BIOLOGICAL SCIENCES

Localization and distribution of CCK-8-, NPY-, Leu-ENK-, and Ghrelin- in the digestive tract of *Prochilodus lineatus* (Valenciennes, 1836)

CARLOS E. BARRIOS, JUAN JOSÉ SANTINÓN, HUGO A. DOMITROVIC, SEBASTIÁN SÁNCHEZ & DAVID R. HERNÁNDEZ

Abstract: This study describes the histological characteristics and distribution of gastrointestinal tract endocrine cells (ECs) of *Prochilodus lineatus* (detritivorous fish) using immunohistochemical procedures. The digestive tract of *P. lineatus* was divided into seven portions: stomach (cardial and pyloric), pyloric caeca, and intestine (anterior, glandular, middle and posterior). A pool of specific antisera against cholecystokinin (CCK-8), -neuropeptide Y (NPY), -ghrelin (Ghre) and -leu-enkephalin (Leu-ENK) to identify ECs were used. According to the morphological characteristics of ECs, two different types were identified and classified as open or closed-type. The number of ECs varied throughout the gastrointestinal tract, though a high abundance was found in the anterior intestine and pyloric caeca. A large number of ECs immunoreactive to CCK-8 and NPY were recorded in the anterior, glandular and middle intestine. ECs immunopositive to Leu-ENK were distributed in the stomach and pyloric caeca. For Ghre, immunopositive ECs were restricted to the glandular intestine. The results of the present study indicate that *P. lineatus* presents an ECs distribution pattern with species-specific particularities. However, CCK showed a distribution similar to that of omnivores, which is possibly related to local signaling functions in order to achieve the correct digestion of the various organisms found in the detritus.

Key words: detritivorous fish, sábalo, endocrine cells, immunohistochemistry, neuropeptide.

INTRODUCTION

The digestive tract (DT) of fish exhibit a diversity of morphological and functional characteristics, varying from short and simple to long and complex (Olsson 2011), and it is fundamentally related to the different environments, diets, and developmental states of the individuals (Angelescu & Gneri 1949, Wilson & Castro 2010). Regardless of these particularities, there exist different types of gastrointestinal neuropeptides synthesized by endocrine cells (ECs) located in the wall and epithelium of the digestive tract (Buddington & Krogdahl 2004, Holmgren & Olsson 2009). ECs are one of the largest endocrine systems in the body that participate in the mechanisms of control of the digestive processes, as well as in the peripheral signaling of food intake and energy homeostasis, similar to that observed in mammals (Lin et al. 2000, Canosa et al. 2005, Volkoff et al. 2005). Several studies demonstrated the occurrence and distribution of ECs through immunohistochemical techniques in the

gastrointestinal tract of various fish species (Pan et al. 2000, Bosi et al. 2004, Ku et al. 2004, Çinar et al. 2006, Vigliano et al. 2011, Hernández et al. 2012, Pereira et al. 2015, Lin et al. 2017). Several neuropeptide distribution patterns were observed according to different gastrointestinal tract morphologies and feeding habits, as observed in carnivores (Bosi et al. 2004, Çinar et al. 2006, Pereira et al. 2015), omnivores (Kiliaan et al. 1992, Pan et al. 2000), and herbivores (Ku et al. 2004, Lin et al. 2017).

Neuropeptides such as cholecystokinin (CCK), neuropeptide Y (NPY), leu-enkephalin (Leu-ENK), and ghrelin (Ghre) are synthesized by endocrine cells of the DT, and play a key role in nutritional homeostasis regulation. CCK is mainly synthesized in the DT and in the brain (Moran & Kinzig 2004), thereby intervening in both digestion processes and peripheral satiety signaling (anorexigenic) (Rubio et al. 2008, MacDonald & Volkoff 2009, Volkoff 2016). NPY is a peptide that presents a primary structure highly conserved among vertebrates, including fish (Jensen 2001). It also has important functions such as energy metabolism regulation, as well as digestive, reproductive, and immune processes, thus highlighting its important role in the regulation of eating behavior as an orexigenic factor (Volkoff et al. 2005, Volkoff 2006, MacDonald & Volkoff 2009, Zhou et al. 2013). Leu-ENK is found in the DT of different fish species (Pan et al. 2000, Vigliano et al. 2011, Hernández et al. 2012, Lin et al. 2017), and its function would be related to responses to inflammatory processes (Dezfuli et al. 2002, 2004). Ghrelin is known as an appetite-stimulating intestinal hormone and it is involved in multiple physiological functions, such as the regulation of food intake, growth, and reproduction (Kaiya et al. 2008).

Prochilodus lineatus a species widely distributed in Latin America and of great commercial importance to this region (Espinach

Ros & Sánchez 2007). *P. lineatus* represents great productive potential for fish farming due to its good growth with foods of low protein content (Croux 1992). This species is strictly detritivorous, with several anatomical and physiological adaptations for the efficient collection and digestion of organic detritus (Bowen 1983). Previous studies described the morphological and histophysiological characteristics of the digestive tract, including intestine length, which exceeds several times the body length and exhibits a complex pattern of intestinal loops (Angelescu & Gneri 1949, Barbieri et al. 1998, Barbieri & Hernández-Blazquez 2002), numerous pyloric caeca (Angelescu & Gneri 1949), and glands in the midgut (Domitrovic 1983, Nachi et al. 1998). However, no records have reported to date on the occurrence of endocrine cells in the digestive tract of detritivorous fish.

This study aimed to determine the characteristics and distribution of some neuromodulators of the *P. lineatus* DT using immunohistochemical techniques. These results can provide useful information to improve our understanding of the relationship between the morphological and functional characteristics of the digestive tract and endocrine signaling mechanisms in a detritivorous species.

MATERIALS AND METHODS

In this study, six healthy adult specimens of *P. lineatus* without sex distinction (average weight and standard length: 152 ± 18.60 g, 195 ± 11.25 mm, respectively) collected from the Northeast Institute of Ichthyology, Faculty of Veterinary Sciences (Corrientes, Argentina) were used. After euthanasia with an overdose of benzocaine (100 ppm), tissue samples were taken from stomach (cardial and pyloric), pyloric caeca and intestine (anterior, glandular, middle and posterior) (Domitrovic 1983, Barbieri et al. 1998, Nachi et al.

