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Does MCH play a role on establishment or maintenance of social hierarchy in Nile tilapia?

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ABSTRACT

Body coloration has a fundamental role in animal communication by signaling sex, age, reproductive behavior, aggression, etc. Nile-tilapia exhibits dominance hierarchy and the dominants are paler than subordinates. During social interactions in these animals, these color changes occur rapidly, and normally the subordinates become dark. In teleosteans, from the great number of hormones and neurotransmitters involved in color changes, melanocyte hormone stimulates (α-MSH) and melanin concentrates hormone (MCH) are the most remarkable. The aim of this project was to investigate the role of MCH in the establishment of hierarchical dominance of the Nile-tilapia. We analyzed the effect of background coloration in the dominance hierarchy. It was then compared to the melanophore sensibility of dominants and subordinates' fishes to MCH; finally, it was checked if the social rank affects the number of these pigment cells in dominants and subordinated fishes.

Fishes which have a social hierarchy established and adjusted individually to the background exhibits paler body coloration when a visual contact was possible, independently of previous social rank and background color. Probably, even recognizing each other, fishes could be defending their new territory. Melanophores of the subordinate fishes were more sensible to MCH than dominants. It suggests that dominants fishes, which are paler than subordinates, could be under a chronic effect of MCH, which could be due a desensitization of melanophores to this hormone. The opposite effect seems to be occurring on subordinate fishes. It was not observed a significant change in the number of melanophores when the fishes were exposed to a prolonged period of agonistic interaction. It is possible that the exposure time for this interaction might not have been sufficient to have any change in the number of these cells of dominants and subordinate fishes.

1. Introduction

The establishment of social hierarchy is a significant feature in several groups of animals, including cichlid fishes. Size, previous occupation of a territory, prior hierarchical position and sex are among the factors that can lead to dominance in a group of fishes [1,2].

In the context of complex social interactions in fishes, color can be considered as an indication of relative hierarchical level among the individuals of a group. As hierarchical relationships may vary over a short period, the very existence of plastic mechanisms associated with these circumstances is crucial. Besides providing for rapid change, coloring has also proved to be suitable for situations where a relationship can last for an even longer period. Due to the high density of melanophores, the males in some species of cichlids present a prominent and dark vertical stripe in the anterior region of the eye, as an indication of dominance or aggression. This eye-bar often disappears in subordinate fish or in situations of flight [3].

Volpato et al. [4] reported variations in body and eye-color patterns in combatant Nile tilapia, as indicative of hierarchical levels between opponents. Sometimes this display is even enough for prior definition of the winner and the loser, thereby avoiding any actual aggressive interaction [5].

This ability to rapidly change color during social interaction, as well as to adapt to variations in ambient lighting conditions and background color, as observed in fishes and other ectothermic vertebrate groups, is due to the translocation of pigment granules within specialized cells (chromatophores) originating from the embryonic neural crest [6]. These cells, with their inherent dendrites, synthesize pigments, which they store in specialized organelles [7].

The most common mechanism of fish color-change involves alterations in the intensity, and even the area, of the melanic colors, black, brown and grey, through either the dispersion, or aggregation, of pigment granules present in pigment cells, or through variations in the amount of pigments. These processes are controlled by either the

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nervous or the hormonal system, or by both, with quicker or slower responses. Several hormones and neurotransmitters, such as melanocyte-stimulating (MSH), melanin-concentrating (MCH), and norepinephrine, are known to regulate chromatophores response, thereby inducing either the aggregation of pigment granules around the nucleus, or their dispersion into dendritic projections [8]. In addition to the quick or physiological responses, these cells can also change their number or size, when the stimulus persists long enough. This process, denominated morphological color-change, also facilitates more efficient animal adaptation to the substrate, as is the case of cryptic fishes.

Variations in body-color in cichlid fish clearly show differences in hierarchical level [1,9], and higher α – MSH plasma levels in subordinate salmonid fishes in relation to the dominant [10]. It is also known that, whereas stress affects plasma levels of α – MSH and MCH in teleost fishes [11,12], substrate coloration directly affects the animal's body-color, due to changes in melanotropin secretion [13].

There are no available data on cichlids as to the involvement of melanotropic hormones during social interactions. Even so, during intense social interaction among individuals of the Nile tilapia, *Oreochromis niloticus*, dominant animals become lighter-colored than subordinates [4,9], an indication of the involvement of α – MSH in the maintenance of hierarchy in this species. On the other hand, the pallor of dominant individuals could be linked to the chronic effect of MCH in melanophores.

