Characterization of the skin mucus in the common octopus *Octopus vulgaris* (Cuvier) reared paralarvae

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Abstract

The *Octopus vulgaris* farming is impaired by the high mortality of the paralarvae during the first month of life. Several factors have been investigated in this regard, but no data exist on the body surface mucus, which represents the interface with the outside environment. This study included morphometric analysis and glycoconjugate characterization of skin mucus in reared *Octopus vulgaris* paralarvae during the first month of life. Four types of mucous cells were distinguished: mucous 1 (m1) and mucous 2 (m2) cells were scattered in the mantle epidermis, mucous 3 (m3) and mucous 4 (m4) in the epithelium surrounding the sucker. Except for the presence of fucosylated and neutral glycoconjugates in all mucous cells, each cell type expressed a characteristic glycopattern. m2 and m4 contained also sulphate and acid non-sulphate glycans, m3 lacked sulphate glycoproteins. Lectin histochemistry showed that mantle mucous cells (m1, m2) expressed GlcNAc and lactosamine terminating glycan, m3 lacked sulphate glycoproteins. However, skin morphological changes have been described for camarobranchiids, phophophores and Kelliker’s organ during the growth of the cephalopods paralarvae and in particular in the genus *Octopus*. Thus, despite the epidermis represents the outermost tissue directly interacting with the environment and the first line of body defense, to date nothing is known about its mucus content along the *O. vulgaris* paralarva body, including the suckers. These structures play a key role in a variety of crucial processes for the paralarvae survival such as the grasping, manipulating and investigating objects and suction process.

Introduction

The cephalopod *Octopus vulgaris* is an important candidate for aquaculture although the farming is impaired due to the high mortality of the small planktonic hatching, conventionally called paralarvae. Several authors have suggested that the transition from yolk utilization to active predation is a critical period in the early life history of cephalopods due to low rates of survival. The high mortality problem has been related to the diet and its nutritional content. A nutritional imbalance in the proteins, lipids, minerals and other nutrients of the artificial feed has been pointed as one of the reasons for this high mortality. Several factors have been investigated about the paralarvae survival such as swimming and feeding behaviour, ontogenic changes in size and shape, arm growth, water circulation, den availability, food type.

The skin of cephalopods is a complex system involved in a number of functions, such as lubrication, protection against mechanical damage, microbes, viruses, or proteolytic degradation, osmotic and ionic regulation, oxygen and nutrient uptake, sensitivity, camouflage and communication. As in other aquatic organisms, several of these functions are mediated by glycoproteins constituting the mucus, a gel-like and viscous substance, secreted by mucous cells. Among the species belonging to phylum Mollusca, the mucus has been extensively studied in gastropods and bivalves. Concerning the cephalopods, mucus secreting cells have been observed in the skin of adults.

However, skin morphological changes have been described for camarobranchiids, phophophores and Kelliker’s organ during the growth of the cephalopods paralarvae and in particular in the genus *Octopus*. Thus, despite the epidermis represents the outermost tissue directly interacting with the environment and the first line of body defense, to date nothing is known about its mucus content along the *O. vulgaris* paralarva body, including the suckers. These structures play a key role in a variety of crucial processes for the paralarvae survival such as the grasping, manipulating and investigating objects and suction process.

This study dealt with the morphological features and the glycoconjugate patterns of the skin from reared *O. vulgaris* paralarvae, in order to provide new insights about this critical period of the early life of this species.

