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Stomach content analysis and length-weight relationship of the Pontic shad *Alosa immaculata* Bennett, 1835 (Pisces: Clupeidae), from the eastern Black Sea coast of Turkey

RAHSAN EVREN MAZLUM AND SEVKIYE AKGUMUS
Recep Tayyip Erdogan University, Faculty of Fisheries, 53100, Rize, Turkey
e-mail: evren.mazlum@erdogan.edu.tr

ABSTRACT

Diet composition of the anadromous Pontic shad *Alosa immaculata* Bennett, 1835 was studied in relation to season and body size, from samples collected from the south-east Black Sea coast of Turkey in spring, autumn and winter during the period from March 2012 - February 2013. Out of 236 samples, 226 stomachs contained food and 24 prey items were identified. *Nematoda* sp. and teleosts were the main prey items with index of relative importance (IRI) of 59.3 and 38.2% respectively. Seasonal analysis showed that the IRI of *Nematoda* sp. was 71.6% in winter, 50.6% in spring and 37.1% in autumn. The stomachs of fishes in the length range of 12.0 -19.9 cm contained a higher proportion of *Nematoda* sp. (IRI up to 74 - 80%). Larger fishes in the length range of 24.0-32.8 cm consumed more teleosts (>45% IRI) than the others. The length-weight relationship showed that females grow in positive allometric pattern while males have isometric growth.

Keywords: *Alosa immaculata*, Diadromous, Feeding ecology, Length-weight relationship, Nematoda, Pontic shad

Introduction

Shads (Family:Clupeidae) are economically important fishes with a signifcant role in the food web as prey item of other piscivorous fish (Juanes *et al.*, 1993; Aprahamian *et al.*, 2003). They are commercially harvested in the Danube River, Black and Azov Seas (Navodaru and Waldman, 2003; Raykov and Triantaphyllidis, 2015; TUIK, 2015). Three species of shads (Caspian shad *Alosa caspia*; Black Sea shad, *Alosa maeotica* and Pontic shad *Alosa immaculata*) are found in the Black Sea (Lenhardt *et al.*, 2012). In Turkey, the total catch volume of shads was 2,581.5 t in 2011 that decreased by 21.2% to 2,034.7 t in 2015 (TUIK, 2015). Data from NAFA (National Agency for Fisheries and Aquaculture) showed a decrease of 65% in the share of *A. immaculata* to the total fish production of Bulgaria from 2010 to 2012 (Raykov and Triantaphyllidis, 2015). The population of *A. immaculata* in the Black and Azov seas has been shrinking since the last decade due to exploitation and pollution, including the construction of dams that prevent their access to rivers for spawning and nursery grounds (Freyhof and Kottelat, 2008). In Hungary, it is regionally extinct and hence listed as a vulnerable species (IUCN, 2017).

Despite being listed as a vulnerable species and its economic importance, limited studies exist on the food and feeding habits of *A. immaculata,* with no record from the Black Sea coast of Turkey. Data on diet composition are useful to understand the predator-prey relationship and identify the food preferences of a fish species. Such information can be used to assess conservative regulations and policies (Lopez-Peralta, 2002; Bandpei *et al.*, 2012).

In this study, the diet of *A. immaculata* Bennett, 1835 was investigated in the south-east Black Sea coast of Turkey during spring, autumn and winter seasons. The dietary preferences of *A. immaculata* was also determined in relation to different length classes in order to analyse the impact of its body size on diet composition. The length-weight relationship was also derived.

Materials and methods

The study area was located around the Trabzon-Rize coast on the south-east Black Sea (Fig. 1). Specimens of *A. immaculata* were caught on a monthly basis from March 2012 to February 2013 across sites 1 and 2 using a commercial purse-seine with mesh size 10-16 mm. Since they migrate to rivers during summer for spawning and nursery grounds (Kottelat, 1997) and also since summer is a closed season (15 April to 1 September) in Turkish Black Sea, no specimen was procured during this period. Immediately after collection, the fish were placed in 70% alcohol and transferred to the laboratory.

