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## SESSION V. PHYSIOLOGY

### The Role of Prolactin in the Adaptation of Tilapia to Hypo- and Hyperosmotic Environments

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#### Abstract

Many studies have shown the essential role of prolactin (PRL) in osmoregulation. When tilapia are transferred to hyperosmotic environments, an increase is observed in plasma ion concentrations, in the rate of ion turnover and in gill Na/K-ATPase activity. Plasma PRL concentrations decrease. Hypophysectomy of freshwater fish causes an imbalance in the hydromineral equilibrium leading to the animals' death. Supplements of PRL allow their survival by re-establishing the sodium net flux. In hyperosmotic environments, hypophysectomy does not lead to the animals' death. In this environment, PRL reduces sodium permeability and causes an increase in plasma sodium concentrations. PRL seems to have an effect on chloride cells but does not decrease gill Na/K-ATPase activity. Stress related to anesthesia, to confinement or to the water's physicochemical characteristics causes an increase in plasma PRL concentrations.

#### Introduction

The fish of the genera *Tilapia*, *Sarotherodon* and *Oreochromis* are characterized by a great tolerance to a wide range of environmental conditions. They tolerate great temperature variations (Chervinski 1982) as well as salinity (Dharmamba and Nishioka 1968 on *O. mossambicus*; Fukusho 1969 on *O. niloticus*) and some species known to be freshwater species are found in estuaries or in the sea (Stickney 1986). The range of tolerance also extends to water quality: anoxia, pH and dissolved nitrogen.

Efforts have been made to develop tilapia culture in marginal areas, such as in brackishwater or in seawater (Payne 1983). However, the intensive develop-

ment of this farming system presents several difficulties. For example, species with a high culture potential such as *O. niloticus* have low euryhalinity, while species with poor growth such as *O. mossambicus* show adequate euryhalinity. Consequently, Doudet (1986) and Morissens (1987) report high mortalities associated with low growth rates in tilapias that are or could be farmed in brackishwater lagoons. These results, which show the low resistance of tilapias to salinity, contradict the laboratory findings. Environmental fluctuations during culture cycles, which are not reproduced in the laboratory and which the animals must resist for long periods of time (temperature, salinity and quality), could account for the observed differences.

In order to understand these mortalities, it is necessary to analyze the influences of the environmental parameters on fish physiology, and especially on the endocrine control of osmoregulation. Knowledge on the subject is still fragmentary (see review of Prunet and Bornancin 1989).

This paper is limited to the study of prolactin (PRL), and reviews both the major bibliographic data and our specific results about this hormone and its role in osmoregulation.

In tilapia, prolactin is found in two forms (Specker et al. 1985; Rentier-Delrue et al. 1989) which are synthesized by two distinct genes (Rentier-Delrue et al. 1989). The complete sequences of both PRL are well known (Yamaguchi et al. 1988; Rentier-Delrue et al. 1989). The heavier of the two, PRL-I (24 kDa, 188 amino acids) contains 11 more amino acids than the other, PRL-II (20 kDa, 177 amino acids).

### Changes in Some Physiological Parameters during the Adaptation of Tilapias to Hyperosmotic Environments

#### *Descriptive Analysis of Some Parameters during the Adaptation to a Hyperosmotic Environment*

In tilapias (*O. mossambicus*) adapted to seawater, the plasma Na and plasma Cl concentrations, and the osmotic pressure are slightly higher (5 to 10%) than those of animals kept in freshwater (Table 1) (Dharmamba et al. 1973; Dharmamba et al. 1975; Dangé 1985; Young et al. 1988). In *O. aureus*, the direct transfer from freshwater to brackishwater (14 ppt) results in an increase in plasma chloride levels, followed by a rapid decrease. After three days

