2-26-2010

The Life History and Energy Budget of *Hippocampus erectus* in Tampa Bay, Florida

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by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
College of Marine Science
University of South Florida

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Date of Approval:
February 26, 2010

Keywords: seahorse, vertebra, lipids, protein, respiration

Acknowledgments

I would like to thank my committee members for all of their advice and guidance. I would also like to think Joe Donnelly, Erica Hudson, Tammy Sorge, and Rob Masserini from the lab at USF for all of their help with lab procedures: respiration, protein, lipid, and ammonia. From the Florida Fish and Wildlife Conservation Commission I would like to thank the Fisheries-Independent Monitoring staff for all of their help with fish collections, along with the extra help I received from the following staff: Bob Heagey (holding tanks for the live specimens), Will Fletcher (gut specimens), Paula Scott (SEM), Janet Tunnell and Allison Amick (sectioning vertebra), Brent Winner (reading vertebra), and Jenna Tortorelli and Michelle Press (help with maintaining the tanks). Without their help this project never would have been as successful.

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ABSTRACT

Seahorses, genus *Hippocampus erectus*, are subject to large and continuously-growing international trade. Concerns with the effects of trade on seahorse population worldwide have led to their international protection by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In order to manage seahorse populations, we first need to understand their basic biology. The purpose of this project was to establish an energy budget for *Hippocampus erectus* from Tampa Bay, Florida. A total of 108 specimens were collected throughout Tampa Bay from 2004 to 2007 using 21.3 m offshore seines and 6.1 m otter trawls. Those specimens were utilized to determine different components of the energy budget: size at maturity, feeding and nutrition within a captive environment, and metabolic rate. Seahorses collected in the study ranged in size from 4 mm to 160 mm, and were 0 to 4 years in age. Conventional methods of aging fish could not be applied to this species. Instead, an alternative method that involved sectioning vertebrae and using Scanning Electron Microscopy (SEM) to enumerate age-specific marks on vertebra was employed. Age data were then paired against length-weight charts to estimate age by length and mass. Length-frequencies were segregated by

sex and compared to the growth data. No sex-specific differences were found. Fish reared in captivity were also used for nutrition, respiration, excretion analysis. *Hippocampus erectus* in captivity were calculated to have a resting respiration rate of 85.9 μl O_2/g/h and an excretion rate of 0.48 mmol NH_3/g/h. The viscera of *H. erectus* and *Artemia* spp. were broken into protein and lipid content by caloric composition. The overall percentage of protein per seahorse was 11% and 17% by lipid concentration. Overall the sum of energy cost of *H. erectus* accounted for 81% of the total energy ingested.

Introduction

Seahorses, genus *Hippocampus*, are subject to a large and continuously-growing international trade. They are primarily used in traditional Chinese medicines, the aquarium industry, and the curio trade. Recently, concerns about the impact of an increasing harvest on seahorse populations worldwide have led to their protection by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). However, their basic biology is poorly known, which makes seahorse populations difficult to manage effectively.

Seahorses are found worldwide in a variety of habitats in tropical to temperate seas (Kuiter 2000). Species such as *Hippocampus kelloggi* occur in waters exceeding 60 m, living among gorgonians and sea whips, whereas other species, such as *Hippocampus kuda*, occur in shallow estuarine and inshore waters (Choo & Liew 2003).

The subject of the present study, *Hippocampus erectus*, is found from the southern tip of Nova Scotia to northern Venezuela and is very common in Florida (Lourie et al. 1999). This species is usually found in shallow areas among seagrass beds, sponges, and macroalgae but has been found as deep as 73 m (Lourie et al. 1999).

A common threat to *H. erectus* is the shrimp trawling industry. In a study of bycatch in the Gulf of Mexico shrimp trawl fishery, *H. erectus* were caught offshore but were not typically reported as bycatch. However, the number of

seahorses captured in shrimp trawls can be substantial. For example, the shrimp fleet that targeted the area off Hernando Beach on the western coast of Florida caught approximately 72,000 seahorses annually. This estimate was based on catch per unit of effort (CPUE) (Baum et al. 2003).

Hippocampus erectus is distinguishable from a similar Florida seahorse, *Hippocampus reidi*. Both species grow to lengths of approximately 190 mm; but the coronet of *H. erectus* has a wide and low triangular-wedge and is ridge-like with raised sharp edges and sharp spines with a moderately long snout. Whereas, *H. reidi*, lacks the distinctive coronet structure and has low, rounded tubercles, two eye spines, and a longer snout (Lourie et al. 1999). Other features of *H. reidi* which distinguish it from *H. erectus* are the yellow body coloration and a peppering of white dots on the body.

Typically, seahorses have a short lifespan relative to other species of fishes. *Hippocampus erectus* lives 4 - 5 years but is a very fecund species. Adults can have broods of 100 - 1550 young and can carry up to three batches in a pouch at one time (Teixeira and Musick 2000, Dzyuba et al. 2006).

Seahorses are visual ambush predators. Their eyes can move independently, enabling the seahorse to maximize its search area (Ocken, 1994). Seahorses generally remain motionless until prey swim close to their mouths, when they rapidly intake water, sucking the prey item out of the water column. While seahorses exploit a variety of habitats, the one similarity among most species of seahorses is their diet, which primarily consists of copepods,

amphipods, and caridean and mysid shrimps (Foster & Vincent 2004; Masonjones & Lewis 2000; Woods 2002).

In a study analyzing feeding and metabolism in seahorses, Woods (2002) investigated the diet of adult wild seahorses, *Hippocampus abdominalis*, from Wellington Harbour, New Zealand. His study's was primary focus was gut contents; and his analyses included gut volume versus total length as well as seasonal variation in diet. The average dry weight of digestive tract contents was estimated as 4.9% of the dry body weight of the seahorses sampled, but was subject to change between larval and juvenile stages. Smaller *H. abdominalis* consumed larger numbers of amphipods than larger seahorses, and diet varied seasonally.

Masonjones (2001) analyzed the reproductive behavior of *H. zosterae* to determine the effects of monogamous pair bonding and reproduction on general metabolism. The result was that mass-specific oxygen consumption rates differed dramatically between fish of different reproductive status. The metabolic rates were almost identical for sexually isolated males and females; however, the oxygen consumption rates dropped for both sexes during courtship.

The studies described above provide some background on various elements of seahorse ecology, life history, and energetics. However, there is as yet no one study that gives a complete energy budget for a seahorse interpreted within the context of its field biology. The purpose of this project was to establish an energy budget for *H. erectus* from Tampa Bay, Florida, by determining size at maturity, analyzing feeding and nutrition within a captive environment, and

determining metabolic parameter through respiration experiments. Growth rate was also determined by calculating a von Bertalanffy growth curve of field caught specimens to complete the energy budget equation.

Materials and Methods

Specimen Collection

A total of 108 *Hippocampus erectus* were collected from Tampa Bay using 21.3 m offshore seines and 6.1 m otter trawls. Specimens used for life history workup were collected monthly from a series of zones in Tampa Bay based on a stratified-random sampling (SRS) design (Figure 1). Otter trawls were used to collect the seahorses in deeper areas of the bay ($\geq$1.3 - 7.6 m) and seines were used to collect seahorses in more shallow (0.3 - 1.3 m) habitats. Specimens needed for the energy budget portion of the project were collected over a one-month period from the Egmont Key and Ft. DeSoto area and kept in a filtered aquarium until acclimated.

