

# CHANGES IN GUT GROSS MORPHOLOGY OF TRAÍRA, *Hoplias malabaricus* (TELEOSTEI, ERYTHRINIDAE) DURING LONG-TERM STARVATION AND AFTER REFEEDING

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## ABSTRACT

Adult traíra (*Hoplias malabaricus*) were submitted to different periods of food deprivation (from 30 to 240 days) and refed for 30 days after 90 and 240 days of starvation. Stomach length remained constant during all the experimental period. However, the intestine length was significantly reduced after 30 days of food deprivation. Normal length was not recovered after refeeding. The number of pyloric caeca did not change significantly. Conversely, caeca thickness decreased after 150 days of starvation and their length decreased after 180 days. After refeeding, however, the pyloric caeca recovered original thickness. In fish refed after 240 days of starvation the length of these structures seemed to present compensatory growth, becoming longer than in the control group.

*Key words:* gut, gross morphology, pyloric caeca, starvation, refeeding.

## RESUMO

### Alterações na morfologia do trato digestório da traíra, *Hoplias malabaricus* (Teleostei, Erythrinidae), durante jejum prolongado e realimentação

Traíras (*Hoplias malabaricus*) adultas foram submetidas a diferentes períodos de privação de alimento (de 30 a 240 dias) e realimentadas por 30 dias após 90 e 240 dias de jejum. O comprimento do estômago permaneceu constante durante todo o tempo experimental. No entanto, o comprimento do intestino reduziu significativamente após 30 dias de jejum. O comprimento normal não foi recuperado após a realimentação. O número de cecos pilóricos não sofreu modificações significativas. Por outro lado, a espessura dos cecos diminuiu após 150 dias de privação de alimento e seu comprimento diminuiu após 180 dias. Após a realimentação, porém, os cecos pilóricos recuperaram a espessura original. Nos peixes realimentados após 240 dias de jejum, os comprimentos dessas estruturas aparentemente apresentaram crescimento compensatório, tornando-se mais longas que as do grupo controle.

*Palavras-chave:* trato digestório, morfologia, cecos pilóricos, privação de alimento, realimentação.

## INTRODUCTION

Buddington *et al.* (1997) revised the structure and function of the intestine of carnivore fish and observed a relationship between the diversity of functional demands placed on an organ system and

the variation of structure. Correspondingly, of all vertebrate organ systems, the gastrointestinal tract and respiratory system are structurally the most variable. The expansive surface area of the intestine represents a selectively permeable barrier that allows nutrient absorption, but excludes most toxic subs-

tances and pathogenic organisms. Additionally, the intestine presents three other important functions: water and electrolyte balance, immunity, and regulation of digestion and metabolism. Intestinal structure can respond rapidly and reversibly to changes in dietary inputs, and is of critical interest to ecologists and importance to culturists.

Pyloric caeca are blind diverticula of the proximal intestine found in many fishes, but their function is unclear. Based largely on structure, several authors (Dawes, 1929; Jansson & Olsson, 1960; Buddington & Diamond, 1986) have suggested that their function is not basically different from that of the proximal intestine. Histologically, they are similar to the intestine, presenting a number of folds and furrows, which increase the absorptive and secretory surface and work as a food reservoir (Williams & Nickol, 1989; Zavala-Camin, 1996; Buddington *et al.*, 1997; Kubitza, 1999). In salmonids, the proximal intestine and its associated caeca are the major site for absorption of lipids (Ostos Garrido *et al.*, 1993) and carotenoids (Guillou *et al.*, 1992). Furthermore, taking into account the position of the pyloric caeca and their absence in stomachless fishes, it is possible that one of the roles of these structures is to increase the bolus pH, optimizing digestion in the anterior portion of the intestine (Zavala-Camin, 1996).

It is well known that gut length is closely related to the properties of food consumed by fish. As an example, phytophagous and detritivore species present longer guts when compared to carnivores, allowing the consumption of food with low digestibility (e.g., Gerking, 1994). Shorter digestive tracts, as observed in carnivores, present a greater number of villi and pyloric caeca, which amplify manifold and compensate for a relatively short intestine (Buddington *et al.*, 1997; Kubitza, 1999).

