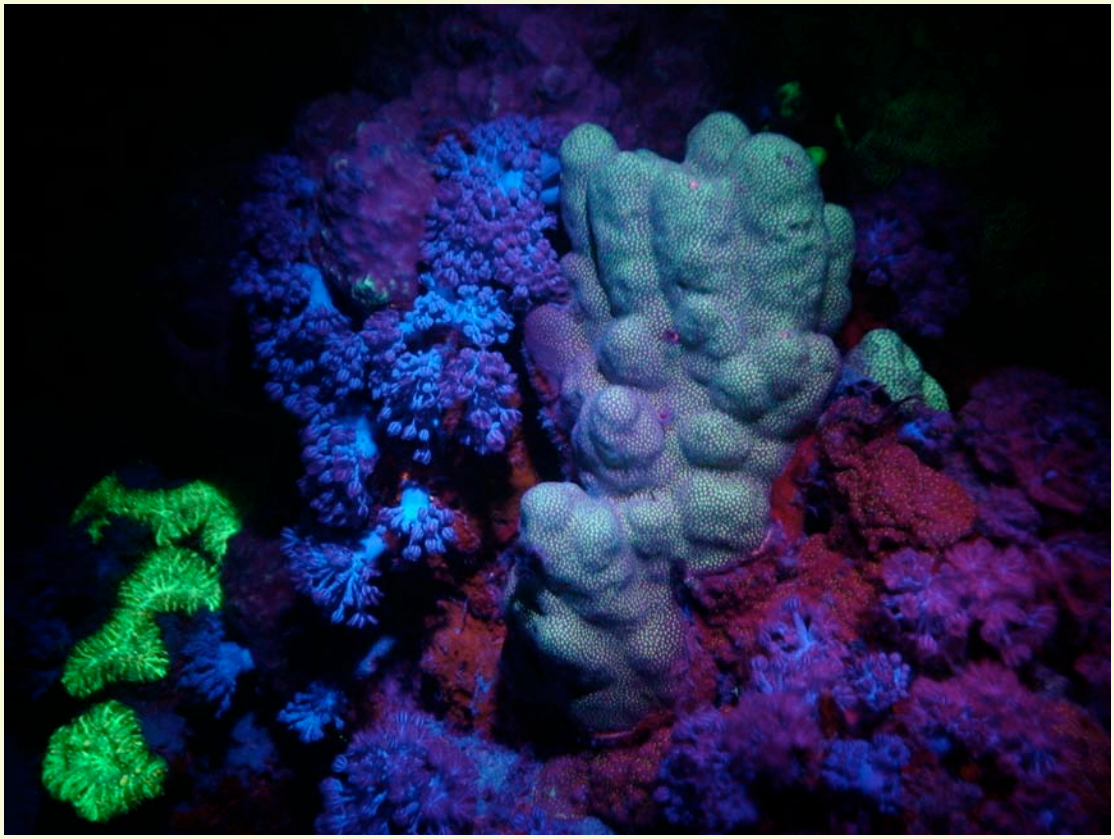
Coral reefs

World Heritage of Mankind

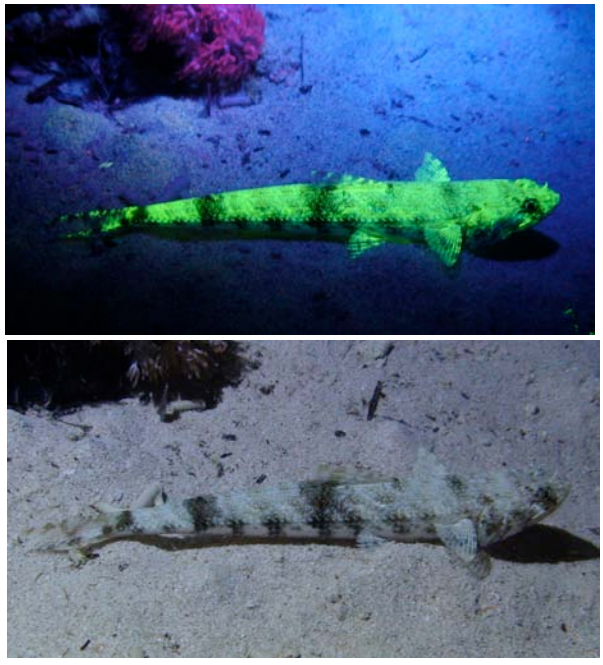
Magic World explored during Fluorescence Night Dives - a technique to monitor the status of an endangered ecosystem



Horst Grunz 2011

Prologe

Coral reefs are under natural and human (anthropogenic) threat worldwide. There is a permanent conflict between ecology and economy. Since coral reefs are important for the biological biodiversity of the marine ecosystem and as shore and coastal protection they deserve our all commitment. There must be strict rules and enforced laws to prevent overfishing and environmental violation (pollution and illegal construction of buildings for tourists and industry). Similar to the tropical reign forests coral reefs are endangered by commercial interests of industry and tourist activities without surveillance. Greenhouse gases (water vapor, carbon dioxide, methane, nitrous oxide and ozone) are responsible for fundamental changes in the physical and chemical properties of the oceans. The average temperature of the upper layers of the ocean has increased by 0.6 Grad Celsius over the past century and the average pH has decreased 0.1 units. Such changes are especially risky for sensitive biotopes as mangrove forests, sea grass areas and corals. Especially the reef-building corals are under extreme stress, since increasing acidification the ocean water decreases the ability of corals to build their calcareous skeletons, which is important for the maintaining the reef structure. Like the rain forest coral reefs are key-habitats for the whole planet. Increased destruction leads to a jeopardy of mankind Therefore the demand for sustainability should be not limited to the platform of local and international conferences only.



Abstract

With special HighTech fluorescence torches during night dives scuba divers will have an outstanding experience and will see the coral reef in magic colors superior to the situation with normal white light or during dives at daytime.
The fluorescence of the corals in species specific manner in rainbow colors results from pigments, which are close related to the Green Fluorescent Protein (GFP). It was first detected in jelly fish *Aequorea victoria.* Three scientist earned the nobel prize of chemistry for the analysis in 2008. GFP is meanwhile a powerful tool in molecular biology to solve complicated processes in living embryos and cell cultures in respect to different diseases including cancer.
The professional divers (marine biologists and professional photographers) can use the illumination with fluorescence torches to perform large scale scanning of reefs or document living corals, various fishes and „lower“ animals (invertebrates) in beautiful colors. We developed HighTech torches, which allow a scannning of large areas. The data will result in a documentation of the state of the corals, since it is easy to distinguish quickly between living and dead corals. Furthermore even tiny newly settled corals can easily be detected. So it is possible to detect the recruitment of the reef and juvenile corals (even 1 mm in diameter about 4-5 meters away) much earlier than ever possible during daytime and with normal white illumination.
This is important for reef check studies to find out if a reef will be recovered after natural or anthropogenic threats.

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Introduction

Coral reefs similar to the tropical reign forest are the most species-rhich ecosystems on earth and are the home of a huge variety of animals and plants, also coined as an extreme biodiversity. Both ecosystems are severely endangered by natural and anthropogenic stress. Therefore everybody should care for the preservation of this unique biodiversity.
Fluorescence during night dives is a valuable tool to check the state of the reef and to distinguish living from dead corals. Living corals show a pattern of species specific pattern of rainbow-like colors, while dead coral reefs look like white/gray rocks similar to concrete. This is a magic experience for both the hobby scuba divers as well for professionals like marine biologist and experienced photographers. In this article we will report about our results in the ElQuadim Bay, ElQuseir, Egypt. The theory of fluorescence and the contruction of fluorescence torches will explained in detail. Furthermore we describe the anatomy, early embryonic development of corals and their importance for the structure of coral reefs in respect of maintainance of marine biodiversity and coastal protection especially of flat islands againsts high waves including Tsunami impacts.
This topic is of general public interest for discussion of impacts of greenhouse effect and warming up of the sea temperature resulting in coral bleaching an final death of the coral reefs.

The biology of corals

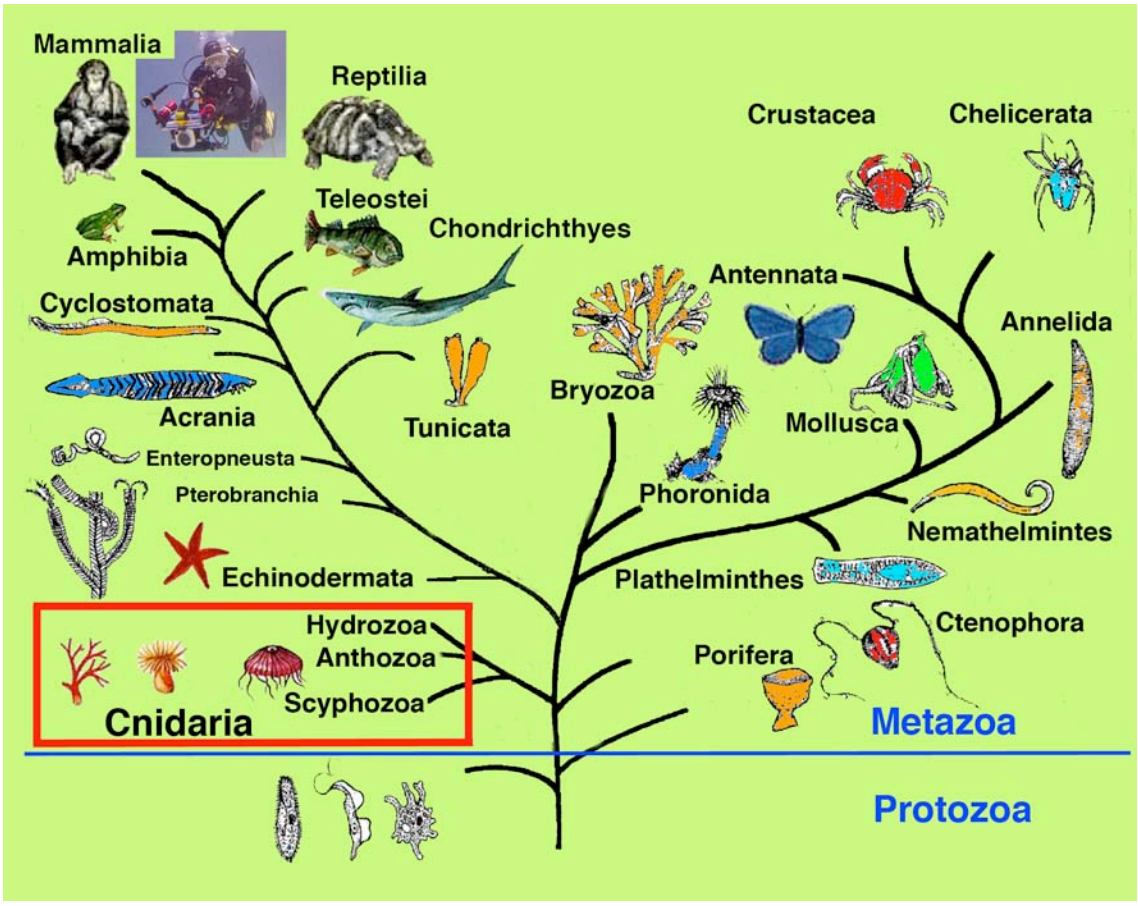


Fig. 1 Phylogenetic tree of the animal kingdom.

The diagram shows in a very simplified way the position of the corals *(Anthozoa)* in the hierarchy (red frame). *Cnidaria* consist of two germ layers separated by a thin membrane, the mesogloea, only. Similar to sponges (porifera) they are the first organisms (in contrast to protozoa) during evolution which have developed a multicellular organization.

Corals belong as the jelly fishes to the cnidaria, which contain stinging cells. The anthozao (especially the stone corals) play an important role as reef building organisms. The coral reef is an indispensable factor to establish and maintain an extended biodiversity. The destruction of these biotops especially by anthropogenic influences (climate change, pollution, tourism, overfishing, dynamite and cyanide fishing) will result in threats of the marine biodiversity and the loss of coast protection (especially of low tropic islands surrounded by coral reefs).

Biology of corals (embryonic development and morphology)

Fig. 2 Schematic diagram of a single coral polyp.
The inset shows the two layers of a tentacle. The outer layer (epidermis) contains the stinging cells, which also give the name to this animal class (cnidaria). In this layer also the chromatophore proteins are situated, which are responsible for the fluorescence of the different species. The inner layer separated from the outer layer by a thin membrane - like structure (mesogloea > blue) contains the symbionts (here shown as ellipses). Below the polyp we can see a part of the calcium skeleton (pink).

modified after Jen Veron: Corals of the World (2000)

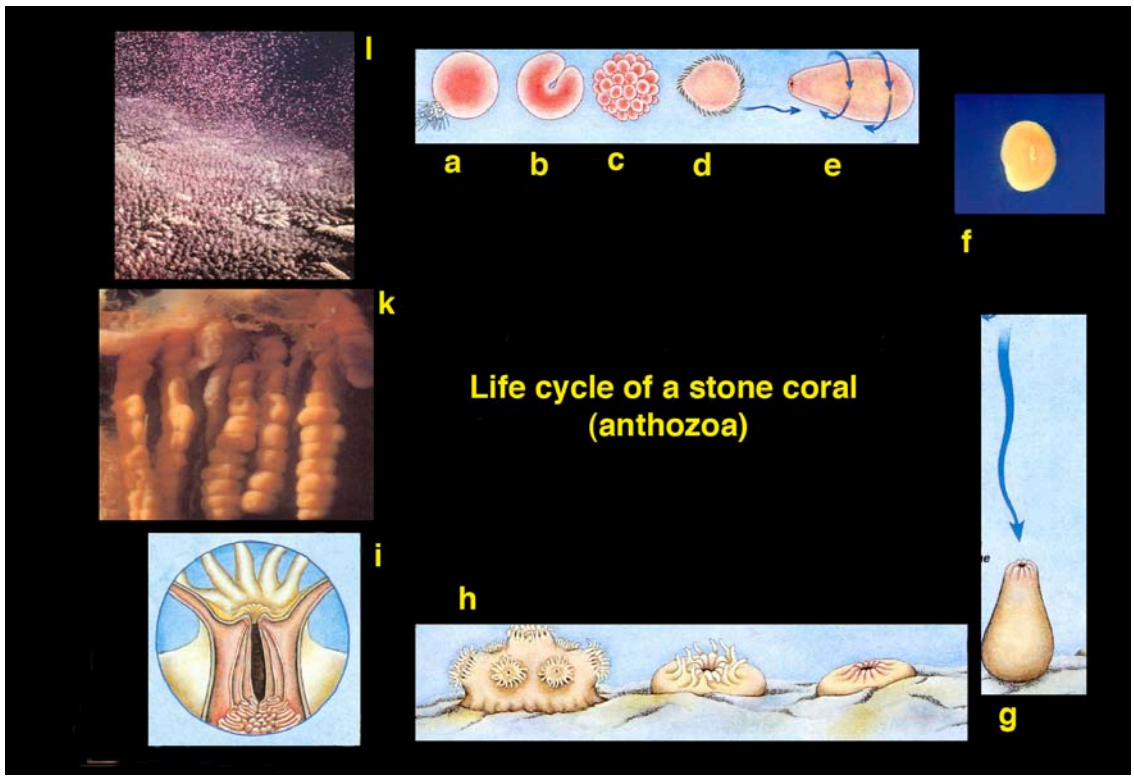


Fig. 3 Early embryonic development of a stone coral.

a) the embryogenesis starts with the fertilization of mature oozytes in the open water

b) the zygote (egg) starts immediately with dividing into 2 cell-, 4-cell, 8-cell-stage

c) 32-64-cell stage

d) early planula larvae with cilia

e) free swimming planula larva showing rotation during movement

f) early polyp stage ready for settlement

g) just settled polyp with its foot side on the substrate and moth field oriented to the open water

h) early coral stock with several polyps formed by vegetative propagation

i) single polyp with gonads (oozytes or sperms), see also k)

k) gonads (here oozytes)

l) spawning colony (many coral stocks simultaneously release oozytes and sperms into the open water during certain priods of the year)

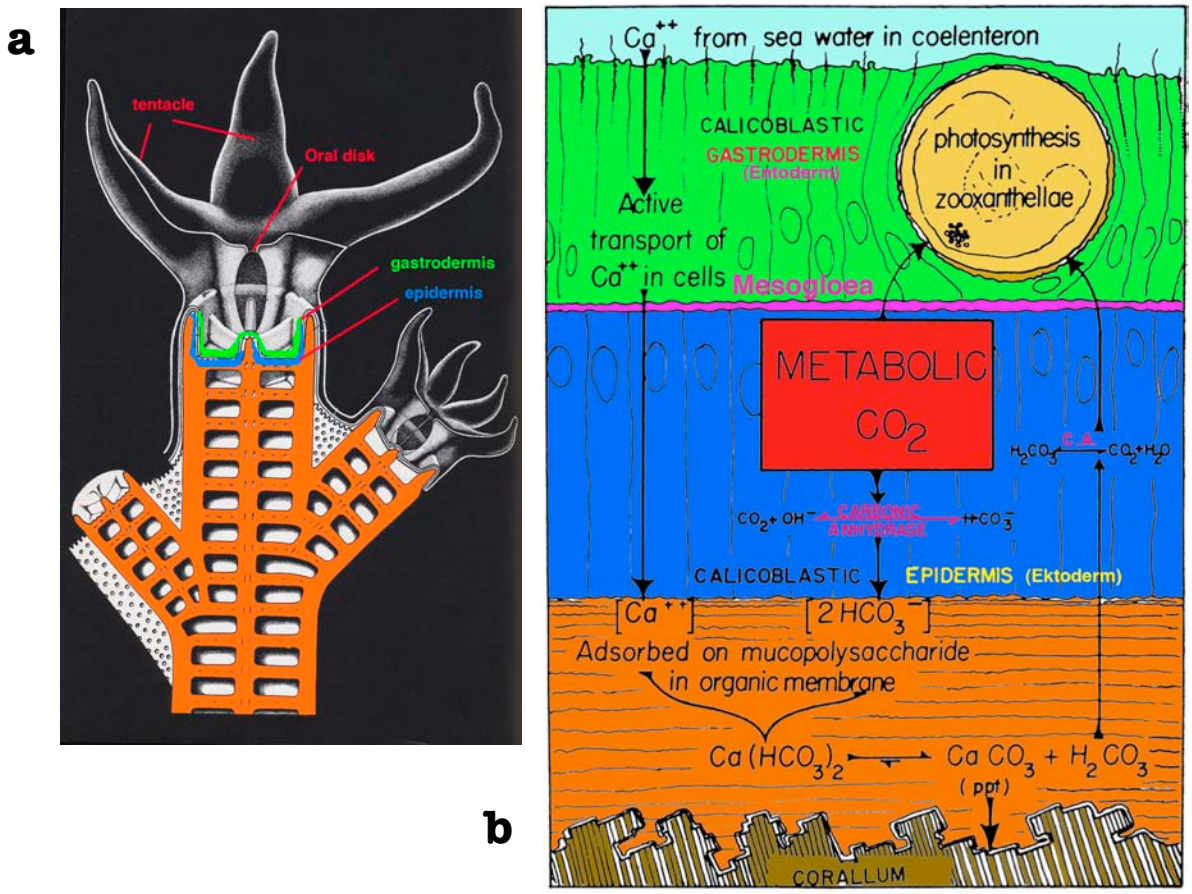


Fig. 4 Schematic diagrams of the process during formation of the calcium skeleton of stone corals (anthozoa)

a) polyps showing the two germ layers (gastrodermis green and epidermis blue), compare with fig. **b**

b) in the gastrodermis (green area) the zooxanthellae (symbiontic algae) are localized. They supply the corals with oxygen, carbonhydrytes and lipids. Furthermore they eliminate carbon dioxide and use it for photosynthesis. Carbonic anhydrase, an important enzyme dramatically enhances these metabolic processes leading for an effective formation of the calcium skeleton (brown). There are strong indications that the inhibition of carbonic anhydrase by low pH (acidification of sea water) could be one of the factors reducing the speed of growth of coral skeleton important to maintain the reef structure.

What is fluorescence?

The term fluorescence is coined in correlation to the fluorescent mineral Fluorit (calciumfluorite, CaF). The emmision of short wavelength light on suitable objects will cause a re-emmision of longer wavelenght. Sometimes ultraviolet light is used (wavelength around 280 nm and lower), which is also called „black light“, since humans cannot see it. This UV light is frequently used in fluorescence microscopy. Under these conditions certain targets glow in special colors. However, for the illumination of

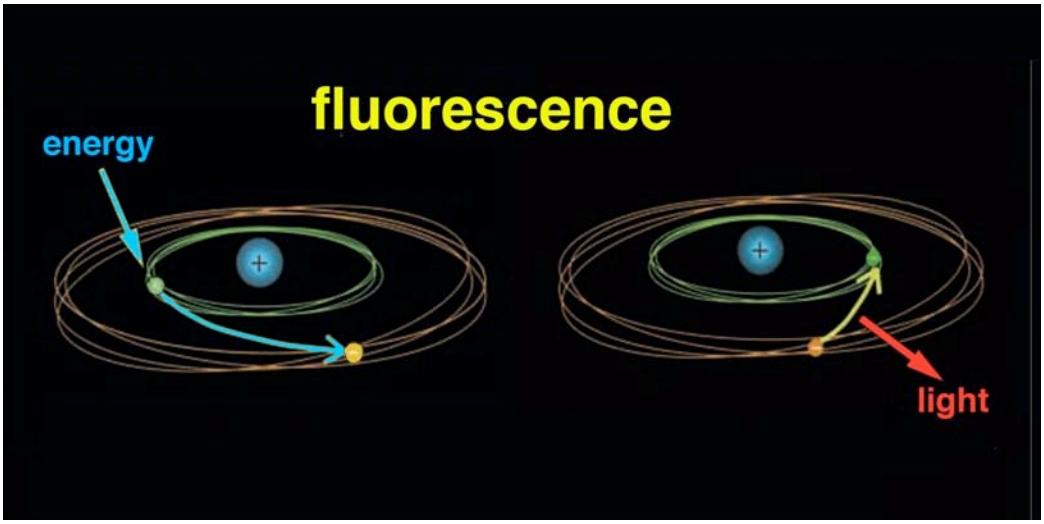


Fig. 5 Fluorescence is the phenomenon by which light (UV or blue light wavelenght with) is absorbed at a target (certain minerals or living corals) and re-emitted at another, longer wavelength.
1. Energy (here blue light) is absorbed by electrons of a fluorescent molecule. 2.The photons are jumping to a higher energy level
3. Immediately, the electron drops back to the ground state, emitting a photon (or a packet of light) - resulting in fluorescence of the molecule

corals and other animals as algae, fishes and invertebrates like sponges, mollusks, bristleworms etc. the extreme short wave light is not suitable. It turned out that blue light (460-470 nm) is the better source. Many inhabitants of the reef glow in different colors of a rainbow pattern, depending on the species specific GFP - like proteins.

