2015

Molecular Identification and Functional Characteristics of Peptide Transporter 1 (PEPT1) in the Bonnethead Shark (Sphyrna tiburo)

Hannah Hart

University of North Florida

MOLECULAR IDENTIFICATION AND FUNCTIONAL CHARACTERISTICS OF PEPTIDE TRANSPORTER 1 (PEPT1) IN THE BONNETHEAD SHARK (*SPHYRNA TIBURO*)

by

Hannah Hart

A thesis submitted to the Department of Biology
in partial fulfillment of the requirements for the degree of

Masters of Science in Biology

UNIVERSITY OF NORTH FLORIDA

COLLEGE OF ARTS AND SCIENCES

August 2015

CERTIFICATE OF APPROVAL

The thesis "Molecular identification and functional characteristics of peptide transporter 1 (PEPT1) in the bonnethead shark (*Sphyrna tiburo*)" submitted by Hannah Hart

Approved by the thesis committee: Date

Dr. Greg Ahearn
Committee Chair

Dr. Jim Gelsleichter

Dr. Andrew Evans

Accepted for the Department of Biology:

Dr. Cliff Ross
Department Chair

Accepted for the College of Arts and Sciences:

Dr. Barbara A. Hetrick
Dean

Accepted for the University:

Dr. John Kantner
Dean of the Graduate School

ACKNOWLEDGEMENTS

First and foremost I would like to thank my advisor and committee members: Dr. Greg Ahearn, Professor at University of North Florida; Dr. Jim Gelsleichter, Associate Professor at University of North Florida; and Dr. Andrew Evans, Assistant Professor at the University of Southern Mississippi Gulf Coast Research Laboratory. Without their help and support this project would not have been possible. Each of them have played such a significant role in helping me to achieve success throughout my graduate career. With their knowledge and assistance this research flourished and we were able to discover novel findings.

I would also like to thank the University of North Florida for funding that helped support this project, as well as the Martha Green scholarship from the Alpha Phi foundation. I would also like to thank Dr. Hannalore Daniel, Professor at the TUM School of Life Sciences, for providing the primary antibody used in the immunohistochemistry aspect of this project.

Last, I would like to thank all of my lab mates that supported me throughout this project, in addition to Dr. Andrew Evans' lab for housing me and helping me to complete the molecular identification portion of my thesis research.

TABLE OF CONTENTS

Content		Page
List of Figures		5
Abstract		6
Component I:		8
	Introduction	8
	Methodology	13
	Results	24
	Discussion	35
Work Cited		39
Vitae		43

LIST OF FIGURES

Content		Page
Figure 1	Map of South Carolina, Georgia and Florida demonstrating sites where animals were collected in the present study.	26
Figure 2	Morphological image of all components of the bonnethead gastrointestinal tract used in the present study.	26
Figure 3	Bonnethead PEPT1 protein aligned with PEPT1 proteins from diverse vertebrate taxa. Lined regions with roman numerals indicate putative transmembrane domains.	27
Figure 4	Bonnethead PEPT2 protein aligned with PEPT2 proteins from diverse vertebrate taxa. Lined regions with roman numerals indicate putative transmembrane domains.	28
Figure 5	Phylogenetic tree built from the alignment of the complete PEPT1 and PEPT2 protein sequences of the bonnethead shark in comparison to other known vertebrate PEPT1 and PEPT2 proteins.	29
Figure 6	Cross sections of GI organs using H&E staining methods demonstrate the histological architecture of the organs (a). Cross sections of negative immunohistochemistry analyses in all components of the GI tract (b). Cross sections of positive immunohistochemistry analyses in all the components of the GI tract. Arrows represent positive staining of PEPT1 (c). All sections were observed at 400x.	30
Figure 7	Time course experiment testing the effect of pH on [10 mM] ^{3}H-Glycylsarcosine uptake. Vesicles were preloaded with 150mM KCl, 20mM HEPES at pH 7.5, and incubated in various pH levels (5.5, 7.5, 8.5). Experiments were conducted three times with three replicates each. Symbols are mean ± SE.	31
Figure 8	Kinetics experiment testing the effect of Glycylsarcosine concentration. Vesicles were preloaded with 150mM KCl, 20mM HEPES at pH 7.5, and incubated in 150mM KCl, 20mM MES at pH 5.5 solutions with various concentrations of ^{3}H-Glycylsarcosine (1mM, 2.5mM, 5mM, 10mM, 25mM). Experiments were conducted three times with five replicates each. Symbols are mean ± SE.	32

ABSTRACT

Many elasmobranchs are considered top predators with worldwide distribution, and in general these fish play an important role in the transfer of energy from the lower to the upper trophic levels within the marine ecosystem. Despite this, little research has been done regarding the rates of prey ingestion, digestion, and the processes of energy and nutrient absorption. Specifically understudied is enzymatic digestion within the intestinal brush border, which functions to break down macromolecules into smaller subunits for luminal absorption across the gastrointestinal epithelium. Given their carnivorous diet, the present study sought to expand knowledge on nutrient intake in elasmobranchs by focusing on the uptake of products of protein metabolism. To accomplish this, sequence encoding Peptide Transporter 1 (PepT1), a protein found within the brush border membrane (BBM) of higher vertebrates that is responsible for the translocation and absorption of small peptides released during digestion by luminal and membrane-bound proteases, was molecularly identified in the bonnethead shark (*Sphyrna tiburo*) using degenerate primers based on conserved portions of known PEPT1 sequences from other vertebrates. Sequence encoding Peptide Transporter 2 (PepT2) was also isolated from the *S. tiburo* scroll valve intestine using the same methodology. PepT1 was then localized using immunocytochemistry with rabbit polyclonal anti-rat PEPT1 in the esophagus, stomach, duodenum, scroll valve intestine, rectum, and pancreas. Vesicle studies were used to identify the apparent affinity of the transporter, and to quantify the rate of uptake by its H^+-dependent cotransporter properties, using ^{3}H-glycylsarcosine as a model dipeptide. The results of this study provide insight into the rate and properties of food passage within *S. tiburo,* and can lead to

future work on topics such as physiological regulation of protein metabolism and absorption and how it may vary in elasmobranchs that exhibit different feeding strategies.

Key Words: Elasmobranch, Peptide transporter 1, Peptide transporter 2, scroll valve intestine, gastrointestinal tract, absorption.

INTRODUCTION

Sharks, skates and rays form a group of saltwater- and freshwater-dwelling fish called elasmobranchs. This group represents primitive vertebrates that evolved at least 400 million years ago, and are considered to be some of the first jawed vertebrates (Moy-Thomas 1938; Maisey 1980; Wilga *et al*. 2001). The jaw is made up of a series of homologous branchial arches as part of the visceral skeleton, which has acquired the function of biting (Moy-Thomas 1938). This morphological and functional development led to more complex feeding mechanisms and allowed for a shift in diet (Moy-Thomas 1938; Wilga *et al.* 2001). This ability to consume comparatively larger and more nutrient-valuable prey called for absorptive modifications within the gastrointestinal system (Holmgren 1999; Wilga *et al.* 2001). As seen in agnathans (jawless vertebrates) the gastrointestinal tract is made up of an esophagus and a gut tube (intestine) that leads to the cloaca. Comparatively however, the gnathostome (jawed vertebrate) GI tract such as that of elasmobranchs is more complex and consists of an esophagus, stomach, duodenum, spiral/scroll valve intestine, and colon (Fishbeck *et al.* 2008).

The elasmobranch spiral/scroll valve intestine is a unique organ that increases surface area without increasing length by the infolding of the mucosa and submucosa. It is commonly thought that due to the presence of this organ elasmobranchs undergo slow food passage, which is often associated with a low rate of consumption, therefore limiting growth and reproductive rates. A study on juvenile lemon sharks examined digestive capability using an indirect method via an inert, naturally occurring marker in their food (Cortes and Gruber 1990). The authors found that lemon sharks are capable of absorbing energy with an average efficiency of about 80%, which is

similar to the absorption efficiency of a carnivorous teleost. However, the rate of digestion was prolonged in comparison, taking between 70 to 100 hours (Cortes and Gruber 1990). Feeding frequency has also been examined in various species of sharks, and it has been found that the rate of food passage varies among species (Bush 2002; Lowe 2001; Wetherbee *et al.* 2004). Species such as the spiny dogfish are known to gorge themselves every 10 to 16 days (Wetherbee *et al.* 2004). Conversely, the scalloped hammerhead feeds much more frequently ranging from every 10 to 11 hours (Bush 2002; Lowe 2001). Moreover, although all elasmobranchs are carnivores, the diet across species can range from microscopic phytoplankton to large pelagic fish. A number of studies have used quantitative diet analysis to understand what elasmobranchs consume and different predator-prey interactions important to sharks (Bethea *et al.* 2007; Cortes *et al.* 2006; NMFS 1999). Through such research and different technological advancements current research has been able to estimate species-specific metabolic rates for a number of elasmobranch species, and such information can be used to better grasp bioenergetics (Carlson *et al.* 2004). However most fish, including sharks, do not initially break down their food prior to ingestion into the gastrointestinal tract. Thus, it is important to go beyond diet itself, to understand the chemical means of digestion and absorption (Papastamatiou and Lowe 2004; Papastamatiou and Lowe 2005; Clements and Raubenheimer 2006; German 2011). For example, Jhaveri *et al.* (2015) examined digestive enzyme activity along the gut of the bonnethead shark (*Sphyrna tiburo)*. This study specifically looked at what compounds the bonnethead shark could digest and applied this information to infer the digestive strategy within the hindguts of *S. tiburo*. Results demonstrated that gut content was concentrated more within the intestinal region (duodenum, spiral valve intestine, and colon), with greater concentrations within the spiral valve intestine in comparison to the duodenum and colon. They also found that pancreatic enzyme

activity was elevated in the duodenum and spiral valve, whereas brush border membrane (BBM) enzymatic activity peaked in the spiral valve and colon. However, microbial enzymes were highest on or within the colon. From such results, they inferred that the spiral valve intestine is the most active, absorptive section of the shark intestinal region. An additional/final conclusion was that the bonnethead shark has adapted a yield-maximizing digestive strategy, meaning that they consume relatively large meals infrequently and thus have enzymatic patterns as described above (Jhaveri *et al*. 2015).