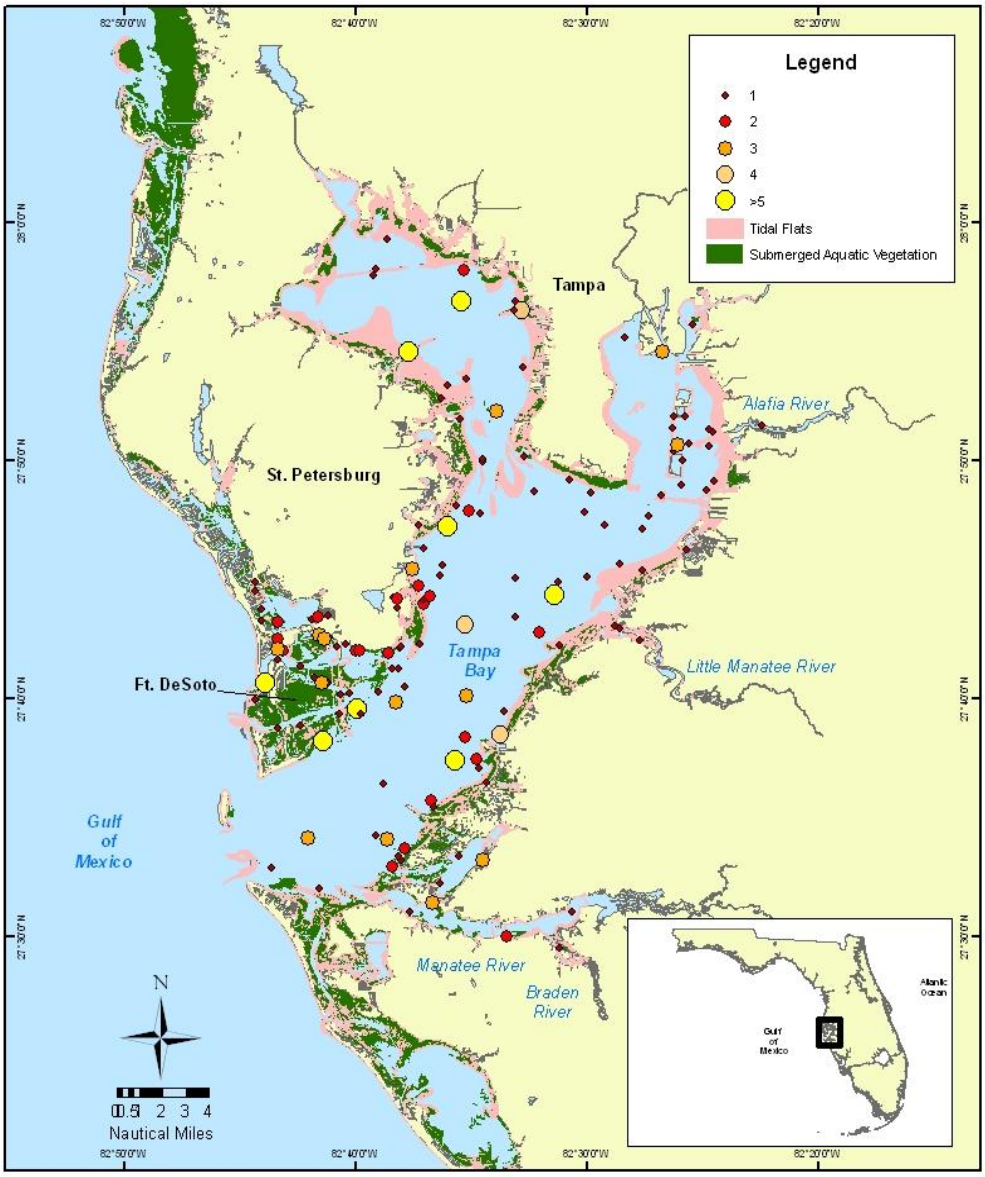

Figure 1: Abundance of *Hippocampus erectus* in Tampa Bay, FL from 2004-2007. Seahorses were collected from locations using seines along the tidal flats and shorelines. Trawls were used to collect samples from deep areas of the bay.

Historical data were used to estimate length frequencies by month. Those data were collected using a variety of gears by the Florida Fish and Wildlife

Conservation Commission's Fisheries-Independent Monitoring program. Sampling gear included haul and purse seines, otter trawls, and seines (Table 1).

Table 1: Fisheries-Independent Monitoring Gear Descriptions from 1996 to 2007 (FWRI 2007).

Gear Sampled	Gear Description	Years Sampled
Trawls	6.1m otter trawl w/ 3.1mm liner & tickler chain. Towed in a straight path (1.8-7.6 m). Towed in an arc (1.0-1.7 m)	1996-2007
Offshore Seines	21.3m center-bag seine, 3.1mm mesh, leads spaced every 150mm, offshore-circular set.	1996-2007
Beach Seines	21.3m center-bag seine, 3.1mm mesh, leads spaced every 150mm, onshore set w/o boat (beach set).	1996-1997

Sample Processing and Sex Determination

Morphological data were collected for each specimen to insure accurate identifications, and total length, from the top of the head to the end of the tail, was used as the standard measurement of size. Each animal was also weighed and sex was determined was determined by the presence or absence of a brood pouch in larger specimens and by dissection in smaller specimens. Embryos from pregnant males were extracted and counted.

Age and Growth (G)

The otoliths of seahorses are extremely small and difficult to find; therefore, this conventional method of aging fish was not used. Instead, vertebral rings were used as a means of age determination. In order to first determine if the rings of the vertebra could be read, the 3rd or 4th vertebrae was

removed from a few specimens and cleaned mechanically. Remaining tissue was removed using 30% Hydrogen Peroxide, and then the vertebra was rinsed three times with nanopure water. Each whole vertebra was then mounted on a disk and sputter coated with gold. These vertebra were then examined for rings using Scanning Electron Microscopy (SEM) (Figure 2a). After the presence of clear rings on the vertebra were established, another vertebra from the same animal was removed from the seahorse and embedded in araldite, sectioned, and mounted in Flo-texx from Fischer Scientific. Growth rings on the vertebrae were read under 40 x magnifications, counted and corroborated, using newborn seahorses as age 0 (Figure 2b).