Fluorescence should be not confused with phosphorescence or bioluminescence. Phosphorescence is a special form of luminescence. While fluorescence immediately ends, when the illumination is terminated, phosphorescent objects glow even second till hours after the shut down of the light.

Fluorescence technique - prerequisites

For the illumination and documentation of fluorescent targets special equipment is essential. Similar to the studies with a fluorescence microscope in cell biology the following prerequisites are necessary:

1. HighTech powerful fluorescence source (emission, excitation)
2. Fluorescent target
3. special filter for analysis (barrier-filter)

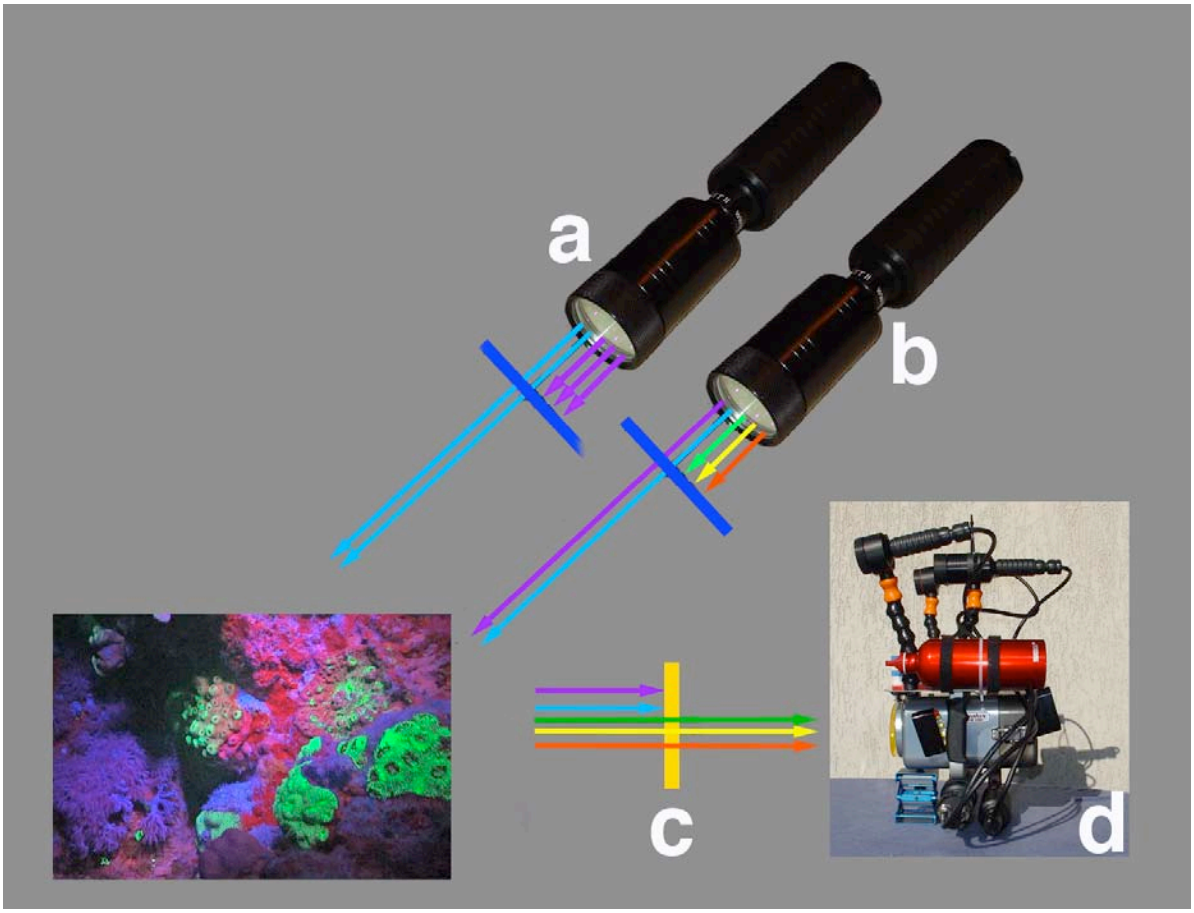
to 1. light with short wavelength (here blue with 460 - 470 nm) will be oriented to the target (corals and other lower animal; fishes)

to 2.: the fluorescent targets re-emitt light with longer wavelenght (yellow, green, red)

to 3.: to prevent the observation of the blue light interfering with the reinbow colors, a yellow barrier filter is place in front of the mask and camera, which allows the transfer of light with a wavelenght higher than 500 nm.

Fig. 6 Equipment for fluorescence studies

a, b Fluorescence torches
a contains blue HighTech LEDs and an additional blue filter (exciting filter) **b** works with white LEDs. The special blue filter eliminates not all wavelength below 500 nm
c yellow barrier filter, which will placed in front of the camera (**d**) and the diving mask.



What is bioluminescence?

Bioluminescence is a phenomena which is caused by chemical reactions within an organism. It is so called cold light. It can be seen in Dinoflagellates, bacteria and fireflies. At least two important chemical substances participate in this process, the Luciferin and the enzyme Luziferase. During catalysis of the Luciferin by Luciferase resulting in Oxyluciferin and the release of yellow-green light. Bacteria with the Luciferin-Luciferase-system are also found in deep sea organisms like the Humpback anglerfish *(Melanocetus johnsonii).*

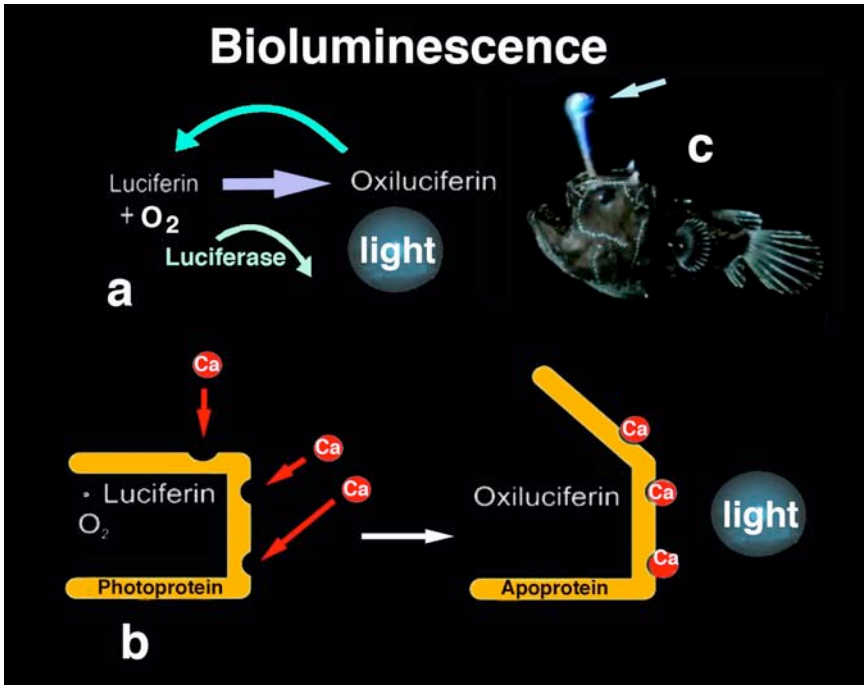


Fig. 7 Bioluminescence is cold light produced by a chemical reaction within an organism (certain bacteria, flagellates or fireflies)

a. At least two chemicals are necessary. Luciferin and the enzyme Luciferase. Luciferase catalyzes the oxidation of luciferin. This resuts in light and an inactive oxyluciferin. For a reiteration of the light emission fresh luciferin must be brought into the system, either through the diet or internal synthesis
b. Sometimes the luciferin and catalyzing protein (equivalent to luciferase), as well as cofactors such as oxygen, are bound together to form a photoprotein. This molecule can be triggered to produce light when a particular type of ion is added to the system (frequently calcium)
c. Deep sea angler fish *Melanocetus johnsonii* emits light from their lantern-like appendix to attract prey. This bioluminescence is a result of symbiosis with bacteria containing the luciferin/ luciferase system

Importance of GFPs (Green Fluorescent Protein) and derivatives

First the GFP was isolated from the jelly fish *Aequorea victoria.* Illumination of this animal with blue light results in a green-yellow color. In molecular biology and cell biology the gene GFP was coupled with other genes coding for proteins of interest, which function should be studied. These constructs can be detected in **living** cells, tissues and organs. So it is possible to detect the migration and position of certain proteins in living organisms. Therefore this technique is meanwhile a keystone of modern cell biology including cancer research. Very spectacular (but from the scientific standpoint rather unimportant) was the demonstration to „illuminate“ by this gene technology whole living animals as flies, fishes, frogs, axolotl *(Ambystoma mexicanum),* mice, cats, rabbits and pork. The „classic“ GFP reacts on emission of 509 nm and glows in green. Roger Tsien, one of the 3 nobel prize winners of 2008, modified the GFP in that the derivates glow in all colors of the rainbow (fig. 9). The different colors result from small changes of the amino acid configuration of the central molecule in the inner part of the beta-barrel structure of the protein (fig. 8). Such GFP-like proteins are found in species-specific way in different corals. This is the reason that shortwave (blue) light will illuminate them in magic colors. The emission spectrum is quite different in the various coral species (fig. 11).

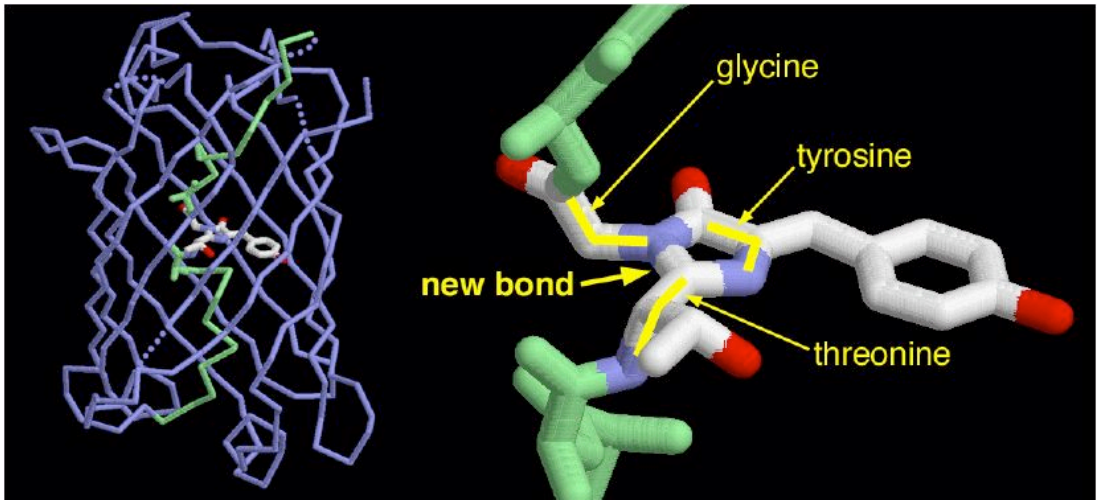


Fig. 8 Structure of Green Fluorescent Protein (GFP) first identified in a jelly fish. The molecule in the center of a basket-like protein is responsible for the green fluorescence when illuminated with light of short wavelength.

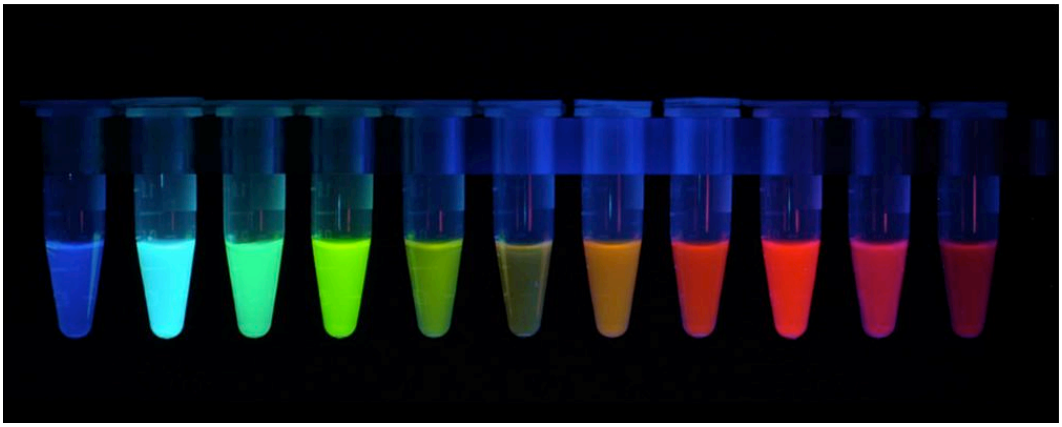


Fig. 9 Modified GFP comparable to different chromatophores in corals. Monomeric or tandem dimeric fluorescent proteins derived from *Aequorea* GFP or *Discosoma* RFP, expressed in bacteria and purified. This photo is a time exposure of fluorescences excited at different wavelengths and viewed through different cutoff filters (modified after Tsien, 2009 Nobel Prize lecture)

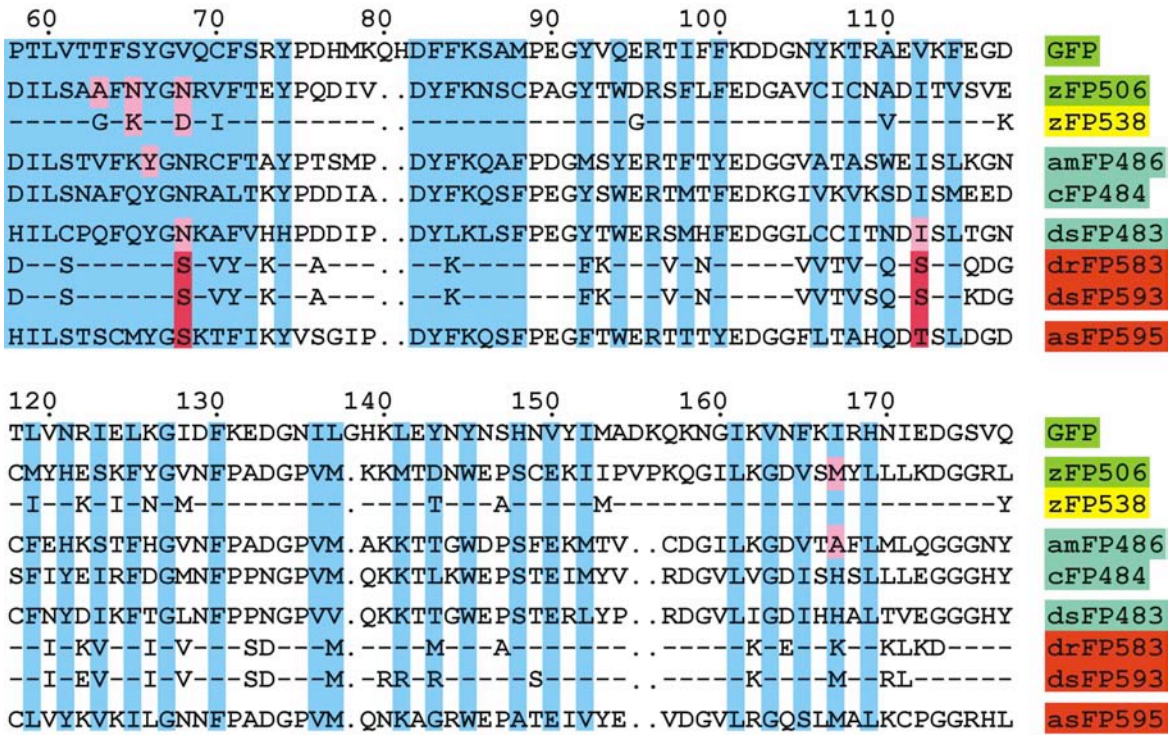
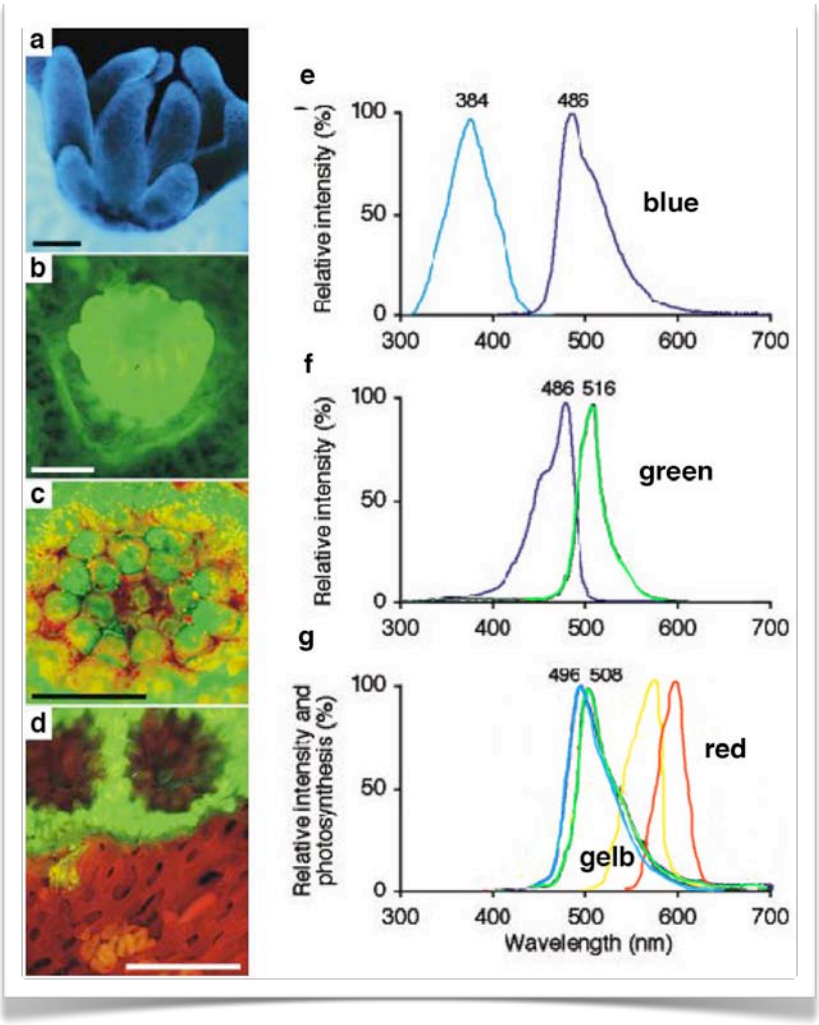


Fig. 10 Multiple alignment of GFP and *Anthozoa* FPs. The numbering is according to GFP, the N- and C-termini of the proteins are not shown. Coral FPs are named designated by lowercase letters identifying the species (e.g., "z" for *Zoanthus sp.,* "am" for *Anemonia majano,* "c" for *Clavularia sp.,* "ds" for *Discosoma sp.,* "dr" for *Discosoma sp.* red, "as" for *Anemonia sulcatd).* Shown is the part of the amino sequence of the proteins, which are responsible for the fluorescence, i.e. yellow, green and red. They are close related to the green GFP.
from: Nadya G Gurskaya, Alexander P Savitsky, Yurii G Yanushevich, Sergey A Lukyanov and Konstantin A Lukyanov *Color transitions in coral's fluorescent proteins by site-directed mutagenesis; BMC Biochemistry (2001) 2:6*

Possible role of GFP-like proteins for the corals

Corals belong as the jelly fishes to the cnidaria (anthocoa). They contain two germ layers, the outer epidermis and the inner layer, derived from the entoderm (also named gastroderm). The gastroderm responsible for the digestion is separeted from the outer layer by a thin layer, the so called mesogloea. The symbionts (algae belonging to the dinoflagellates) are situated in the endoderm derivative (gastroderm), which are essential for the survival of the corals. They are responsible for the different intensity of brown color of the corals. In several publications it is postulated that the green, red and yellow colors of the corals orign from these symbionts. However, the GFP-related proteins, which are responsible for the beautiful rainbow colors are located in the outer layer (epidermis) of the coral polyp. The symbiontic algae situated the endoderm derivative supply the coral polyps with carbonhydrates (sugar), fats (lipids) and oygen by photosynthesis. There are still speculations about the role of the chromophores (GFP-like) proteins in the epidermis of the coral polyps. They could convert light with short wavelength into long wavelenght light, which could be used for photosynthesis by the symbiontic algae. This may be important for corals deeper than 5 meters, where only blue parts of the light spectrum are present. Other scientists argue that the chromoproteins function as protection against the sun in those corals, which are situated close to the water surface, where the intensity of UV is especially high. In deeper regions of the reef the conversion of short to long wavelenght light could be essential for the symbionts of the coral polyps.
Unclear up till now is the fact that certain fishes still in 15 meters operate with autofluorescent red signal colors, while the naked human eye is unable to see other colors than green or blue. Apparently these fishes can recognize the head or sideline area as signals for recognition of predators, for breeding behaviour and for defence of their territories against competitors.

Fig 11 Main types of fluorescent pigments (FPs) in coral polyps. These pigments are found in blue, green, yellow and red combinations (**a, b, c, d**) with overlapping excitation and emission spectra (**e, f, g**). **a,e**, Mainly blue, in *Acropora nobilis*. **b, f**, Mainly green, in *Pocillopora damicornis*. **c,g**, Emissions of outer blue/green and underlying yellow FPs in 'sun' Porites cylindrica. Coral photosynthetic action spectrum (red line) shows that much of the energy is emitted at wavelengths not usable in photosynthesis. **d**, Sub-surface red FPs in green *Montipora digitata*.



modified after Anya Salih, Anthony Larku, Guy Cox, Michael Kühl & Ove Hoegh-Guldberg; NATURE | VOL 408 | 2000

Why night dives with fluorescence torches?