Although diet composition and general aspects of enzymatic digestion within the elasmobranch gut have been studied, studies regarding the breakdown of dietary macromolecules and their absorption across intestinal epithelium within the BBM are significantly lacking. Microscopically, the epithelial cells and microvilli that make up the BBM in the elasmobranch GI tract are remarkably similar to those of the mammalian and teleost gastrointestinal tracts (Crane 1978). Based on previous studies, it is known that the mammalian BBM increases surface area for nutritive absorption, and contains enzymes near transporters that facilitate the absorption of digested nutrients into the intestine (Schmitz *et al*. 1973; Hauser *et al.* 1980). However, in regards to elasmobranchs, little information is available about the functional properties of the components of the gastrointestinal tract. In one of the few studies on this topic, Crane (1979) demonstrated the presence of potential BBM D-glucose transporters within small dogfish (*Scyliorhinus canicula*) spiral valve intestine. That study showed that glucose uptake by *S. canicula* spiral valve BBM is stimulated by a Na^+ gradient and inhibited by the monosaccharide α- methylglucoside, an alternative substrate for glucose transporters, or by phlorizin, a specific inhibitor of intestinal glucose transporters. Such information provides insight into the cellular

mechanisms of the elasmobranch intestine, and similarities to the teleost and mammalian digestive mechanisms.

Sharks have a carnivorous diet, and therefore protein absorption is an important aspect to understand. However, in contrast to the modest research conducted on sugar uptake, there are no publications regarding the mechanisms by which peptides cross the epithelial cells of the spiral/scroll valve and other digestive organs. It is known that dietary proteins can be degraded into free amino acids within the digestive tract. However, a great deal of studies provide evidence that within the mammalian and teleost intestine, most dietary proteins are broken down into small peptides rather than being broken down to single amino acids (Shimakura *et al.* 2006). Thus intestinal peptide transport is an important aspect to understand and such knowledge will provide further insight into the ability of sharks to absorb amino acids or short peptides for energy usage, and also lead to further research on topics such as the physiological regulation of protein metabolism and absorption (Matthews, 1975; Daniel, 2004).

Research has shown that peptide absorption is markedly influenced by an inwardly directed proton gradient, stimulated by an inside-negative membrane potential and inhibited by an inside-positive membrane potential; implying that the transport of peptides across a membrane is associated with the transfer of positively charged ions (Leibach *et al.*, 1996). Peptide transporter 1 (PEPT1 or SLC15A1) and Peptide transporter 2 (PEPT2 or SLC15A2) are both a critical peptide transporters within the mammalian and teleost digestive and renal tracts that depend on this driving force. These transporters have also been identified as part of the major facilitator superfamily of 12 transmembrane domain transporter proteins. However, although these two

peptide transporters share similarities in function and structural characteristics, the distribution of PEPT1 and PEPT2 differ. PEPT1 has been successfully identified and described in a number of teleost and mammalian species within the gastrointestinal tract, rectum, gallbladder, pancreas, nuclei of smooth muscle cells, the liver, and throughout the renal tract (Leibach and Ganapathy 1996; Verri *et al.* 2010). However, studies report that PEPT2 is predominately located in the kidney BBM, brain, gonads, lungs, gallbladder, and liver (Leibach and Ganapathy 1996; Saito *et al.* 1996). PEPT2 has not been detected in the mammalian or teleost intestinal tract. It is important to note that studies have also shown that PEPT2 possesses a higher affinity than PEPT1 for diverse dipeptides (Leibach and Ganapathy 1996). In 2003, Verri *et al*. confirmed the presence of PepT1 in fish by successfully cloning and functionally characterizing it within the *Danio rerio* intestinal BBM. Specifically, PEPT1 is a Na^+- independent, H^+ - dependent cotransporter protein present in the intestinal BBM that is responsible for the translocation and absorption of di- and tripeptides released during digestion by luminal and membrane- bound proteases.

As there are consistencies seen in the distribution of PEPT1 and PEPT2 across vertebrates overall, we expect to find PepT1 to have the same range of expression in elasmobranchs, specifically in the spiral valve intestine and other GI organs. The purpose of this study was to molecularly identify the presence of PepT1 within the elasmobranch spiral valve intestine and to histologically assess the distribution of this transporter in all components of the GI tract and the pancreas. In addition, this study will report the uptake capabilities of the intestinal peptide transporter. For this particular study bonnethead sharks (*Sphyrna tiburo*) were used as the model species due to their abundance and availability.

METHODOLOGY

Sample collection

S. tiburo were collected from sites ranging from Charleston, South Carolina to Cape Canaveral, Florida (Fig. 1) using gillnet and longline fishing. Animals were euthanatized using IACUC-approved methodology, and the gastrointestinal tract was obtained by dissection (Fig. 2). Small portions of the scroll valve intestine were sampled and stored in RNAlater at -4˚C for use in molecular studies. Samples (~2-3 mm) of the gastrointestinal tract and accessory organs (esophagus, stomach, pancreas, duodenum, scroll valve intestine, and rectum) were also obtained and fixed in 10% formalin in elasmobranch-modified saline for about 48 hrs., then rinsed and transferred to 70% ethanol for long-term storage until used for histology and immunocytochemistry. Last, whole intestines were obtained from some animals and stored at -80˚C until used for vesicle experiments.

Fig. 1: Map of South Carolina, Georgia and Florida demonstrating sites where animals (representative of blue circles) were collected in the present study. Animals were collected mainly in coastal waters of Charleston, SC, Jacksonville, FL, and Cape Canaveral, FL.

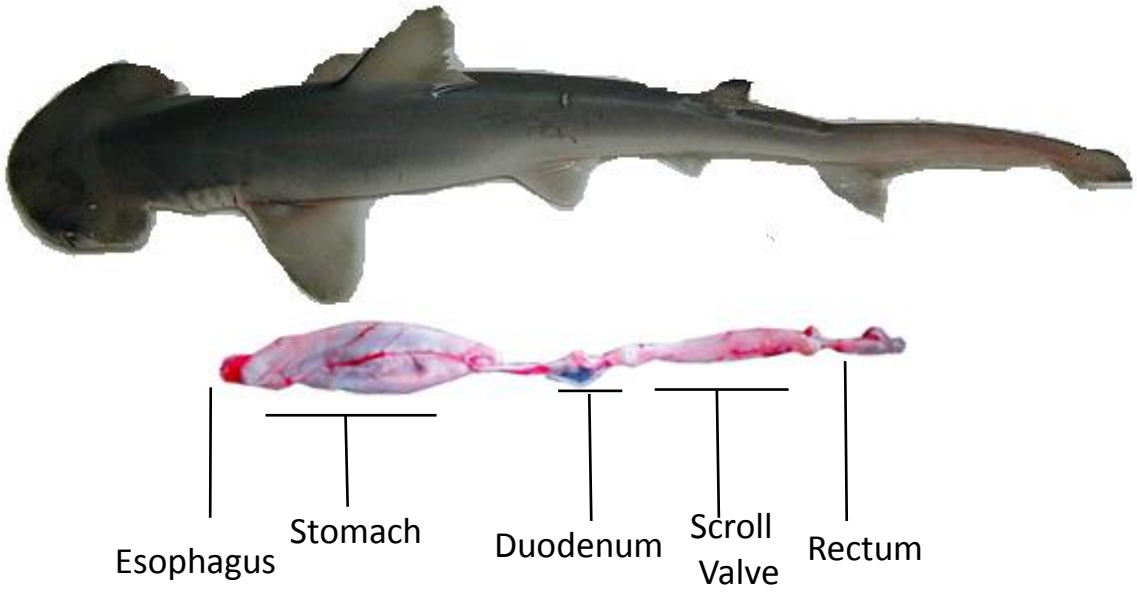

Fig. 2: Morphological image of the bonnethead gastrointestinal tract.

Molecular Identification

1. Isolation of *S. tiburo pept1* and *pept2* cDNA sequences

To isolate total RNA, approximately 50 mg of scroll valve intestine was minced, placed in 750 µL of Trizol reagent and homogenized. The homogenate was centrifuged for 1 minute at 12,000 x g to pellet any remaining solid tissue, with 700 µl of the supernatant subsequently placed into a microcentrifuge tube. The Direct-Zol RNA Miniprep kit (Zymo Research, Irvine, CA) was then used for total RNA extraction. The concentration and purity of RNA was determined using a NanoDrop spectrophotometer and gel electrophoresis. Reverse transcription of 1 µg of total RNA into cDNA was conducted using Superscript III reverse transcriptase (Life Technologies) following the manufacturer's instructions.