Materials and Methods

Animals

Paralarvae of *Octopus vulgaris* from Maricolutta Mattinatense (Mattinata, FG, Italy) in partnership with Acquacoltura Jonica (San Vito, TA, Italy) were collected during aquaculture trials financially supported by F.P. 2007-2013 - Reg. CE n. 1198/2006 - “Reproduction and breeding of the common octopus, Octopus vulgaris”. Mated *O. vulgaris* females (n=12) were kept in captivity using the technology described by Moxica et al. Briefly, about two thousands of recently hatched (day 0) paralarvae were transferred to a 1 m³
Sampling and histology processing

Fifty paralarvae were collected from June to August 2013 at 1-4, 11-14 and 21-28 days after hatching and labelled as P01, P02 and P03, respectively. According to guidelines of European Directive 201/63/UE concerning the humane killing of animals in research and to Villanueva and Norman, after collection the paralarvae were anesthetized in a 2% ethanol-seawater and fixed in 4% (v/v) PBS-buffered paraformaldehyde for 24 h at 4°C. Then the samples were dehydrated through a graded alcohol series and embedded in paraffin wax. Serial sections (4 µm thick) were cut and, after dewaxing with xylene and hydration in an ethanol series of descending concentrations, were stained with Hematoxylin-Eosin for morphological and morphometric studies and, by means of conventional histochemical procedures or lectin histochemistry for glycoconjugate characterization.

Conventional histochemistry

For the general staining of carbohydrates Periodic acid-Schiff (PAS) was performed. Acidic glycans were detected with Alcian Blue pH 2.5 (AB pH 2.5). Combined High iron diamine-Alcian Blue pH 2.5 (HD-AB pH 2.5) was used to stain simultaneously sulphated (brown-black) and non-sulphated (blue) acidic glycans. Details were about histochemical techniques can be found in Scillitani et al. All the chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

Lectin histochemistry

Binding of ten FITC-labelled lectins (Vector Laboratories, Burlingame, CA, USA) was tested to investigate the composition and distribution of oligosaccharidic chains in mucins (Table 1 and references therein). Sections were incubated for 1 h at room temperature with the lectin solution in HEPES and subsequently rinsed in the same buffer and mounted in Fluoromount (Sigma-Aldrich). Each experiment was repeated twice on each sample.

Controls for lectin labelling were performed by i) substitution of lectin with HEPES alone; ii) incubation in lectin with inhibitory sugar added (0.2-0.5 M); iii) binding to the egg envelope of the toads Bombina pachypus and Bufo bufo, the mucus of which are demonstrated to be labelled by the tested lectins. Lectin-binding was observed in epifluorescence under 495 nm light emission. Photos were taken by a Nikon DMX 1200 camera coupled with a Nikon Eclipse 600 photomicroscope (Nikon Instruments SpA, Calenzano, FI, Italy).

Morphometry and statistical analysis

Four types of PAS positive muciparous cells (two in the mantle and two in the arms skin) were detected during the investigated periods (P01, P02, P03). The number of each muciparous cell type was counted on 30 microphotograph fields randomly detected in PAS-Hematoxylin stained sections using a 60x lens, and the cell abundance was expressed as mean numbers of mantle muciparous cells/100 µm and as number of mucous cells per sucker. Image analysis was performed by ImageJ software. Statistical evaluations were performed separately for each cell type. Descriptive statistics (arithmetic mean, standard deviation, sample variance, standard error, kurtosis, skewness) were computed for each sample. The normality of sample variances was tested by Shapiro-Wilk tests and the homogeneity of sample variances was tested by Levene’s tests. The significance of variation among mean values was evaluated by both parametric one-way Analysis of Variance (ANOVA) and non-parametric Kruskal-Wallis test (KW). Post-hoc pairwise comparisons allowed to detect the pairs of sample values which differed significantly and were performed with both parametric Tukey’s Honestly-Significant-Difference (THSD, related to ANOVA) and non-parametric Dwass-Steel-Chritchlow-Fligner.

