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The fluffy sea anemone *Metridium senile* in periodically oxygen depleted surroundings

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Abstract

In SCUBA-diving monitored field experiments (Flensburg Fjord, 15 m, 1981) and parallel aquarium tests, the behavior of *Metridium senile* under anoxic conditions and its oxygen-deficiency resistance were studied. When oxygen is lacking the fluffy sea anemone diminishes body surface, and then, successively, shuts off most energy consuming activities. By means of this strategy, 50% of the tested individuals survived 3 weeks of total anoxia.

Introduction

The deeper layers of calm or stagnant waters tend to become oxygen depleted by bacterial decomposing activities. This process can be accelerated by the input of nutrient-rich sewage.

In the Flensburg fjord an extremely stable thermocline exists at a depth of 6 to 12 m during summer, causing the complete isolation of a cold, lightless, deep-water body – at least, in the inner part of the fjord. Towns like Flensburg, Glücksburg, Gråsten and Sønderborg add important amounts of nitrates and phosphates to this fjord (Kändler, 1963; Gemeinsames Komitee Flensburger Förde, 1974).

Thus the essential conditions are fulfilled for the creation of an oxygen depleted habitat – a situation which actually can be observed every summer between May and October (Fig. 1). In general the length of this anoxic period for a given depth shortens parallel to the pollution gradient, but in the opposite direction: from Flensburg harbour to the open Baltic.

The observation made in winter 1980/1981, that adult *Metridium senile* occurred at depths which had been anoxic for several weeks the previous summer, initiated this study. This could either be due to a recolonization of these zones by adult animals in autumn, or to a remarkable resistance of *Metridium senile* to O_2-depletion. (Studies on recolonization will be published later.)

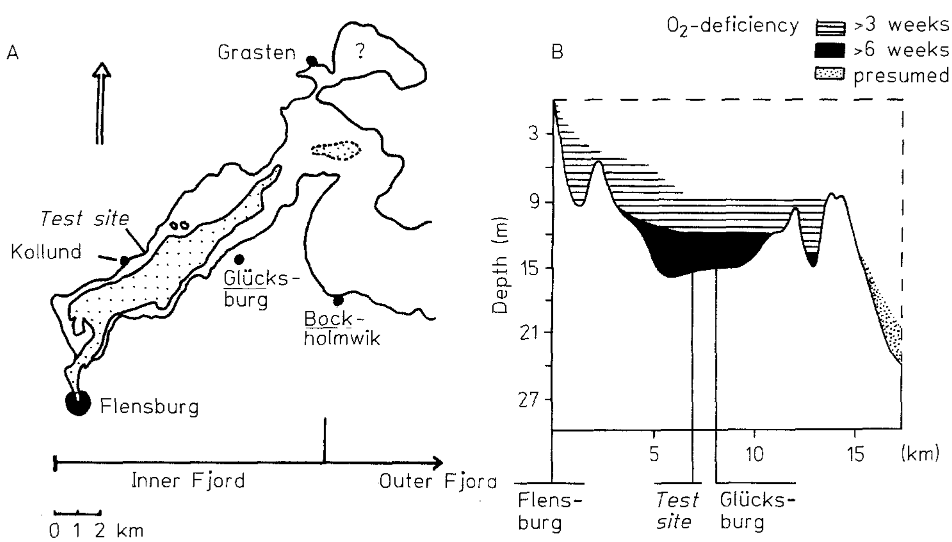

Fig. 1. (A) Areas in the inner Flensburg Fjord, where deeper water layers were O_2-deficient for more than three weeks in summer 1981 (0.5 cm = 1 km). (B) Longitudinal profile of the inner Flensburg Fjord (from Flensburg harbour to mid-fjord) showing the water body which was O_2-deficient for at least three weeks in summer 1981

In the literature there is little to be found on the resistance of the fluffy sea anemone to anoxic conditions. The more important general facts on this topic (responses of marine animals to low O_2-concentrations, respiratory physiology of marine evertebrates, etc.) are discussed in Wieser and Kanwisher (1959), Brafield and Chapman (1965), Sassaman and Mangum (1970, 1972, 1973), Beattie (1971), Lenhoff *et al.* (1971), Saz (1971), Von Oertzen and Schlungbaum (1972), Hochachka *et al.* (1973), Mangum and Van Winkle (1973), Dries and Theede (1974), Jones *et al.* (1977), Mangum (1980).