For degenerate PCR, degenerate primers were designed based on conserved portions of known PEPT1 sequences from chicken (NM_204365.1), salmon (NM_001146692.1), eel (AB7762417), and zebrafish (NM_198064). Four combinations from the mentioned taxa were created, two different forward and two different reverse primers, to maximize the possibility of at least one combination annealing to bonnethead cDNA and amplifying a portion of *pept1*. PCR was performed using six different cycling parameters, to increase the range of annealing temperatures tested. Cycling parameters included: 95˚C for 2 min followed by initial annealing temperatures of 52.5, 53.3, 54.9, 57.2, 60.1 or 62.5 (a different starting temperature for each reaction) for 30 sec and then 72 ˚C for 1 min. For the first 25 cycles, the annealing temperature was decreased each cycle by 0.5˚C, followed by 15 cycles (for 40 total) at an annealing temperature of 40 ˚C for all reactions. Following the PCR a 1% gel electrophoresis was run to determine the optimal

temperature and combination of primers, with products of the appropriate size isolated, cloned and sequenced as described below.

The primers that yielded the largest product were 5'-GAGTTCTGYGARMGDTTCTCCTACT-3' as the forward primer and 5'-TGGTCAAANARDGYCCAGAACAT-3' as the reverse primer. Products from PCR reactions using this primer combination were ligated into the pGEM-T vector (Promega) and used to transform competent *Escherichia coli*. Twenty-five µl of transformed cells were then plated on agar plates coated with isopropyl-beta-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal) for blue-white screening and incubated at 37 ˚C overnight. White (positive) colonies were then selected, placed in 2 mL of LB broth and incubated overnight at 37˚C with shaking at 250 rpm. Overnight incubations were transferred into capped tubes and centrifuged at high speed for about 30 sec. The supernatant was poured out and the pellet then resuspended in 600 µl of sterile water The Zyppy Plasmid Miniprep Kit (Zymo Research) was then used to isolate plasmid DNA from for sequencing preparation. Selected plasmids were then sequenced by Genewiz, and sequences were analyzed using the CLC Main Workbench 7 software (CLCbio, Qiagen). Although the target cDNA was *pept1*, the methods described above yielded clones positive for both *pept1* and *pept2* sequences, likely due to high sequence similarity; therefore full length cDNA sequences were obtained for both genes using rapid amplification of cDNA ends (RACE).

To obtain the remaining 3' and 5' coding sequence as well as the 5' and 3' untranslated regions of *pept1* and *pept2*, the FirstChoice RLM-RACE kit (Life Technologies) was used. Total RNA from scroll valve intestine was extracted as described above, and 5' and 3' RACE-ready cDNA was then generated following the manufacturer's instructions. 5' and 3' RACE PCR products were then obtained using nested PCR reactions that included kit-specific primers and species-

specific primers (Table 1) for *pept1* and *pept2* designed using the cDNA fragments isolated as described above. RACE products were then cloned and sequenced as described above, and sequences were assembled using CLC Main Workbench 7.

	outer	inner
pept1 RACE 5’	CCAAGCCAGGAGTCAGCAATGATT	CGTGGTAAACGGCTGTAGCGAGA
pept1 RACE 3’	CTTTGGAGTCCCAGCTGCTCTGAT	TTCATGGACTGGGCTTCAGAGAA
pept2 RACE 5’	ACGATGGACAGCACAATGATGGTT	CGTGGTAGATGGCAGTGGAAAGAT
pept2 RACE 3’	CAGGAAGTTTGCTCTCGACCATCA	CAATCGGTGGAAACATCGCAGAA

Table 1: Bonnethead species-specific primers used to obtain the remaining 5’ and 3’ coding sequence and untranslated regions using inner and outer RACE PCR reactions. Primers were designed based on cDNA fragments isolated from the bonnethead shark.

2. Sequence and phylogenetic analyses

BLAST analysis was used to confirm the identity of the presumed *S. tiburo* PepT1 and PepT2 protein sequences (http://www.ncbi.nlm.nih.gov/BLAST). *S. tiburo* sequences were then aligned with other elasmobranchs, teleost, and higher vertebrates using the Clustal W algorithm in CLC Main Workbench for analysis of gene homology (Figures 3 and 4). Potential transmembrane domains for both *pept1* and *pept2* were defined using TMprep computational resources (http://www.bioinformatics.utep.edu/BIMER/tools/transmembrane.html). For phylogenetic analysis, both *S. tiburo* protein sequences were aligned with PEPT1 and PEPT2 sequences from diverse vertebrate taxa using the Clustal W algorithm in *MEGA* version 6 (Tamura *et al.* 2013); phylogenetic relationships were then determined using the Neighbor-Joining method with 2000 iterations to generate a bootstrap consensus tree (Figure 5).

Histology

Fixed tissue samples were processed for routine paraffin histology as described by Gelsleichter *et al.* (2003). Once embedded in paraffin, samples were sectioned (5 µm) using a rotary microtome and mounted on poly-L-lysine-coated slides. They were then stained with Harris hematoxylin and eosin to examine general cellular architecture.

Immunocytochemistry

Gastrointestinal tract and accessory organ samples were fixed and sectioned as described above. Immunocytochemistry was then performed to examine the cellular location of PEPT1 using a rabbit polyclonal anti-rat PEPT1 (SLC15a1, Millipore, Berlin, Germany) and the Vector ImmPRESS anti-rabbit kit (Vector Laboratories, Burlingame, CA). Tissue sections were

incubated in a limonene-based solvent for deparaffinization, rehydrated by submerging in a descending series of graded alcohol concentrations (100%- 95%), and then rinsed for 10 minutes in a running tap water bath. Sections were then incubated in an antigen retrieval solution (10 mM sodium citrate, pH 6.0) at 95˚C for 20 minutes to expose any epitopes of the target antigen that may have been masked by the fixation process. Sections were then removed from the bath and brought to room temperature. They were then rinsed in reverse osmosis (RO) water, followed by phosphate buffered saline (PBS), and then blocked for nonspecific reactivity with primary antibodies by overnight incubation in 2% normal goat serum in PBS (Vector laboratories) at 4˚C. After blocking, slides were washed with PBS and endogenous peroxidase activity was then quenched by incubation in a 1:1 mixture of 3% hydrogen peroxide and methanol for 15 minutes. Sections were then rinsed again in two separate baths of PBS. Sections were then incubated overnight in primary antibody diluted 1/1000 in a PBS solution containing 0.1% gelatin and 0.1% sodium azide (G-PBS). Negative control sections were incubated in diluent only. Following incubation, sections were rinsed in a PBS bath containing 0.05% TWEEN-20 (PBS-T), followed by two additional PBS rinses. Afterwards, slides were incubated for approximately 30 minutes with secondary antibody, anti-rabbit Ig. Following this incubation, sections were rinsed in three separate PBS baths and then incubated in the chromogen 3.3'-diaminobenzidine (DAB), using the ImmPACT DAB Peroxidase (HRP) substrate kit. Slides were rinsed in tap water and then counterstained in 2% methyl green (Vector laboratories) for 15-60 minutes at 37˚C. Afterwards, they were rinsed briefly in tap water, dehydrated in an ascending series of graded alcohols (95%-100%), cleared using limonene-based solvent, and then mounted using Cytoseal 60 (Thermo Scientific, Fair Lawn, NJ). Microscopy was then used to examine the distribution of immunoreactive PEPT1 in the bonnethead digestive system.

Vesicle experiments

1. Preparation of Brush Border Membrane Vesicles:

Whole intestines stored at -80˚C were thawed on ice in PBS. Subsamples of intestine were then cut and scraped using a razor blade to free epithelial cells into 60 mL of a 300 mM mannitol, 20 mM Tris HCl, 50 mM EGTA, 1 mM PMSF buffer adjusted to pH 7.0 (buffer 1). For Brush Border Membrane Vesicle (BBMV) purification, an experimental design used on Mozambique tilapia (*Oreochromis mossambicus)* intestine (Thamotharan *et al.* 1996) was implemented on *S. tiburo*. Similar methodology was used by Crane et al. (1979), showing that elasmobranch BBMV can be isolated using this approach. The expanded technique has two additional washing steps for discarding stored cytoplasmic digestive enzymes (Thamotharan *et al.* 1996). Purified BBMV were then used for transport measurements.

2. Transport measurements

Transport experiments were conducted using the scroll intestine BBMV and the Millipore filtration technique (Thamotharan *et al.* 1996). [^{3}H] Glycylsarcosine (Moravek Biochemicals, Brea, California 92821) (Gly-Sar) was used in 120 min uptake experiments by mixing 20 µL of membrane suspension with 180 µL of radiolabelled incubation medium. Composition of incubation medium varied with the nature of each experiment. Effect of pH on uptake of radiolabeled Gly-Sar in intestinal BBMV was examined using incubation media made up of 150 mM KCl and 20 mM HEPES adjusted to a pH of 7.5 or a pH of 8.5, and a third medium contained 150 mM KCl and 20 mM MES adjusted to pH 5.5. Measurements were taken at intervals of 0.25, 1, 2, 5, 10, 60, and 120 min.

Kinetics of [^{3}H] Gly-Sar influx at 1 min was then examined using media composed of 150 mM KCl, 20 mM HEPES at concentrations of 1, 2.5, 5, 10, and 25 mM Gly-Sar.