In several studies (O'Connell, 1976; Ehrlich *et al.*, 1976; Theilacker, 1978; Yin & Blaxter, 1986; Segner *et al.*, 1987; Uriarte & Balbontín, 1987), gut morphology was used as a clue to larvae condition during the transition phase from endogenous to exogenous feeding, when the larvae often experience a critical period of starvation. The digestive tract and its associated glands are the first organs impaired by food deprivation (Theilacker, 1978). In adult fish, starvation can produce degeneration and atrophy of a number of digestive tract structures (Love, 1970, 1980; Noaillac-Depeyre & Gas, 1974; Gas & Noaillac-Depeyre, 1976; Peters, 1982; Kuperman

& Kuz'mina, 1994; Collins & Anderson, 1995; Hall & Bellwood, 1995; Baevefjord & Krogdahl, 1996).

The morphology of the oro-pharyngeal cavity of traíra, *Hoplias malabaricus*, indicates its ability to swallow whole preys, especially nektonic organisms (Menin & Mimura, 1991). The gut length/total body length ratio (gut quotient) characterizes traíra as an essentially piscivore species (Barbieri *et al.*, 1994), which is corroborated by studies on its feeding habits (Knöppel, 1970; Paiva, 1974; Caramaschi, 1979; Bistoni *et al.*, 1995). However, during annual drought periods, this species usually overcomes severe food scarcity (Lowe-McConnell, 1975). Some authors have found that traíra presents great tolerance to food deprivation (Paiva, 1974; Machado *et al.*, 1989; Rios *et al.*, 2002). However, studies on morphological changes induced by starvation are lacking for this species.

The main purpose of the present study was to evaluate the effects of long periods of food deprivation, and subsequent refeeding, on the gut length, and number and dimensions of pyloric caeca.

## MATERIAL AND METHODS

### *Experimental animals*

Adult specimens of *H. malabaricus* ( $251.7 \pm 6.5$  g) were collected in the Mogi-Guaçu River basin (near São Carlos, SP, Brazil). The fish were maintained for at least 3 weeks in 500 L tanks supplied with a continuous flow of normoxic water, at constant temperature ( $25 \pm 1^\circ\text{C}$ ) and 12/12 photoperiod.

### *Feeding treatment*

During the acclimation period, traíras were fed weekly on small live fish (*Astyanax*, *Prochilodus*, *Geophagus*, and *Oreochromis*) of a size appropriate for being completely swallowed. After acclimation, fish received the same type of food, but at controlled levels (2% of biomass  $\cdot$  day<sup>-1</sup>) during 30 consecutive days. The control group was sampled just after this feeding period. Eight groups of 10 individuals were subjected to 30, 60, 90, 150, 180, and 240 days of food deprivation. Two groups were kept under starvation for 90 days and two groups for 240 days, and one of each of these latter was refed for 30 days after the starvation period (2% of biomass  $\cdot$  day<sup>-1</sup>).

### *Gut sampling and morphometry*

Fish were sacrificed by rapid decapitation, weighed, and measured for the following: (1) gut

length (from cardiac sphincter to anus), (2) stomach length (from the cardiac to the pyloric sphincter), and (3) intestine length (from the pyloric sphincter to the anus). Organ lengths were expressed as a ratio of standard length ( $L_S$ ; distance from the edge of the snout to the end of the caudal peduncle) and not of total length ( $L_T$ ), as done, e.g., by Barbieri *et al.* (1994), since traíra caudal fin are often impaired. The ratios are dimensionless and named as: gut quotient ( $Q_G$  – gut length/ $L_S$ ), stomach quotient ( $Q_S$  – stomach length/ $L_S$ ), and intestine quotient ( $Q_I$  – intestine length/ $L_S$ ).

