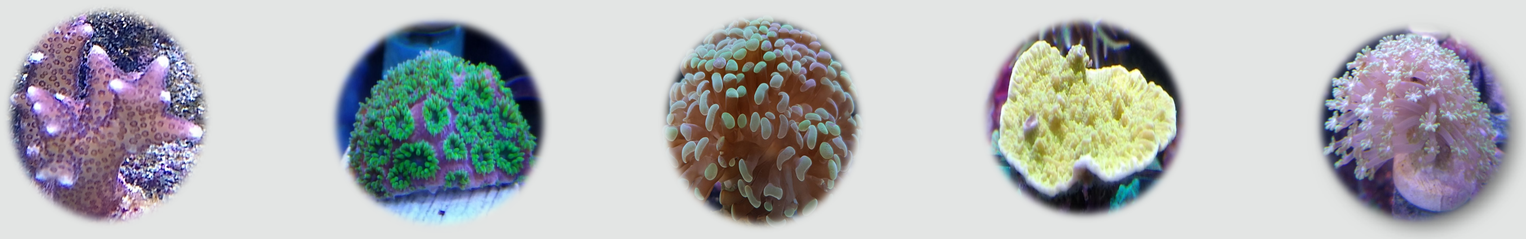
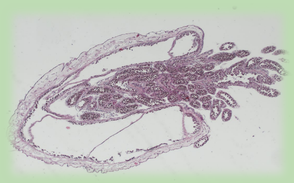


Approaches to Standardize Methods for Identifying the Negative Effects of Stressors on the Biodiversity of Coral Ecosystems



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It is very apparent that climate change significantly affects most ecosystems resulting in potentially dramatic changes of food webs. Further chemical pollutants affect ecosystems in a significant way. One of the most endangered ecosystems are coral reefs. Up to now, special focus has been given to the effect of skincare chemicals on corals. But there is still a knowledge deficit in identifying and quantifying other risks. Increasing run-off of pesticides from agriculture and industrial wastewater are origins for contaminants in the coastal water.

Ecotoxicological research is useful for assessing potential risks and simulating impacts under laboratory conditions. Building new test systems which can quantify potential risks to the environment is an important tool for avoiding biological breakdown in a multicomplex system. We are currently working on new test methods and the identification of suitable test species to assess ecotoxicological risks to coral reefs. We present test approaches under laboratory conditions to generate reliable and comparable toxicity endpoints.

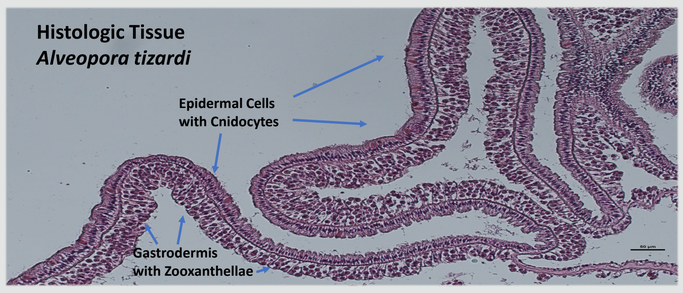
Test conditions

Temperature:	25°C±1°C
pH-value:	8.2±0.2
Calcium:	410 mg/L
Magnesium:	1020 mg/L
Salinity:	35.4 g/L
Phosphate:	0.1 mg/L
Nitrate:	10 mg/L
Carbonate Hardness:	7.0 (°dkH)
Oxygen content:	8.40 mg/L