In these two separate experiments, uptake of [^{3}H] Gly-Sar was terminated by injecting 20 µL of the reaction into 2 mL of a stop solution (composed of same solution as the incubation medium without the radiolabelled dipeptide). The solution was then filtered using a Millipore filter (0.65 µm pore diameter) and washed with an additional 3 mL of stop solution. Filters were then placed in 3 mL of Beckman Volume scintillation cocktail and counted using a Beckman LS-6100 scintillation spectrometer. The average of each set of replicates was determined and graphed in SigmaPlot10.0. A one-way ANOVA was then ran to statistically test the significance between the mean values of GlySar uptake among the given ion gradients where the overshoot is present.

RESULTS

Sequence analysis

The bonnethead *pept1* cDNA was 4,055 bp, with an ORF of 2,157 bp encoding a putative protein of 718 aa. The sequence also included 455 bp of 5' untranslated region (UTR) sequence and 1,443 bp of 3'UTR sequence with a polyadenosine mRNA tail. Hydropathy analysis predicts 12 potential transmembrane domains (TMD) with an extracellular loop between TMD IX and X (Fig. 3).When compared to previously characterized PEPT1 sequences from other vertebrates using BLAST, the predicted bonnethead sequence shows high sequence identity, ranging from 60 to 67.3 percent.

The *pept2* cDNA was also isolated from the bonnethead shark intestine. The sequence was 2,549 bp, with an ORF of 2,169 bp encoding a putative protein of 722 aa. The sequence included 91 bp of 5' UTR sequence and 289 bp of 3'UTR sequence with a polyadenosine mRNA tail. Hydropathy analysis predicts 13 potential TMD with an extracellular loop between TMD X and XI (Fig. 4). When compared to previously characterized PEPT2 sequences from other species using BLAST, the predicted bonnethead sequence shows high sequence identity, ranging from 61.8 to 66 percent.

Percent identity between the two isolated intestinal peptide transporters was only 51%. Also, the phylogenetic reconstruction of the vertebrate peptide transporter proteins assigned the two isolated bonnethead shark peptide sequences to separate clades and within the PEPT1 and PEPT2 branches of the phylogenetic tree, which also demonstrated early divergence of the elasmobranch protein sequences from those of the teleost and mammalian groups (Fig. 5).

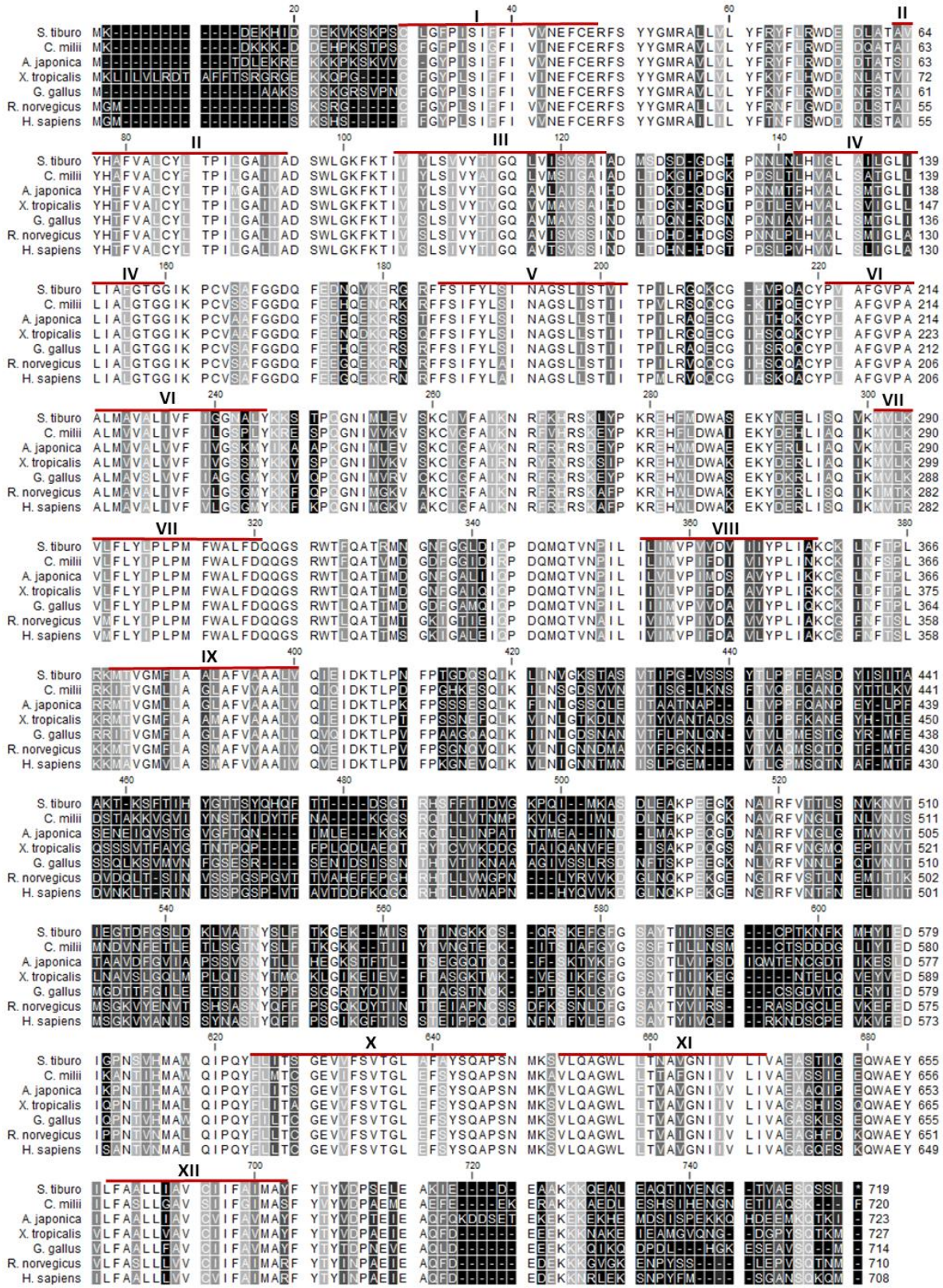

Fig. 3: Bonnethead PepT1 aligned with PEPT1 proteins from diverse vertebrate taxa: *Callorchinchus milii, Anguilla japonica, Xenopus tropicalis, Gallus gallus, Rattus norvegicus, and Homo sapiens.* Darker shading indicates decreasing sequence conservation across taxa. Line region with roman numerals indicates predicted TMDs.