1998). The procedures adopted with the animals in this research were in accordance with the ethical principles of animal experimentation, and approved according to protocol N° 0033 by the Ethics and Biosafety Committee of School of Veterinary Sciences of the Northeast National University (UNNE) of Argentina.

Light microscopy and immunohistochemistry

Samples were fixed in Bouin's solution (12 h) and then embedded in paraffin wax after processing in a graded ethanol series. Microtome sections (1-3 µm thick) were collected on slides pretreated with silane (3-amino-propyltriethoxysilane; Sigma Chemical, St Louis, MO, USA), allowed to dry overnight and then de-waxed and hydrated. To assess digestive structures by light microscopy, sections were then stained with haematoxylin and eosin (H&E). For immunohistochemistry, all incubations were performed in a humid chamber with primary antibody for 16-20 h at 4 °C, and all washing procedures consisted of three successive 5 min immersions in 0.1 M phosphate-buffered saline (PBS; 8 mM Na_2HPO_4, 3 mM NaH_2PO_4, 150 mM NaCl). Endogenous peroxidase activity was blocked by incubation in peroxidase blocking solution (3% H_2O_2 in PBS) for 30 min, and after a rinse in PBS, the sections were treated with 3% skim milk powder for 15 min to block non-specific antibody binding. Subsequently, the samples were incubated with the primary antibodies listed in Table I, washed with PBS, and incubated with biotinylated secondary antibody followed by streptavidin peroxidase conjugate (CytoScan™ HRP Detection System, Cell Marque) both at room temperature for 20 min. Finally, the sections were treated with DAB chromogen (3-3' diaminobenzidine), then immersed in deionized water to stop the reaction, counterstained with haematoxylin, dehydrated, and coverslipped. As positive control, sections of pig intestine were used. On the other hand, negative control slides were sections in which the primary antibody was replaced by PBS.

The recorded values were obtained from the total number of endocrine cells, manually counting the cross sections independent of each portion of the DT and for each antibody, and reported as average values from 1000 µm of intestinal perimeter (Hall & Bellwood 1995). The number of endocrine cells of each section was classified into the following grades: no detected (–), 1 ~ 10 cells (+), 11 ~ 20 cells (++), 21 ~ 30 cells (+++); more than 30 cells (++++). The images were obtained using a Leica DM500 microscope with Leica ICC50 digital camera equipped with an image analysis system: Leica Application Suite 3.4.1.

RESULTS

According to the morphological characteristics, two types of endocrine cells located between the enterocytes of the intestinal epithelium were observed. Open-type ECs exhibit an elongated shape and are wider in the zone occupied by

Table I. List of primary antisera used in this study.

Antibodies against	Donor	Working dilution	Source (Code)
Cholecystokinin	Rabbit polyclonal	1:250	Abcam™ Labs (ab27441)
Neuropeptide Y	Rabbit polyclonal	1:250	Abcam™ Labs (ab30914)
Leu-enkephalin	Rabbit polyclonal	1:1500	Abcam™ Labs (ab22619)
Ghrelin	Mouse monoclonal	1:350	Abcam™ Labs (ab57222)

the nucleus, and exhibits a granular content in the supranuclear cytoplasm (Figure 1). Closed-type ECs are round in shape and located in the basal region of the epithelium (Figure 2). In addition, some nerve cells with an irregular contour defining their typical stellate shape with cytoplasmic projections were observed. The distribution of each EC type exhibited high variation along the digestive tract (Table II).

CCK-immunoreactive endocrine cells

Immunoreactivity to CCK was detected in cells of the epithelial layer of the pyloric caeca and anterior intestine (Figure 1a, b). The highest density of CCK-IR open-type ECs was observed in the pyloric caeca, while the number of ECs decreased from anterior toward the caudal segments of the gut, being absent in posterior intestine (Table II). Notably, no CCK-IR ECs were found in the stomach of *P. lineatus*.

NPY- immunoreactive endocrine cells

Large amounts of NPY-IR ECs were detected, mainly in the pyloric caeca, but also in the anterior, glandular, and middle portions of the intestine (Table II). Similar to the distribution observed in CCK, only open-type cells were observed in the intestine (Figure 1c, d). However,
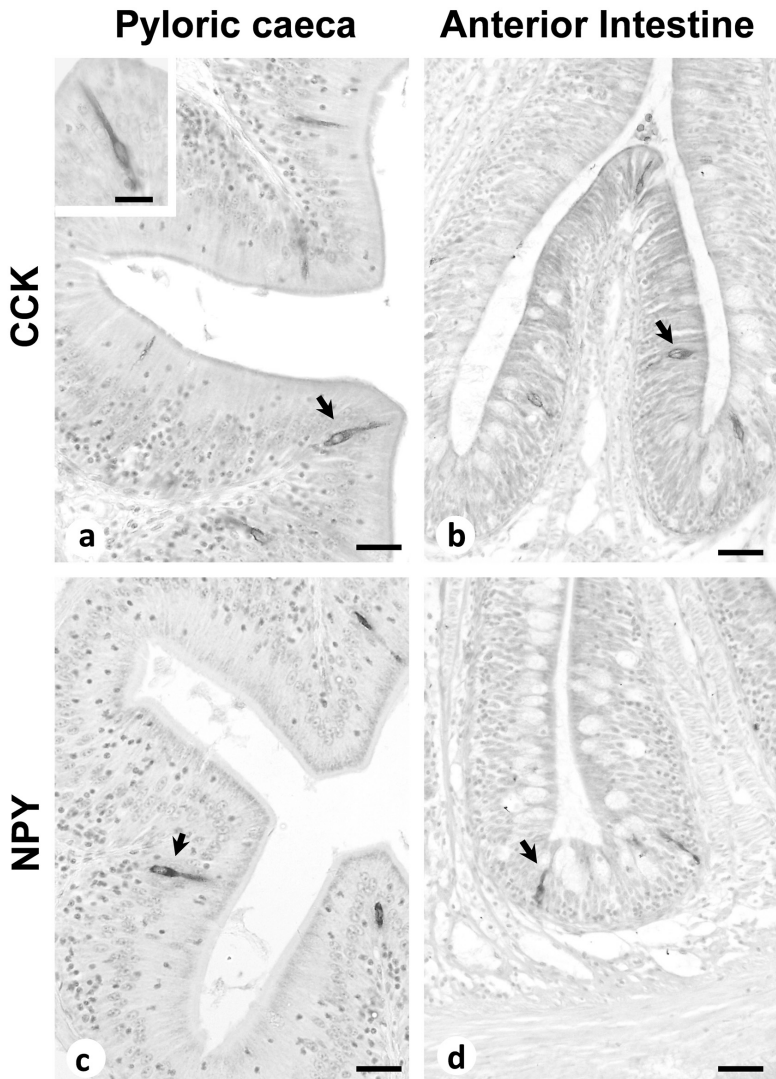