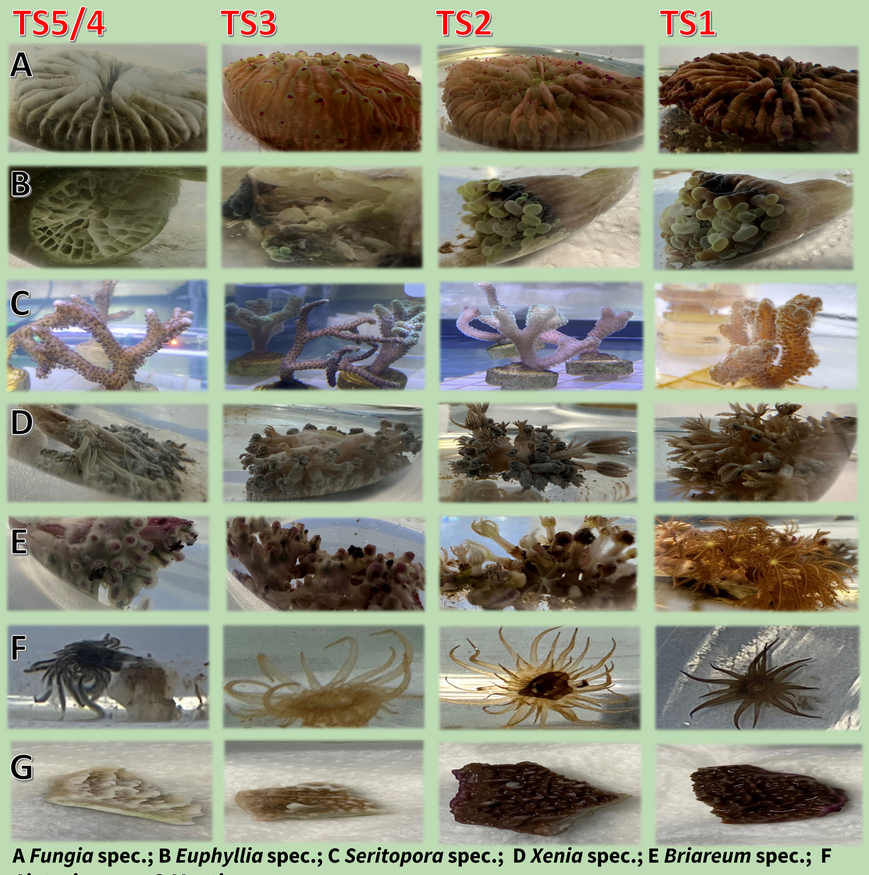
Acute Toxicity Testing up to 96 hours

Test item 3,5-Dichlorophenol (3,5-DCP: concentration 0.625 (TS 1), 1.25 (TS2), 2.50 (TS3), 5.00 (TS4), 10.0 (TS5) mg/L.

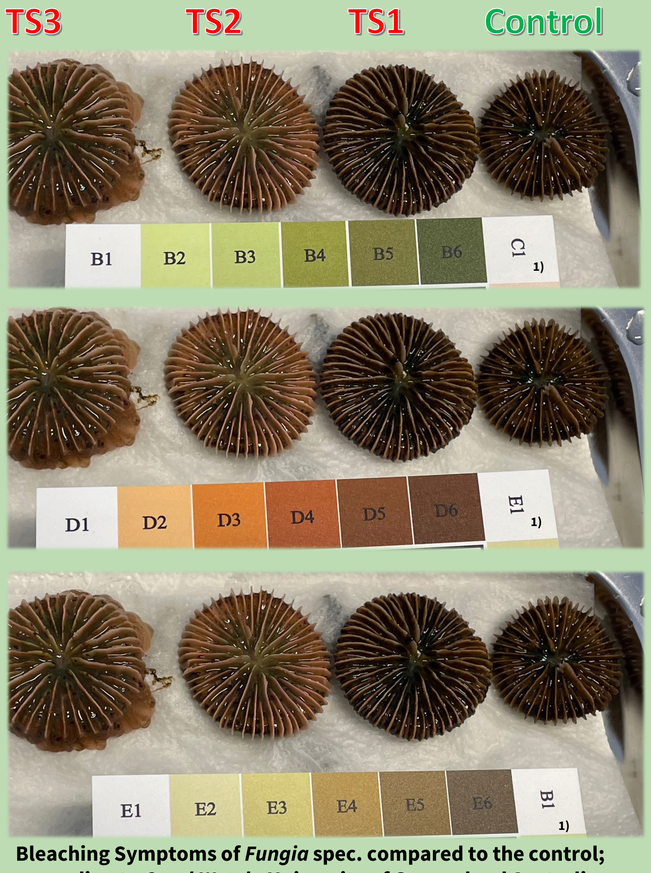
Species selected from different taxa: *Fungia* spec.; *Euphyllia* spec.; *Seritopora* spec.; *Xenia* spec.; *Briareum* spec.; *Montipora* spec.; *Acropora spec.* and as reference species *Aiptasia* spec.



Results



A *Fungia* spec.; B *Euphyllia* spec.; C *Seritopora* spec.; D *Xenia* spec.; E *Briareum* spec.; F *Aiptasia* spec; G *Montipora* spec.

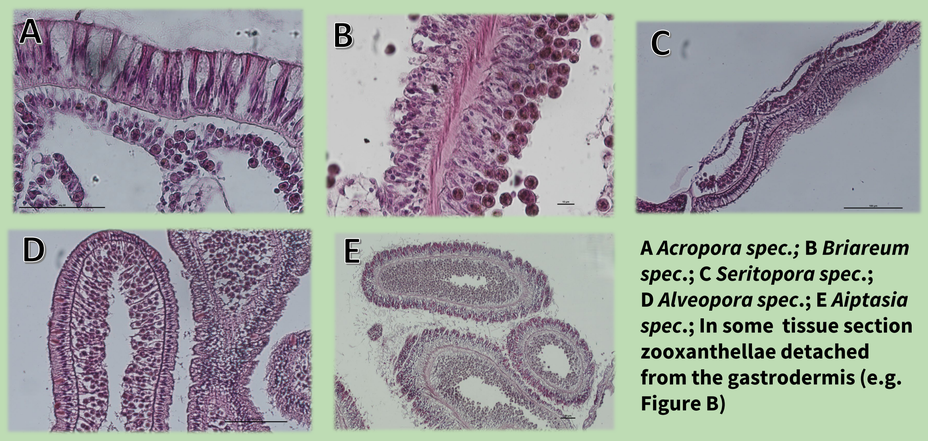


Bleaching Symptoms of *Fungia* spec. compared to the control; according to *Coral Watch*; University of Queensland Australia.