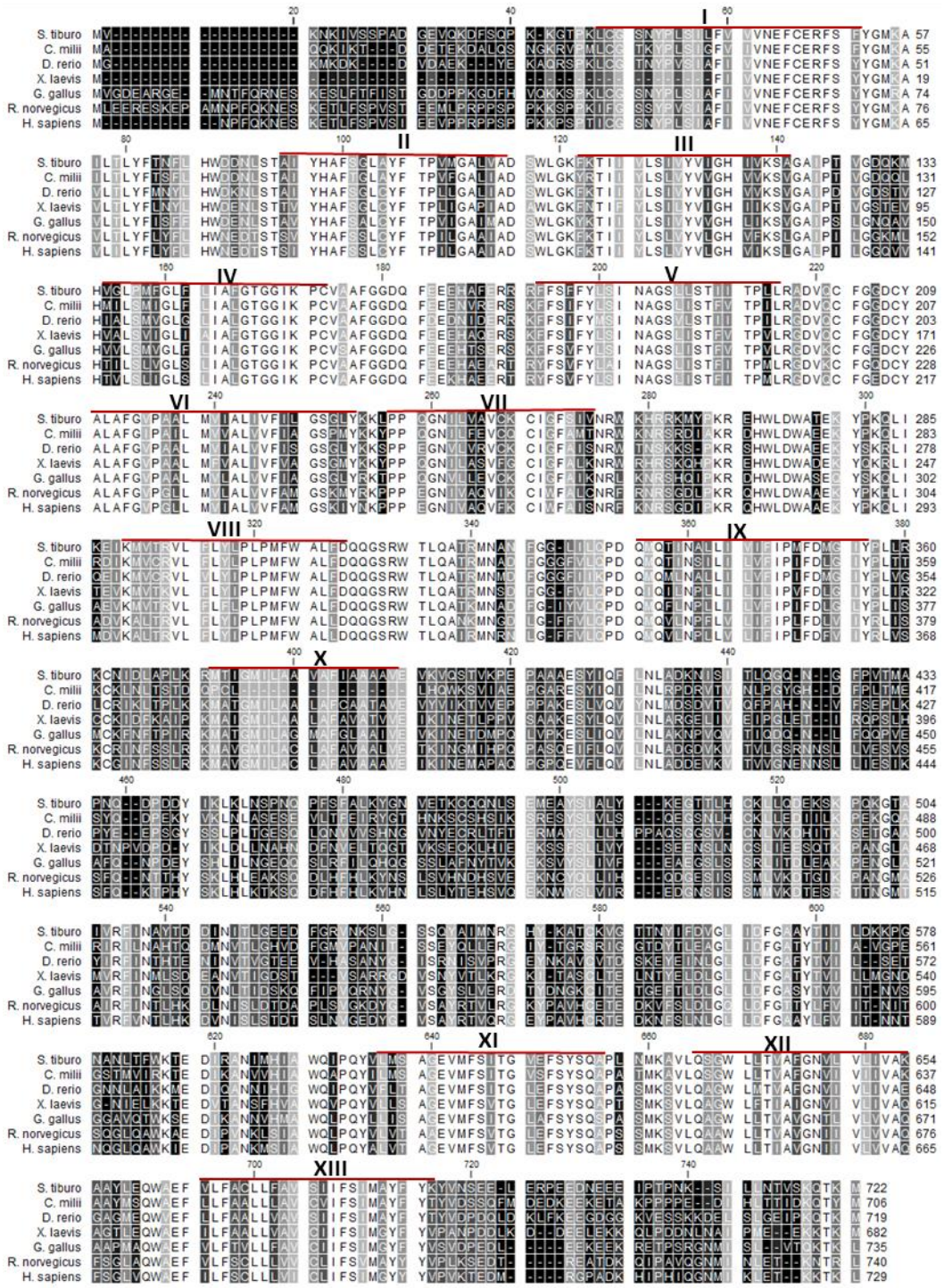

Fig. 4: Bonnethead PepT2 aligned with PEPT2 proteins from diverse vertebrate taxa: *Callorchinchus milii, Danio rerio, Xenopus laevis, Gallus gallus, Rattus norvegicus, and Homo sapiens.* Darker shading indicates decreasing sequence conservation across taxa. Lined region with roman numerals indicates predicted TMDs.

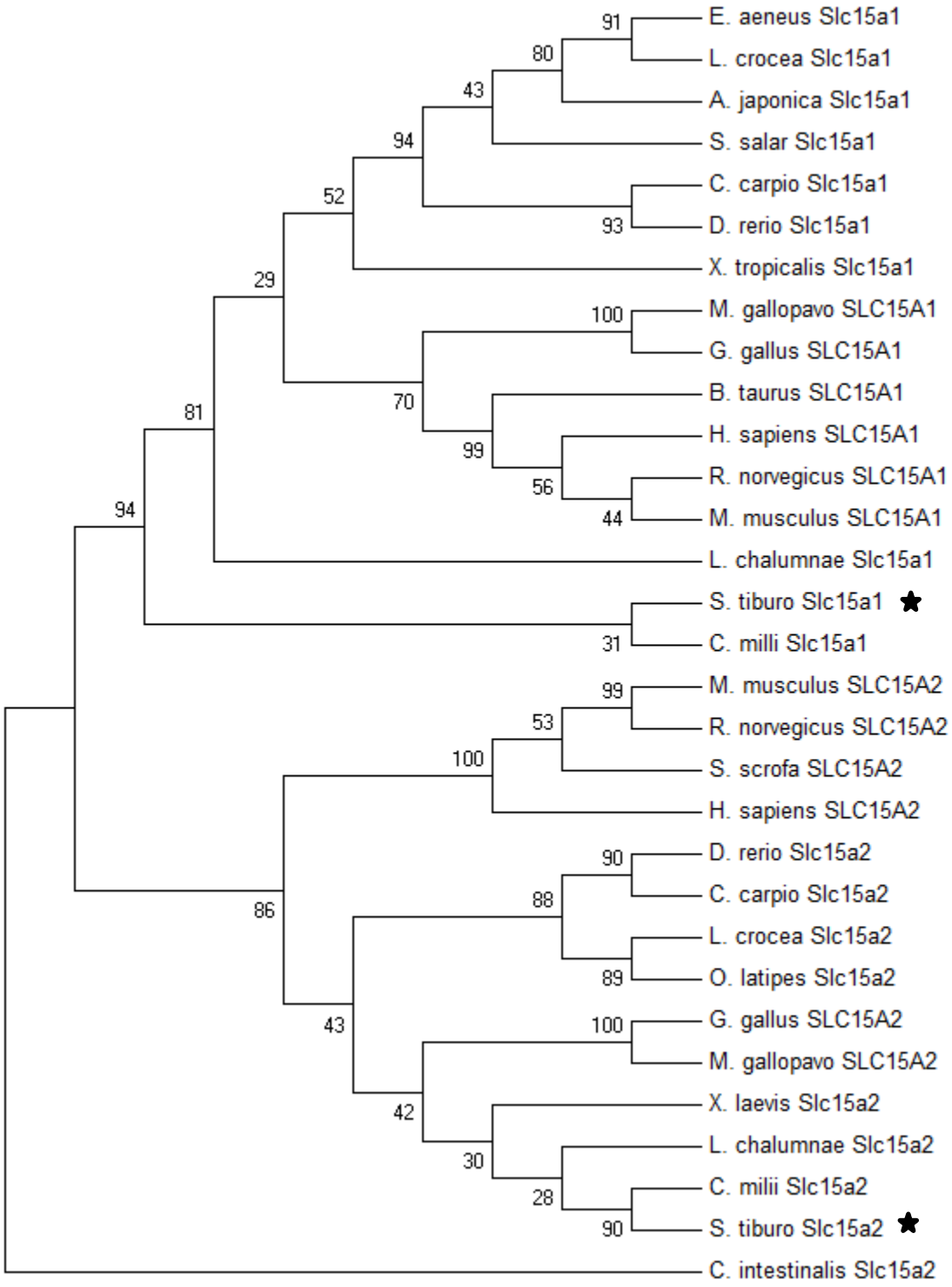

Fig. 5: Phylogenetic analysis of bonnethead PepT1 (Slc15a1*)* and PepT2 (Slc15a2). Relationships were inferred using the Neighbor-Joining method in Mega4 (Tamura *et al.* 2007). PepT1 sequences include *Callorhinchus milii* (XP_007904487.1), *Meleagris gallopavo* (NP_001290095.1), *Gallus gallus* (NP_989696.1), *Rattus norvegicus* (NP_ 476462.1), *Mus*

musculus (NP_444309.2), *Bos taurus* (NP_001092848.1), *Homo sapiens* (NP_005064.1) *Latimeria chalumnae* (XP_005992366.1) *Anguilla japonica* (BAM67012.1), *Xenopus tropicalis* (XP_002935692.2), *Cyprinus carpio* (AEX13747.1), *Epinephelus aeneus* (AFP33141.1), *Larimichthys crocea* (NP_001290295.1), *Danio rerio* (NP_932330.1), and *Salmo salar* (NP_001140154.1). PepT2 sequences include *Mus musculus* (NP_067276.2), *Homo sapiens* (NP_066568.3), *Gallus gallus* (AGZ02797.1), *Danio rerio* (NP_001034917.1), *Xenopus laevis* (NP_001080398.1), *Larimichthys crocea* (KKF11892.1), *Oryzias latipes* (XP_004081581.1), *Cyprinus carpio* (ADM48102.1), *Latimeria chalumnae* (XP_006004055.1), *Sus scrofa* (NP_001090983.1), *Rattus norvegicus* (NP_113860.2), *Meleagris gallopavo* (XP_010712188.1), and *Callorhinchus milii* (XP_007907469.1). The tree is rooted using the PepT2 sequence from *Ciona intestinalis* (XP_002121251.1). Numbers at branch points indicate the percentage of 2000 bootstrap replicates supporting the division.

Localization

The GI and accessory organs consisted of simple columnar epithelium supported by a smooth muscle mucosa and submucosa (Fig. 6a). Specifically within the stomach, the epithelial cells were shown to extend into gastric pits lined by the mucous cells (Fig. 6a). Within the intestinal region, the duodenum and scroll valve, the epithelial cells were organized into villous forms and contained a thick layer of microvilli making up the brush border membrane (Fig. 6a). Moreover, the rectum of the bonnethead shark was found to contain a large amount of goblet cells, and the epithelium made up colonic crypts (Fig. 6a). Last, the pancreas contained acini, which are ovoid-elliptical clusters of acinar cells (Fig. 6a).

Immunohistochemistry was conducted on all components of the bonnethead gastrointestinal system from 10 individuals and PepT1 was detected in multiple areas. Staining of such organs reveals the epithelium of the esophagus, stomach, duodenum, scroll valve intestine, rectum and pancreatic acinar cells as distinctly immunopositive, implying the presence of PepT1. However, staining of the stomach and scroll valve intestine is much more intense than that of the other gastrointestinal organs (Fig. 6c). Moderate immunopositive staining was detected within the duodenum, rectum, and pancreatic acinar cells in comparison to staining within the stomach and scroll valve intestine (Fig. 6c). There was also minimal immunopositive staining observed within the esophagus, as minor distinct differences were observed between the control and non-control slides (Fig. 6b & 6c).