Figure 1. Photomicrograph of *Prochilodus lineatus* endocrine cells. – a and b. Open-type CCK-8- IR endocrine cells of the pyloric caeca and anterior intestine. – c and d. Open-type NPY- IR endocrine cells of the pyloric caeca and anterior intestine. Scale bars: low-power magnification views = 30 μm; insets = 10 μm.

no NPY-IR ECs were found in other portions of the DT, such as the stomach and posterior intestine.

Leu-ENK-immunoreactive endocrine cells

In the epithelial layer of the cardial and pyloric stomach, only open-type Leu-ENK-IR ECs were detected, while closed-type endocrine cells were observed in the pyloric caeca (Table II) (Figure 2a, b). Moreover, immunoreaction to Leu-ENK was observed in nerve cells surrounding the gastric glands (Figure 2c, e), as well as in neurons of the myenteric plexus. Leu-ENK IR neurons presented an irregular outline defining their typical stellated shape with cytoplasmic projections (Figure 2d, f). Nerve fibers distributed in the lamina propria-submucosa and muscle layers throughout the DT also showed immunoreactivity to Leu-ENK antisera.

Ghre-immunoreactive endocrine cells

Ghre-IR open-type ECs were restricted to the glandular intestine (Figure 2g), and exhibited a triangular shape with large secretory granules distributed throughout the cell (Figure 2h). These cells were found in the base and middle portion of the glands.
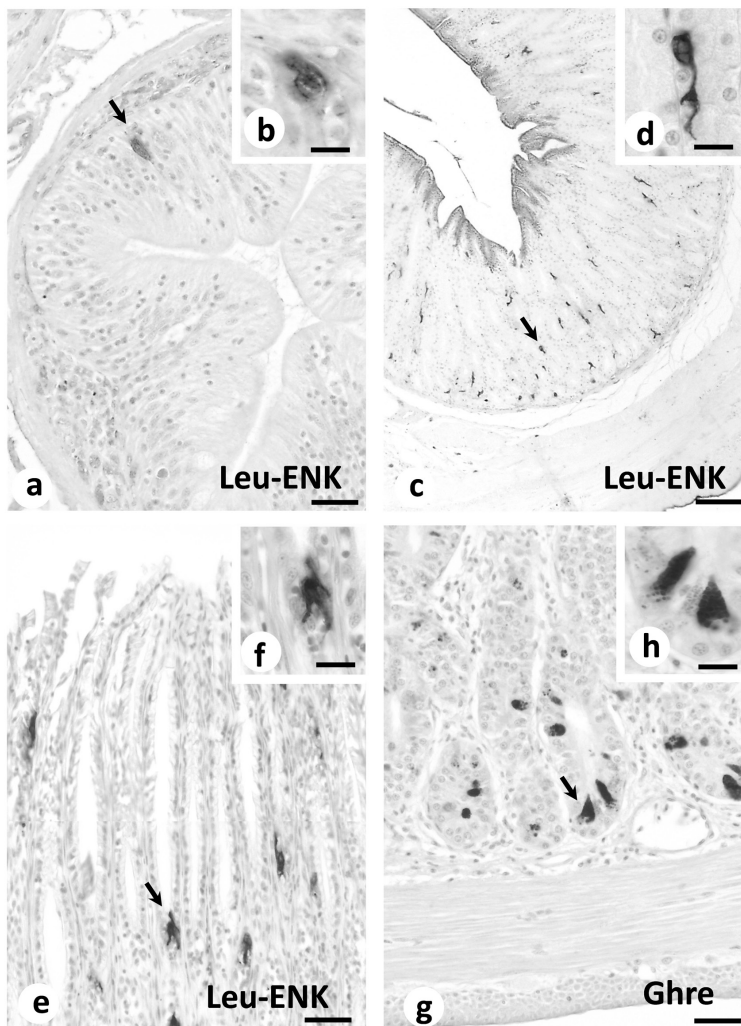

Figure 2. Comparative photomicrographs of *Prochilodus lineatus* digestive tract endocrine cells. – a and b. Pyloric caeca presenting closed-type Leu-ENK-IR endocrine cells (arrow). – c and e. Leu-ENK-IR endocrine cells located surrounding the gastric glands (arrow). – d, f, and h. High-power magnification showing different IR endocrine cells. – g. Ghre-IR endocrine cells observed in the epithelium of the glandular intestine (arrow). Scale bars: low-power magnification views = 30 μm; insets = 10 μm.

DISCUSSION

The occurrence and distribution of ECs was analyzed in the DT of several fish species, which showed considerable variation in morphology and physiology independent of trophic habit (Vigliano et al. 2011, Pereira et al. 2015, Lin et al. 2017). This could indicate that variations found among species would be the result of adaptations to environmental conditions and nutritional requirements (Olsson et al. 2011). However, several studies attempted to generalize the regional distribution patterns of ECs. In this sense, Rønnestad et al. (2007) proposed a distribution model of CCK ECs in fish larvae related to the macroscopic anatomy of the digestive tract. In this model, species with straight gut would have a distribution pattern of CCK ECs throughout the gut, whereas in larvae with rotated gut, CCK ECs would be highly concentrated in the anterior segment of the intestine. Therefore, in species with rotated gut, CCK-producing cells would exhibit a strategic distribution related to the capture of chemical signals of food coming from the stomach in order to achieve a highly functional feedback control of the digestive process. In *P. lineatus* (fish with coiled gut), we observed a distribution pattern similar to fish with rotated gut. This was similar to the pattern observed in other species with coiled guts, such as *Carassius auratus* (Kiliaan et al. 1992), *Oreochromis mossambicus* (Kiliaan et al. 1992), *Zacco platypus* (Ku et al. 2004), *R. quelen* (Hernández et al. 2012), *O. niloticus* (Pereira et al. 2017), and *Chanos chanos* (Lin et al. 2017). In contrast, in fish with straight gut, the disseminated distribution pattern was attributed to action on the control of digestive processes by receiving signals from undigested food that quickly reach the posterior intestine (Kamisaka et al. 2005, Hartviksen et al. 2009, Gräns & Olsson 2011). However, several species

