ISSN: 0001-5113 AADRAY	ACTA ADRIAT., 46 (2): 193 - 199, 2005	UDC: 594.56:616.993 (262) Original scientific paper

Aggregata infection in the common octopus, *Octopus vulgaris* (Linnaeus, 1758), Cephalopoda: Octopodidae, reared in a flow-through system

Ivona MLADINEO* and Mladen JOZIĆ

Laboratory of Aquaculture, Institute of Oceanography and Fisheries, P.O. Box 500, 21000 Split, Croatia

****Corresponding author, e-mail: mladineo@izor.hr***

*Along with the introduction of the common octopus (*Octopus vulgaris*) to rearing systems in the Mediterranean by fattening or experimental paralarval production, emergence of diseases has become a concern. The most devastating infection of reared octopus stock is the coccidian parasite* Aggregata *sp. (Apicomplexa: Aggregatidae) that causes weight loss, excitation and behavioral changes, and miliar subcutaneous parasitic cysts. During eight months of experimental fattening of common octopus in the aquaculture facilities of the Institute of Oceanography and Fisheries (Split, Croatia), 7% mortality was attributed to infection by* Aggregata *sp. Although the detailed life cycle of the genus* Aggregata *is not yet completely elucidated, it is known that this heteroxenous coccidian uses crustaceans for merogony, which comprise 30% of the cephalopod's diet. For the moment, only adequate zooprophylactic measures can prevent the emergence of the infection in the rearing system. Preventing introduction of adult specimens into the rearing system is a critical zooprophylactic measure, along with stocking only juvenile cephalopods that are uninfected by the coccidian through the food web in the natural environment and are, thus, unable to introduce the infection into the rearing system. The second measure involves avoiding crustaceans in the rearing diet.*

Key words: common octopus (*Octopus vulgaris*), rearing, coccidia, *Aggregata* sp.

INTRODUCTION

The common octopus, *Octopus vulgaris* (Linnaeus, 1758), is among the first cephalopod candidates for introduction to aquaculture because of its ubiquitous distribution, adaptability to rearing conditions, high growth rate, and nutritive characteristics (70% protein; IGLESIAS *et al.,* 2000). Adopting a variety of rearing and nutritive strategies, Spanish researchers today lead in the experimental rearing of the common octopus from egg to adult stage. The bottleneck in large-scale production is finding a balanced diet for paralarvae (NAVARRO & VILLANUEVA, 2003). The fattening of octopus is a commercially important process in a growing number of Mediterranean countries (IGLESIAS *et al.,* 2000). Fattening consists of catching adult specimens and feeding them for 10-12 months until the weight has considerably increased. This procedure was experimentally developed in the Laboratory of Aquaculture of the Institute

of Oceanography and Fisheries in Split, Croatia, with satisfactory growth results. However, a small percent of mortalities occurred.

Mortalities caused by infective agents are among the most limiting factors during the development of rearing processes for new aquaculture candidates since pathogens can more easily spread in rearing conditions than in natural environments. Three *Aggregata* species have been isolated from European waters, with slight morphological differences in cyst and sporozoite appearance, size, and number (SARDELLA *et al.,* 2000). The common octopus is parasitized by *A. octopiana* (ESTÉVEZ *et al.,* 1996; PASCUAL *et al.,* 1996), the cuttlefish *Sepia officinalis* by *A. eberthi*, and the flying squid *Todarodes sagittatus* by *A. sagittata* (GESTAL *et al.*, 2000). Based on electron microscopy, *A. spinosa* is synonymous with *A. octopiana* (GESTAL *et al.,* 1999).

The detailed life cycle of *A. octopiana* is not yet completely described. The coccidian is heteroxenous, showing merogony in decapod crustaceans as an intermediate host and gametogony and sporogony in cephalopods as the final host, causing infection of the final host through the food chain (GESTAL *et al.,* 2002).

MATERIAL AND METHODS

Wild common octopi (*O. vulgaris*) were caught in January 2004 in the southwestern waters of Brač Island in the Adriatic Sea. The specimens were experimentally reared in the aquaculture facility of the Institute of Oceanography and Fisheries in Split. Thirty individuals (250-500 g) were placed in concrete tanks of 4 m^3 with a flow-through system, natural photoperiod, and plastic suspensors and artifacts on the bottom for shelter. The octopi were fed daily frozen or fresh teleost species such as *Sardina plichardus, Trisopterus minutus, Micromesistius poutassou,* and *Trachurus trachurus* (70%) and fresh crustaceans, mostly the warty crab *Eriphia verrucosa* and lesser spider crab *Maja crispata* (30%).