Illumination in the night with fluorescent torches converts the reef into a magic world. Our fluorescent torches brighten an area of about 20 - 30 square meters. So far commercial available torches are effective in an area of 50 x 50 cm only. The hobby scuba diver will be impressed by a new underwater world. One has the impression to visit an underwater flower garden.

On the other hand the scientist is able to use the technique of flluorescence as a tool for analysis of the state of the coral reef. As previously mentioned living corals only glow in fluorescent light in yellow, yellow-green and red.violett colors. In contrast dead coral stocks appear in a concrete grey color. The death of coral ployps and whole coral reefs is correlated to different environmental impacts, which causes the loss of symbiontic algae from their hosts. After the death of corals remains only the white-gray skeleton. The color, which is corellated with the GFP-like proteins is lost forever. Under certain positive conditions (reduction of environmental stress) coral reefs can regenerate. For such processes the reef needs very often long periods. Also for such studies HiTec fluorescence is extremely valuable. In large-scale analysis it is possible to detect even tiny new settled polyps. These newcomers can be detected with the high power fluorescent light as tiny spots in the dark surrounding.

Setting up of the fluorescence equipment

As fluorescence source we used a torch containing HighTech LEDs. These are 4 blue high power OSTAR SMT LEDs in multichip technology (4 x 9 MegaCd/qm), i.e. 16 HighTech LEDs. In our recent construction (fig.18) we used 3 blue LUMINUS HighTech LEDs with 1200 Lumen. The first commercially available torches contained 1 LED only.

The diving mask will be covered with a special yellow filter from the US factory Night.Sea. A similar filter was placed in front of the housing for the cam corder.

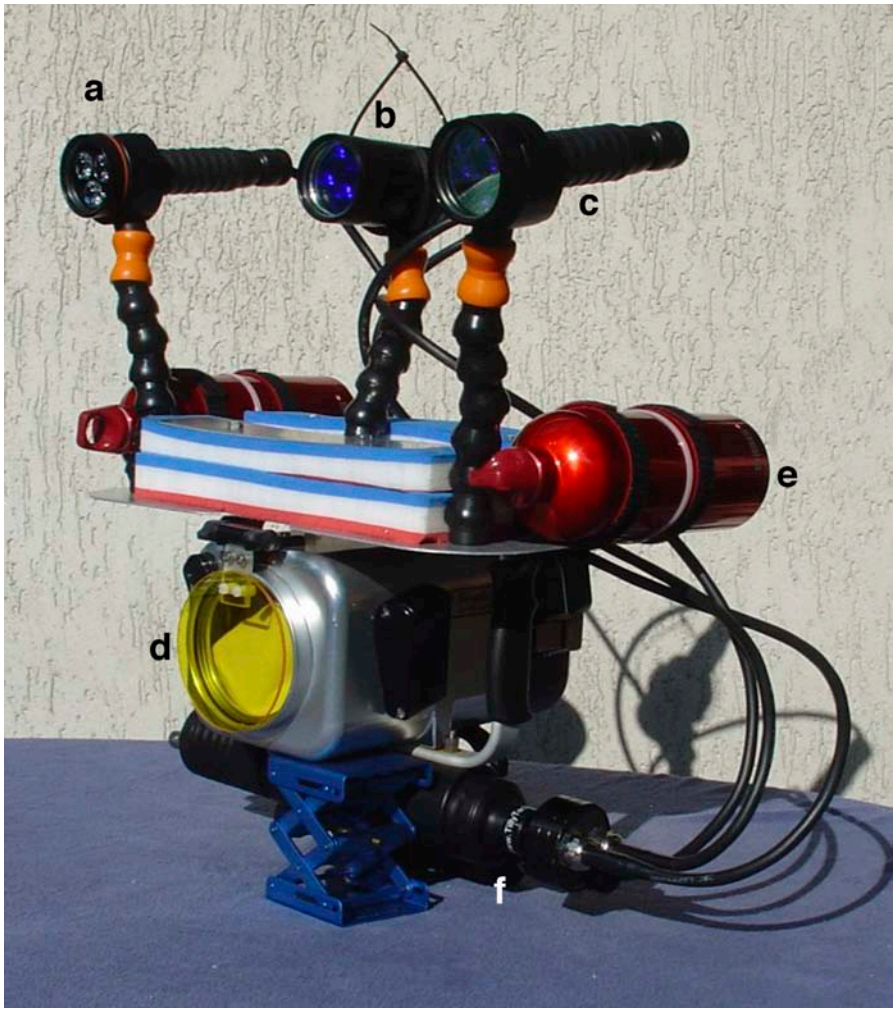


Fig. 12 Sealux® Housing

Fig. 13 The author close to the coral reef in the ElQuadim Bay, ElQuseir, Egypt

Fig. 14 Documentation during day time in a depth of 20 meters

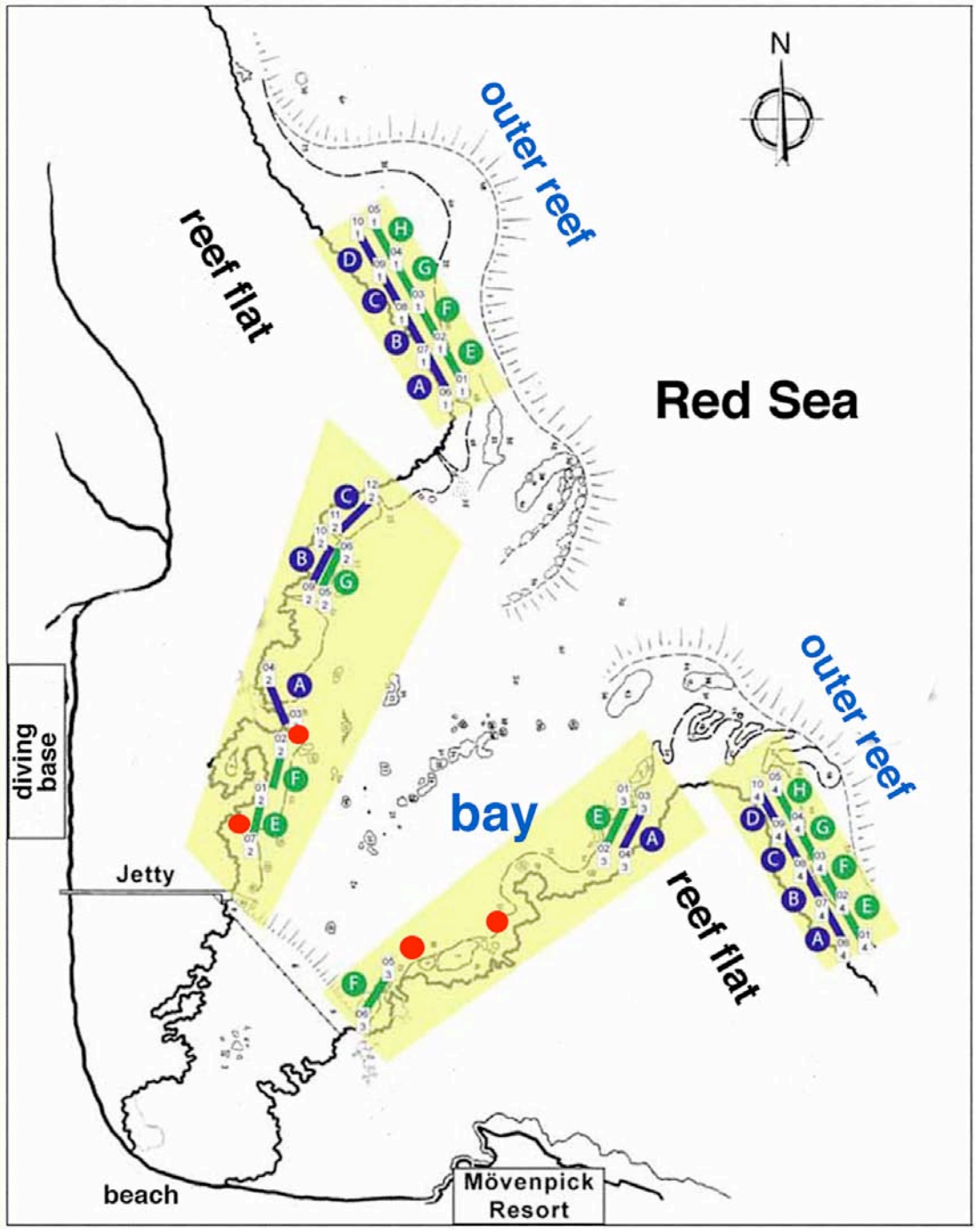


Fig. 15 The El Quadim bay, El Quseir, Egypt

The Fringing Reef (outer reef) is interrupted by the bay, which was a former harbor during the Roman time. The red points mark the area, where we have performed our fluorescence studies in a depth of 15 - 25 meters.

fig. modified after H. Heiss et al. (2005)

Fig. 16 Shown are 4 modules OSRAM SMTs, each with 4 LEDs (alltogether 16 LEDs). Also one ALU - reflector can be seen

Fig. 17 Explosion diagram of the fluorescent torch. The black body was a former HID housing of TillyTec - Modular Power Lightsystem

Fig. 18 HiTec Fluorescence with 3 blue LUMINUS LEDs

a) top view on the 3 high power LEDs

b) top view with the 3 mounted reflectors

c) explosion diagram. The black housing is from a former HID torch of TillyTec® - Modular Power Lightsystem

Perspectives

Fluorescence during night dives is a fascinating alternative technique to dives during day and night with normal light especially for ambitious divers and underwater photographers. The marine biologist can document the state of the reef, which gives information if the reef is still intact or if it is already endangered or totally destroyed (for example by fishing with dynamite or cyanide). Such reports are of general interest for the information of the general public, journalists and politicians to draw attention to this labile ecosystem and to promote approaches for sustainability.

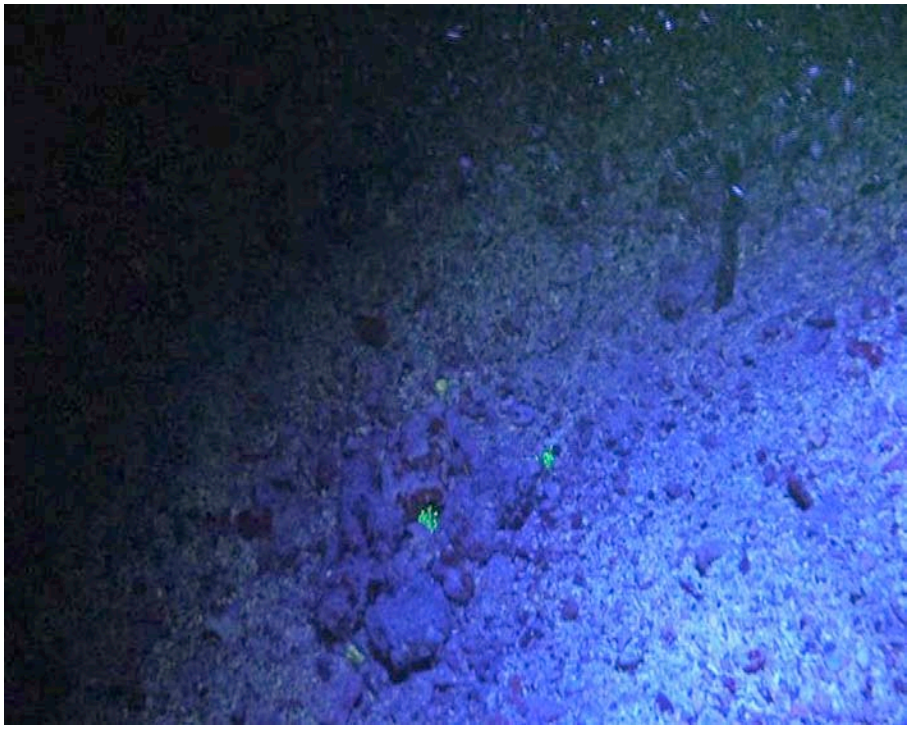


Fig.19 Illumination of an area of 4 x 5 meters with high power fluorescent light.

Tiny young coral polyps can be identified. With this large scale scanning method the successful settlement of newcomers can be documented during night dives. During daytime it is very hard or even impossible to identify the small primary coral colonies.

Acknowledgments

The results were received during night dives 2009 and 2011 in the ElQuadim bay , ElQuseir, Egypt.
We thank especially Johann Vifian, Director of the SUBEX Diving Centers (headquarter Hurghada) for his support. We gratefully acknowledge that he continously showed outstanding interest in our work and made available the diving and living infrastructure of the diving base in El Quseir. Furthermore we are indepted to Stefan Piesker, dive center manager, and his team for the logistic organization of day and night dives.

watch also the YouTube movies:

http://www.youtube.com/watch?v=ZGWcoM7Apyc
http://www.youtube.com/watch?v=nLMAyYHNeeQ
http://www.youtube.com/watch?v=O9GfctqCGKE
http://www.youtube.com/watch?v=COmbc4kLbwU

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For further informations visit my homepage http://www.uni-due.de/zoophysiologie/
Do not hesitate to contact me for further discussions: horst.grunz et uni-due.de

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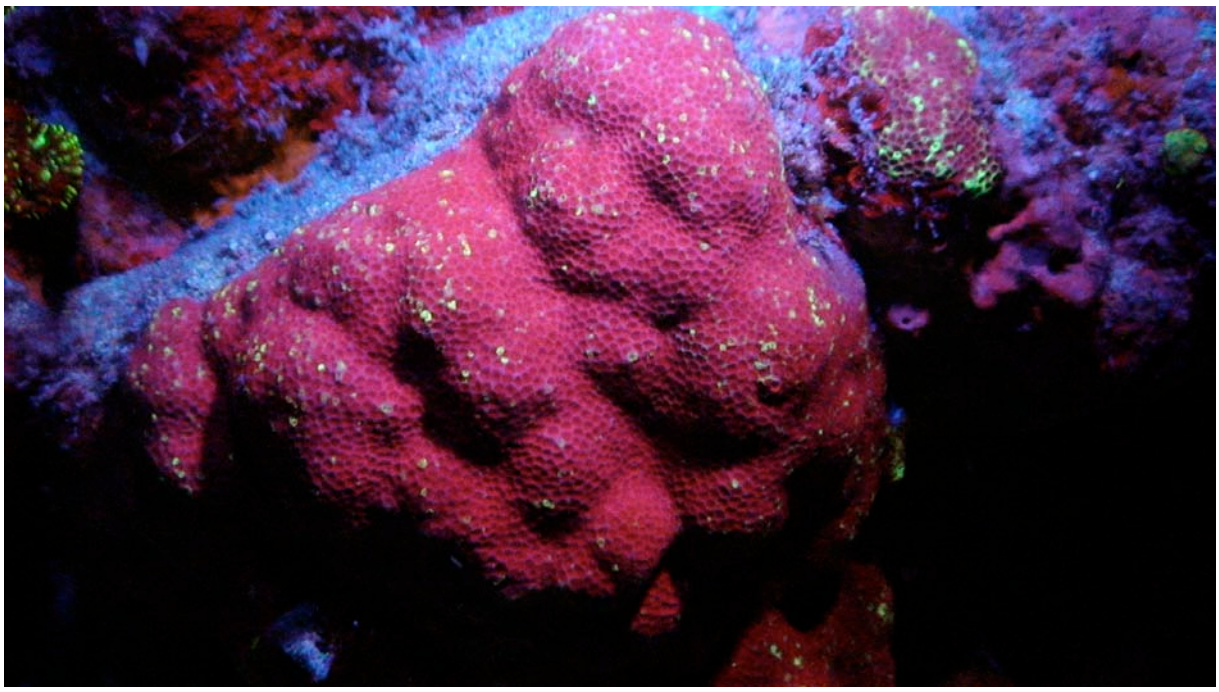
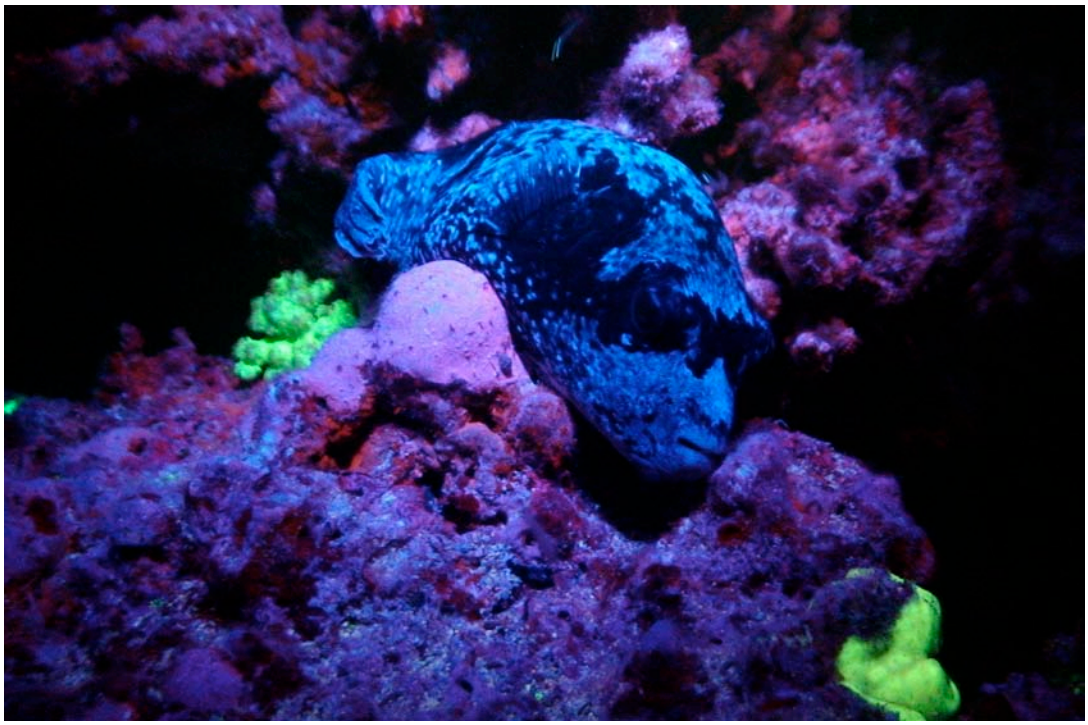
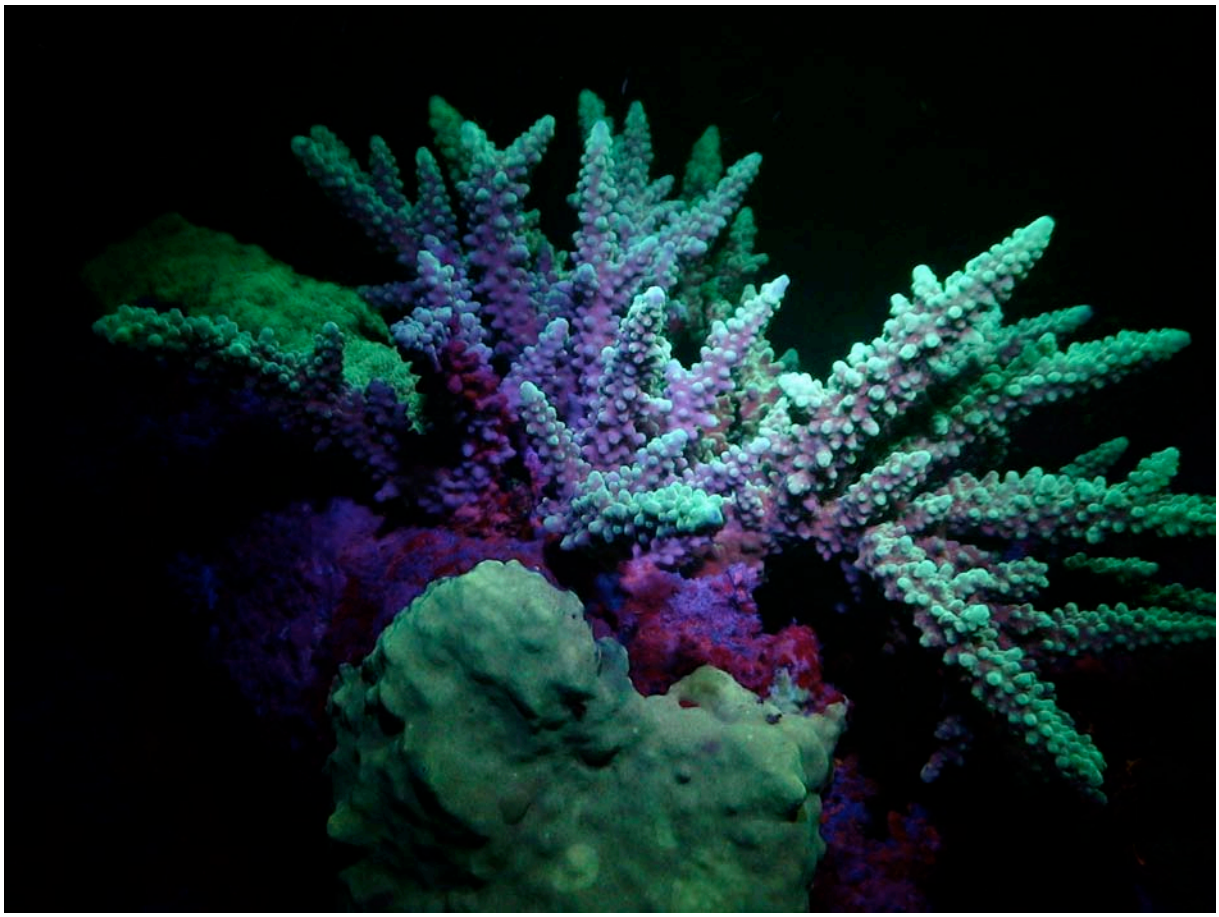
Appendix

