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REPORT

Separation and viability of gill and hepatopancreatic cells of a mangrove crab *Ucides cordatus*

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Abstract The gills contain essential cells for respiration and osmoregulation, whereas the hepatopancreas is the site of digestion, absorption, and nutrients storage. The aim of this work was to separate and characterize gill and hepatopancreatic cells of the mangrove crab, *Ucides cordatus*. For gills, the methodology consisted of an enzymatic cellular dissociation using Trypsin at 0.5%, observation of cellular viability with Tripan Blue, and separation of cells using discontinuous sucrose gradient at concentrations of 10%, 20%, 30%, and 40%. The hepatopancreatic cells were dissociated by magnetic stirring, with posterior separation by sucrose gradient at the same concentrations above. For gills, a high cellular viability was observed (92.5±2.1%), with hemocyte cells in 10% sucrose layer (57.99±0.17%, *$P<0.05$), principal cells in the 20% sucrose layer (57.33±0.18, *$P<0.05$), and thick cells and pillar cells in the 30% and 40% sucrose layers, respectively (39.54±0.05%, *$P<0.05$; and 41.81±0.04%, *$P<0.05$). The hepatopancreatic cells also showed good viability (79.22±0.02%), with the observation of embryonic (E) cells in the 10% sucrose layer (67.87±0.06%, **$P<0.001$), resorptive (R) and fibrillar (F) cells in the 20% and 30% sucrose layers (44.71±0.06%, **$P<0.001$, and 43.25±0.01%, *$P<0.05$; respectively), and blister (B) cells in the 40% sucrose layer (63.09±0.03%, **$P<0.001$). The results are a starting point for in vitro studies of heavy metal transport in isolated cells of the mangrove crab *U. cordatus*, subjected to contamination by metals in the mangrove habitat where they are found.

Keywords *Ucides cordatus* · Gills · Hepatopancreas · Enzymatic dissociation · Isolated cells

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Crustaceans are frequently used as bioindicators, and as biomonitors, providing temporal and geographic variations in the availability of contaminants through cumulative concentrations in their body or tissue (Rinderhagen et al. 2000).

Contact between the crustaceans and the environment occurs through the gills. The gills are associated with gas exchange, ionic regulation, enzymatic functions, and ammonia excretion (Freire et al. 2007).

The gill structures possess specialized and differentiated cells which consist of the following types (Lawson et al. 1995; Freire et al. 2007): (1) thick cells contain microvilli, characterizing an ion transporting cell; (2) principal cells possessing a respiratory function and (3) pillar cells that possess microtubules that support the gill axis and allow the passage of hemolymph; and (4) hemocytes that are in the hemolymph or in the intracellular septum of the gill lamella.

The hepatopancreas is composed of four cell types: blister cells (B), embryonic cells (E), resorptive cells (R), and fibrillar cells (F) (Gibson and Barker 1979; Hopkin and Nott 1980; AL-Mohanna and Nott 1986, 1989; Icely and Nott 1992). These cells are involved on digestion, absorption, secretion, and detoxification (Mulford and Villena

Table 1. Cellular viability in the first and second h of the each experiment performed (*P<0.05, ANOVA)

Experiment	Viable cells (first hour)	Unviable cells (first hour)	Viable cells (second hour)	Unviable cells (second hour)
1	84.6%	15.4%	83.6%	16.4%
2	90.4%	9.6%	86.4%	13.6%
3	98.0%	2.0%	62.3%	37.7%
4	91.2%	9.0%	79.7%	20.3%
5	95.1%	5.0%	92.5%	7.5%
6	96.0%	4.1%	95.1%	5.0%
Average	92.5±2.1%*	7.5 ± 2.0%	83.3±4.8%*	16.7±4.8%

2000). The E cells are able to originate the other cell types. The B cells play a role in intracellular digestion and excretion of products and xenobiotics. The R cells are responsible for nutrients absorption, whereas the F cells are secretory, releasing digestive enzymes.

So, both the gills and the hepatopancreatic cells interact with the external medium, being directly affected by changes in the environment. In this sense, it is important to characterize and study the physiological processes of these cells. The objective of this study was to separate the gill and hepatopancreatic cells of *Ucides cordatus* in different sucrose concentrations, characterize them in different fractions, and measure their viability at the end of the separation process.

For the experiments, the animals were collected in Itanhaém, São Paulo coast, and brought to Universidade Presbiteriana Mackenzie (São Paulo campus) where they were acclimatized. The crabs were kept in tanks filled with sea water at 20 ppt, gravel, water filter, and pieces of brick for the emersion of the animals.

