Vol. 4: 219-221, 1988 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published July 27

NOTE

Gill structure of cultured *Salmo trutta fario* related to sampling techniques

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ABSTRACT: A scanning electron microscope and histological study of the trout *Salmo trutta fario* gill was undertaken following 3 different killing techniques (concussion, decapitation and prolonged anesthesia with MS 222) and 2 different fixation procedures (glutaraldehyde and formaldehyde). Fifty % of fish killed by concussion and 20 % of fish sacrificed by decapitation displayed gill lamellar dilations (telangiectasis), whereas no anesthetized fish showed altered gill structure. No differences could be detected when comparing the effects of the 2 fixatives. We conclude that salmonids should not be killed either by concussion or decapitation if gills are to be examined histologically.

The fish gill is very sensitive to physical and chemical alterations of the aquatic medium. Hocutt & Tilney (1985) have described gill lesions (oedema, epithelial desquamation and fusion of lamellae) caused by heat. Pollution (acid stress, un-ionized ammonia, heavy metals, pesticides, etc.) has been shown to alter chloride cell structure and induce lamellar epithelium desquamation and/or filament epithelium hyperplasia (Leino & McCormick 1984, Soderberg et al. 1984, Crespo & Sala 1986, Sinhaseni & Tesprateed 1987). There is a close relationship between gill morphological alterations and stress (Peters & Hong 1985) or lack of polyunsaturated fatty acids in the diet (Bell et al. 1985), and several infectious agents have been described in association with proliferative gill diseases and gill necrosis (Kovács-Gayer 1984, Daoust & Ferguson 1985). Gill morphology is therefore a good indicator of the water quality and the general health condition of cultured fish (Peters et al. 1984).

Telangiectasis (gill lamellar dilations) has been reported by several authors as a consequence of NH_3 exposure in channel catfish *Ictalurus punctatus* and rainbow trout *Salmo gairdneri* (Smart 1976, Soderberg et al. 1984, Soderberg 1985). Recently, Herman & Meade (1985) proved that appearance of telangiectasis in the gill lamellae of lake trout *Salvelinus namaycush* was due to the method used to kill the fish. However, results were somewhat controversial since other salmonid species (*Salmo salar* and *S. gairdneri*) were not affected by killing techniques. Hughes (1979) suggested that some of the undulations on the rainbow trout gill lamellae viewed in the scanning electron microscope (which corresponded to telangiectasis) might be related to contraction during fixation.

The purpose of the present study was to investigate the effects of concussion (a sharp blow on the skull), decapitation and prolonged anesthesia with MS 222 on the gill structure of cultured brown trout *Salmo trutta fario* and to compare the effects of 2 fixative solutions (glutaraldehyde and formaldehyde).

Fish were obtained from a fish farm of the Department of Natural Environment, Spain (Generalitat de Catalunya), supplied with well-aerated unpolluted running water (pH = 7.7; T = 8 °C). Thirty fish of ca 250 to 300 g body weight were killed following the 3 different methodologies (10 fish per group). The handling procedure before killing was identical for all fishes. Gill arches were immediately removed and placed in fixative solutions: 5 of each group in 5 % glutaraldehyde and the remaining 5 in 10 % formaldehyde, both in 0.1 *M* Na cacodylate buffer (pH = 7.3). Samples were dehydrated through ethanol series, embedded in hydroxyethyl methacrylate, cut at 3 to 4 µm, stained with toluidine blue and viewed under the light microscope. At least 15 filaments per gill were studied and telangiectasis in each filament was quantified in serial sections. Some ethanol-dehydrated samples were critical-point dried with liquid CO_2, coated with a thin layer of gold and viewed in a Super III A Isi scanning electron microscope.

Single, very small capillary dilations were observed in gill lamellae, independently of sampling techniques. However, these were not considered as true telangiec-

 0177-5103/88/0004/0219/$ 03.00

tasis and could not be seen in the scanning electron microscope. No fish anesthetized with MS 222 displayed an altered gill structure (Fig. 1a). Of the fish killed by concussion, 50 % displayed more than 4 telangiectases per filament (Fig 1b), and of the fish killed by decapitation, 20 % showed lamellar gill dilations. Telangiectasis could be found at the tip of the lamellae or at their base and the pillar cell system was often completely disrupted (Fig. 2). Neither oedema nor epithelial desquamation were detected in any of the groups. Occasionally, 2 or 3 lamellae were fused but this was not related to sampling techniques. No differences could be detected when comparing the effects of the 2 fixatives, glutaraldehyde and formaldehyde.

