of cultivation).

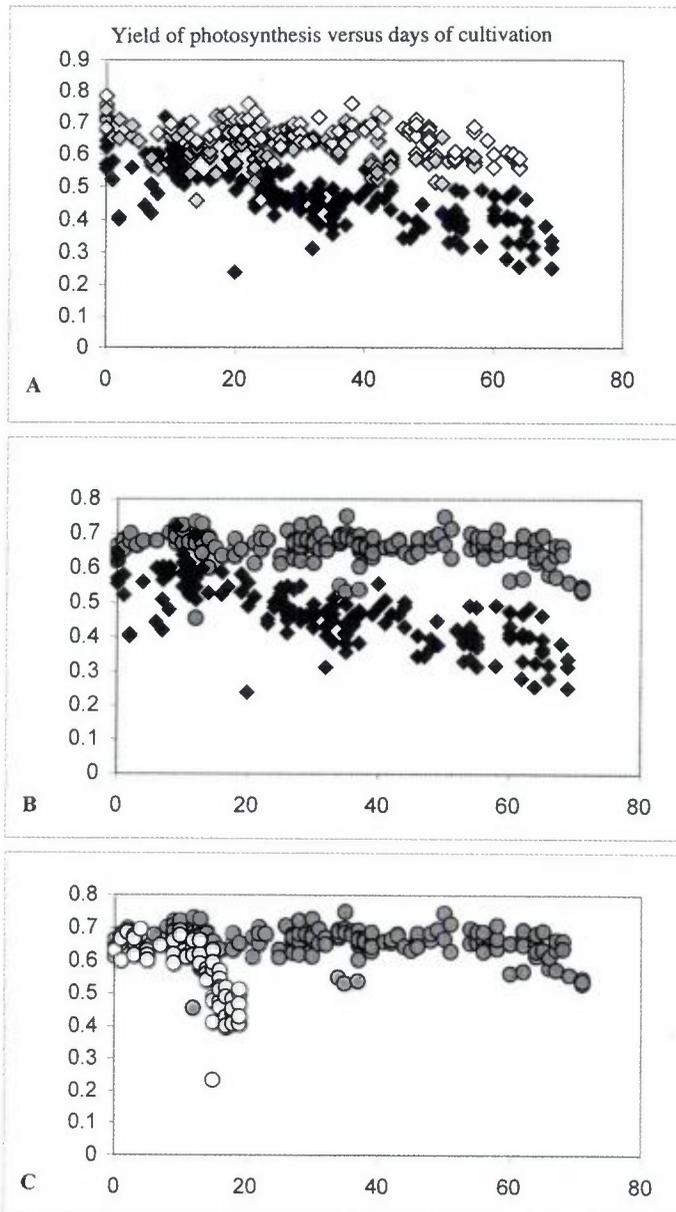


Figure 2. Yield of photosynthesis (Φ_{IIe}) versus time. A: *Phyllodesmium briareum* Pb1 starved (dark grey), Pb8 (white) and Pb10 (light grey) fed on *Briareum violacea*. B: *Phyllodesmium briareum* Pb1 (diamonds, dark grey) and *Pteraeolidia ianthina* Pte1 (circles, light grey), both under starving conditions in light. C: *Pteraeolidia ianthina* Pte1 kept in light (circles, light grey) and *P. ianthina* Pte2 kept in light for 9 days and then moved to darkness (circles, white).