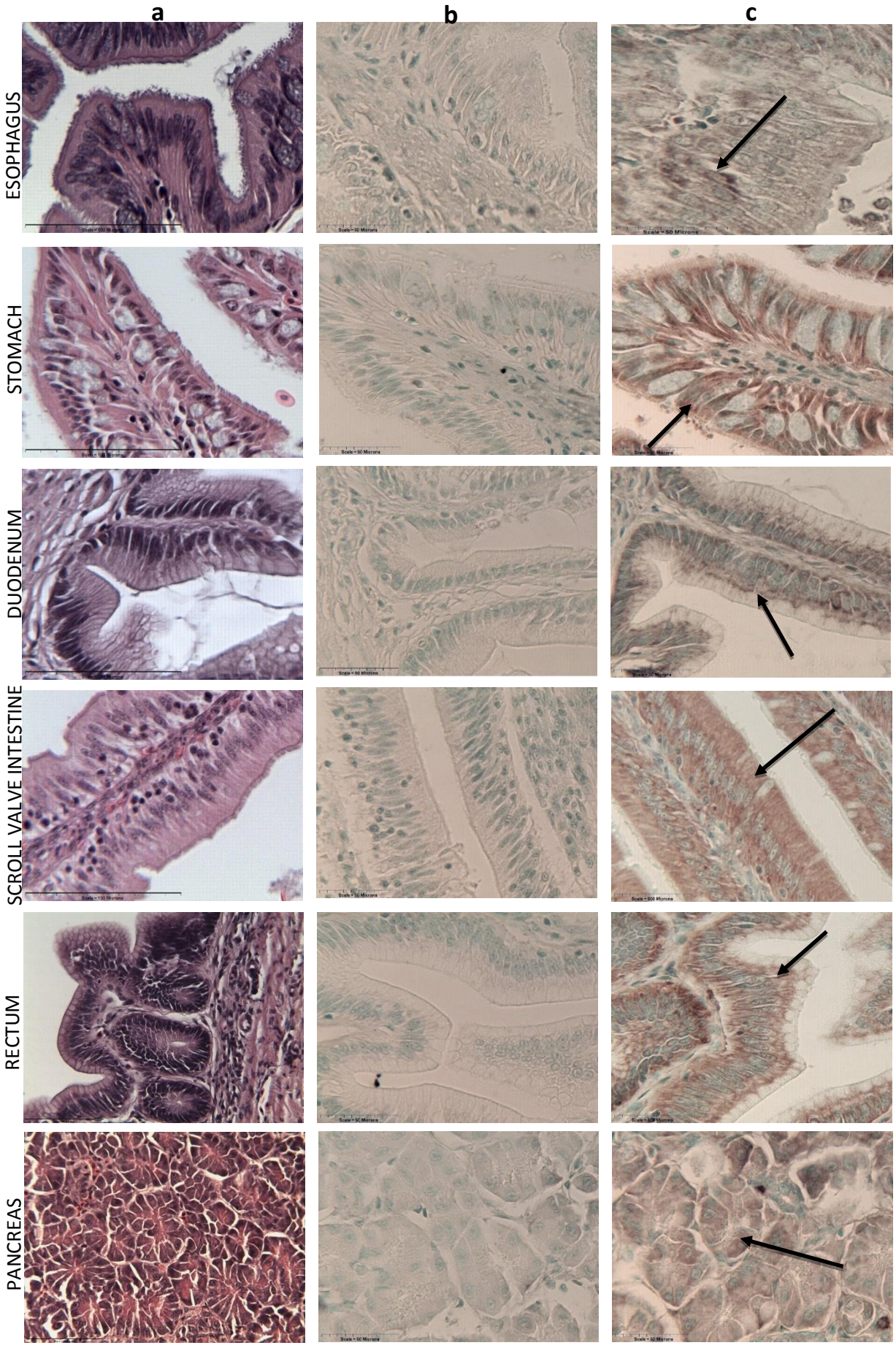
a
b
c
ESOPHAGUS
STOMACH
DUODENUM
SCROLL VALVE INTESTINE
RECTUM
PANCREAS

Fig. 6: Cross sections of GI organs using H&E staining methods demonstrate the histological architecture of the organs (column a). Cross sections of negative immunohistochemistry analyses in all components of the GI tract (column b). Cross sections of positive immunohistochemistry analyses in all the components of the GI tract. Arrows represent positive staining of PepT1 (column c). All sections were observed at 400x.

Function

Figure 7 illustrates the effects of a pH gradient on the time course of 1mM [^{3}H] Glycylsarcosine uptake by the bonnethead scroll valve BBMV. This time course was characterized by an overshoot at 1 minute that was highest when the pH inside was 7.5 and the pH outside was 5.5 (Fig. 7). No overshoots were seen when the outside pH was 7.5 or 8.5. Overshoots in the presence of proton gradient suggest that the transmembrane concentration gradient of hydrogen ions provides the driving force for the uptake of peptides by the scroll valve brush border membrane vesicles.

Influxes (1 min uptakes) of [^{3}H] Gly-Sar into the scroll valve BBMVs were measured over a concentration range of 1- 25mM [^{3}H] Gly-Sar in the presence of an inwardly-directed proton gradient (inside pH 7.5 and outside pH 5.5). Influxes for this concentration range were used in the Michaelis-Menten kinetics equation ($J_{oi} = \{(J_{max} * [S])/(K_m + [S])\}$), where J_{oi} is peptide influx, J_{max} is maximal influx, K_m is the Gly-Sar concentration at ½ J_{max}, and S is Gly-Sar concentration. This analysis yielded Michaelis- Menten values for a saturable, low- affinity system with a K_m = 6.18 ±1.66 mM and a J_{max} = 6077.86 ± 632.57 pmol/ mg protein per 1 min (n=3).

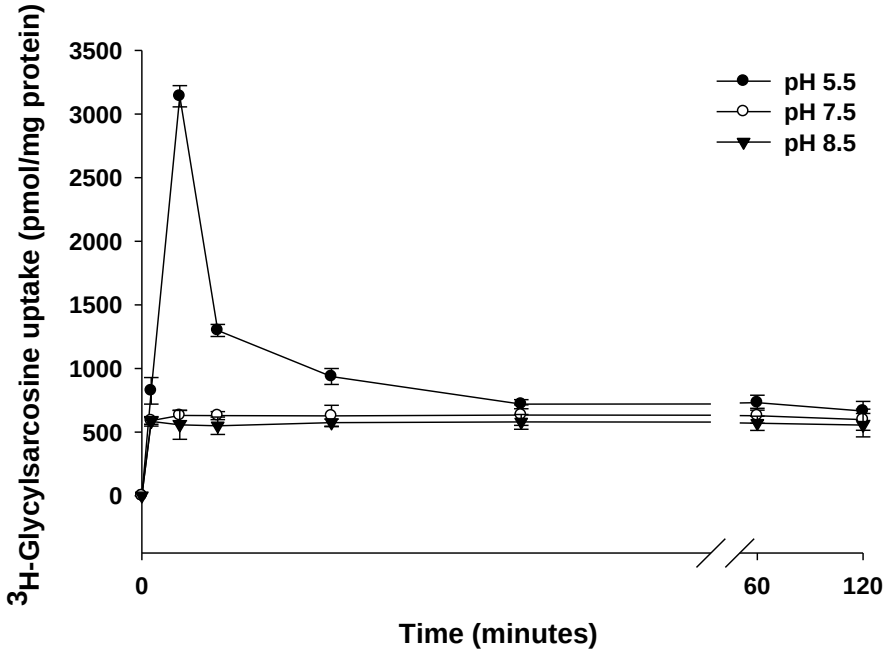

Fig. 7: Time course experiment testing the effect of pH on 1 mM ^{3}H-Glycylsarcosine uptake. Vesicles were preloaded with 150 mM KCl, 20 mM HEPES at pH 7.5, and incubated in various pH levels (5.5, 7.5, 8.5). Experiments were conducted three times with three replicates each. Symbols are mean ± SE.

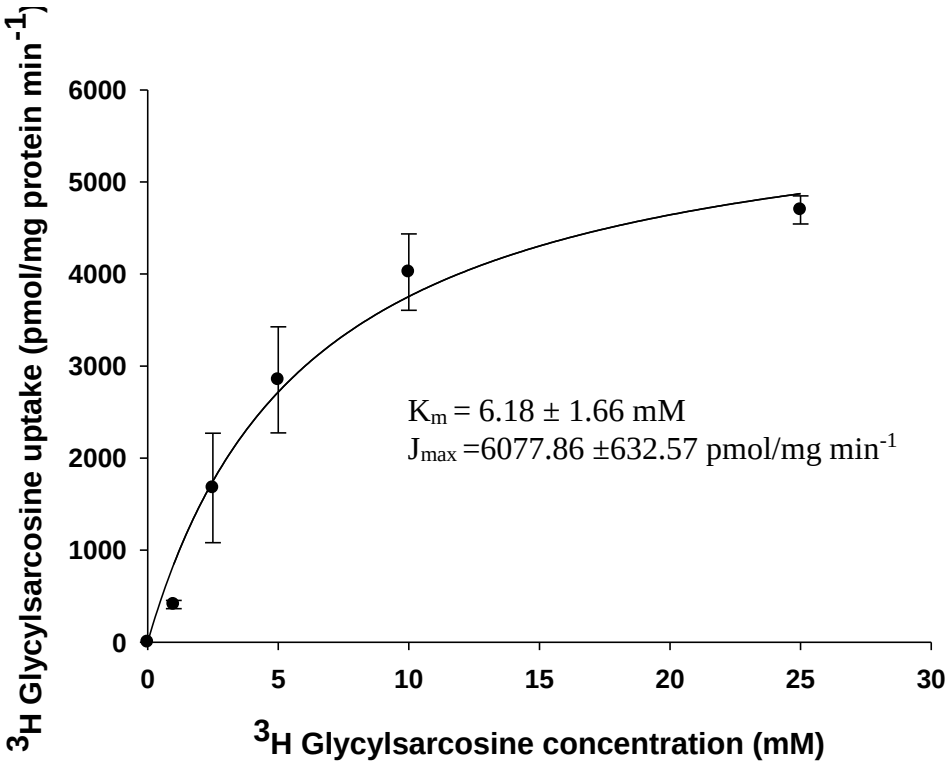

Fig. 8: Kinetics experiment testing the effect of Glycylsarcosine concentration. Vesicles were preloaded with 150 mM KCl, 20 mM HEPES at pH 7.5, and incubated in 150 mM KCl, 20 mM MES at pH 5.5 solutions with various concentrations of ^{3}H-Glycylsarcosine (1 mM, 2.5 mM, 5 mM, 10 mM, 25 mM). Experiments were conducted three times with five replicates each Symbols are mean ± SE.