The aim herein was to investigate the role of melanotropic hormones during social interaction among Nile tilapia, with emphasis on MCH.

2. Material and methods

Fish of the same size were randomly isolated (1 fish per aquarium) for 7 days, in glass aquaria with a grey colored bottom (40 × 20 × 25 cm) and aeration, a 12:00 L: 12:00 D photoperiod, 250 Lux, and 23 °C ± 2, to so minimize hierarchy that had been previously established in the maintenance tank. Commercial food was given to the proportion of 5% of fish biomass.

Following isolation, the fishes were transferred to another aquarium similar to the above, but which was divided equally in 2, by an opaque screen, to so eliminate visual or direct contact, but to maintain the chemical. Individual identification was by variegated caudal fin cutting. Under these conditions, two fishes were then randomly paired per aquarium. During the first 3 days, pairing was for 30, 20 and 20 min, respectively, thereby facilitating the establishment of clear social ranking. The number of clashes that occurred during confrontation was quantified throughout, taking into consideration biting as a whole, frontal confrontation (mutual pushing with the mouth), and side confrontation (mutual sideways body-rubbing). To evaluate whether there was change in the number of melanophores of subordinate and/or dominant fishes, for a longer period, the same procedure was done, and the number of melanophores evaluate at the 1st, 5th and 10th day. The data were analyzed by Wilcoxon testing ($p < 0.05$).

Specimens of Nile tilapia have dark vertical and horizontal stripes along both sides of the body. From the operculum to the base of the caudal fin, there are 8 vertical and 2 horizontal stripes. The total number of stripes was transformed into a percentage, with 100% being considered as the highest degree of darkening. In order to increase homogeneity, data were transformed by extracting the square root of each value added to a constant ($k = 0.5$). The number of stripes was calculated at time zero and after 20 min of contact, to so facilitate checking any change in color during confrontation. Darkening percentages between dominant and subordinate individuals were compared by two-way ANOVA, followed by t-testing for dependent samples ($p < 0.05$).

After a clear dominant-subordinate relationship had been established by the 4th day, the pairs were removed to other tanks as described above, with a black and white, or either a white or a black background color. Experimental conditions were: 1 – SDWB – subordinate and dominant in a white background; 2 – SWDB – subordinate in a white and dominant in a black background; 3 – SBDW – subordinate in a black and dominant in a white background; 4 – both subordinate and dominant in a black background. The specimens remained isolated for 1 h. After removing the opaque screen, the number of confrontations and the color were quantified before and after 30 min, as described. Confrontation was analyzed by Wilcoxon testing, and body color by one-way ANOVA followed by LSD testing. Differences were considered significant for $p < 0.05$.

On the 4th day, scales were excised from identical regions of the body of paired subordinate and dominant specimens in a grey background, and then placed in physiological solution (in mM: NaCl 128; KCl 2.7; $CaCl_2$ 1.8; glucose 5.6; $NaHCO_3$ 2.5; pH 7.4), prior to fixing on lamina in a closed system, where hormone doses (10^{-12} M to 10^{-8} M) were perfused accumulatively. The MCH (Bachem Co.) effects on pigment cells were measured with a special microscopic ocular furnished with a numeric scale, randomly focalized on melanophores dendritic processes. Values were transformed as percentages of aggregation. EC_{50}s were calculated from dose-response curves through non-linear regression, whereupon the curves were compared by two-way ANOVA followed by t-testing ($p < 0.05$).

3. Results

In tanks with a grey-colored background, the outcome of agonistic encounters between pairs revealed a clear dominant/subordinate hierarchy, with a higher number of attacks by the former than the latter (Fig. 1). However, when transferred to tanks with either black or white backgrounds, agnostic behavior continued, but even so, independent of hierarchy or background color, hence without statistical significance (Fig. 1).

