



Intestinal description of serotonin and melatonin content at different stages life cycle of *Salmo salar* and *Onchorynchus kitsuch*.

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Coloquio Internacional "Brechas de Investigación en larvicultura de peces"

Sede Puerto Montt
Instituto de
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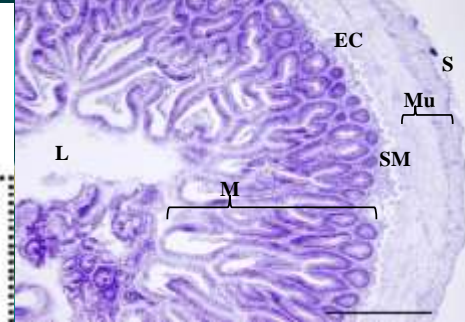
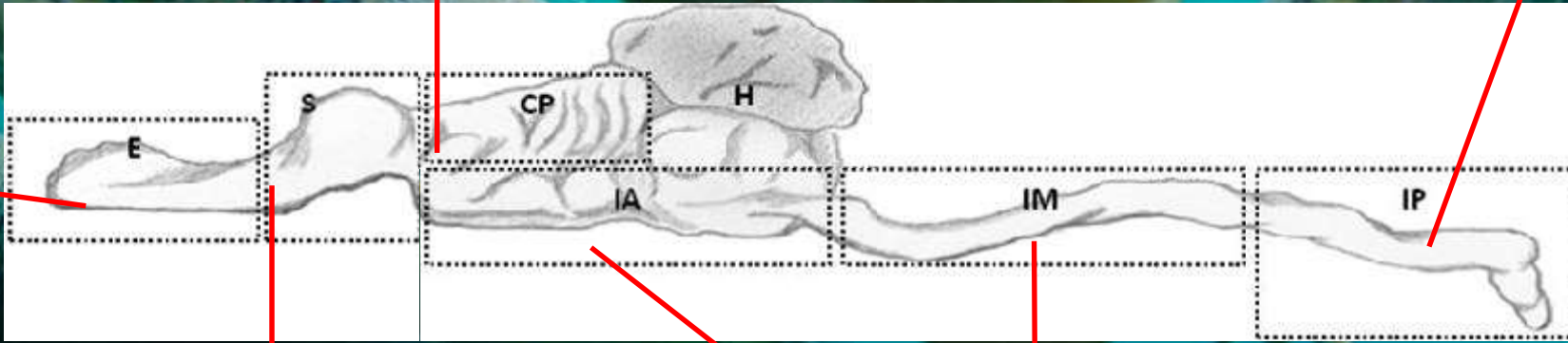
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Gastrointestinal tract

Enzymatic digestion,
nutrient absorption,
storage and
fermentation

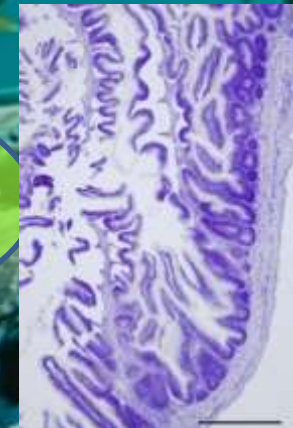
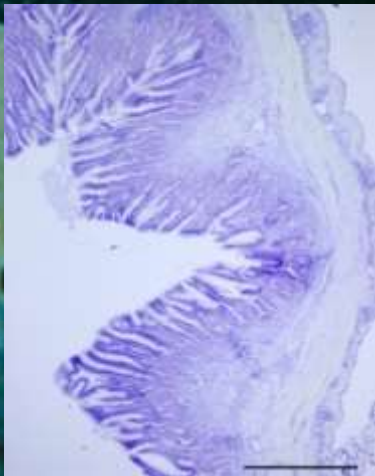
Stores
undigested
food remains