Cellular dissociation was performed through the enzymatic method, where 15 mL of the extraction solution (NaCl 395 mM; KCl 10 mM; $NaHCO_3$ 2.5 mM; NaH_2PO_4 2.5 mM; Hepes 3.75 mM; glucose 1 mM; EDTA 0.9 mM)

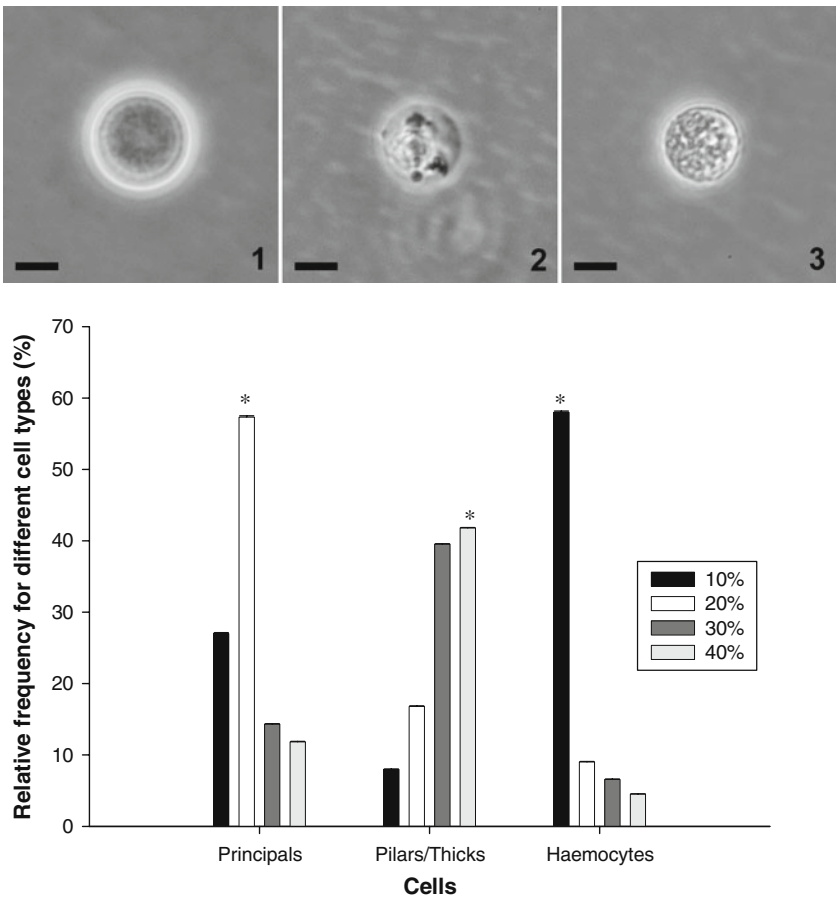

Figure 1. Hemocyte cell represented by number *1*. Principal cell represented by number *2*, and pillar/thick cell represented by number *3*; and the relative frequency for the different gill cells, according to the sucrose gradient of 10%, 20%, 30%, and 40% (*P<0.05, ANOVA).

was mixed with 150 μL of Trypsin enzyme (0.5%). The gills were soaked in the solution and kept in ice, for 15 min. They were minced with scissors, and they were taken to the shaker in bath at 30°C, 1.8 g for 15 min. After that, the minced gills were filtered in thin mash of 30 μm and taken to the centrifugation, at 115×*g*, 5°C for 5 min. The filtered gills were reserved for a second digestion.

After the first centrifugation, some of the cells that sedimented (pellet) were resuspended in extraction solution and kept in ice. A second digestion was performed using the filtered and reserved gills from the first digestion.

Hepatopancreatic cells dissociation was done using a magnetic stirring. The hepatopancreas was taken from the animal and put in a beaker with 15 mL of the same extracting solution used for gills. They were put in the magnetic stirring for 30 min. Next, the solution was filtered in 30-μm mash and taken to the centrifugation for 5 min, at 115×*g*. The pellet was resuspended and stored, and later used for the separation by a discontinuous sucrose gradient.

The cellular viability was tested using the Tripan Blue method, where in 200 μL of cells were added 20 μL of Tripan Blue (Tennant 1964), and put in the Neubauer chamber. After that, the viability was quantified by the visualization of the stained cells (unviable) and translucent cells (viable) in four quadrants, with the result multiplied by 10^4.