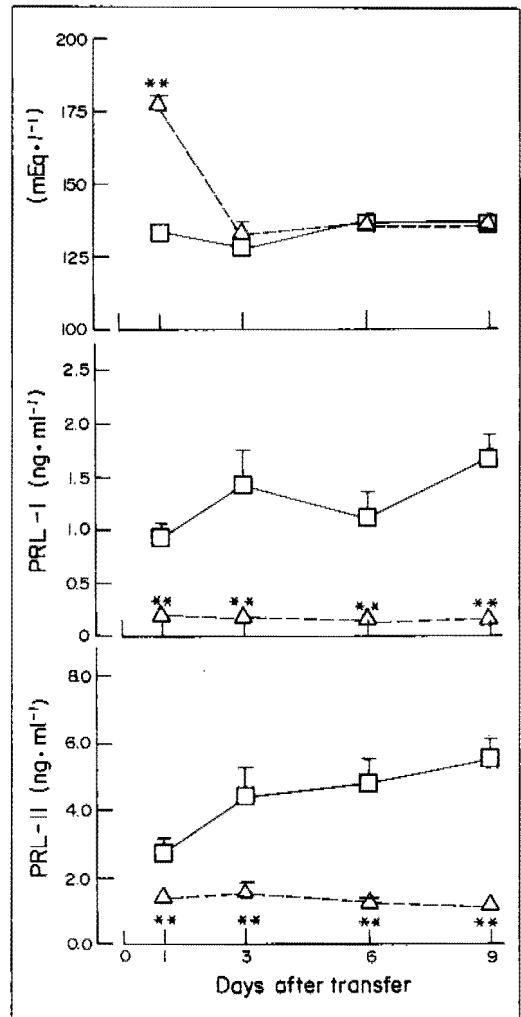


Fig. 1. Changes in plasma Cl, PRL-I and PRL-II levels in the adaptation of *Oreochromis aureus* to a brackish environment (14 ppt). Experiments were conducted on animals of 70 g mean body weight. The samples, taken at different dates after the beginning of treatment, were always taken at the same hour. Each point represents nine animals. □: control animals in freshwater; Δ: animals transferred from freshwater to brackishwater (14 ppt). \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ .

in brackishwater, the chloremia of the animals transferred is no longer significantly different from that of the animals kept in freshwater (Fig. 1).

The adaptation of *O. mossambicus* to brackishwater results in an increase in gill Na/K-ATPase activity as shown either by direct measurement (Table 1)

Table 1. Effect of salinity on plasma Na and Cl concentrations, gill Na/K-ATPase activity, transbranchial potential and Na fluxes (Dharmamba et al. 1973, 1975; Dangé 1985; Young et al. 1988) in *Oreochromis mossambicus*. Values are means  $\pm$  standard errors with numbers of determination in brackets.

	Environment		
	Freshwater	1/3 seawater	Seawater
Plasma values ( $\mu\text{Eq}\cdot\text{l}^{-1}$ )			
Na	158.5 $\pm$ 2.2(12)	163.2 $\pm$ 2.5(9)	163.8 $\pm$ 2.0(11)
Cl	139.0 $\pm$ 1.0(12)	149.5 $\pm$ 2.2(9)	146.2 $\pm$ 1.7(11)
NA/K-ATPase activity of the microsomal fraction of gill epithelium ( $\mu\text{mol}$ of phosphate/hour:milligram of proteins)	1.5 $\pm$ 0.1(12)	2.8 $\pm$ 0.7(9)	3.5 $\pm$ 0.8(11)
Transbranchial potential (Int-Ext) mV		14.7 $\pm$ 1.8(3)	35.2 $\pm$ 3.7(3)
Na flux $\mu\text{Eq}\cdot 100\text{ g}^{-1}\cdot\text{h}^{-1}$			
inflow	13.6 $\pm$ 2.5(8)	116 $\pm$ 29 (6)	746 $\pm$ 79 (6)
outflow	8.4 $\pm$ 1.3(8)	123 $\pm$ 56 (5)	1,651 $\pm$ 171 (8)

(Dharmamba et al. 1975; Dangé 1985; Young et al. 1988) or by fluorescence measurements on the opercular membranes using fluorescent ouabain (McCormick 1990).

Finally, during adaptation to seawater, the transbranchial potential and the transepithelial potential, which are positive compared to the external environment, increase strongly in *O. mossambicus* (Table 1) (Dharmamba et al. 1975; Young et al. 1988).