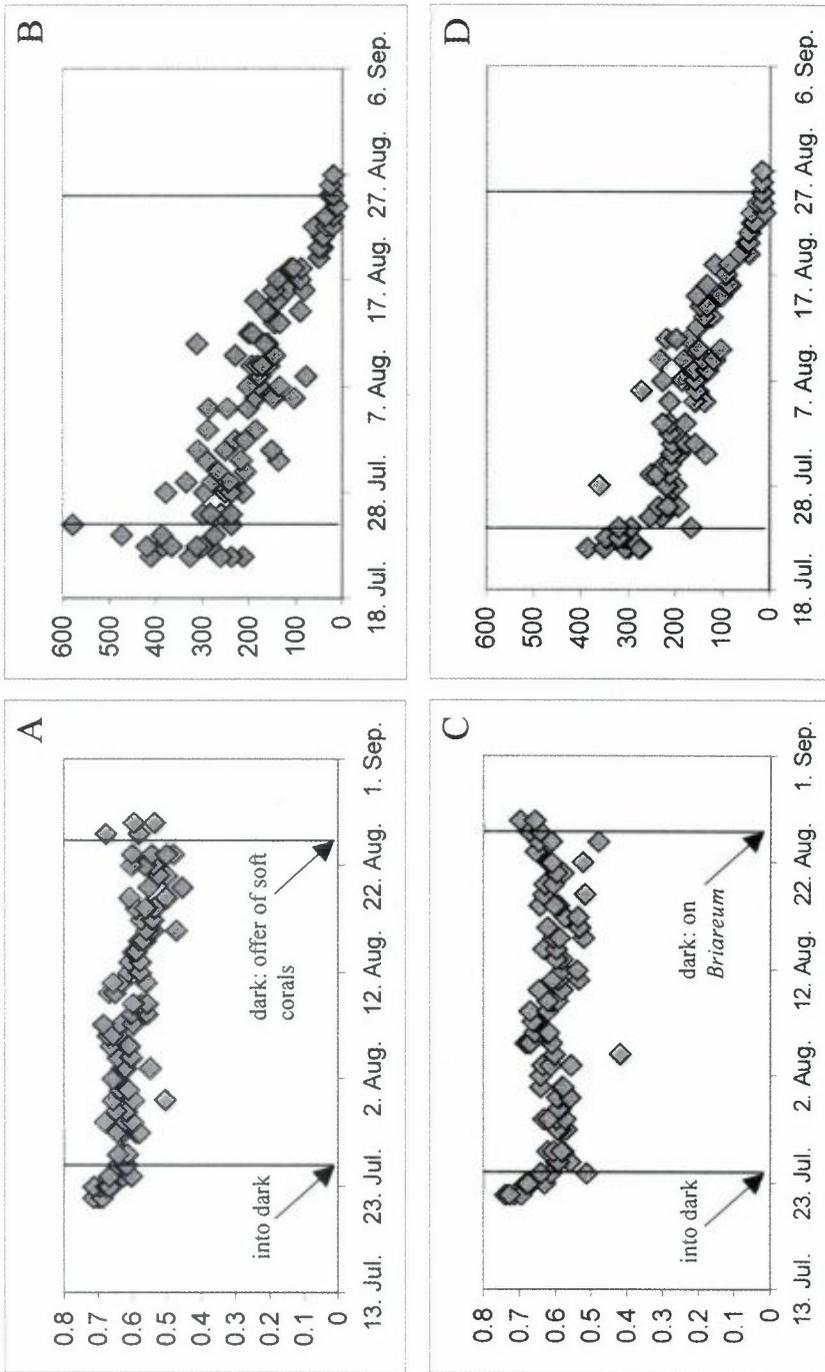


Figure 3. Φ_{1e} versus time (A, C, E, G) and F_0 versus time (B, D, F, H) of different juvenile specimens of *Phylloidesmium briareum* kept in total darkness for some time (compare Table 1). First vertical line indicates change from light into darkness. A and B: *P. briareum* Pb5, second line indicates offer of different soft corals. C and D: Pb4, second line indicates offer of *Briareum violacea* in darkness.