The gill and hepatopancreatic cells were separated in four different sucrose concentrations: 10%, 20%, 30%, and 40%, respectively. For this, the sucrose was diluted in the extracting solution to the desired concentration.

The tube was taken to centrifugation for 2 min, at 7×*g*, and then, the gills and the hepatopancreatic cell layers were aliquoted and visualized in conventional microscopy.

Analysis of variance (ANOVA) and post hoc test were used to compare the different cells types in the different sucrose gradients. Differences were accepted at $P<0.05$.

Gills cells enzymatic dissociation resulted in a viability of 92.5±2.1%, $N=6$. It was observed significant cell viability for both the first and the second h of the experiment, with an increase in cell death after the second hour of the experiment, although not significant (Table 1). Here, the enzymatic dissociation of the gills reflected in high cell viability because Trypsin digests protein binding regions between the cells, allowing their dissociation with no cell damage (Ringwood et al. 2003).

Figure 1 represents the cell types present in the gill and the gill separation in SG. The hemocyte cells were more frequent in 10% SG (*$P<0.05$, ANOVA). The principal

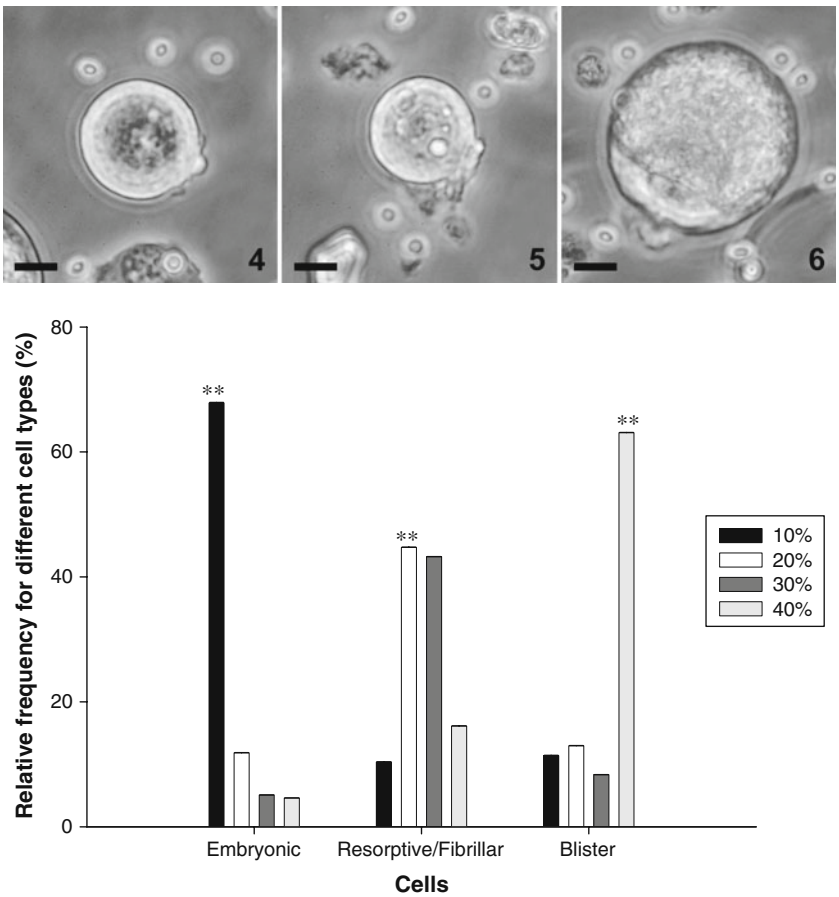

Figure 2. Embryonic cell (E) represented by number *4*. Resorptive/Fibrillar cell (R/F) represented by number *5*, and blister cell (B) represented by number *6*; and the relative frequency for the different hepatopancreatic cells, according to the sucrose gradient of 10%, 20%, 30%, and 40% (*$P<0.001$, ANOVA).

cells are more frequent in 20% SG (*P<0.05, ANOVA). The pillar/thick cells were more frequent in 30% and 40% SG (*P<0.05, ANOVA).

For hepatopancreatic cells, the results are represented in Fig. 2. The E cells were more frequent in 10% SG (**P<0.001, ANOVA). The R/F cells were found in higher frequency in the 20% and 30% SG (**P<0.001, ANOVA; *P<0.05, ANOVA, respectively). The B cells, on the other hand, were more frequent in 40% SG (**P<0.001, ANOVA). The cells appeared in different layers because each group of cells has different densities and amount of granules (Chavez-Crooker et al. 2001).

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