In the laboratory, each specimen was weighed to the nearest 0.01 g and total length (L_T) was recorded to the nearest 0.1 cm. Based on body size, the fishes were categorised into length classes of 4 cm intervals. The stomach contents were recovered and identified to the lowest possible taxonomic level. Following Kitsos *et al.* (2008), the stomach fullness was categorised as empty (0%), moderately full (25%), half full (50%), quite full (75%) and very full (100%). The contribution of each prey type in the diet of *A. immaculata* was assessed by calculating index of relative importance (% IRI) using the formula (Cortes, 1997):

$$IRI = (\%N + \%W) \times \%F = > \%IRI = \frac{IRI}{\sum IRI} \times 100$$

where %N = percentage of prey groups' numerical frequency, %W = percentage of prey groups' weight and %F = percentage of prey groups' occurrence (Hyslop, 1980). Seasonal and length-based variations in the diet composition were studied through dendrogram analysis using Minitab 17 Statistical Software.

The length-weight relationship for *A. immaculata* was determined by log transformation of the exponential equation $W = a L_T^{\ b}$ (where *a* is the intercept and *b* is the slope) and performing least squares regression analysis with MS Excel software. The statistical deviation of *b* from the isometric value of 3.0 was tested by *t*-test (Pauly, 1984).

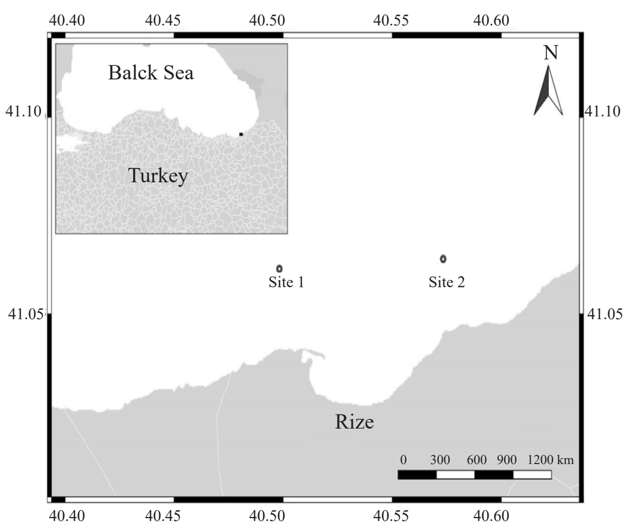

Fig. 1. Map of the study area

Results

Length frequency distribution and sex ratio

A total of 236 samples of *A. immaculata* were analysed, of which 176 were females in the size range of 13.5-32.8 cm total length (L_T) and 60 were males (12.5-28.7 cm L_T). The mean (± S.E) length of males (18.2±0.6) was significantly smaller than that of females (23.6±0.4) (*t*-test, $p<0.001$). The size-frequency distribution of females and males also significantly differed (Kolmogorov-Simirnov test: d = 0.45985, $p<0.00$). In females the dominant size was 28-30 cm while in males it was 18-20 cm (Fig. 2). The sex ratio (female: male) of *A. immaculata* in the south-east Black Sea was 1:0.34, which deviated significantly from 1:1 (χ^2= 57.02, $p<0.01$).

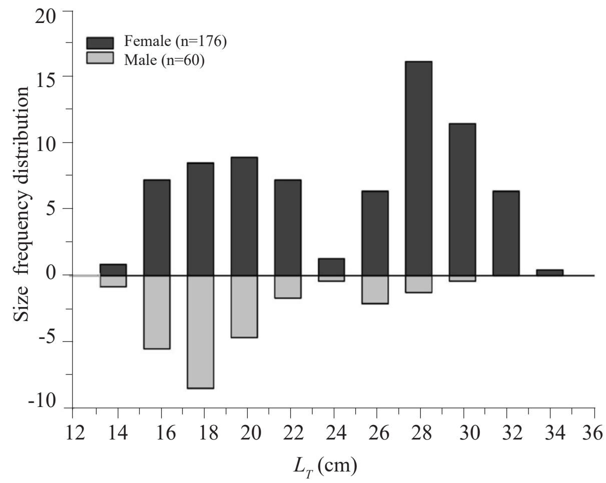

Fig. 2. Size frequency distribution of females and males of *A. immaculata* in the south-east Black Sea coast of Turkey (Total length, L_T)

Stomach fullness

Overall, 4.24% of the stomachs were empty, 27.54% moderately full, 25% half full, 25.42% quite full and 17.8% very full. The larger number (>50%) of moderately full stomachs were found during winter (Fig. 3). The qualitative dietary analysis included only non-empty stomachs.

