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Understanding the cephalopod immune system based on functional and molecular evidence

C. Gestal*, S. Castellanos-Martínez [1]

Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain

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ABSTRACT

Cephalopods have the most advanced circulatory and nervous system among the mollusks. Recently, they have been included in the European directive which state that suffering and pain should be minimized in cephalopods used in experimentation. The knowledge about cephalopod welfare is still limited and several gaps are yet to be filled, especially in reference to pathogens, pathologies and immune response of these mollusks. In light of the requirements of the normative, in addition to the ecologic and economic importance of cephalopods, in this review we update the work published to date concerning cephalopod immune system. Significant advances have been reached in relation to the characterization of haemocytes and defensive mechanisms comprising cellular and humoral factors mainly, but not limited, in species of high economic value like *Sepia officinalis* and *Octopus vulgaris*. Moreover, the improvement of molecular approaches has helped to discover several immune-related genes/proteins. These immune genes/proteins include antimicrobial peptides, phenoloxidases, antioxidant enzymes, serine protease inhibitor, lipopolysaccharide-induced TNF-α factor, Toll-like receptors, lectins, even clusters of differentiation among others. Most of them have been found in haemocytes but also in gills and digestive gland, and the characterization as well as their precise role in the immune response of cephalopods is still pending to be elucidated. The assessment of immune parameters in cephalopods exposed to contaminants is just starting, but the negative impact of some pollutants on the immune response of the common octopus has been reported. This review summarizes the current status of our knowledge about the cephalopod immune system that seems to be far from simply. On the contrary, the advances gained to date point out a complex innate immunity in cephalopods.

1. Introduction

Cephalopods are a small Class of the Phylum Mollusca. Currently more than 700 species (octopuses, squids, cuttlefish and nautiluses) are recognized occupying all the world's oceans, from the intertidal areas to the deep sea. Interest in cephalopods has increased considerably in the last years, mainly because they are the subject of important fisheries with high market value. Since the decline of traditional fisheries, cephalopods have gained attention in aquaculture practice. Among cephalopods, the common octopus *Octopus vulgaris* and the cuttlefish *Sepia officinalis* are good candidate species in European aquaculture, and especially in Mediterranean countries (Spain, Portugal and Italy) because of their short life cycle and fast growth, readily adaptation to captivity conditions, high feed efficiency, high reproductive rate, and high nutritional value and market price. In America, the candidate species are *Octopus maya, Octopus mimus, Robsonella fontaniana, O. vulgaris*, *Octopus bimaculoides* and *Enteroctopus megalocyathus*. The aquaculture of these holobenthic and merobenthic species is still under experimentation because, as in the rest of the cephalopods, the feeding habits of paralarvae have not been resolved [1]. A state of the art of cephalopod culture today providing scientific reference for aquaculture and on-growing different cephalopod species all around the world has been published recently [2].

Nowadays the common octopus is cultured in Spain in on-growing cages in a semi-open system (Fig. 1). This system relays on captured wild individuals but the lack of hatcheries that could supply with juveniles remains a major problem. Thus, to close the complete cycle in captivity is still a challenge for the aquaculture.

* Corresponding author. Tel./fax: +34 986 231930x290.
E-mail address: cgestal@iim.csic.es (C. Gestal).

[1] Present address: Departamento de Recursos del Mar, Centro de Investigación y de Estudios Avanzados del IPN Unidad Mérida, Antigua carretera a Progreso Km. 6, 97310 Mérida, Yucatán, Mexico.