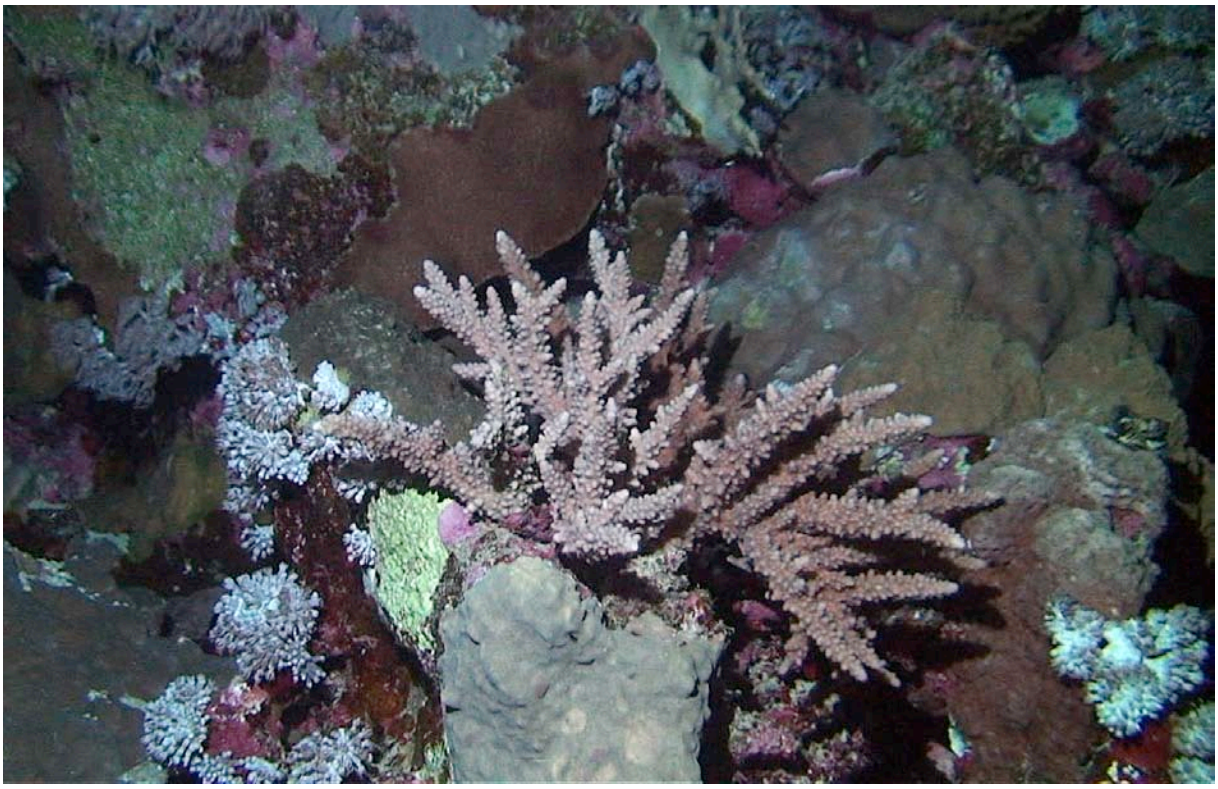
The tables show the comparison of similar or even identical areas illuminated with normal light and with fluorescent source.
It should be pointed out that our HiTec Fluorescent Torches are able to illuminate a very large area - sometimes 7 x 5 meters. This holds true also for our torch with HiTec white LEDs. This is important when large scale scanning during reef check projects will be performed during night dives.
see also my Youtube movie http://www.youtube.com/watch?v=ZGWcoM7Apyc



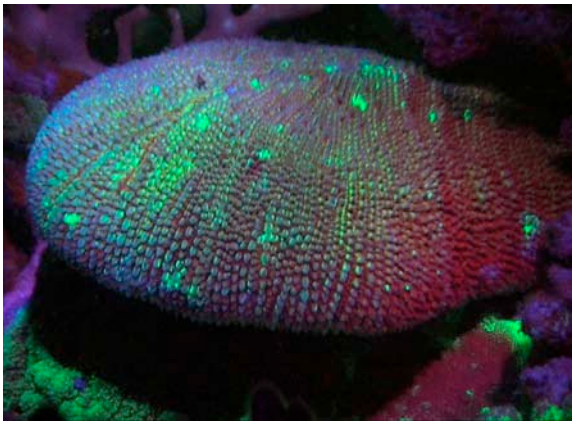


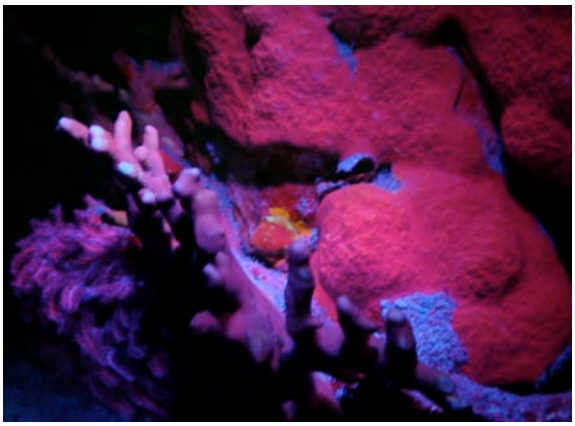
Sleeping puffer fish

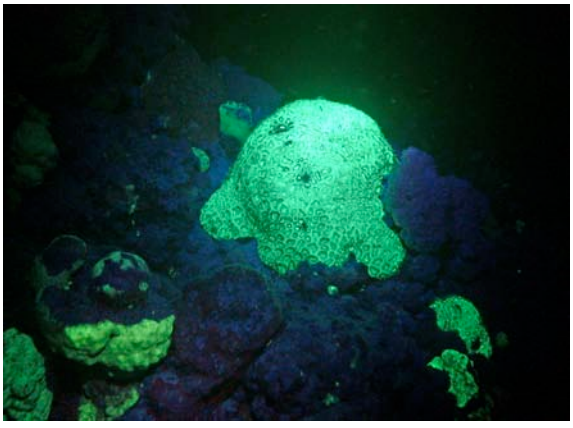


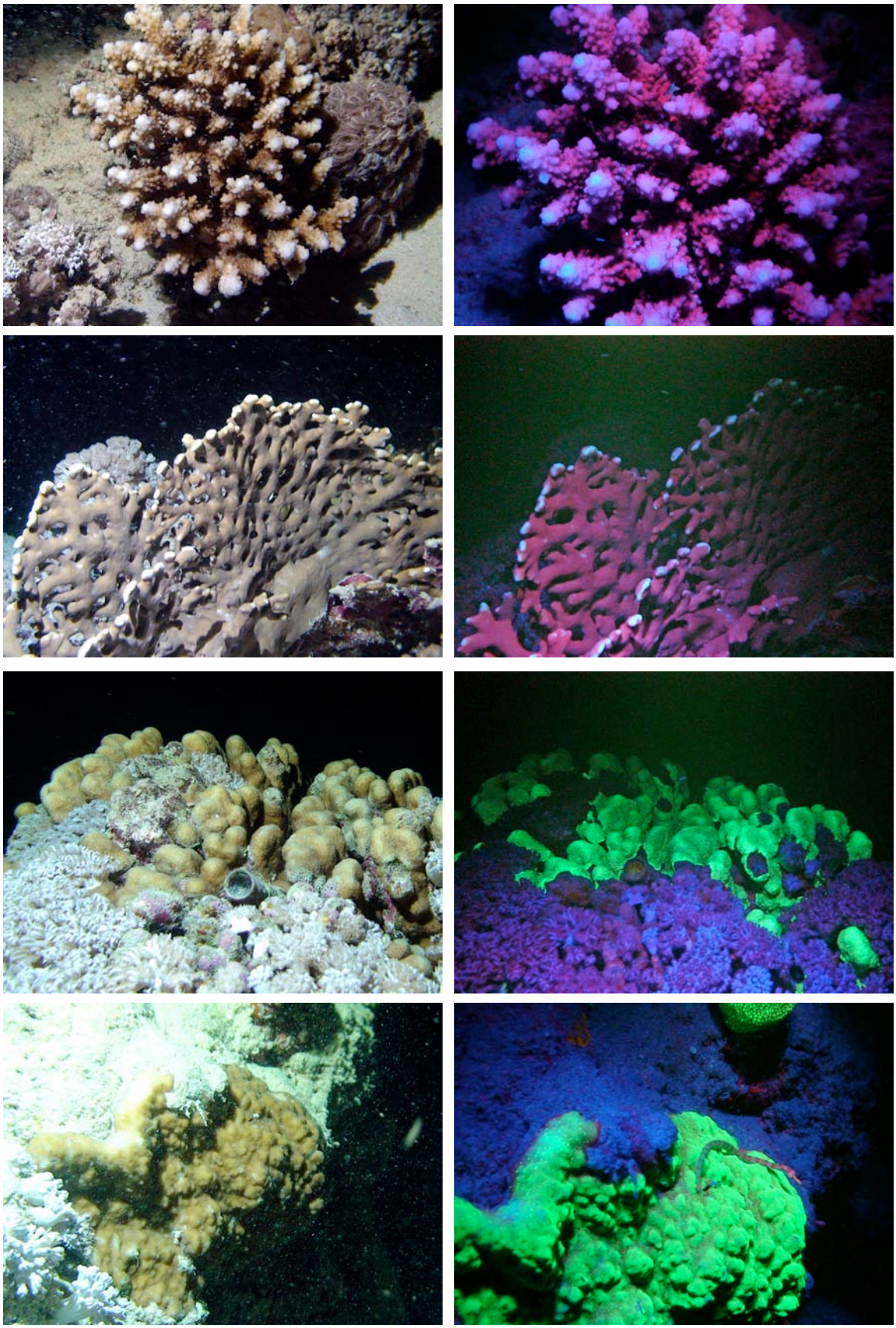


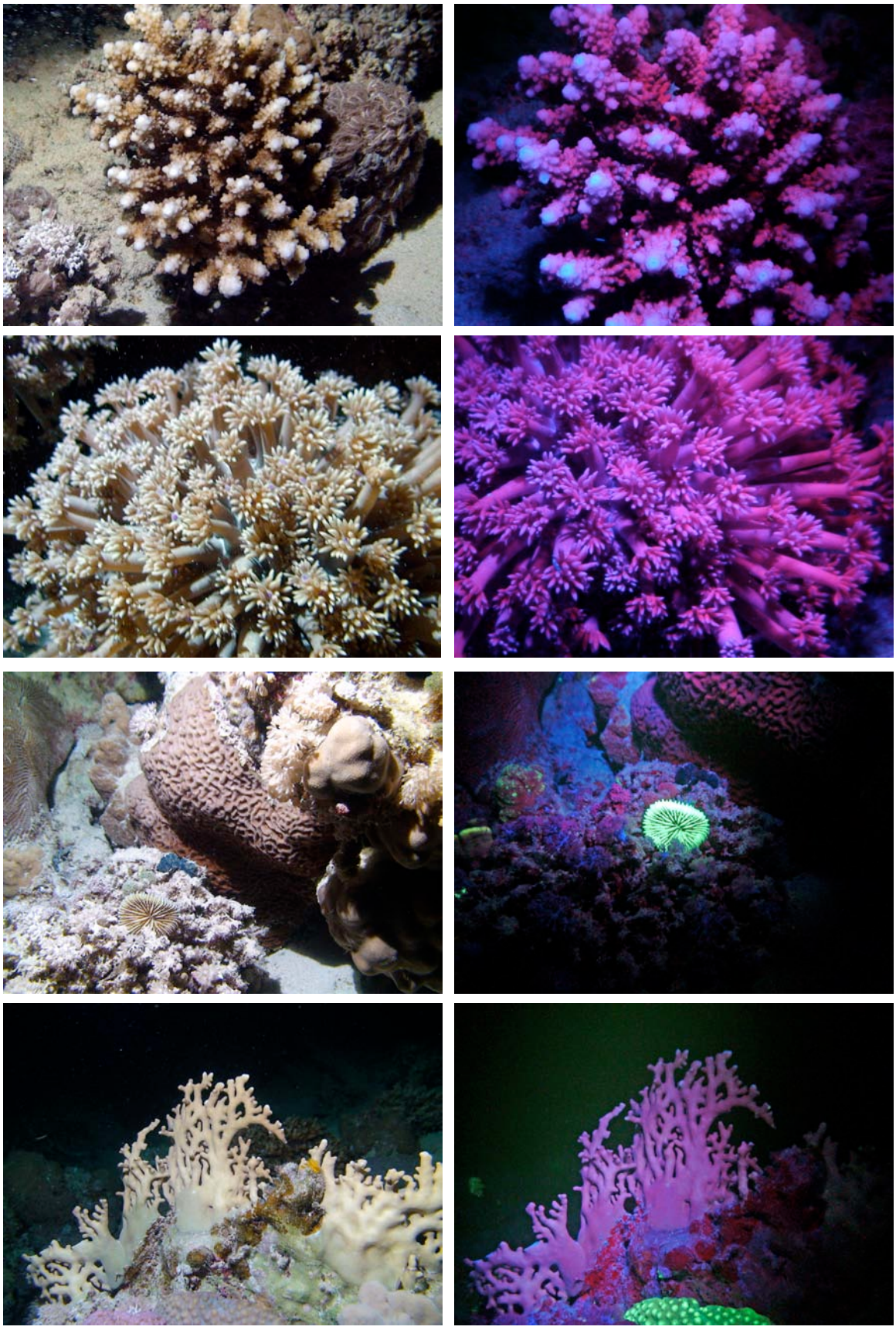


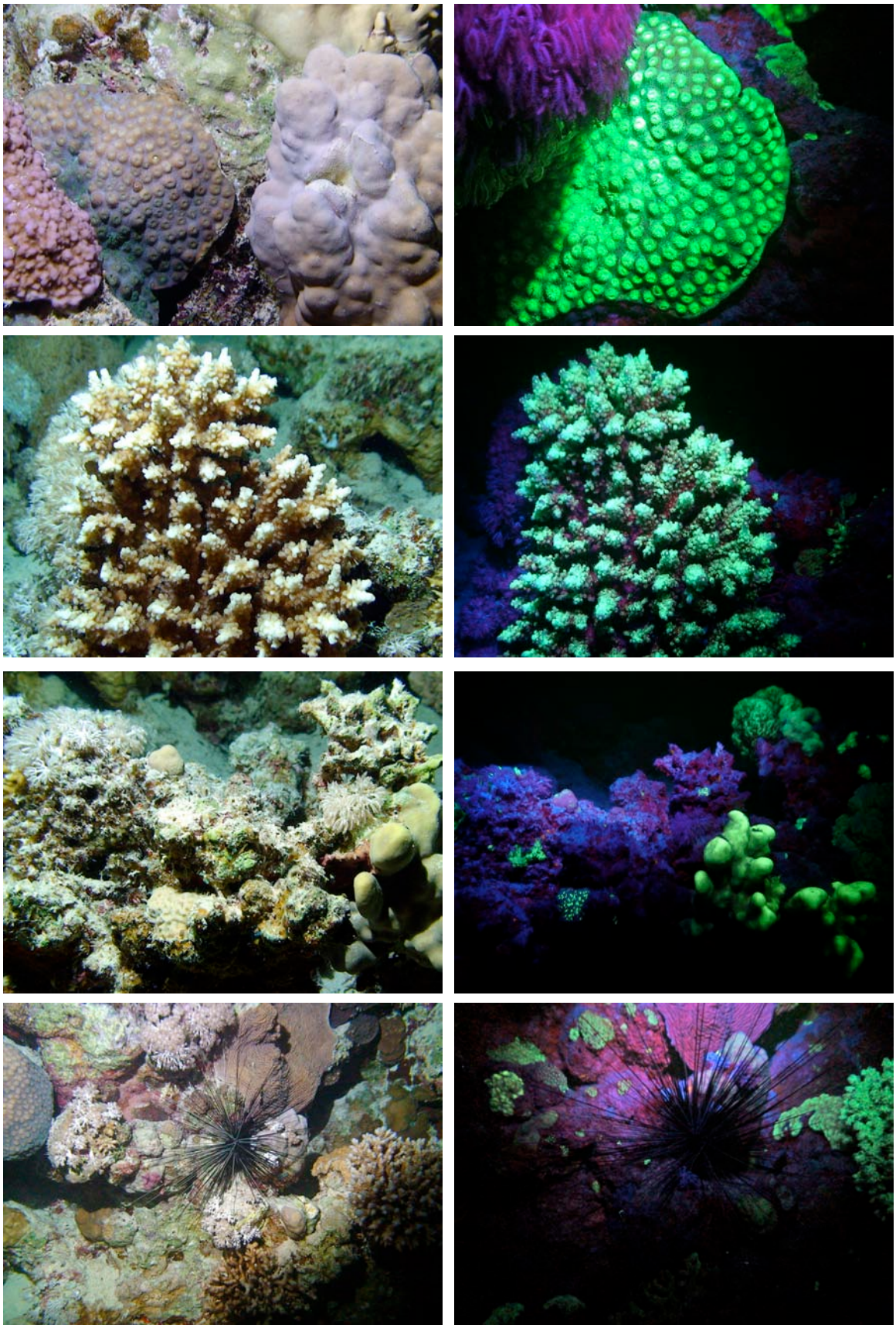





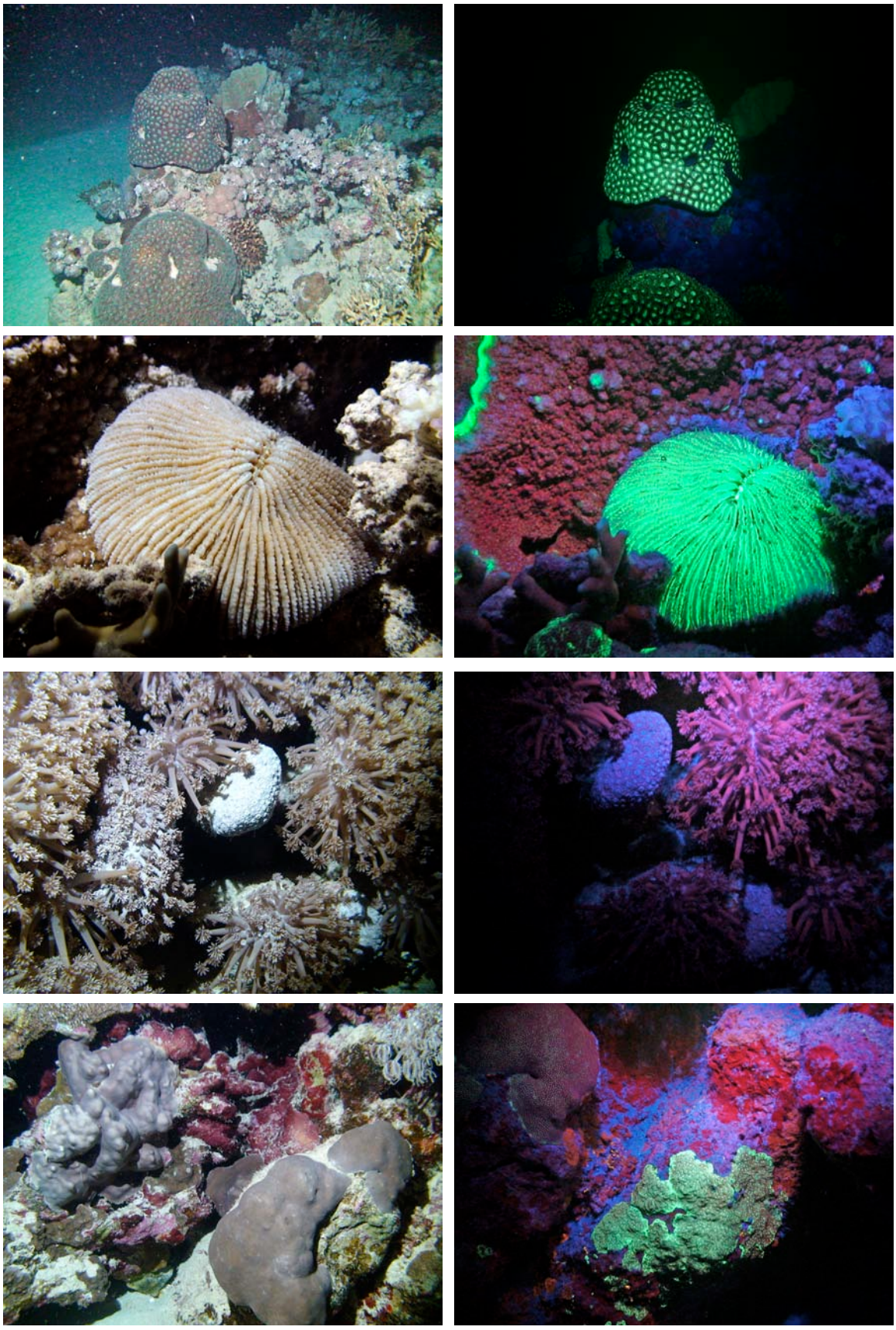




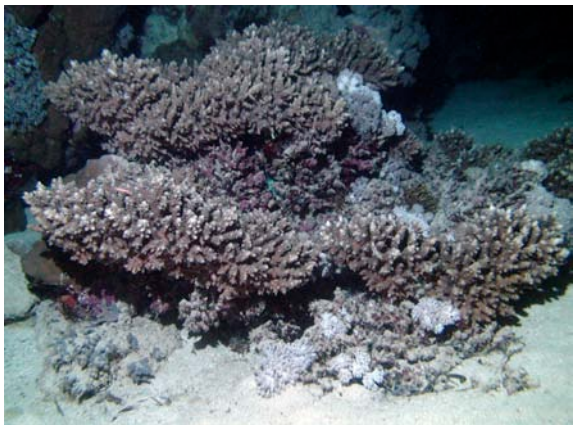
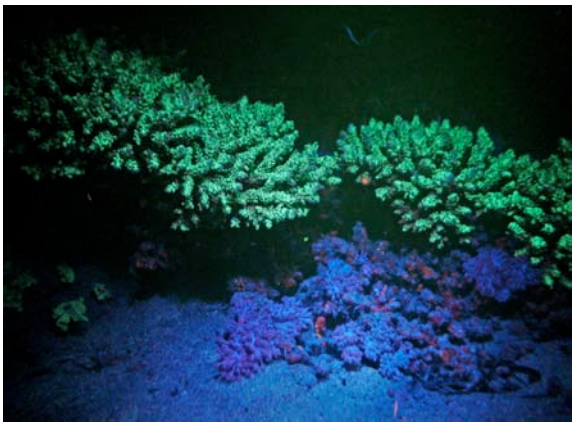