#### **Analysis of Ion Movements Involved in Plasma Ion Variations**

Experiments were conducted on Na fluxes in order to understand better the variations observed in plasma ion concentrations. In *O. mossambicus* reared in freshwater, the rate of Na turnover is  $0.15\% \cdot \text{hour}^{-1}$  (Dharmamba et al. 1973) with a positive net flux of sodium (inflow). This Na turnover value increases to  $26\% \cdot \text{hour}^{-1}$  in animals adapted to seawater (Dharmamba et al. 1973).

This increase in turnover in seawater can be explained by an increase in the

sodium (and chloride) outflow at the gill level, which is multiplied 10 times for diluted seawater (1/3 part) and 200 times for pure seawater (Table 1) (Dharmamba et al. 1975). The increase in Cl fluxes at the opercular membrane level is just as strong in seawater (Table 2) (Foskett et al. 1981).

In addition to this modification in ion fluxes, the adaptation of *O. mossambicus* to seawater results in reduced water permeability compared to freshwater fish (Potts et al. 1967).

#### **Concomitance between Ion Movement Variations and Gill Cell Modification during Adaptation to a Hyperosmotic Environment**

The increase in chloride excretion described previously, and demonstrated by Foskett et al. (1982), can be correlated to a modification of chloride cells at the gill and opercular membrane levels. These structures are rich in chloride-excreting chloride cells (Foskett and Scheffey 1982). For example, chloride cells are small and poorly developed in freshwater, but in seawater

Table 2. Fluxes of  $^{36}\text{Cl}$  measured under short-circuit current at the level of the opercular membranes isolated in *Oreochromis mossambicus* adapted to different environments (Foskett et al. 1981) Values are means  $\pm$  standard errors with numbers of determination in brackets.

$\mu\text{Eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$	Freshwater	After 10 days adaptation to seawater
Outflow	0.26 $\pm$ 0.07(6)	2.60 $\pm$ 0.30(5)
Inflow	0.18 $\pm$ 0.04(6)	0.50 $\pm$ 0.08(5)
Net flux	0.07 $\pm$ 0.07(-)	2.10 $\pm$ 0.30(-)

they are rich in mitochondria and show a well developed tubular system (seat of the Na/K-ATPase activity) in connection with the basolateral membrane (Foskett and Scheffey 1982; Foskett et al. 1982).

Foskett et al. (1982) describe two periods in the relationship between the increase in chloride excretion and the modifications observed at the chloride cell level. The first period in the increase in chloride excretion, which lasts three days, seems to be related to an increase in the number of chloride cells. The second period in the increase of excretion, noted after three days, seems to be due to an increase in chloride cell diameter and, therefore, to an increase in secretion in each cell.

These electrophysiological and microscopic studies allow us to conclude that the increase in transopercular ion fluxes observed during adaptation to seawater are closely related to an increase in the number of differentiated chloride cells which are responsible for the excretion of chlorides.

#### **Plasma Endocrine Parameters**

In endocrine terms, the adaptation of *O. mossambicus* to brackishwater results in a decrease in the circulating levels of prolactin. Such decrease occurs as soon as salinity drops 10% below seawater salinity (Nicoll et al. 1981).

Studies conducted on *O. aureus* transferred to brackishwater (14 ppt) show that the drop in PRL-I and PRL-II circulating

levels occurs in 24 hours (Fig. 2) (RIA titration: Aupérin et al. 1994). The evolution of both PRL-I and PRL-II plasma levels follows the same pattern, but the PRL-II concentration is higher than the PRL-I concentration after two weeks adaptation in brackishwater. This seems to indicate a coordinated regulation of the secretion of both PRL forms.

These results concur with the studies conducted in vitro on *O. mossambicus*, which show that the hypophyses of animals reared in freshwater synthesize and secrete more PRL than those of animals reared in seawater (Nagahama et al. 1975; Grau et al. 1981).

Therefore, the adaptation of tilapias to seawater results in a slight increase in plasma ion concentrations, an increase in their turnover rate, an increase of the gill Na/K-ATPase activity and a decrease in plasma PRL concentrations. It also results in an increase in the transmembrane potential which is held responsible for the passive Na outflow.

