—研究論文—
Scientific Paper

Gill Structure of the Antarctic Fishes *Notothenia (Gobionotothen) gibberifrons* and *Trematomus newnesi*, Nototheniidae Stressed by Salinity Changes and Some Behavioral Consequences

Edith FANTA*, Márcia Fabiana LUVIZOTTO* and Ana Aparecida MEYER*

異なる塩分に対する南極産ノトセニア科魚類 *Notothenia (Gobionotothen) gibberifrons* と *Trematomus newnesi* の鰓上皮層細胞の形態変化および一般形態と行動上の変化

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要旨: 南極沿岸の浅海域では，海水の凍結，融解に伴う塩分濃度の変化が顕著である．ここに生息する魚類が，いかに塩分の濃度の変化に適応しているかを調べるために，南極半島，キングジョージ島，アドミラルティ湾産の *Notothenia (Gobionotothen) gibberifrons* と *Trematomus newnesi* を異なる塩分環境下で実験的に飼育した．鰓上皮層にある塩分細胞，粘液分泌細胞の形態変化を観察したところ顕著な変化が認められた．合わせて，体色などの外部形態の変化，行動の変化についての観察も行った．

***Abstract*:** In coastal Antarctic regions, melting and freezing affect water salinity. The aim of this study was to compare morphological changes of branchial epithelial cells in two species of Antarctic fishes, *Notothenia (Gobionotothen) gibberifrons* and *Trematomus newnesi,* and to detect behavioral consequences. They occur in Admiralty Bay (King George Island) during the summer, in a mean salinity of 32‰. Tests were conducted in the salinity range between 26‰ to 38‰ in experiments. Chloride cells in *T. newnesi* are roundish or elongated, with a small apex, at 32‰, thin with a large apex at low salinity, and roundish with small intrusions at the surface at 38‰. Chloride cells are elongated in *N.(G.) gibberifrons* at normal and low salinity, and increase in number, size and activity at 38‰ with large intrusions at the apical surface. In both species, mucous cells increase in volume, number and activity with salinity increase, being less active at low salinity. Epithelial detachment are observed at high salinity in *T. newnesi* and in low salinity in *N.(G.) gibberifrons*. Behavioral consequences of branchial cellular changes were observed: linear decrease of respiratory frequency with salinity increase; swimming decrease with salinity changes, except that at 26‰ forced swimming occurs and at 38‰ lethargy is interrupted by brisk jumps; paleness occurs with salinity decrease and darkness with salinity increase. These symptoms seem to be secondary consequences of hypoxia and ionic changes.

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1. Introduction

The major environmental factors in the Antarctic marine ecosystem are either homogeneous in space and time or predictable in their variations (CLARKE, 1983).

One of these factors is the sea water salinity that is around 34‰, being slightly higher close to the sea bottom. On the other hand the surface waters south of the Antarctic convergence are characterized by lower salinities showing considerable variation due to ice formation and melting. The surface waters on the continental shelves are quite similar to those over the deep ocean with the exception that often the salinity values are less than 34.5‰. Salinities up to about 35‰ are found on the continental shelves in the Weddell and Ross Seas (FOSTER, 1984).

In bays and coastal Antarctic regions, such as in Admiralty Bay of King George Island, the salinity changes through the year, as a consequence of freezing and melting, caused by seasonal temperature variability and perhaps slight global climatic changes. This may bring the water salinity locally down to values as low as 26‰ during the summer, particularly close to glacial melt river influence zones (Fig. 1). On the other hand, the cold winter causes an increase in the salinity, due to sea water freezing.

Antarctic fish such as Nototheniidae, living in such regions, are very attractive subjects for morpho-physiological studies on the effect of environmental impact.

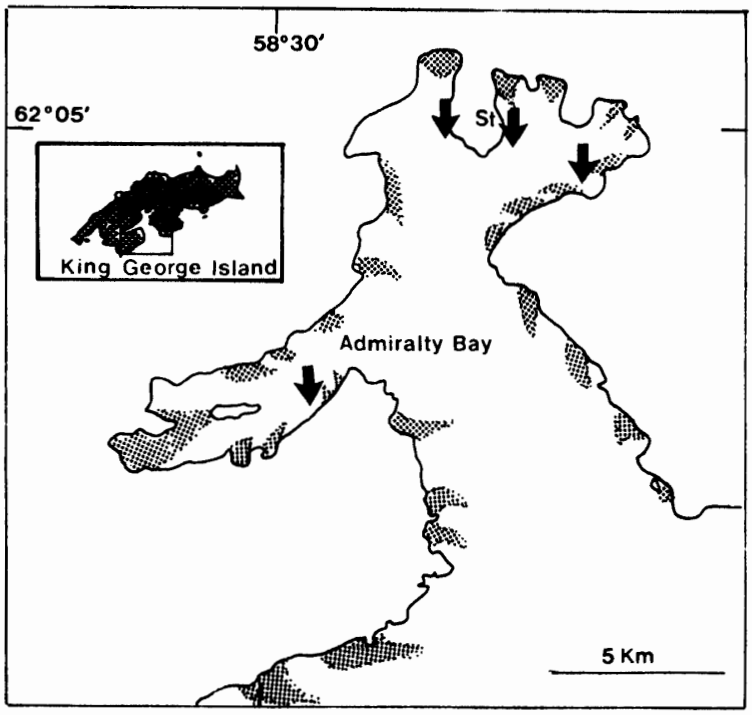

Fig. 1. Admiralty Bay, King George Island, showing some of the melting ice influence zones where salinity is lower every summer (dotted) and collection sites of Notothenia (Gobionotothen) gibberifrons and Trematomus newnesi (arrows).

