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, sygnathid fish have relatively undifferentiated, tube-shaped digestive tracts (Tipton and Bell, 1988; Ryer and...
Boehlert, 1983) and availability of some digestive enzymes in the digestive tracts can be 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.**
Regression analysis was examined for the relationships between gut length and total length (GL=a TL), (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.
The relationships between gut length and prey preference of pipefish species has been studied. Figure 5 shows the stomach fullness (%) in samples for three species: Syngnathus acus, Syngnathus typhle, and Nerophis ophidion. The results indicate that the gut length varies with total length (TL) and prey composition in the habitat where species inhabit.

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

<table>
<thead>
<tr>
<th>Prey Items</th>
<th>Syngnathus acus</th>
<th>Syngnathus typhle</th>
<th>Nerophis ophidion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mysidaceae</td>
<td>-</td>
<td>77.083</td>
<td>-</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>1.179</td>
<td>5.714</td>
<td>6.410</td>
</tr>
<tr>
<td>Copepoda</td>
<td>61.48</td>
<td>28.571</td>
<td>78.717</td>
</tr>
<tr>
<td>Euphasidacea</td>
<td>0.277</td>
<td>0.277</td>
<td>-</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>8.743</td>
<td>10.00</td>
<td>11.282</td>
</tr>
<tr>
<td>Decapod rustacea*</td>
<td>27.68</td>
<td>27.68</td>
<td>0.256</td>
</tr>
<tr>
<td>Cirripedia</td>
<td>0.485</td>
<td>-</td>
<td>2.564</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>7.142</td>
<td>-</td>
<td>3.703</td>
</tr>
<tr>
<td>Bivalvia</td>
<td>0.069</td>
<td>1.428</td>
<td>18.518</td>
</tr>
<tr>
<td>Cladocera</td>
<td>-</td>
<td>0.694</td>
<td>5.560</td>
</tr>
<tr>
<td>Insecta</td>
<td>0.069</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>Fish eggs/larvae</td>
<td>-</td>
<td>2.08</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>GL</th>
<th>RGL±SE</th>
<th>GL=a TL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngnathus acus</td>
<td>134</td>
<td>11.95±0.83</td>
<td>0.097±0.08</td>
<td>0.0002 TL 2.264</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Syngnathus typhle</td>
<td>70</td>
<td>23.60±2.82</td>
<td>0.137±0.02</td>
<td>0.0076 TL 1.560</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Nerophis ophidion</td>
<td>15</td>
<td>15.38±5.36</td>
<td>0.106±0.04</td>
<td>0.0017 TL 1.825</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

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 omnvores 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
The 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, Cepholo macrephythaalma, 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 agastic 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 reflects the food resources in habitat where they live.

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References