Sassaman and Mangum (1972, 1973) tested the resistance of *Metridium senile* to anoxia at 22° to 24 °C. They found that not one individual could endure longer than 5 d of O_2-depletion. If this were true, the above mentioned presence of sea anemones in previously O_2-deprived water-bodies could only be explained by a recent and surprisingly numerous immigration of adults into this area. In 1981 and 1982 I studied the behavior and survival of *M. senile* in an anoxic milieu by *in-situ* experiments (Flensburg Fjord, SCUBA-diving), parallelled by aquarium experiments at Kiel University, Germany (FRG).

Material and methods

In-vitro experiments

The aquarium experiments took place in a water-circulation system (Fig. 2), which consisted of a pump (15 l h^{-1}; 1 h d^{-1}), a main water reservoir (25 l) and a series of 6 to 9 experimental chambers (1.5 l). Each of these chambers could be taken out of the system individually without interfering with the experiment. One to ten test individuals were placed in each vessel. After an appropriate adaption time of 1 to 2 d under oxic conditions, the system water was oxygen-depleted by an intensive N_2-flushing in the main tank in 5 to 6 h (with system water circulating). The O_2-concentration during experimentation never exceeded 0.5 ml O_2 l^{-1}. This value is tolerable as an upper limit as has been shown by pilot experiments and in comparison to the *in-situ* experiments (here O_2-concentration was 0 ml O_2 l^{-1}) (see also Sassaman and Mangum, 1972). Water temperature was 6° to 7 °C.

At least weekly, 25 l of system water were replaced by fresh, but O_2-free, seawater. Thus the (presumed) accumulation of harmful excretions was diluted 2 to 3 fold. Oxygen concentration was measured daily by the Winkler method. The individuals were not fed, as previous experiments had shown that a three month fast did not affect their vitality. Controls in an identical experimental system, but with high O_2-concentrations, showed perfectly normal behavior.

The habitus of test individuals was codified numerically (in order to plot behavioral changes against time, see Figs. 3 and 4) and noted daily. Every day, during the crucial phase (see Results), one vessel was taken out of the test system and plugged into the circulating, O_2-rich seawater to monitor recovery or death of the sea anemones after an ever longer period ($n+1$ d) of oxygen starvation. All in all, 81 *Metridium senile* were tested by this method.

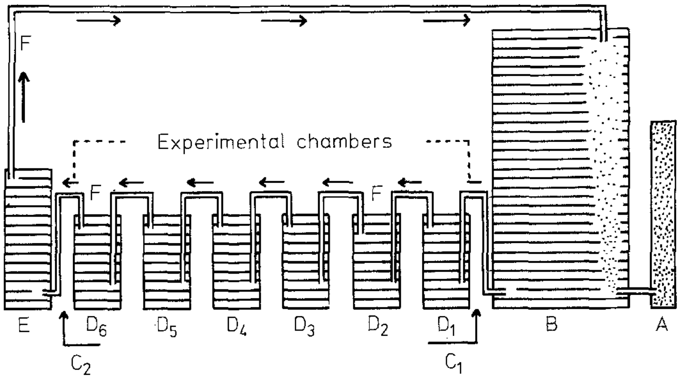

Fig. 2. *Metridium senile.* The water circulation system of the aquarium experiments: A = N_2-pressure tank; B = main water reservoir (25 l); C_1 and C_2 = points of daily oxygen monitoring; D_1, D_2, etc. = experimental chambers (1.5 l, 1 to 10 test anemones); E = pump (ca 15 l/h); F = glass tubes

In-situ experiments

All work was carried out by SCUBA-diving from the end of May to October 1981. The experiment site was situated near Kollund (Denmark) in the inner part of the Flensburg Fjord at a depth of 15 m. During the test period water temperature ranged from 7° to 9 °C, salinity lay between 18 and 22‰ S and oxygen was absent for more than three months below the thermocline (10 to 11 m). Eutrophication is high (for water quality information see Gemeinsames Komitee Flensburger Förde, 1974).