Trematomus newnesi and *Notothenia* (*Gobionotothen*) *gibberifrons* are near-bottom species living in depths of 20 to 80 m; both are very common in Admiralty Bay during the summer. While swimming they may come in contact with different water salinities. Whether in such cases, in nature, they show any avoidance reaction is not known. In any case, they must be able to deal with the influence of higher or lower salinity for short periods of time, in order to survive and eventually escape.

It is well known that fish gills are important osmoregulatory organs, as well as being respiratory organs. Therefore they have some cells specialized in ion changes, the chloride cells (HOSSLER, 1980; LAURENT, 1982, 1984) as well as mucous cells (HUGHES and BYCZKOWKA-SMYR, 1974; BOYD *et al.*, 1980). If the water salinity changes, it is expected that these cells may change morphologically (MALLAT, 1985) and functionally in order to maintain the homeostasis of the animals.

Thus, the aim of this research was to describe and compare the morphological changes in branchial epithelial cells, mainly in some specialized cells like chloride and mucous cells, when the fishes have to deal with salinity changes. As some pathologies may cause secondary and tertiary effects, behavioral manifestations or symptoms can be identified.

2. Material and Methods

The Antarctic Nototheniidae fishes *Notothenia (Gobionotothen) gibberifrons* and *Trematomus newnesi* were caught in a mean salinity of 32‰ , at 50 to 80 m depth, in Admiralty Bay, King George Island, South Shetlands, during the summer, through gill-nets.

Acclimation was in the laboratory of the Antarctic Station Comandante Ferraz, in 1000 liter tanks, in a salinity of 32‰ (±0.5‰), pH 7.8 (±0.3), temperature of 0°C (+0.5°C), photoperiod of 12 hours light/12 hours dark. The fish were fed amphipods "ad libitum". The tests were performed in 10 liter tanks, in the same environmental conditions as during the acclimation. The salinity was controlled with an electronic conductivimeter. The salinity was lowered by dilution of the control water with filtered melt ice water, in proportions leading to final salinities of 30‰, 28‰ and 26‰; the salinity was increased by mixing the local sea water with concentrated commercial artificial sea water in the amount that was necessary to obtain the salinities 34‰, 36‰ and 38‰.

The behavior was monitored every hour by direct observations and video-filmed for later evaluation of all symptoms along the salinity gradient on a short time scale.

Each experiment ended after 6 hours, during which time the fish were kept at one constant salinity. The animals were decapitated, the second gill arch was dissected out and rinsed briefly with filtered water of the same salinity as that used in the experiment and prepared for morphological studies. All individuals were classified (FISHER and HUREAU, 1985), and measured, the mean total length being 4.4 cm (± 1.1 cm) for *N.* (*G.*) *gibberifrons* and 6.5 cm (± 0.2 cm) for *T. newnesi.*

For morphological observation, the gill arches were fixed in Bouin's mixture for 8 hours, then dehydrated through a graded alcohol series, cleared with xylene and embedded in Paraplast Plus$^{(r)}$ using routine procedures. The 3 μm thick sections were

stained with Hematoxilin-Eosin, Alcian Blue at pH 0.5 and 2.5, Alcian Yellow, Hematoxilin-Phloxin, Novelli and PAS (CULLING *et al.*, 1985), in order to allow identification of all cells in the branchial epithelium. The preparations were photographed using an Olympus BH-2 Microscope.

3. Results

The gills of both species are basically organized as is usual for teleosts, the arches being slightly curved, with two main regions: the pharyngeal and the respiratory. In the respiratory region, both species have primary lamellae that support leaf shaped secondary or respiratory lamellae. They are slightly longer and broader in *N.* (*G.*) *gibberifrons* (Fig. 5a), and thinner and shorter in *T. newnesi* (Fig. 2a). They are lined