with straight digestive tracts and carnivorous feeding habits vary widely in relation to the regional distribution patterns of CCK-producing cells. In this sense, high densities of CCK-IR cells were reported in the esophagus of *Pseudophoxinus antalyae* (Şenol & Çinar 2006); in stomach and pyloric caeca of *Salminus brasiliensis* (Pereira et al. 2015), *Godus moruha* (Jönsson et al. 1987), and *Oncorhynchus mykiss* (Barrenechea et al. 1994, Jensen et al. 2001); in the anterior intestine of *Dicentrarchus labrax* (Diler et al. 2011), *Coreoperca herzi* (Lee et al. 2004), *Salmo trutta* (Bosi et al. 2004), and *S. brasiliensis* (Pereira et al. 2015); and in the posterior intestine of *Oligosarcus hepsetus* (Vieira-Lopes et al. 2013). In the present study, the high density of CCK-IR cells observed in the anterior intestine and pyloric caecum would represent a key location, since this region is strongly related to diffuse acinar pancreatic tissue (Sverlij et al. 1993, Rotta 2003). In this place, ECs would release CCK in response to intraluminal nutrients, consequently stimulating the release of pancreatic digestive enzymes towards the intestinal lumen (Einarsson et al. 1997). In addition, this is valid when considering the existence of a retrograde peristalsis mechanism (Rønnestad et al. 2000) favoring the filling of pyloric caeca with chyme and mixing with the digestive secretions of this region (Gräns & Olsson 2011). Thereby, our results are logical when considering the biological role of CCK on pancreatic enzymes release, gallbladder contraction, gastrointestinal motility stimulation, and gastric emptying inhibition (Volkoff et al. 2005, Gorissen et al. 2006, Rønnestad et al. 2017). In addition, the distribution of CCK ECs in *P. lineatus* exhibits similarity to omnivorous species. This could be related to the diversity of organisms consumed by the detritivores, where phytoplankton, zooplankton, benthic micro- and macroflora, necton macroflora, coprogenic

material, and organic allochthonous material are the main food source of this species (Gneri & Angelescu 1951, Sverlij et al. 1993).

In fish, NPY is widely distributed in the CNS (Rodríguez-Gómez et al. 2001, Pérez Sirkin et al. 2013, Hosomi et al. 2014) and in the digestive system (Vigliano et al. 2011, Pereira et al. 2015, Lin et al. 2017). In the CNS, hypothalamic neurons are the main site of NPY production, which play a key role in increasing food consumption (López-Patiño et al. 1999, Silverstein & Plisetskaya 2000, Kiris et al. 2007), whereas the DT is the main peripheral NPY producer organ and exerts primarily inhibitory effects on secretion, intestinal motility, and blood flow (Uesaka et al. 1996, Shahbazi et al. 2002, Gomez et al. 2012), and induces immune activation or suppression (Carpio et al. 2007, Farzi et al. 2015). In contrast to CCK, the regional distribution of NPY in DT shows minor variations between species. Several studies mentioned that NPY-IR ECs were primarily observed in the anterior intestine and pyloric caeca of fish, as seen in carnivorous (Al-Mahrouki & Youson 1998, Çinar et al. 2006, Min et al. 2009, Vigliano et al. 2011, Pereira et al. 2015), omnivorous (Al-Mahrouki & Youson 1998, Pereira et al. 2017), or herbivorous (Lin et al. 2017). In the present study, NPY-IR ECs were localized in the epithelial mucosa throughout the intestine, with the exception of the rectal portion. The highest reactivity was observed in the anterior intestine and pyloric caeca, being similar to that observed in most fish species. Moreover, the regional distribution of NPY in the DT of *P. lineatus* would be related to local signaling functions and peripheral monitoring for appetite hypothalamic regulating center (Vigliano et al. 2011, Babichuk & Volkoff 2013, Pereira et al. 2015, Hernández et al. 2018).

Leu-ENK is a pentapeptide associated with the regulation of the inflammatory process, as well as the modulation of intestinal peristalsis

(Radulovic et al. 1996). In addition, previous studies reported that fish infected with parasites showed increased immunostaining of Leu-ENK in affected areas, revealing a possible role in the modulation of the inflammatory process (Dezfuli et al. 2002, 2004, Bosi et al. 2005). Previous studies described the occurrence of closed-type or open-type Leu-ENK ECs distributed in the epithelium of the DT, as well as in the neuronal bodies or nerve fibers of the DT wall. In *C. chanos* (Lin et al. 2017), closed-type Leu-ENK-IR ECs were found in the pyloric caeca, whereas open-type ECs were observed in the anterior intestine region. In *Cyprinus carpio*, *Ctenopharyngodon idellus*, *Mylopharyngodon piceus* (Pan et al. 2000), *Odontesthes bonariensis* (Vigliano et al. 2011), and *R. quelen* (Hernández et al. 2012), only open-type Leu-ENK IR ECs were observed, and these were distributed in the epithelial layer of the intestine. In the present study, closed-type Leu-ENK-IR ECs were observed in the stomach and pyloric caeca, as well as in nerve fibers of the myenteric plexus. This distribution could be related to the immunomodulatory action necessary in strategic sites of the digestive tract, and with the peristalsis modulation that would help to displace food through the long intestinal tract characteristic of this species.