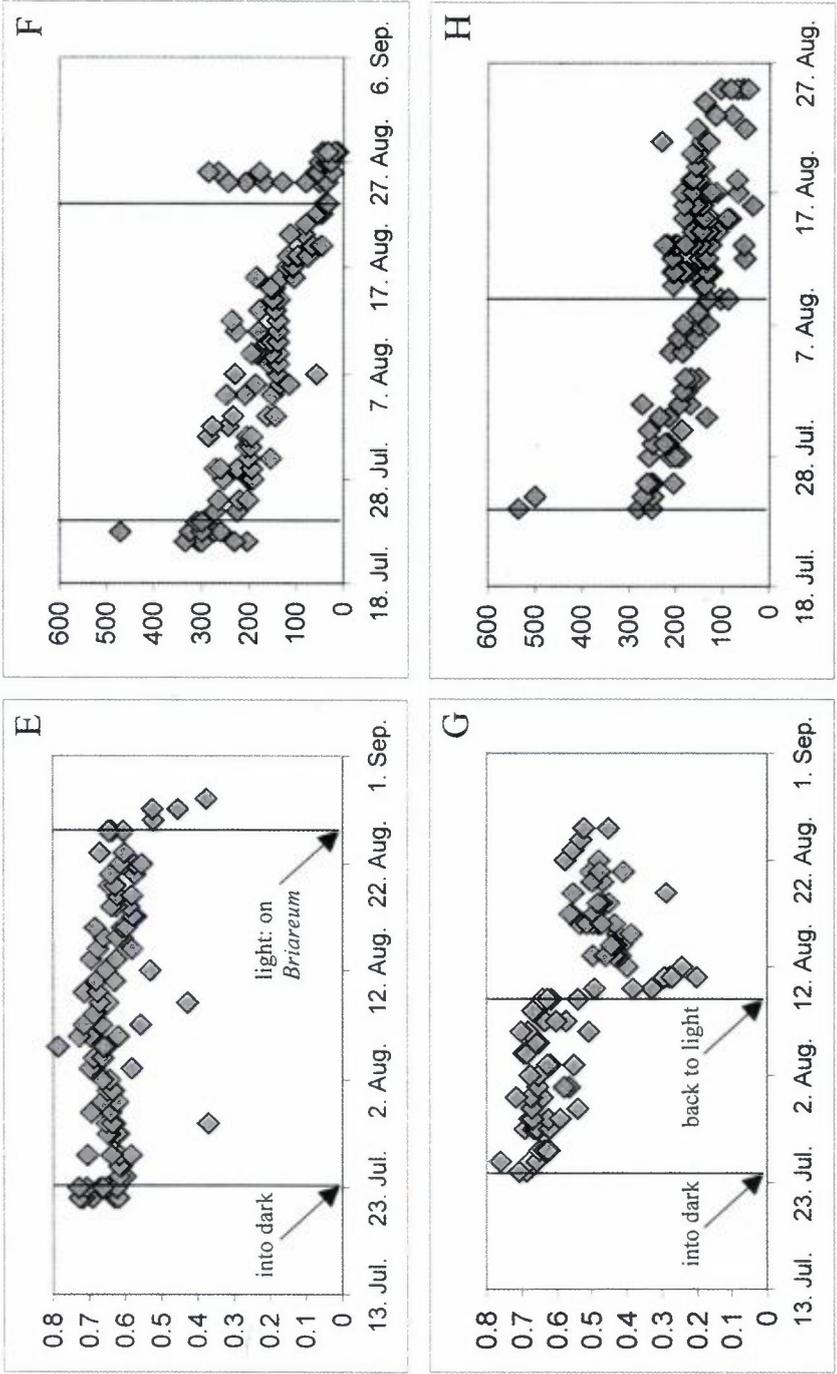


Figure 3. Φ_{I0} versus time (A, C, E, G) and F_0 versus time (B, D, F, H) of different juvenile specimens of *Phylloidesmium briareum* kept in total darkness for some time (compare Table 1). First vertical line indicates change from light into darkness. E and F: *P. briareum* Pb3, second line indicates moving back into light and offering *Briareum*. G and H: *P. briareum* Pb9, second line indicates moving back into light without food offer.

The ground fluorescence (F_0) in the starving adult specimens of *Phyllodesmium briareum* and *Pteraeolidia ianthina* did not decrease considerably more than the yield values. Similar results can be observed for the fed specimens of *P. briareum* (Pb8 + Pb10). The F_0 values somewhat fluctuate (probably dependant on the distance between optical fiber and animal) but stayed in average on the same level for the whole time of the experiments (not shown here).

All starving specimens of *Phyllodesmium briareum* kept in darkness (Pb2, 3, 4, 5, 6, 9) show a slight decrease of Φ_{IIe} similar as the ones kept in light, but the F_0 of all specimens in the dark dropped to nearly zero (<10 mV) after approximately 30 days (Fig. 3).

The Φ_{IIe} and F_0 versus time curves of Pb5 show the typical course of the Φ_{IIe} - and F_0 values (Figs. 1A,B) of the animals kept in darkness. Additionally, different soft corals (*Sarcophyton* sp., *Sinularia* sp., *Xenia* sp.) were offered to Pb5 to check the food specificity of this species living under dark conditions. No feeding was observed and no increase of the F_0 values, that would indicate an uptake of fresh zooxanthellae, was measured.

Pb4 (Figs. 3C,D) was kept under similar conditions as Pb5, but instead of other soft corals, its proper food source (*Briareum violacea*) was offered. Again, there was no increase of F_0 values that would have indicated feeding and incorporation of zooxanthellae.