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**Table 1. Lectin used, their sugar specificities and the inhibitory sugars used in control experiments.**

<table>
<thead>
<tr>
<th>Lectin abbreviation</th>
<th>Source of lectin</th>
<th>Binding specificity</th>
<th>µg/mL</th>
<th>Inhibitory sugar concentration</th>
</tr>
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<tbody>
<tr>
<td>WGA</td>
<td>Triticum vulgare</td>
<td>(GlcNAcβ1,4)n</td>
<td>20</td>
<td>0.5 M GlcNAc</td>
</tr>
<tr>
<td>GSA II</td>
<td>Griffonia simplicifolia</td>
<td>GlcNAc</td>
<td>10</td>
<td>0.5 M GlcNAc</td>
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<tr>
<td>SNA</td>
<td>Sambucus nigra</td>
<td>Neu5ac2,6Gal/GalNAc</td>
<td>20</td>
<td>0.2 M Neu5ac</td>
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<tr>
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<td>Arachis hypogaea</td>
<td>Galβ1,3GlcNAc</td>
<td>10</td>
<td>0.2 M Gal</td>
</tr>
<tr>
<td>RCA I</td>
<td>Ricinus communis</td>
<td>Galβ1,4GlcNAc</td>
<td>20</td>
<td>0.2 M Gal</td>
</tr>
<tr>
<td>SBA</td>
<td>Glycine max</td>
<td>GalNAc</td>
<td>10</td>
<td>0.2 M GalNAc</td>
</tr>
<tr>
<td>Con A</td>
<td>Canavalia ensiformis</td>
<td>D-Man, D-Glc</td>
<td>20</td>
<td>0.1 M MucM</td>
</tr>
<tr>
<td>AAA</td>
<td>Aleuria aurantia</td>
<td>Fucα1,6GlcNAc-βNAsn; Fucα1,3, Fucβ1,4</td>
<td>10</td>
<td>0.2 M L-Fuc</td>
</tr>
<tr>
<td>UEA1</td>
<td>Ulex europaeus</td>
<td>Fucα1,2</td>
<td>10</td>
<td>0.2 M L-Fuc</td>
</tr>
<tr>
<td>LTA</td>
<td>Tetragonolobus purpuratus</td>
<td>L-Fucα1,6GlcNAc; L-Fucα1,2Galβ1,4; L-Fucα1,3 Galβ1,3; GlcNAcβ1,6R</td>
<td>20</td>
<td>0.2 M L-Fuc</td>
</tr>
</tbody>
</table>

Ann, araripine; Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; MucM, methyl-α-mannopranoside; Man, mannose; Neu5Ac, N-acetylleucaminic (sialic) acid.
(DSCF, related to Kruskal-Wallis) tests. Significance for probability computed from tests was set at P<0.01. Statistical computations were generated by the Real Statistics Resource Pack software release 4.3. All data were expressed as means ± standard error (SE).

Results

Morphology

The gross outer morphology of *O. vulgaris* paralarvae displayed relatively short arms, a large buccal mass, two large eyes and several chromatophores distributed in the skin covering the mantle and arms (Figure 1A).

The epidermis covering the mantle of paralarvae showed a simple columnar epithelium constituted by epitheliocytes and mucous cells (Figure 1B). The epidermis was crossed by several Kölliker’s organs (Figure 1B). The head portion of paralarva body contained well developed and differentiated arms. The ventral side of each arm showed at least three suckers which were constituted by two “chambers”: the infundibulum and the acetabulum (Figure 1C).

The infundibulum was in the outer part and the acetabulum in the inner part of the sucker (Figure 1C). The infundibulum is the exposed portion of the sucker and the acetabulum is a spheroidal cavity that opens into the infundibulum through an orifice (Figure 1C). The epithelium covering the infundibulum was simple cuboidal, whereas the epithelium lining the acetabulum as well as the space interposed between adjacent suckers had a simple squamous appearance (Figure 1C).