Effects on Corals

Collapse of the polyps | **Edema and loss of polyp movement** | **Retracted polyps** | **Mucus formation** | **Necrosis** | **Bleaching**

Histologic Tissue Section of different Coral Species and Anemones



A *Acropora spec.*; B *Briareum spec.*; C *Seritopora spec.*; D *Alveopora spec.*; E *Aiptasia spec.*; In some tissue section zooxanthellae detached from the gastrodermis (e.g. Figure B)

Percentage Reduction in the Health of Corals and Aiptasia treated with 3,5-DCP

3,5-DCP		*Xenia spec.*	*Briareum spec.*	*Acropora spec. 1*	*Acropora spec. 2*	*Euphyllia spec.*	*Montipora spec.*	*Fungia spec.*	*Seritopora spec.*	*Aiptasia spec.*
Treatment	Conc. [mg/L]	48-72 hours	48-72 hours	48-72 hours	48-72 hours	48-72 hours	48-72 hours	48-72 hours	48-72 hours	72-96 hours
control	0.00	0%	0%	0%	0%	0%	0%	0%	0%	0%
TS1	0.625	0%	30% Observed effects: Less colourful, pale	0%	0%	0%	0%	0%	50% Observed effects: Less colourful, pale	50% Observed effects: Less colourful, pale
TS2	1.25	0%	100% Observed effects: Less colourful, pale, deformation of polyps	0%	50% Observed effects: Necrosis, mucus formation	50% Observed effects: Partly retracted polyps	50% Observed effects: Partly bleaching	40% Observed effects: Less colourful, pale	100% Observed effects: Less colourful, pale, partly contracted polyps	100% Observed effects: Less colourful, pale, partly contracted polyps
TS3	2.50	100% Observed effects: Partly retracted polyps, fast contraction of polyps	100% Observed effects: White polyps, deformation of polyps	100% Observed effects: Necrosis, mucus formation	100% Observed effects: Necrosis, mucus formation	100% Observed effects: retracted polyps	100% Observed effects: Partly necrosis, mucus formation	100% Observed effects: Pale, edema and loss of polyp movement	100% Observed effects: Less colourful, white polyps	100% Observed effects: Less colourful, white polyps
TS4	5.00	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Necrosis, collapse of polyps
TS5	10.0	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Bleaching, necrosis, collapse of polyps	100% Observed effects: Necrosis, collapse of polyps
EC_{50}	[mg/L]	1.78	0.664	1.78	1.25	1.25	1.25	1.29	0.625	0.625

Note: *Acropora spec. 1* = firmly attached corals with well-developed foot; *Acropora spec. 2* = freshly prepared fragments

Discussion

Tests with the reference substance 3,5-DCP show that corals react quite sensitive compared to other test species such as algae or macrophytes. It became clear that corals are indicator species, especially corals that live in symbiosis with algae. It was shown that the zooxanthellae were released within a short period of time. This led to coral bleaching in acute tests over 72-96 hours. In addition to bleaching, the observed effects were polyp collapse, edema, loss of polyp movement, retracted polyps, mucus formation and necrosis. It should be mentioned that laboratory tests with corals and anemones, e.g. *Aiptasia spec.*, may identify potential risks to coral reef ecosystems. However, it should be noted that species from well-adapted laboratory cultures should be preferred in order to minimize the response to environmental changes and conserve natural resources. Corals react quickly to changes in temperature and light as well as to chemical changes in the test water. Therefore, only corals from our own stock cultures under controlled environmental conditions were used for the tests. Further the adaptation and damage of corals should be taken into account. The first tests with *Acropora* species showed that handling of corals can significantly increase or decrease the response to chemical contaminants. In addition, anemones appear to be a good alternative for screening of effects on coral reefs. Exposure of Aiptasia over 96 hours showed similar effects compared to tests with corals over 48-72 hours. Therefore, the recommended time for acute testing with anemones is 96 hours. In addition, anemones can also be tested for longer exposure scenarios. In contrast to corals, this can be done in static systems with lower water volumes. The results show that potential chemical stressors for coral reefs can be identified under laboratory conditions and included in the risk assessment.

Special Thanks to Johann Kirchhauser, Natural History Museum of Karlsruhe; Hannah Manns and Christian Voolstra, University of Konstanz for the scientific exchange. 1) Coral Health Chart; University of Queensland Australia

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