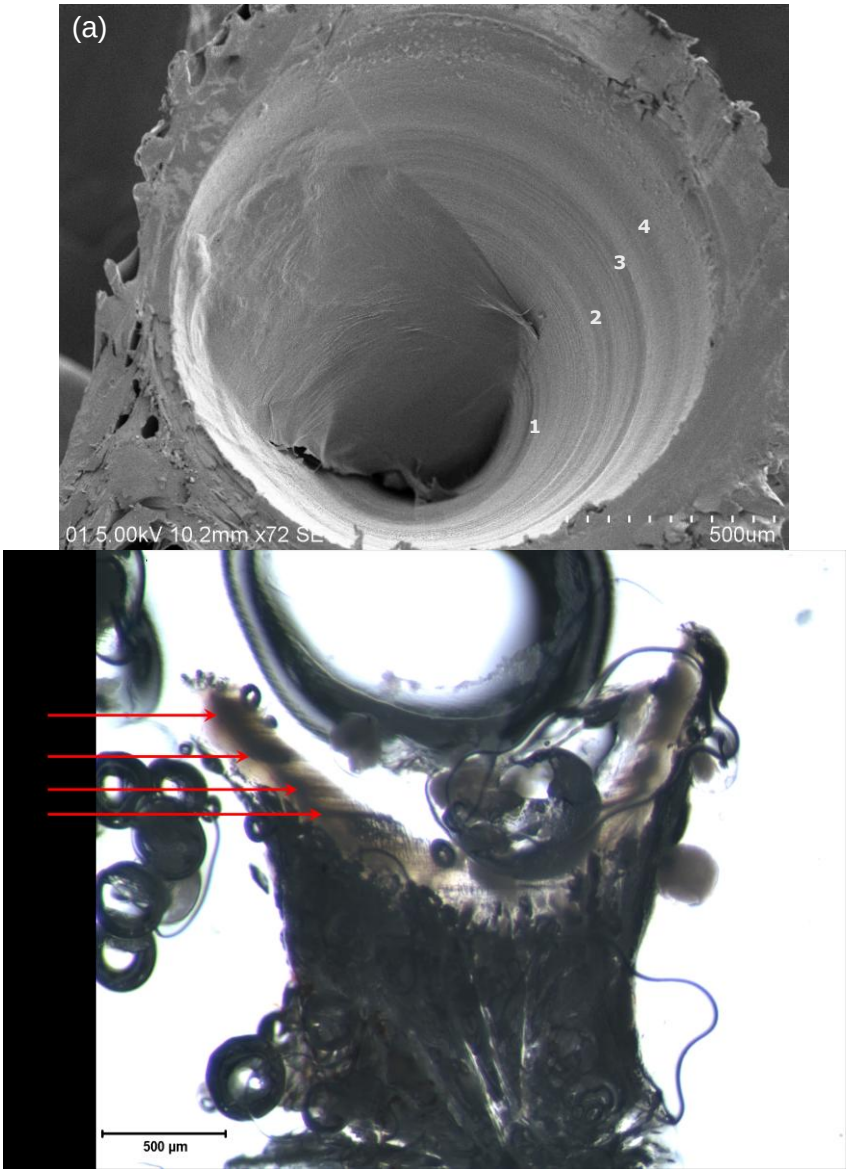

Figure 2: Scanning electron microscope images of annuli in (a) a whole vertebra from a 149 mm *Hippocampus erectus* (b) and a sectioned portion of vertebra.

Age-and-growth was then determined by enumerating annual growth marks on sectioned of vertebrae and comparing those data to length-frequency for validation. A second independent read was done to confirm annual marks. If the two readings did not agree then the vertebra was not used in the age-and-

growth study. A growth curve was calculated using the Von Bertalanffy equations based on the annul rings from the vertebra. I used the equation below, which is the simplest version of the Von Bertalanffy growth curve (Helfman et al. 1997) and can be applied to most fish species.

$$L_t = L_\infty (1 - e^{-kt})$$

L_t = length at time t
L_∞ = maximum length observed
k = growth coefficient

Energy Budget

Before any laboratory experiments were conducted, each seahorse was given 72 hr. to acclimate to tank conditions and avoid any further stress to the animal. Twenty- three specimens were collected from Tampa Bay (Figure 1) and used in a series of experiments that were conducted to determine energy usage for a range of sizes. The experiments were predicated on the energy budget equation below from Withers (1992):

$$I = G + M + E$$

I = the amount of energy ingested or consumed
G = the amount of energy used for growth and reproduction
M = the amount of energy used for oxygen consumption
E = the amount of energy lost to excretion

Ingestion (I)

Ingestion rate was determined by measuring daily food consumption. Two methods were used to determine daily food consumption. In the first, laboratory-based method, *H. erectus* were fed *Artemia* spp. every four hours until full. The

second method computed ingestion rate based on feeding periodicity and gut fullness over 24 hr periods to estimate a daily ration and a caloric value of the gut content.

Laboratory Determinations

Before feeding experiments in a laboratory setting could be conducted using frozen “prey” (mysids), the seahorses needed to be conditioned to eat non-live food. The natural preference for live food is also what makes seahorses difficult to maintain in captivity. Conditioning was achieved by turning off the filter whenever it was time to feed the animals and then slowly incorporating mysids in with live *Artemia* spp.

Five *H. erectus* were kept in a 150 gal. tank at 24ºC and a salinity of 29 ppt. A12/12 L-D cycle was maintained, with lights on from 0800 to 2000 hr. In both feeding experiments *H. erectus* were fed until satiation (n=5). In the case of *Artemia* spp., feedings were scheduled every four hours until the seahorses were full, but the fish would only feed during light periods (0800 – 7 to 12 brine shrimp; 1200 – 2 to 3 shrimp; 1600- 8 to 9 shrimp). In the experiments using frozen mysids, each seahorse only ate 3-4 mysids twice a day (0800 and 1600 hrs.) before gut fullness was achieved. Then when each individual was placed back in the general holding tank, the individual would eat until fullness was achieved then sink to the bottom of the tank and remain in a resting position.

Wild Specimens

Randomly selected wild specimens collected for diet analyses were fixed in 10% buffered formalin and fixed for 48 hr. Because seahorses have

rudimentary stomachs, the digestive tract was not removed in the field (or prior to fixation). This was done to maintain the integrity of the digestive tract and minimize the loss of stomach contents. In order to remove all formalin from the samples each underwent three rinse –soak cycles: *i.e.*, rinse and soak in tap water for a minimum of three hours per cycle. After the seahorses were rinsed they were transferred to 50% isopropyl alcohol. Stomachs were opened to check for fullness and parasites. Digeneans trematodes that were sometimes found outside of the gut cavity were not considered prey items. Prey items were sorted and identified to the lowest possible taxonomic categories.

Energy content of the food was determined using the proximate composition method described below.

Proximate Composition

Individual seahorse viscera were ground and separated to calculate protein and lipid content. Each homogenate was prepared at a 9:1 tissue dilution (Bligh and Dyer, 1959; Lowry et. al., 1951). A sample of 50 μL of homogenate and 750 μL of water were mixed prior to each assay. No part of the gonad was included in the analysis to avoid biases in energy content between the sexes.

Protein Extraction: Using the protein assay method of Lowry et. al. (1951), 80 μL of homogenate was pipetted into three 10 x 75 mm test tubes. The protein was then hydrolyzed by adding 120 μL of 0.1 N NaOH to each test tube and then heating the samples at 100°C for 10 minutes. Each sample was cooled to room temperature, and a 1.2 ml copper solution of sodium carbonate, cupric sulfate, and sodium tartrate (100:1:1) was added and mixed immediately. After

10 minutes, 120 μL of phenol reagent was added and allowed to stand for 30 minutes. Then 1 ml of solution was transferred to 1.5 ml cuvettes and read on the spectrophotometer at 750 nm.

Lipid Extraction: Using the lipid assay methods by Bligh and Dyer (1959), Marsh and Weinstein (1966) and Reisenbichler and Bailey (1991) two 200 μL samples of homogenate were pipetted into 16 x 100 mm test tubes. Lipids were extracted using a mixture of methanol, chloroform and water in an initial ratio of2:1:0.8 and a final ratio of 1:1:0.9. Next the particulate material was isolated from the chloroform layer using a centrifuge filter with a 0.45 μm Teflon membrane. The remaining chloroform was evaporated from the test tubes overnight. The next day the lipids were charred into carbon by adding 1 ml of concentrated sulfuric acid and heated at 200°C for 20 minutes. After the tubes were cooled to room temperature, 10 ml of tap water was added to each test tube. Then 1-ml of solution was transferred to 1.5 ml cuvettes and read on the spectrophotometer at 375 nm.