The Φ_{IIe} - and F_0 -data of specimen Pb3 (Figs. 3E,F) are similar to the ones of all dark cultivated individuals of *Phyllodesmium briareum*, but after putting the animal back to light into an aquarium with *Briareum violacea*, this specimen fed on the coral. The Φ_{IIe} (Fig. 3E) decreased quickly from 0.6 to 0.35 (5 days after the return to light conditions) and the F_0 (Fig. 3F) increased within one day up to 270 mV after introducing *B. violacea*. After one day on *Briareum violacea* the F_0 decreased again to nearly zero.

Pb9 was cultivated like the other starving and dark cultivated specimens but after 16 days, it was brought back into light. The Φ_{IIe} - and F_0 -data (Figs. 3G,H) show similar tendencies in the beginning, but after being back into light (still under starvation) a decrease of the Φ_{IIe} can be measured which then stabilises around yield values of 0.5 later on (Fig. 3G). The observed decrease of F_0 in the beginning of the experiment was stopped when the animal was brought back to the light. F_0 stabilized at ~150 mV (Fig. 3H).

Lifespans and behaviour of the investigated specimens of Phyllodesmium briareum and Pteraeolidia ianthina

Both (adult) specimens of *Pteraeolidia ianthina* survived the whole time of the experiments until they were preserved: Pte1 (71 days), Pte2 (20 days).

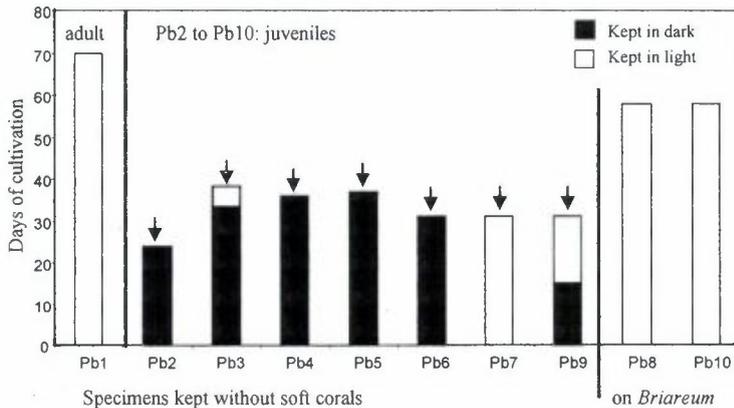


Figure 4. Life spans of juvenile and adult specimens of *Phyllodesmium briareum*, kept under different conditions (see Table 1). Specimens that died naturally are marked with an arrow. Pb1, Pb8 and Pb10 lived until experiments had to be finished.

Fig. 4 summarizes the life spans of the 10 investigated specimens of *Phyllodesmium briareum*. All juveniles that were kept under starving conditions (Table 1, Fig. 4) died after about 35 days, no matter whether they were kept in light or in darkness. Pb8 and Pb10, that were kept on colonies of *Briareum violacea* survived the whole experimental time. The specimen that was already adult in the beginning of our experiments (Pb1) was kept under starving conditions in the light and survived 70 days, until the end of experiments (Fig. 4). Although the starving adult specimen of *Phyllodesmium briareum* (Pb1) survived until the end of the experiments and although there was still photosynthetic activity, the animal lost the brownish colour of the zooxanthellae, became pale and did not grow (Fig. 5A). In the end of the experimental period, the slug became smaller and thinner. The starving juvenile in light (Pb7) went pale much quicker than Pb1 and died after only 32 days. It did not grow at all.

The two juvenile specimens that were cultivated on *Briareum violacea* (Pb8 and Pb10) grew fast in size. Although the starving individual Pb1 was a few millimetres longer than these two in the beginning of the experiments, in the end Pb8 was double the size of Pb1, dark brownish in colour and healthy looking, that means with a lot of thick cerata and no shrinking process visible (Fig. 5A). In general, the fed specimens were brown in colour and looked healthy during the whole time. They were mainly sitting between the polyps of *B. violacea*, were well camouflaged and did not move much. They were able to re-grow autotomized cerata and often laid egg clutches, mainly between the polyps.