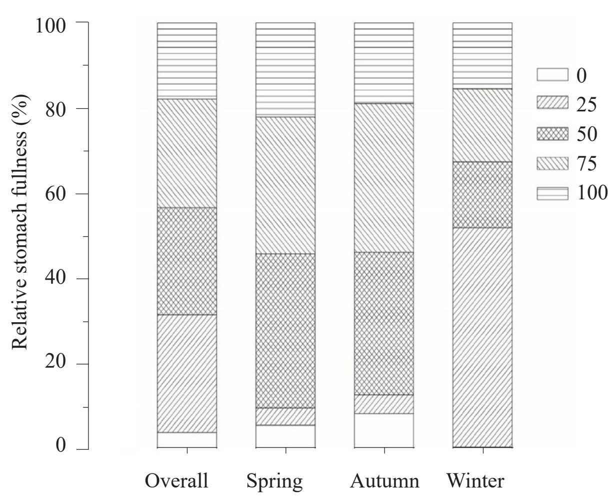

Fig. 3. Stomach fullness in *A. immaculata* in the south-east Black Sea coast of Turkey

Diet composition

In all, 24 prey items were found in the stomachs of *A. immaculata* belonging to four prey categories *viz.,* zooplankton, fish eggs (ichthyoplankton), teleostei and insecta. The *Nematoda* sp. represented 59.3% IRI of the stomach contents while the second most abundant prey item was teleosts, constituting 38.2% IRI of the diet (Table 1).

Table 1. Prey categories found in the stomachs of *A. immaculata* in the south-east Black Sea coast of Turkey. Stomach content expressed as % IRI

Prey groups	Overall (n=236)	Spring (n=50)	Autumn (n=69)	Winter (n=117)
Zooplankton				
Arthrophoda				
Copepoda				
Pseudocalanus sp.	0.0017	0.011	0.005	
Ostracoda	0.0004	0.011		
Isopoda	0.0592	0.011	0.031	0.097
Dynamene sp.	0.0018	0.047		
Decapoda				
Decapod larvae	0.0043		0.046	
Cumacea				
Cumacean sp.	0.0004		0.005	
Amphipoda				
Gammarus sp.	0.0004			0.001
Corophium sp.	0.0004		0.005	
Tanaidacea				
Tanaidacea sp.	0.0028		0.025	
Chaetognatha				
Sagittwa setosa	0.0004		0.005	
Appendicularia				
Oikopleura dioica	0.0017		0.021	
Nematoda				
Nematoda sp.	59.2513	50.64	37.11	71.555
Cnidaria				
Ctenophora				
Planula larvae	0.0004		0.005	
Mollusca				
Gastropoda				
Tricolia pullus	0.002		0.018	
Ichthyoplankton				
Fish eggs	2.0457	3.167	2.423	1.329
Teleostei				
Engraulis encrasicolus	24.6537	21.903	33.047	18.854
Merlangius merlangus	0.2798	0.069	2.116	
Sprattus sprattus	4.8097	17.009	3.992	2.881
Syngnathus acus	0.0012	0.041		
Trachurus trachurus	8.4550	7.092	16.023	5.279
Insecta				
Diptera sp. pupa	0.0004			0.001
Diptera sp.	0.0006		0.006	
Others				
Sand grains	0.4252		5.109	
Plastics	0.0017		0.005	0.001

Influence of season, sex and body size on diet composition

A wide variety of prey items were found during autumn (19 prey types) followed by spring (11). In winter, only nine different prey types were recovered from the stomachs of *A. immaculata* (Table 1). The predominant prey items were *Nematoda* sp. and isopoda from zooplankton and *Engraulis encrasicolus*, *Sprattus sprattus* and *Trachurus trachurus* from teleostei. Fish eggs (ichthyoplankton) were found in all seasons. Over 92% IRI of the total stomach contents were composed of zooplankton and teleosts (Fig. 4a). The presence of plastics in the stomach contents was observed during autumn and winter while sand grains were observed only in autumn with 5.1% IRI.