Regarding the coloration of the body, the data are presented in Fig. 2. It is observed that dominant and submissive fish, in grey substrate (3rd day), did not present significant difference in body color prior to the beginning of social interaction. However, after 20 min of clashes, the dominant ones were significantly lighter compared to the submissive ones. On the 4th day, with the animals adjusted to the colorations of the respective substrates. The body color was measured at time zero and after 30 min of visual contact. In the initial period, the fish presented the body cohort related to the substrate coloration. That is, dark animals on dark substrates and light animals on light substrates (Fig. 2). Interestingly, when visual contact between aquarium walls was allowed, both dominant and submissive cleared, regardless of the previous hierarchical degree and color of the substrate to which they were previously adjusted (Fig. 2).

It was also analyzed whether dominant and submissive fish, exposed to the chronic effect of social hierarchy, present a difference in the number of melanophores related to the hierarchical degree. Fig. 3 shows the hierarchical relationship between dominant and subordinate fish over 10 days of confrontation. However, between dominant and subordinate fish, no significant difference was observed in relation to the number of cells with the hierarchical rank, during the same period of confrontation (Fig. 4).

From the analysis of hormone response to MCH, wherein hierarchy had already become well-established at the 3rd day (Fig. 5), hormone-induced dose-dependent pigment aggregation in melanophores was analyzed. Notably, the pigment cells of subordinate fish ($EC_{50} = 3.88 \times 10^{-12}$ M), are more sensitive to MCH than in dominant individuals ($EC_{50} = 7.99 \times 10^{-11}$ M). EC_{50} of subordinate fishes was 20 times lower than in dominant ones (Fig. 6).

4. Discussion

Hierarchical relationships are complex mechanisms involving endogenous and exogenous factors, as well as previous experience,

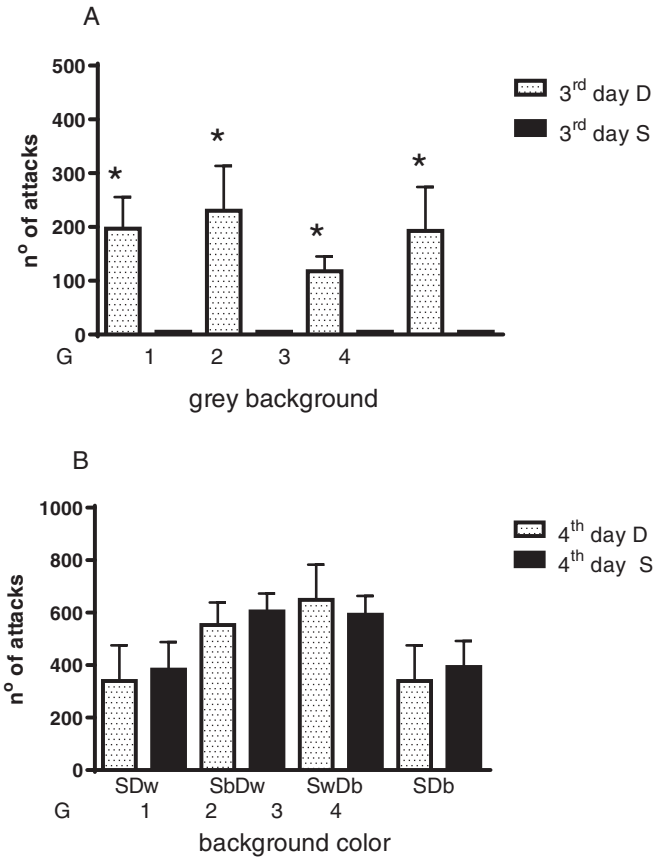


Fig. 1. Average frequency (± standard deviation – SD) of Nile tilapia attacks as a function of the color of the substrate. S = subordinate; D = dominant. In chart A – dominant animals, on grey background, and in B the same groups 1 to 4 of chart A correspond respectively to the SDw, SbDw, SwDb and SDb where SDw = subordinate and dominant on white background; SbDw = subordinate on black and dominant in a white background; SwDb = subordinate on white and dominant on a black background; SDb = subordinate and dominant on black background. In A, *means statistically different ($p \leq 0.05$). There was no significant difference between the means of the groups in B. Each group is composed of 5 pairs of specimens.

leading to individual dominance or submissiveness. Although hierarchical and behavioral relationships in Nile tilapia have been well reported in the literature [1,4,9,14], the hormone modulation involved in determining species social-hierarchy still remains practically obscure.