Diseased octopi were examined for gross pathology and autopsied. Fresh smears of cysts from different organs were mounted on slides and observed under a light microscope. Coccidian oocysts, sporocysts, and sporozoites from the fresh smears were described and measured. Infected tissues (muscular epidermal tissue, intestine, gills) were dissected, fixed in modified Davidson fixative, and processed for routine histology. Briefly, tissues were dehydrated in increasing alcohol concentrations, cleared in chloroform, and embedded in paraffin blocks (Histowax, Leica). Blocks were cut into 5-µm sections, stained with hematoxylin-eosin, and observed under a light microscope. Microphotographs were measured using DP-soft Olympus software for image processing.

RESULTS

In 7% of the reared and diseased cephalopods, a superinfection of the coccidian parasite *Aggregata* sp. (Apicomplexa: Aggregatidae) was demonstrated, indicating the necessity for a more elaborated nutritional plan to avoid or at least drastically decrease the percentage of crustaceans in the octopus diet.

Mortalities began mainly in July-August in animals that had exhibited changes in feeding habits. Food consumption in such octopi decreased approximately two months before the mortalities began, ceasing completely one month before death. These animals also displayed changes in behavior, becoming excited, often leaving shelters and swimming in the tank, and becoming incapable of color adaptation and camouflage. They were inactive in the shelters and unresponsive to outside stimuli a few days before dying. Occasionally aggressive behavior was noticed, even when the density of animals of similar body weights was low.

Dead octopi weighed 1380-1510 g, gaining only three times their initial weight in eight months. The integument of diseased octopi had areas of large exfoliation and external lesions, with miliary disseminated yellow cysts measuring 278 x 259 µm in the epidermis and noticeable to the naked eye (Fig. 1).

Even though no swabs were taken for bacteriological purposes, external lesions could have been the result of secondary bacterial

