

A SIMPLE METHOD FOR FRESH-WATER FISH LYMPHOCYTE CULTURE

Alberto Sérgio Fenocchio¹ and Luiz A.C. Bertollo²

ABSTRACT

We describe a technique of whole peripheral blood lymphocyte culture for application in studies of fish cytogenetics. This method is relatively simple, shows high repeatability and produces good-quality chromosome preparations.

INTRODUCTION

Fish cytogenetics is a promising research area in terms of karyotypic evolution and cytotaxonomy. However, most of the studies in this area are almost completely based on direct cytological preparations.

Tissue or cell culture techniques started to be utilized in fish cytogenetics during the 1960's. Starting from a few pioneering studies, such as those by Roberts and Labat *et al.* (in Kligerman and Bloom, 1977), new methods have been described and adapted in order to replace direct preparation techniques, thereby avoiding sacrifice of the animal, making it possible to repeat treatments in the same individual, and especially improving the quality of chromosome preparations.

Several factors, however, had a negative effect on the generalized use of these techniques. In the specific case of lymphocyte cultures, the greatest difficulties are related to composition of media, mitotic activators, qualitative variation of nutrients (fetal calf serum, FCS, in particular), gas requirements of cells in culture, and the nucleate nature of red blood cells (Blaxhall, 1983a,b; Hartley and Horne, 1983, 1985).

¹ Universidade Nacional de Misiones, Misiones, Argentina.

² Departamento de Ciências Biológicas, Universidade Federal de São Carlos, 13560 São Carlos, SP, Brasil. Send correspondence to L.A.C.B.

The cell culture method described in the present paper is relatively simple and of easy application, with no need for complex equipment or installations. It is based on techniques recently described for fish lymphocyte cultures (Blaxhall, 1983a,b; Hartley and Horne, 1983, 1985) associated with routine methods utilized in the Human Cytogenetics Laboratory of the Faculty of Medicine of Ribeirão Preto (USP).

PROCEDURE

Composition and preparation of the culture medium

The culture medium consists of TC 199 (Gibco Laboratories) containing Hanks salts and L-glutamine, 20% inactivated human serum, 5 mg/l penicillin, 10 mg/l streptomycin, and 30 ml/l phytohemagglutinin. The medium is sterilized by filtration through a 0.2- μ Millipore filter and distributed into cylindric 40-ml bottles (10 ml/bottle) which are stored frozen at 0°C until the time for use.

Blood extraction

Peripheral blood (0.3-0.4 ml/10 ml culture medium) is obtained from the dorsal vessels by vertically puncturing the axilla of the anal fin until the hemal canal is reached. A 2- to 5-ml syringe and a 27 x 5 needle are used after sterilization and heparinization (Liquemine Roche) (Figure 1).

Blood inoculation

Blood is added dropwise to the surface of the culture medium until it reaches a concentration of approximately 0.3-0.4 ml/10 ml medium. The flask is shaken gently to separate the cells.

Culture development

a) Mean culture time is 72 hours; however, depending on the extent of growth and on the amount of blood (less than 0.3 ml), culture time may be increased to 120 hours; b) temperature: 27.5 to 30°C; c) colchicine addition: 0.1 ml of a 0.017% aqueous solution of colchicine is added after the appropriate culture time and allowed to act for about 90 minutes.

Harvesting of material

The flask is shaken gently to resuspend the cells and the material is transferred