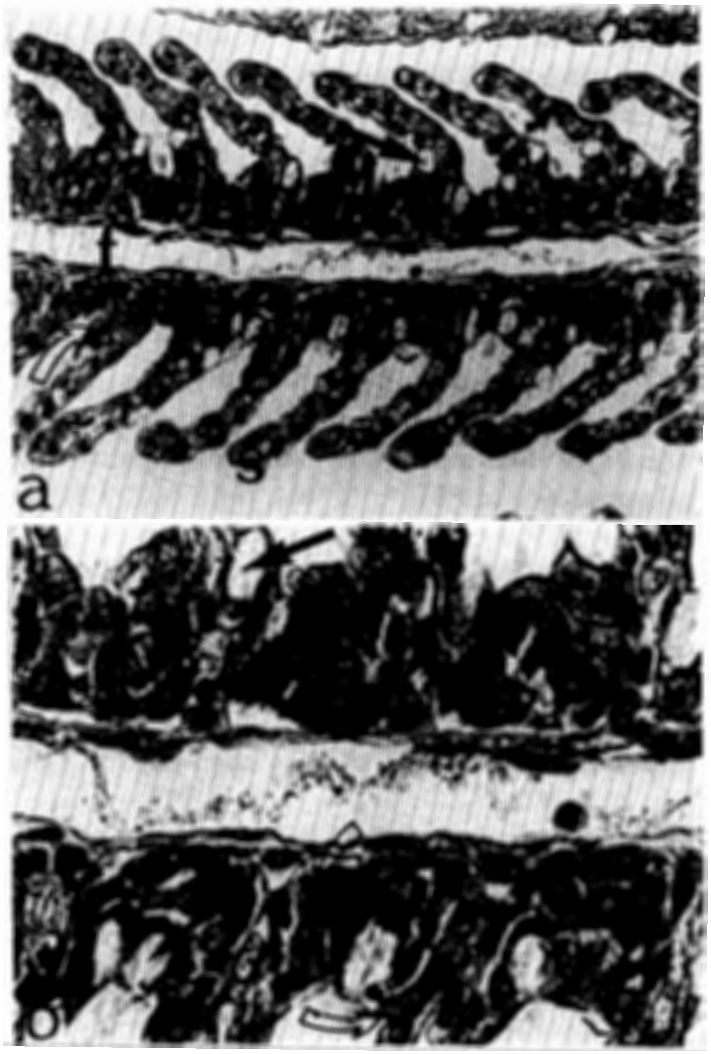

Fig. 2. Branchial epithelium of Trematomus newnesi at salinity 32‰. a: part of respiratory lamellae (s) coming out of the filament or primary lamella (f) showing the pillar cells (small arrow), mucous cells (long arrow), chloride cells (white arrow) and the interlamellar space (^)(400×); b: detail of the interlamellar space with mucous cells (long arrow) and chloride cells (white arrows) (1000×).

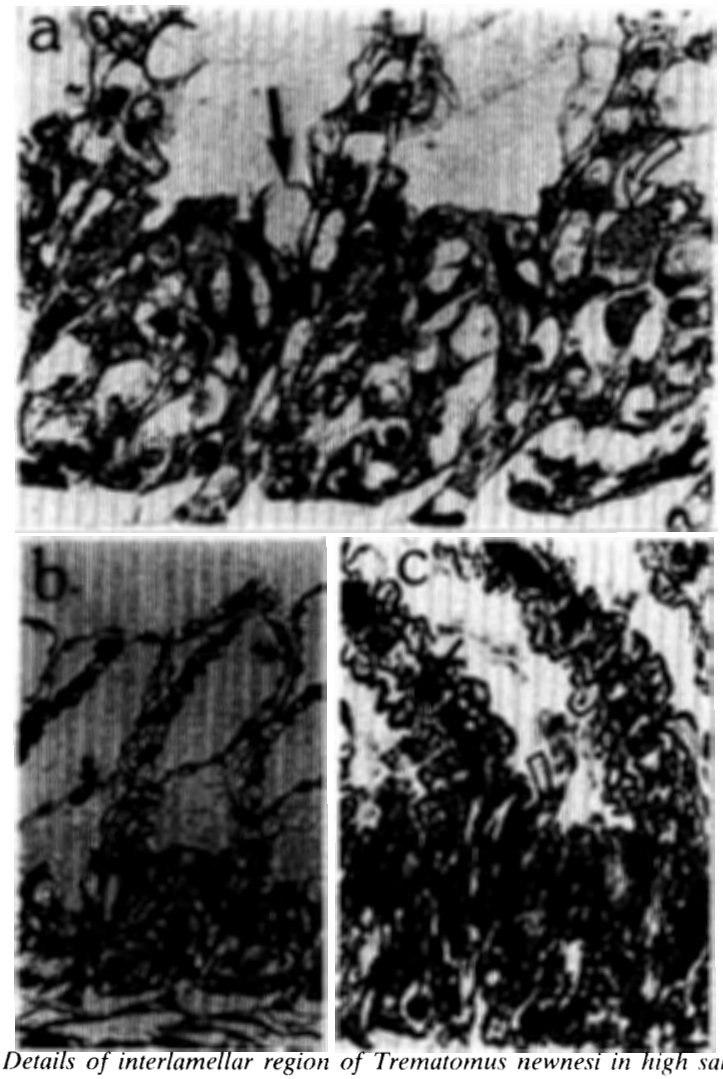

Fig. 3. Details of interlamellar region of Trematomus newnesi in high salinity. a: salinity 38‰, detail of the mucous cell (long arrow) and chloride cells (white arrow) (1000×); b: detachment in secondary lamella epithelium (small arrow) (400×); c: at 34‰ corrugation and swelling of secondary lamellae epithelial cells (*), large pillar cells (small arrow) and chloride cells (white arrow) (1000×).

by an epithelium that is one or two layers thick; internally, pillar cells define blood spaces. The interlamellar space is large in *N.* (*G.*) *gibberifrons* (Fig. 5a, 5b) and narrow in *T. newnesi* (Fig. 2a, 2b). It is lined by a stratified epithelium in which, besides the common epithelial cells, two specialized types occur: chloride cells and mucous cells. Shapes, sizes and distribution, as well as the number of cells, vary in both species and along the salinity gradient.

