Since the discovery of the sex steroids in marine bivalves, the most important questions in bivalves endocrinology include "*where are the steroids present in bivalve tissues coming from? Do they possess a veritable factory for estrogen production or rather accumulate steroids from the ambient environment? What is the role of steroids and aromatisation pathway in this group of organisms*?".

Aromatase is a key enzyme in the biosynthesis of estrogens in all vertebrate groups. It belongs to the family of cytochromes P450 (CYP19) and is commonly found in endoplasmic reticulum (microsomes) of tissues such as female and male gonads, adipose tissue and brain. The most effective and sensitive method for measuring aromatase activity involves the usage of an isotopically labelled [1β-^{3}H] androstendione substrate and the measurement of the amount of $^{3}H_2O$ water produced as an end product of aromatisation. Aromatase (CYP19) gene orthologue first appeared in a direct ancestor of the chordates (*Branchiostoma lanceolatum*) and, so far, there is no information about cyp19 gene in invertebrates available. Additionally, the activity of this enzyme measured in invertebrates using an isotopic method was at a very low level (determining its values of androgens conversion to estrogens (from 0.01% in mussels to 3% in sea snails). The results of our studies, however, highlighted that marine bivalves, and in particular *Mytilus trossulus*, can be as efficient at aromatising androgens as vertebrates. This process is, however, temperature dependent and occurs both, in steroidogenic (gonads) and non-steroidogenic (gills) bivalves tissues.

The aim of the current project is to investigate the efficiency of androgens aromatisation by Baltic bivalves using the blue mussels, *M. trossulus*, as a model species. We will assess whether the aromatization of steroids is as efficient in molluscs living in a polluted zone (the vicinity of a sewage treatment plant outlet) and in a not polluted one. During the sampling of biological material it is planned to collect bottom water for determination of total estrogenicity of environmental samples. Endocrine Disruptors (EDs) pose a serious risk to marine organisms and may lead to reproduction disorders, developmental changes and, consequently, to sterility and the extinction of species. The group of EDs that can regulate mollusc reproductive processes interacts through receptors and thus activates signalling pathways leading to neurohormonal regulation of the gonadal maturation and gametes production. However, a particular group of EDs can directly affect the process of estrogen biosynthesis by inducing or inhibiting their synthesis (the amount of androgens increase). Regulation must therefore be based on the level of activation or inhibition of the protein that function as aromatase. In order to confirm the influence of EDs on the regulation of the aromatisation process in bivalves, an exposure experiment is planned in which the model bivalve species *Mytilus trossulus* (blue mussel) will be incubated with 17α-ethinylestradiol (synthetic estrogen) or testosterone (masculinising effect). The obtained results will be compared with *in vitro* analyses, where subcellular fractions will be incubated also in the presence of EDs compounds. Another aim of our project is to analyse the effect of model EDs on transcriptomic variability in different bivalves tissues by RNA-seq method. The evaluation of the obtained transcriptomic profiles will allow to narrow the area for searching for genes responsible for the aromatization process in *M. trossulus*. As some microorganisms have enzymes allowing them to metabolise hormones and thus be involved in various aromatisation steps, we also plan to characterize the microbiome of bivalve tissues and water (with feed) using metagenome sequencing and metatranscriptomics (RNA-seq).

Our main goal is therefore to provide key information about steroids origin in marine bivalves by analysing aromatisation pathway in the blue mussels and to assess its potential to be a target for EDs present in marine environments. A critical aspect of the conducted research will be characterization of an enzyme catalyzing aromatisation in bivalves and testing whether or not its activity is involved in sexual determination and maturation of bivalves. Also, the role of steroids and the aromatization as such will be investigated by testing their effects during windows of susceptibility recognized in marine bivalves. Identification of susceptible to hormonal dysfunctions stages in bivalves development will therefore be also achieved.