Cluster analysis revealed a high percentage of similarity (79%) between spring and winter, indicating similar diets. The spring and winter clusters were separated from autumn with 31% dissimilarity (Fig. 5a).

Female *A. immaculata* was found to consume a wider variety of prey items (21 prey types) than male (11 prey types). *Nematoda* sp. were the predominant prey group found in the stomach contents of both sexes with IRI of 52.6% for female and 75.9% for male. The other most abundant prey items were *T. trachurus* (8.1% IRI), *S. sprattus* (7.4% IRI) and *E. encrasicolus* (7.2%) in male and *E. encrasicolus* (30.8%), *T. trachurus* (8.7%) and *S. sprattus* (4.3%) in female *A. immaculata*.

The IRI of *Nematoda* sp. in stomach contents of smaller *A. immaculata* in the length classes of 12.0-15.9 and 16.0-19.9 cm was 80 and 74% respectively, while that of teleosts was 19.3 and 25.6% respectively (Table 2, Fig. 4b). The larger fishes (20.0-23.9 cm and above) consumed relatively higher amount of teleosts (IRI 34.1-51.2%) than the small sized fishes. The IRI of *Nematoda* sp. ranged between 41.3% and 46.1% in the larger fishes. The larger fishes were also found to consume a relatively wide variety of zooplankton (Table 2).

According to dendrogram analysis, the diets of fishes in the 12.0-15.9 and 16.0-19.9 cm length classes were extremely similar (93.75% similarity) followed by high similarity between 24.0-27.9 and 28.0-31.9 cm length classes (86.24%). These higher length classes were separated from 20.0-23.9 length class with 22.6% dissimilarity, while the 12.0-15.9 and 16.0-19.9 length classes were separated from the others with 35.8% dissimilarity (Fig. 5b).

Length-weight relationship

The allometric coefficient *b* was estimated for females and males separately and for sexes pooled (Table 3). The

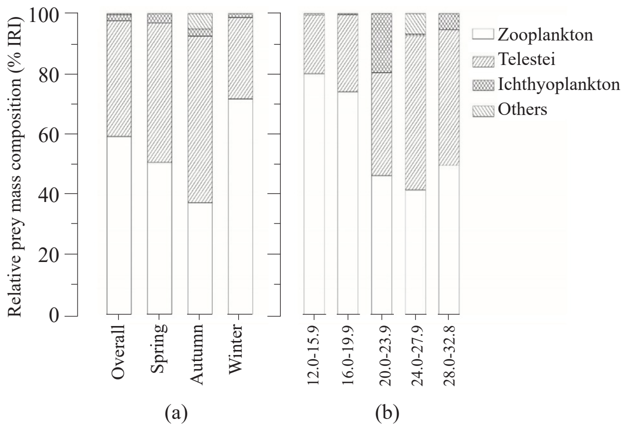

Fig. 4. Relative contribution of prey group in the stomach contents of *A. immaculata* in the south-east Black Sea coast of Turkey by (a) season and (b) length classes (total length, cm).

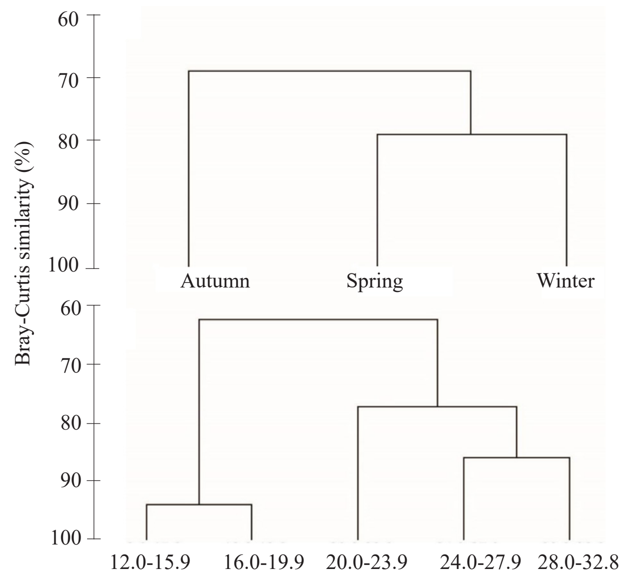

Fig. 5. Dendrogram (based on Bray-Curtis similarity method) of similarities in diet of *A. immaculata* in the south-east Black Sea coast of Turkey based on % IRI across (a) seasons and (b) size (total length, cm)

