

## RNA isolation from marine invertebrates

Diploma Thesis

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### Abstract

Marine invertebrates, e.g. sponges, bryozoans or sea squirts differ a lot in their living from terrestrial organisms. Therefore, they offer a good starting-point when looking for new natural substances. The exploration of these, e.g. the search of new enzymes, is increasingly done on levels of molecular biology and genetics. The present study is based on this idea.

It deals with the question, if and how good RNA can be isolated from different marine organisms using different methods. Three ways of treatment and RNA isolation were carried out and compared. The respective disadvantages and advantages of these methods were listed.

Sampling took place in the coastal area of the island of Elba/Italy. For this project five invertebrates of five different phyla were chosen:

Mollusca	- <i>Berthella aurantiaca</i>
Cnidaria	- <i>Actinia equina</i>
Porifera	- <i>Agelas oroides</i>
Tunicata	- <i>Halocynthia papillosa</i>
Annelida	- <i>Spirographis spallanzoni</i>

Sample treatment took place at the field station of the HYDRA institute for Marine sciences, Fetovaia, Elba/Italy and in Stuttgart/Germany.

The RNA isolation methods carried out in this study are based on the phenol-chloroform-extraction (CHROMCZYNSKI&SACCHI, 1986) whereas the method was modified, depending on the organism used.

In the first approach RNA was isolated immediately after sampling of the organisms. For the second method an RNA stabilizing solution (RNAlater) was used. It allows the conservation of organisms for a longer period of time without any influence on the RNA-activity. Immediately after sampling the organisms were conserved in RNAlater solution. They were transported to Stuttgart/Germany, where the RNA was extracted. In the third approach, living organisms were transported to Stuttgart, where they were kept in aquaria for a subsequent RNA isolation.

The three methods were evaluated in two ways:

First, in the quantitative approach, the amount of biomass was compared to the amount of isolated RNA. Secondly, the isolated RNA extracted with the different methods was evaluated qualitatively using the ratio (absorption of RNA at 260nm and 280 nm) and the appearance of the nucleic acid in the agarose gel. Using these two approaches it was not only possible to make a statement about the efficiency of a method, but also about the purity of the RNA.

The study showed, that only very little amounts of RNA (if at all) could be isolated from the organisms conserved in the RNAlater solution under the prevalent conditions.

In comparison, the isolation of RNA at the field station immediately after sampling showed a lot of advantages. Using this method, the highest amounts of RNA with satisfying quality were found. On the other hand, best results, regarding the quality of the RNA, were achieved with isolation of RNA from the organisms held in aquariums. However, the organisms used for RNA isolation at the field station do not suffer from stress due to the transport and maintenance in aquariums.

Looking at the challenges concerning the problems with sample processing at a field station, difficulties could be minimized due to the experience gained. This method therefore could be used as an excellent way when searching for new natural substances.