Lubricates
and
facilitates
food
passage



Start of food
decomposition

Chemical digestion and
absorption of proteins,
fats and carbohydrates

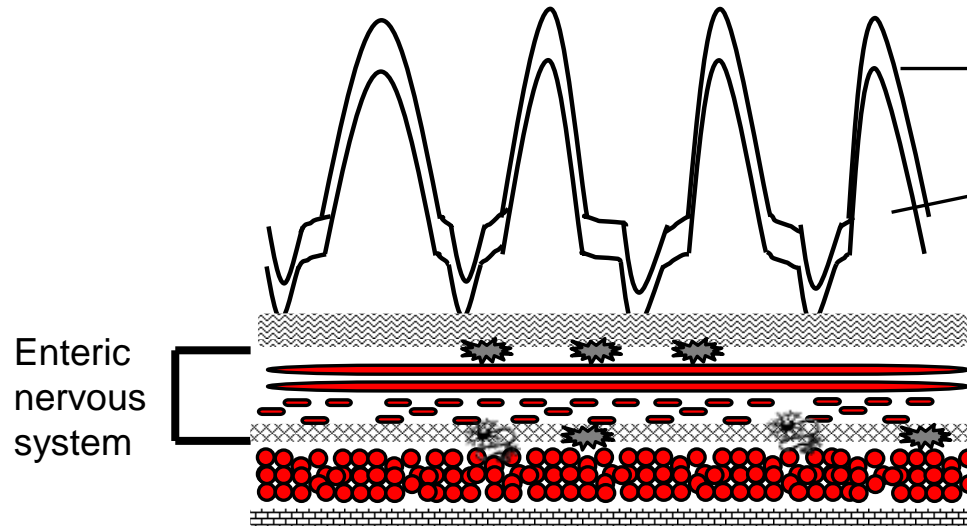


Nervous and hormonal regulation

➤ Optimize digestion and absorption



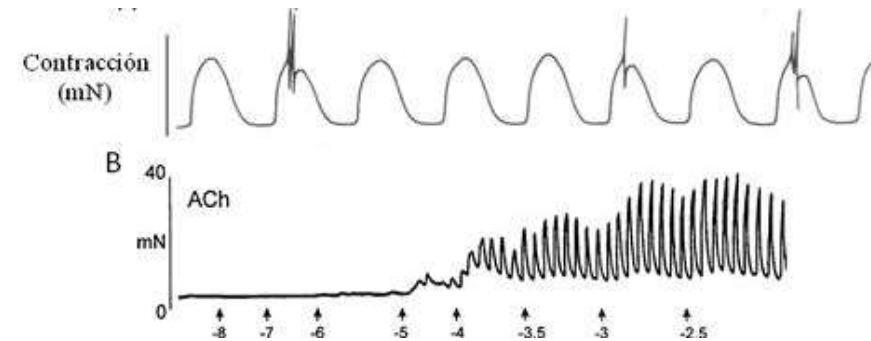
Motility



Intrinsic miogénic Activity



Contráctil tone



Neurotransmitters and hormones in GIT

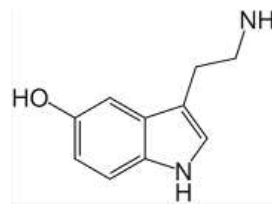
ACh

- * Motility control
 - Involved in transporting water and electrolytes
 - Liberated in vague afferences
 - Receptors in fish

Histamine, substance P, neurokinin A, colecistoquinin,...

Neuropeptides (VIP, PACAP,...)

Serotonin (5HT)