From data presented here we may conclude that telangiectasis in *Salmo trutta fario* is due to trauma, capillary dilations probably being caused by an unusual amount of blood flowing from the afferent branchial artery or by a decreased outflow in the efferent branchial artery when fish are stunned by a blow on the head or decapitated. An acurate diagnosis on the farm or in the wild is necessary to control disease outbreaks. Taking into account that fish are generally killed by concussion or decapitation (Roberts & Shepherd 1979), histopathological studies might be

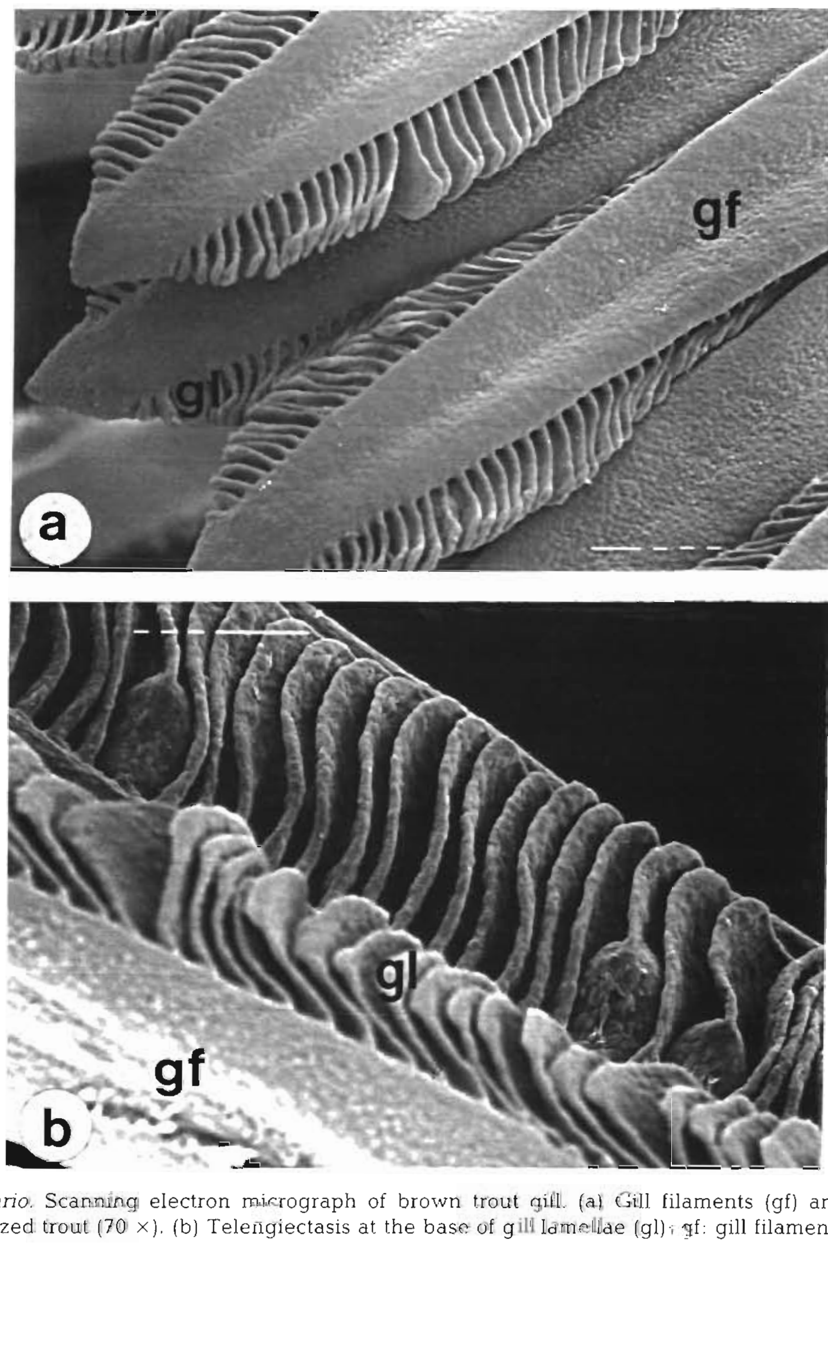

Fig. 1. *Salmo trutta fario*. Scanning electron micrograph of brown trout gill. (a) Gill filaments (gf) and gill lamellae (gl) of anesthetized trout (70 ×). (b) Telengiectasis at the base of gill lamellae (gl); gf: gill filament (135 ×)

Fig. 2. *Salmo trutta fario*. Telangiestasis affecting the whole lamella of the trout either stunned by a blow on the head or decapitated (250 ×)

misleading. Fish (at least salmonids) should not be killed following traumatic techniques if gills are to be studied histologically.

Acknowledgements. We thank Mr Marino Guillén (Sección Territorial del Medi Natural de la Generalitat de Catalunya) for his invaluable collaboration. This work received financial support from C.A.I.C.Y.T., GR 85-0016.

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Responsible Subject Editor: Professor O. Kinne; accepted for printing on May 10, 1988