Water content was determined by comparing the difference between the wet weight and the dry weight of the animal and then calculating the overall percentage.

$$\% \text{ Water} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

Metabolism (M)

Oxygen-consumption rates were determined by allowing individuals to deplete the oxygen in a sealed water-jacketed chamber filled with filtered (0.45 μm pore-size) seawater. Temperature was maintained at 24°C ($\pm$ 0.1°C) using a circulating heated water-bath. Oxygen partial-pressure was continuously monitored using a Clark polarographic oxygen electrode (Clark 1956), as an individual reduced oxygen levels to intermediate (~80 mm Hg) partial-pressures. Electrodes were calibrated using air- and nitrogen-saturated seawater at the experimental temperature (Childress 1971). The time required for consumption of oxygen to intermediate levels varied from 5 to 8 hr. Streptomycin and Neomycin (each 25 mg l^{-1}) were added to the seawater to minimize microbial growth.

The cylindrical chambers were constructed of Lucite and contained a perforated Lucite false-bottom with an added extension bar that isolated the fish from a stirring bar. A low stirring speed was used to minimize disturbance. In order to reduce additional stress, an extension was added on to each plate to act as a holdfast for the seahorse and all experiments took place in the dark, with brief periods of observation in low light.

Data were recorded using a computer-controlled digital data-logging system. Each oxygen probe was scanned once per minute, its signal averaged over a period of 1s, and then recorded. Data were reduced by first averaging the 30 recorded values in each 30 min increment of an entire 5 to 8 hr experiment, producing between 10 and 16 30-min points per run. Data obtained during the

first hour were discarded due to the high levels of activity of fish immediately after introduction into the respirometer. All 30 -in points thereafter, down to an oxygen partial-pressure (P_{O_2}) 80 mm Hg, were average to produce a routine rate for each individual. Maximum rates were the maximum 30 min rate and minimum rates the minimum 30 min rate recorded for each individual after the first hour, but above a P_{O_2} of 80 mm Hg (Figure 3). Usually, maximum rates were recored near the beginning of an experiment and minimum rates were recorded during middle portions of a run.

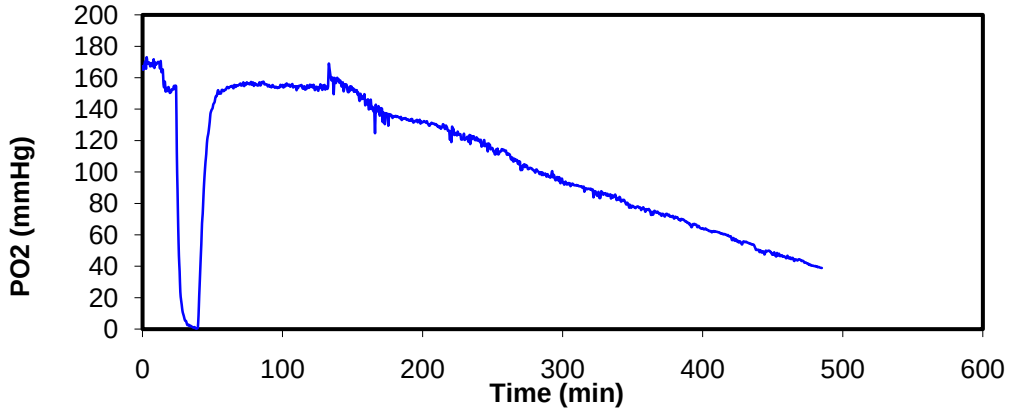

Figure 3: Oxygen consumption verses mass in *Hippocampus erectus*. This curve is typical of those observed in various experimental trials.

Excretion (E)

Excretion was calculated for each individual by removing a 20 ml water sample from each chamber after a run was completed. Each water sample was then diluted and analyzed for ammonia, nitrate, and nitrite with a Technicon® Auto Analyzer II (AAII). This nutrient analyzer uses a special flow technique named "continuous flow analysis (CFA)" or more correctly "segmented flow

analysis (SFA)". Change in ammonia concentration was calculated by using the difference in the ammonia before and after the run and then dividing by the run-time per animal. Each animal was also reweighed and measured after run to check for change in mass.

Results

Life History and Sex Determination

Seahorses ranged in length from 19 - 161 mm and in mass from 0.03 - 22.1 g. Many juvenile seahorses collected, which suggests effective sampling of small seahorses by small seines and trawls (Figure 4). More seahorses were captured in the lower bay in the Fort DeSoto and outer Bishop's Harbor regions than in other regions of Tampa Bay (Figure 1).

The combined length/mass regression for seahorses was $y=5x10^{-6}X^{(2.97)}$ ($Y=aX^{b}$). The slope or "b-value" of the curve was 2.97 based on 77 seahorses (Figure 5a). When the regression curve was analyzed by sex using student's t-test there were no significant differences between the sexes ($t=0.03256$, $P<0.05$), (Figure 5b). There was a female to male ratio of 1:1.5.

Size at maturity was estimated at 90 - 100 mm for males and 85 - 100 mm for females. Males began developing a brood pouch around 89 mm, but mature brood pouches were not found at sizes smaller than 120 mm in length. Males were consided fully mature when eggs were found in the brood pouch. Maturity in female seahorse was determined by the first signs of hydrated eggs in the gonad. Females began showing signs of maturity at 92 mm, and were fully mature at 120 mm, coinciding with the maturity of males. Sex determination in all smaller specimens was determined by dissection.

The total number of embryos in a pouch or clutch size ranged from to 122 - 1,212 individuals. The average clutch size of the embryos were proportional to the total length of the seahorse. For example, the smallest clutch size (122 embryos) was extracted from a seahorse at 132 mm and the largest clutch size (1,212 embryos) was extracted from a seahorse at 161 mm. The embryos from the largest seahorse were also the closest to birth and had an average size of 4.9-mm.

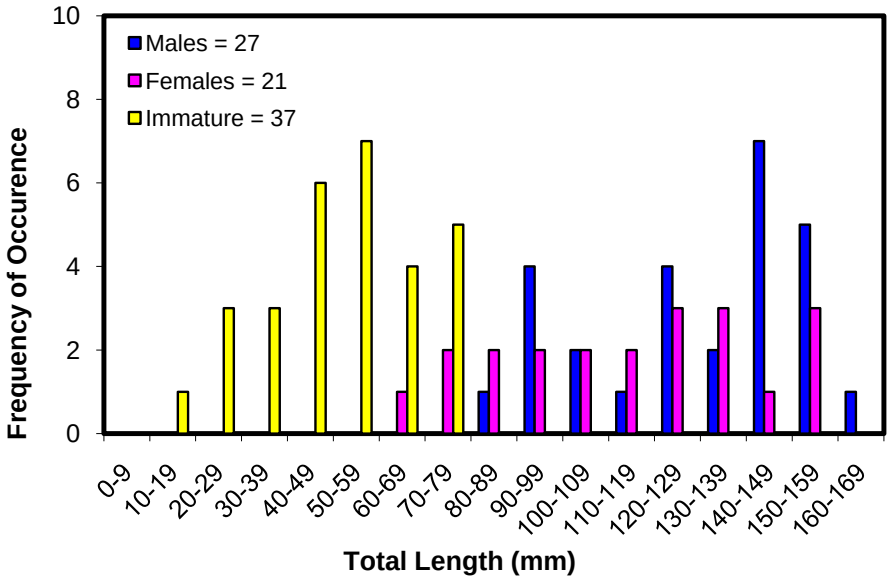

Figure 4: Combined length frequency distributions of wild caught *Hippocampus erectus* collected in Tampa Bay between 2004 and 2007 sorted by sex and size.