Following prolonged investigation of the physiology of pigmentation in teleost fish, modulation in pigment cells turned out to be hormone-mediated. Outstanding among these is the role of alpha-MSH, as a darkening agent in all the vertebrates investigated [15–18]. On the contrary, MCH biological activity for inducing pigment-granule-aggregation only occurs in the teleost and dipnoan. Even so, its role as neurotransmitter and modulator in several physiological processes has been documented in all the vertebrates investigated to date, this including the genus *Oreochromis* [19–26].

Observations on the adaptation of vertebrate body to substrate color have been under way since the eighteenth century. Evidently, an increase in both cell number and the amount of inner melanin occurs after longtime exposure to dark substrates, whereas after adaptation to a light-colored background for extended periods there is a decrease in both [27,28].

In the present study, variations in substrate color were used to manipulate body-color in specimens of Nile tilapia. Data showed that adaptation to a variety of background colors occurred in about 1 h. Regardless of hierarchical level, both dominant and submissive fish adapted to the respective substrate, with or without display of the vertical and horizontal dark stripes. Surprisingly, and without exception, when eye contact occurred, even though in separate aquariums, all changed to a lighter color, independent of prior adaptation. In a similar study of social interaction among specimens of *Salevinus alpinus*, this adaptation to light and dark backgrounds was also observed [10]. Although the authors found no significant differences in body-color between dark-substrate-adapted dominant and submissive individuals, in a light one, the color in submissive fish took on a darker tone, whereas in the dominant this remained light.

One possible explanation for our results seems to be territory defense. When studying the cichlid *Pterophyllum scalare*, Chellappa and colleagues [29] noted that prior residents, even though smaller than intruders, were invariably the winners in territorial disputes against invaders, thence implying that precedence overrules body-size. This was not the case in a neutral territory, whence the largest animals were overwhelming. As in the present study, aquariums with different-colored substrates also comprise neutral territories, territory defense occurred independent of visual recognition.

Hence the implication that, in Nile tilapia the control of body-color seems to follow an order of priorities, whereby territorial defense assumes greater importance than adaptation to the substrate.

Melanotropins act directly on chromatophores through the mediation of specific receptors. In the present study, MCH-activity was observed in melanophores of fishes with a previously established hierarchy, with pigment-aggregation-induction in both dominant and submissive individuals. Since the EC50 dose-response curve in submissive fish was 20 times lower than in the dominant, melanophore sensitivity was apparently higher in the former. Clarity in dominant individuals is likely due to the chronic effects of MCH-activity, a possible indication of this lesser sensitivity, whereas insusceptibility to these effects in the usually darker, submissive animals clearly indicates greater MCH-sensitivity.

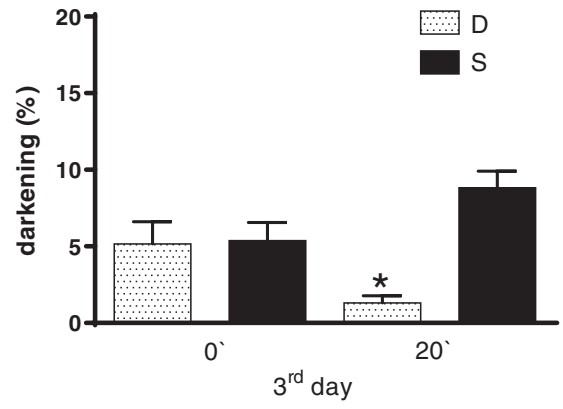
The MCH-role as modulating agent in the release of alpha-MSH in the pituitary gland of *Oreochromis mossambicus* [25], bolsters this hypothesis. A decrease in the plasma levels of alpha-MSH was noted in trout injected with MCH [30]. This level was higher in submissive fish than in dominant in the salmonid, *Salevinus alpinus* [10].

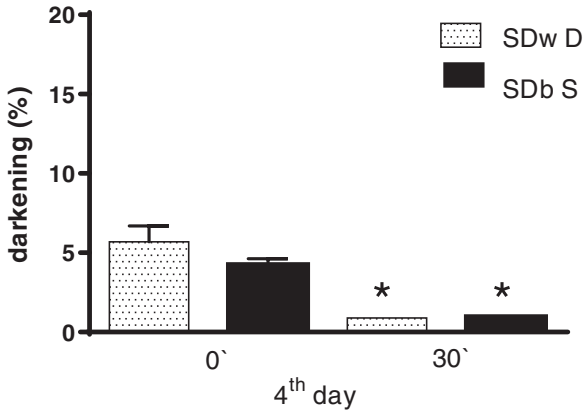
Worthy of note; teleost fish present innervated melanophores [8,15]. During social interactions, color changes occur rapidly, only a question of seconds to minutes. Such changes could already be under way due to the prior release of catecholamines from nerve endings, these inducing the aggregation, or dispersion, of pigment granules. Subsequently, the role of melanotropic hormones would be to maintain body coloration according to the assumed hierarchical level.