The hormone ghrelin is considered an orexigenic factor that is highly conserved among vertebrates (Kaiya et al. 2008). Previous studies demonstrated that ghrelin concentrations increase under fasting conditions (Murashita et al. 2009) and decreases following feeding (Kojima & Kangawa 2005, Cummings 2006, Olszewski et al. 2008). However, some controversial results have been reported. Thus, studies conducted on *O. mykiss* suggest that ghrelin levels decrease in fasted fish (Jönsson et al. 2007), and that they possibly possess anorexigenic roles (Jönsson et al. 2010). In non-mammalian vertebrates, ghrelin expression has been detected in

Table II. Regional distribution and immunoreaction intensity of endocrine cells in the digestive tract of *Prochilodus lineatus*.

Antisera	Stomach		Pyloric Caeca	Intestine			
	CR	PR		AI	GLI	MI	PI
CCK-8	–	–	++++	++++	+	++	–
NPY	–	–	++++	+++	+	+	–
Leu-ENK	+	+	+	–	–	–	–
Ghrelin	–	–	–	–	++	–	–

Note: CR, cardial region; PR, pyloric region; AI, anterior intestine; GLI, glandular intestine; MI, middle intestine; PI, posterior intestine. Intensity grades for the immunoreactions of endocrine cells: (–) not detected; (+) 1-10 cells; (++) 11-20 cells; (+++) 21-30 cells; (++++) more than 30 cells.

different organs; however, the DT seems to be the main production site (Kaiya et al. 2008). In *O. mykiss* (Sakata et al. 2004), *Anguilla japonica* (Kaiya et al. 2006), and *Paralichthys dentatus* (Breves et al. 2009) ghrelin was identified only in stomach, while in *Salmo salar* (Murashita et al. 2009) it was identified in stomach, pyloric caeca, and intestine. Nevertheless, the highest ghrelin expression in the herbivorous carp (*C. idellus*) was observed in the anterior intestine (Feng et al. 2013), while the highest expression in *Megalobrama amblycephala* (Ji et al. 2015) was observed in the posterior intestine. In the present study, ghrelin immunoreactivity was only observed in the glandular cells of the glandular intestine. Likely, this distribution could be related to the biological function of ghrelin in appetite regulation. However, the observed variations in ghrelin expression would exhibit species-specific distribution patterns (Feng et al. 2013).

The present study provides valuable information regarding the distribution of different neuromodulators of the digestive tract in *P. lineatus*, which show some similarity with the distribution pattern of neuropeptides found in omnivorous fish. However, the regional variation observed among the distribution patterns of neuropeptides in species with the same trophic habit is likely the result of environmental changes and physiological adaptations, affecting the dietary behavior of fish in evolutionary terms (Volkoff 2016, Soengas et al. 2018).

REFERENCES

AL-MAHROUKI AA & YOUSON JH. 1998. Immunohistochemical studies of the endocrine cells within the gastro-entero-pancreatic system of Osteoglossomorpha, an ancient teleostean group. Gen Comp Endocrinol 110(2): 125-139.

ANGELESCU V & GNERI FS. 1949. Adaptaciones del aparato digestivo al régimen alimentario en algunos peces iliófagos del Río Uruguay y Río de la Plata. Rev Inst Nac Inv C Nat 1(6): 161-272.

BABICHUK NA & VOLKOFF H. 2013. Changes in expression of appetite-regulating hormones in the cunner (*Tautogolabrus adspersus*) during short-term fasting and winter torpor. Physiol Behav 120: 54-63.

BARBIERI RL & HERNÁNDEZ-BLAZQUEZ FJ. 2002. Análise ultra-estrutural da absorção intestinal de macromolécula protéica com o uso de peixe como modelo experimental. ConScientiae Saúde 1: 21-30.

BARBIERI RL, LEITE RG, DE ALMEIDA STERMAN F & HERNANDEZ-BLAZQUEZ FJ. 1998. Food passage time through the alimentary tract of a brazilian teleost fish, *Prochilodus scrofa* (Steindachner, 1881) using radiography. Braz J Vet Res Anim Sci 35(1): 32-36.

BARRENECHEA MA, LOPEZ J & MARTÍNEZ A. 1994. Regulatory peptides ingastric endocrine cells of the rainbow trout *Oncorhynchus mykiss*: general distribution and colocalizations. Tissue Cell 26: 309-321.

BOSI G, DI GIANCAMILLO A, ARRIGHI S & DOMENEGHINI C. 2004. An immunohistochemical study on the neuroendocrine system in the alimentary canal of the brown trout, *Salmo trutta*, L. 1758. Gen Comp Endocrinol 138(2): 166-181.

BOSI G, DOMENEGHINI C, ARRIGHI S, GIARI L, SIMONI E & DEZFULI BS. 2005. Response of the gut neuroendocrine system of *Leuciscus cephalus* (L.) to the presence of *Pomphorhynchus laevis* Müller, 1776 (Acanthocephala). Histol Histopathol 20(2): 509-518.

BOWEN SH. 1983. Detritivory in neotropical fish communities. Environ Biol Fishes 9: 137-144.

BREVES JP, VEILLETTE PA & SPECKER JL. 2009. Ghrelin in the summer flounder: immunolocalization to the gastric glands and action on plasma cortisol levels. Comp Biochem Phys A Mol Integr Physiol 152(2): 268-272.

BUDDINGTON RK & KROGDAHL Å. 2004. Hormonal regulation of the fish gastrointestinal tract. Comp Biochem Phys A Mol Integr Physiol 139(3): 261-271.

CANOSA LF, UNNIAPPAN S & PETER RE. 2005. Periprandial changes in growth hormone release in goldfish: role of somatostatin, ghrelin, and gastrin releasing peptide. Am J Physiol Regul Integr Comp Physiol 289(1): 125-133.

CARPIO Y, LEÓN K, ACOSTA J, MORALES R & ESTRADA M. 2007. Recombinant tilapia Neuropeptide Y promotes growth and antioxidant defenses in African catfish (*Clarias gariepinus*) fry. Aquaculture 272(1-4): 649-655.

ÇINAR K, SENOL N & OZEN MR. 2006. Immunohistochemical study on distribution of endocrine cells in gastrointestinal tract of flower fish (*Pseudophoxinus antalyae*). World J Gastroenterol 12(42): 6874-6878.

CROUX MJP. 1992. Comportamiento y crecimiento de *Prochilodus lineatus* (Pisces, Curimatidae) en condiciones controladas. Rev Asoc Cienc Nat Litoral 1(23): 9-20.

