

Towards the development of standard toxicity test protocols for corals

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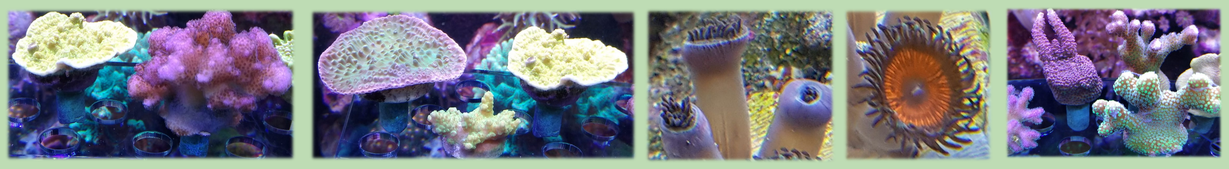
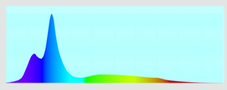
Chemical pollutants may affect corals at very low concentration levels. In focus are skincare products. Beside this run-off of pesticides from agriculture and industrial wastewater are further origins for contaminants in costal water. However, the risk assessment which is generally performed for any registration of chemical products does not yet cover corals for the most contaminants. Due to the sensitivity and significance of already threatened corals in marine environments, it is essential to include corals as a test organism and to develop a standard toxicity test protocol, which is unavoidable for the generation of reliable and comparable toxicity endpoints. The sensitivity of corals to chemical pollutants is species and life-stage dependent and hence the risk assessment should consider coral larvae and nubbins (branch tips consisting of several polyps). For the risk assessment using nubbins, growth rates (e.g., length and biomass), necrosis (loss of tissue) and coral bleaching should be assessed. Further, parameter including light intensity, pH, temperature, salinity, alkalinity, calcium-, nitrate- and phosphate concentrations should be determined. We screen coral species of interest for ecotoxicological testing with a focus on doubling biomass during the test period, which is essential to evaluate effects on growth. Besides growth rates, the species needs to be relevant, widespread, and sensitive and the coefficient of variation (CV) for the control group be as low as possible.

The growth parameters that are based on metric data will assist in refining risk assessments. Toxic endpoints that are based on visual observations such as necrosis, bleaching, polyp contraction, change in color, loss of tissue and mucous formation can also be assessed.



Material & Methods

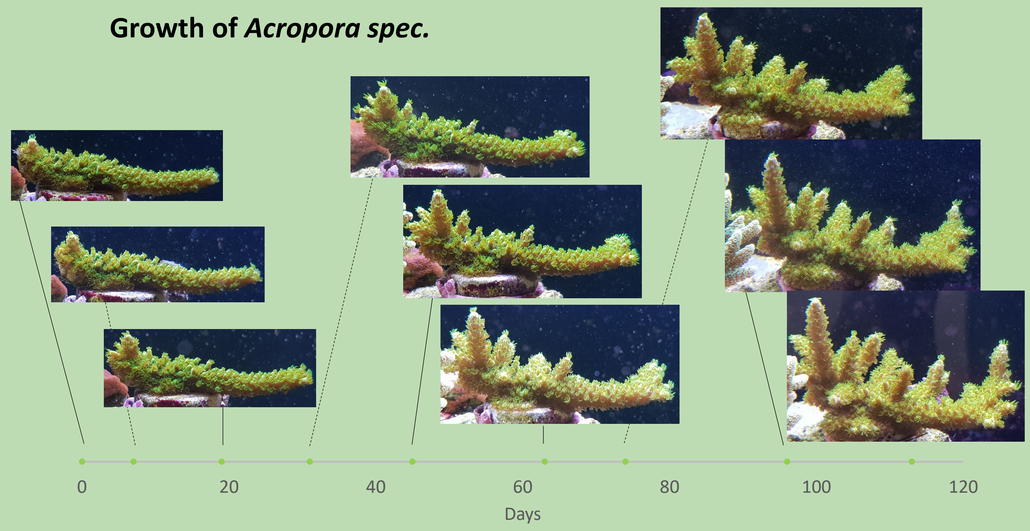
Temperature:	25°C±1°C
pH-value:	8.2±0.2
Carbonate hardness (°dkH):	6.7±0.3
Calcium:	420-430 mg/L
Magnesium:	1230-1290 mg/L
Oxygen content:	8.02-8.48 mg/L
Salinity:	34.4-34.8 g/L
pH-value:	8.2±0.2
Phosphate:	0-0.05 mg/L
Nitrate:	0.2-0.5 mg/L
Light spectrum:	

Doubling Time: $T_d = \ln 2/\mu$ where μ is the average specific growth rate

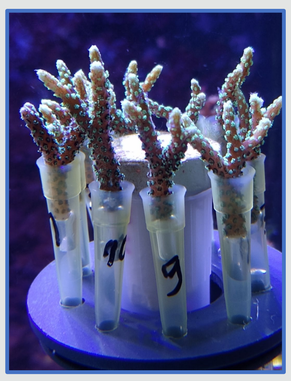
Test Design: based on OECD Guideline 239

Validity criteria:
Mean fresh weight at least double during the exposure phase of the test. In addition, control corals must not show any visual symptoms of bleaching and should be visibly free from contamination by other organisms such as algae and/or bacterial films.
The mean coefficient of variation for yield based on measurements of fresh weight does not exceed 35% between replicates