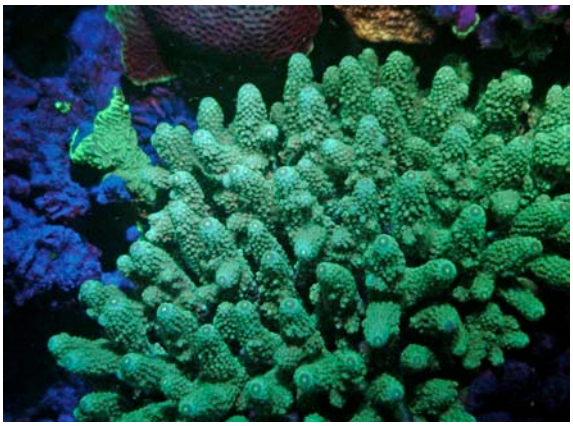


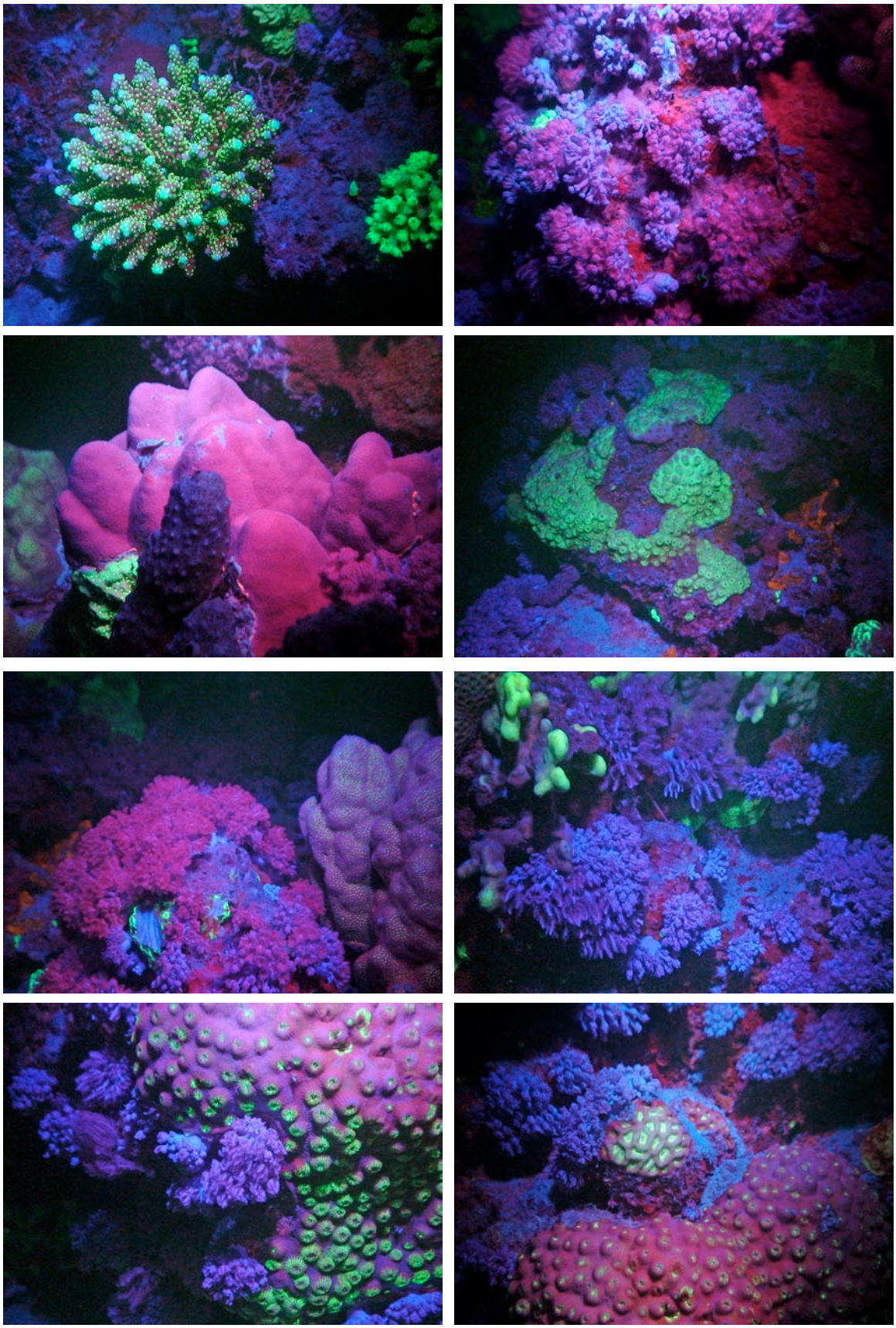
Lower row: The sea urchin shows now fluorescence

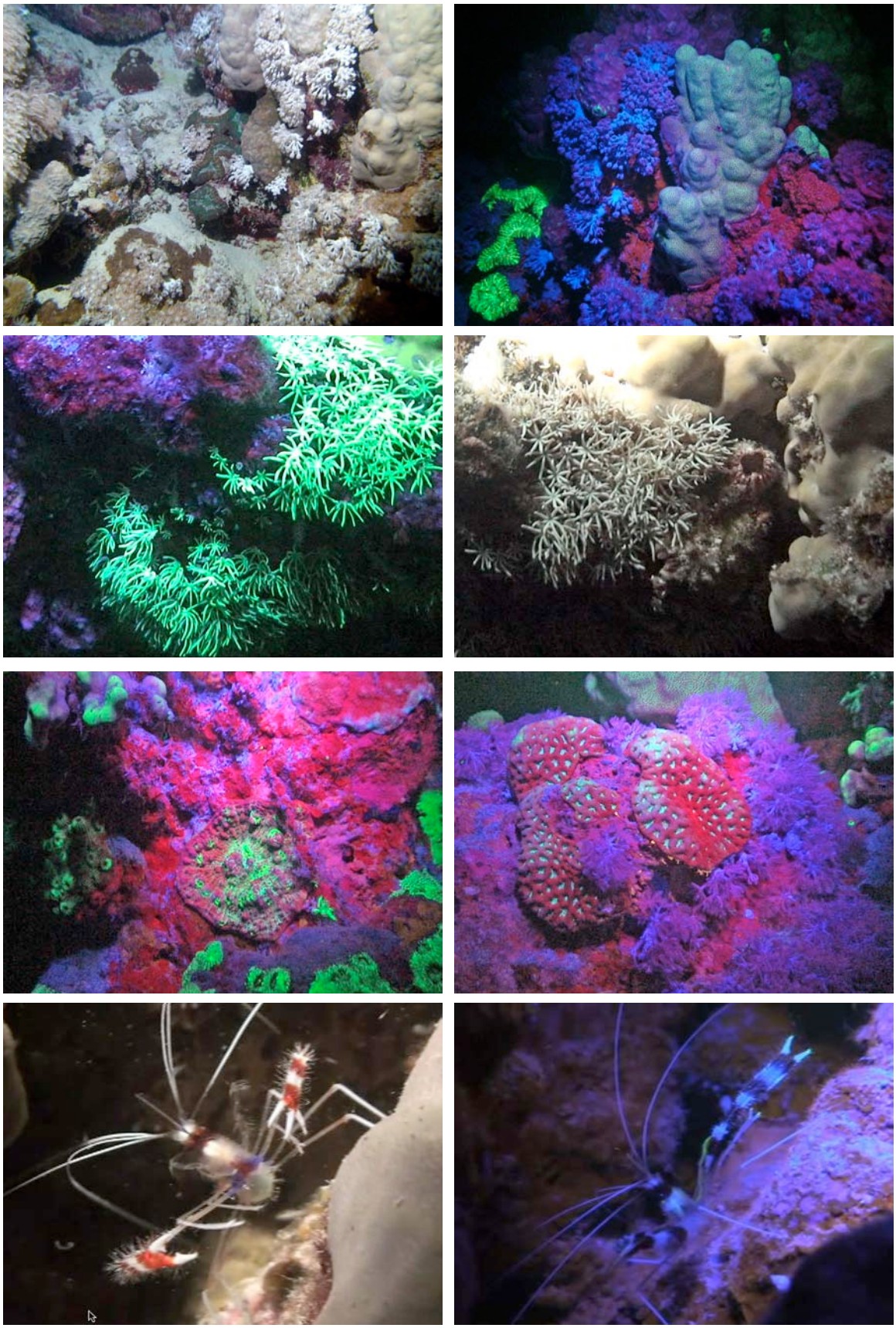




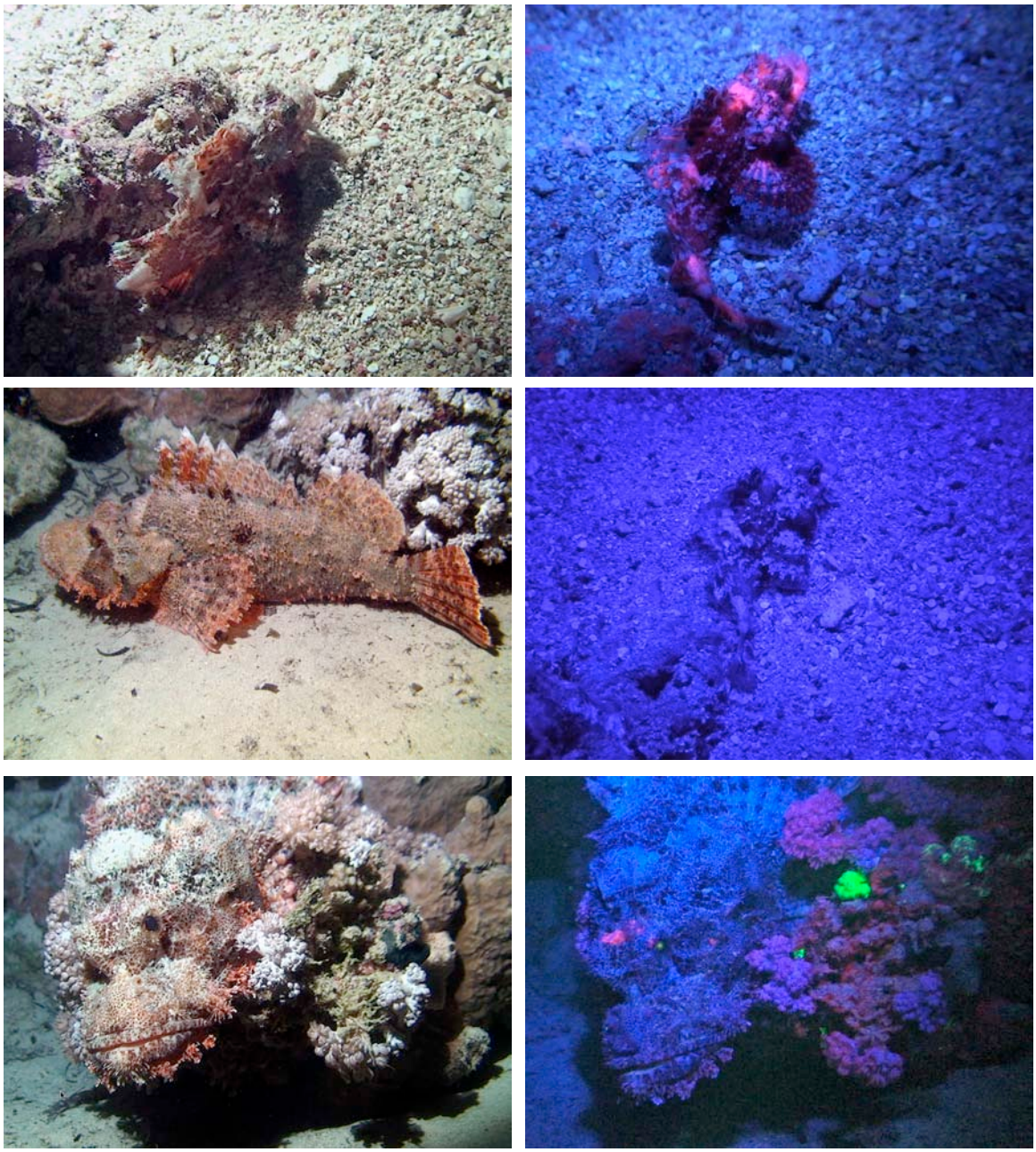









Banded Shrimp see also the YouTube movie
http://www.youtube.com/watch?v=nLMAyYHNeeQ



Scorpion fish

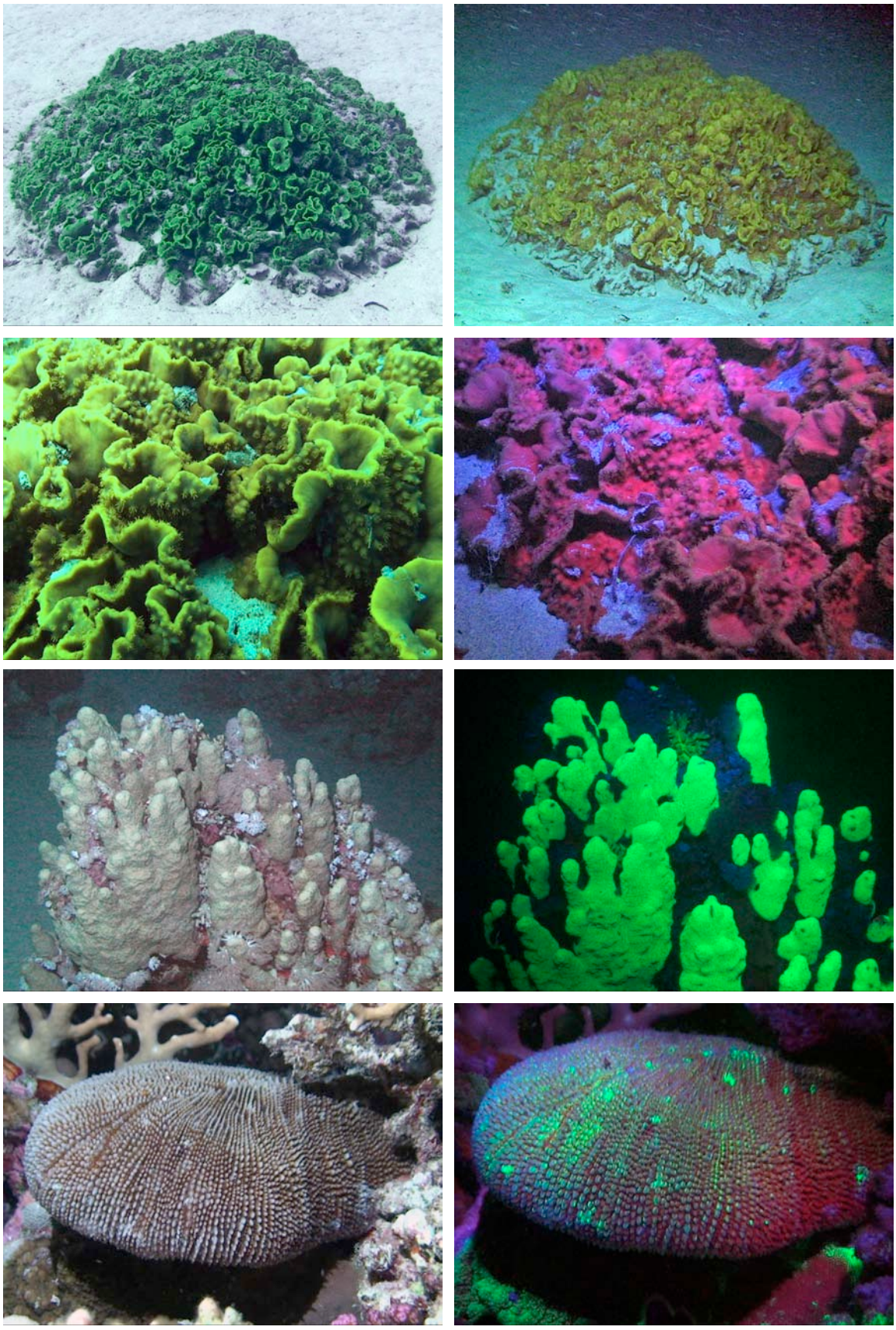
Upper row: young specimen; normal light left; right: fluorescence
middle row: left adult animal; right: young specimen in blue light without barrier filter
lower row: adult animal with and without fluorescence; in contrast to the youngster the adult shows two pink spots on his head only

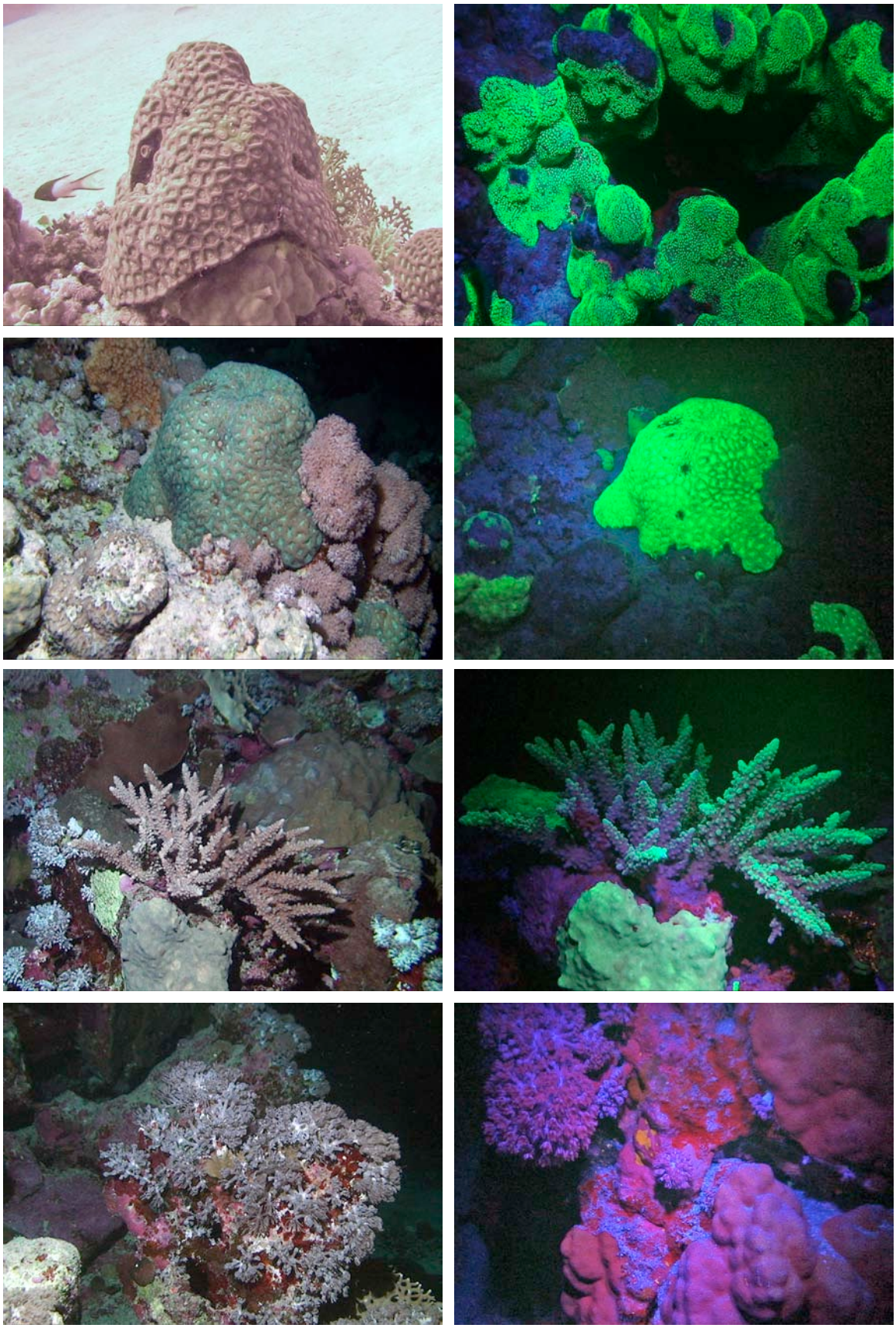
see also my YouTube movie http://www.youtube.com/watch?