Test individuals were collected with their substratum (mussels, stones, a plastic dish, etc.) in the shallower and oxic depths (5 to 8 m) of the same station and immediately transferred under water to the test site (15 m) and fixed to an artificial support, 20 to 70 cm above the sediment. (Phytoplankton sedimentation rates being extremely high – up to 10 cm wk^{-1}, personal observation – the position of the test individuals was chosen so that near bottom currents acted as a continuous cleaning flow.) This procedure assured minimal stress to test individuals prior to the beginning of the experiment, because they never left their natural medium, they were not scratched off their substratum, and the whole transfer only took about 15 min.

The monitoring of O_2-concentration was not necessary as long as the diver could smell hydrogen sulfide (H_2S) through the mask and/or the sediment surface was black (activity of anaerobic sulfur bacteria), as was the case throughout these experiments.

Every 3 to 7 d I noted the habitus of each individual and tested the touch-contraction reflex. Those anemones were considered dead, which showed a "habitus-state" below 0.3 (for code explanations see Results) or showed no reaction to touch.

In four experimental series, 40 *Metridium senile* were tested.

Results

The two categories of experimentation (*in-situ* and *in-vitro*) provided results which are so similar that there is no need to present them separately.

Behavior under anoxic conditions

During an oxygen-deficiency period the fluffy anemones pass through a series of form changes, which, in all experiments, showed such a high degree of conformity and constancy that I like to regard this as a typical behavioral sequence of *Metridium senile* under such conditions (Fig. 3). To facilitate analysis the major steps have been attributed a numerical value between 1.0 and 0.0. Fig. 4 illustrates the form changes exhibited by the test anemones during O_2-starvation. In the chronological order it is:

1.0: healthy, expanded individual;
0.9: tentacles retracted;
0.8: complete contraction;
0.7 to 0.6: progressive relaxation of longitudinal muscles;
0.5: reduction of ciliary activity, loss of inner hydrostatic pressure;
0.4: relaxation of pedal muscle, detachment from substrate (0.5 and 0.4 are "alternative" states between 0.6 and 0.3, with a slight preference to 0.5);
0.3: pedal muscle activity and ciliary activity have completely stopped;
0.2: sphincter is relaxed;
0.1: tentacles decaying;
0.0: death.

This sequence seems to illustrate the progressive loss of vitality of the test organisms.

While one can encounter each of these "habitus-stages" occasionally in a healthy population, under anoxic conditions all individuals pass through these states in a more or less synchronous fashion (the standard error for Fig. 4 is ca ±0.1).

If conditions return to normal (i.e. oxic) when the anemones occupy state 0.1, some rare individuals may recover, discard their damaged organs and rebuild them. On the average, however, the critical line between recovery and death is near to 0.3 (loss of hydrostatic pressure and pedal muscle activity).

Oxygen deficiency resistance

In an aquarium experiment the behavior and development of 42 fluffy sea anemones during and after a 19-d absence of O_2 were followed individually (Fig. 5, temperature, 6° to 7 °C).