estimated values of *b* (mean±95% Confidence Interval, CI) obtained from males, females and pooled data were 3.099±0.300, 3.276±0.091 and 3.287 ± 0.106 respectively and were not statistically different. The value of *b* for males did not significantly deviate from the isometric value of 3.0 while the *b* values obtained for females and sexes pooled were found to deviate significantly from 3.0, suggesting isometric growth in males and positive allometric growth in females (Table 3).

Discussion

Diet composition

The study showed that zooplankton and teleosts together made up 97.5% IRI of the total stomach contents of *A. immaculata*. These results are in accordance with previous studies (Moskvin, 1940; Kottelat and Freyhof, 2007). The teleost species identified in the diet (*Engraulis* sp. and *Sprattus* sp.) are also found to be in line with the findings of Kottelat (1997). However, while Moskvin (1940) and Kottelat (1997) reported the main prey among zooplankton to be nekton and crustaceans, the present study recorded *Nematoda* sp. as the dominant zooplankton in the diet of *A. immaculata*. The significant contribution of nematodes in the diet of grey mullet (Osteichthyes, Mugilidae), detritivorous estuarine fishes and flounder (*Platichthys flesus* L.) have been reported earlier (Lasserre *et al.*, 1976; Aarnio *et al.*, 1996; Laffaille *et al.*, 2002). Generally, the contribution of nematodes to the diet of fish have been underestimated (Gee, 1989). The nematodes have soft-bodies supporting their fast digestion and hence, their actual contribution to the fish diet has been underestimated (Nikolsky, 1963; Aarnio *et al.*, 1996). According to Gee (1989) fish should be examined immediately after capture in order to assess the actual contribution of nematodes in their diet.

Several studies have shown that the majority of *Alosa* spp. consume zooplankton and small fishes (Assis *et al.*, 1992; Johnson and Dropkin, 1996; Kottelat and Freyhof, 2007; Azeroual, 2010) and a few of them such as *Alosa kessleri* and *Alosa braschnikowi* are known to be exclusively piscivorous having 85-98.6% of fish in the diet (Vetchanin, 1984; Coad, 1999; Bandpei *et al.*, 2012). The presence of *Nematoda* sp. along with sand grains indicates that *A. immaculata* could be a bottom feeder in the sea.

In the present study, the diet of smaller size *A. immaculata* had a higher proportion of *Nematoda* sp. (74.0-80.1%) with 19.3-25.6% of teleosts. The larger size fishes consumed relatively more teleosts than smaller size *A. immaculata*. This could be attributed to mouth size as prey size increases with fish size (Hambright, 1991; Khan *et al.*, 2013, 2014).

In this study, the maximum variety of different prey types in the diet of *A. immaculata* was seen during autumn. In the south-east Black Sea coast of Turkey, the diet of European anchovy, *E. encrasicolus* also showed a wide variety of prey types in autumn (Mazlum *et al.*, 2017) that might indicate the maximum abundance of different prey groups during this season.

Size-frequency distribution, sex ratio and length-weight relationship

The size-frequency distribution analysis revealed that most of the specimens in females and males belong to

Table 2. Diet compositions of *A. immaculata* in relation to sex and body size (% IRI).