CUMMINGS DE. 2006. Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav 89(1): 71-84.

DEZFULI BS, GIARI L, SIMONI E, SHINN AP & BOSI G. 2004. Immunohistochemistry, histopathology and ultrastructure of *Gasterosteus aculeatus* tissues infected with *Glugea anomala*. Dis Aquat Organ 58(2-3): 193-202.

DEZFULI BS, PIRONI F, GIARI L, DOMENEGHINI C & BOSI G. 2002. Effect of *Pomphorhynchus laevis* (Acanthocephala) on putative neuromodulators in the intestine of naturally infected *Salmo trutta*. Dis Aquat Organ 51(1): 27-35.

DILER D, ÇINAR K & ZORLU S. 2011. An Immunohistochemical Study on the Endocrine Cells in the Stomach and Intestine Regions of the *Dicentrarchus labrax*, L., 1758. FÜ Sağ Bil Vet Derg 25(1): 1-6.

DOMITROVIC HA. 1983. Histología del tracto digestivo del sábalo (*Prochilodus platensis*, Holmberg, 1880, Pisces, Prochilodontiae). Physis 41: 57-56.

EINARSSON S, DAVIES PS & TALBOT C. 1997. Effect of exogenous cholecystokinin on the discharge of the gallbladder and the secretion of trypsin and chymotrypsin from the pancreas of the *Atlantic salmon*, *Salmo salar* L. Comp Biochem Physiol C Toxicol Pharmacol 117(1): 63-67.

ESPINACH ROS A & SÁNCHEZ RP. 2007. Proyecto Evaluación del Recurso Sábalo en el Paraná. Informe de los resultados de la primera etapa (2005-2006) y medidas de manejo recomendadas. In: Secretaría de Agricultura, Ganadería, Pesca y Alimentos (Ed), Serie Pesca y Acuicultura: Estudios e Investigaciones Aplicadas. Buenos Aires, Argentina, 80 p.

FARZI A, REICHMANN F & HOLZER P. 2015. The homeostatic role of neuropeptide Y in immune function and its impact on mood and behaviour. Acta Physiol 213: 603-627.

FENG K, ZHANG GR, WEI KJ & XIONG BX. 2013. Molecular cloning, tissue distribution, and ontogenetic expression of ghrelin and regulation of expression by fasting and refeeding in the grass carp (*Ctenopharyngodon idellus*). J Exp Zool A Ecol Genet Physiol 319(4): 202-212.

GNERI FS & ANGELESCU V. 1951. La nutrición de los peces ilíofagos en relación con el metabolismo general del ambiente acuático. Rev Mus Argent Cienc Nat 2: 1-44.

GOMEZ GA, ENGLANDER EW & GREELEY GH. 2012. Postpyloric gastrointestinal peptides. In: Johnson LR (Ed), Physiology of the gastrointestinal tract, Academic, New York, USA 5: 155-198.

GORISSEN MH, FLIK G & HUISING MO. 2006. Peptides and proteins regulating food intake: a comparative view. Anim Biol Leiden Neth 56: 447-473.

GRÄNS A & OLSSON C. 2011. Gut Motility. In: Farrell AP (Ed), Encyclopedia of Fish Physiology: From Genome to Environment, Academic Press, San Diego 2: 1292-1300.

HARTVIKSEN MB, KAMISAKA Y, JORDAL AEO, KOEDIJK RM & RØNNESTAD I. 2009. Distribution of cholecystokinin-immunoreactive cells in the gut of developing atlantic cod *Gadus morhua* L. larvae fed zooplankton or rotifers. J Fish Biol 75(4): 834-844.

HERNÁNDEZ DR, BARRIOS CE, SANTINON JJ, SÁNCHEZ S & BALDISSEROTTO B. 2018. Effect of fasting and feeding on growth, intestinal morphology and enteroendocrine cell density in *Rhamdia quelen* juveniles. Aquac Res 49(4): 1512-1520.

HERNÁNDEZ DR, VIGLIANO FA, SÁNCHEZ S, BERMÚDEZ R, DOMITROVIC HA & QUIROGA MI. 2012. Neuroendocrine system of the digestive tract in *Rhamdia quelen* juvenile: an immunohistochemical study. Tissue Cell 44: 220-226.

HOLMGREN S & OLSSON C. 2009. The neuronal and endocrine regulation of gut function. In: Bernier et al. (Eds), Fish Physiology. Fish Neuroendocrinology, Academic Press, Burlington 28: 467-512.

HOSOMI N, FURUTANI T, TAKAHASHI N, MASUMOTO T & FUKADA H. 2014. Yellowtail neuropeptide Y: molecular cloning, tissue distribution, and response to fasting. Fish Sci 80: 483-492.

JENSEN J. 2001. Regulatory peptides and control of food intake in non mammalian vertebrates. Comp Biochem Phys A Mol Integr Physiol 128(3): 471-479.

JI W, PING HC, WEI KJ, ZHANG GR, SHI ZC, YANG RB, ZOU GW & WANG WM. 2015. Ghrelin, neuropeptide Y (NPY) and cholecystokinin (CCK) in blunt snout bream (*Megalobrama amblycephala*): cDNA cloning, tissue distribution and mRNA expression changes responding to fasting and refeeding. Gen Comp Endocrinol 223: 108-119.

JÖNSSON AC, HOLMGREN S & HOLSTEIN B. 1987. Gastrin/CCK-like immunoreactivity in endocrine cells and nerves in the gastrointestinal tract of the cod, *Gadus morhua*, and the effect of peptides of the gastrin/CCK family on cod gastrointestinal smooth muscle. Gen Comp Endocrinol 66(2): 190-202.

JÖNSSON E, FORSMAN A, EINARSDOTTIR IE, KAIYA H, RUOHONEN K & BJÖRNSSON BT. 2007. Plasma ghrelin levels in rainbow trout in response to fasting, feeding and food composition, and effects of ghrelin on voluntary food intake. Comp Biochem Physiol A Mol Integr Physiol 147(4): 1116-1124.

JÖNSSON E, KAIYA H & BJORNSSON BT. 2010. Ghrelin decreases food intake in juvenile rainbow trout (*Oncorhynchus mykiss*) through the central anorexigenic corticotropin releasing factor system. Gen Comp Endocrinol 166: 39-46.