➤ Enzyme TPH limitant step

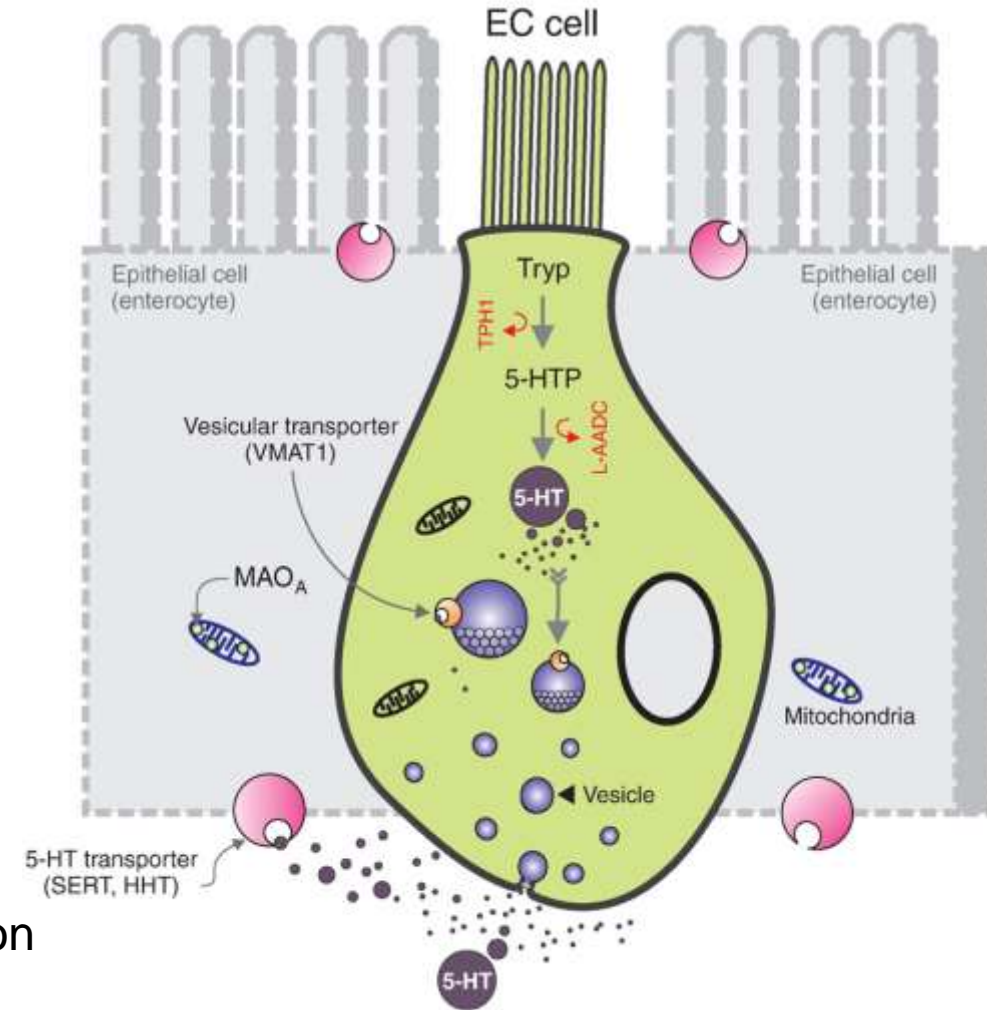
➤ 5HIAA inactive metabolit

Intestinal Serotonin

- Mammals: enterocromafin cells (EC)
- Release in response to mechanical / chemical stimuli of food.

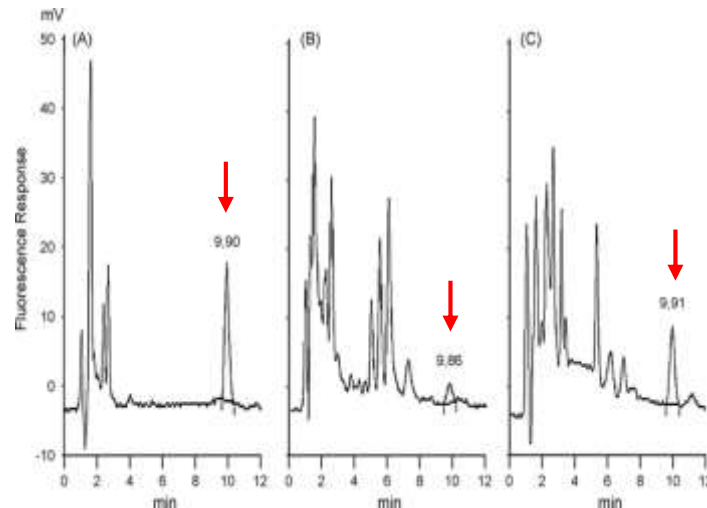
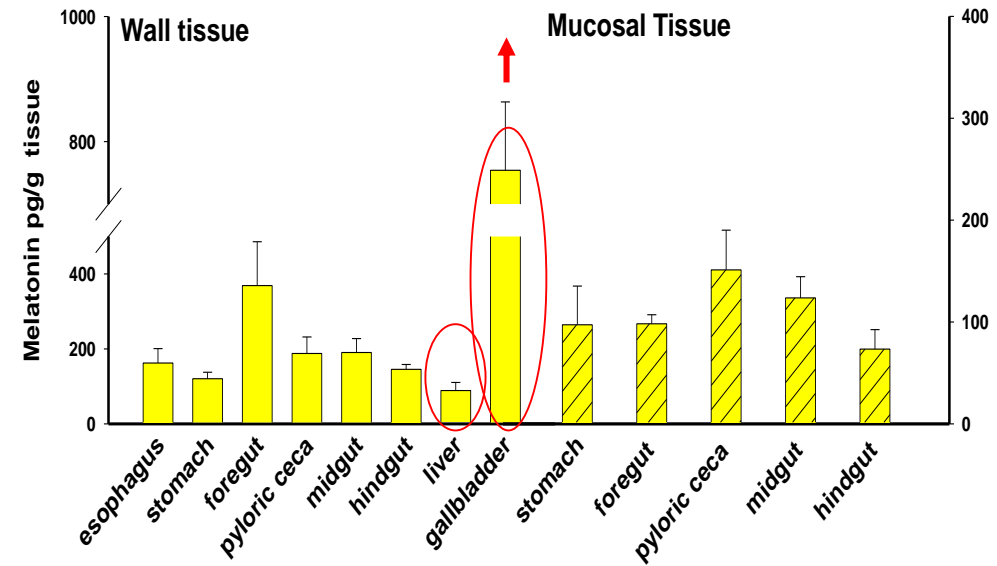
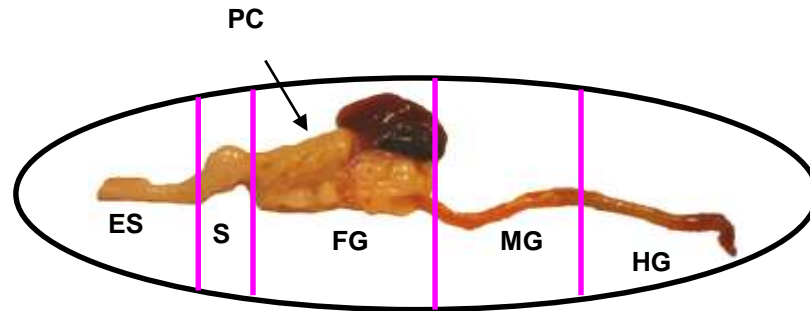
Functions

- Stimulates electrolyte and mucus secretion
- Cyclic motility pattern modulator
 - Mammals
 - ¿Fishes?