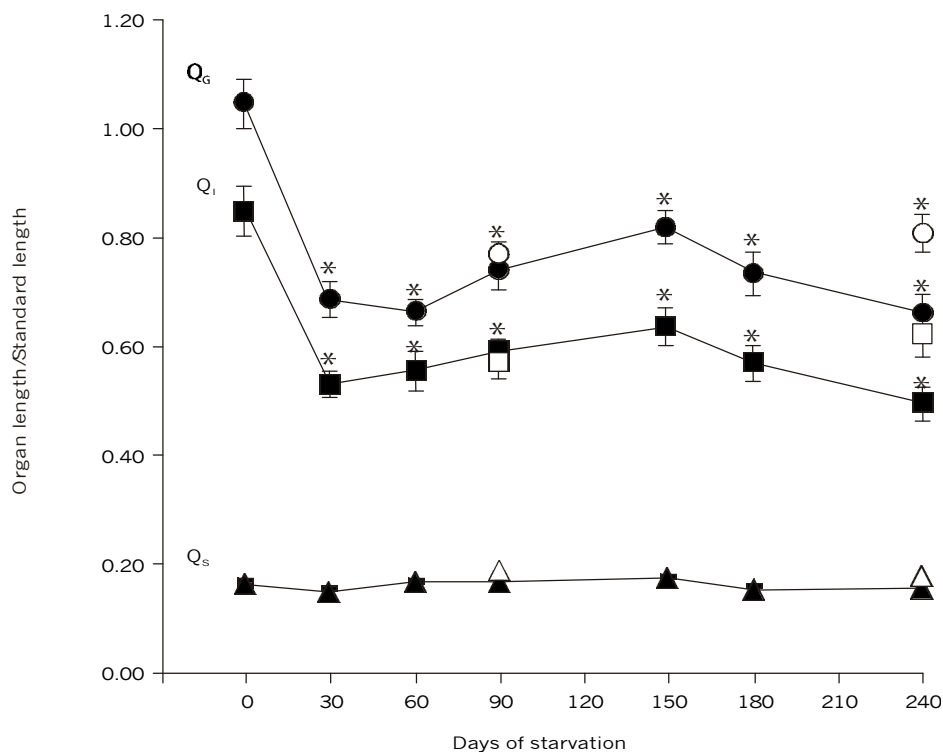
The digestive tracts were fixed in 10% formaline. The number, length, and thickness (external diameter at the base) of pyloric caeca were obtained under a stereoscopic microscope (Zeiss Stemi SV6 with a millimetric scale in one of the ocular lenses). About 50% of randomly chosen caeca were used to measure their size. Length and thickness of pyloric caeca were expressed as a percentage of  $L_S$ .

### Statistics

One-way analysis of variance (ANOVA), complemented by Bartlett's test for homogeneity of variances and the Tukey-Kramer multiple comparisons test, were employed to analyze the data of each group (GraphPad InStat – GraphPad Software Version 3.0). Data were expressed as mean  $\pm$  SEM and considered significantly different when  $p < 0.05$ .

## RESULTS

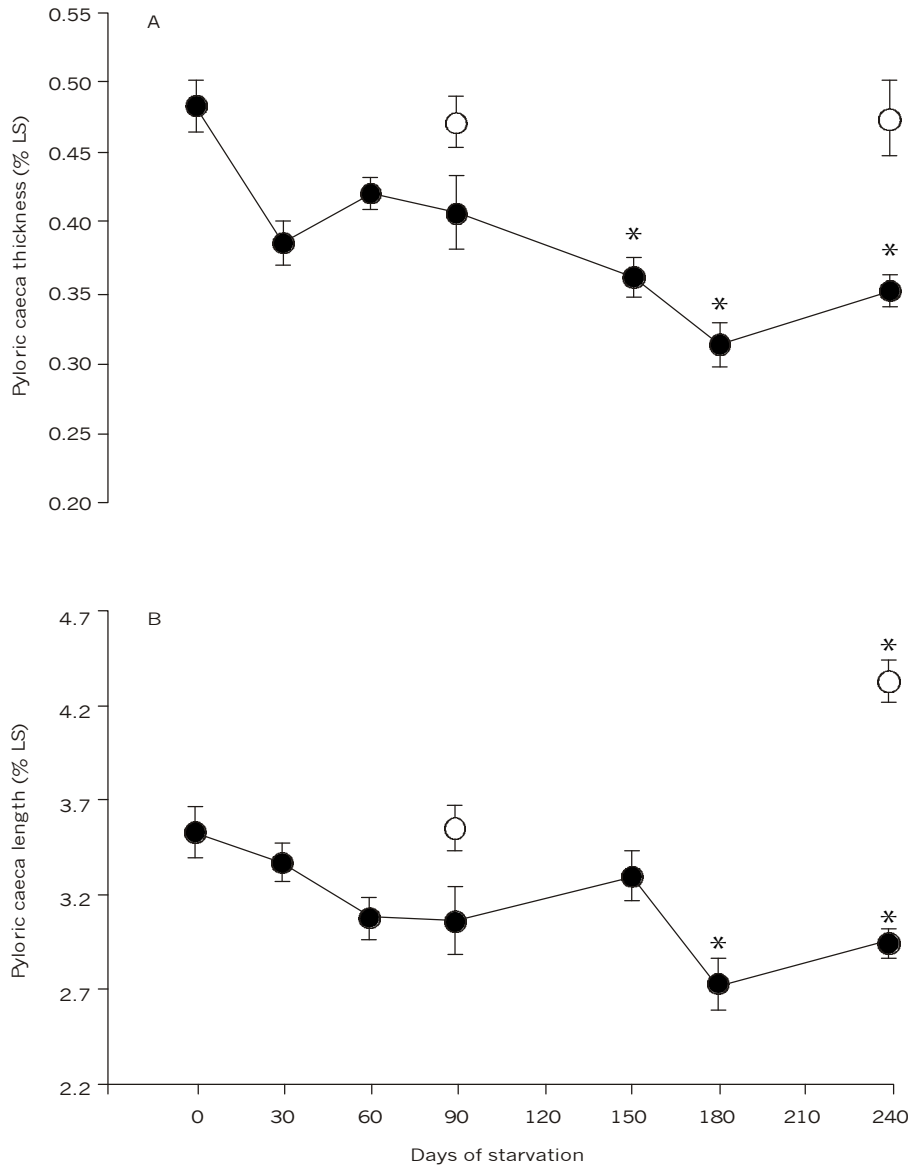
After 30 days of starvation, total gut quotient ( $Q_G$ ) decreased about 31% ( $p < 0.001$ ; Fig. 1). The same tendency was observed for the intestine quotient ( $Q_I$ ), which decreased about 33% ( $p < 0.001$ ; Fig. 1). The size of these structures remained reduced during the longer periods of food deprivation. Refed fish did not recover the gut and intestine lengths. Stomach quotient ( $Q_S$ ) did not change significantly during starvation and refeeding (Fig. 1).