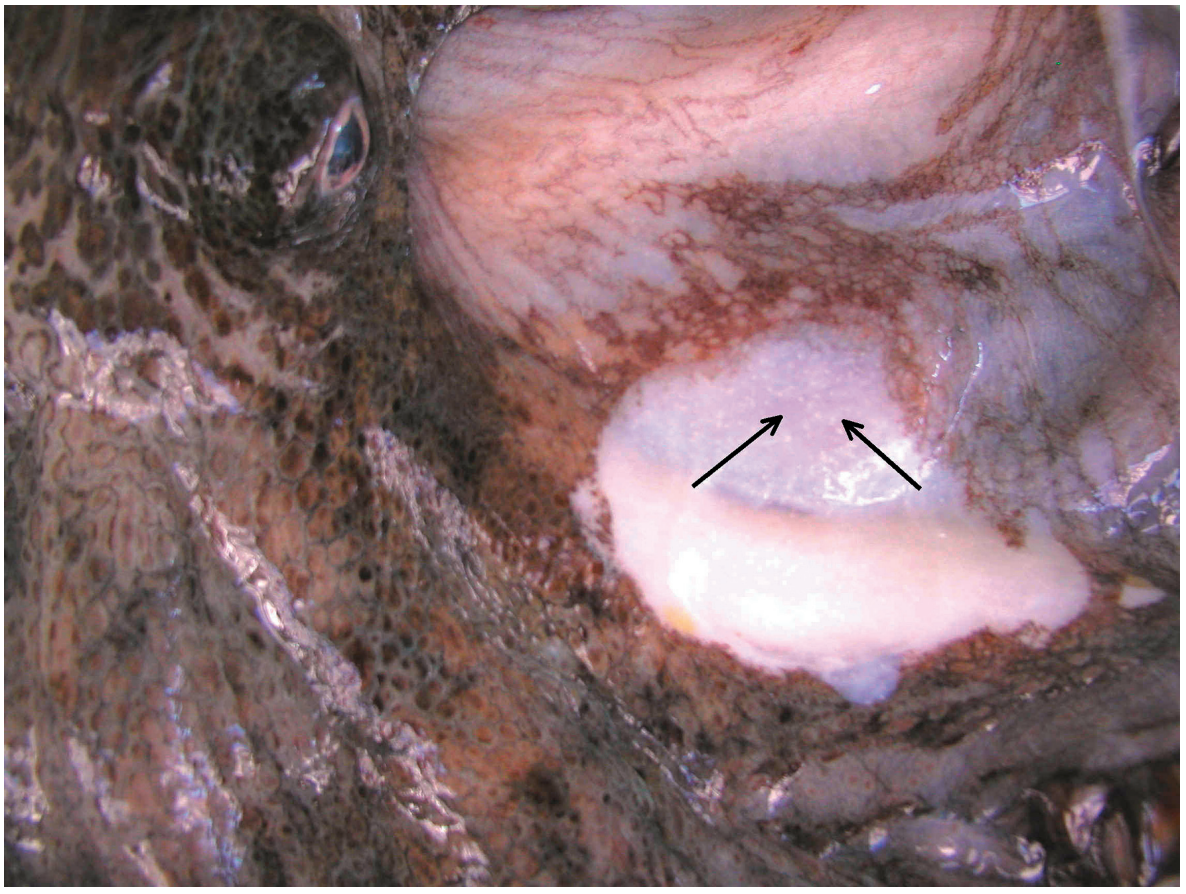

Fig. 1. Area of exfoliation of the mantle, showing miliary disseminated yellow cysts in the epidermis, noticeable to the naked eye

infection. Cysts were found throughout the cephalopod body, gills, and digestive tract, especially in spiral ceca. A large number of subcutaneous cysts, together with the presence of cysts of different sizes in the *lamina propria* of the digestive tract, indicated superinfection. Cyst walls revealed tissue reaction at the sites of the coccidian infection, however, they were thin and had no extensive connective tissue proliferation or hemocytic infiltration.

Sporocysts were numerous, uniform, spherical to slightly ovoid, and ranged 10.77-13.12 (mean 11.56±0.54) by 11.13-13.58 µm (mean 12.08±0.64 µm; n = 30; Fig. 2). The sporocyst walls were 0.48-1.02 µm (mean 0.66±0.16 µm). Four to five sporozoites (n = 30; Fig. 3), measuring 1.51-2.69 µm (mean 2.06±0.33 µm) in thickness, were curled within a sporocyst. As they were not isolated from the sporocyst, their length was impossible to measure.

Histological sections of intestinal mucosa revealed complete substitution of tissue by a large number of cysts. The process was most evident in cecal tissue, showing strong deterioration and substitution with mature cysts (Fig. 4). No glandular elements were evident subepithelially. *Lamina mucosa* lost their microvillous epithelial surface, showing numerous development stages of sporogony. Connective tissue of *lamina propria* had a loose and spongy structure, with scarce cellular or connective elements and abundant proliferating cysts of different sizes. Cysts were present in gill and subcutaneous tissue, eliciting no extensive tissue response and having a lower intensity than in digestive tissue.

Fig. 2. Wet mount of sporocysts. Arrow shows curled sporozoites within the sporocysts, x 1000, scale bar 20 µm

