

Melano-macrophage centres in whitemouth croaker, *Micropogonias furnieri*, as biological indicators of environmental changes

G. J. MACCHI*†, L. A. ROMANO‡ AND H. E. CHRISTIANSEN*

*Instituto Nacional de Investigación y Desarrollo Pesquero, cc. 175, (7600), Mar del Plata,

†Consejo Nacional de Investigaciones Científicas y Técnicas and ‡Centro de Investigaciones Médicas Albert Einstein, Luis Viale 2831, Buenos Aires, Argentina

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Quantitative analysis of the melano-macrophage centres in the *Micropogonias furnieri* were made. The results obtained showed a stimulation of the mononuclear phagocytic system related to the presence of contaminants.

Key words: *Micropogonias furnieri*; melano-macrophage centres; biological indicators; pollution.

Melano-macrophage centres (MMC) are physiological structures of the mononuclear phagocytic system, exclusive to the higher Teleostei. Their function is to remove by phagocytosis foreign particles or products from cell degradation (Ellis *et al.*, 1976; Agius, 1979). Ellis (1980) and Herraiz & Zapata (1986) also suggested an important role in the capture of antigenic material. Comparative studies have shown variations in size, number and pigmentation of these centres in relation to age, health and nutritional condition of organisms, as well as environmental changes due to pollutants (Agius, 1981; Agius & Roberts, 1981; Agius, 1983; Brown & George, 1985). The present study analyses quali-quantitatively the MMCs in the spleen of *Micropogonias furnieri* (Desmarest, 1823) in different areas of distribution of this species.

The material was obtained from four coastal areas: Uruguay (35°S–54°W), El Rincón (39°S–61°W), Samborombón Bay (36°S–57°W) and South Samborombón (36°30'S–56°30'W). The last two localities receive significant contamination from rivers, notably the Río de La Plata and the Salado. The spleen of 90 adult female *M. furnieri* were excised and fixed in 10% formalin. Sections cut for histological examination were stained according to the H-E, PAS, Prussian Blue, Fontana Masson, Masson Trichromic and Ziehl Neelsen techniques. The estimations of number and relative surface areas of MMCs were made using a Zeiss Integrating Eyepiece following the methodology of Weibel and Gomez (1962). The results were compared with an ANOVA model and Scheffé's test of contrasts. The Fontana Masson technique gave positive results, pinpointing the constant presence of melanin pigments in the MMCs. PAS-positive amorphous material was observed, possibly as a deposit secondary to phagocytosis. With the Prussian Blue the irregular presence of ferric pigment could be demonstrated, the iron being the result of the haemocatheretic activity. The Ziehl Neelsen method gave weak results, indicating scarcity of ceroid pigment. The relative surface area values of MMCs showed a marked increase in fish collected from the Samborombón zone, with a maximum for those inside the bay (Table I). The number of centres were higher in this zone, but an increase inside the bay was not observed. This shows that the relative surface area variation is caused mainly by the larger size of structures and not by the numerical increase. The ANOVA showed highly significant differences between the means of relative surface area ($P < 0.01$). The Scheffé's contrast test showed significant differences between El Rincón, Uruguay and between Samborombón Bay, south Samborombón, while the difference was highly significant for the means of the two first v. the latter areas (Table II).

TABLE I. Mean values and dispersion obtained for the variates relative surface area (I) and number of MMCs (II)

Variates		El Rincón	Uruguay	S. Samborombón	Bahia
I	\bar{X}	6.54	9.68	14.61	20.87
	S	8.17	16.41	33.58	61.53
II	\bar{X}	6.01	7.84	9.96	9.33
	S	4.75	3.97	8.37	5.95
	n	17	28	25	20

TABLE II. Results of Scheffe's contrast test between relative surface area means of each zone. 1 (El Rincón), 2 (Uruguay), 3 (South Samborombón), 4 (Samborombón Bay)

1-2	3-4	$\bar{X}_{1-2} - \bar{X}_{3-4}$
0.41	0.37	0.80
*	*	**

* $0.1 < P < 0.05$; ** $P < 0.01$.

These results conform with those obtained previously by analysing the prevalence of ovarian pathologies of *M. furnieri* in the same areas (Macchi *et al.*, in press). In this paper a high proportion of affected individuals was observed in the Samborombón zone, and these anomalies were related to the presence of contaminants in the area. The increment in size of the MMCs, associated with a stimulation of the organism's immune system, tends to support the hypothesis presented in Macchi *et al.* (in press), and also agrees with the results obtained by Wolke *et al.* (1985) in winter flounder, *Pseudopleuronectes americanus* Walbaum.

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References

- Agius, C. (1979). The role of melano-macrophage centres in iron storage in normal and diseased fish. *Journal of Fish Diseases* **2**, 337-343.
- Agius, C. (1981). The effects of splenectomy and subsequent starvation on the storage of hemosiderin by melano-macrophages of rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **18**, 41-44.
- Agius, C. (1983). On failure to detect haemosiderin in the melano-macrophages of the dogfish, *Scyliorhinus canicula* (L), after prolonged starvation. *Experientia* **39**, 64-67.
- Agius, C. & Roberts, J. R. (1981). Effects of starvation of melano-macrophage centres in fish. *Journal of Fish Biology* **19**, 161-169.
- Brown, C. L. & George, C. J. (1985). Age dependent accumulation of macrophage aggregates in the yellow perch, *Perca flavescens* (Mitchill). *Journal of Fish Diseases* **8**, 135-138.
- Ellis, A. E. (1980). Antigen trapping in the spleen and kidney of the plaice, *Pleuronectes platessa* L. *Journal of Fish Diseases* **3**, 413-426.

- Ellis, A. E., Munro, A. L. S. & Roberts, R. J. (1976). Defence mechanisms in fish. I. A study of the phagocytic system and the fate of intraperitoneally injected particulate material in plaice (*Pleuronectes platessa*). *Journal of Fish Biology* **8**, 67–78.
- Herraez, M. P. & Zapata, A. G. (1986). Structure and function of the melano-macrophage centres of goldfish, *Carassium auratus*. *Veterinary Immunology and Immunopathology* **12**, 117–126.
- Macchi, G. J., Aubone, A. & Christiansen, H. E. (in press). Incidencia de patologías en ovarios de corvina rubia, *Micropogonias furnieri*. Su relación con distintas zonas costeras de Uruguay y la Provincia de Buenos Aires. *Atlántica. Rio Grande*.
- Weibel, E. R. & Gomez, D. M. (1962). A principle for counting tissue structures on random section. *Journal of Applied Physiology* **17**, 343.
- Wolke, R. E., Murchelano, R. A., Dickstein, C. D. & George, C. J. (1985). Preliminary evaluation of the use of macrophage aggregates (MA) as fish health monitors. *Bulletin of Environmental Contamination and Toxicology* **35**, 222–227.