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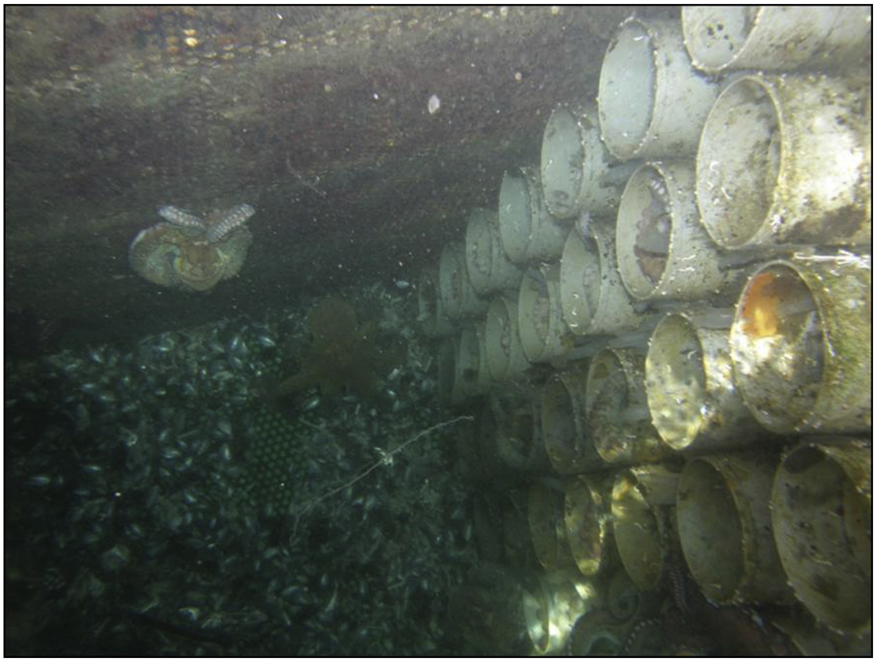

Fig. 1. *Octopus vulgaris* reared in on-growing cages in Ria of Vigo (Galicia, Spain). Courtesy of Rubén Chamorro.

At present, life cycle from hatching to settlement or beginning of the benthonic adult phase is not commercially viable [3] due to the paralarval high mortality. Currently special attention is focused on the optimization of octopus early stages in rearing conditions, not only for the high economical interest of their aquaculture, but also due to the requirement of the new European Directive 2010/63/EU to minimize the use of captured animals from the wild to be used in experimentation. These studies on early stages are providing relevant knowledge not only on nutritional physiology, but also on the effect of environmental stress on the immune-competence status of paralarvae and the identification of biomarkers of welfare and health as key factors that could help to resolve successfully the culture of this species [4].

In addition, cephalopods have a high value as experimental animals for biomedical and behavioral research [5,6]. In fact, the class Cephalopoda is considered the most complex one in the phylum Mollusca and it is call as “advanced invertebrate” or “exceptional invertebrate class” [7]. They have evolved many characteristic features such as highly differentiated multi-lobular brains, a vertebrate-like eye, close circulatory system, a sophisticated set of sensory organs and fast jet-propelled locomotion [8,9] that make them interesting models for research.

1.1. Cephalopods as subjects of the European Directive

The recent inclusion of “all live cephalopods” in the Directive 2010/63/EU that regulates the use of animals for scientific purposes (European Parliament and Council of the European Union 2010) [10] represents the first time that an entire class of invertebrates has been included in laboratory animal legislation throughout the EU. The decision was based on the evidence for sentience and capacity to experience pain, suffering, distress and lasting harm [11–13]. Until now only vertebrates have been included in the National legislation regulating experimentation on living animals. The only exception was the inclusion of the species *Octopus vulgaris* in a revision of the UK legislation, but no studies had been conducted under this legislation. Outside Europe, cephalopods have been included in various National legislations covering research, such as Canada, New Zealand, Australia, Switzerland and Norway [14].

The inclusion of cephalopods in the European Directive, based on the reinforcement of the 3Rs (reduction, refinement and replacement) policy, obliges cephalopod researchers and technicians to promote the best welfare practices during aquaculture procedures, maintenance and experimentation on cephalopods. However, the lack of knowledge on different aspects of this class, including general care and principles of good practice, welfare, health and housing requirements, among others, requires the description of guidelines for different species. Currently, few references concerning ethical considerations and welfare in cephalopods used in experimentation were published [12,15]; The first guideline for the care and welfare of cephalopods in research [16] has been recently developed as a result of a joint international initiative between researchers as an interdisciplinary network of experts with the objective of improving procedures and foster scientific exchanges to integrate knowledge on welfare practices. This guideline cover topics such as implications of the Directive for cephalopod research, project application requirements and authorization process, the application of the 3Rs principles, the need for harm-benefit assessment and severity classification, as well as species specific requirements and scientific procedures. Although a great effort in publishing new experimental data have been observed in last years, the knowledge about cephalopod welfare is limited and scattered among laboratories, and there are gaps of scientific knowledge regarding several aspects of health assessment and well-being.

1.2. The past and present of the cephalopod immune system

One of the most important issues for aquaculture and also for experimental aquarium maintenance is the animal health. High population densities, animal manipulation, and culture conditions are associated with stress, which affects the immune-competence and favors the development of infectious diseases. To avoid

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