DISCUSSION

Multiple aspects of this study, including the molecular identification, immunohistochemistry, and BBMV experiments support the identification of cDNAs encoding two individual and functional *S. tiburo* peptide transporters of the PTR family. The two encoded proteins, designated as PepT1 and PepT2, represent the products of orthologous genes in other vertebrate species. When compared to other known members of the PTR family in vertebrates, the predicted bonnethead PepT1 and PepT2 share significantly higher overall identity to other known PEPT1 and PEPT2 sequences, respectively, and are assigned to the expected monophyletic groups of the reconstructed phylogenetic tree. The analysis of domains also reveals that the bonnethead PepT1, like other vertebrate PEPT1 proteins, includes 12 TMDs and the overall major area of difference lies within the large extracellular loop between TMDs 9 and 10. However, unlike PEPT2 in other vertebrates, the bonnethead PepT2 sequence consists of 13 predicted TMDs rather than 12, and the overall major area of difference lies within the large extracellular loop between TMDs 10 and 11 instead of between TMDs 9 and 10. However, TMD 1 is very weak with a score of 524; therefore it is possible that this TMD is not a major structural component and that there are in fact 12 TMDs with an extracellular loop between TMDs 9 and 10 as seen in other vertebrate PEPT2 proteins. This idea is further suported by a lysine present half way down what would be TMD 7, which will later be discussed in detail.

PepT1 in the bonnethead shark was localized primarily in the epithelial cells of multiple gastrointestinal organs. The presence of this transporter within the stomach, duodenum, scroll valve intestine, and rectum gives insight into the absorptive qualities of such organs. With PepT1 present in the epithelial linning of such organs, it can be concluded that in each of these organs

there is some level of absorption of dietary peptides as they digest their prey throughout the entire gastrointestinal tract. Also, the minimal expression of PepT1 within the esophagus provides further support that elasmobranchs typically engulf their prey whole and therefore there is minimal need for peptide absorption in the esophagus, with initial break down instead occuring within the elasmobranch stomach. The elasmobranch stomach is the first organ used for major digestion through the release of hydrochloric acid (HCl), which converts the inactive zymogen pepsinogen into the active protease enzyme pepsin, initiating the digestion and absorption of protiens (Papastamatiou & Lowe 2005, Papastamatiou 2007). Within the stomach the prey is processed into chyme, an acidic fluid consisting of gastric juices and partly digested food, and then passed into the intestine (Camilleri *et al.* 1986). Once chyme enters the shark spiral/scroll valve intestine, it has been found that pancreatic enzyme activity largely decrease moving down the gut while brush border enzyme activities peak, suggesting that the spiral/ scroll valve intestine is the primary site of absorption (Jhaveri *et al*. 2015). Therefore, supporting that the intensity of PepT1 expression within the stomach and scroll valve intestine epithelial lining is likely correlated with the importance of absorption of dietary peptides within these organs. Unfortunately, the localization of PepT2 was not explored in this study as it was not expected that the *pept2* sequence would be isolated. However, the differential distribution of these two peptide transporters has been explored in a number of vertebrates, and PEPT1 is characterized as mainly the intestinal peptide transport system (Winckler *et al.* 1999; Verri *et al.* 2003; Shimakura *et al.* 2006) with PEPT2 characterized as the renal peptide transporter (Leibach and Ganapathy 1996; Saito *et al.* 1996). However, both transporters are also important in other organs and parts of the body. For instance, both PEPT1 and PEPT2 are found within the renal proximal tubules. It is thought that this organ may play a significant role in conserving peptide-bound amino acid and

amino nitrogen via the peptide transport process, which may otherwise be lost in urine. Therefore, the presence of both PEPT1 and PEPT2 maximizes the amount of peptides conserved before leaving the body (Schlagheck and Webb1984; Matthews 1991; Seal and Parker 1991; Gardner 1994). With this in mind, unlike the teleost and mammalian intestine, the presence of both *pept1* and *pept2* mRNA within the bonnethead scroll valve intestine may be critical for absorbing the necessary amount of peptides needed to carry out life. The scroll valve intestine has been described as the most active and absorptive section of the shark intestinal system (Jhaveri *et al*. 2015) due to its unique structure that conserves space within the body cavity by the infolding of the mucosa and submucosa in a spiral or scroll-like fashion. It may be necessary for elasmobranchs, which are known to consume large meals, to hold those meals for an extended period of time in the stomach (Wetherbee *et al.* 1987; Holmgren and Nilsson 1999; Papastamatiou 2007) and to have multiple active peptide transporters within the intestine. This arrangement may maximize the amount of peptides absorbed before leaving the body, enabling these large cartilaginous fish to abosorb the nutrients nessesary to carry out life.

The funtional aspects of mammalian peptide transporters have been well-examined in the past decade, using BBMV techniques, which have allowed detailed characterization of the kinetics and ion- dependent properies of such transporters. The functional results from this study provide new insight into the mechanism and driving force for dipeptide transport in a shark scroll valve intestine. These data show that the uptake of [^{3}H]Gly-Sar in *S. tiburo* was stimulated by a proton gradient, in the absence of sodium. This is further supported by the presence of a lysine amino acid half way down TMD 7, specifically at amino acid 294 or PepT1 (Fig. 3) and at amino acid 297 of PepT2 (Fig. 4). Meredith (2009) found that the mutation of this residue to anything other than a positively-charged residue (such as arginine or lysine) abolishes the stimulation of

transport by a proton electrochemical gradient. This author identified that the loss of this positively charged residue is linked to the stoichiometry (1proton: 1 dipeptide) of a proton coupled transport system. Therefore, rather than a true channel being formed, to allow peptide transit, there is a small slipage of ion during the conformational change which occurs and prevents the translocation of the peptide to the other side of the membrane (Meredith 2009).

Gly-Sar uptake by intestinal BBMV in the bonnethead shark appears to be mediated by a low-affinity, high-capacity type carrier system. This low-affinity carrier system exhibited consistent quantitative K_m kinetic constant binding values with those of substrate binding from mammalian and teleost studies ranging from 0.2-10 mM (Thamotharan *et al.* 1996; Daniel *et al.* 1991; Skopicki *et al.* 1991).

In conclusion, the identification of both *pept1* and *pept2* mRNA within the bonnethead scroll valve intestine provides a new understanding of the elasmobranch gastrointestinal system, and gives insight into the absorptive capabilities of this unique organ. With this information along with the distribution and functional qualities of the PepT1 protein, we can conclude that the scroll valve intestine, stomach, duodenum, and rectum all appear to play significant roles in peptide absorption. It is important to continue to research such topics within elasmobranchs, because the speed of a physiological response and rate at which digestion occurs determine whether the response is relevant to daily variations in an individual animal's foraging behavior, growth and development, and evolutionary potential. There is an overall interest in sharks, and to better grasp their ecological role it is important to understand what they are eating, digesting, and excreting back into the environment in order to better predict how sharks function within their environment and implement appropriate management strategies.

REFERENCES

Bethea, D.M., Hale, L., Carson, J.K., Cortes, E., Manire, C.A., Gelsleichter, J. (2007). Geographic and ontogeneic variation in the diet and daily ration of the bonnethead shark, *Sphyrna tiburo*, from the eastern Gulf of Mexico. Mar. Bio. Vol. 152: 1009-1020.

Bush, A.C., Holland, K.H. (2002). Food limitation in a nursery area: estimates of daily ration in juvenile scalloped hammerhead, *Sphyrna lewini*, in Kaneohe Bay, Oahu, Hawaii. J. Exp. Mar. Biol. Ecol. 278: 157-178.

Camilleri, M., Brown, M.L., Malagelada, J.R. (1986). Impaired transit of chime in chronic intestinal pseudoobstruction. Gastroenterology. 91(3): 619-626.

Carlson, J.K., Goldman, K., Lowe, C. (2004). Metabolism, energetic demands, and endothermy. In: Carrier J.C., Musick, J.A., Heithaus, M.R. (Ed.) Biology of sharks and their relative. CRC Press, Boca Raton, FL, pp. 203-224.

Clements, K.D., Raubenheimer, D. (2006). Feeding and nutrition. In: Evans, D.H. (Ed.), The Physiology of Fishes. CRC Press, Boca Raton, FL, pp. 47-82.

Cortes, E., Manire, C.A., Hueter, R.E. (1996). Diet, feeding, habits, and diel feeding chronology of the bonnethead shark, *Sphyrna tiburo*, in southwest Florida. Bull. Mar. Sci. 58: 353-367.

Crane, R.K., Boge, G., Rigal, A. (1979). Isolation of brush border membranes in veicular form from the intestinal spiral valve of small dogfish (*Scyliorhinus canicula*). Biochimica et Biophysica Acta. 554: 264-267.

