We investigated the esterase activity of carbonic anhydrase (CA) on total esterase activity level in mussels sampled from 19 locations along the Croatian coast of Adriatic Sea. The results of total esterase activities in winter were lower than in summer at almost all investigated locations. CA activities determined in gills of mussel sampled in winter ranged from 1.75% to 24.65% of total esterase activity. CA activities in summer samples were practically not detectable. Despite in vitro research showed potential application of CA activity in bioassay and biomarker studies for application of CA as a biomarkers of environmental pollution further research are needed.

**Keywords:** Adriatic Sea, Ecotoxicology, Enzymes, Mollusca, Pollution

**INTRODUCTION**

Carbonic anhydrase (CA), a ubiquitous enzyme in the bacteria, plant, and animal kingdoms catalyses the reversible hydration of CO$_2$ to produce H$^+$ and HCO$_3^-$ using zinc as a cofactor. To date 15 CAs or CA-like proteins have been identified in mammals. In humans, for CAII isozyyme, turnover number for CO$_2$ hydration is the highest known for any enzyme, while for the other isozymes activities are lower in the order CAI > CAIV > CAI > CAIII. There are several CAs which have shown esterase activity, enzymes known to hydrolyse endogenous substrates, and the majority hydrolyse lipid ester substrates. In in vitro conditions, the esterase activity of CA was determined, both in physiological and pathological conditions. Additionally, besides Na,K-ATPase, CA represents a key enzyme involved in the adaptation of marine organisms to environmental conditions [1]. Experimental studies performed on Crustacea gills have shown two main isozymes of CA located in membrane-bound and in cytosolic fractions, functionally similar to mammalian CAIV and CAII [2]. So far no report has dealt with the esterase activity of CA on total esterase activity level in mussel.

**MATERIAL AND METHODS**

In this study the esterase activity of CA on total esterase activity level was investigated in mussels sampled from 19 locations along the Croatian coast. The gills were the target tissue because the respiratory, ionic transport and pH regulatory enzyme function of CA and it's potential usage as a biomarker of environmental pollution was the main topic. Previously it was suggested the possible application of CA activity inhibition as an in vitro bioassay for the detection of heavy metals in pollution monitoring using the mussel *Mytilus galloprovincialis* Lamarck, 1819 [3,4]. Total esterase activity was measured in the cytosolic fraction of gills homogenates by colorimetric end point reaction using p-nitrophenyl acetate as enzyme substrate, and CA activity was estimated by the same enzymatic reaction using acetazolamide as a specific CA inhibitor.

**RESULTS**

The results of total esterase activities in winter (March; average value 0.137±0.057) were lower then determined for the summer season (August; 0.153±0.036) at almost all investigated locations (Fig. 1). CA activities determined in gills of mussels sampled in winter ranged from 1.75% to 24.65% of total esterase activity. CA activities in summer samples were practically not detectable. This is preliminary research; it is difficult to assume a direct relationship between pollution at investigated sites with determined CA activities, especially in the summer period.

**REFERENCES**