In *T. newnesi* the respiratory lamellae have a smooth surface, as they are lined by squamous cells. The blood spaces are defined by pillar cells with great roundish nuclei (Fig. 2a). When the salinity is lowered, aneurysms are formed in some regions of the secondary lamellae (Fig. 4a), and blood congestion is seen in some of the primary lamellae, as well as corrugation at the epithelial surface (Fig. 4c). Epithelial lifting is

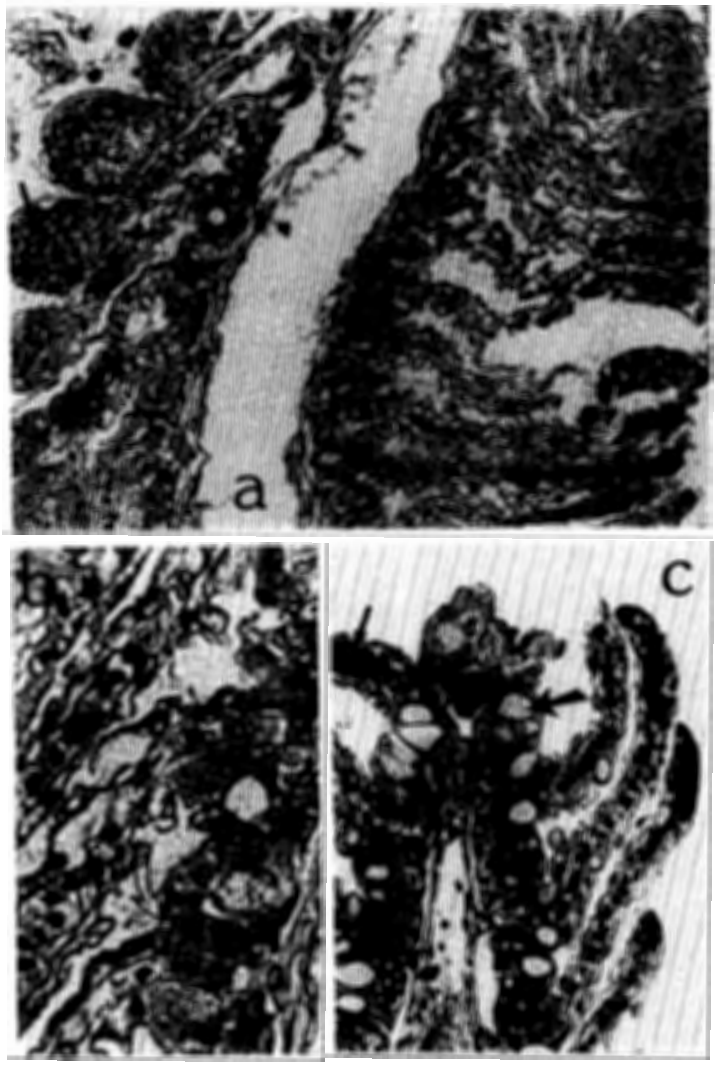

Fig. 4. Trematomus newnesi at low salinity. a: at 28‰, secondary lamellae with aneurisms and blood congestion (arrow) (400×); b: detail of interlamellar region with chloride cells (white arrow) (1000×); c: mucous cells (large arrow) and blood congestion (small arrow) (400×).

seen at lower salinities (Fig. 4a,b), but the detachment is total at the highest salinity (38‰) (Fig. 3b). A slight surface corrugation occurs at 34‰ (Fig. 3a)

N.(G.) gibberifrons has respiratory lamellae lined by roundish cells, causing an irregular lamellar surface. In proportion to the epithelial cells, pillar cells are small (Fig. 5a, 5b). As the salinity increases to 38‰, these features remain the same, but the cells are slightly swollen (Fig. 6a, 6b). At lower salinities, even in 30‰, epithelial detachment in various degrees of intensity is observed in many secondary lamellae and in the surface cell layer of the primary lamellae in the interlamellar space (Fig. 7a, 7b). The epithelial cells of the secondary lamellae become thin, and shrinkage of the interlamellar space is observed (Fig. 7a).

The chloride cells of *T. newnesi* are roundish or elongated, with a small apex, occupying a great proportion of the thickness of the epithelium at the control salinity

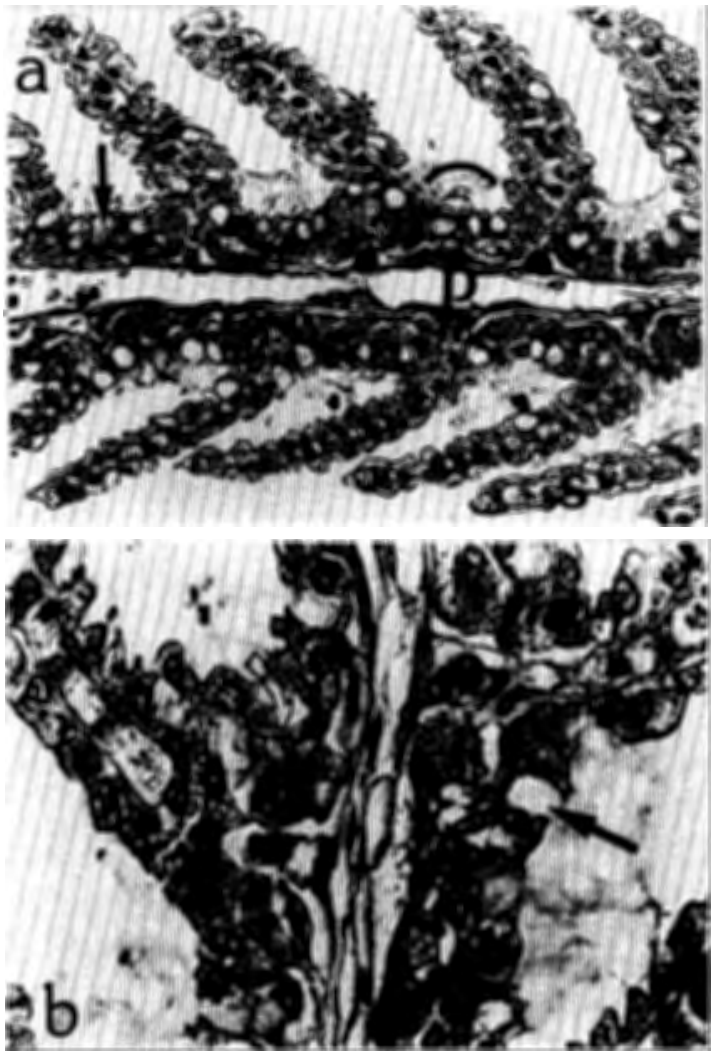

Fig. 5. Notothenia (G.) gibberifrons at controll salinity of 32‰. a: primary lamella or filament (p) with many secondary lamellae (s), and the position of mucous cells (arrow), epithelial cells (*) and the interlamellar region (arch)(400×); b: detail of interlamellar region with mucous cells (arrow) and chloride cells (white arrow), as well as pillar cells (short arrow) (1000×).