Conventional histochemistry

The results of histochemistry methods are summarized in Table 2. The epitheliocytes of both the mantle and arms showed no cytoplasmic staining with all the used histochemical methods. As for the skin, apical surface it displayed PAS positivity in the mantle and suckers (Figure 2 A,B), whereas it showed AB 2.5 staining in the mantle (Figure 2C) and HID reactivity in the cuticle of the sucker infundibulum. Four different types of mucus-containing cells (named m1, m2, m3, m4) were distinguishable based on their appearance, localization and histochemical reactivity (Figure 2, Table 2). m1 and m2 cells showed columnar shape.
and were in the mantle (Figure 2 A,C,E). m1 cells were coarse-grained PAS positive cells (Figure 2A), whereas m2 cells were fine-grained cells displaying strong PAS positivity (Figure 2A). Some m2 cells showed moderate affinity with both AB 2.5 (Figure 2C) and HID (inset of Figure 2E) staining. m3 and m4 cells were found in the epithelium surrounding the suckers. m3 cells were roundish in shape and were scattered in the epithelium surrounding the infundibulum (Figure 2B,D,F) and strongly stained with PAS (Figure 2B) and AB 2.5 (Figure 2 D,F). m4 were round cells scattered in the epithelium surrounding the acetabulum zone of the sucker (Figure 2B,D,F). They displayed PAS (Figure 2B) and AB 2.5 (Figure 2D) positivity, although some of them exhibited HID positivity (Figure 2F). No staining variation was detected in the mantle and arm structures during the investigated periods. Kölliker’s organs showed weak staining with the used methods (Figure 2 A,C,E).

Lectin histochemistry

The results of lectin histochemistry are summarized in Table 2 and illustrated in Figures 3 and 4.

N-acetylglucosamine residues (WGA, GSA II)

WGA bound to all the mucous cell types (Figure 3 A,B) as well as to the cuticle of the sucker infundibulum (Figure 3B). GSA II reacted with the m1 and m2 cells of the mantle (Figure 3C), whereas did not react with the mucosal cells and epithelium lining the arms (Figure 3D).

Sialic acid residues (SNA)

SNA, specific for NeuNAcα2,6 Gal/GalNAc terminal residues, linked the m2 and m4 cells but not the other mucous cells and the mantle and arms surface (Figure 3E, F).

Terminal galactose residues (PNA and RCA I)

PNA reacted with the apical surface of the epitheliocytes lining the mantle (Figure 3G) and with the cuticle of sucker infundibulum (Figure 3H). This lectin did not reveal binding sites in any mucous cells. RCA I bound m1 and m2 cells in the mantle (Figure 3I), m3 cells in the arms (Figure 3J) as well as the mantle surface and the cuticle lining the sucker infundibulum (Figure 3K).

N-acetylgalactosamine residues (SBA)

SBA displayed binding sites in m2 cells of the mantle (Figure 3L) and no reactivity in the arms (Figure 3M).

Terminal/internal mannose/glucose residues (Con A)

Con A did not bind the mantle whereas revealed high mannose glycans in the arms at level of the m3 cells and the cuticle of sucker acetabulum (Figure 4A).

Terminal fucose residues (AAA, UEA I, LTA)

AAA linked to all mucous cell types as well as to the surface of the mantle and sucker infundibulum (Figure 4 B,C). UEA I bound the m1 and m2 cells (Figure 4D) as well as the internal surface of the sucker (Figure 4E). LTA showed a similar binding pattern except for the lack of reactivity in the sucker acetabulum (Figure 4F).

The lectin histochemistry reactions did not change during the investigated periods and they were considered reliable because the negative-control procedures gave no reactivity (Figure 4G).
Figure 3. Lectin histochemistry of *Octopus vulgaris* paralarvae. A) WGA binding sites in m1, m2. Kölliker’s organ of the mantle from a P01 paralarva. B) WGA reactivity in the infundibulum cuticle, m3 and m4 cells of a P01 paralarva sucker. C) GSA II staining of m1 and m2 cells from a P02 paralarva. D) GSA II negativity with sucker from same paralarva of C. E) SNA binding sites in m2 but not m1 cells from mantle of a P01 paralarva. F) SNA reactivity of m4 cells in same paralarva of E. G, PNA staining of mantle surface of a P03 paralarva. H, PNA affinity with the infundibulum cuticle of a P03 paralarva. I) RCA I staining of mucous cells and surface lining the mantle of a P01 paralarva. J) RCA I binding sites in m3 and infundibulum cuticle of a sucker from same paralarva of I. K) SBA binding with m1 cells of P01 paralarva mantle. L) SBA did not react with arms of the same paralarva of K. a, acetabulum; arrowhead, m3 cell; i, infundibulum; ic, infundibulum cuticle; k, Kölliker’s organ; m1, coarse-grained mucous cell; m2, fine-grained mucous cell; P01, P02, P03, 1-4, 11-14 and 21-28 days after hatching samples; large arrow, mantle apical surface; small arrow, m4 cell. Scale bars: A-L) 20 µm; insets in A,C,E,K) 10 µm.
Statistical analysis