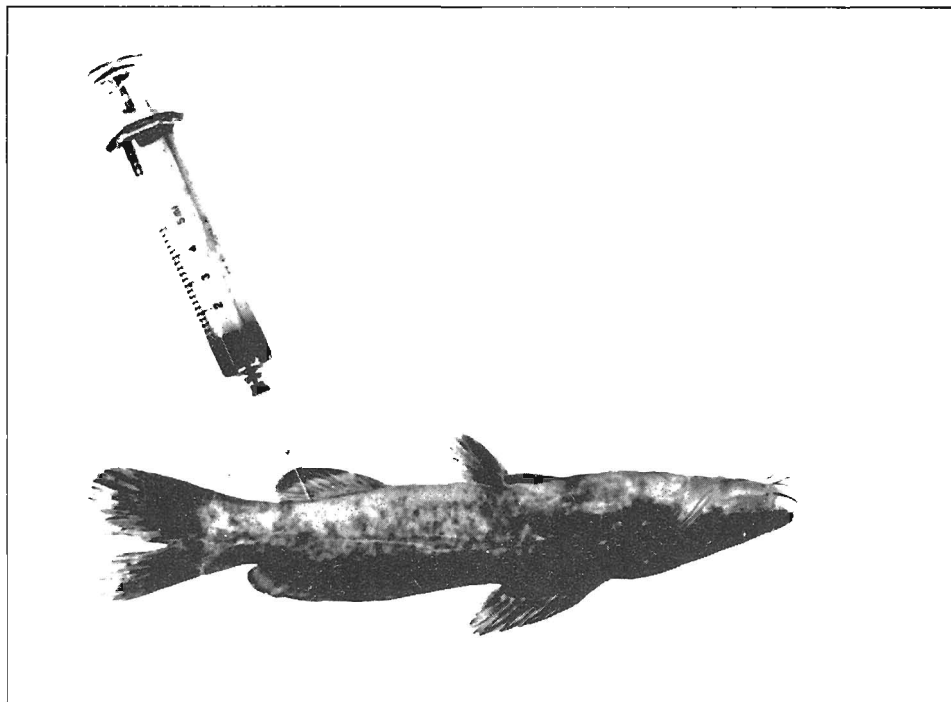


Figure 1 - Extraction of peripheral blood for lymphocyte culture from a fish, using a needle introduced into the axilla of the anal fin (for details, see text).

to a centrifuge tube. Centrifugation is carried out for 10 minutes at 600-800 rpm and the supernatant is discarded. The material is then hypotonized and fixed and slides are prepared by the usual air-drying procedure.

RESULTS AND DISCUSSION

The methodology described above gave good results when applied to the following species of Neotropical fish: *Rhamdia hilarii*, *Rhamdia* sp., *Hoplias* cf. *malabaricus*, *Geophagus brasiliensis*, *Serrasalmus spilopleura*, and *Hypostomus plecostomus* (Figure 2), and to three species of the genus *Leporinus*, i.e. *L. obtusidens*, *L. elongatus* and *L. friderici* (Gobbo C., personal communication).

In *Rhamdia hilarii* and *Rhamdia* sp., culture time occasionally lasted 120 hours and mean time of colchicine action was as much as 3 hours, resulting in frequent production of tetraploid cells as can be seen in Figure 2F.

Blaxhall (1983a) emphasized the influence of animal serum on the variability observed in fish lymphocyte cultures. On the other hand, Hartley and Horne (1985),

