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The relationships between gut length and prey preference of three pipefish (*Syngnathus acus*, *Syngnathus typhle*, *Nerophis ophidion Linnaeus*, 1758) species distributed in Aegean Sea, Turkey

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Introduction

Both external morphology (such as shape, size and position of mouth, etc.) and internal morphology (such as stomach shape, gut size and its length, etc.) give important clues about feeding ecology of fish (Karachle and Stergiou, 2010a). Similarly, ecomorphological correlations are important for fish feeding (Eggold and Motta, 1992). Pipefish populations are an important component of deltaic shallow brackish water environments and *Syngnathus acus*, *Syngnathus typhle* and *Nerophis ophidion* are among the most important representative of such habitats. Feeding apparatus of these pipefishes as well as feeding types are more specialized than the other fishes inhabit these habitats (Oliviera *et al*., 2007; Leysen *et al*., 2011). Pipefishes are known to be epifaunal predators and can be defined as gape-limited pipet-feeders with their sit-and-wait or ambush behaviours (Howard and Koehn, 1985; Tipton and Bell, 1988; Vizzini and Mazzol, 2004). Hunting kinetic of pipefish is provided by a sudden movement of the muscles located in head (Le Page, 2012). Those sudden movements of hipaxial and epaxial muscles contractions are very important in feeding bio-mechanism of pipefish with small mouth structure (Wassenbergh and Aerts, 2008). In this way, the mouth opening can be adjusted by providing a powerful vacuum effect towards the prey (Kendrick and Hydnes, 2005; Flammang *et al*., 2009). Abundance and availability of prey groups in gut contents are related to pipefishes inhabiting areas such as shallow waters, sea-grass beds~~,~~ and vegetated bottoms (Tipton and Bell, 1988).

Some researchers have been shown that Amphipoda, Isopoda, Copepoda and small crustacean groups are important dietary items for pipefish (Taşkavak *et al*., 2010). Besides, syngnathid fish have relatively undifferentiated, tube-shaped digestive tracts (Tipton and Bell, 1988; Ryer and

Boehlert, 1983) and availability of some digestive enzymes in the digest tracts can been related to prey selection (Blanco *et al.,* 2016). It has been reported that gut length provides an important information on fish species' food habits (Karachle and Stergiou, 2010a). Generally, gut content researches were mostly focused on feeding habits and prey groups by the time intervals (seasons and/or months) or sexes in pipefish species (Ryer and Boehlert, 1983; Taşkavak *et al*., 2010; Gurkan *et al*., 2011), whereas, studies on the habitat type effects and gut size or shape are still scarce (Karachle and Stergiou, 2010b). In general, morphometric data such as the relationship between gut length and total length are important for especially ecomorphological studies (Motta, 1988). In Beloniformes and Syngnathiformes that inhabit sea grass beds, digestive tract is related to feeding habits as well as its habitats and body shapes (Verigina, 1991; Karachle and Stergiou, 2010b). Among the studies given above, only two (Gurkan *et al*., 2011) were related to Aegean Sea.

In this study, the relationships of prey selection between gut lengths as well as relationships of gut length between total lengths were determined in three pipefish species captured in the coast of Aegean Sea, Turkey.

Materials and methods

All pipefish samples studied here were obtained from two research projects encoded as SUF 002-and SUF 017. Fish specimens were collected from two different locations in the coasts of Aegean Sea, Turkey (Fig. 1).

In order to detect dietary shifts, morphometric measurements of 142 *S. acus*, 80 *S. typhle* and 21 *N. ophidion* specimens have been used. After capturing, specimens were preserved in 10 % formalin. To determine if dietary shifts are correlated with feeding morphology, total length (TL, mm), gut lengths (GL, mm), total weight (W, g) and gut weight of each specimen was measured in laboratory. Later, fish samples were dissected in laboratory and gut contents were sorted out by group levels under the binocular microscope, and analysed using numerical occurrence (NO %) and frequency occurrence (FO %) methods (Leonard *et al,* 2010). An index of fullness was also calculated. Since the syngnathids have a relatively undifferentiated gastrointestinal tract, stretched gut length of the pipefish specimens was measured between oesophagus and intestine with 0,01mm with digital caliper (Teixeira and Musick, 1995) (Figs. 2,3,4).