Descriptive statistical analysis was performed on the abundance of PAS-reactive m1, m2, m3 and m4 cell types and values obtained (expressed as M1, M2, M3 and M4, respectively) are showed in Tables 3 and 4. Figure 5 shows the histograms comparing mean values and standard errors for M1-M4 in the three samples. M1 and M2 are significantly different among samples. The M1 increases from P01 to P02 but decreases again in P03, whereas M2 decreases from P01 to P03. The relative abundance of the two cell types indicates that in P01 sample M2 outnumbers M1, but they attain similar values in P02 and P03. The deviations from normality tested by Shapiro-Wilk tests (not shown) were not significant except for M2 in P02 (W=0.7994, P=0.0006). Homogeneity of variances, assessed by Levene’s tests (not shown), was revealed in M1 but not in M2 (P=0.0034). One-Way ANOVA and Shapiro-Wilk tests were performed on both M1 and M2 values among the samples and all the results are resumed in Table 5. Both tests revealed a high significant difference between M1 and M2 means among samples. The results of post-hoc comparisons of means between samples by THSD and DSCF tests are in Table 6. Most pairwise comparisons indicate significant differences, except for P01-P03 (THSD only) and P02-P03 (both THSD and DSCF).

The abundance of m3 e m4 sucker cells (indicated as M3 and M4, respectively) did not show deviations from normality tested by Shapiro-Wilk tests (not shown) and their variances had homogeneous values by Levene’s tests (not shown). The mean values did not vary significantly among samples (Figure 5 and Table 4). Thus, post-hoc comparisons were not performed.

Discussion

The body surface of aquatic organisms is covered with a protective mucous layer which represents the first interface between the animal and the environment. Therefore, the evaluation of the glycoconjugates composition of the skin mucus could give new data about the biology of octopus paralarvae which, due to high mortality rate, represent an impairment for the aquaculture. In this study, for the first time, we describe the glycan profile of the epidermis and suckers.
in the reared *O. vulgaris* paralarvae.

The cephalopod epidermal mucus-secreting cells have received little attention compared to other mollusks. However, the diversity of mucous cells types found in the studied cephalopod species, and the presence of a thin mucus-gel film surrounding the animal body underlines its importance in the post-hatchling life.

Morphological investigations revealed that the mantle was lined with the epidermis, which was a simple columnar epithelium constituted of epitheliocytes and two different types of mucous secreting cells: m1 and m2 cells. Both these secretory cells contained neutral glycoproteins (PAS staining) which presumably ended with GlcNAc (WGA and GSA II reactivity), lactosamine (RCA I affinity), and fucose (AAA, UEAI, and LTA binding). However, these two cell types produced different glycans since m2 cells synthesized also carboxyl (AB 2.5 positivity) and/or O-sulphate esters (HID staining) glycoproteins containing GalNAc and sialic acid (SBA and SNA binding, respectively).