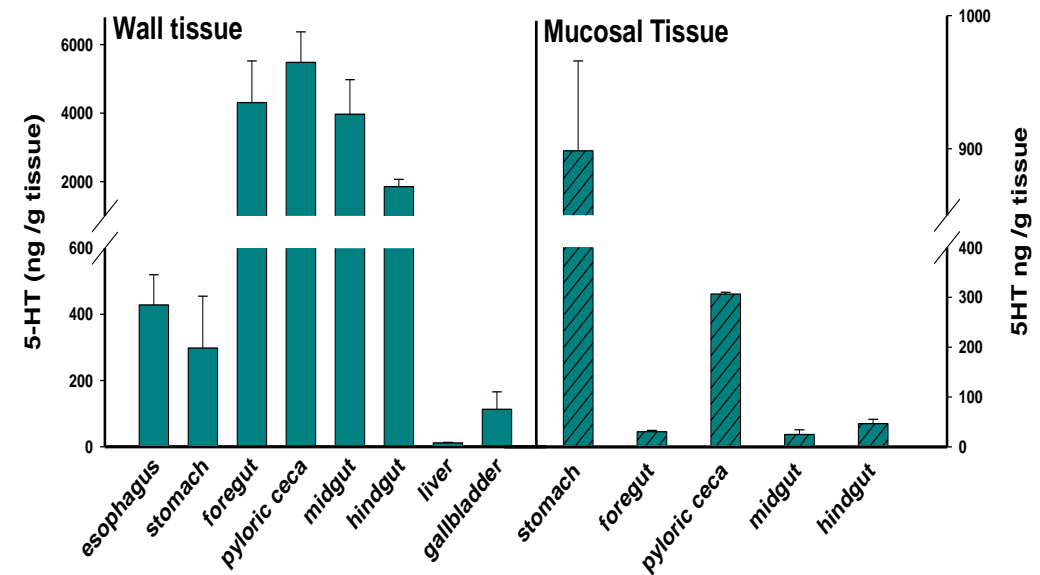


¿Melatonin?

Melatonin levels in intestinal tissues and bile



HPLC measures

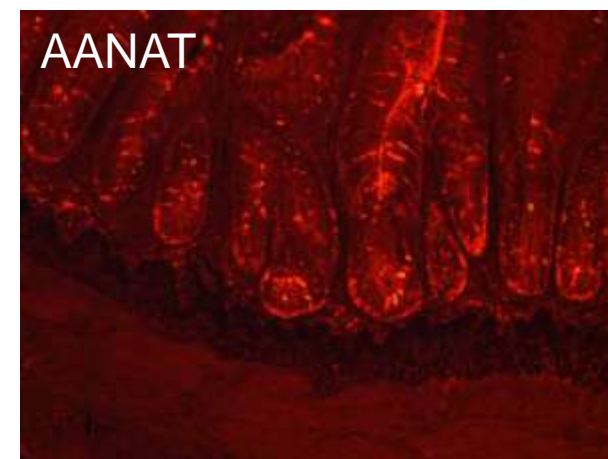
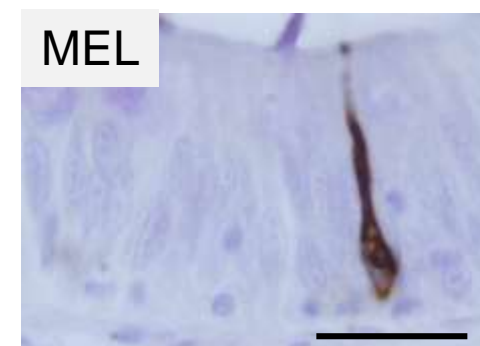
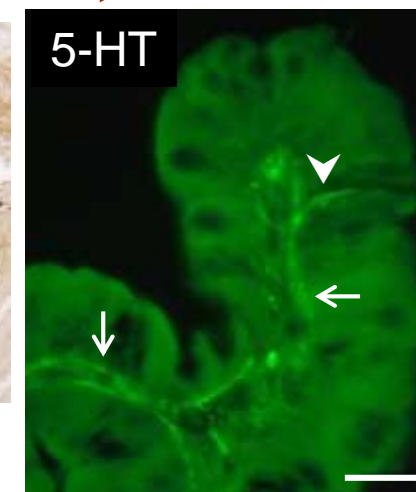
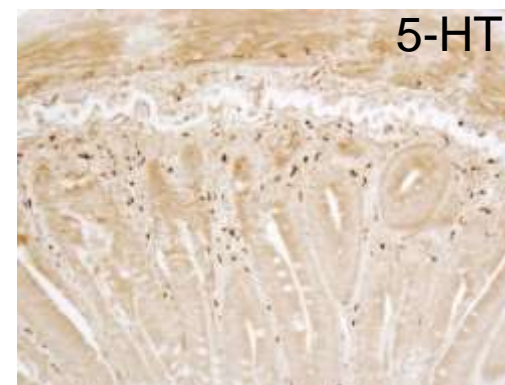
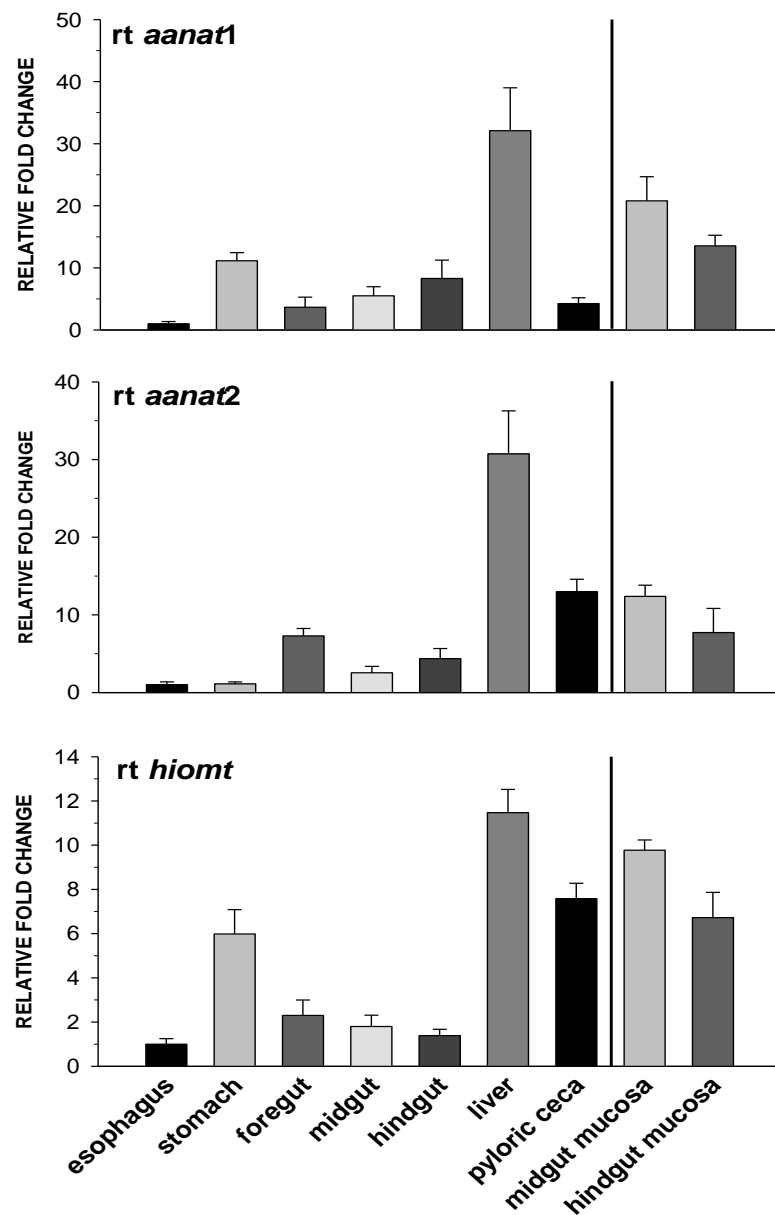