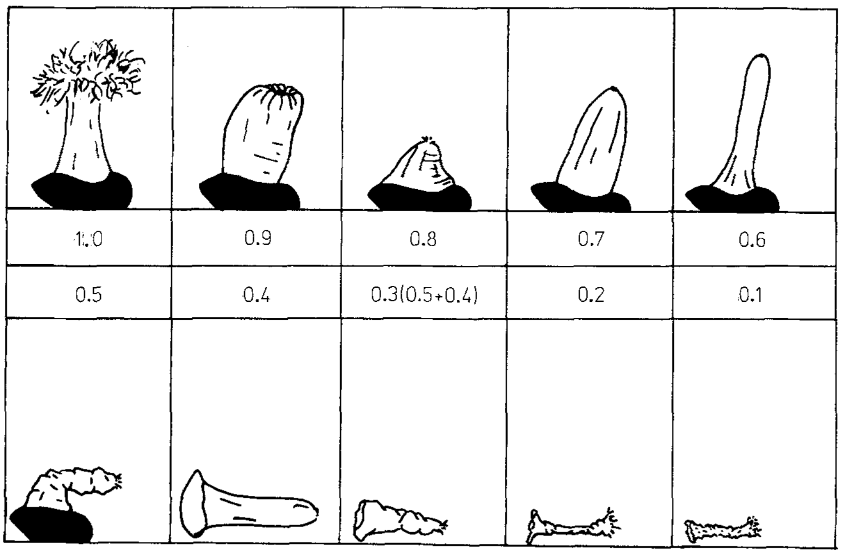

Fig. 3. *Metridium senile.* Change of habitus under anoxic conditions (sketches drawn after photographs; 0.5 and 0.4 are alternative steps, see text)

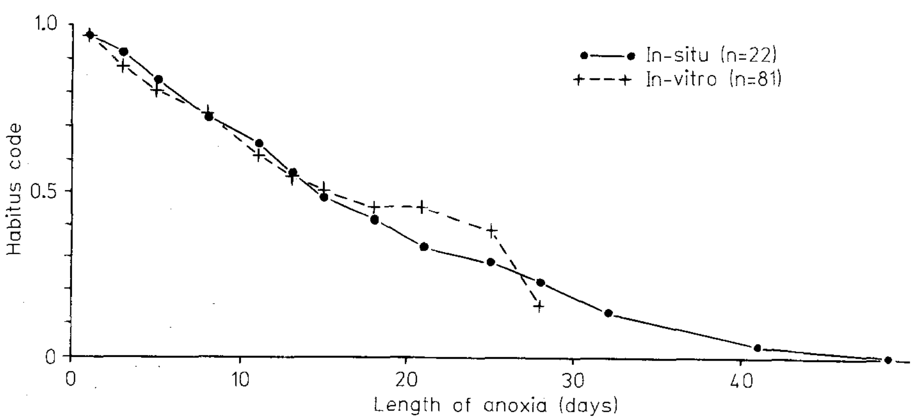

Fig. 4. *Metridium senile.* Change of habitus as a function of time during an O_2-free period (standard error is ca 0.1)

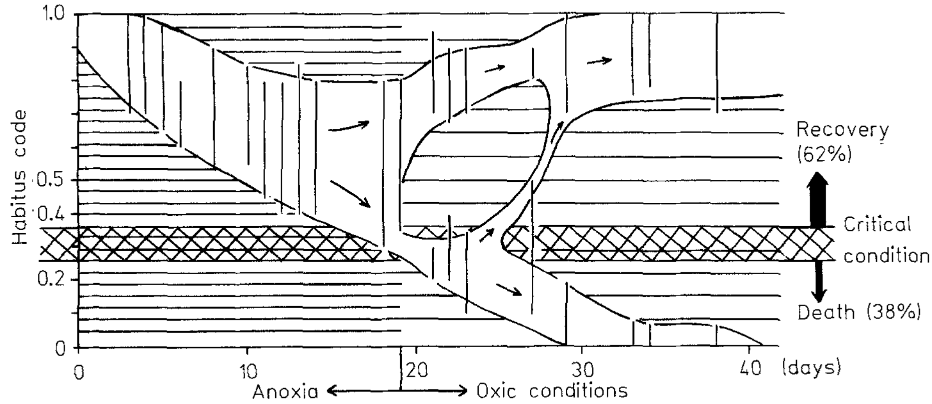

Fig. 5. *Metridium senile.* Development of a group of 42 *M. senile* during and after an O_2-free period of 19 d (*in vitro*). The habitus spectrum, that is the difference between the fittest and the weakest anemone (of a behavioral group) on a given day, is represented by the vertical bars; see text