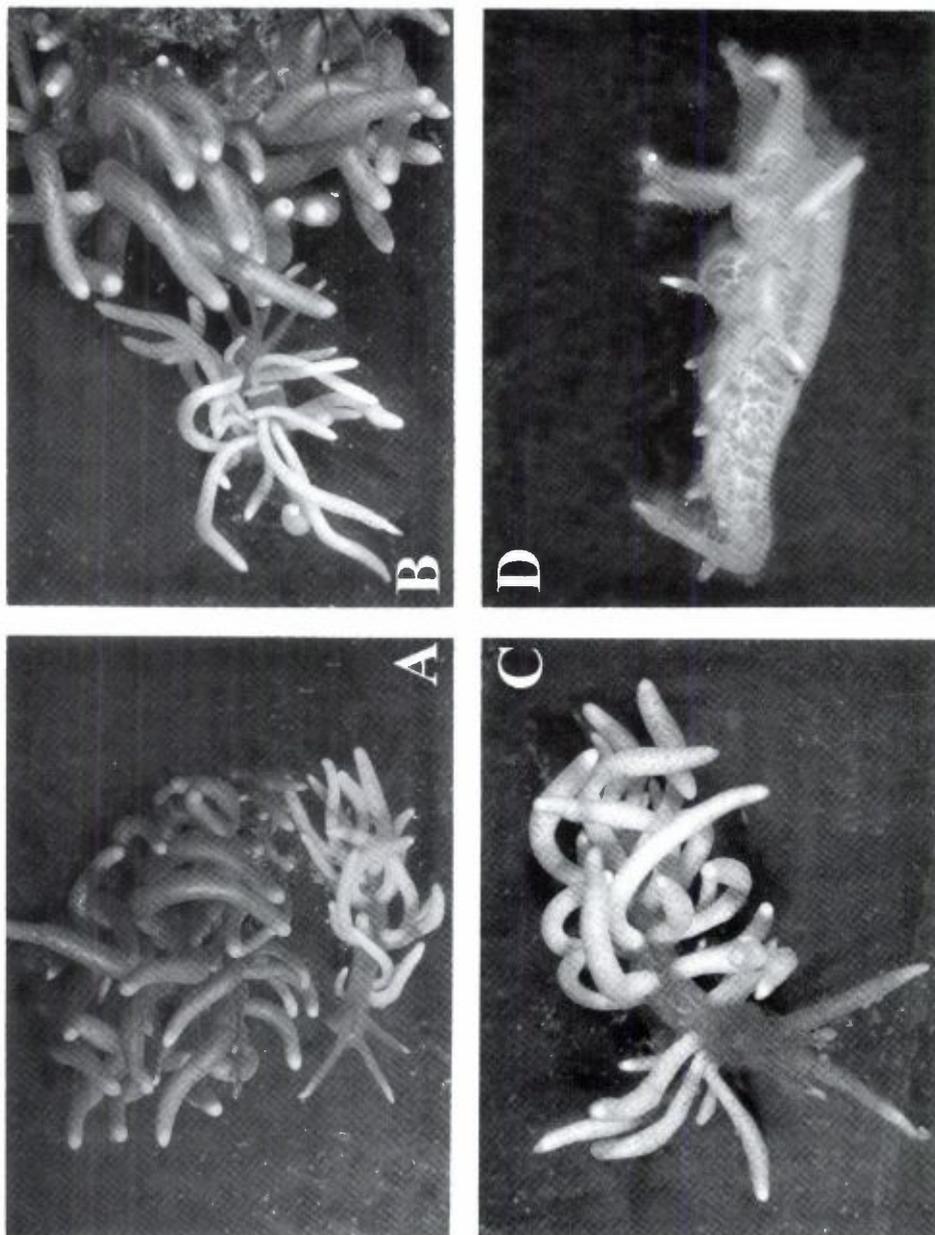


Figure 5. Living aspects of different specimens of *Phyllodesmium briareum* (see Table 1). A: *P. briareum* Pb1 (small specimen, 25th day without food in light) and *P. briareum* Pb8 (13th day in light on *Briareum violacea*). B: *P. briareum* Pb2 (small specimen, 15th day without food in darkness) and *P. briareum* Pb8 (16th day in light on *Briareum violacea*). C: *P. briareum* Pb4 (18th day without food, 16th day in darkness). D: *P. briareum* Pb4 (36th day without food, 34th day in darkness).

The juvenile specimens of *Phyllodesmium*, that were kept in darkness (Pb2, 3, 4, 5, 6, 9), all went pale quickly. The cerata of these specimens went pale much faster than the rest of the body. Especially inside the foot there were still single brownish patches of zooxanthellae to see. During the first days of their captivity in darkness all of these juveniles produced a lot of brownish faeces that did not show any photosynthetic activity measured by the Diving-PAM. In the end of the cultivation all specimens in darkness were nearly transparent and the branched ducts of the mid-gut were clearly visible. The slugs lost size much faster than the starving specimens in light and developed local swellings over their body. Additionally they autotomized more and more cerata but were not able to re-grow them.