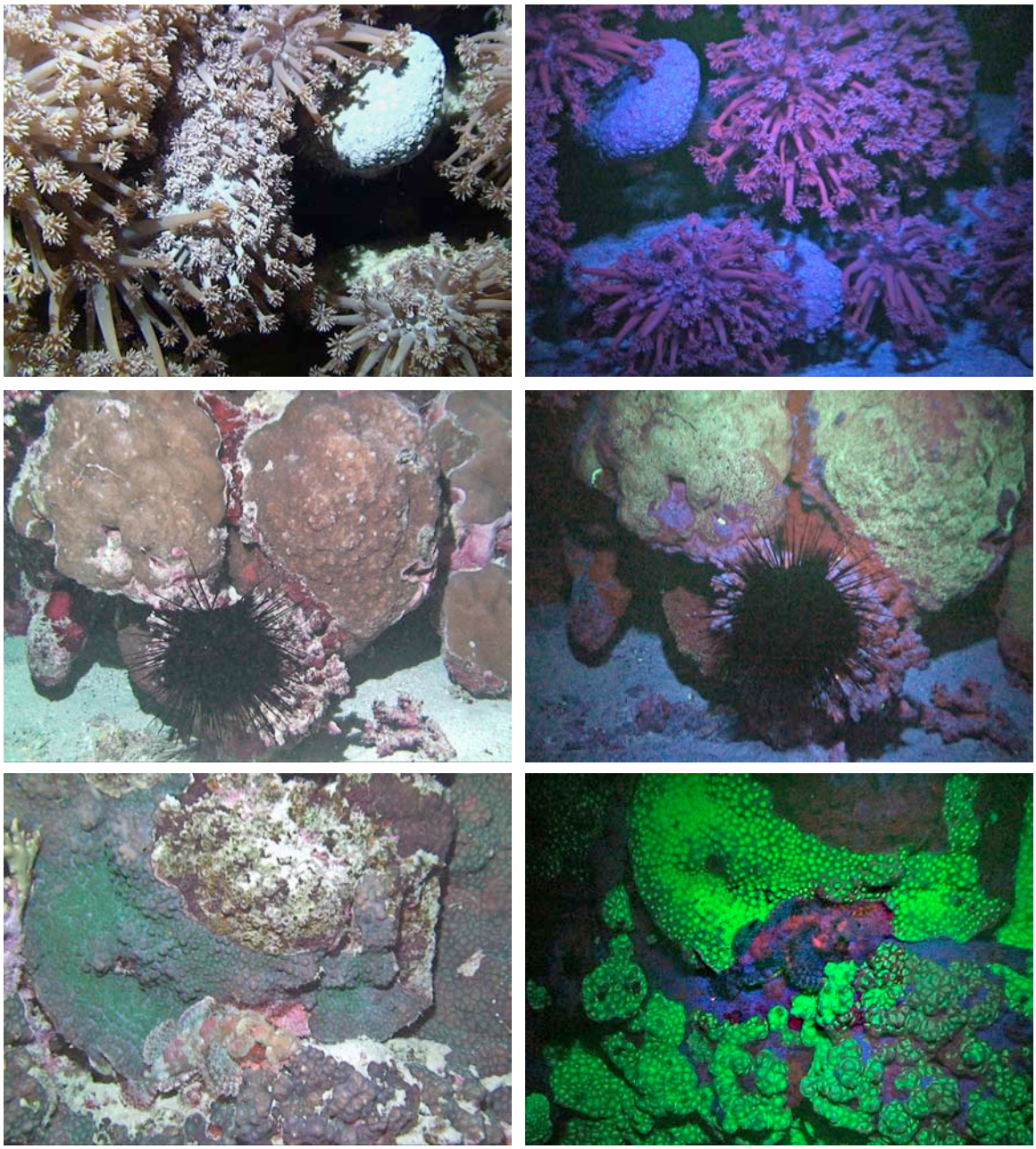
Lionfish

upper row left: specimen at daytime
right column shows different animals during night dives with fluorescence; significant are the light blue spots on the fins and in the zone of the side line, the special sensory organ of fishes

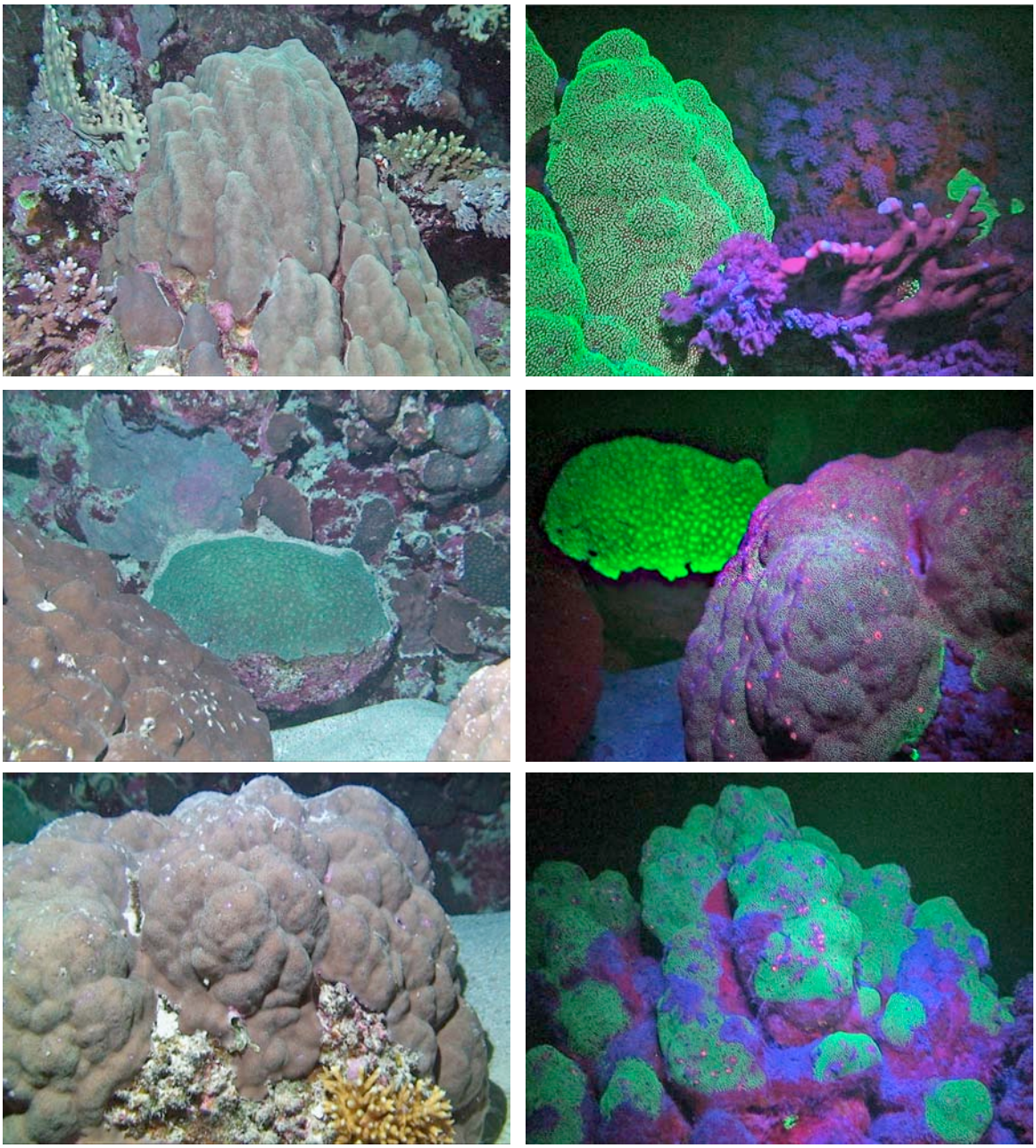
see also the you tube movies
http://www.youtube.com/watch?v=O9GfctqCGKE
http://www.youtube.com/watch?v=nLMAyYHNeeQ





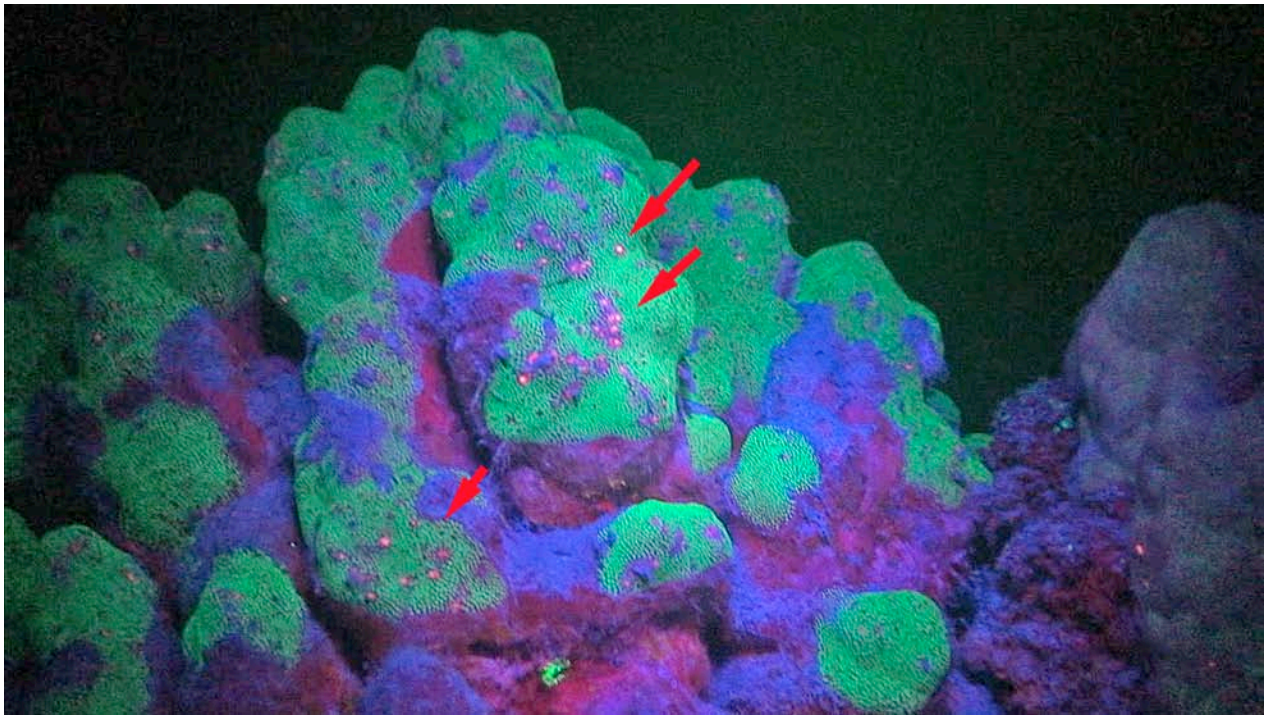


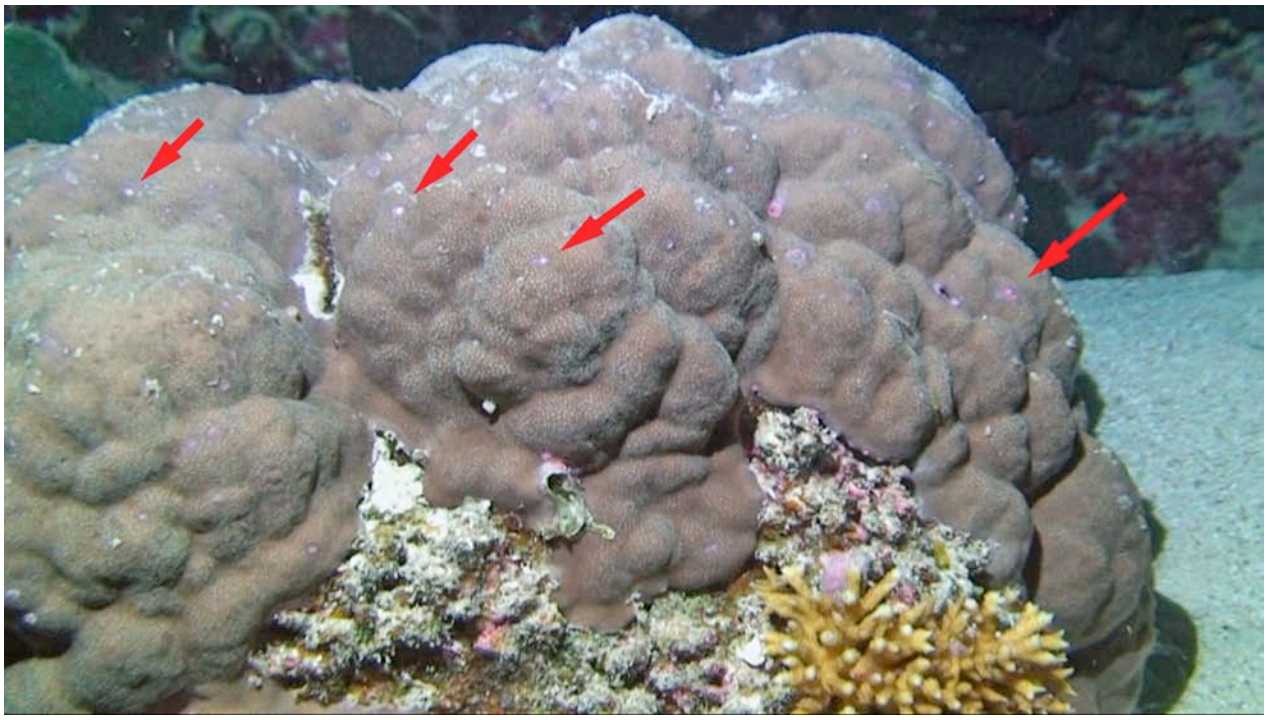
Lower row: a scorpionfish can easily be detected with the fluorescence technique, because it can be discriminated from its green fluorescent surrounding.
middle row: the sea urching shows no fluorescence



In the middle and lower row the activity of parrot fishes are shown.
It is much easier to detect the effect with fluorescence as small pink spots. You can see this much better in the next table.

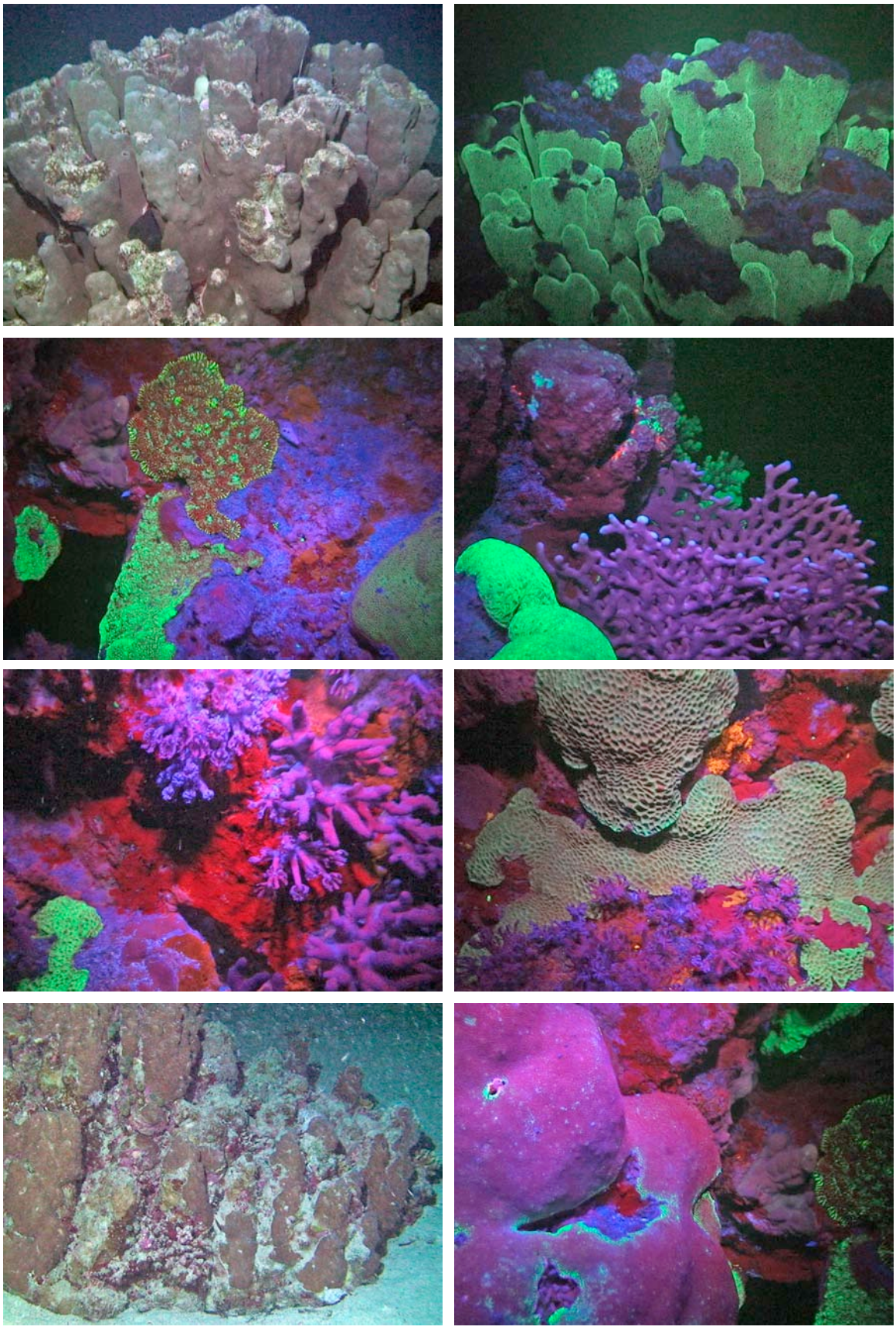
see also my YouTube mpvie about a parrot fish in action >
http://www.youtube.com/watch?v=COmbc4kLbwU



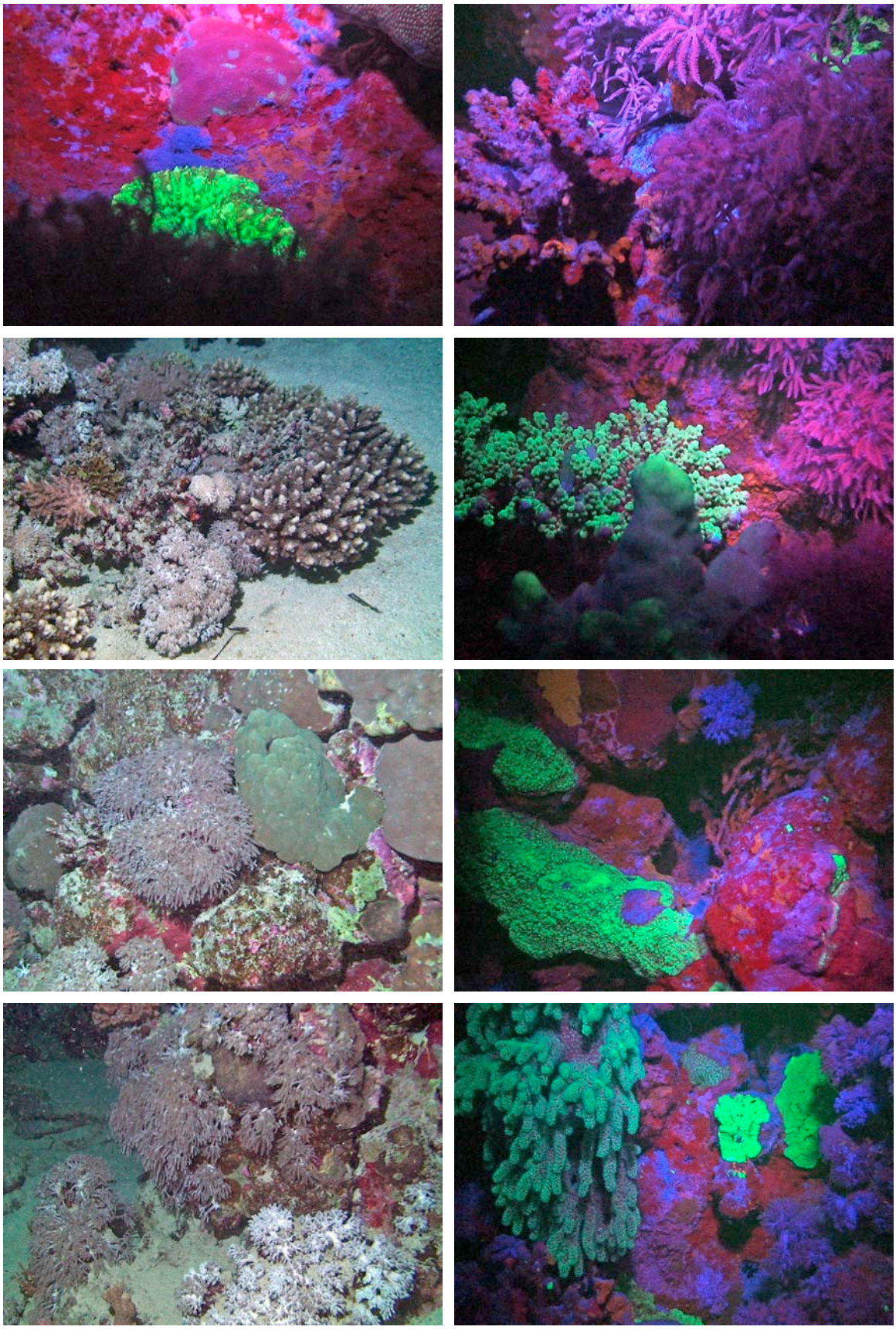


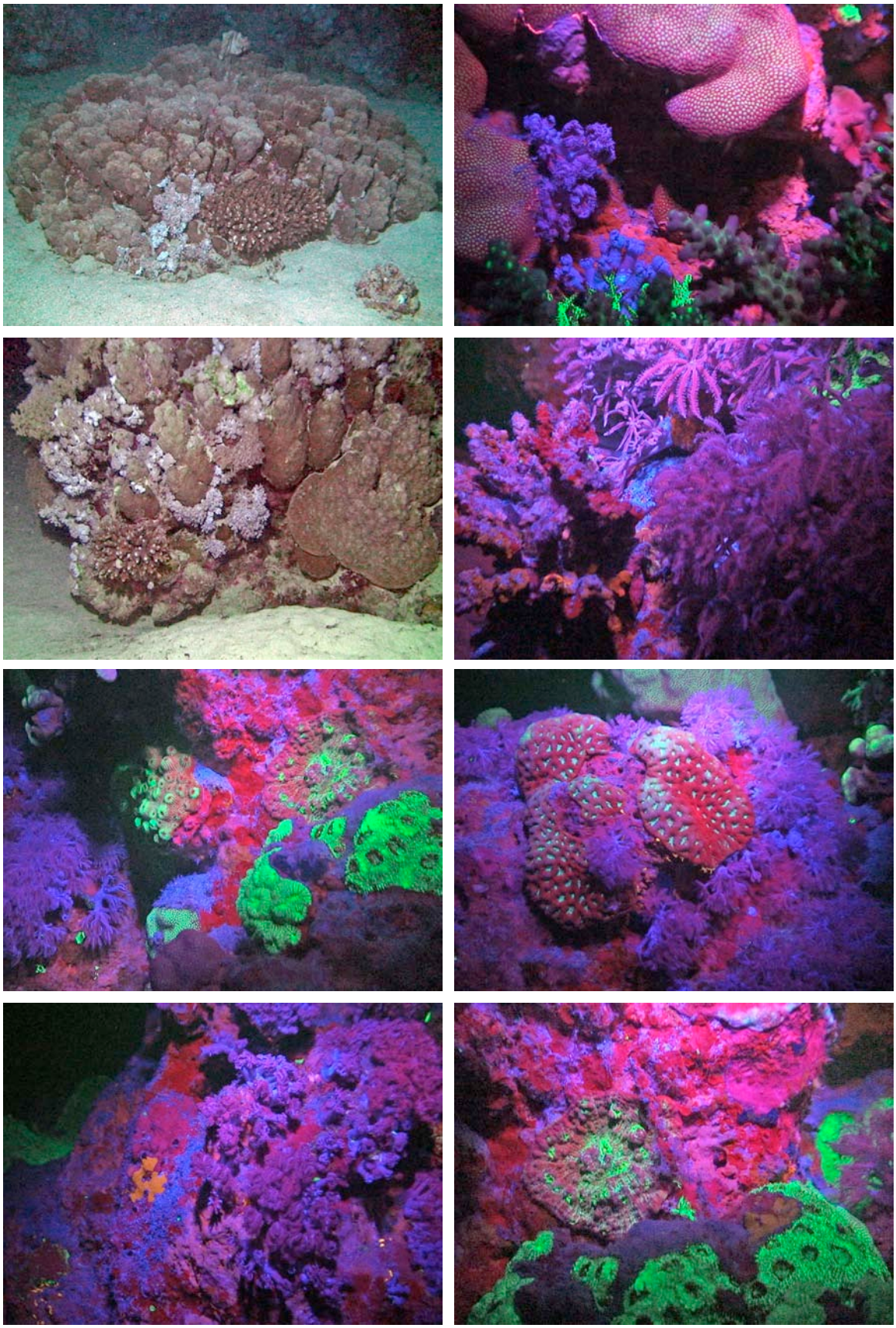
Bites of parrot fishes.
The effect can be detected much easier during night dives with fluorescence (red arrows)

