NOTAS CIENTÍFICAS

A DIRECT METHOD FOR WHITE BLOOD CELL COUNT IN FISHES

MARIA DA SILVA PITOMBEIRA (1)

JOSÉ MURILO MARTINS (2)

The red blood cells of fishes are nucleated. When the leukocyte count is performed using the usual direct method in a counting chamber, the nuclei of the hemolysed red cells are easily mistaken with white blood cells. Direct methods using special diluents were developed but the indirect method has been found to be more accurate (McKnight, 1966) .

A direct method for counting fish leukocytes without the use of special diluents was developed by us. It is an adaptation of Maspes & Jamra direct method for counting platelets (Maspes & Jamra, 1955) .

METHODS AND MATERIAL

Direct method procedure: 1) Draw 3-5 cc of blood at the level of gills (or other suitable place) with a syringe wet with heparin. Blood with any sign of hemolysis must be discarded. 2) Determine the hematocrit using the Wintrobe tube at 3,000 r.p.m. for 30 minutes. Note the plasma hematocrit. 3) Put 3 cc of blood in a test tube 12 x 75 mm and centrifuge at 500 — 800 r.p.m. for 1 — 3 minutes. The supernatant plasma must contain an homogeneous suspension of leukocytes and thrombocytes. If a small layer of white cells is observed or if the fall of red cells is higher than 20% the blood must be shaken again and recentrifuged in a smaller period of time. It is not recommended to wait for the sedimentation of the blood cells, because it is very slow in fishes. 4) Withdraw carefully the supernatant plasma free of red cells with a Pasteur pipette. 5) Dilute the plasma with the Türk's fluid in a Thoma erytrocyte diluting pipette (1/200 dilution). Shake well the pipette and fill the Neubauer counting chamber. Wait a few minutes for the sedimentation of the cells. 7) Count the white blood cells in the central reticulum (1 x 1 mm) . 8) The number of leukocytes per mm³ of blood must be calculated according to the formula

$$N = \frac{n \times d \times hmc.pl \times 10}{100}$$

being N = number of leukocytes per mm³ of blood; n = number of leukocytes counted in 1 mm² ; d = dilution used (200) ; hmc.pl = hematocrit of the plasma in percentage; 10 = depth of the camera in micra. 9) Platelets are also nucleated but they are easily distinguished from white cells.

In order to detect the accuracy of the method we collected blood from 90 specimens of Spanish mackerel, *Scomberomorus maculatus* (Mitchill), and performed the white blood cell count by the direct and indirect methods.

The *indirect method* was performed counting in a stained slide with an oil immersion lens the number of leukocytes in relation to 5,000 erythrocytes. A previous direct method red cell count is necessary. The number of white blood cells per mm³ is obtained according to the formula

$$N = \frac{n \times rbc}{5{,}000}$$

being N = number of leukocytes per mm³ of blood; n = number of leukocytes counted in relation to 5,000 red blood cells; rbc = direct method red blood cell count per mm³ of blood.

COMMENTS

The result of the simultaneous white blood cell counts obtained by the direct and indirect methods was submitted to statistical analysis. Paired comparison method was used on account that the distribution of values was not a normal curve. It was concluded that the results obtained by the two methods are equivalent and the difference between them is not significant ($t = 1.12$) at the level of 5% of significancy with 89 degrees of freedom.

The direct method does not require a well distributed peripheral blood slide and does not have the disadvantage of doing a tiresome counting of 5,000 red blood cells. It is easier to be done, quicker and may be used whenever the fish is large enough to collect 3 — 5 cc of blood.

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(1) — Estação de Biologia Marinha — Universidade Federal do Ceará — Fortaleza, Ceará, Brasil.

(2) — Instituto de Medicina Preventiva — Universidade Federal do Ceará — Fortaleza, Ceará, Brasil.

A CASE OF ATROPHIED PEREIOPOD IN THE ABDOMEN OF A SPINY-LOBSTER **PANULIRUS ARGUS** (LATREILLE)

RAIMUNDO SARAIVA DA COSTA

Estação de Biologia Marinha
Universidade Federal do Ceará
Fortaleza — Ceará — Brasil

The scientific literature does not record cases of pereiopod appearing in the abdomen of spiny-lobsters of the family Palinuridae. In this paper we record a case of an atrophied periopod appearing in the abdomen of a spiny-lobster of the species *Panulirus argus* (Latreille).

Figure 1 — Ventral view of a female spiny-lobster tail of the species *Panulirus argus* (Latreille), showing one atrophied pereiopod in the pleopod of the fourth abdominal segment, as well as the deformities in the shape of the exopodites.

Among spiny-lobster tails received from Sabiaguaba (Itapipoca — Ceará — Brazil) on February 26, 1966, there was one from an ovigerous female with an atrophied pereiopod. That tail measured 174 mm from the anterior dorsal edge of the first abdominal segment, to the posterior edge of the telson.

The atrophied pereiopod, in this case, substitutes the endopodite of the pleopod of the fourth abdominal segment on the left side, the dactylopodite and protopodite being quite evident. Also, partial deformations in the shape of the pleopod under reference are noticed, as well as in the exopodite of the pleopod of the third abdominal segment of the right side (figures 1 and 2).

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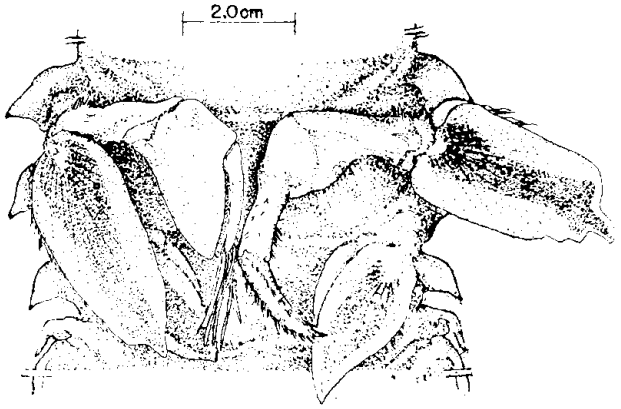

Figure 2 — Detailed drawing of atrophied pereiopod and the deformities in the shape of the exopodites, in a female spiny-lobster tail of the species *Panulirus argus* (Latreille).