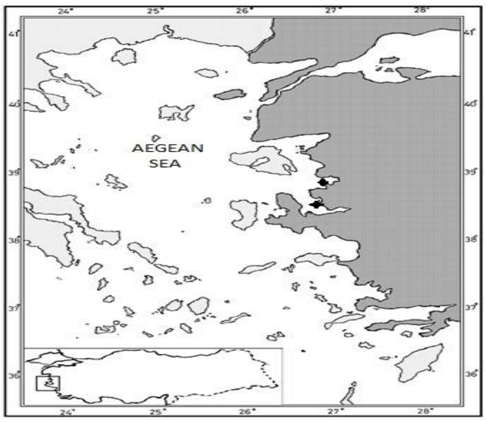

Figure 1: Sampling area.

Figure 2: Digestive tract in *Syngnathus acus* (*Photos: S.Gurkan*).

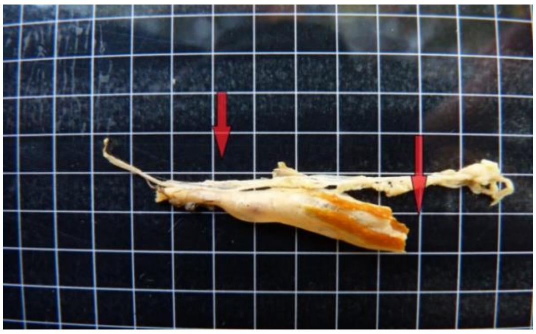

Figure 3: Digestive tract of *Syngnathus typhle.*

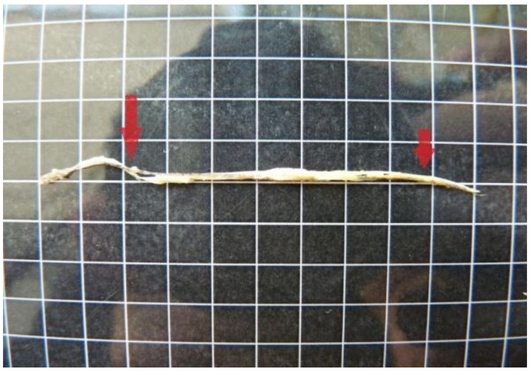

Figure 4: Digestive tract of *Nerophis ophidion*

Regression analysis was examined for the relationships between gut length and total length ($GL=a\ TL^b$), (Hyslop, 1980). Also, *t-test* has been used for the relationships between gut length (GL) and relative gut length (RGL) (Zar, 1999). In this study, a statistical approach between fish size and prey size for selectivity of the pipefish samples was not taken into consideration.

Results and discussion

Our data show that the ratio of the empty stomachs among the examined specimens was 32.5% in *S. typhle*, 25.3% in *S. acus* and 19.04% in *N. ophidion*. However, among two sympatric pipefish, the gut content ratio of *S. acus* (74.64%) was higher than *S. typhle* (67.5%) (Fig. 5). In Table 1, prey compositions, numerical occurrence (NO%) and frequency occurrence (FO%) of prey groups in stomach contents were showed. *S. acus*, *N.ophidion*, *S.typhle* in the study area fed on benthic and epibenthic invertebrates such as Copepoda, Copepoda and Ostracoda, Mysidaceae and decapod crustaceae, respectively. All of pipefish species tend to capture small zooplanktonic prey groups. Taşkavak *et al*. (2010) and Gurkan *et al*. (2011) stated that small zooplanktonic organisms were the most important prey in the diets of *S. acus* and *N. ophidion* in the Turkish coasts of Aegean Sea.

Little information is known about feeding behaviour and prey compositions of *S. typhle* in the Mediterranean Sea (Vizzini and Mazzola, 2004; Oliviera *et al.*, 2007), however, while the benthic and planktonic crustaceans were given for the food groups of *S. typhle* (Moreira *et al.*, 1992) in the Europe coasts (Germany, France and Portugal), those prey groups are not known for this species in Aegean Sea.