#### **Effects of Hypophysectomy and Prolactin Supplement in Animals Reared in Different Salinity Environments**

The studies of Handin et al. (1964) and Dharmamba et al. (1967) on hypophysectomized tilapias (*O. mossambicus*) have shown that these animals were unable to survive in

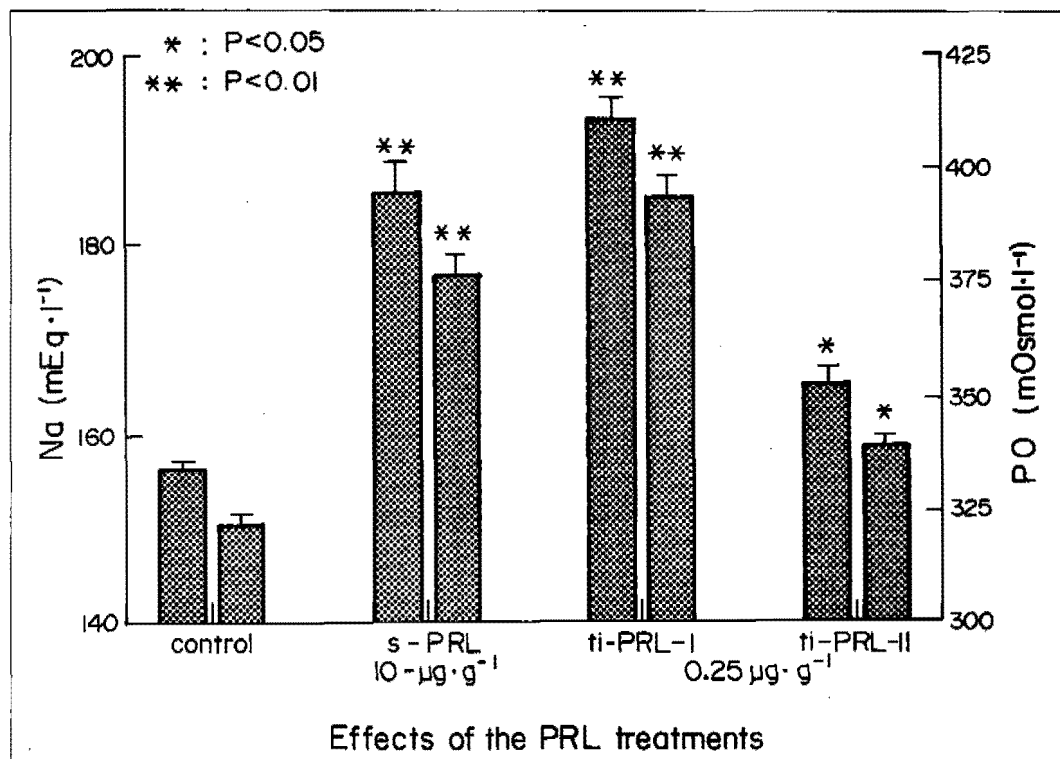


Fig. 2. Effect of sheep (s) or tilapia (ti) PRL treatments on the osmotic pressure (OP) and plasma Na concentrations in Nile tilapia (*Oreochromis niloticus*) adapted for 15 days to brackishwater (20 ppt) at the time of the first injection. PRL was injected every other day, 1 hour after daybreak (12/12) with a total of four injections. Injected doses are: sPRL: 10 µg·g<sup>-1</sup> of wet weight per injection; tiPRL: 0.25 µg·g<sup>-1</sup> of whole weight per injection (ti PRL's I and II were provided by F. Rentler-Delrue). \*: P<0.05; \*\*: P<0.01.

freshwater, but that treatments using sheep prolactin (sPRL) allow their survival. These first results have led to many other studies showing the role of prolactin in osmoregulation.

#### **Hypophysectomized Animals Reared in Freshwater**

The culture of hypophysectomized *O. mossambicus* in a Ringer environment (isotonic environment) allows the survival of the animals. The transfer to freshwater causes 100% mortality in 10 days (Dharmamba et al. 1967; Dharmamba 1970).

These mortalities in freshwater are due to an imbalance of the hydromineral equilibrium, which results in a continuous decrease in osmotic pressure (OP) and in Na concentrations as compared to the control animals reared in freshwater or to hypophysectomized animals kept in Ringer (Dharmamba et al. 1967; Dharmamba 1970).