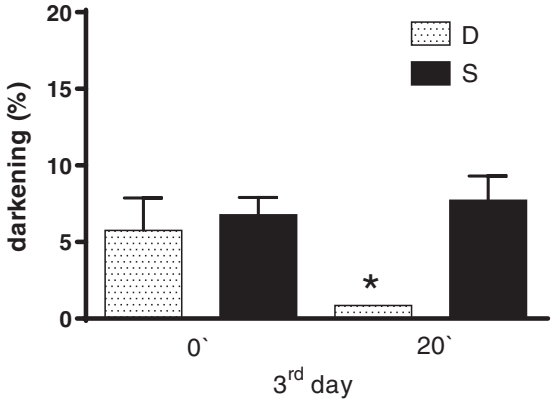
In an attempt to elucidate the effect of MCH on body coloration during agonistic interaction in Nile tilapia, the question was whether the chronic effect of a social hierarchy comprising dominant and submissive fishes would result in changes in the number of melanophores. As already mentioned, in these fishes changes in body coloration during social interactions are very fast. When separated individuals, with well-established hierarchical levels (by the 3rd day), were again paired, in less than 1 min (data not shown) both quickly assumed coloration of the pre-assumed level, i.e., dominant light and submissive dark. Herein, this was also noticed during prolonged periods of agonistic interaction.

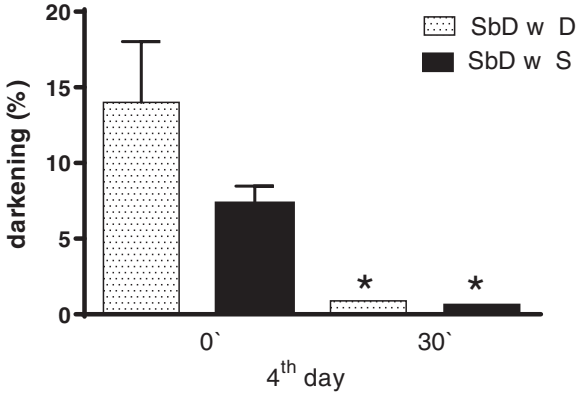
Thus, when hierarchy had been well-established after 5 to 10 days of social interaction, a comparison was made of the number of cells per scale in both dominant and submissive fish. No significant difference between the two was found, in either the number of melanophores, nor in the total number of cells.

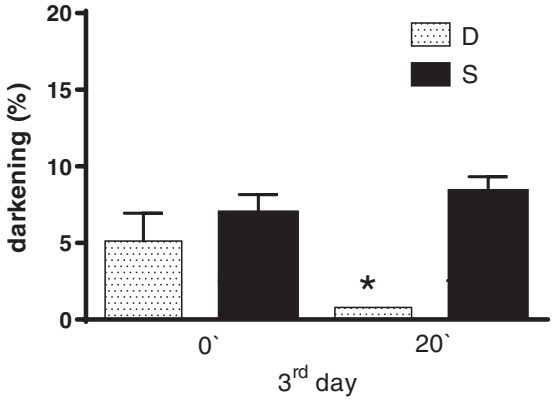
When studying the teleost *Verasper moseri*, Amiya and colleagues [31] noted drastic differences in body coloration of individuals adapted to a white background in relation to those to a dark one. In the former, there was a significant decrease in pigmented area, when compared to the latter. In the same study there were significant differences in the plasma level of MCH, and its immunoreactivity, in the brain of fishes adapted to a white substrate, compared to those to a dark one. Both