This different glycosylation pattern could be related to differential functions of the mucins secreted by m1 and m2 cells. Interestingly, GalNAc is important for the O-glycosidic linkage of the saccharidic chains to the protein backbone (typical to secreted mucins) and is often subterminal to sulphated and carboxylated residues. Concerning sialic acid, this sugar contributes to the net negative charge of glycans because it is typically found at the terminal position of glycans and it is involved in several important functions, such as intercellular interactions, transport of ions and molecules, viscosity of mucus, proteo-
tion from microorganisms or interactions with symbionts, and its alterations characterize several pathologies in vertebrates including human. The presence of the sialic acid is an unusual finding in mollusks, where it is usually substituted by muramic acid. Differently from the other monosaccharides, the backbone of this sugar can exhibit pronounced chemical diversity in structure and linkage so that all the known sialic acids constitute the sialome. Thus, the sialome analysis could be a useful tool for monitoring health conditions of paralarvae from hatching to settlement. Furthermore, the sulphated and fucosylated glycoproteins (HID, AAA, UEA I and LTA reactivity) could play an important role in microbial interactions. A morpho-functional interpretation of our results requires comparison with literature data, but this is difficult because of both the absence of such investigations and the different terminology used in reported studies. Ultrastructural analysis of the O. vulgaris epidermis revealed the presence of three type of mantle secretory cells (type 1, 2, and 3) but no data concerning the type of secreted mucosubstances. In sepioïds, the skin of Euprymna scolopes exhibited two types of secreting cells such as ovate cells and goblet cells producing sulphate and neutral glycoproteins, respectively. Moreover, in the Sepia esculenta skin three types of secreting cells (A, B, and C cells) were observed. All these cells secreted neutral glycoconjugates and type A produced also acidic glycans.

The morphometric analysis showed that the trends of m1 and m2 cells number were significantly different, because m1 cells increased during the first two weeks and decreased during the fourth week after hatching, whereas m2 cells gradually reduced during the first month after hatching. However, interestingly the number of both mucous cell types decreased at the end of the month. This suggests two different hypotheses: i) the mucus secretion at level of the mantle changes at the end of the first month after hatching; ii) the reduction in the number of mucous cells could cause some

| Table 5. Results of One-Way ANOVA and Kruskal-Wallis tests for comparisons of means between M1 and M2 numbers of mucous cells per 100 μm of the Octopus vulgaris paralarvae mantle skin. |
|---|---|---|---|
| M1 Test | SS | df | MS |
| One-Way ANOVA Sources Between groups 3.9659 2 1.9829 |
| Within groups 6.1505 85 0.0724 |
| Total 10.1163 87 0.1163 |
| F | 27.4042 |
| P | 0.0000 |
| Kruskal-Wallis H | 2 | 32.6394 |
| P | 0.0000 |
| M2 Test | SS | df | MS |
| One-Way ANOVA Sources Between groups 64.7213 2 32.3607 |
| Within groups 41.4948 85 0.4882 |
| Total 106.2161 87 1.2209 |
| F | 66.1892 |
| P | 0.0000 |
| Kruskal-Wallis H | 2 | 56.3832 |
| P | 0.0000 |
| M3 Test | SS | df | MS |
| One-Way ANOVA Sources Between groups 0.0002 2 0.0003 |
| Within groups 0.0200 76 0.0003 |
| Total 0.0202 78 0.0003 |
| F | 0.3923 |
| P | 0.6773 |
| Kruskal-Wallis H | 2 | 0.7498 |
| P | 0.6873 |
| M4 Test | SS | df | MS |
| One-Way ANOVA Sources Between groups 0.0004 2 0.0003 |
| Within groups 0.0069 76 0.0003 |
| Total 0.0073 78 0.0003 |
| F | 2.4013 |
| P | 0.0974 |
| Kruskal-Wallis H | 2 | 3.9857 |
| P | 0.1363 |

SS, sum of squares; df, degrees of freedom; MS, mean squares; F, value of Fisher’s statistics; H, value of H statistics; P, value of probability associated to each test.
change in the glycan/molecular composition of mucous layer of the skin which can be related to the high mortality rates. However, the *O. vulgaris* paralarvae used in this study showed a growth pattern in line with the data reported in previously studies (the body length ranged from 1.7±0.4 mm to 3.1±0.5 mm at four days and twenty-eight days from hatching, respectively) and they exhibited the same morphological features described on wild animals with no signs of anatomical damage.