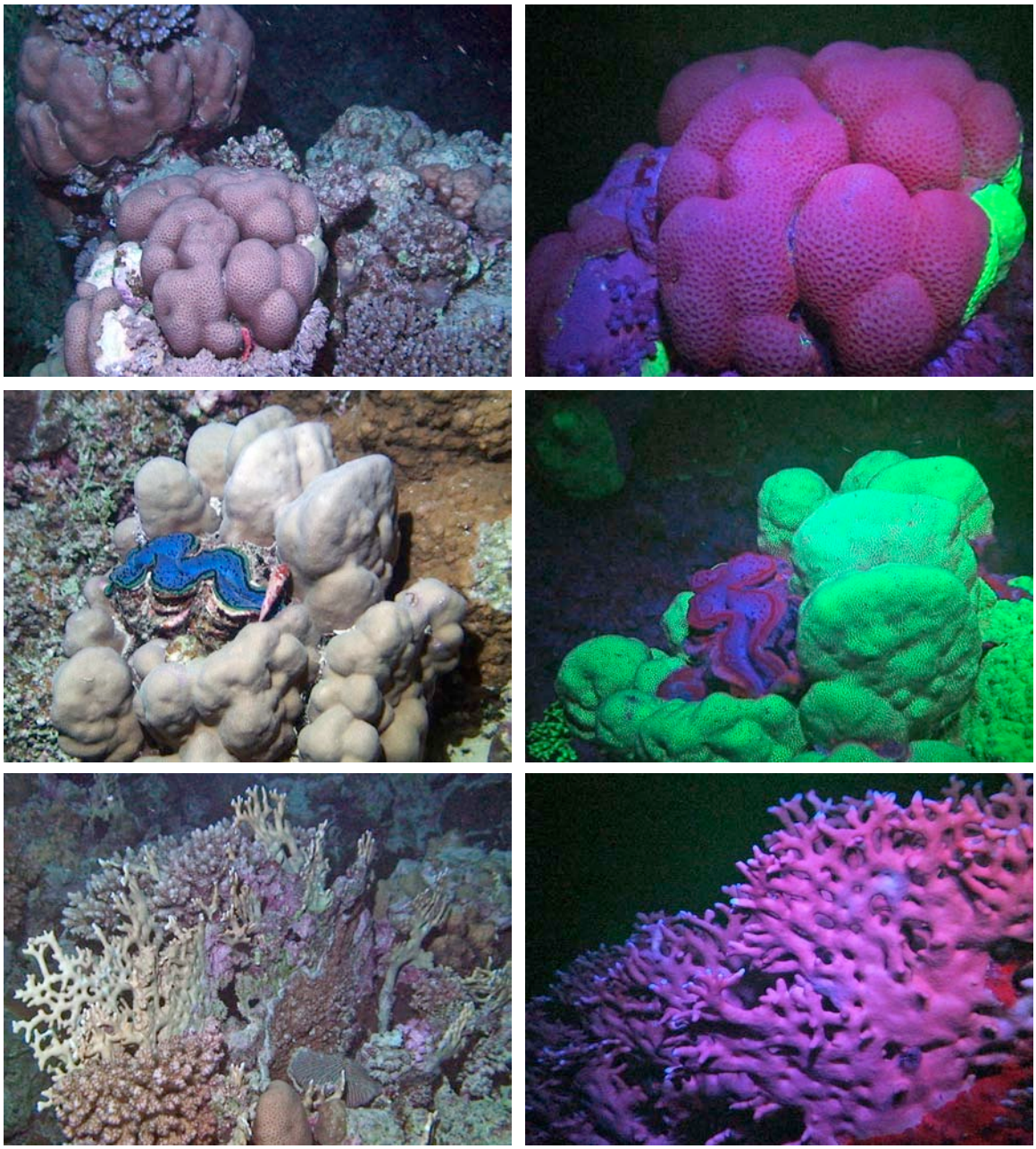
see also my YouTube mpvie about a parrot fish in action >
http://www.youtube.com/watch?v=COmbc4kLbwU



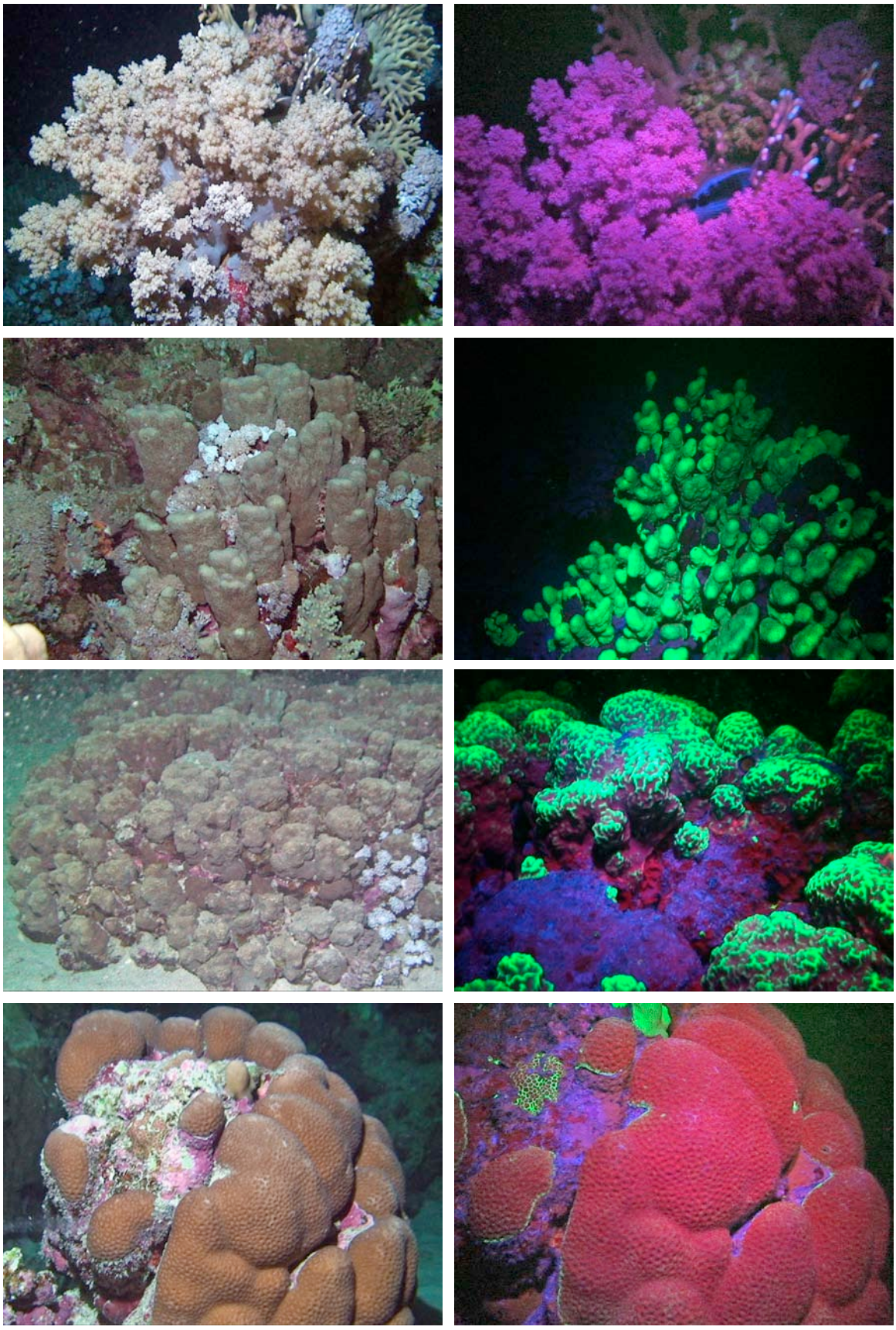
Horst Grunz Magic World of coral reef with HiTec Fluorescence







middle row: **giant clam**
with normal white light and blue light source



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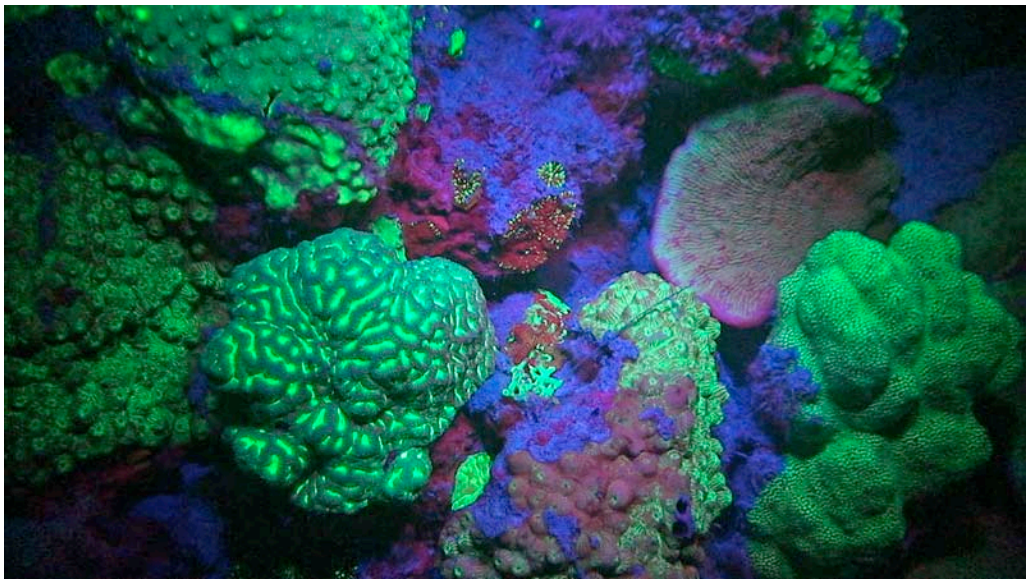
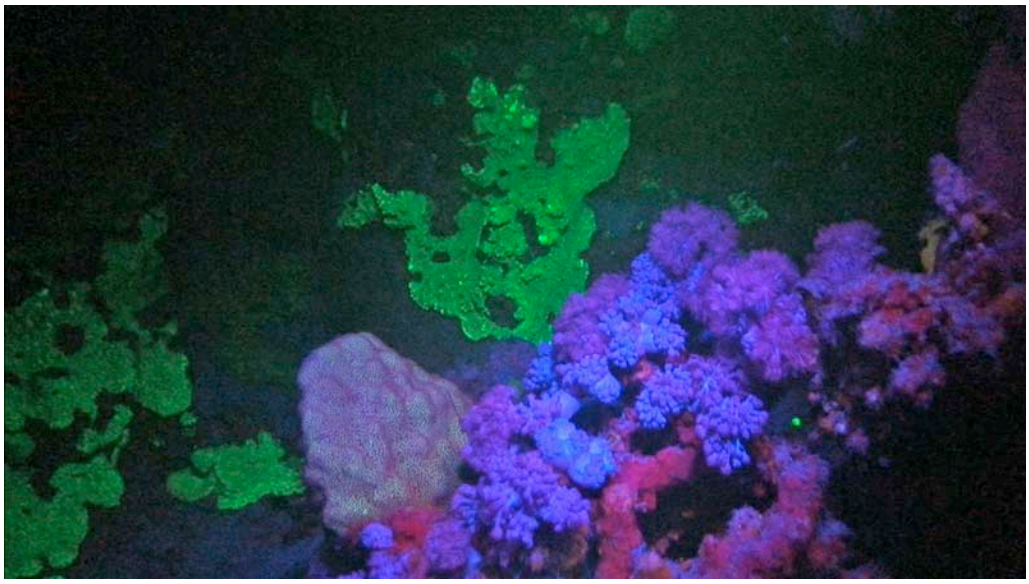
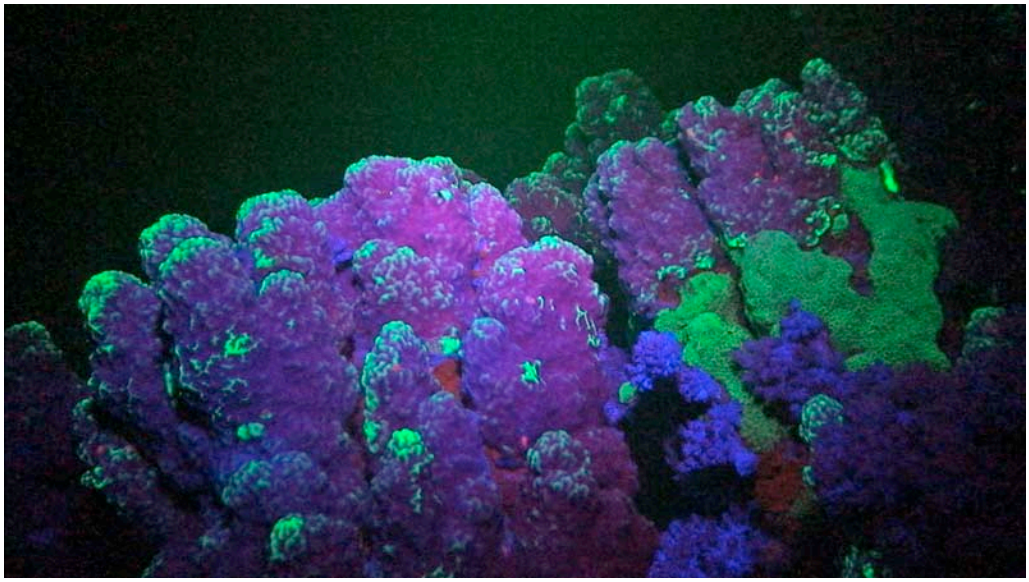
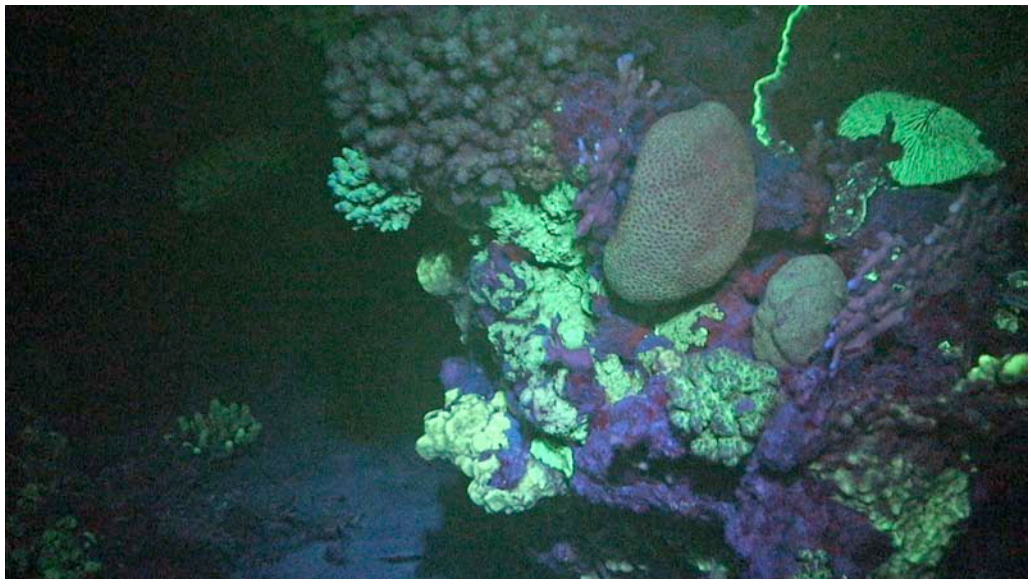
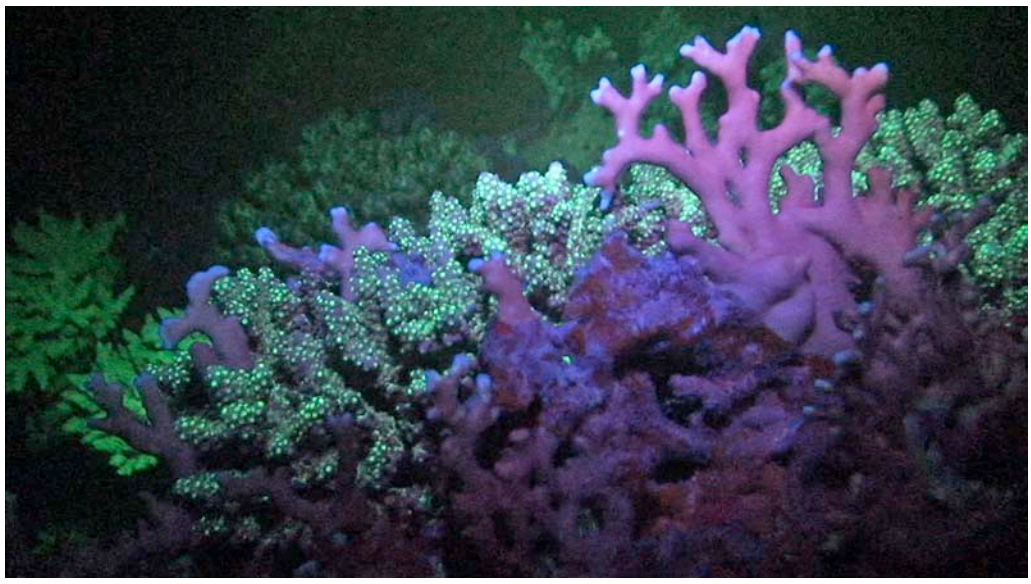