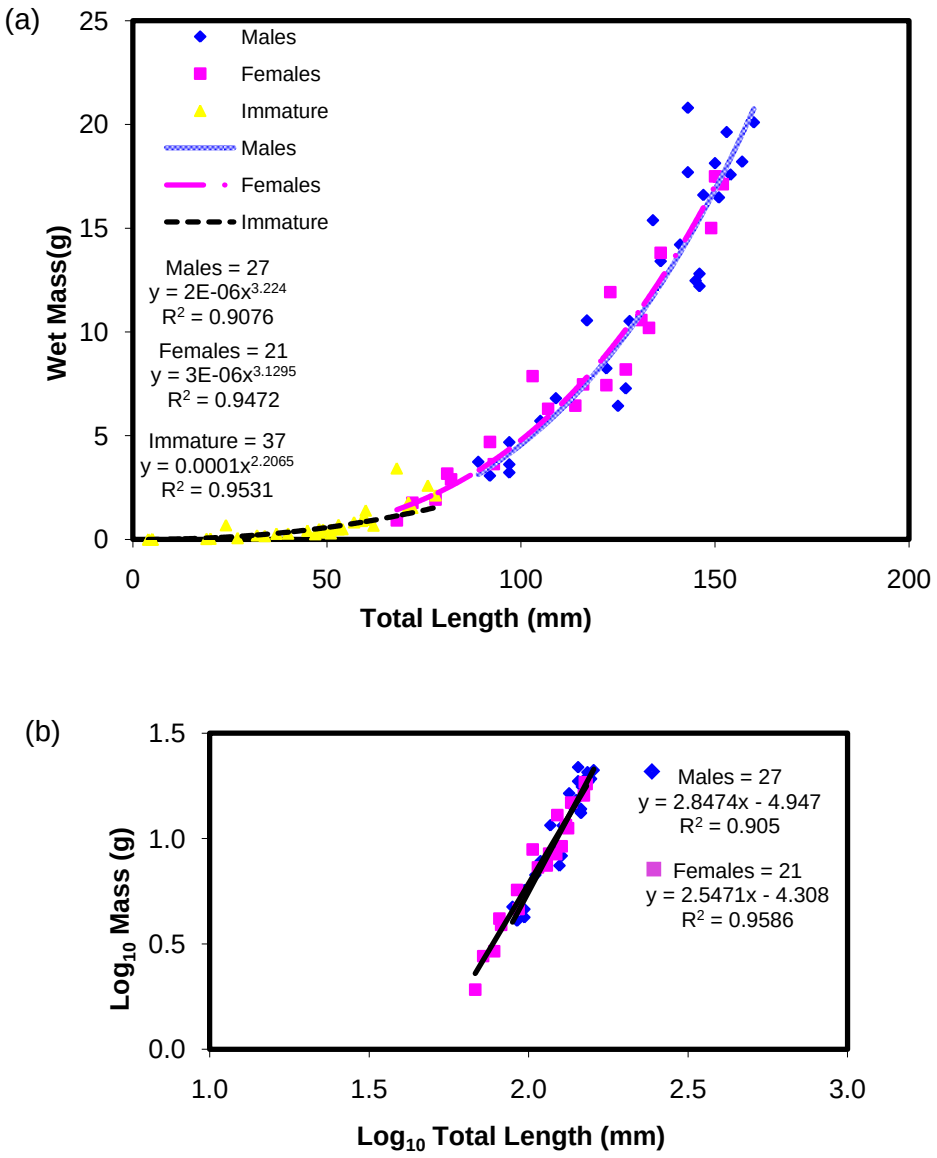

Figure 5: Length versus mass of *Hippocampus erectus*: (a) untransformed data, all specimens combined; (b) $\log_{10}$ transformed data for males and females

Energy Budget

Growth (G)

Growth is typically expressed as a function of length; however, for the purpose of this study growth was calculated as a function of mass. A von

Bertalanffy growth curve was calculated based on yearly growth using the vertebrae (Figure 6). Seahorses plotted at six months were determined by size from previous growth studies and then compared to the data in this study. The growth constant k was calculated at 0.74 yr^{-1} (L_0 = 4.9 mm, L_∞ = 157 mm). This equation was a good fit for individuals from 1.5 to 4.5 years old.

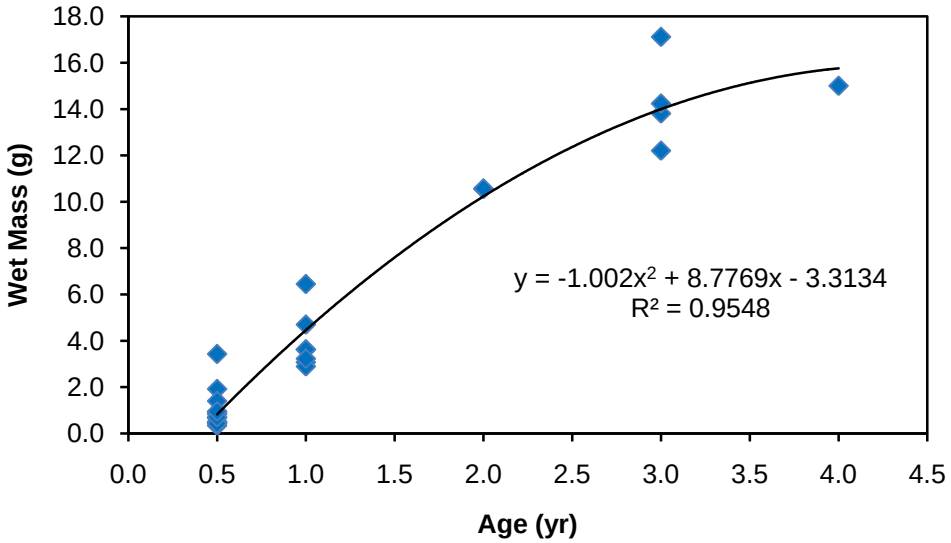

Figure 6: A von Bertalanffy growth curve of age versus mass in *Hippocampus erectus* based on annual growth. Growth rate determined from annual rings in sectioned vertebrae. The size at 0.5 years of age was determined by size from previous studies and then compared to the data collected during this study.

Ingestion (I)

Seahorses are exclusively suction feeders, and with the small size of their snout and mouth this limits their food options to a small prey size. Wild caught specimens were found to ingest a variety of prey items ranging from algae to isopods (Table 2a). Gut content analysis in wild caught *H. erectus* showed that amphipods were the predominant food items found in the guts and accounted for 73.2% of the prey items (Table 2b). In the combined total of amphipods, 27.6%

could not be identified because they were too badly digested.

Paramicrodeutopus myersi made up 16.4% of all amphipods collected by volume from *H. erectus'* digestive tracts (Table 2c).