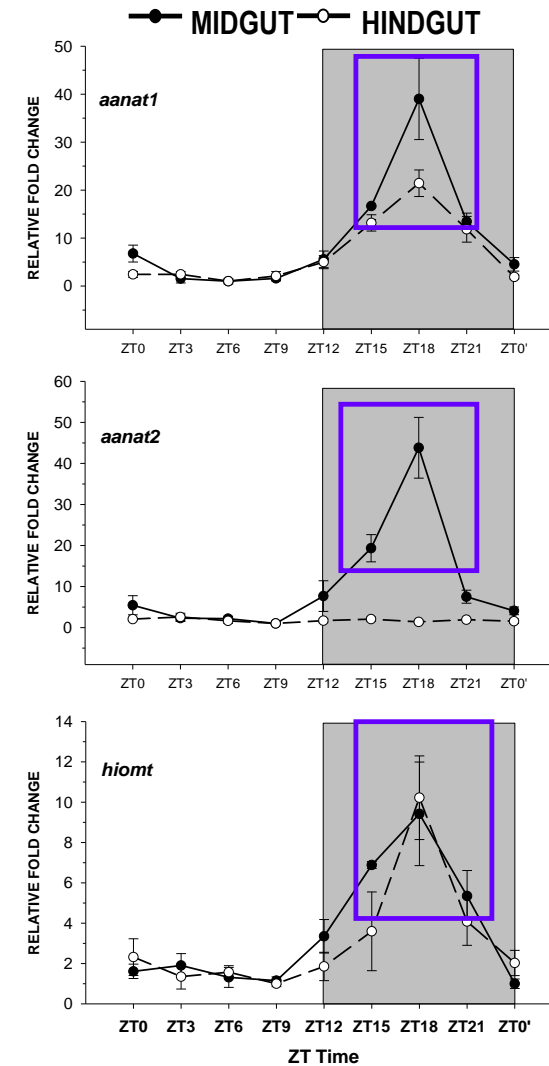
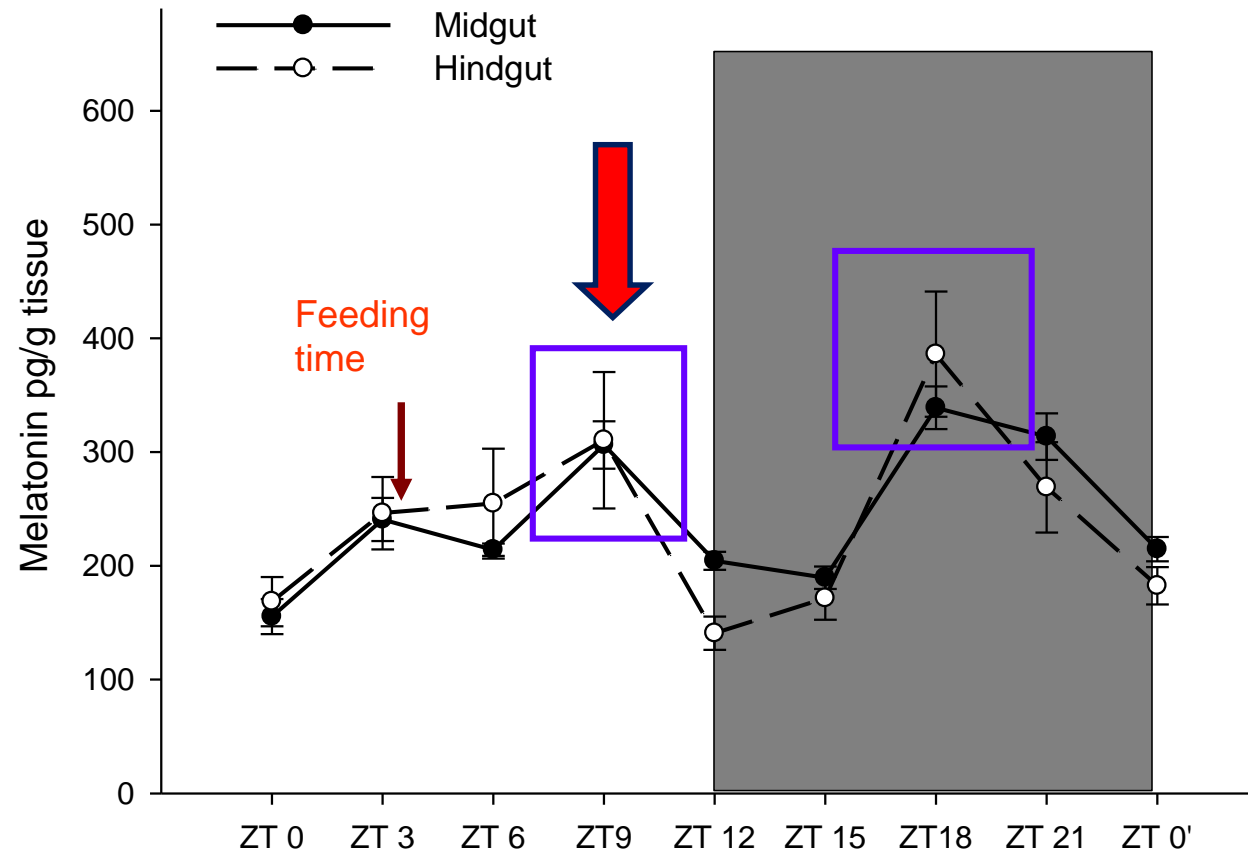
mRNA content (qRT-PCR)