In general, the slugs kept in dark were less active than the ones kept in sunlight and showed unusual behaviour: They were often drifting near the water surface with spread-out cerata – a behaviour that was never observed for the specimens kept in light.

Fig. 5C presents the dark acclimated specimen Pb4 after 16 days in darkness. Fig. 5D shows the same animal after 34 days in darkness. In Fig. 5B two juveniles (Pb8 and Pb2) kept under different starving and light conditions after 2 weeks are presented. Pb8 which was cultivated in light on *Briareum violacea*, is double the size of Pb2 which was kept in darkness under starvation.

In general, *Phyllodesmium briareum* seemed to avoid high irradiances in full sunlight and was more active in moderate irradiances, e.g., when the sky was cloudy or during morning or evening hours. *Briareum violacea* was also sensitive to high irradiances. It retracted its polyps not only in night time but also in direct sunlight.

The two starving individuals of *Pteraeolidia ianthina*, Pte1 + Pte2 were much more active at higher irradiances than *Phyllodesmium briareum* and did not avoid direct sunlight. Pte1 stayed dark brown nearly until the end of the experiment but lost colour and size, from 40 mm length to 17 mm, suddenly a few days before preservation, i.e., after approximately 65 days of cultivation. Pte2 kept its brownish colour until it was preserved, even after cultivation in darkness for 11 days.

4. Discussion

Φ_{IIe} -E-curves

The data of the present study confirm the assumption of Hoegh-Guldberg and Hinde (1986), Rudman (1991) and Wägele and Johnsen (2001) that *Pteraeolidia ianthina* and *Phyllodesmium briareum* are nudibranchs with a highly evolved photosynthetic relationship with zooxanthellae. This study, based on PAM-

measurements also shows significant inter- and intraspecific differences of the photosynthetic parameters α , P_{\max} and E_k .

The P_{\max} - and E_k values of the starving adult *Phyllodesmium briareum* (Pb1) and in general the values of all specimens of *P. briareum* are much lower than the ones of *Pteraeolidia ianthina*. The other specimen of *Phyllodesmium briareum* (Pb7) that was kept under the same conditions (starvation in light), was a juvenile and showed similar results in the tests as Pb1, but the P_{\max} and E_k values were generally lower.

Comparing these data with the ones of the *Phyllodesmium briareum* specimens kept in light, but on their food source *Briareum violacea* (Pb8 + Pb10), there are significant differences to the starving animals. Both fed slugs showed high values of P_{\max} . The results for the E_k values are similar. The P_{\max} values of the fed specimens (Pb8 + Pb10) are more similar to the ones of *Briareum violacea* ($90 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), whereas those of the starved specimens Pb1 and Pb7 are considerably lower. The E_k value of *Briareum violacea* was high compared to the data of the nudibranchs. Only *Pteraeolidia ianthina* showed higher values.

Low values of P_{\max} and E_k indicate low light adapted or acclimated photosynthetic active organisms, high values the opposite (Wägele and Johnsen, 2001). These data presented here match the locality where we found the investigated species. *Pteraeolidia ianthina* was collected in the intertidal zone in areas with exposure to high irradiances, whereas *Phyllodesmium briareum* was collected between 15 and 20 m, areas with lower light conditions ($50\text{--}100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at noon on a bright day).

Pteraeolidia ianthina is known to forage on several hydrozoan species (Kempf, 1984; Willan, 1989; Hadfield, 1976; Gosliner, 1980), but we do not know what kind of prey our specimens were feeding on. Therefore, we do not know the source of the zooxanthellae. It is still unclear how many species of the genus *Symbiodinium* and zooxanthellae in general are involved in symbiotic relationships with nudibranchs. Hoegh-Guldberg and Hinde (1986) identified one species in *Pteraeolidia ianthina* as *Symbiodinium microadriaticum* Freudenthal, 1962 by morphological means. Marin and Ros (1991) assumed that the *Symbiodinium* they found in different nudibranchs also belong to this species. Contrarily to this assumption, Blank and Trench (1985) identified at least four different species in cnidarians on the basis of chromosome numbers and physiological behaviour. The current discussion in the literature distinguishes at least seven different phylogenetic clades or strains of *Symbiodinium*, based on data on ribosomal DNA (LaJeunesse, 2001; Rodriguez-Lanetty, 2003). Five of these clades are known to occur in corals. Unfortunately, there are no detailed investigations about *Symbiodinium* in octocorals. It is known that different clades of *Symbiodinium* can be associated with

individual coral colonies simultaneously (Rowan and Knowlton, 1995; van Oppen et al., 2001).