The analysis of Na fluxes shows that the hypophysectomy of tilapia transferred to freshwater results in an inversion of the sodium net flux: from an inflow to an outflow. This inversion is due to a large increase in the passive outflow

and a decrease in the inflow (Table 3) (Dharmamba and Maetz 1972).

These results concur with the decrease in gill Na/K-ATPase activity, and in the decrease in transepithelial potential responsible for the passive Na outflow observed in hypophysectomized animals (Young et al. 1988).

#### **Hypophysectomized Animals Reared In Freshwater and Supplemented with Prolactin**

Daily injections of sheep prolactin (10  $\mu\text{g}\cdot\text{g}^{-1}$  of wet weight) in hypophysectomized *O. mossambicus* allow the survival of these animals in freshwater for several days. The osmotic pressure and the plasma Na concentrations in these animals are, based on the number of days of treatment, slightly lower than or equal to those in operated yet not hypophysectomized control animals (Dharmamba et al. 1967; Dharmamba 1970).

Similar results were obtained with injections of tilapia PRL (PRL-I, PRL-II or a mix of the two forms) before the transfer of hypophysectomized animals to freshwater. Thus these treatments allow the maintenance of plasma Na and Cl concentrations, the OP, and the transepithelial potential in the control animals (Specker et al. 1985; Young et al. 1988). Specker et al. (1985) did not observe

any difference between the two forms of PRL in the retention of Na.

The analysis of the Na fluxes in hypophysectomized animals transferred to freshwater shows that injections of sheep PRL in *O. mossambicus* reestablish a net inflow by decreasing the outflow, but do not seem to act on the inflow (Table 3) (Dharmamba and Maetz 1972).

#### **Intact Animals In Hyperosmotic Environments**

In tilapias (*O. mossambicus*) adapted to seawater, injections of sheep prolactin over a period of five days produce a 40 to 50% increase in plasma Na and Cl concentrations and an increase in osmotic pressure (Table 4) (Clarke 1973; Dharmamba et al. 1973; Dharmamba and Maetz 1976; Herndon et al. 1991). In *O. niloticus* adapted to the brackishwater (20 ppt), injections of tilapia prolactin (PRL-I or PRL-II) also cause an increase in plasma Na and Cl concentrations and in the OP (Fig. 2).

This increase in plasma Na after the injections of PRL can be explained by a modification in the hydrogen and sodium balance. For example, the results of Wendelaar Bonga and Van Der Meij (1981) on tilapias (*O. mossambicus*) adapted to a calcium-poor, 9 ppt saline environment (isoosmotic) indicate that sheep prolactin reduces the osmotic

Table 3. Na Flux ( $\mu\text{Eq}\cdot 100\text{ g}^{-1}\cdot\text{h}^{-1}$ ) at the gill level in *Oreochromis mossambicus* after six to seven days in freshwater: Effect of the hypophysectomy and the sheep PRL supplement (10  $\mu\text{g}\cdot\text{g}^{-1}$ , one injection per day for five days). Values are means  $\pm$  standard errors (Dharmamba and Maetz 1972).

	Inflow	Outflow	Net flux
Whole animals (8)	13.63 $\pm$ 2.49	8.43 $\pm$ 1.27	+5.19 $\pm$ 1.66
Hypophysectomized animals (6)	5.58 $\pm$ 2.83	21.56 $\pm$ 3.18	-15.98 $\pm$ 2.32
Hypophysectomized animals (7) + NaCl (0.9%)	9.42 $\pm$ 0.89	14.81 $\pm$ 1.35	-5.40 $\pm$ 1.40
Hypophysectomized animals (13) + sPRL (10 $\mu\text{g}\cdot\text{g}^{-1}$ )	9.87 $\pm$ 1.24	8.74 $\pm$ 0.94	+1.13 $\pm$ 0.81

Table 4. Osmotic pressure (OP), plasma Na concentration and Na Inflow and outflow ( $\mu\text{Eq}\cdot\text{hour}^{-1}\cdot 100\text{g}^{-1}$ ) in *Oreochromis mossambicus* adapted to seawater. Effect of the hypophysectomy and the sheep PRL supplement ( $10\ \mu\text{g}\cdot\text{g}^{-1}$ , one Injection per day for five days) (Dharmamba et al. 1973; \*: Dharmamba and Maetz 1976; and \*\*: Dharmamba et al. 1975). Values are means  $\pm$  standard errors with number of determinations in brackets.