Tissue distribution

Histological evidences



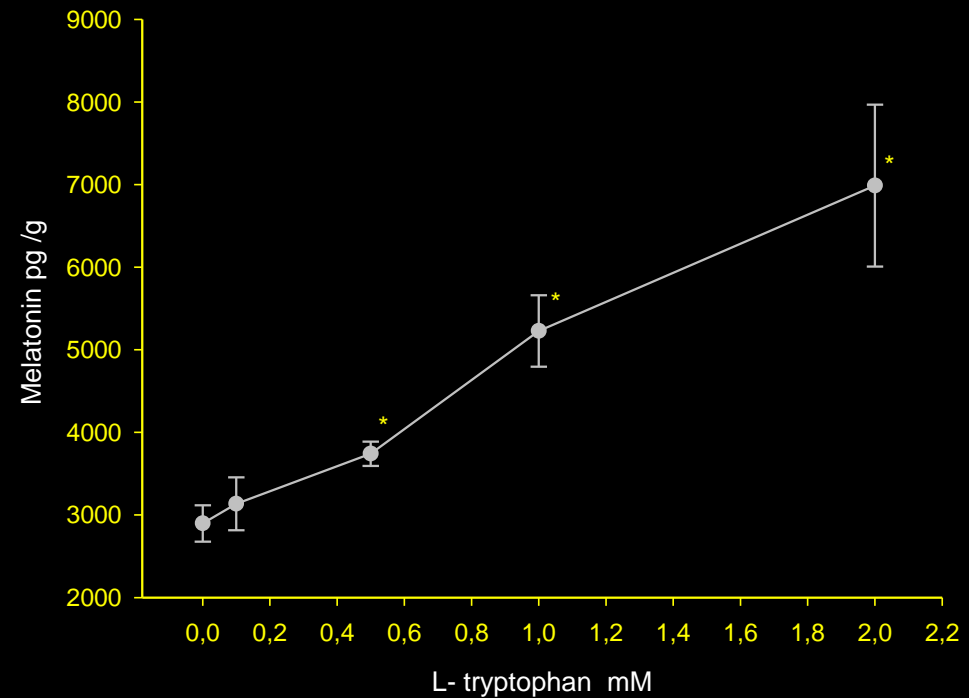
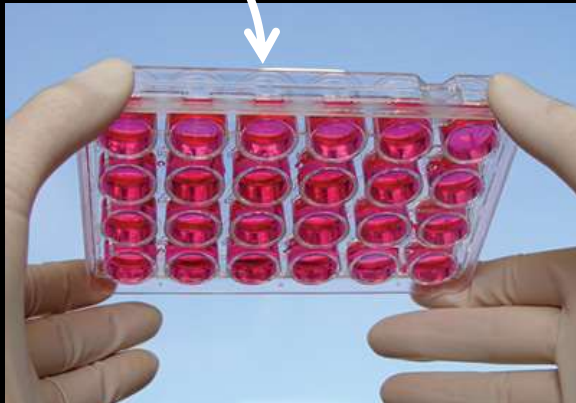
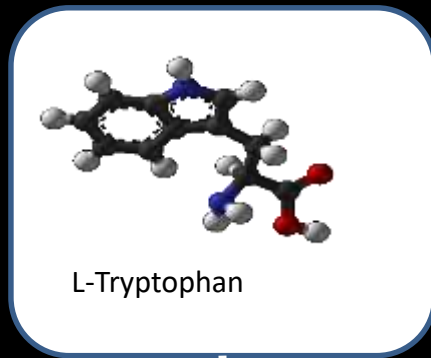
Intestinal melatonin contents increase at the end of the light phase and in the middle of the night