Table 2a: Volume and frequency of occurrence of prey items in wild caught *Hippocampus erectus* collected in Tampa Bay. F(%) is the frequency of occurrence and V(ml) is volume in milliliters. Digenea were included as prey if they were confined within the alimentary tract.

Major Taxon	Family	Scientific Name	V(ml)	F(%)
Algae			0.28	11.11
Amphipoda			658.11	100.00
	Ampeliscidae	*Ampelisca* sp. C	80.00	11.1
		Ampelisca spp.	1.00	55.6
	Ampithoidae	*Cymadusa compta*	18.00	11.1
	Aoridae	*Globosolembos smithi*	11.00	11.1
		Rudilemboides naglei	2.00	11.1
	Caprellidae	*Caprella scaura*	5.00	22.2
		Dulichiella lecroyae	80.00	33.3
		Dulichiella spp.	20.00	44.4
	Ceraphronoidea	*Paramicrodeutopus myersi*	108.00	22.2
	Eusiridae	*Eusiridae*, unidentified	0.88	55.6
	Hyalidae	*Hyalidae*	14.00	11.1
		Parhyale hawaiensis	6.00	11.1
	Isaeidae	*Microprotopus raneyi*	0.57	11.1
		Photis sp. C	9.00	11.1
		Photis sp. F	12.00	22.2
	Ischyroceridae	*Ericthonius brasiliensis*	13.42	33.3
		Ischyroceridae, unidentified	2.00	22.2
		Jassa spp.	45.13	11.1
	Melitidae	*Dulichiella appendiculata*	18.68	11.1
	Lysianassidae	*Shoemakerella cubensis*	3.00	11.1
		Melitidae, unidentified	2.42	11.1
	Neomegamphopidae	*Neomegamphopidae*	24.00	33.3
		Amphipoda, unidentified	182.0	100.0
	Total		658.11	100.0
Clupeiformes			4.00	11.1
	Clupeidae	Clupeidae - unidentified	4.00	11.1
Copepoda			2.00	11.1
		Copepoda - unidentified		
Decapoda			224.95	100.0
	Alpheidae	*Alpheus normanni*	93.00	22.2
		crab - unidentified	1.00	11.1
		crab - unidentified megalopa	6.00	22.2
		shrimp, unidentified	6.00	33.1
	Hippolytidae	*Hippolyte zostericola*	12.00	11.1
		Hippolytidae	1.00	11.1

	Palaemonidae	*Periclimenes americanus*	4.00	11.1
	Penaeoidea	*Penaeoidea*	8.00	11.1
		Sicyonia parri	27.00	11.1
	Pinnotheridae	*Pinnixa* spp.	0.94	11.1
	Porcellanidae	Porcellanidae (porceline crabs)	49.00	55.6
	Processidae	Processidae (night shrimps)	17.00	11.1
Gastropoda			2.00	11.1
		Gastropoda - unidentified		
Isopoda			6.00	11.1
	Serolidae	*Heteroserolis mgrayi*		
Malacostraca			1.00	11.1
	Tanaidacea	*Tanaidacea* - unidentified		
Digenea			1.66	11.1
		Digenea, unidentified		

Table 2b: Volumetric contribution of major prey taxa for *Hippocampus erectus* *Hippocampus erectus* from Tampa Bay.

Major Taxon	PV(%)
Algae	0.03
Amphipoda	73.12
Clupeiforms	0.44
Copepoda	0.22
Decapoda	24.99
Gastropoda	0.22
Isopoda	0.67
Malacostraca	0.11
Digenea	0.18

Table 2c: Total Volumetric contribution of amphipod families to the diet *Hippocampus erectus* from Tampa Bay.

Major Taxon	Family	% Volume
Amphipoda	Ampeliscidae	12.31
	Ampithoidae	2.74
	Aoridae	1.98
	Caprellidae	15.95
	Ceraphronoidea	16.41
	Eusiridae	0.13
	Hyalidae	3.04
	Isaeidae	3.28
	Ischyroceridae	9.20
	Melitidae	2.84
	Lysianassidae	0.82
	Neomegamphopidae	3.65
	Unidentified	27.66

Artemia spp. were found to be a richer source of essential fatty acids (21%, Table 3), but the mysids had higher concentrations of proteins (10.2%), this made this food source a better choice to maintain the seahorses throughout the energy budget experiments.

Table 3: Nutritional breakdown of the viscera from *Hippocampus erectus* and the food sources. A*rtemia* spp. used during tank experiments. *Artemia* spp. values were based on the wet weight. Variables for breakdown included: WW = Percent Wet Weight, %AFDW = Percent Ash Free Dry weight, %PV = Percent Protein Concentration by Wet Mass, %LV = Percent Lipid Concentration by Wet Mass, Energy value = kilocalories per gram.

Species	%WW	%AFDW	%PV	%LV	Energy Value (kcal g^{-1})
Hippocampus erectus (viscera)	69	49.2	11.2	17	30.5
Artemia spp. (based on 200 individuals)	9.24	2	3.9	1.7	0.7

In order to calculate growth over a one year period first the amount of food per day needed to be calculated and then calculated by the change in mass over a six month period. If we were to look at an individual *H. erectus* at 127 mm and approximately 2 yr. in age the average amount of energy needed for growth over a one year period would be 13,356 J. The high amount of energy needed is most likely due to their rudimentary stomachs, which do not allow for long term retention of nutrients. Therefore seahorses and pipefishes need a constant food supply in order to maintain the rest of their metabolic needs.

Metabolism and Respiration (M)

In order to avoid biasing the results of this experiment only non-pregnant males and females were used in the respiration experiments. *Hippocampus*

erectus in captivity were determined to have an average resting respiration rate of 0.74 ml O_2 hr^{-1}. The resting metabolic rate of adult *H. erectus* when plotted vs mass resulted in a linear regression of y= 1.26x +1.68 with a slope of b=1.26 (R^2 = 0.80) (where Y was $\log_{10}$ oxygen consumption rate and x was $\log_{10}$ mass) (Figure 7). The volume of oxygen was calculated as $y=46.7\ x^{0.30}$ with a slope of b=0.30 (R^2 = 0.15).

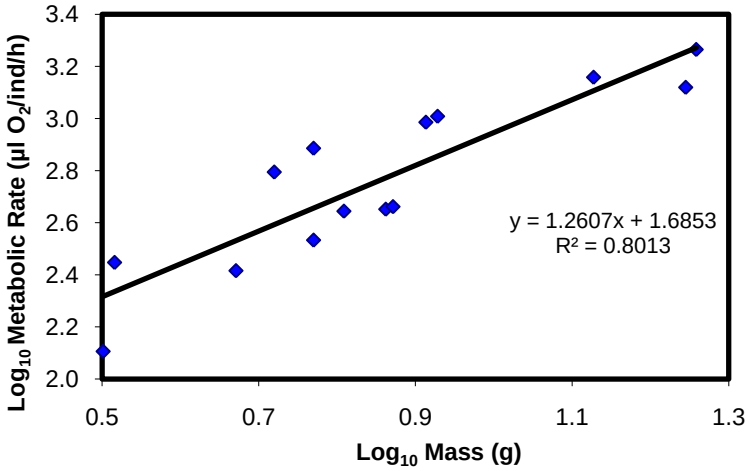

Figure 7: Resting metabolic rate of *Hippocampus erectus* based on linear regression of $\log_{10}$ mass (g) versus $\log_{10}$ oxygen consumption rate (µl O_2 h^{-1}). Rates were calculated from animals held in an oxygen respiration chamber.