KAIYA H, MIYAZATO M, KANGAWA K, PETER RE & UNNIAPPAN S. 2008. Ghrelin: a multifunctional hormone in non-mammalian vertebrates. Comp Biochem Physiol A Mol Integr Physiol 149(2): 109-128.

KAIYA H, TSUKADA T, YUGE S, MONDO H, KANGAWA K & TAKEI Y. 2006. Identification of eel ghrelin in plasma and stomach by radioimmunoassay and histochemistry. Gen Comp Endocrinol 148(3): 375-382.

KAMISAKA Y, DRIVENES Ø, KUROKAWA T, TAGAWA M, RØNNESTAD I, TANAKA M & HELVIK JV. 2005. Cholecystokinin mRNA in Atlantic herring, *Clupea harengus* – molecular cloning, characterization and distribution in the digestive tract during the early life stages. Peptides 26(3): 385-393.

KILIAAN A, HOLMGREEN S, JONSSON AC, DEKEER K & GROOT J. 1992. Neurotensin, substance P, gastrin/cholecystokinin, and bombesin in the intestine of the tilapia (*Oreochromis mossambicus*) and the goldfish (*Carassius auratus*): lmmunochemical Detection and effects on electrophysiological characteristics. Gen Comp Endocrinol 88(3): 351-363.

KIRIS GA, KUMLU M & DIKEL S. 2007. Stimulatory effects of neuropeptide Y on food intake and growth of *Oreochromis niloticus*. Aquaculture 264(1-4): 383-389.

KOJIMA M & KANGAWA K. 2005. Ghrelin: structure and function. Physiol Rev 85(2): 495-522.

KU SK, LEE JH & LEE HS. 2004. Immnohistochemical study on the endocrine cells in gut of the stomachless teleost, *Zacco platypus* (Cyprinidae). Anat Histol Embryol 33(4): 212-219.

LEE JH, KU SK, PARK KD & LEE HS. 2004. Immunohistochemical study of the gastrointestinal endocrine cells in the *Korean aucha* perch. J Fish Biol 65(1): 170-181.

LIN X, VOLKOFF H, NARNAWARE Y, BERNIER NJ, PEYON P & PETER RE. 2000. Brain regulation of feeding behavior and food intake in fish. Comp Biochem Physiol A Mol Integr Physiol 126(4): 415-434.

LIN X, WANG P, OU Y, LI J & WEN J. 2017. An immunohistochemical study on endocrine cells in the neuroendocrine system of the digestive tract of milkfish *Chanos chanos* (Forsskal, 1775). Aquac Res 48(4): 1439-1449.

LÓPEZ-PATIÑO MA, GUIJARRO AI, ISORNA E, DELGADO MJ, ALONSO-BEDATE M & DE PEDRO N. 1999. Neuropeptide Y has a stimulatory action on feeding behavior in goldfish (*Carassius auratus*). Eur J Pharmacol 377(2-3): 147-153.

LUKIW WJ. 2006. Endogenous signaling complexity in neuropeptides – leucine- and methionine-enkephalin. Cell Mol Neurobiol 26(4-6): 1003-1010.

MACDONALD E & VOLKOFF H. 2009. Neuropeptide Y (NPY), cocaine andamphetamine regulated transcript (CART) and cholecystokinin (CCK) in winter skate (*Raja ocellata*): cDNA cloning, tissue distribution and mRNA expression

responses to fasting. Gen Comp Endocrinol 161(2): 252-261.

MIN H, KAI-YU W & YU Z. 2009. Immunocytochemical identification and localization of diffuse neuroendocrine system (DNES) cells in gastrointestinal tract of channel catfish (*Ictalurus punctatus*). Agric Sci China 8(2): 238-243.

MORAN TH & KINZIG KP. 2004. Gastrointestinal satiety signals II. Cholecystokinin. Am J Physiol Gastrointest Liver Physiol 286(2): 183-188.

MURASHITA K, KUROKAWA T, NILSEN TO & RØNNESTAD I. 2009. Ghrelin, cholecystokinin, and peptide YY in Atlantic salmon (*Salmo salar*): Molecular cloning and tissue expression. Gen Comp Endocrinol 160(3): 223-235.

NACHI AM, HERNANDEZ-BLAZQUEZ FJ, PHAN M, BARBIERI RL & LEITE RG. 1998. Intestinal Histology of a Detritivorous (iliophagous) Fish *Prochilodus scrofa* (Characiformes - Prochilodontidae). Ann Sci Nat Zool 2: 81-88.

OLSSON C. 2011. Gut anatomy and morphology: Gut anatomy. In: Farrell AP (Ed), Encyclopedia of Fish Physiology: From Genome to Environment, San Diego, USA. Academic Press 2: 1268-1275.

OLSZEWSKI PK, SCHIOTH HB & LEVINE AS. 2008. Ghrelin in the CNS: From hunger to a rewarding and memorable meal? Brain Res Rev 58(1): 160-170.

PAN QS, FANG ZP & ZHAO YX. 2000. Immunocytochemical identification and localization of APUD cells in the gut of seven stomachless teleost fishes. World J Gastroenterol 6(1): 96-101.

PEREIRA RT, COSTA LS, OLIVEIRA IR, ARAÚJO JC, AERTS M, VIGLIANO FA & ROSA PV. 2015. Relative distribution of gastrin-, CCK-8-, NPY-and CGRP-immunoreactive cells in the digestive tract of dorado (*Salminus brasiliensis*). Tissue Cell 47(2): 123-131.

PEREIRA RT, DE FREITAS TR, DE OLIVEIRA IRC, COSTA LS, VIGLIANO FA & ROSA PV. 2017. Endocrine cells producing peptide hormones in the intestine of Nile tilapia: distribution and effects of feeding and fasting on the cell density. Fish Physiol Biochem 43(5): 1399-1412.

PÉREZ SIRKIN DI, SUZUKI H, CÁNEPA MM & VISSIO PG. 2013. Orexin and neuropeptide Y: Tissue specific expression and immunoreactivity in the hypothalamus and preoptic area of the cichlid fish *Cichlasoma dimerus*. Tissue Cell 45(6): 452-459.