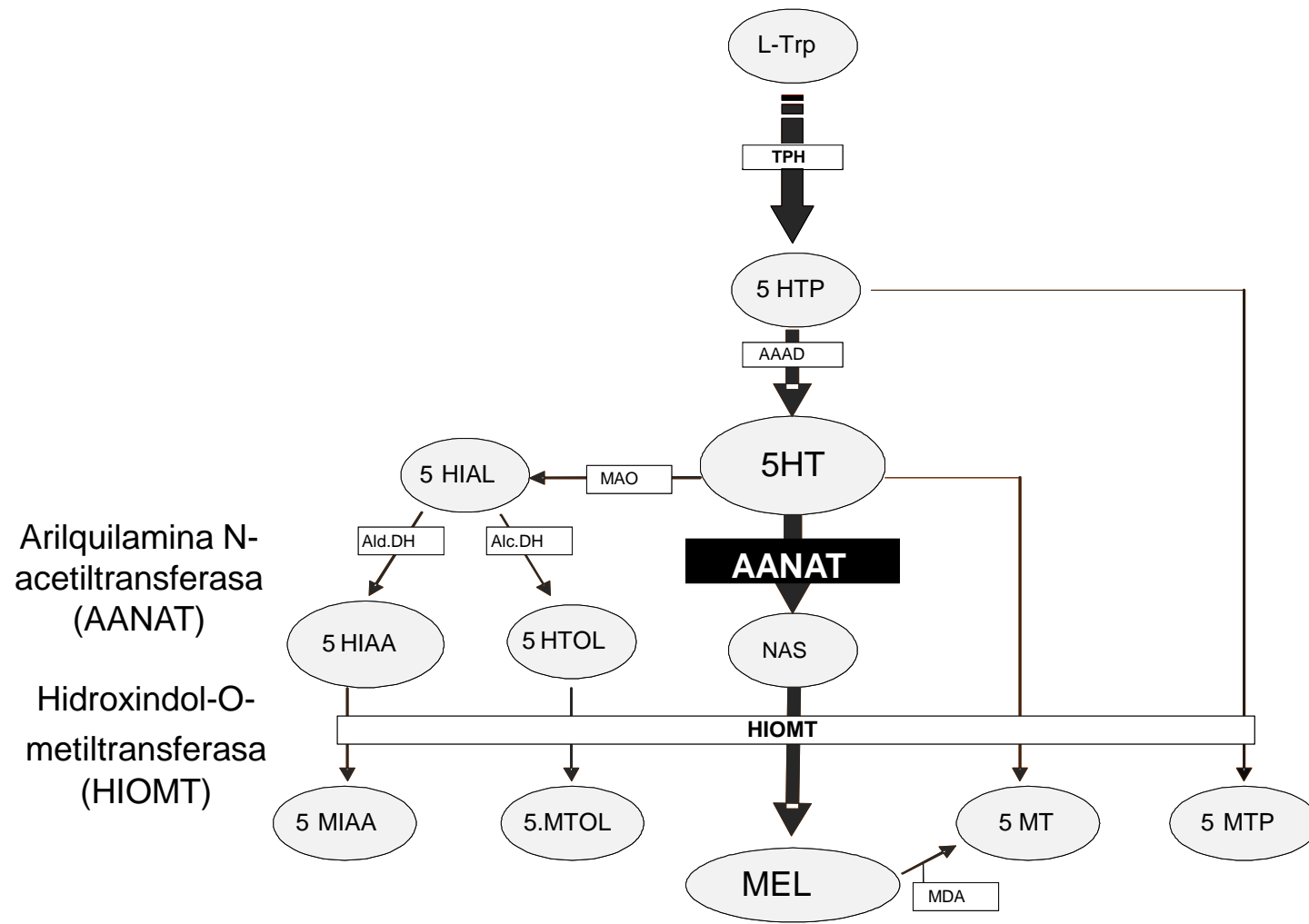


AANAT and HIOMT enzyme expression show clear daily fluctuations peaking at night

Tryptophan increases melatonin production on trout intestine *in vitro*

In fish, recent studies have shown that L-tryptophan (L-trp) increases the production of melatonin *in vivo* and *in vitro* in trout intestinal tissues.





Indolic Metabolism in pineal organ. Melatonin synthesis

Melatonin in the TGI: presence, origin and distribution

Mammals

- First Detections (70s)
- Local and independent synthesis: pinealectomy
Presence of crucial enzymes in the synthesis process
- Variations between species and intestinal region

Teleost

Levels described in sturgeon, carp and trout

Recent in vitro studies have demonstrated their local production in TGI

Presence of aanat2 expression in trout; Variations between layers (mucosa, wall) and tissues.

Presence of expression aanat1, aanat2 and hiont in carpin.

Regulación

Melatonin intestinal receptors

- **Mammals:**

- ✓ Found in intestinal mucosa and villi
- ✓ Depending on the intestinal region
- ✓ MT1 (mucosa, submucosa); MT2 (mucosa, muscular layer)

- **Teleost:**

- ✓ MT1 (sole)
- ✓ Pharmacological evidence (Trout, Carpín)



Characterization of melatonin synthesis in the gastrointestinal tract of rainbow trout (*Oncorhynchus mykiss*): distribution, relation with serotonin, daily rhythms and photoperiod regulation

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Abstract Melatonin is synthesized in peripheral locations of vertebrates, including the gastrointestinal tract (GIT). In teleost, information regarding this topic is scarce. Here we studied the presence and synthesis of melatonin at the rainbow trout GIT. Different sections of trout GIT (from esophagus to hindgut) were dissected out and assayed for contents of melatonin, serotonin (5-HT) and its metabolite, 5-hydroxyindole acetic acid, as well as for *aanat1*, *aanat2* and *hiomt* mRNA abundance. A trout group was pinealectomized to evaluate changes in plasma and gut melatonin content. Finally, the daily profile of melatonin and 5-HT content, and *aanat1*, *aanat2* and *hiomt* mRNA abundance were analyzed in gut of trout kept under normal lighting, and then under constant