

The sea urchin, a versatile model for eco-toxicity studies and ecological experimental research

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Abstract

Echinoderm early developmental stages represent a good tool for toxicity testing in different fields, ranging from environment to food contamination, and in full respect of the 3Rs objectives (Reduction, Refinement, Replacement of animal experiments), that will lead to the reduction of vertebrate use for toxicity testing. Further, sea urchins are key species in a wide range of marine habitats, as they are able to structure algal community. Experiments and observations aiming at the characterization of anthropogenic or climate changes effect on their settlement, population structure, feeding behaviour and reproductive condition, may be useful to describe future scenarios regarding the whole marine community. The present paper represents a short review of the possible applications of eco-toxicity bioassays using *Paracentrotus lividus* gametes and embryos. Further, examples of ecological researches, involving sea urchins, aiming at the definition of future scenarios will be presented.

Introduction

Early developmental stages of echinoderms, in particular sea urchins, represent a convenient experimental model to investigate the mechanisms driving development and differentiation in higher organisms, including mammalians. This is supported by the fact that recently, the results of the project "Sea Urchin Genome", carried out by the California Institute of Technology, revealed a high degree of mammalian-urchin homologies. Thus, sea urchin early development and differentiation has been proved to be a suitable alternative model for the analysis of risk represented by environmental pollutants on environmental and human health and food contamination [1-4]. Further, such a model fully respects the 3Rs objectives (Reduction, Refinement, Replacement of animal experiments) [i.e. 5], that will eventually lead to the reduction of high vertebrate use for toxicity testing. Sea urchin is one

of the few organismic models considered promising by the European Agency for Alternative models (ECVAM). Further, sea urchins role in marine ecology is particularly relevant. Several sea urchins species, for example *Paracentrotus lividus* and *Arbacia lixula* in the Mediterranean Sea [i.e. 6, 7], and *Diadema antillarum* in the Atlantic Ocean [8] are considered key species of photophilic assemblages. In fact, thanks to their grazing activity, whose power is strongly linked to population structure, they are able to handle the shift from photophilic algal communities to coralline algae dominated ones [i.e. 9]. In this way, the whole ecosystem functioning is potentially affected, the established trophic chains are altered and a wide range of marine organisms (i.e. fishes, corals) are affected, whose populations dynamics is linked to the structure of algal communities [7]. Thus, in order to describe theoretical future scenarios of marine ecosystems, related to the occurring global changes, marine ecologists often focus their attention on sea urchins response to potentially conditioning factors, such as sea water acidification and temperature increase [10] and interference of predator alien species or harmful micro and macro-algae. In this short review, we supply examples of toxicity tests, using the Mediterranean sea urchin *P. lividus*, related to different events and to different contaminants, including pesticides, nanoparticles and pre-chemical analysis of sediments. We will further describe how it has been possible to develop a theoretical future scenario of the Mediterranean sea in relation to global changes by experimentation regarding sea urchins population dynamics.

Toxicity tests

Sea urchin embryonic development has been studied since more than one century, and the complex nets of intercellular communications leading the different events is well known, as well the possibility for environmental molecules and their residuals to interfere with such communications, causing developmental anomalies. The good characteristics of the model are: ease of use, as the sea urchin fertilization occurs outside the maternal body; high number of gametes and embryos, allowing the statistical elaboration of the tests results; speed of responses, mainly for some tests such as the spermiotoxicity test; possibility of standardization intra- and inter-laboratory; cost-effectiveness; high throughput of results. The biomarkers allowed by this model are numerous, and easily quantifiable; e.g. the number of eggs fertilized by control

and exposed sperms, the rate of cell proliferation during the first developmental phases, the number of anomalous larvae (generic toxicity tests) or more specific targets, such as the class of embryonic and larval anomalies [4], intracellular calcium release [11] transcription factors delivery to the nucleus [12].

These tests have been used to characterize the biological effects and targets of agricultural pesticides, such as carbamates and organophosphates [13]; in addition, a number of environmental emerging risks has been quantified and identified by use of the spermio- and embryo-toxicity test, such as cell proliferation enhancement caused by different types of electromagnetic fields [14, 15], radioactivity in suspect areas (unpublished data), metallic nanoparticles from combustion [16].

Experimental ecology

The population structure of sea urchins is conditioned by recruitment, strongly related to larval development, metamorphosis success and juvenile survival, and by adult fitness, primarily linked to food availability and presence of competitors. Thus experimental researches aiming at the characterization of stressors effects on sea urchins population dynamics have to focus on all their life stages, taking in account also inter and intra-specific interactions.

A. lixula and *P. lividus* role in barren grounds formation and maintenance has been deeply studied, both through experimental [17] and descriptive studies [18] and it has been suggested that the former species plays a more relevant role, potentially furthermore increased by the absence of *P. lividus*. In fact, this species grazing on photophilic algae is more competitive although the effect produced on algal communities seems to be less relevant than the coexisting species one. Further, *A. lixula* population fitness, in terms of gonadic production and size frequency distribution features, on barren habitat underlines that this species is better adapted to live on coralline algae dominated environments [6]. In lab experiments regarding temperature effect on larval development, and substrate selectivity for metamorphosis of the two species [19] further underlined *A. lixula* preference for high temperatures (>22°C) and for calcareous or naked rocky substrates, while for *P. lividus* no substrate preference have been reported and lower temperatures (<20°C) seem to favour larval growth and survival. Such findings, taking in account the increasing temperatures of the Mediterranean Sea, lead to hypothesize that *A. lixula* population are going to be increased while *P. lividus* ones may be reduced. Thus, it has been suggested the trigger of a feed-back loop in which barren areas will grow in number and in extension and *A. lixula* settlement will be facilitated.

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