Daniel, H., Morse, E.L., Adibi, S.A. (1991). The high and low affinity transport system for dipeptides in kidneys brush border membrane respond differently to alterations in pH gradient and membrane potential. J. Biol. Chem. 263: 917-924.

Fishbeck, Dale W., and Aurora M. Sebastiani. *Comparative Anatomy: Manual of Vertebrate Dissection*. 2nd ed. Englewood, CO: Morton Pub., 2008. Print.

Gardner, M.L.G. (1994). Absorption of intact proteins and peptides. In: Johnson, L.R. (Ed.) Physiology of the Gastrointestinal Tract. CRC Press, New York, New York, pp. 1795-1820.

Gelsleichter, J., Rasmussen, L.E.L., Manire, C.A., Tyminski, J., Chang, B., Lombardi-Carlson, L.
(2003). Serum steroid concentrations and development of reproductive organs during puberty in male bonnethead sharks, *Sphyrna tiburo*. Fish Physiology and Biochemistry. 26:389-401.

German, D.P., Weintraub, M.N, Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol. Biochem. 43: 1387-1397.

Holmgren, S., Nilsson, S. (1999). Digestive system. In: Hamlett W.C. (Ed.), Sharks, skates, and rays; the biology of elasmobranch fishes. The Johns Hopkins University Press, Baltimore, MD, pp. 144-173.

Jhaveri, P., Papastamatiou, Y.P., and German, D.P. (2015). Digestive enzyme activities in the gut of bonnethead sharks (*Sphyrna tiburo*) provide insight into their digestive strategy and evidence for microbial digestion in their hindguts. Comp. Biochem. Physiol. A. In Press.

Leibach, F.H., Ganapathy, V. (1996). Peptide transporters in the intestine and the kidney. Annu. Rev. Nutr. 16: 99-199.

Lowe, C.G. (2001). Metabolic rates of juvenile scalloped hammerhead sharks (*Sphyrna lewini*).

Mar. Biol. 139: 447-453.
Maisey, J.G. (1980). An Evaluation of Jaw Suspension in Sharks. *American Museum of Natural History*. 2706: 1-17.
Matthews, D.M. (1975). Intestinal absorption of peptides. Physil. Rev. 55: 537-608.
Matthews, D.M. (1991). Protein Absorption. In: Wiley-Liss (Ed.) Development and Present state of the subject. New York, New York.
Meredith, D. (2009). The mammalian proton-coupled peptide cotransporter PEPT1: sitting on the transporter-channel fence? Phil. Trans. R. Soc. B. 364: 203-207.
National Marine Fisheries Service (NMFS) (1999a). Fishery management plan of the Atlantic tunas, swordfish and sharks. Silver Spring, National oceanic and atmospheric administration, US Department of Commerce, Washington.
Papastamatiou, Y.P. (2007). The potential influence of gastric acid secretion during fasting on digestion time in leopard sharks (*Triakis semifasciata*). Comp. Biochem. Physiol. A. 147: 37-42.
Papastamatiou, Y.P., Lowe, C.G. (2004). Postprandial response of gastric pH in leopard sharks (*Triakis semifasciata*) and its use to study foraging ecology. J. Exp. Biol. 207: 225-232.
Papastamatiou, Y.P., Lowe, C.G. (2005). Variations in gastric acid secretion during periods of fasting between two species of shark. Comp. Biochem. Physiol. A. 141: 201-214.
Saito, H., Terada, T., Okuda, M., Sasaki, S., Inui, K. (1996). Molecular cloning and tissue distribution of rat peptide transporter PEPT2. Biochimica et Biophysic Acta. 1280: 173-177.
Schlagheck, T.G., Webb, K.E. (1984). Characterization of peptides from the gastrointestinal tract of calves. Fed. Proc. 43: 671.
Seal, C.J., Parker, D.S. (1991). Isolation and characterization of circulating low molecular weight peptides in steer, sheep, and rat portal and peripheral blood. Comp. Biochem. Physiol. 99B: 679-685.
Skopicki, H.A., Fisher, A.K., Zikos, D., Bloch, R., Flouret, G., Peterson, D.R. (1991). Multiple carrier-mediated transport of glycyl-L-proline in renal BBMV. Am. J. Physiol. 261: 670-678.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA5: Molecular evolutionary genetics analysis version 6.0 Molecular Biology and Evolution: 30: 2725-2729.
Thamotharan, M., Gomme, J., Zonno, V., Maffia, M., Storelli, C., Ahearn, G.A. (1996). Electrogenic, proton-coupled, intestinal dipeptide transport in herbivorous and carnivorous teleosts. Amer. Physiol. Soc. 939-947.
Verri, T., Kottra, G.m, Romano, A., Tiso, N., Peric, M., Affia, M., Boll, M., Argenton, F., Daniel, H., Storelli, C. (2003). Molecular and functional characterization of the zebrafish (*Danio rerio*) PEPT1- type peptide transporter. *FEBS Letters.* 549: 115-122.
Verri, T.m Romano, A.m, Barca, A., Kottra, G., Daniel, H., Storelli, C. (2010). Transport of di- and tripeptides in teleost fish intestine. *Aquaculture Research.* 41: 641-653.

Wetherbee, B.M., Gruber, S.H. (1993). Absorption efficiency of lemon shark *Negaprion brevirostris* at varying rates of energy intake. Copeia 1993. 416-425.
Wetherbee, B.M., Gruber, S.H., Ramsey, A.L. (1987). X- radiographic observations of food passage through digestive tracts of lemon sharks. Trans. Am. Fish. Soc. 116: 763-

767.
Wilga, C.D., Hueter, R.E, Wainwright, P.C., Motta, P.J. (2001) Evolution of Upper Jaw Protrusion Mechanisms in Elasmobranchs. *American Zoology*. Vol. 41 (6): 1248-1257.

Hannah R. Hart
Department of Biology
University of North Florida
1 UNF Drive
Jacksonville, FL 32246

EDUCATION

B.S. in Biology
Florida Institute of Technology, Melbourne, FL

RESEARCH EXPERIENCE

University of North Florida
Graduate Research, August 2013- December 2015

- Physiological and molecular analysis of the peptide transporter 1 in the elasmobranch spiral valve intestine

Florida Fish and Wildlife Conservation Commission, *FWRI Crustaceans*
Research Biologist I, September 2015- Present

- Coordinate with commercial fishermen to set and collect crab traps for life history analysis
- Blue crab dissection and tissue sample extraction (blood, gonads, and walking legs)
- Data entry and analysis for reproductive study on the Northeastern Blue crab population

Florida Fish and Wildlife Conservation Commission, *Fisheries Independent monitoring*
Research Biologist I, May 2014- Present

- Run data analysis and compile data for annual reports
- Data entry and Compile/send out monthly reports
- Assist with scientific fisheries surveys using seine and trawl nets
- Fish identification, dissections, and tissue sample extractions (otoliths, gonads, stomach content, and other tissue samples)

Florida Institute of Technology
Undergraduate Research, 2012-2013

- Meta-analysis concerning the temporal and spatial patterns of exotic and native species of catfish within the St. John's River

ORAL PRESENATIONS

Hart, H., Evans, A., Gelsleichter, J., Ahearn, G. July 2014. The molecular identification and functional characteristics of peptide transporter 1 (PEPT1) in the bonnethead shark (*Sphyrna tiburo*). American Elasmobranch Society, Reno, NV.

POSTER PRESENTATIONS

Hart, H., Evans, A., Gelsleichter, J., Ahearn, G. July 2014. The molecular identification and functional characteristics of peptide transporter 1 (PEPT1) in the bonnethead shark (*Sphyrna tiburo*). Society of Integrative and Comparative Biology, West Palm Beach, FL.

Hart, H., Evans, A., Gelsleichter, J., Ahearn, G. July 2014. The molecular identification and functional characteristics of peptide transporter 1 (PEPT1) in the bonnethead shark (*Sphyrna tiburo*). University of North Florida Research Week, Jacksonville, FL.

Hart, H., Evans, A., Gelsleichter, J., Ahearn, G. July 2014. Transport of di- and tripeptides in the *Sphyrna tiburo* spiral valve intestine. American Elasmobranch Society, Chattanooga, TN.

PUBLICATIONS

Works in Progress:

"The molecular identification and functional characteristics of peptide transporter 1 (PEPT1) in the bonnethead shark (*Sphyrna tiburo*)"

A molecular and physiological analysis on an elasmobranch gastrointestinal tract with a focus on the peptide transporters.

TEACHING AND OUTREACH EXPERIENCE

University of North Florida

Graduate Teaching Assistant, January 2015- May 2015

- Independely taught two Biology 1 laboratories
- Created lesson plans, held weekly office hours, and presented curriculum for weekly classes
- Maintained lab and prepped material for upcoming lab experiments
- Graded all course materials (labs, quizzes, writing assignments, and tests)

Expanding Your Horizons

- Assist in teaching scientific modules to young woman that include hands on learning exercises

Brevard County Zoo Intern/ volunteer

- Directed and helped assist with learning modules for high school students on the importance of conservation and biological research

REFERENCES

Jim Gelsleichter
Assistant Professor, Department of Biology
University of North Florida

Andrew Evans
Assistant Professor, Department of Coastal Sciences
Gulf Coast Research Laboratory

Greg Ahearn
Professor, Department of Biology
University of North Florida