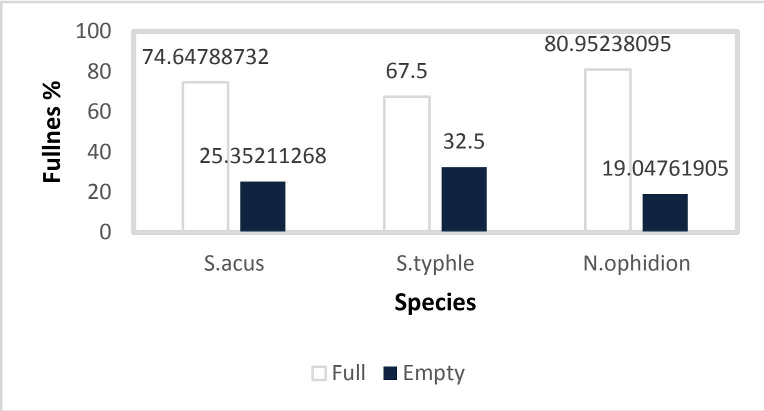

Figure 5: Stomach fullness (%) in samples.

Table 1: The prey items and frequency and numerical occurrence (%) in the gut contents.

	Syngnathus acus		*Syngnathus typhle*		*Nerophis ophidion*	
	N%	**F%**	**N%**	**F%**	**N%**	**F%**
Mysidaceae	-	-	77.083		-	-
Ostracoda	1.179	5.714	-	-	6.410	
					96.269	
Copepoda	61.48	28.571	2.777	7.547	78.717	
					22.222	
Euphasidacea	0.277	0.277	-	-	-	-
Amphipoda	8.743	10.00	8.333	18.867	11.282	
					3.703	
Decapod	27.68	27.68	9.027	54.716	0.256	
rustacea*					14.814	
Cirripedia	0.485		-	-	2.564	
	7.142				3.703	
Gastropoda	-	-	-	-	0.769	
					18.518	
Bivalvia	0.069		-	-	-	-
	1.428					
Cladocera	-	-	0.694	5.560	-	-
Insecta	0.069	0.00	-	-	-	-
Fish eggs/larvae	-	0.00	2.08	0.00	-	-

*: Decapod crustacean eggs and larvae

The relationship of GL-TL regression of each species is given in Figs 6, 7 and 8. Accordingly, GL value of *S. typhle* containing stomach contents such as large fish eggs and fish larvae is greater than two other pipefish species in similar size. According to the results given in Table 2, the total length and gut length of three species have a strong statistical relationship ($p<0.05$). The GL rate that increase with total length (TL) may depend on prey composition in the habitat where species inhabits. Similarly, it was indicated that the GL rate is also affected by species' habitat and gut shape (Karachle and Stergiou, 2010b).

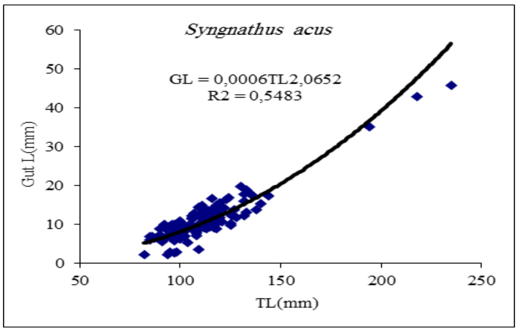

Figure 6: Regression between body length and the gut length in *Syngnathus acus*.

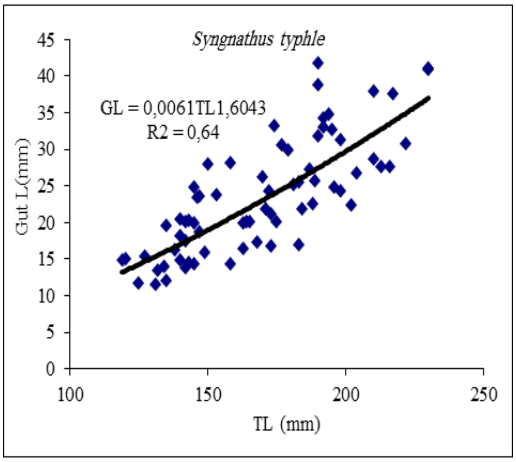

Figure 7: Regression between body length and the gut length in *Syngnathus typhle.*