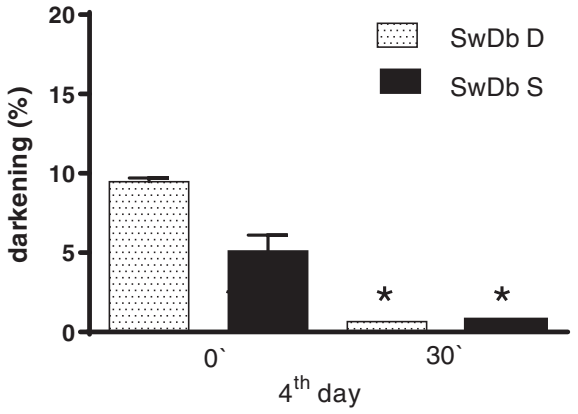


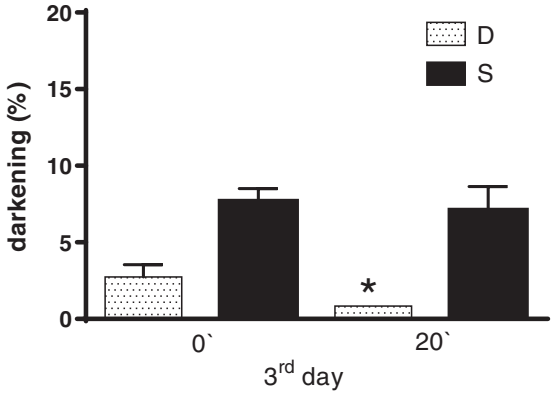












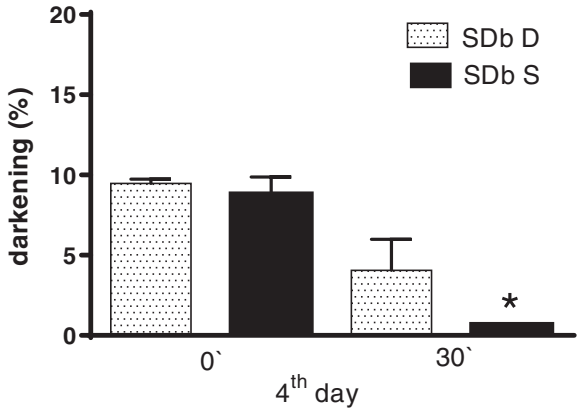


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Fig. 2. Body color in relation to hierarchical degree and substrate color in Nile tilapia. Mean data (n = 5; ± mean standard error – MSE) of the percentage of dark stripes in the body (the higher the percentage, the darker the animal). Data measured before social interaction (0 min) and after this interaction (20 or 30 min), on two consecutive days (3rd and 4th). On the 3rd day the contact was physical, with the animals paired in the same aquarium. On the 4th day, there was only visual matching, with the animals in separate and contiguous aquariums. *Indicates significant difference between dominant and subordinate at the same time. SDw = subordinate and dominant on white background; SwDb = subordinate on white and dominant on a black background; SbDw = subordinate on black and dominant in a white background; SDb = subordinate and dominant on black background. On the 3rd day the substrate was grey, and on the 4th day the substrates were white or black.

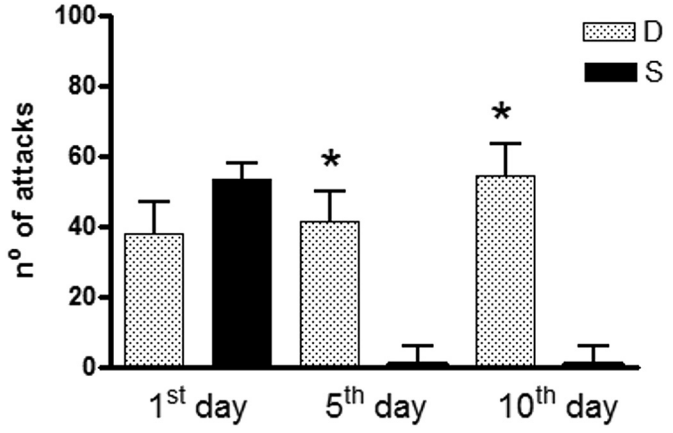

Fig. 3. Mean number (n = 5; ± MSE) of attacks due to degree of dominance and periods of encounter. S = subordinate; D = dominant. *Significant difference on the day of observation ($p < 0.05$).