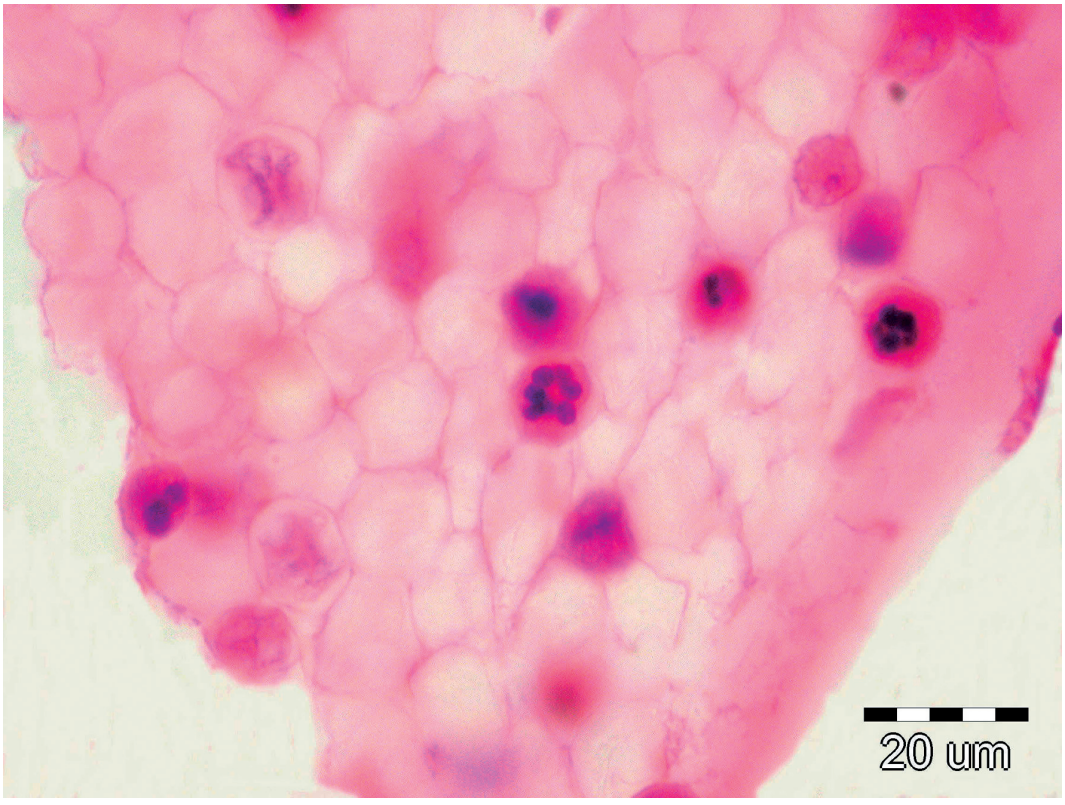

Fig. 3. Hematoxylin-eosin stained sporozoites within the sporocysts from the spiral ceca section, x 1000, scale bar 20 µm

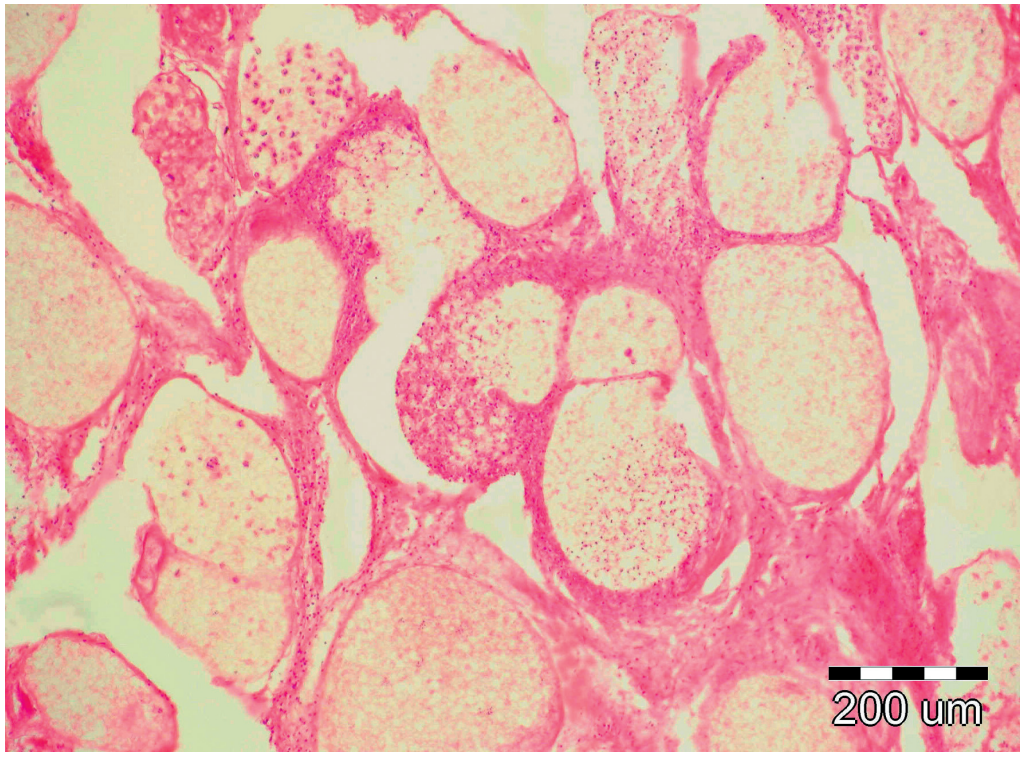

Fig. 4. Histological section of cecal portion completely filled with yellowish Aggregata *cysts (hematoxylin-eosin), x 400, scale bar 20 µm*

