SYMBIODINIUM SP. CAN STAY ALIVE THROUGH THE GUT AND IN THE FAECES OF CNIDARIA PREDATORS. THE CASE OF *CORALLIOPHILLA MEYENDORFFI* AND *ANEMONIA VIRIDIS*.

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Abstract
The gastropod *Coralliophilla meyendorffi* is a common predator of the zooxanthellate anemone *Anemonia viridis*. Zooxanthella from the anemones are an important constitutent of the mollusc faeces. Cell integrity, occurence of flagellated forms, live/dead proportions and mitotic index of *Symbiodinium* collected from the faeces and cultivated in vitro were examined and compared to that of algae *in hospite*. The results show that most algae withstand digestive processes of the predator, staying alive and dividing actively in the faeces. Motile (lagellated) algae arise from dividing cells and escape the fecal pellets.
Impact on coral bleaching recovery is discussed.
Keywords: Cnidaria, Dinoflagellates, Symbiosis, Predation, Fecal Pellets

Introduction
Many eukaryotic microalgae live in symbiosis with marine invertebrates. One of the most widely distributed and abundant intracellular algae are the zooxanthellae (dinoflagellates of the genus *Symbiodinium*) in symbiosis within cells of reef forming corals and of other cnidaria [1]. The increased frequency of disruption of this symbiosis (coral bleaching) is a major threat for coral reefs future [2]. In order to recover, bleached cnidaria can, either let proliferate the algae not previously expelled (5 to 25 % of the initial stock) or catch free *Symbiodinium* in the environment [3,5,7]. Both processes potentially result in algae community changes, favouring environmentally better adapted strains (adaptive theory of bleaching [8,9]). As "free" zooxanthellae are quite rare in the plankton [1], one can look for other possible sources of symbionts. Here we investigate the potential role of undigested algae embedded in the faeces of cnidaria predators as source of zooxanthellae for recolonization of bleached hosts.

Material and methods
In June 2009, 15 live *Anemonia viridis* (Forskal, 1775) and 15 *Coralliophilla meyendorffi* (Calcara, 1845) were collected by scuba diving (3-8 m depth) close to the oceanographic station STARESO (Calvi, Corsica) and kept separate for acclimatization during one month in free flowing aquaria in the laboratory under in situ light and temperature conditions (18 to 22°C, maximum light intensity 1970 lumen m^{-2}). Experiments were performed in July 2009. Anemone extracted zooxanthellae were collected by gentle homogenization of 0.5 g (FW) of anemone tentacles in 10 ml of 0.2 µm filtered SW with a potter grinder, then purified from anemone debris by filtration (50 µm pore size) and centrifugation (500 x g), resuspended and cultured under in situ illumination and temperature in F/2 medium. Faeces zooxanthellae were recovered by grinding freshly collected faeces of *Coralliophilla* exclusively fed with *Anemonia* for 1 week, then purified and cultivated the same way. Algae density in culture was monitored in both cases for 12 days (5 replicates) by epifluorescence counts. Mitotic indexes (MI, as % of "doublets", N >500 cells) and % dead cells (Trypan blue permeation test, N>300 cells) were recorded every day at 9h00 in culture samples. Apparition of motile (flagellated) cells was monitored along nycthemeral cycles (during the log phase of both cultures) during 48 hours every 2 hours.

Results and discussion
Most zooxanthellae found in the faeces are alive (> 80 %) and able to multiply quickly. Actively growing cultures were obtained from algae either directly extracted from anemones or from faeces of *Coralliophilla* exclusively fed with *Anemonia viridis*. There are no significant differences between the kinetics of both growth curves : lag phase last approximately 6 days and logarithmic growth for another 6 days, culminating with densities around 200 to 250 10^3 algae ml^{-1} (inoculum was 25 10^3 cell ml^{-1}).
The MI of faeces isolated algae is significantly lower than the MI of anemone extracted ones during the 3 first days of culture (0.8 to 1.2% compared to 2.0 to 2.8%) ; there is no significant difference during the following days. MI is classically low in zooxanthellae, close to 2 to 5 % of the population according to strains, light and nutrients supply [4,6]. Maximum MI occurs after 8 days of culure (2.9 to 3.4%). Division occur mainly during the very first hours of "light" period (from 6h30 to 9h00 in our conditions, 100 to 600 lumen m^{-2}) and at dusk (18h30 to 21h00, 400 to 100 lumen m^{-2}).
Division peaks were followed by apparition of motile algae (8h30 to 10h30 and from 20h30 to 21h30). There were virtually no flagellated forms during the night (light intensity < 50 lumen m^{-2}), and very few ones (< 0.2 % during the most illuminated hours of the day, > 600 lumen m^{-2}). This conforms to previous observations under natural conditions [6]. Motile cells also escaped from undisturbed fecal pellets even if most cannot get free from mucous threads in which algae are embedded.
% dead cells was much higher just after isolation from *Coralliophilla* faeces (10 to 25% of the population at day 1) compared to anemone extracted ones (2 to 7%). From day 3 to day 12, the % dead cells in culture remained constant around 2% of the algae population for both conditions.

Conclusion
Most symbiotic zooxanthellae from *Anemonia viridis* consumed together with animal tissues by the gastropod *Coralliophilla* withstand digestion and are recovered alive, actively dividing and potentially motile in the faeces of the predator. Observations by Fit & Trench [5] on *Cassiopeia* scyphistome show that live *Symbiodinium* cells introduced in the coelenteron resist digestion by the cnidaria and are internalized (endocytosis) by endodermal cells. So, fecal pellets, once resuspended in the POM could be a source of algae for recolonization of bleached host. Moreover, flagellated cells produced by undigested zooxanthellae in faeces are another potential source as these motile algae are attracted to potential hosts [4], if freed from mucous threads. It should be quite interesting to check the hypothesis that faeces and fecal pellets of cnidaria predators may constitute a reservoir of live zooxanthellae able to be consumed and internalized during recovering phases post-bleaching.

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