RADULOVIC J, MANCEV Z, STANOJEVIC S, VASILJEVIC T, KOVACEVIC-JOVANOVIC V & PESIC G. 1996. Modulation of humoral immune response by central administration of leucine-enkephalin: effects of mu, delta and kappa opioid receptor antagonists. J Neuroimmunol 65(2): 155-161.

RODRÍGUEZ-GÓMEZ FJ, RENDÓN-UNCETA C, SARASQUETE C & MUNOZ-CUETO JÁ. 2001.Distribution of neuropeptide Y-like immunoreactivity in the brain of the Senegalese sole (*Solea senegalensis*). Anat Rec 262(3): 227-237.

RØNNESTAD I, GOMES AS, MURASHITA K, ANGOTZI R, JÖNSSON E & VOLKOFF H. 2017. Appetite-controlling endocrine systems in teleosts. Front Endocrinol 8: 73.

RØNNESTAD I, KAMISAKA Y, CONCEIÇÃO LEC, MORAIS S & TONHEIM SK. 2007. Digestive physiology of marine fish larvae: Hormonal control and processing capacity for proteins, peptides and amino acids. Aquaculture 268(1-4): 82-97.

RØNNESTAD I, ROJAS-GARCIA CR & SKADAL J. 2000. Retrograde peristalsis, a possible mechanism for filling the pyloric caeca? J Fish Biol 56(1): 216-218.

ROTTA MA. 2003. Aspectos gerais da fisiologia e estrutura do sistema digestivo dos peixes relacionados à piscicultura, Embrapa Pantanal. Documentos 53: 1517-1973.

RUBIO VC, SÁNCHEZ-VÁZQUEZ FJ & MADRID JA. 2008. Role of cholecystokinin and its antagonist proglumide on macronutrient selection in European sea bass Dicentrarchus labrax, L. Physiol Behav 93(4-5): 862-869.

SAKATA I, MORI T, KAIYA H, YAMAZAKI M, KANGAWA K, INOUE K & SAKAI T. 2004. Localization of ghrelin-producing cells in the stomach of the rainbow trout (*Oncorhynchus mykiss*). Zoolog Sci 21(7): 757-762.

SHAHBAZI F, HOLMGREN S, LARHAMMAR D & JENSEN J. 2002. Neuropeptide Y effects on vasorelaxation and intestinal contraction in the Atlantic cod *Gadus morhua*. Am J Physiol Regul Integr Comp Physiol 282(5): 1414-1421.

ŞENOL N & ÇINAR K. 2006. Immunohistochemical localization of cholecystokinin and histamine in gastrointestinal tract in flower fish (*Pseudophoxinus antalyae*). Süleyman Demirel Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi 1: 26-34.

SILVERSTEIN JT & PLISETSKAYA EM. 2000. The effects of NPY and insulin on food regulation in fish. Am Zool 40: 296-308.

SOENGAS JL, CERDA-REVERTER JM & DELGADO MJ. 2018. Central regulation of food intake in fish: an evolutionary perspective. J Mol Endocrinol 60(4): 171-199.

SVERLIJ SB, ESPINACH ROS A & ORTI G. 1993. Sinopsis de los datos biológicos y pesqueros de sábalo *Prochilodus lineatus* (Valenciennes, 1847). FAO Sinopsis sobre la Pesca, Roma: FAO 154: 64.

UESAKA T, YANO K, SUGIMOTO S & ANDO M. 1996. Effects of neuropeptide Y on ion and water transport across the seawater eel intestine. Zoolog Sci 13(3): 341-346.

VIEIRA-LOPES DA, PINHEIRO NL, SALES A, VENTURA A, ARAÚJO FG, GOMES ID & NASCIMENTO AA. 2013. Immunohistochemical study of the digestive tract of *Oligosarcus hepsetus*. World J Gastroenterol 19(12): 1919-1929.

VIGLIANO L, MUÑOZ D, HERNÁNDEZ P, CERUTTI R, BERMÚDEZ & QUIROGA MI. 2011. Immunohistochemical study on the gut neuroendocrine system of juvenile pejerrey (*Odontesthes bonariensis*). J Fish Biol 78: 901-911.

VOLKOFF H. 2006. The role of neuropeptide Y, orexins, cocaine and amphetamine-related transcript, cholecystokinin, amylin and leptin in the regulation of feeding in fish. Comp Biochem Physiol A Mol Integr Physiol 144(3): 325-331.

VOLKOFF H. 2016. The neuroendocrine regulation of food intake in fish: a review of current knowledge. Front Neurosci 10: 1-31.

VOLKOFF H, CANOSA LF, UNNIAPPAN S, CERDÁ-REVERTER JM, BERNIER NJ, KELLY SP & PETER RE. 2005. Neuropeptides and the control of food intake in fish. Gen Comp Endocrinol 142(1-2): 3-19.

WILSON JM & CASTRO LFC. 2010. Morphological diversity of the gastrointestinal tract in fishes. In: Grosell et al. (Eds), Fish Physiology, Academic Press, Burlington 30: 1-55.

ZHOU ET AL. 2013. Neuropeptide Y stimulates food intake and regulates metabolism in grass carp, *Ctenopharyngodon idellus*. Aquaculture 380-383: 52-61.

CARLOS E. BARRIOS
http://orcid.org/0000-0001-7071-3805

JUAN JOSÉ SANTINÓN
http://orcid.org/0000-0003-3373-8717

HUGO A. DOMITROVIC
https://orcid.org/0000-0001-9039-0636

SEBASTIÁN SÁNCHEZ
http://orcid.org/0000-0002-8093-5759

DAVID R. HERNÁNDEZ
https://orcid.org/0000-0001-8375-3021

Instituto de Ictiología del Nordeste, Facultad de Ciencias Veterinarias, UNNE, Sargento Cabral 2139, Corrientes (3400), Argentina

Correspondence to: **David Roque Hernández**
E-mail: dhernandez@vet.unne.edu.ar

Author contributions

C.E.B. designed and provided the fishes, analyzed data and co-wrote the paper. J.J.S. and D.R.H. performed immunohistochemical procedures, analyzed the samples and co-wrote the paper. H.A.D. supervised the research, provided final approval of the version to publish. S.S. provided critical revision of the article.