If we were to look at an individual *H. erectus* approximately 2 yr. of age with a length of 127 mm, the standard respiration rate per hour for that individual would be estimated at 0.97 ml hr^{-1} (Table 4). When calculating the amount of energy needed for metabolism in a 24 hr. period, the same individual would require 365.9 J of energy. If this value were then extrapolated over one year, the amount of energy utilized would be 6,678 J.

Excretion (E)

In order to estimate the amount excreted by an individual, the ammonia concentration from each 20 ml sample was analyzed. The change in ammonia was calculated by taking the difference of the ammonia levels and dividing by the run time per animal. The average amount of ammonia excreted by an individual *H. erectus* was calculated 0.41 mmol NH_3 g^{-1} h^{-1}. These specimens had an oxygen to ammonia ratio ($O:NH_3$) of 3.02 (Table 4). The energy value in carnivorous fishes is typically estimated at 27% of the total energy budget (Brett and Groves, 1979).

Table 4: Oxygen consumption and excretion values rates IN *Hippocampus erectus*. Rates calculated by measuring the partial pressure of oxygen vs. mass then removing a 20 ml sample of water to calculate the amount of ammonia NH_3 excreted by each individual animal. VO_2 = Maximum volume of oxygen measured by microliters of oxygen per gram of wet mass per hour; RR = Respiration Rate by milliliters of oxygen by hour; NH_3= amount of ammonia by millimoles.

Run & Channel	Wet Mass (g)	VO_2 (µl O_2 $gWM^{-1}h^{-1}$) mean	max	min	Mean RR (ml O_2/h)	Respiration Rate (ml O_2 h^{-1}) max	min	STDEV	NH_3 (mmol)	Ratio O:NH_3
Run 1 Ch 1	3.17	40.25	77.31	2.86	0.13	0.25	0.01	0.09	15.06	2.36
Run 8 Ch 2	3.28	109.95	176.91	58.97	0.28	0.66	0.02	0.26	10.55	3.34
Run 1 Ch 2	4.69	55.56	124.83	9.13	0.26	0.59	0.04	0.23	8.63	3.29
Run 8 Ch 3	5.25	118.73	217.49	57.65	0.62	1.14	0.30	0.22	12.77	3.38
Run 4 Ch 4	5.89	57.95	111.78	17.65	0.34	0.66	0.10	0.18	9.27	3.28
Run 7 Ch 4	5.89	130.53	316.76	36.11	0.77	1.87	0.21	0.38		2.12
Run 8 Ch 4	6.44	68.47	100.91	50.45	0.44	0.65	0.32	0.11	16.86	2.83
Run 3 Ch 4	7.28	61.60	119.97	18.87	0.45	0.87	0.14	0.35	7.37	3.62
Run 5 Ch 5	7.44	61.67	98.13	7.27	0.46	0.73	0.05	0.22	17.54	2.80
Run 2 Ch 2	8.19	118.10	192.50	47.43	0.97	1.58	0.39	0.51	17.40	2.73
Run 6 Ch 4	8.48	120.22	299.86	31.10	1.02	2.54	0.26	0.72	7.69	4.15
Run 3 Ch 2	13.41	107.34	222.65	45.92	1.44	2.99	0.62	0.82	27.08	2.57
Run 3 Ch 5	17.58				1.32	5.30	0.23	1.41	15.66	3.05
Run 4 Ch 2	18.13	101.48	239.97	15.95	1.84	4.35	0.29	1.23	5.17	4.94
Mean x =	8.22	88.61	176.85	30.72	0.74	1.20	0.21	0.48	13.16	3.02

Discussion

Life History

While males do get slightly larger than the females, there was no significant size difference between the sexes in this study. Both sexes mature around the same size, but the brood carrying males do not appear until 120 mm. It could then be inferred that females also do not become reproductively active until 120 mm as well. Previous studies by Beverton and Holt (1959) on *H. erectus* suggested that the size at maturity was closer to 70 mm, and in a study by Baum et al. (2003) *H. erectus* were shown to mature at 56 mm. The size range in this study was consistent with the size range in a study by Foster and Vincent (2004), where they began to see breeding behavior in *H. erectus* at age 6 months to 1 year old, which is in the size range of 56 - 85 mm.

While most fishes have a tradition spawning season, *H. erectus* appears to have a yearlong spawning period, with a higher recruitment time in February and May. Reproductive males and females were also found in the summer months, but there was a lack of young-of-the-year recruits, < 45mm, recorded from July-September (Figure 8).

According to Foster and Vincent (2004) the breeding season for *H. zosterae* correlated more with day-length than with temperature; however, the duration of the male's pregnancy was found to be directly correlated with water

temperature. Their observations were applied to *H. erectus* by assuming that somatic growth in *H. erectus* also correlated with temperature.

When looking at the vertebral rings it appeared that seahorses were laying down annual rings on the vertebrae similar to those documented in otoliths (Figure 2). This projected time line coincides with the length at age data collected by Curtis and Vincent (2006); however the data in that study were not validated.

The calculated k constant of 0.74 yr^{-1} used in the Von Bertalanffy growth curve was comparable with the wild caught and aquarium raised seahorses using the equation by Takahashi (2003). However, there was a significant difference using the calculated k constant in the 6 months to 1 yr old animals. The juvenile specimens in this experiment matched the observed growth at 6 months to a year by Foster and Vincent (2004). The calculated model in this study was a better fit with minimal differences for individuals from 1.5 yr to 4.5 yr, and could be effectively applied calculate stock assessments in wild caught *H. erectus* (Figure 6).

The calculated k constant of 0.74 yr^{-1} in this present study was also comparable to the study by Matlock (1992), which was based on field data, where the growth constant K was estimated at 0.92 yr^{-1} (L_∞ = 214.0 mm). However, when compared to the von Bertalanffy growth constant K from other studies on *H. erectus*, the growth constant K from the study by Beverton and Holt (1959) and cited by Lourie et al. (1999) had an estimated growth constant K of 2.5 yr^{-1} (L_∞ = 140 mm). In spite of this value, when *H. erectus* were observed in

the tank the size at approximately six months to one year was 56 - 92 mm in length, which is similar to the wild caught data in this study (51 - 97 mm).

Other syngnathid species such as *Hippocampus comes* had a growth constant K value of 1.7 and in a study by Takahashi et al. (2003) on *S. biaculeatus* the growth constant K was estimated at 0.0076 d^{-1} or 2.8 yr^{-1} (L_0 = 21 mm and L_∞ = 260 mm). This study was based on laboratory-reared juveniles over 20 days.