	OP (mOsmol $\cdot\text{l}^{-1}$ )	Plasma Na concentration ( $\mu\text{Eq}\cdot\text{l}^{-1}$ )	Inflow ( $\mu\text{Eq}\cdot\text{h}^{-1}\cdot 100\text{g}^{-1}$ )	Outflow ( $\mu\text{Eq}\cdot\text{h}^{-1}\cdot 100\text{g}^{-1}$ )
Whole animals In seawater (5)	293 $\pm$ 7	165.7 $\pm$ 4.5	746 $\pm$ 79 (6)**	1,651 $\pm$ 17 (8)**
Whole animals In seawater + Injections of NaCl (5)	294 $\pm$ 9	161.7 $\pm$ 2.5	739 $\pm$ 141.2	1,275 $\pm$ 199.5
Whole animals In seawater + injections of sheep PRL	414 $\pm$ 10 (7)	230.6 $\pm$ 5.2 (7)	191 $\pm$ 23.6 (5)	321 $\pm$ 61.8(5)
Controls In seawater (7)		153.6 $\pm$ 4.8*		
Hypophysectomized animals in seawater (6)		175.7 $\pm$ 1.5*		

water inflow and outflow at the gill level. In the same species adapted to seawater, the increase in plasma Na after a sheep prolactin treatment causes 70 to 75% decrease in the Na turnover (Dharmamba et al. 1973). This coincides with the decrease in sodium permeability (Young et al. 1988) which results in a fivefold reduction of the Na inflow, and a fourfold reduction of the outflow (Table 4). Under these conditions, the net outflow is inhibited by 75% (Dharmamba et al. 1975).

These studies support the results of Foskett et al. (1982) which indicate that in tilapias (*O. mossambicus*) adapted to seawater, sheep PRL inhibits, in a dose-dependent manner, the excretion of chloride and conductance at the opercular membrane level. Similar effects are observed during injections of PRL from the rostral *pars distalis* of the PRL-rich adenohypophysis.

Foskett et al. (1982) also suggest that the inhibition of chloride (and conse-

quently Na) secretion after an injection of PRL occurs due to a decline in the population of sodium-excreting chloride cells and/or a decline in the active transport to the remaining cells. In fact, recent studies (Herndon et al. 1991) have shown that a treatment using sheep prolactin inhibits chloride cell differentiation. For example, the number of chloride cells at the opercular membrane level does not vary, but the average size (diameter and depth) decreases. These chloride cells are therefore no longer in contact with both the external and internal environments. The effect of prolactin seems to be to inhibit hypertrophy and the differentiation of new chloride cells (Herndon et al. 1991).

Jointly, these results suggest that sheep prolactin inhibits the gill Na/K-ATPase in animals adapted to seawater (Dharmamba et al. 1973). However, under the conditions used by Young et al. (1988) (two injections to hypophysectomized animals kept in 25%



seawater), the two prolactin isoforms do not change the Na/K-ATPase activity. These results support those of Herndon et al. (1991) (Table 5), who did not observe any variation in the Na/K-ATPase activity after five injections of sheep prolactin.

These two sets of results (decrease in the number of chloride cells and absence of variation in the Na/K-ATPase activity) seem to be contradictory and suggest a complex effect of prolactin on chloride cells.

### ***Hypophysectomized Animals In Hyperosmotic Environments***

Hypophysectomy performed on *O. mossambicus* reared in seawater does not significantly modify Na and Cl concentrations as found in whole animals reared in the same environment (Table 4) (Dharmamba and Maetz 1976), but it produces an inhibition of 50% of the Na outflow (Dharmamba and Maetz 1976).

Whatever the environment salinity, sheep PRL or its equivalent leads to an increase in OP and plasma Na and Cl concentrations. But the action of prolactin on sodium movements seems to be different depending on whether animals are kept in fresh- or seawater. In freshwater, prolactin acts only on the outflow, whereas in seawater, it reduces both inflow and outflow.