(32‰) (Fig. 2b). In low salinities (28‰) they become thin, narrow in the basal and larger in the apical region, occupying the total thickness of the stratified epithelium. They are granulated in the middle and basal regions and contain non-condensed nuclei. At the contact apical surfaces of the cells small intrusions may be seen (Fig. 4b). When the salinity increases (34‰) they become roundish, as big as the mucous cells, with a great contact surface of the cell with the environment, where many intrusions are seen, and with big granules in the upper part of the cell at 38‰ (Fig. 3a).

In *N.(G.) gibberifrons* the apex of the elongated chloride cells is slightly protruding at the control salinity (32‰), the size being half or all of the epithelial thickness (Fig. 5a, 5b). The chloride cells become elongated at low salinities,

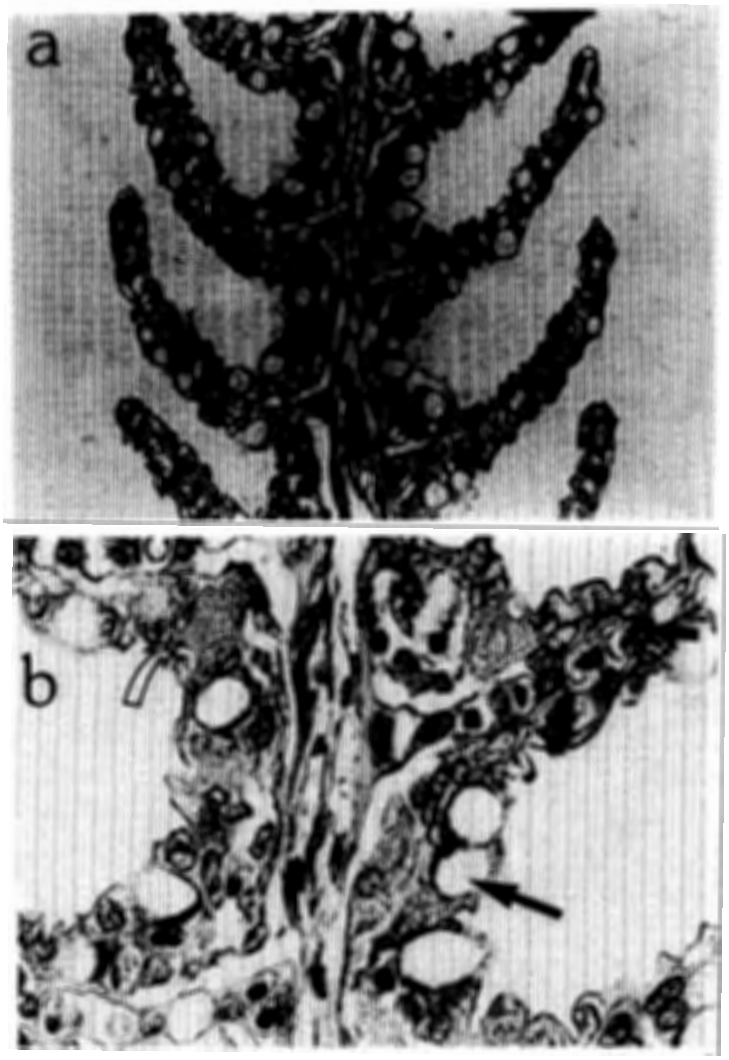

Fig. 6. Notothenia (G.) gibberifrons in high salinity. a: branchiae at 38‰ (400 ×); b: detail of interlamellar region with mucous cells (long arrow), chloride cells (white arrow) and pillar cells (short arrow) (1000×).

extending from the basal to the apical region of the epithelium in the interlamellar region, with a small apical region and a basal nucleus (Fig. 7b). Granulation can be seen in the basal region. From 34‰ up, their number and activity increase with salinity increase. They become large in the apical region (Fig. 6b), some being smaller at the apex, apparently due to the presence of many mucous cells at the surface. They are as long as the epithelium is thick. The granules were heavily stained with Novelli indicating high activity, and were concentrated mainly in the medium and apical regions. The apical region of the chloride cells had large intrusions (Fig. 6b). Chloride cells are also observed in the basal region of the respiratory lamellae (Fig. 6a).