darkness. Melatonin was detected in all GIT regions with higher concentrations in the muscular wall than in the mucosa, a similar trend to that of 5-HT. In contrast, transcripts of melatonin synthesis enzymes were more abundant in the mucosa. Pinealectomy did not affect melatonin levels in midgut and hindgut either at day or at night. Additionally, no daily rhythms could be defined for melatonin content in gut tissues but increases during late light phase and at midnight occurred. However, *aanat1*,

aanat2 and *hiomt* mRNA abundance showed clear daily rhythms with peaks at night. These rhythms remained with a 3-h phase advanced peak in fish exposed to constant darkness. Our results provide clear evidence for a local synthesis of melatonin in trout GIT that might be influenced by the content of 5-HT in the tissue. The process is affected by environmental light cycle and is likely to be under circadian regulation.

Keywords Melatonin · Gastrointestinal tract · Serotonin · Rainbow trout · Daily rhythms · Gene expression

Introduction

Melatonin is a rhythmic hormonal signal that in vertebrates is synthesized and released mainly from the pineal photoreceptor cells to the blood and cerebrospinal fluid. In most species studied so far the daily profiles of melatonin synthesis and its plasmatic levels are characterized by a large increase at night whereas basal levels are detected during the daytime (Reiter 1993; Falcón et al. 2010). This cyclic signal is well conserved throughout phylogeny, thus playing an important role in the circadian organization and synchronization of a wide number of daily (locomotor activity, feeding, sleep-awake,...) and seasonal (reproduction, growth, hibernation,...) processes that adjust with environment changes (Pévet et al. 2006; Falcón et al. 2010). In teleost, the photic regulation of melatonin synthesis in pineal organ is relatively well known. Melatonin is produced from serotonin (5-hydroxytryptamine, 5-HT) through two enzymatic steps catalyzed by arylalkylamine *N*-acetyltransferase (AANAT; EC 2.3.1.87) that converts 5-HT into *N*-acetylserotonin (NAS), and hydroxyindole-*O*-methyltransferase (HIOMT; EC 2.1.1.4) that acts on

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