**Fig. 1** — Relative length of the gut of traíra, *H. malabaricus*, in relation to days of starvation. Symbols: ● – Gut quotient ( $Q_G$ ), ■ – Intestine quotient ( $Q_I$ ), and ▲ – Stomach quotient ( $Q_S$ ) after each starvation period; ○ – Gut quotient ( $Q_G$ ), □ – Intestine quotient ( $Q_I$ ), and △ – Stomach quotient ( $Q_S$ ) of fish refed after 90 and 240 days of starvation. Statistics: \* – significant difference in relation to the control values ( $p < 0.001$ ). Values are mean  $\pm$  SEM;  $n = 10$ .

The number of pyloric caeca remained constant during all periods of food deprivation ( $95.9 \pm 3.3$ ). However, pyloric caeca thickness decreased gradually, becoming significantly reduced (about 29%) after 150 days of starvation ( $p < 0.001$ ; Fig. 2A) as was caeca length (about 20%) after 180 days of

starvation ( $p < 0.001$ ; Fig. 2B). In the refed fish, the caeca recovered original thickness and length. In fish refed after 240 days of starvation, the length of these structures actually became significantly longer (about 23%;  $p < 0.001$ ) than in the control group.



**Fig. 2** — (A) Pyloric caeca thickness and (B) length of traíra, *H. malabaricus*, in relation to days of starvation. Symbols: ● — after each starvation period and ○ — fish refed after 90 and 240 days of starvation. Statistics: \* — significant difference in relation to the control values ( $p < 0.01$ ). Values are mean  $\pm$  SEM;  $n = 10$ .

## DISCUSSION

Some authors believe that reduced nutrient concentration in the gut lumen as well as lack of direct stimulation by food are partially responsible for mucosal atrophy (McLesse & Moon, 1989). Gut mucosa seems to be extremely dynamic and to respond very quickly to food availability. The larvae midgut of some fish species is extremely vulnerable to food deficiency of food, usually presenting changes after 1 day of starvation (Theilacker, 1978).

Since stomach length did not present changes related to food deprivation, gut length variation could be attributed to intestinal length decrease. Reductions in gut weight, length, or diameter are often observed in fish chronically deprived of food (Angelescu & Gneri, 1949; Gas & Noaillac-Depeyre, 1976; Love, 1980; Kuperman & Kuz'mina, 1994; Collins & Anderson, 1995; Baeverfjord & Krogdahl, 1996).