However, known examples of mixed zooxanthellae communities are limited (Rowan and Knowlton, 1995; Toller et al., 2001; van Oppen et al., 2001; Diekmann et al., 2002; LaJeunesse, 2002). Although some studies have found a lack of correlation between *Symbiodinium* phylotypes and photo-physiology (Savage et al., 2002), the distribution of zooxanthellae in some coral species correlates with light intensity and quality on different spatial scales. Light intensity and spectral composition are thus suggested to affect the composition of the zooxanthella community within individual colonies (Ulstrup and van Oppen, 2003). These data on corals are helpful for understanding the mutualism between the investigated nudibranchs and *Symbiodinium* because their only source of zooxanthellae are cnidarians.

Our results on the two different nudibranchs suggest that different ecotypes of *Symbiodinium* or even strains showing different photosynthetic characteristics are involved. *Pteraeolidia ianthina* houses a "highlight-adapted" type of *Symbiodinium*, *Phyllodesmium briareum* a "lowlight-adapted" one. *P. briareum* avoids high irradiances by escaping to shaded places and is more active in moderate irradiances. Interestingly, *Briareum violacea* also retracts its polyps in very high irradiances. This indicates that *P. briareum* and *B. violacea* may house similar light-sensitive zooxanthellae. In contrast to that, *P. ianthina* is also very active in direct sunlight irrespective of high irradiances.

When *Phyllodesmium briareum* feeds on *Briareum violacea*, a selective retention of different clades or types of *Symbiodinium* is possible, that would explain the differences of the E_k values between the soft coral and the fed slugs (see Table 2). One or more types of *Symbiodinium* that are more adapted to higher irradiances are probably not able to survive inside the slugs, but inside the coral. Thus the P_{max} - and E_k values of the fed specimens of *Phyllodesmium briareum* are more similar to the ones of *Briareum violacea* and higher than in the starving specimens, because they always had the opportunity to get "fresh" zooxanthellae from the coral.

Although the values in *Briareum violacea* are higher than in the starving animals of *Phyllodesmium briareum*, they are distinctly below the ones of *Pteraeolidia ianthina*. There seems to be no ecotype or strain of *Symbiodinium* adapted to extreme high irradiances in *Briareum violacea*, that was mainly found in depths of more than 13 meters around Lizard Island. Wägele and Johnsen (2001) indicate a much higher value of P_{max} and E_k in *Phyllodesmium briareum*. This might be due to the only few measurements and self-shading-effects in that study. High degree of self shading will give lower E_k values (Johnsen et al., 1997).

P-T-curves and F₀-T-curves

The results of the long-term experiments also show intra- and interspecific differences in Φ_{IIe} - and F_0 values over time. The Φ_{IIe} of the fed specimens of *Phyllodesmium briareum* (Pb8 + Pb10) was high (0.7–0.6) and did not decrease in comparison to the starved animals Pb1 and Pb7. This is certainly a consequence of permanent uptake of "fresh" zooxanthellae from *Briareum violacea*. These specimens can keep *Symbiodinium* cells photosynthetically active, but may replace them when new zooxanthellae are available. The decrease of the Φ_{IIe} in starving animals suggests that the portion of still photosynthetically active zooxanthellae is getting smaller and the animal is not able to keep all of them fully photosynthetic active over a long period. Nevertheless, the number of zooxanthellae seems to be stable over time indicated by nearly no changes in F_0 , even in the starving animals.

The interspecific differences between the starving specimens of *Phyllodesmium briareum* and *Pteraeolidia ianthina*, shown by the Φ_{IIe} plotted versus time, indicate that *P. ianthina* is able to keep the photosynthetic performance of its zooxanthellae stable for a long period (Pte1: at least 71 days). This confirms the results of Kempf (1984) that this species is able to survive at least some months exclusively on the photosynthetic products of its zooxanthellae. Their and our results suggest that *Pteraeolidia* has a stable, highly evolved and effective mutualistic symbiosis with *Symbiodinium*.