Slightly elongated mucous cells in *T. newnesi* are always present in the interlamellar region. They may be very close to each other in this region and isolated in the secondary lamellae (Fig. 2a). They form a continuous layer at the tip of the primary lamellae, at all salinities. They react positively to PAS, Alcian blue at pH 2.5

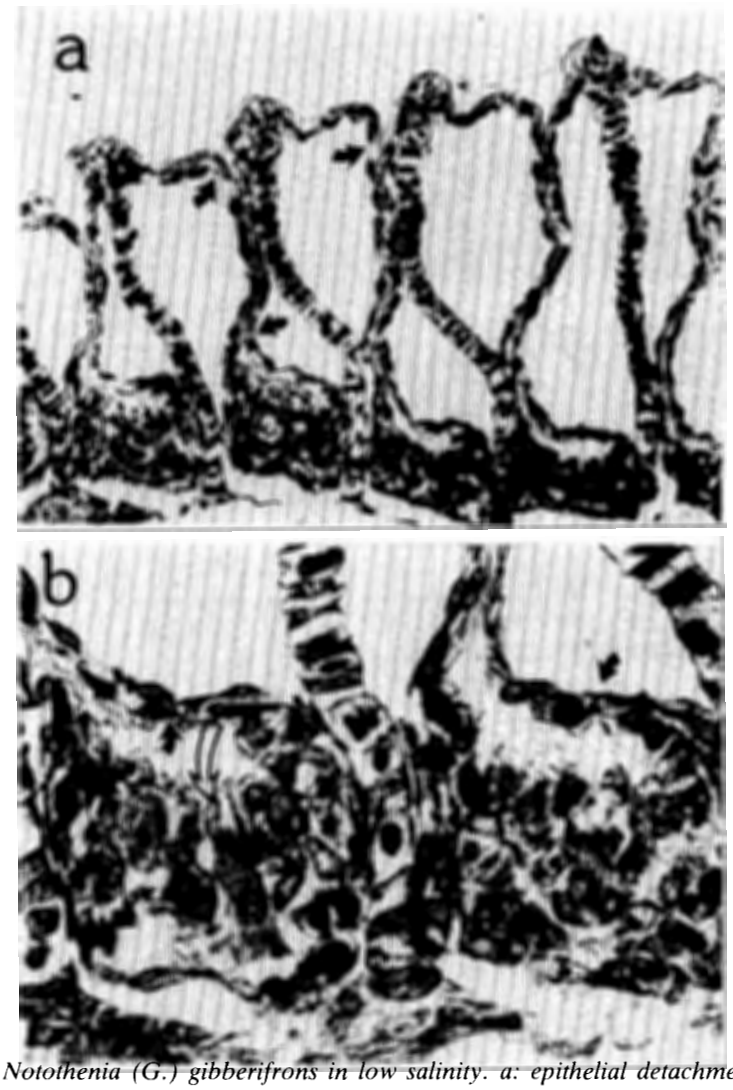

Fig. 7. Notothenia (G.) gibberifrons in low salinity. a: epithelial detachment at 30‰, in primary and secondary lamellae (small arrows) (400×); b: detail of interlamellar region showing epithelial detachment (small arrows), chloride cells (white arrow) and high, thin pillar cells (small arrow) (1000×).

and Alcian Yellow, and weakly to Alcian Blue at pH 0.5. Thus, the secretory product of these cells contains carboxylated and sulfated carbohydrates, and neutral, unsaturated and aldehyde glycol lipids. At higher salinities, their number increases (Fig. 3b, 3c), but the secretory product remains the same. At 38‰ the elongated mucous cells protrude at the lamellar surface. At lower salinity the cells are roundish and small (Fig. 4b), and not so active. Their number increases closer to the tip of the primary lamellae (Fig. 4c).

In *N.(G.) gibberifrons* a great number of globous mucous cells are present at the primary and the secondary lamellae (Fig. 5a). Mucous cells in the respiratory lamellae become frequent as the salinity increases, even in the secondary lamella, at salinity 38‰ (Fig. 6a). At high salinities they become spherical and very active. They

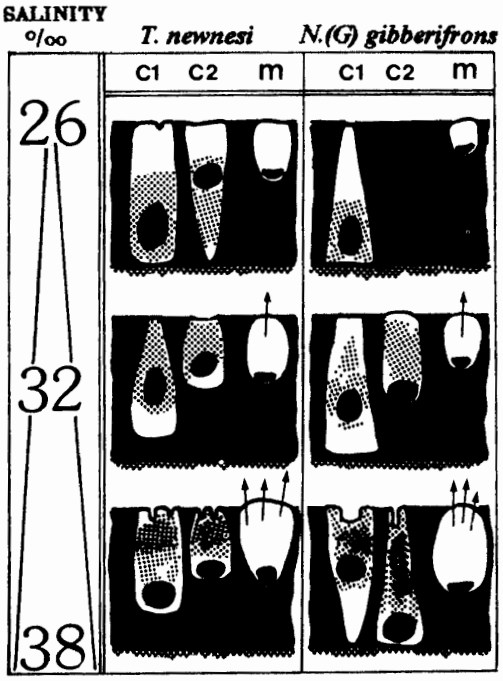

Fig. 8. Comparative schematic view of chloride cells (C1 and C2) and mucous cells (m) in medium, high and low salinity for both species, Notothenia (G.) gibberifrons and Trematomus newnesi. Notice the intensity of secreting activity (arrows), and the presence and distribution of mitochondria or granules (dotted).

are close to the surface, occupying half the epithelial thickness in the interlamellar region (Fig. 6b). At 30‰ they almost disappear from the secondary lamellae, and the few remaining in the interlamellar region are small (Fig. 6a, 6b). They react intensively to PAS, Alcian Blue at pH 2.5, positively to Alcian Yellow, and weakly to Alcian Blue at pH 0.5, having a different secretory nature than in *T. newnesi* as they contain only very small amounts of sulfated carbohydrates.

Figure 8 summarizes the main changes in size, shape, position and characteristics of chloride and mucous cells in both species, at different salinities.