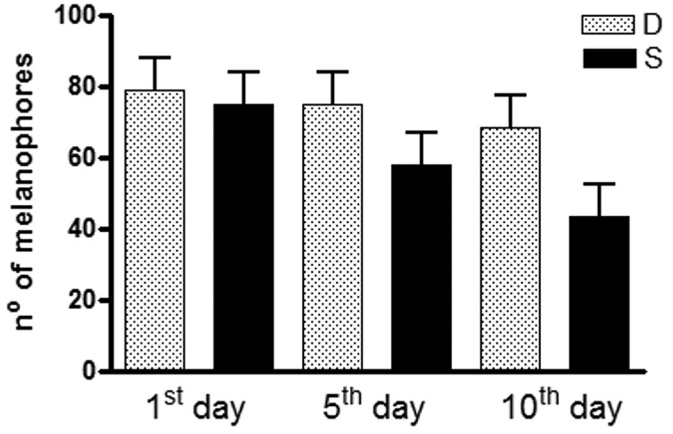

Fig. 4. Mean number (n = 5; ± MSE) of dominant (D) and subordinate (S) Nile tilapia melanophores as a function of time. There was no significant difference between the means in each day of confrontation.

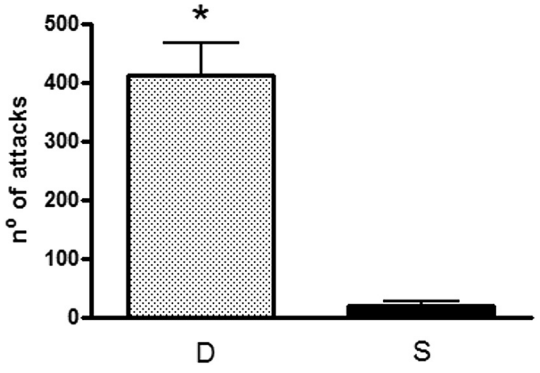

Fig. 5. Mean number (n = 5; ± SD) of attacks emitted by dominant and subordinate fish of Nile tilapia, on the 3rd day of pairing. *Statistically different. S = subordinate fish; D = dominant fish.

were higher in specimens adapted to a white background, thereby implying that MCH had induced a decrease in pigmented area.

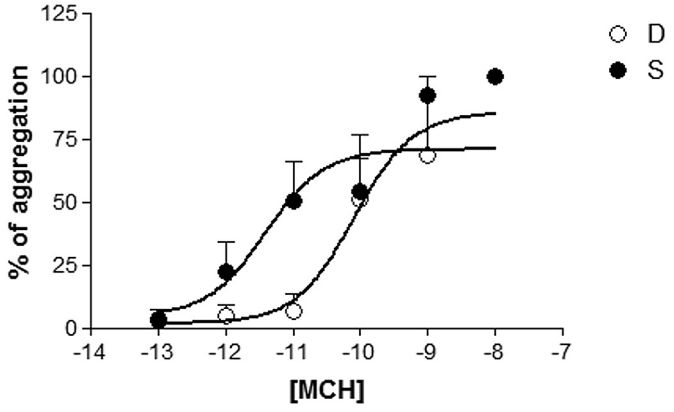

Fig. 6. Dose-response curves for MCH in melanophores of dominant and subordinate fish of Nile tilapia. Each point is the mean (n = 5; ± MSE). S = subordinate fish; D = dominant fish. The curves differ statistically from each other.

Yamanome and colleagues [32] also noted high reduction in the pigmented area of *V. moseri* individuals, previously injected intraperitoneally with MCH. Thus, these two studies demonstrate the effect of both endogenous and exogenous MCH in the control of body-color.

Perhaps the time of background adaptation chosen for the present study, was insufficient to conduce to any change in the number of melanophores. According to the literature, in several studies on morphological color-change, the animals were kept in experimental substrates for longer periods, sometimes weeks or even months [6,26,27]. Hence, the period adopted in this study could actually be too short to observe any change in the number of cells. However, we must stress that our analysis was performed in animals exposed to chronic effect of social hierarchy. This condition may have interfered with the physiological processes that lead to change in the number of pigment cells, in the course of substrate adaptation.

5. Conclusions

There is an order of priorities among factors that modulate body coloration, in which hierarchical struggles overlap the effects of substrate coloration. MCH seems to be involved in the variation of coloration dependent on the hierarchical degree, since the sensitivity to pigment aggregation induced by this hormone in the melanophores of submissive fish was greater in relation to that occurred in the dominant animals. It is assumed that this effect derives from the chronic effect of the MCH on the melanophores of the fish. In social interactions of a few days, less chronic, the effects observed in coloration cannot be attributed to changes in the number of melanophores in the scales.

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