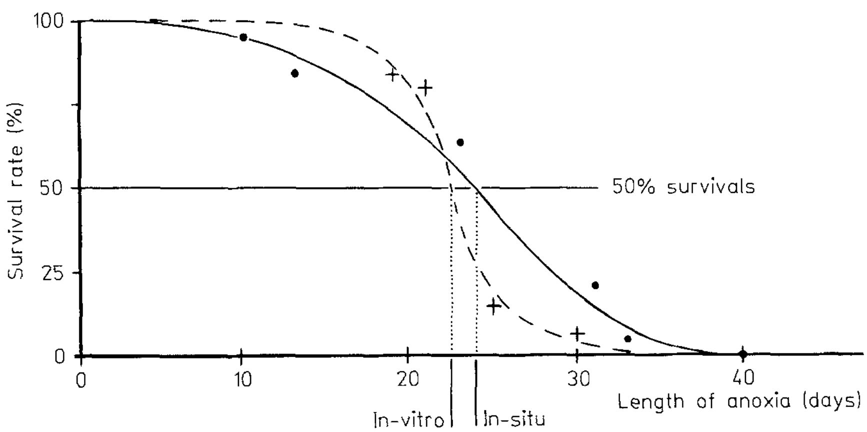

Fig. 6. *Metridium senile.* Mean LD_{50}-graphs of the *in-situ* and the *in-vitro* experiments (●—● = *in-situ:* 7° to 9 °C; +– – –+ = *in-vitro:* 6° to 7 °C)

During the oxygen-free period the band of "habitus-states" slowly sinks and widens: All anemones pass through the typical behavioral sequence described previously. However, some seem more sensitive to the stress, while others are tougher. On Day 19 there are five "condition-units" between the strongest (0.8) and the weakest (0.25). Apart from these extremes, the bulk of the test individuals were grouping around 0.4 (not discernible on Fig. 5). When the chambers were plugged into oxygen-rich seawater on Day 19, the anemones split up into two behavioral groups: 60% recovered rapidly, while the rest continued with a downward trend. On Day 23 a few *Metridium senile* separated from the latter and joined, after a rather steep recovery, the group of survivors. On Day 40, 38% of the test organisms had died, the rest were in a healthy condition.

Figure 6 summarizes and illustrates the results of the *in-situ* and *in-vitro* experiments on the O_2-deficiency resistance of *Metridium senile:* In the Flensburg Fjord deep O_2-free layers, 50% of the test organisms were dead after 22 to 24 d of O_2-starvation, the toughest individuals died on Day 40.

Under simulated aquarium conditions, the LD_{50}-value is 21 to 22 d, the endurance limit being 35 d.

Discussion

The behavioral sequence typical of *Metridium senile* when oxygen is absent is described here, to my knowledge, for the first time.

During prolonged oxygen starvation, two successive strategies are used:

(1) diminution of body surface by tentacle retraction, then closing of the sphincter, finally complete body contraction (0.9 to 0.8). This first phase is a common sea anemone reaction, designed to protect the individual from harmful influence or contact.

(2) Then, after the first 7 to 9 O_2-free days, the anemone switches off successively most energy-consuming activities: work of longitudinal muscles (resulting in 0.7 and 0.6), ciliary and pedal muscle activity (0.5 to 0.3) and sphincter contraction (0.2). When the tentacles start decomposing (0.1), a few individuals (estimated 1 to 5%) may recover if returned to favorable conditions.

If oxygen returns before ciliary activity stops *and* before the anemone detaches (before 0.3), most individuals recover (more than 60%). Once hydrostatic pressure *and* attachment are lost (below 0.3), recovery is rare. Curiously, diminution of ciliary activity alone or pedal muscle relaxation alone do not seem to be lethal. Apparently only the co-existence of both phenomena signals a total exhaustion and the loss of the last energy reserves.