DISCUSSION

Sporocysts of the isolated *Aggregata* sp. fitted within the usual size range of isolated *A. octopiana* cysts from common octopus, even though they differed in an important identification parameter: the number of sporozoites. *Aggregata* sp. from the Adriatic octopus harbored only four to five sporozoites, which in *A. octopiana* can reach eight (GESTAL *et. al.,* 1999*,* 2002). Because of this characteristic, which constitutes one of the main rules for taxonomic diagnosis of eimerians (DAVIES & BALL, 1993), the isolated *Aggregata* resembled *A. eberthi*, an eimeriorin parasite of common cuttlefish (*S. officinalis*), more than *A. octopiana*. The similar number of sporozoites and the larger sized sporocysts are common to the newly isolated species *A. sagittata* from the flaying squid (*T. sagittatus*). Against the classification of the isolated *Aggregata* sp. as *A. eberthi*, however, stands the fact that the isolated oocysts were morphologically more similar to *A. octopiana* as they are spherical and not ovoid as in the case of *A. eberthi.* A precise classification could be based on SS rRNA gene sequence or SDS-PAGE and immunoblotting (RODRIGUEZ *et al.,* 2003).

The conspicuous taxonomy of the isolated *Aggregata* sp. and the massive superinfection represent a serious threat for experimental or commercial octopus rearing. Although it is very likely that the octopi brought the parasite from the wild environment, intensive feeding with crustaceans, the apicomplexan intermediate hosts during rearing, exceeded the sustainable infection level, resulting in superinfection and mortality. While the mortality rate seemed low, it was observed in experimental conditions with only 30 octopus specimens. In intensive commercial rearing, the infection could be manifested at a much higher rate and the fact that in nature there is 100% prevalence of *Aggregata* sp. in adult octopi (PASCUAL *et al.,* 1996) should be seriously taken into account.

Commercial rearing of the common octopus could suffer great economical losses from the infection because, in addition to mortality, the eimerian causes low weight gains as noted in

this study. IGLESIAS *et al.* (2000) reported that small octopi of 300-500 g brought for rearing usually gain 2.5 kg in only four months. In this study, the weight of the infected octopi only tripled after eight months.

Live but infected octopi have noticeable disseminated yellowish cysts throughout the mantle, cannot be sold to fish markets, and are declared unsuitable for human consumption based on the by-laws of veterinary-sanitary inspections for the control of animals before slaughtering and products of animal origin (NARODNE NOVINE, 1991) and article 13, point 2 of the Food Law (NARODNE NOVINE, 2003).

To attenuate or completely prevent the emergence of *Aggregata* sp. in reared octopi, zooprophylactic measures are the only measures that can be successfully employed; no available or applicable chemotherapeutics exist. First, only the smallest juvenile octopi should be chosen for rearing and, second, their diet should consist of balanced meals without crustaceans. On-going experimental rearing in our laboratory, which includes trial feeding with slaughterhouse wastes, will help clarify this topic.

REFERENCES

DAVIES, A.J. & S.J. BALL. 1993. The biology of fish coccidian. Advanced Parasitol., 32: 294-366.

ESTÉVEZ, J., S. PASCUAL, C. GESTAL, M. SOTO, H. RODRIGUEZ & C. ARIAS. 1996. *Aggregata octopiana* (Apicomplexa: Aggregatidae) from *Octopus vulgaris* off NW Spain. Dis. Aquat. Org., 27: 227-231.

GESTAL, C., S. PASCUAL, L. CORRAL & C. AZEVEDO. 1999. Ultrastructural aspects of the sporogony of *Aggregata octopiana* (Apicomplexa, Aggregatidae), a coccidian parasite of *Octopus vulgaris* (Mollusca, Cephalopoda) from NE Atlantic. Eur. J. Protistol., 35: 417-425.

GESTAL, C., A. GUERRA, E. ABOLLO & S. PASCUAL. 2000. *Aggregata sagittata* n. sp. (Apicomplexa: Aggregatidae), a coccidian parasite from the European flying squid *Todarodes sagittatus* (Mollusca: Cephalopoda). Syst. Parasitol., 47: 203-206.