As the salinity changes, as a consequence of all morphological changes at the respiratory lamellae the respiratory frequency tends to increase at lower salinities and decrease at higher salinities in *N. (G.) gibberifrons* (Fig. 9). In *T. newnesi*, the respiratory movements are visually not detectable even at normal salinity.

The behavioral symptoms were identified, comparing the behavior of control animals with that of the experimental ones. Both species had the same basic kind of symptoms (Fig. 10), but their intensity varied. The following behavioral features were considered: body colors like pale, light, medium and dark; keeping the mouth open, without closing it to pump water; yawning, noted only when its frequency became high; surfacing, meaning that the fish remained with the mouth at the surface for longer periods than usual; deep respiration, noted only when the respiratory movements of mouth and operculae were exagerated; resting, meaning that the animal was motionless at the bottom; sleeping, meaning deep resting without any reaction to external stimuli; aggressiveness which was normally very low and manifested through frontal display and fin raising; forced swimming, considered when the fish swam constinuously, which is completely abnormal for both species; briskness, referring to a sudden turn to change swimming direction; contortions, or binding movements of the whole body; jumping, brisk strong swimming movements

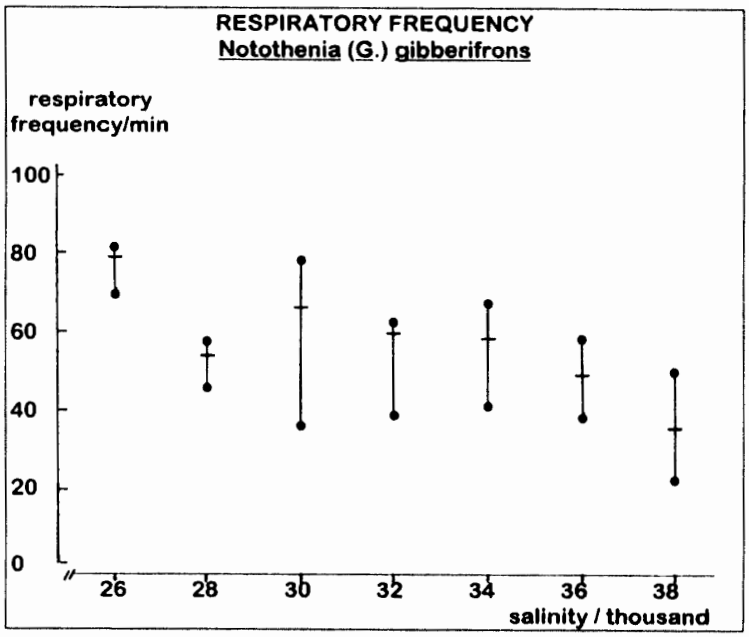

Fig. 9. Respiratory frequency of Notothenia (G.) gibberifrons in all salinities, showing the highest, lowest and mean values.

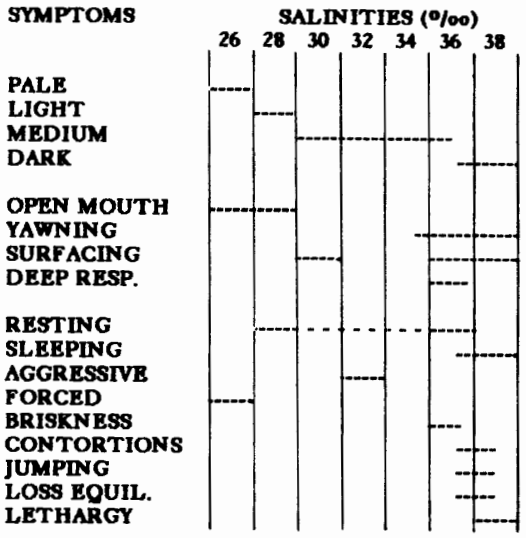

Fig. 10. Behavioral and other symptoms of both species with the lowering and increase of salinity.

for short distances; loss of equilibrium, detected as the animals fell to one side or the other, or kept the head or tail lower than the rest of the body; lethargy preceding death, characterized by absence of motion, the animal being carried passively by the water movement, without reaction.

In a general way, for both species (Fig. 10), the higher the salinity, the darker the fish and the more frequent the resting periods, including sleeping. Increased depression of the central nervous system was observed at 38‰. In *T. newnesi*,

yawning was observed frequently at high salinities, the mouth being kept open constantly at low salinities. At lower salinities, long resting times were also observed, but at 26‰ *T. newnesi* showed a forced swimming and escape reaction. Aggressiveness was completely lost in both situations. Loss of coordination, brisk movements, jumping and contortions were all observed with salinity increase, but only for *T. newnesi*. *N.* (*G.*) *gibberifrons* remained with low motility the whole time.

4. Discussion

Salinity is a factor that influences the distribution of many fish species (FITZGERALD, 1985). Euryhaline teleosts respond to changes in salinity rapidly, and stenohaline fish, which cannot respond, die within a few hours due to demineralization if transferred from sea water to fresh water, and dehydration if transferred from fresh water to sea water (MOTAIS and GARCIA ROMEU, 1972).

Through evaluation of the content of the ion-transporting enzyme in the chloride cells it was seen that, in around 6 hours, the gill filament epithelium of *Mugil cephalus* responded to changes in salinity (HOSSLER, 1980). Even considering that several days would be required to complete the process, in this short time significant changes were detected morphologically in the gills of both species.