Prey groups	Sex-specific diet composition		Size-specific diet composition Length classes (cm)				
	Female (n=176)	Male (n=60)	12–15.9 (n=30)	16–19.9 (n=75)	20–23.9 (n=26)	24–27.9 (n=58)	28–32.8 (n=47)
Zooplankton							
Arthrophoda							
Copepoda							
Pseudocalanus sp.	0.0008	0.0059	0.03				0.009
Ostracoda	0.0008			0.003			
Isopoda	0.1025				0.077	0.012	0.586
Dynamene sp.		0.0259		0.004	0.077		
Decapoda							
Decapod larvae	0.0074					0.079	
Cumacea							
Cumacean sp.	0.0008						0.009
Amphipoda							
Gammarus sp.	0.0008						0.009
Corophium sp.	0.0008						0.009
Tanaidacea							
Tanaidacea sp.	0.0045						0.038
Chaetognatha							
Sagitta setosa	0.0008						0.009
Appendicularia							
Oikopleura dioica	0.0031						0.034
Nematoda							
Nematoda sp.	52.5715	75.9472	80.075	74.022	46.103	41.340	43.394
Cnidaria							
Ctenophora							
Planula larvae	0.0008					0.009	
Mollusca						0.032	
Gastropoda							
Tricolia pullus		0.0449					
Ichthyoplankton							
Fish eggs	2.8038	0.4265	0.546	0.375	19.496	0.483	7.754
Teleosteı							
E. encrasicolus	30.8048	7.1746	11.680	12.606	25.810	27.505	31.476
M. merlangus	0.2378	0.4582		0.03		1.258	0.531
S. sprattus	4.3132	7.3938	7.113	9.071	6.653	4.972	1.28
Syngnathus acus		0.0251		0.017			
T. trachurus	8.7150	8.0876	0.556	3.868	1.706	17.513	14.843
Insecta							
Diptera sp. pupa	0.0008					0.009	
Diptera sp.	0.0010						0.009
Others							
Sand grains	0.4261	0.4102			0.077	6.790	
Plastics	0.0031			0.003			0.009

28-30 and 18-20 cm length classes respectively, which are consistent with the findings of Yılmaz and Polat (2011). They reported the dominant size group as 31 cm for females and 17 cm for males.

In this study, the sex ratio of *A. immaculata* significantly deviated from 1:1 which is in line with the sex ratio of 1:0.67 reported by Yılmaz and Polat (2011). However, Erguden *et al.* (2011) reported a sex ratio of

Table 3. Comparison of parameters of the length-weight relationship (LWR) for *A. immaculata* from the south-east Black Sea coast, with estimates from earlier studies

Sex	Total length (cm)		Parameters of *LWR*						
	n	Min-Max	*a*	*b*	95% CI (*b*)	r^2	Pauly *t*-test	p	Reference
♀	176	13.5-32.8	0.003	3.276	0.091	0.966	5.992	<0.001	Present study
♂	60	12.5-28.7	0.006	3.099	0.300	0.908	0.745	>0.05	
B	236	12.5-32.8	0.003	3.287	0.106	0.963	6.317	<0.001	
♀	438	10.2-38.8	0.003	3.303	0.027	0.993	-	<0.05*	Yılmaz and Polat (2011)
♂	292	11.4-35.5	0.004	3.249	0.037	0.990	-	<0.05*	
B	730	10.2-38.8	0.003	3.285	0.021	0.992	-	<0.05*	
♀	294	14.0-34.2	0.010	2.970	0.066	0.955	-	-	Erguden *et al.* (2011)
♂	273	13.2-34.1	0.007	3.070	0.079	0.951	-	-	
B	567	13.2-34.2	0.008	3.040	0.050	0.952	-	-	
♀	1039	11.6-31.2	0.021	3.390	-	0.984	-	-	Samsun (1995)
♂	851	11.0-31.6	0.025	3.340	-	0.979	-	-	
B	1890	11.6-31.6	0.002	3.390	-	0.983	-	-	
B	-	9.0-36.0	0.063	2.550	-	-	-	-	Kolarov (1991)
B	191	24.2-37.7	0.071	2.488	-	0.780	-	0.03*	Yankova *et al.* (2011)

n: Number of specimens measured, B: both sexes; *: significant

1:0.93 for *A. immaculata* that did not reflect a significant deviation from 1:1.

The present study suggested a positive allometric growth for females and for sexes pooled data (including males and females) which was consistent with other studies from the Black Sea (Samsun, 1995; Erguden *et al.*, 2011; Yılmaz and Polat, 2011). However, in the present study, male *A. immaculata* exhibited an isometric growth pattern, which might be the result of a smaller sample size (n=60) for males. Interestingly, some studies from Bulgarian waters have also reported negative allometric growth in this species (Kolarov, 1991; Yankova *et al.*, 2011).

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