Thus, the second part of the behavioral sequence is most characteristic for the circumstances described. During my 3-yr diving activities in the Flensburg Fjord, a general occurrence of the "habitus-states" 0.7 to 0.1 was *always* connected with a lack of O_2. Vice versa, if, for instance, most individuals of a *Metridium senile* population look like 0.7 or 0.6 (Fig. 3), one may be quite sure that O_2 has been absent for at least one week, and still is.

Under the environmental conditions of the Flensburg Fjord (6° to 9 °C, 17 to 22‰ S), 50% of a population of the fluffy sea anemone can survive a three week long oxygen deficiency. Some of the toughest individuals are still alive after 40 O_2-free days.

These values exceed fivefold those found by Sassaman and Mangum (1972) for water temperatures between 22° and 24 °C, some 15 C° warmer than the Flensburg Fjord deeper layers. Can the difference in the values of O_2-deficiency resistance be solely due to this temperature difference? In this context, the temperature might have an influence on two levels: the "physical" one, as O_2-solubility in seawater (but here oxygen concentrations are extremely small and the experimental medium is all but O_2-saturated) and the "biochemical" one, i.e. the metabolism of the test individuals. Normally, higher temperatures accelerate the metabolic rate by a quantifiable factor (Q_{10}). But in this case, in the absence of oxygen, the anemone metabolism is not a "normal" one.

Under the summer conditions at the bottom of the Flensburg Fjord the anemones of the O_2-free water layers do not feed, because tentacles are retracted from the second or third day onward and, in any case, there should not be many living planktonic organisms in the black anoxic waters several meters beneath a very stable thermocline. Thus (if there is no important uptake of dissolved organic compounds such as sugar, amino acids, etc.) survival is intimately linked with the preservation of energy reserves (see interpretation of behavioral sequence above). If a higher warm water metabolism of Sassaman and Mangum's (1972) anemones were responsible for a more rapid exhaustion of stocks (by anaerobic pathways, the existence of which is doubtful), the Q_{10}-value would be 3.2 for the temperature range from 7° to 23 °C.

However, Newell and Northcroft (1967), studying the metabolism of contracted inactive *Metridium senile* (corresponding to my "habitus-stages" 0.8 and lower), proved the existence of a "maintenance metabolism" with ciliary and muscle activity reduced to a minimum – independent of temperature. Consequently the O_2-demand is constant and the Q_{10} is 1 for the tested temperature range and for this physiological state.

Thus either the metabolism of *Metridium senile* comes to a standstill under anoxic conditions, or the fluffy anemone is capable of switching to anaerobic pathways, in which case its activity is nevertheless reduced (from the first week onward, see Fig. 4) to the above mentioned "maintenance-metabolism" insensitive to changing temperatures. In either case, the discrepancy between my results and those found by Sassaman and Mangum (1972) cannot be explained solely by different experimental temperatures.

As for another important physical factor, Shumway (1978) studied the influence of salinity changes on the metabolism of *Metridium senile:* when salinity drops, the anemones contract and become inactive. Their reduced respiration rate, as a specific response to a more or less sudden change of salinity, may reflect the equivalent of the maintenance metabolism described by Newell and Northcroft (1967). However, the Flensburg Fjord anemones and the individuals used by Sassaman and Mangum (1972) were *adapted* to their respective experiment salinities. Consequently there was no stress (due to salinity "change") to cause a defensive response with a reduction of activity. Thus lower salinity in my experiments is not likely to be responsible for a higher O_2-deficiency resistance. Probably there are other factors responsible, e.g. a physiological (or genetic) difference between the tested anemone populations or differences in the experimental conditions (e.g. the consequences of a rather limited amount of water in the tests of Sassaman and Mangum, etc.).

It would certainly be helpful to compare such populations directly and to study the influence of temperature on the O_2-deficiency resistance.

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