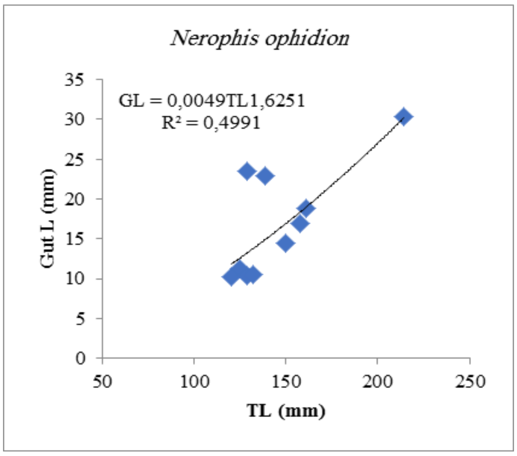

Figure 8: Regression between body length and the gut length in *Nerophis ophidion.*

Table 2: Mean gut lengths (GL±SE) and relative the gut lengths (RGL±SE) of samples.

Species	N	GL	RGL±SE	GL=a TL^b	*p*
Syngnathus acus	134	11.954±0.83	0.097±0.08	0,0002 TL $^{2.264}$	$p<0.05$
Syngnathus typhle	70	23.605±2.82	0.137±0.02	0.0076 TL^{1,560}	$p<0.05$
Nerophis ophidion	15	15.383±5.36	0.106±0.04	0.0017 TL^{1,825}	$p<0.05$

p<0,05: statically difference

The RGL values calculated for 219 specimens of three pipefish species are given in Table 2. If the mean GL and RGL values were taken into consideration, *S. typhle* have the highest value, the lowest values were computed for *S. acus*. It was stated that the RGL value smaller than 1 shows carnivorous diet, between 1 and 3 omnivores feeding, whereas the RGL value bigger than 3 shows diet based on vegetative material or detritus (Karachle and Stergiou, 2010a). Besides, the species' feeding habits can be estimate with the indices. When we take into consideration the GL and RGL index values of the specimens examined, it is seen that the computed values are compatible with those reported by (Karachle and Stergiou, 2010a) for ranking of the feeding relationship. Compositions of prey items in gut contents are given Table 1. However, it was showed that each three pipefish species consumed zooplanktonic preys as carnivorous type feeding. (Taşkavak *et al*. (2010) and Gurkan *et al*. (2011) stated that small zooplanktonic preys were main prey groups in *S. acus* and *N. ophidion*. According to (Karachle and Stergiou, 2010b), this type of indices for the determination of general feeding habits of the species is useful. Similarly, the obtained results in this study support low index values explaining the exact feeding habit of the pipefish species.

Gut length (GL) must increase allometrically since isometric intestine growth for both freshwater and marine fishes would provide reduced capacity of metabolic needs of any given fish (Karachle and Stergiou, 2010b). Accordingly, stomach type and intestine morphology of fish were related with feeding habits, and they are important properties (Karachle and Stergiou, 2010b). It is known that fish may have tube-shaped, flat, prolonged or shortened guts. However, in some fish species such as *Conger conger*, *Cephola macrophythalma*, Syngnathiformes and Beloniformes which are outside of the general framework, it is estimated that the gastrointestinal structures are not only closely related to the feeding habit but also to the body structure that is shaped according to the habitat types they live (Karachle and Stergiou, 2010b). Therefore, feeding habits are associated with increased or decreased gut length that may change depending on fish size and shape. In this study, *S. typhle* with a longer gut length than the other pipefish species explains the situation of being rich in organic gut content such as benthic zooplankton. This situation may also show that prey items in gut contents were retained longer in digestive tract. Gut length of fishes varies and body mass must be taken into account in the comparison of the gut lengths of small and large individuals of the same species, in the ones having similar gut structure with different body mass, or the ones having different gut structure with different body structure (German and Horn, 2006).

Given the claim Karachle and Stergiou (2010b) that those longer guts allow food to spend more time in digestive tract, therefore, more nutrients to be absorbed. On the other hand, it has been indicated that fish with a shorter intestine ingesting feed with lower organic content, increased the area of absorption (Oliviera *et al*., 2007). Since pipefishes and seahorses are regarded as agastric species, the absence of a true stomach in such fish group is associated with typically microphagous feeding habits (Grassel *et al*., 2011).

The findings of the present study show that three pipefish having with similar body shape and digestive track, different prey type they consume is related to the absorbing process of nutrients, nutrient content and feed quality (Wagner *et al*., 2009; Karachle and Stergiou, 2010b). The morphology of feeding apparatus of three pipefishes directly r eflects the food resources in habitat where they live.

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