These results suggest that the decrease in plasma PRL concentrations is a necessary condition to ensure the animals' survival and an optimal salt excretion at the gill level in seawater animals. However, the effect of hypophysectomy in tilapias adapted to seawater, which leads to an inhibition in the Na outflow, suggests that other endocrine mechanisms are involved.

### **Effects of Stress on Prolactin Levels**

Two types of stress must be distinguished: stress related to farming conditions (stocking density, manipulation of the animals, etc.), which causes modifications in plasma PRL concentrations in salmonids (Avella et al. 1991), and stress related to the physicochemical characteristics of the environment (acidic pH, pollution by heavy metals, etc.) which causes an increase in hypophyseal prolactin cell activity in *O. mossambicus*.

#### ***Stress Related to Farming Conditions***

Two types of experiment have provided the means to trace the plasma PRL-I and PRL-II levels. In *O. niloticus*, an analysis of the following effects was conducted:

Table 5. Number and size of chloride cells at the opercular membrane level and gill Na/K-ATPase activity in *Oreochromis mossambicus* adapted to seawater. Effect of the sheep(s) PRL supplement ( $10 \mu\text{g}\cdot\text{g}^{-1}$ , one injection per day for five days) (Herndon et al. 1991). Values are means  $\pm$  standard errors.

	Animals injected with NaCl	Animals injected with s-PRL
Number of chloride cells ( $\text{cells}\cdot\text{cm}^{-2}$ )	6,979 $\pm$ 1,825	7,499 $\pm$ 1,258
Size of chloride cells ( $\mu\text{m}^2$ )	325 $\pm$ 55	130 $\pm$ 17
Gill Na/K-ATPase activity ( $\text{mmol ADP}\cdot\text{mg prot}\cdot\text{hour}^{-1}$ )	9.9 $\pm$ 1.4	11.0 $\pm$ 1.8

1) the effect of the sampling method: rapid sampling without anaesthesia upon release from the culture tank. This technique was used as a control. Also a sampling of the animals after anaesthesia in 4 min (0.5 ml phenoxyethanol·l<sup>-1</sup> of water) or in 1 min (1 ml phenoxyethanol·l<sup>-1</sup> of water); and

2) the effect of stress when animals (five fish in a 10x40x45 cm space) were confined in a reduced volume of water for 1 hour, then anesthetized in 4 min.

PRL circulating levels (Fig. 3) are higher (but not significantly different) in animals anesthetized in 1 min than in control animals. In contrast, stressed animals (showing plasma cortisol concentrations [93.1±6.3 ng·ml<sup>-1</sup>] significantly higher

than those in control animals [2.4±1.5 ng·ml<sup>-1</sup>] or animals anesthetized in 4 min (cortisol concentrations = 113.8±10.6 ng·ml<sup>-1</sup>) show PRL circulating levels significantly higher than those of control animals. However, the increase in PRL circulating levels in stressed animals is not significant when compared to animals anesthetized in 4 min.

#### Environment-related Stress

The exposure of *O. mossambicus* to acidic pH (pH 3.5) causes severe stress leading to a continuous decline in the plasma osmotic pressure (Wendelaar Bonga et al. 1984). This reduction is accentuated in the first six days after