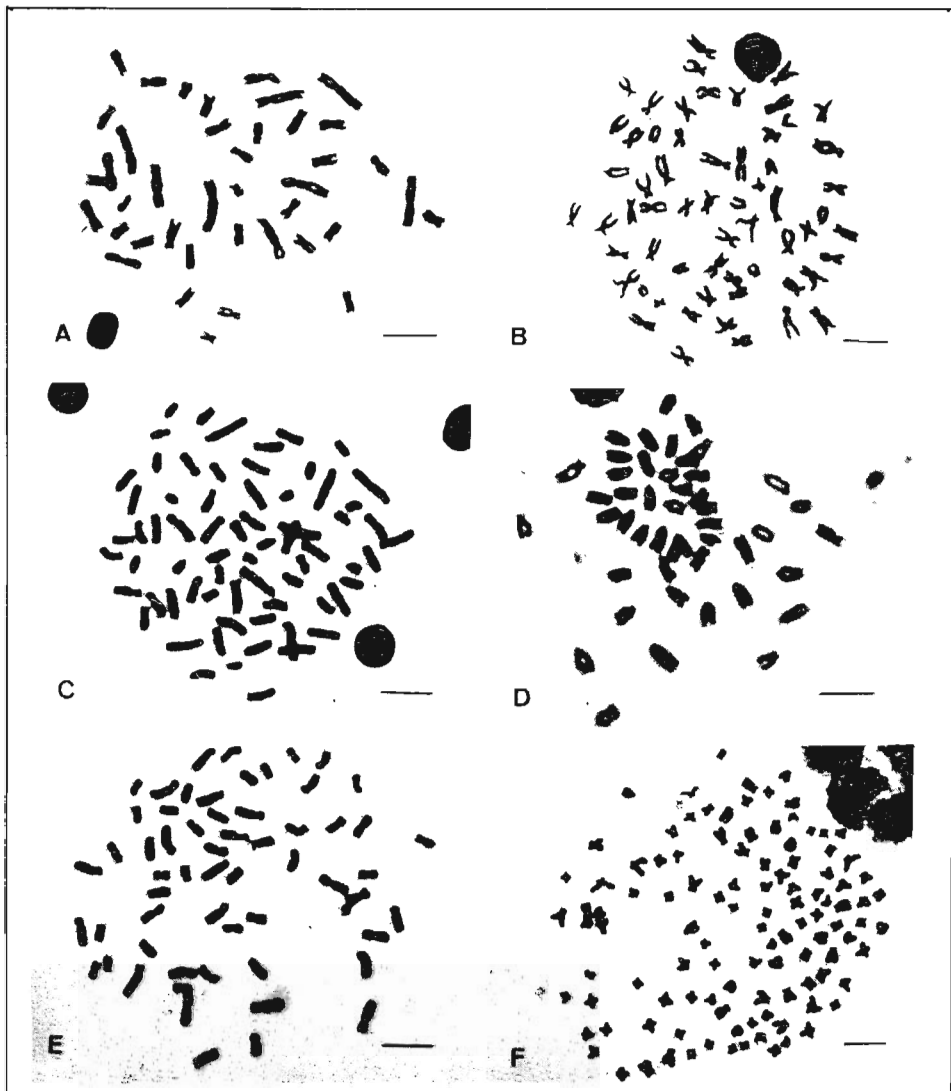


Figure 2 - Fish metaphase spreads obtained by the lymphocyte culture technique described in the present study. A, *Hoplias cf. malabaricus* ("traira", a mullet-like fish); B, *Serrasalmus spilopleura* (piranha); C, *Hypostomus plecostomus* (armored catfish); D, *Geophagus brasiliensis* ("acar", a sunfish); E, *Rhamdia hilarii* ("bagre", a catfish; diploid cell); F, *Rhamdia hilarii* (tetraploid cell). The bars represent approximately 5 μ .

in comparative tests observed a better general response of cells in culture to lipopolysaccharide (LPS), a B lymphocyte activator, whose action considerably exceeded that

REFERENCES

- Blaxhall, P.C. (1983a). Factors affecting lymphocyte culture for chromosome studies. *J. Fish Biol.* 22: 61-76.
- Blaxhall, P.C. (1983b). Lymphocyte culture for chromosome preparation. *J. Fish Biol.* 22: 279-282.
- Hartley, S.E. and Horne, M.T. (1983). A method for obtaining mitotic figures from blood leucocyte cultures of rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* 22: 77-82.
- Hartley, S.E. and Horne, M.T. (1985). Cytogenetic techniques in fish genetics. *J. Fish Biol.* 26: 575-582.
- Kligerman, A.D. and Bloom, S.E. (1977). Rapid chromosome preparations from solid tissues of fishes. *J. Fish. Res. Board Can.* 34: 266-269.

(Received June 25, 1988)