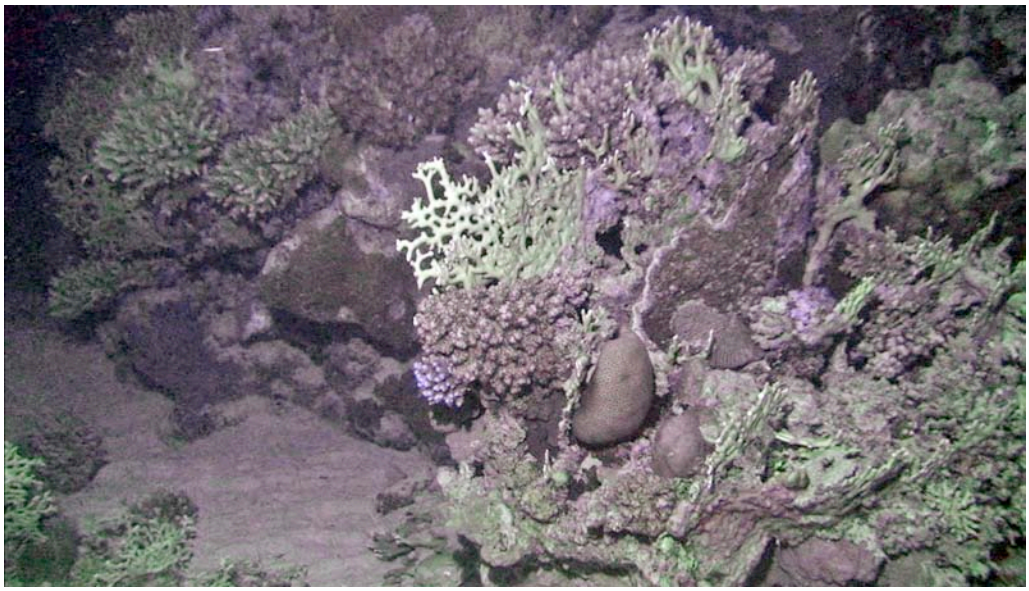


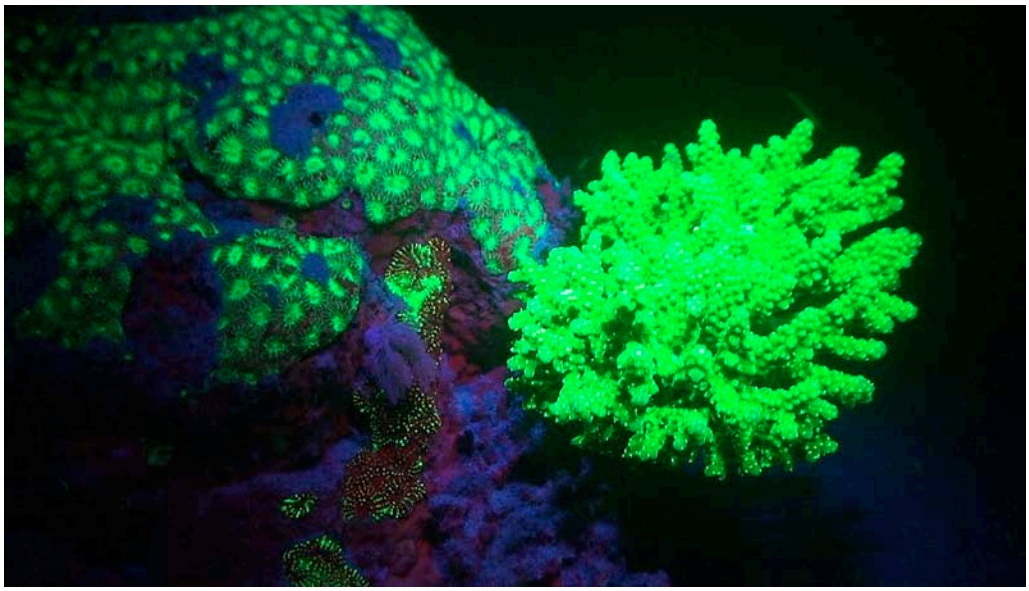


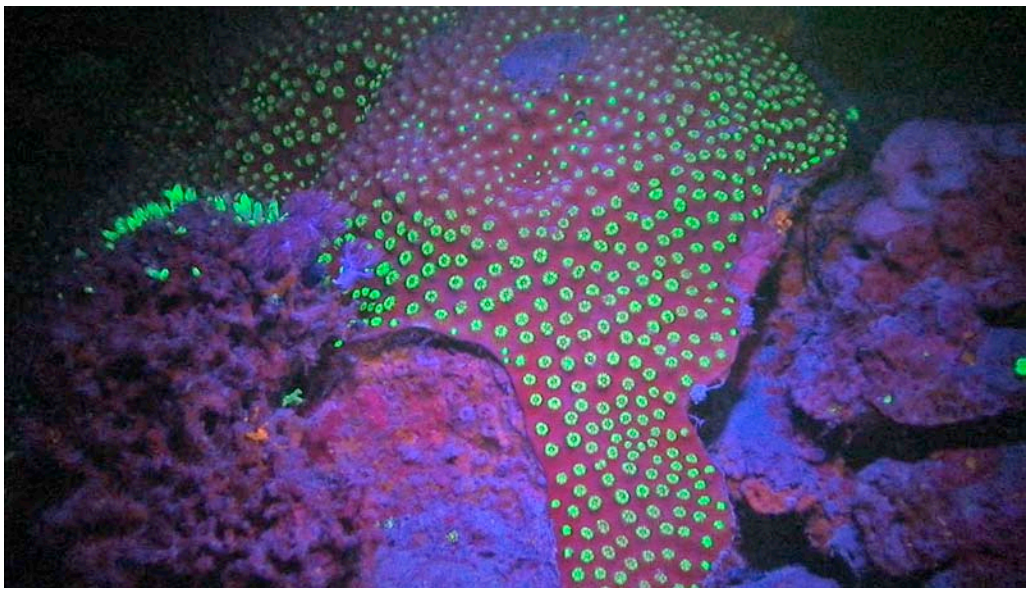


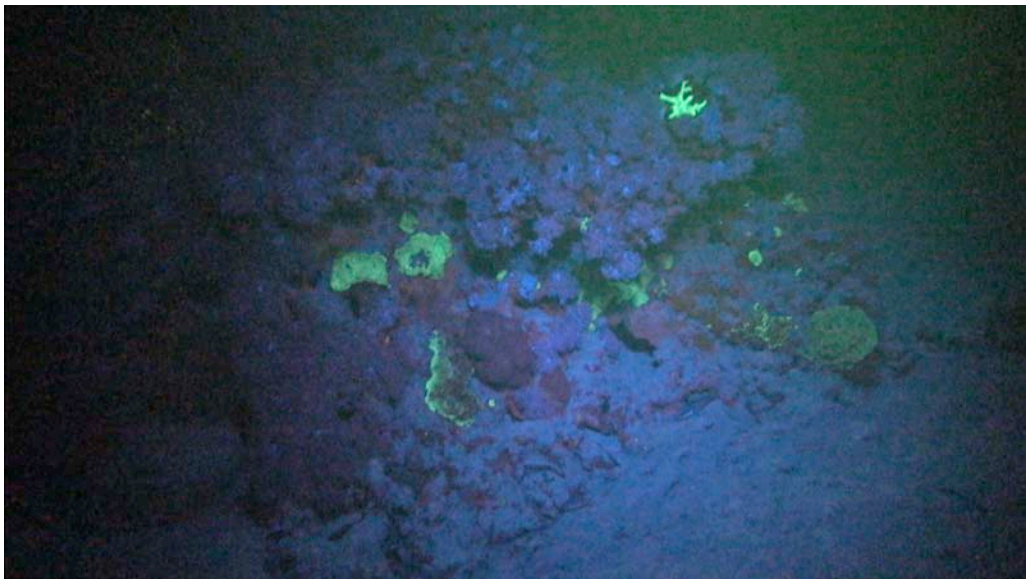
upper row: Documentation of the effect of our HiTec torch with one single LUMINUS LED (2250 lumen),which allows an illumination of 5 x 7 meter.

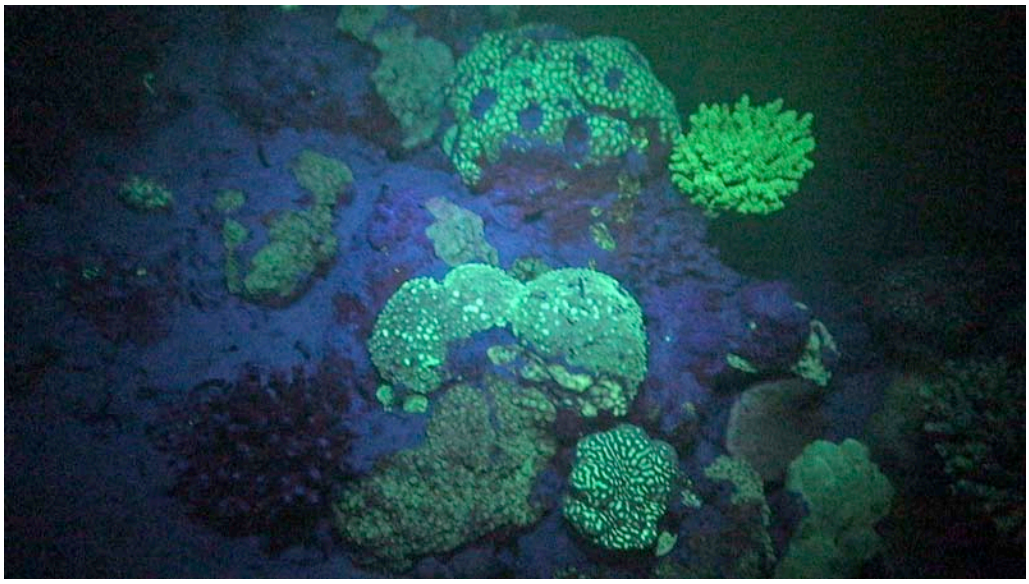
Horst Grunz Magic World of coral reef with HiTec Fluorescence

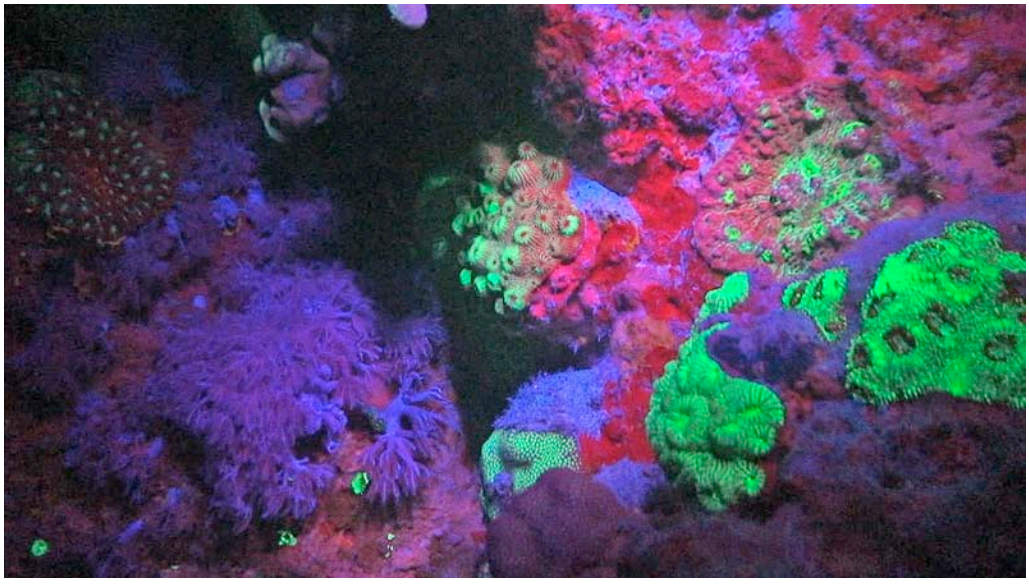






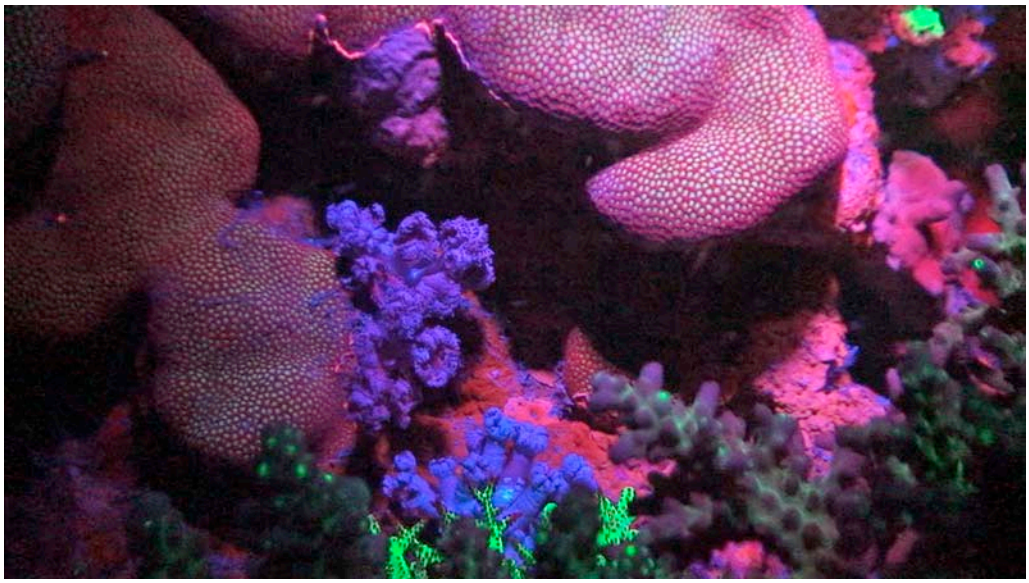


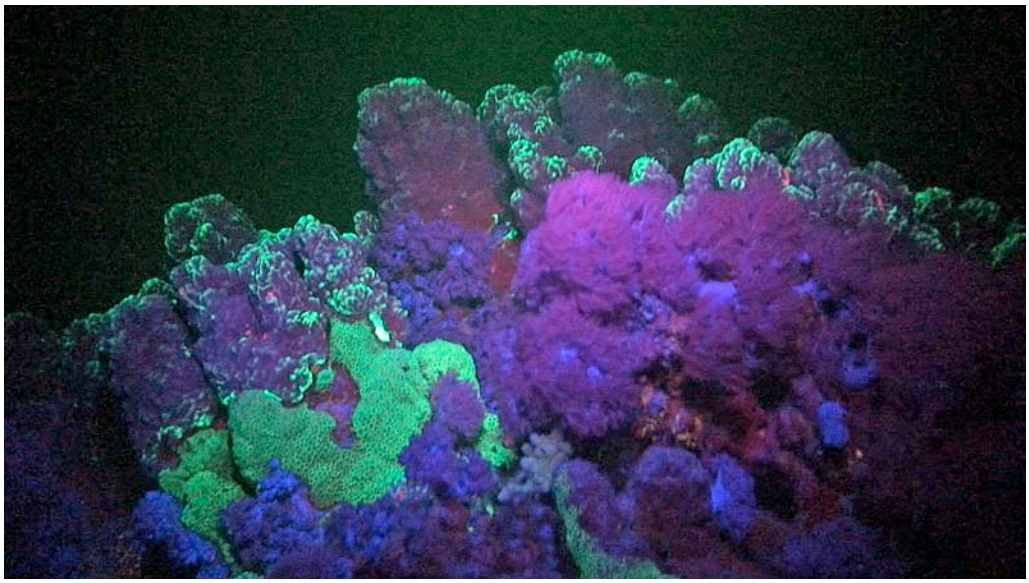


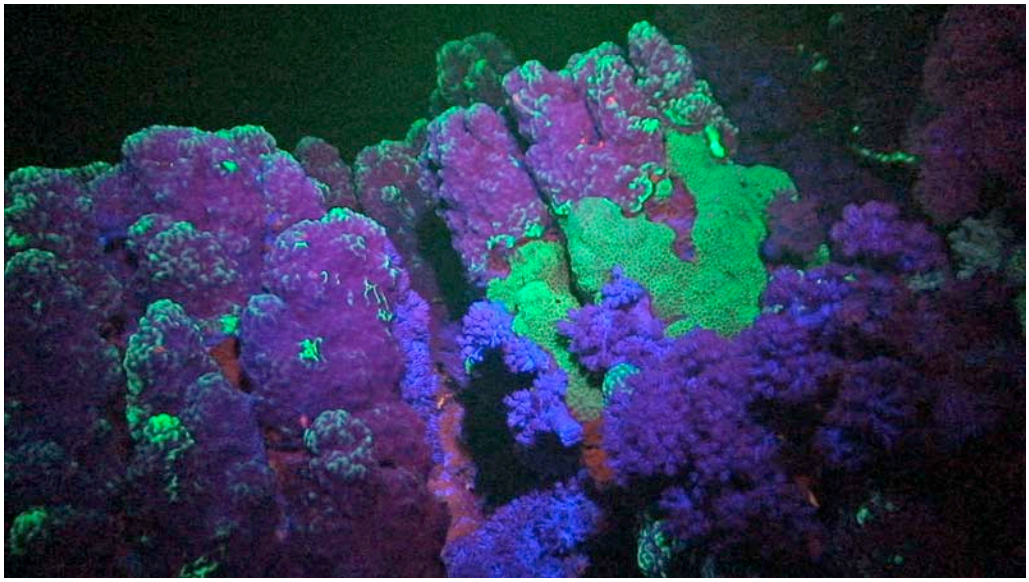


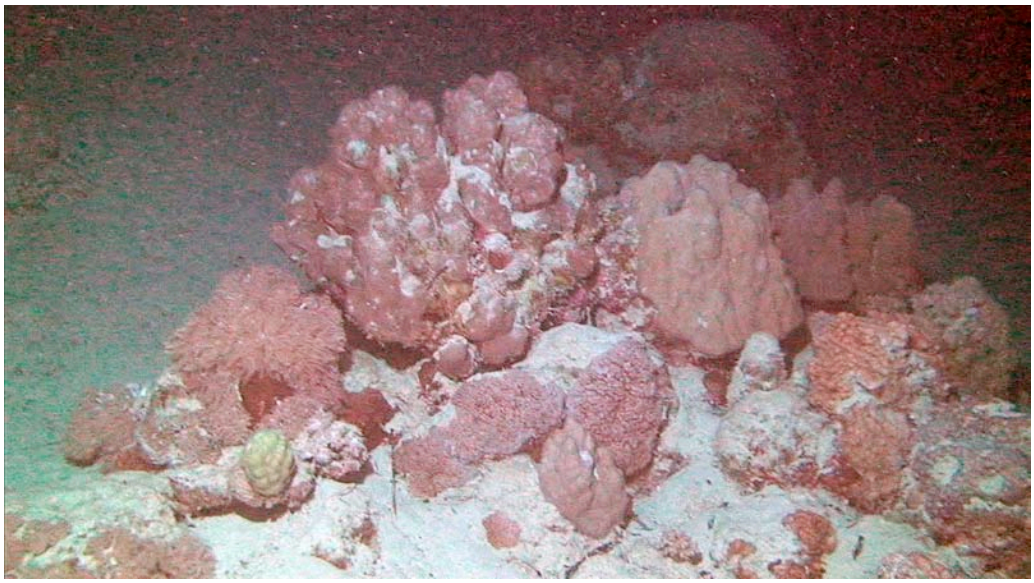
Documentation of the effect of our HiTec fluorescence torch, which allows an illumination of 5 x 7 meter. Especially corals with yellow/ green fluorescence can be detected in a big distance

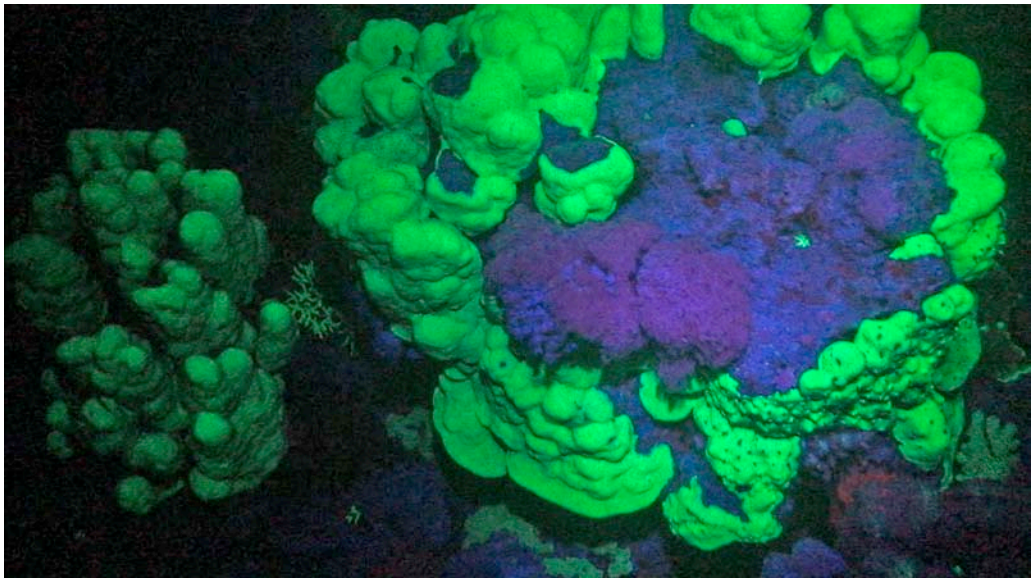
Horst Grunz Magic World of coral reef with HiTec Fluorescence











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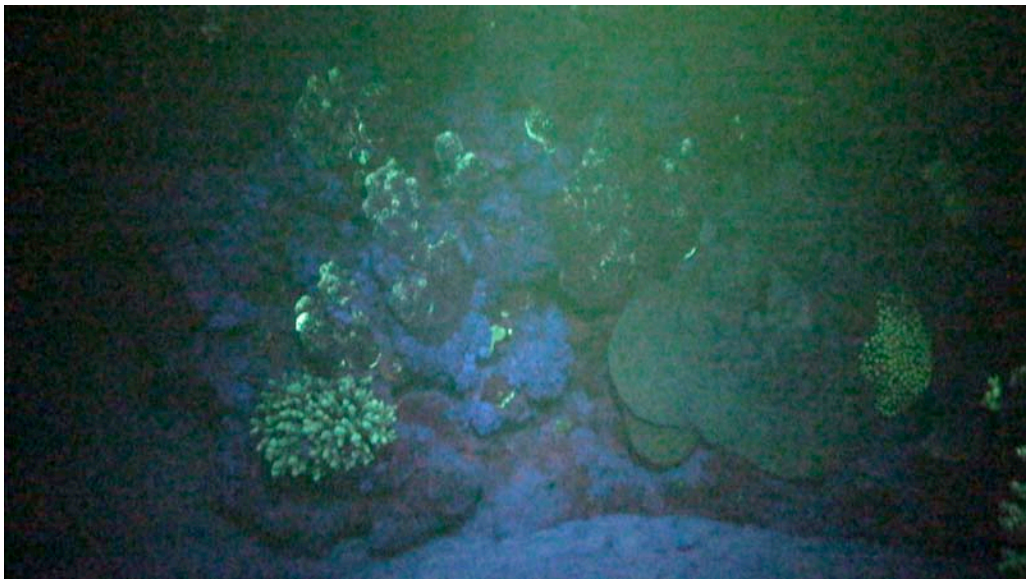

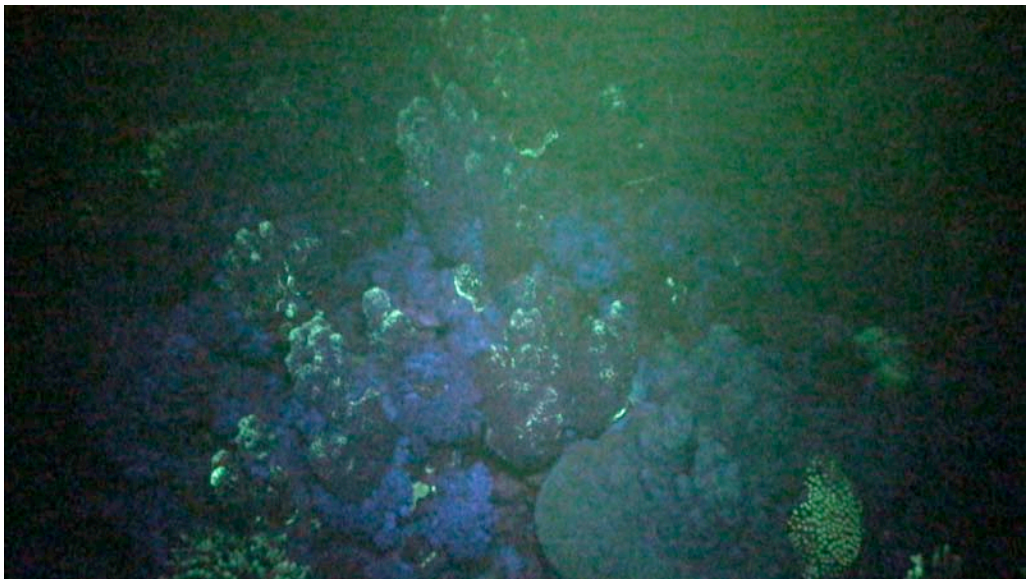

Documentation of the effect of our HiTec fluorescence torch, which allows an illumination of 5 x 7 meter. Especially corals with yellow/ green fluorescence can be detected in a big distance

Horst Grunz Magic World of coral reef with HiTec Fluorescence