The rather slight decrease of Φ_{IIe} values in a specimen of *Phyllodesmium briareum* (Pb1) after more than 70 days of starvation from 0.7 to 0.6 also indicate the presence of a mutualistic symbiosis with zooxanthellae, but because of the decrease of the Φ_{IIe} the symbiotic relationship seems to be less stable and effective than in *Pteraeolidia ianthina*. The grade of branching of the digestive gland is high in *P. briareum* (Rudman, 1991) as well as in *P. ianthina*. The latter shows special adaptations of the digestive glands for housing zooxanthellae, namely the "tubules" described by Wägele and Johnsen (2001). This confirms the hypothesis of Rudman (1991) that a high grade of branching of the digestive gland indicates a symbiosis with *Symbiodinium*.

The fast decrease of the non-fed specimen of *Pteraeolidia ianthina* (Pte2) after 4 days in darkness (Fig. 2C) suggests that the probably high light-adapted zooxanthellae of *Pteraeolidia* are not able to survive several days in low-light or darkness. Comparing the PAM data between the different dark cultivated specimens of *Phyllodesmium briareum*, they show the same tendencies in Φ_{IIe} and F_0 versus time. Φ_{IIe} lies around 0.7 or decreases very slightly to 0.6, whereas the F_0 drops fast from around 300 mV to zero. This indicates that the number of photosynthetically active zooxanthellae decreases in darkness, but the remaining zooxanthellae are functional. Either the *Symbiodinium* cells are digested by the animal or they die because of the

lack of light. Under dark conditions the slugs shrink considerably. They lose their colour and get pale, an additional indication that the number of zooxanthellae decreases. In general, the animals obviously suffer more under starving conditions in darkness than in light, probably because of the missing nutrients produced by photosynthetic active zooxanthellae.

We assume that the zooxanthellae inside the dark cultivated slugs switch to a heterotrophic state and would then use metabolites of the nudibranch (Melkonian, pers. comm.). Since most of the zooxanthellae are situated in the midgut branches of the cerata, an autotomizing of the cerata would reduce the number of zooxanthellae in the slugs considerably. This assumption is supported by the observation that the starving dark cultivated specimen Pb4 did not accept the offered colony of *Briareum violacea*. Uptake of new zooxanthellae was avoided. Pb5, kept under the same conditions as Pb4, refused the offer of other soft corals. This may also indicate the avoidance of uptake of fresh zooxanthellae, but may also be interpreted as a narrow food spectrum of *Phyllodesmium briareum*.

The data of the dark cultivated specimen Pb9 after returning into light (Figs. 3G, H) show that there were still living zooxanthellae. The F_0 stabilized around 150 mV, indicating a stabilisation of the number of photosynthetically active zooxanthellae. The short drop of the Φ_{IIe} after returning into light may be a response of the zooxanthellae which were not able to cope with the quick switch to higher irradiances. The increase of the Φ_{IIe} two days later indicates chloroplast recovery and acclimation to higher irradiances. Additionally, there were more and more brownish patches to see in the body, especially in the foot. The Φ_{IIe} of the dark cultivated individual Pb3 show similar results as Pb9: the Φ_{IIe} decreases after returning the slug back to light. The sudden increase of the F_0 in Pb3 can be explained by the offer of *Briareum violacea* that allowed the uptake of fresh zooxanthellae by feeding. The dramatic decrease of F_0 only one day later suggests that the zooxanthellae were not able to survive as symbionts inside the slug – probably they were digested because of the bad state of the animal.

Fig. 4 shows that all juveniles of *Phyllodesmium briareum* that were kept under starving conditions died after about 35 days – no matter whether they were kept in light or darkness. Therefore we assume that the symbiotic relationship in juveniles is not developed enough to maintain metabolism or even growth without any other food. The two juveniles kept on *Briareum violacea* (Pb8 + Pb10) survived until they were preserved and showed a significant growth, so actually they were adults at the end of the experiments. We would most likely get different results for the non-fed experiments in darkness with adult animals, because of the probably underdeveloped symbiosis in juveniles. Therefore, the same experiments need to be repeated with adults.

According to the results presented here we assume that the energetic output of this mutualistic relationship with photosynthetic units (here zooxanthellae) helps the adult animals to overcome a period of food shortage (Marin and Ros, 1992). It also lengthens the period for search of adequate mating partners, whereas in juvenile specimens the mutualism is not established enough to nourish the animals over a longer period of time.

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