

13.
Organophosphate-resistant acetylcholinesterase in *Mytilus galloprovincialis*: identification of a resistance Ace gene in cerebral ganglion, gills and adductor muscle

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Acetylcholinesterases (AChE, EC 3.1.1.7) are serine hydrolases, whose primary function in animals is the specific hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses. As such they are target of organophosphate and carbamate insecticides, that modify and inactivate the enzyme, and finally lead to paralysis and death. Past studies on mollusks cephalopods and bivalves reported the presence of AChE forms with high substrate specificity, high catalytic efficiency, and low sensitivity to diisopropylfluorophosphate (DFP). In the most widely used sentinel species in pollution monitoring studies of the marine environment, the Mediterranean mussel *Mytilus galloprovincialis*, the AChE form associated to membrane does not undergo inhibition to DFP.

The present study investigate Ace gene in three different organs of *M. galloprovincialis*: pedal and cerebral ganglia, gills and adductor muscle. The entire Ace gene was amplified in three overlapping fragments. Sequence analysis showed an open reading frame encoding a protein of 624 amino acids with an high homology of 50% with Ace of *Loligo opalescens* and less as 20–41% with others. The general structure of AChE seems conserved in the mussel AChE: three amino acid residues on the catalytic domain as well as Trp which binds the quaternary ammonium of Ach. The deduced amino acids sequence showed also in the acyl pocket the presence of a polar amino acid [N327] in a strategic position commonly occupied in the sensitive forms by a non polar one [F(288) in *Torpedo*]. Such substitution might be responsible of the high substrate specificity toward ASCh of mussel AChE, the absence of hydrolysis of BSCh and the resistance to DFP.

The newly obtained sequence of *M. galloprovincialis* AChE will open to the possibility of studying the evolution and geographic origin of different, resistant and not resistant, AChE alleles.

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14.
Closely related crabs from opposite niches adopt different mechanisms to adjust oxygen transport

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The successful colonization of new environments is often achieved through adaptations or key innovations of existing physiological or biochemical mechanisms. The oxygen supply in marine invertebrates represent a complex and deeply integrated system which plays a fundamental role in animal adaptive plasticity. In particular, species which inhabit highly stochastic environments as shallow water or intertidal bands, have to cope with extremely different regimes of oxygen availability and effectively maintain a stable aerobic metabolism.

Within this framework, we have focused on comparative physiology of Portunid Crabs hemocyanin, to evaluate the role molecular heterogeneity and functional plasticity of oxygen transport regulation. The

analyses concerned the Mediterranean green crab *Carcinus aestuarii*, that inhabits shallow brackish waters with soft bottoms and mussel beds and two obligate aquatic species, the grey swimming crab *Liocarcinus vernalis*, that populates sandy bottoms from lower shore to a depth of –40 m, and the swimming crab *L. depurator*, distributed below the thermocline on fine sandy and muddy bottom. To assess the adaptive regulation of oxygen transport among these species, we have considered the effects that physiological and environmental modulators (lactate, pH and temperature) play on hemocyanin functioning. The biochemical parameters undergo to drastic changes during hypoxic events and show significant variations when animals move from aerobic to anaerobic metabolism. At molecular level we have verified that both lactate and pH play a central role in modulating the hemocyanin oxygen-binding properties and we have singled out the effect of each parameter on the oxygen affinity in resting and hypoxic conditions. From this study, a clear separation between subtidal and intertidal species emerges, evidencing the physiological plasticity that tolerant species exhibit when subjected to stressful conditions and underlying the importance that the oxygen availability has played in the actual ecological distribution. On the contrary, the hemocyanin of the three species does not manifest diverse sensitivities to the temperature effect, indicating a lower response to physical factors in these closely related species.

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POSTER PRESENTATIONS

15.
Activation of 5-HT(1A) receptor induces glycogenolysis in the rainbow trout brain (*Oncorhynchus mykiss*).

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It has been demonstrated that serotonin (5-HT) stimulates brain glycogenolysis in mammals and different 5-HT receptors (5-HT₁, 5-HT₂ subtypes) are involved in the observed decrease in brain glycogen. Previous studies from our laboratory showed that, in the rainbow trout, central 5HT could be a regulatory neurotransmitter of brain energy expenditure activating local glycogenolysis. To expand our research and characterizing 5-HT receptor subtypes implicated on brain glycogen metabolism, the aim of the present study was to investigate the effect of 5HT(1A) receptor activation on brain glycogen levels in the rainbow trout. Reductions in brain glycogen content were obtained following the intraperitoneal administration of the selective 5-HT(1A) agonist 8-OH-DPAT. A significant decrease was observed at the highest dose and the effect was blocked when fish were previously injected with two 5-HT(1A) receptor antagonists (NAN190, WAY100365) From our results we conclude that 5-HT(1A) receptor activation promotes glycogenolysis *in vivo* and may be involved in the serotonergic regulation of trout brain energy balance.

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16.
Changes in the ecdysone response pathway after exposure to different endocrine-disrupting chemicals in the aquatic larvae of *Chironomus riparius*

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