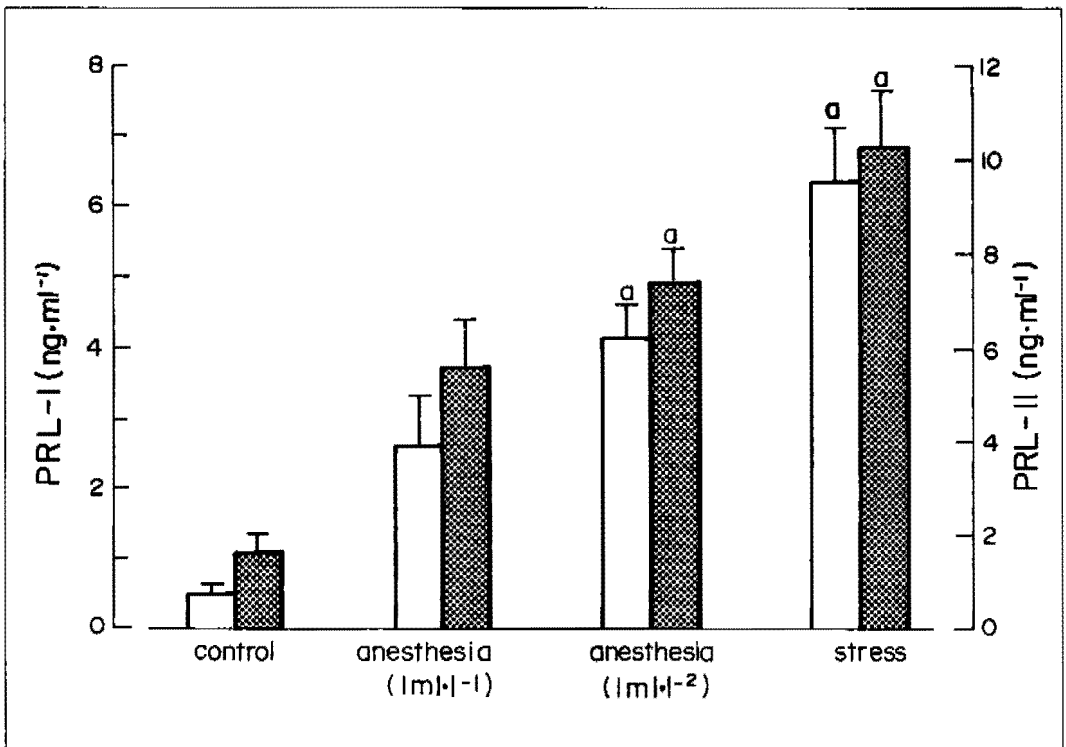


Fig. 3. Effect of anaesthesia and stress on plasma PRL-I (bars with no shading) and PRL-II (shaded bars) concentrations in *Oreochromis niloticus*. Animals were adapted to farming conditions for seven days. Two doses of anesthetic (phenoxyethanol) were used. Stress was produced by confining the animals in a reduced space in the aquarium for periods of 1 hour. a:  $P < 0.05$  compared to control animals.

the transfer, then a partial restoration occurs after 14 days. The authors describe a rapid change (in 24 hours after the transfer to an acidic environment) in the ultrastructure of the prolactin cells, and after 14 days, an increase in the size of the rostral *pars distalis*, from 31% to 47% of the total hypophysis volume, suggesting an increase in the synthesis and release of PRL.

Subsequent to a three-month adaptation in acidic pH (4.5), there is no longer any difference between plasma Na concentrations in animals adapted to acidic pH and those in the control animals (Flick et al. 1989).

These authors indicate that at the beginning of the experiment, the sodium inflow is reduced by 55% and the outflow by 70% of the value found in control animals. In contrast, they describe a restoration of the outflow after 10 days. At the end of this period, the net flux reaches zero, while there is an inflow of  $93 \text{ nmol} \cdot \text{hour}^{-1} \cdot \text{g}^{-1}$  in animals with pH 7.0.

These results support the observations made previously, i.e., that the increase in prolactin secretion observed in the adaptation to acidic pH seems to be causing the decrease in Na outflow observed in these animals.

The effect of cadmium pollution also causes a temporary decline in plasma electrolytes as well as an increase in prolactin cell activity. But such an increase is only temporary, and it ceases when metallothionines appear in the liver and the gills (Fu et al. 1989; Pratap et al. 1989). These detoxifying proteins apparently protect osmoregulating mechanisms against cadmium.

## Conclusion

This study shows that prolactin plays an important role in freshwater tilapias.

It is essential to their survival because it reduced the Na outflow. On the other hand, the absence of prolactin in animals adapted to a hyperosmotic environment also seems essential, since this hormone causes a retention of the plasma Na, which leads to their death. In this environment, prolactin acts by reducing Na inflow and outflow.

Incidents of stress (manipulation, confinement, modification of the quality of farming conditions) lead to a significant and rapid increase in plasma PRL levels. If these factors also cause a similar increase in plasma PRL concentrations in brackishwater, it is likely that it will lead to an imbalance of the hydromineral equilibrium in these animals. Such an imbalance, if it persists in a hypertonic environment, could cause death and could account for the unexplained mortalities in some cultures in brackishwater lagoons. The hypothesis of an increase in plasma PRL concentrations following environmental fluctuations during culture cycles should be studied further.

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