The mantle surface exhibited neutral glycan and acidic non-sulphated glycoproteins (PAS and AB 2.5 positivity) as well as glycoproteins terminating with Galβ1,3GalNAc (PNA), Galβ1,4GlcNAc (RCA I) and fucose linked α1,6 to GlcNAc or α1,3 to N-acetyllactosamine (AA). This suggests that both m1 and m2 cells participate in the production of mucus layering the mantle surface. In sepiaoid the epidermal mucus layer has been related to the sticking mantle surface. In sea snails the epidermal mucus has been related to lubrication and adhesive properties of mucus have been observed in other mollusks. Glue mucus in gastropodes is more rich in sulphated and acetylated sugars than trail mucus.

Since in *O. vulgaris* the relative importance of lubrication in swimming and adhesive interactions with the substrate probably change from a planktonic to a bottom-dwelling life style, we could hypothesize that the variation in abundance of m1 and m2 cells observed from early to older post-hatchings could significantly affect these ecological requirements.

The arms exhibited suckers consisting of the infundibulum and acetabulum chambers which were connected through a constricted orifice. The epithelium lining the infundibulum consisted of cuboidal cells, whereas that covering the acetabulum was cuboidal or stricated orifice. This cell type is particularly well developed surrounding the suckers of reared *O. vulgaris* paralarvae. The m3 cells were scattered in the epithelium surrounding the infundibulum. This cell type contained neutral as well as acidic non-sulphated mucins constituted by mannosylated (N-linked) glycan (Con A affinity) containing GlcNAc (WGA reactivity) and terminating with lactosamine (RCA I staining) as well as with fucosylated residues binding AAA.

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The detected glycoproteins could be involved in the constitution of mucus surrounding the sucker rim to improve the watertight seal during the adhesion process. However, their differential composition suggests a different role of m3- and m4-secreted glycoproteins in this process. Moreover, the release of sulphated glycoproteins on the surface of the aquatic animal epithelium has been related to lubrication that is necessary in protecting the body against mechanical damages as well as in increasing of the viscosity to trap bacteria and other pathogens (reviewed by Rai et al.). Interestingly, the morphometric analysis revealed that the number of m3 and m4 cells is constant during the first month of life after hatching. This suggests that these cells, on the contrary of m1 and m2 cells, are not affected by the hatching conditions.

As for the sucker surface, the infundibulum surface was lined with the cuticle containing neutral and sulphated mucins which were constituted of GlcNAc, Galβ1,3GalNAc, lactosamine, and all investigated fucosylated glycanas.

Interestingly, the cuticle covering the acetabulum expressed a different glycan pattern compared to the infundibulum, since it lacked acidic glycanas and expressed highly mannosylated glycanas.

In conclusion, the present study provides for the first time an insight into the glycoconjugates expressed in the secretory cells and the surface lining the mantle and the suckers of reared *O. vulgaris* paralarvae. In addition, the results provide information on the changes in the number of mucous cells scattered along the mantle of skin of *O. vulgaris* paralarvae during the first month of life. The presented results add information which could be useful in sustain pure as well aquaculture experimental researches aimed to better understand the physiology of this species during this critical stage of the life cycle.

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**Table 6. Results of Tukey’s Honestly-Significant-Difference (THSD) and Dwass-Steel-Chritchlow-Fligner (DSCF) post-hoc tests for comparisons of means between M1 and M2 numbers of mucous cells per 100 µm of the Octopus vulgaris paralarvae mantle skin.**

<table>
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<tr>
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<th>S1</th>
<th>S2</th>
<th>THSD</th>
<th>DSCF</th>
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<tr>
<td></td>
<td>Value</td>
<td>P</td>
<td>Value</td>
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</tr>
<tr>
<td>M1</td>
<td></td>
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</tr>
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<td>P02</td>
<td>1.891</td>
<td>0.000</td>
<td>-25.136</td>
<td>0.000</td>
</tr>
<tr>
<td>P03</td>
<td>0.390</td>
<td>0.173</td>
<td>-3.149</td>
<td>0.067</td>
</tr>
</tbody>
</table>

S1, S2, sample; P, value of probability associated to each test.

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**References**