A number of histopathological and ultrastructural changes were verified in the gut of fish subjected to periods of starvation. Absorptive surface reductions by regression or distortion of villi or microvilli (Gas & Noaillac-Depeyre, 1976; Segner *et al.*, 1987; Hall & Bellwood, 1995) or mucosal thickness reductions due to decreased heights of epithelial cells (Hall & Bellwood, 1995) were observed in different species. There have also been noted in starved fish a widening of intercellular spaces and loosening of cell contacts (O'Connell, 1976; Theilacker, 1978; Peters, 1982; Nonotte *et al.*, 1986; Segner *et al.*, 1987); cell dissociation and disruption in mucosa (Uriarte & Balbotín, 1987); swelling of mitochondria and dilation of reticular system (Gas & Noaillac-Depeyre, 1976; Peters, 1982; Somasundaram *et al.*, 1985; Segner *et al.*, 1987); vacuolization (Peters, 1982; Eckmann, 1985; Segner *et al.*, 1987); an autophagic process (Gas & Noaillac-Depeyre, 1976; Peters, 1982; Segner *et al.*, 1987); size reduction and disappearance of supranuclear vacuole and decrease in number of microvillous invaginations (O'Connell, 1976; Theilacker, 1978; Iida & Yamamoto, 1984; Segner *et al.*, 1987).

Gut epithelium presents continuous cell replacement. Although the constant enterocyte turnover is costly in terms of energy and nutrient expenditures, it may be justified because it removes cells that might have been invaded by pathogens (Buddington *et al.*, 1997). In grass carp, enterocytes can remain on the villi for 15-20 days (Stroband & Debets, 1978),

but in *Carassius auratus*, cell replacement rate is much higher, with complete renewal of mucosal cells estimated to take approximately 6 days (Vickers, 1962). During food deprivation, fish reduce cell component turnover in order to save energy (Jobling, 1994). This can suggest that changes observed in the gut of traíra, as well as in other starved fishes, is a reflex of structure renewal rate modification, leading to gradual tissue degeneration.

A decrease in the number of epithelial cells could result from either a cell proliferation rate reduction in the crypts of the intestine, or an increase extrusion rate of either old or dead cells (Hall & Bellwood, 1995). Both would result in decreased mucosal fold heights and total mucosal surface area reduction. Likewise, decreased epithelial cell heights may result from a shortening of existing epithelial cells or through the production of shorter cells, resulting in mucosa thickness reduction (Hall & Bellwood, 1995).

In this study, the number of pyloric caeca in starved traíra remained approximately constant. However, their length and thickness decreased in response to food deprivation. The size of caeca of starved *Pseudopleuronectes americanus* also decreased (McLesse & Moon, 1989) and degenerated in salmon during spawning migration, when the species was deprived of food (Love, 1970). In traíra, changes in pyloric caeca occurred later than in the intestine. In general, the anterior portion of the intestine seemed to be less affected than the posterior portion. Gas & Noaillac-Depeyre (1976) observed the persistence of microvilli in the proximal section of the intestine of carp (*Cyprinus carpio*). The microvilli, however, disappeared completely from the middle section after a 13-month starvation period.

Conversely, while intestine length of traíra remained reduced after refeeding, the thickness of pyloric caeca was recovered and overcompensation in the length of these structures was observed. The longer pyloric caeca presented by refed traíra after 240 days of starvation suggest an adaptation to enlarging the absorptive surface, thus compensating for intestine reduction. In carp, the renewal of the anterior part of the intestine was also quicker than that of the posterior part (Love, 1980).

Elliot (1972) found that the digestion rate of refed *Salmo trutta* presented no changes in fish previously starved for periods shorter than 7 days, but a reduced digestion rate was observed when fish

were deprived of food for periods of longer than 10 days. Zavala-Camin (1996) related these data to digestive tract retraction and degeneration due to disuse.

In *Macquaria ambigua* food consumption occurred immediately in the refeeding period, which the authors attributed to epithelial regeneration (Collins & Anderson, 1995). Similarly, the ready digestion of food by refed traíra indicates that, despite the observed changes, the digestive tract remained functional during food deprivation. However, it is not possible to affirm that digestion occurred at normal rates. Therefore, further studies are required.

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