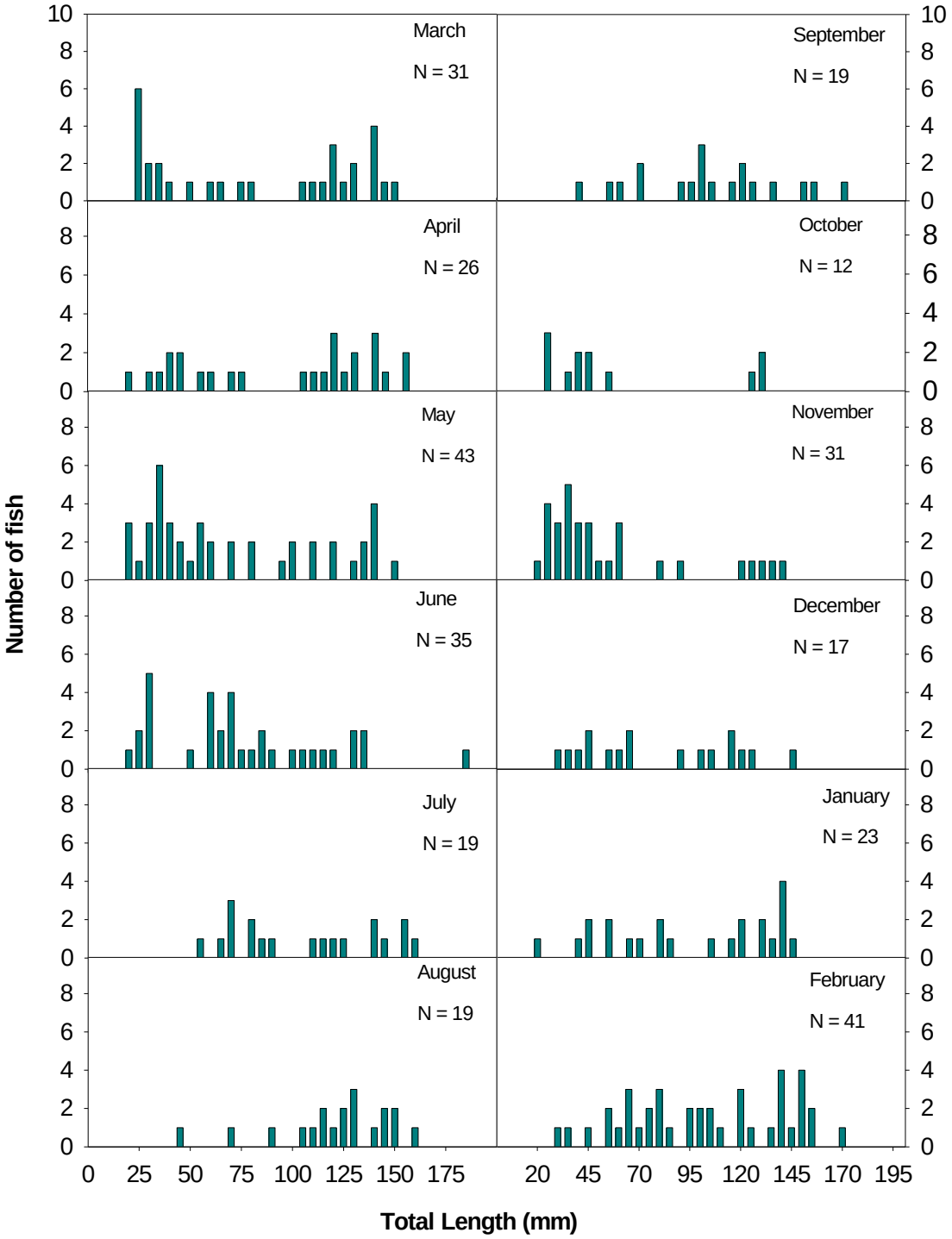

Figure 8: *Hippocampus erectus* collected by month in Tampa Bay. Data collected by offshore seines and trawls used in the Fisheries-Independent Monitoring Program (FWRI from 1996-2007). See methods and Table 1 for gear description.

Energy Budget

Hippocampus erectus were tested for the sum of energy costs broken down by growth, metabolism and excretion. The sum of energy cost of *H. erectus* accounted for 91% of the total energy ingested. However, the final sum total does not take into account energy expanded due to locomotion or reproductive needs such as egg production or gonad growth or other nutritional calculations (i.e. carbohydrates and structural organics).

Ingestion (I) =	Growth (G) +	Metabolism(M) +	Excretion(E)
100%	13%	41%	27%
13,356 J	1,776 J	5,376 J	3,606 J

The respiration rate for metabolism energy costs were calculated as a resting rate. When compared to the model by Brett and Groves (1979) under carnivorous fishes ($M = 43.8 \pm 7.0$), where the metabolic percentage was estimated at 50% of the total energy, the fish in this study were calculated at a metabolic percentage of 41%. Unlike most fish, seahorses use their tails to remain at rest and do not need to use their dorsal or pectoral fins to remain stationary. Other factors explaining the low metabolic rate could be due the minimal retention of stomach contents due to their rudimentary digestive tract. As explained in the methods section above, the individuals were not fed before the respiration experiments were performed. This explains why the excretion levels were low when compared to other species of fish. The addition of the bar inside the respiration chamber allowed the seahorse to remain in a relaxed state and therefore not excrete large amounts of ammonia.

When compared to other pipefishes of similar mass such as *Hippocampus hippocampus* (89.0 µl/g/h) and *Syngnathus acus* (133.9 µl/g/h), the respiration rate of *H. erectus* was most similar to other seahorse species (Table 4). Another observation is that none of the males used in this experiment were pregnant which also could explain why the metabolic rate was low. According to a study by Berglund et al. (1986) on pipefishes, *Siphonostoma typhle* and *Nerophis ophidion*, if the energy loss due to zygote respiration was greater than 1.2 J, then the pregnant males must be contributing energy to the embryos.

Table 5: Oxygen consumption rates versus size and temperature for member of the family Syngnathidae.

Species	Weight (g)	Oxygen consumption (µl/g/h)	Temperature (°C)	Data Source
Hippocampus erectus (Lined seahorse)	8.2	89.5	24.0	* Present Study
Hippocampus hippocampus (Short-snouted seahorse)	10.0	89.0	18.0	Altman and Dittmer, 1971
	3.1	102.8	20.0	Winberg, 1960
	2.9	112.5	20.0	Winberg, 1960
	3.1	160.1	25.0	Altman and Dittmer, 1971
	1.8	173.2	22.2	Winberg, 1960
Syngnathus acus (Greater pipefish)	7.6	133.9	18.5	Winberg, 1960
	7.0	192.5	18.5	Winberg, 1960

When *H. erectus* viscera were broken down by lipid and protein composition, 17% of this species was comprised of lipids and 11.2% protein as a function of wet mass (Table 3). This was comparable to the lipid content of other fishes, such as *Oncorhynchus mykiss* which was tested at 13%, but much higher than *Salmo gairdneri* which tested at approximately 7% lipid (Cowey and Sargent 1975 and Weatherley and Gill 1983).

The breakdown of protein content of *H. erectus* was much lower than a more active fish, such as *Salmo salar* at 24%, but closer in comparison to *Salmo gairdneri* with a protein value of 18% Weatherley and Gill 1983). In the experiment by Cowey and Sargent (1975) *S. salar* was given 40 g of an isolated fish protein as the efficient dietary protein level.

Overall the chemical composition tested does not account for the organic composition of the endoskeleton in *H. erectus*, which has a 3.3% ash weight, indicative of a low organic content.

According to the Fisheries-Dependent Monitoring Program (FWRI 2007), as of 2007, 59,879 seahorses (*Hippocampus* spp.) were reported from commercial landings around the state of Florida. Until recently the basic biology was poorly known, which makes it difficult to manage seahorse populations effectively. However, after looking at the required nutritional needs and growth model, *H. erectus* does not fully mature until 120 mm. As of present there is no size or slot limit for this species. CITES is proposing a minimum slot limit of >100mm, which does not allow the individual to fully mature and should be changed to >120 mm.

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