Growth of *Acropora spec.*



Results



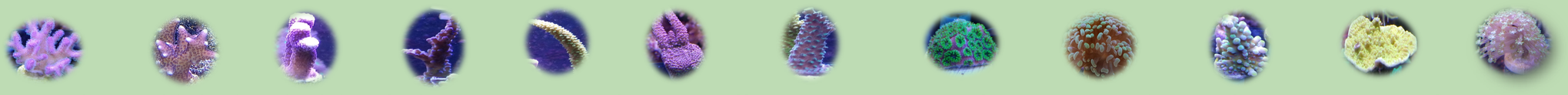

Seriatopora caliendrum Yield (fresh weight; 5-8 replicates over 28 days)					
Batch	1	2	3	4	5
CV%	64.8	42.3	23.7	26.0	32.7

First results indicate that coral testing could be adopted to validity criteria of OECD guideline 239. For doubling of biomass test duration has to be prolonged to at least 28 days. A coefficient of variance below 35% could be reached if start material does not differ in weight by more that 20%. Preferably coral nubbins of approximately 1 g should be selected. For first tests, *Seriatopoa caliendrum* was selected due to their good growth rates and they are less fragile than other species within this family.

Species (screening in Microcosm)	doubling time [d]	Species (screening in Microcosm)	doubling time [d]
Acropora anthocercis	160.1	*Montipora confusa*	171.9
Acropora aspera	64.9	*Montipora delicatula*	>365
Acropora austrea	109.6	*Montipora digitata*	>365
Acropora efflorescens	>365	*Montipora hispida*	256.3
Acropora granulosa	>365	*Montipora porites*	182.8
Acropora hyacinthus	>365	*Pocillopora (var. bicolor)*	111.4
Acropora nana	306.6	*Pocillopora damicorni (var. pink)*	>365
Acropora tenuis	237.0	*Pocillopora elegans*	66.1
Acropora tumida (batch 1)	>365	*Porites attenuata*	167.0
Acropora tumida (batch 2)	59.1	*Porites densa*	96.3
Alveopora tizardi (batch 1)	8.1	*Porites spec.*	266.9
Alveopora tizardi (batch 2)	>365	*Recordea florida*	>365
Alveopora tizardi (batch 3)	104.5	*Seriatopora caliendrum (batch 1)*	28.6
Capnella imbricata	62.8	*Seriatopora caliendrum (batch 2)*	20.5
Caulastraea furcata	117.4	*Seriatopora caliendrum (batch 3)*	15.6
Cyphastrea spec.	175.2	*Seriatopora caliendrum (batch 4)*	38.7
Duncanopsammia axifuga	>365	*Seriatopora hystrix (batch 1 var. green)*	55.2
Echinopora lamellosa	206.8	*Seriatopora hystrix (batch 2 var. green)*	34.1
Erythropodium caribaeorum	27.3	*Seriatopora hystrix (batch 3 var. green)*	55.2
Erythropodium caribaeorum (var. dark green)	77.9	*Seriatopora hystrix (var. pink)*	>365
Euphyllia baliensis	61.2	*Stylophora pistillata (var. milka)*	36.8
Homophyllia australis	>365	*Stylophora pistillata (var. minth green)*	63.1
Hydnophora rigida	131.3	*Turbinaria peltata*	191.7
Leptastrea spec.	>365	*Zoanthus spec.*	170.9

Note: Corals were screened in a microcosm with potential influence of other species. The optimal test conditions could not be chosen for every coral. The focus of this first screening was to select fast growing species at the chosen test conditions. In case these test conditions are adopted, other species could be chosen that show faster growth. This could be observed in parallel test units. Parameters for a good growth of corals are divers and complex and should be monitored preferably daily during testing.

Discussion



Optimal growth conditions can be established in the lab, which is important in detecting effects in comparison of treatment and control groups. In case of extended test durations to 1-2 months, some coral species could be suitable to fulfil validity criteria according to OECD 239 to generate robust and reproducible data to calculate endpoints. These endpoints could then be growth rates and yield based on fresh weight, polyp number or total length of corals, however destructive determination of dry weight should be avoided. Specifically for stony corals, the dry weight of the calcium skeleton will not allow a direct correlation to the polyp weight. It must be assumed that only a good growing polyp allows the coral to develop a strong calcium skeleton. Further, coral dry weight cannot be measured at test start and is therefore only related to representative corals. Beside coral fresh weight, at least one additional growth parameter should be determined (e.g., polyp number or total length of coral). To measure fresh weight, water should be gently removed from the corals and care should be taken to calculate the exact weight of the coral without any surrounding debris. Optionally, the exact weight of any material used to adhere the coral to, has to be determined in advance. This procedure would allow to determine the weight of the coral at several assessment points during testing until doubling of biomass is reached.

During preliminary tests, it became apparent that great care must be taken on the start material. Corals with an initial weight of under 1 g are preferred, especially when using stony corals. The ratio between polyp and the calcium carbonate skeleton seems to be essential for doubling the biomass.

Further, only corals that were adapted to the initial test conditions for at least two weeks should be used. For species with large polyps (LPS), the test could be adapted to the OECD 221. In this case, polyps can be counted and when doubling of numbers is achieved, the size endpoints can be calculated on polyp number and fresh weight. Based on these preliminary results, coral growth parameters can be established and can assist in risk assessment of chemicals on corals.

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