Fish gills are rather similar among fish classes (HUGHES, 1981, 1984; LAURENT and DUNEL, 1980) and, as multi-functional structures (PAYAN *et al.*, 1984), their epithelial cells are specialized, assuming respiratory, osmoregulatory, excretory and secretory roles, integrating these functions and adjusting them to the needs of the organisms. Therefore, drastic morpho-functional changes in these cells may cause important disorders in the whole organism, as secondary and tertiary consequences. Some of them are visible as behavioral symptoms.

Epithelial lifting is one of the most common non specific irritant induced gill lesions (MALLATT, 1985). Even having been observed at all salinities for *Nototheniops nudifrons* (LUVIZOTTO, 1994), it occurred at high salinity for *T. newnesi*, being rarely seen in *N.* (*G.*) *gibberifrons*, and only at very low salinity. It may be a consequence of the higher or lower effectiveness of the action of chloride cells.

In *N.* (*G.*) *gibberifrons* as well as in *T. newnesi*, higher cellular activity is seen at higher salinities, when the granules corresponding to the mitochondria are evident, closer to the surface, showing great production of energy to allow cellular metabolism, as has been observed for other fishes (HOSSLER, 1980; MAETZ and BORNANCIN, 1975).

At all salinities, chloride cells were in contact with both the basal and the apical regions of the epithelium in *N.* (*G.*) *gibberifrons*. The contact with the connective tissue may help, eventually, to allow faster nutritional input from their capillaries, allowing immediate and more intense cell activity.

On the other hand, when the chloride cell has a larger contact surface with the environment, showing even superficial foldings, as mainly in *N.* (*G.*) *gibberifrons*, this will bring the outer membrane closer to the mithochondria. So, the whole system seems to be more efficient. The functional significance of the changes in the epithelial pores, corresponding to chloride cell apical pits, seems to be not completely

understood (BIERTHER, 1970; HOSSLER *et al.*, 1979). But when the cell apex corresponds to a pore at the epithelial surface, the cell surface where excretion of the ions occurs is closer to the mitochondria that have to generate energy for the process, making it faster. On the other hand, when absorption of ions is needed, a larger and protruding surface will make faster absorption possible due to the increased surface. The deepening of the apical pit corresponds to what is believed to be a general response for fish (HOSSLER, 1980; BIERTHER, 1970; MAETZ and BORNANCIN, 1975).

Thus, if in a given species such as *T. newnesi* the surface is increased in high salinity, when excretion is needed, the mechanism is not so efficient as in *N. nudifrons* (LUVIZOTTO, 1964) or in *N. (G.) gibberifrons*, with drastical consequences for the former species. At low salinity, *T. newnesi* has elongated chloride cells, and a relatively large surface, and, beside from some local aneurisms, the changes observed are not so intense. These drastic changes start at salinity as low as 26‰ as opposed to 30‰ as in *N. (G.) gibberifrons*. One can notice that in both species the mitochondria lie close to the cell basis at low salinity.

Hyperplasia of the chloride cells (GALAT *et al.*, 1985; NEWSTEAD, 1967) is not detected, but this may be due to the fact that increase in number of chloride cells has been usually observed during adaptation of a euryhaline teleost from freshwater to sea water. In the case of the Antarctic fish, marine species were kept in salt water the whole time, even if the salt concentration changed. Thus, it seems that in such a case there is no need for new cells but only for increased activity.

At the same time, an incredible increase in number and activity of the mucous cells is seen in both species at high salinity. This proliferation and hyper secretion of mucous cells is another common lesion described for the gill (MALLAT, 1985), and has also been seen in other Antarctic fish such as *Nototheniops nudifrons* (LUVIZOTTO, 1994). But, one can notice that it happens only at high salinity. At low salinity the cells become few and very small.

The significance of the mucus may be related to the function of the chloride cells where a net transepithelial sodium dependant movement of chloride is detected (ZADUNAISKI, 1984). The acid mucopolyssacharides at the chloride cell surface may execute the function of an electrolytic carrier, acting in a manner similar to an ionic exchanger (CONTE, 1969). This may be the reason for the greater mucus production that was observed at high salinities for both species.

All those mechanisms activated in such a short time as 6 hours cannot avoid that the internal medium changes, leading to some structural changes that have functional consequences, such as for example epithelial detachment, aneurysms in the secondary lamellae, swelling or thin epithelial cells in the respiratory lamellae. But, as MALLAT (1985) considered a general consequences of all environmental aggressions, the lesions that can be detected show specific details, differing from one species to an other. Many authors have found that under any given set of exposure conditions, each kind of gill lesion tends to vary in intensity and within a single fish tends to show differing extent of alteration. Different fish also tend to be affected in varying degrees, as was seen for the species studied here: the amount and the activity of chloride cells increased with salinity increase in both species, but their shape and the contact of the cell apex with the environment varied.

All these events that occured at branchial level caused a general decrease in the activity of both species, perhaps in an attempt to save energy. All behavioral changes observed are secondary consequences of hypoxia and changes in blood quality.

Lower salinities, even as low as 26‰, may be locally possible if the fish come close to a fresh water (such as ice melt) river that enters the bay,and seem to affect the animals less than higher salinity; this is reasonable as these species never will face such extreme salinities as 38‰. For realistic salinity values 34‰, or even 36‰, the observed changes are not so intense, contributing to greater survival of the studied species.

For short periods both species are able to survive drastic salinity changes: *T. newnesi* seems to be less adapted to salinity changes than *N. (G.) gibberifrons*. The first species adapt better to lower and the second to higher salinity.

Acknowledgments

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