GESTAL, C., A. GUERRA, S. PASCUAL & C. AZEVEDO. 2002. On the life cycle of *Aggregata eberthi* and observations on *Aggregata octopiana* (Apicomplexa, Aggregatidae) from Galicia (NE Atlantic). Eur. J. Parasitol., 37(4): 427-435.

IGLESIAS, J., F.J. SANCHEZ, J. OTERO & C. MOXICA. 2000. Ongrowing, reproduction and larvae rearing of octopus (*Octopus vulgaris* Cuvier), a new candidate for aquaculture in Galicia (NW Spain). Cah. Opt. Mediterran., 47: 313-321.

NARODNE NOVINE. 1991. By-law on the methods of veterinary-sanitary inspection and control of animals before slaughtering and products of animal origin (NN RH 52/91).

NARODNE NOVINE. 2003. Food Law (NN RH 117/03, 130/03).

NAVARRO, J.C. & R. VILLANUEVA. 2003. The fatty acid composition of *Octopus vulgaris* paralarvae reared with live and inert food: deviation from their natural fatty acid profile. Aquaculture, 219: 613-631.

PASCUAL, S., C. GESTAL, J.M. ESTEVEZ, H. RODRIGUEZ, M. SOTO, E. ABOLLO & C. ARIAS. 1996. Parasite in commercially-exploited cephalopods (Mollusca, Cephalopoda) in Spain: an update perspective. Aquaculture, 142: 1-10.

RODRIGUEZ, H., C. CRESPO, M. SOTO, C. ARIAS, R. IGLESIAS & J.M. GRCIA-ESTEVEZ. 2003. Antigenic relationships between *Aggregata octopiana* and *A. eberthi* two parasites of cephalopods. Acta Protozool., 42: 191-195.

SARDELLA, N.H., M.E. RE & J.T. TIMI. 2000. Two new *Aggregata* species (Apicomplexa: Aggregatidae) infecting *Octopus tehuelchus* and *Enteroctopus megalocyathus* (Mollusca: Octopodidae) in Patagonia, Argentina. J. Parasitol., 86 (5): 1107-1113.

Received: 22 February 2005
Accepted: 9 November 2005

Aggregata sp. infekcija u hobotnici, *Octopus vulgaris* (Linnaeus, 1758), Cephalopoda: Octopodidae, uzgojenoj u protočnom sustavu

Ivona MLADINEO* i Mladen JOZIĆ

Institut za oceanografiju i ribarstvo, P.P. 500, 21 000 Split, Hrvatska
**e-mail: mladineo@izor.hr*

SAŽETAK

Zajedno s trendom uvođenja hobotnice (*Octopus vulgaris*) u uzgoj u Sredozemlju putem "fatteninga" ili eksperimentalne produkcije ličinki, suočeni smo s brigom o mogućem izbijanju bolesti u uvjetima uzgoja. Najštetnija infekcija za uzgojenu hobotnicu pokazala se ona kokcidijom *Aggregata* sp. (Apicomplexa: Aggregatidae), koja uzrokuje gubitak težine, prenadražljivost, promjene ponašanja i diseminirane milijarne potkožne ciste. U pogonu akvakulture Instituta za oceanografiju i ribarstvo u Splitu, tijekom eksperimentalnog "fatteninga" hobotnice, 7% mortaliteta tijekom 8 mjeseci vezano je za infekciju *Aggregata* sp. Iako životni ciklus roda Aggregatida nije u potpunosti rasvijetljen, poznato je da ova heteroksena kokcidija koristi rakove za merogoniju, koji čine 30% obroka uzgojenih glavonožaca. Trenutno samo odgovarajuće zooprofilaktičke mjere mogu spriječiti izbijanje infekcije u uzgoju. Izbjegavanje uvođenja odraslih jedinki u uzgoj bila bi jedna od osnovnih zooprofilaktičkih mjera, uz izlov samo juvenilnih glavonožaca koji nisu susreli kokcidiju kroz hranidbenu mrežu u prirodi, pa tako nisu sposobni uvesti infekciju u uzgoj. Druga mjera bi se sastojala od izbjegavanja rakova u obroku.

Ključne riječi: hobotnica (*Octopus vulgaris*), uzgoj, kokcidija, *Aggregata* sp.