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## Assessment of the body indices and *Anguillicola crassus* infection status in European eel (*Anguilla anguilla*) in estuarine systems in Greece

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**ABSTRACT:** The European eel, is a catadromous species, usually recorded from benthic freshwater ecosystems to brackish and coastal habitats. The aims of the present study were to examine the morphometric features of European eel population in five estuarine systems in Northern and Western Greece, to investigate the prevalence of *Anguillicola crassus* and to identify the risk factors associated with an effect to parasitism. The total length of the examined eels (1,343) ranged from 320 mm to 991 mm and the total weight ranged from 46 g to 2,760 g. Condition factor (K) ranged from 0.02 in Mazoma lagoon to 0.44 in Vistonis and Evros estuarine systems. From the regression results on  $L_t$ - $W_t$  relationship, the coefficient of determination ( $r^2$ ) was found to have a strong correlation between  $L_t$ - $W_t$  ( $p < 0.01$ ). The overall prevalence of the parasitism was 27.1% (CI: 26.0-31.0). Among the different sampling sites, the eels collected from Vistonis estuarine system had the highest prevalence (63.5%) (CI: 57.8-68.8), following the two lagoons from Mesolonghi lagoon complex: Kleisova lagoon (36.6%) and Tholi lagoon (35.6%). The multivariate binary logistic model analysis demonstrated that the significant factors associated with the infection of *A. crassus* into the Greek eel population, were the Condition factor K, gender, life stage and eels' origin from Vistonis estuarine system. A high level of *A. crassus* parasitism in various habitats from the study area was located and the nematode found to be abundant in all *A. anguilla* habitats in Greece.

**Key words:** *Anguilla anguilla*; *Anguillicola crassus*; growth; risk factors; LWR; Greece

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## INTRODUCTION

*Anguilla anguilla* (Linnaeus, 1758) is a carnivorous predator species that spends most of its life in freshwater, brackish, and coastal habitats (Arai and Chino, 2012). When it reaches sexual maturation, its individuals transform to the silver stage and migrate to the Sargasso Sea, where they spawn and die (Tesch, 2003). *A. anguilla* feeds on small fish, crustacean, and planktonic invertebrates (De Meyer et al., 2016; Ayala et al., 2018).

An extensive worldwide reduction in the numbers of *Anguilla* sp. has been noted in the last decades (Aprahamian et al., 2021). The European eel has already been cited on the International Union for Conservation of Nature's Red List of critically endangered species (Jacoby and Gollock, 2014), while the Japanese eel *Anguilla japonica*, Temminck & Schlegel 1846, and the American eel *Anguilla rostrata*, (Lesueur, 1817) have also confirmed a severe population decline (Henderson et al., 2012; Clains et al., 2014; Dekker and Casselman, 2014; Bernotas et al., 2016; Poole et al., 2018). The critical levels of the eel populations in Europe imposed the establishment of measures for stock recovery based on European Council Regulation (EC) No 1100/2007 and management plans for eel fisheries (ICES, 2021).

Several reasons have been proposed to explain the drop in eel population, comprising: habitat loss (Bevacqua et al., 2015), pollution (Genç and Yilmaz, 2017), increased migration barriers (Rahel and McLaughlin, 2018), changes in oceanographic conditions (Knights, 2003), reduction of available prey in freshwater habitats (Smith et al., 2018), alien fish species invasions (Bevacqua et al., 2011), overfishing (Pujolar et al., 2011) and infections (McConville et al., 2018; Barry et al., 2017). Especially about parasitisms, the recent infection of the European eel by the nematode parasite *Anguillicola crassus* appears to have the potential to affect the maturation, the reproduction and the osmoregulatory processes in this fish species (Silva et al., 2018).

It has been proven that *A. crassus* -infected eels lose energy (Newbold et al., 2015), have damaged swimbladder function (Wurtz et al., 1996) and limited swimming performance (Palstra et al., 2007). As a result, the probability of eels to complete a prosperous spawning migration is reduced and concerning the Mediterranean population, only a small proportion of mature eels can successfully reach the spawning areas (Capoccioni et al., 2014).

During the last twenty years, eel landings have had a significant decline in Mediterranean countries (ICES, 2018) and the species survival is imperative due to its socioeconomic importance and its role in the biodiversity conservation (MacNamara et al., 2014; Aschonitis et al., 2017; Kitada et al., 2019). Based on the above, the present contribution's aims were: i) to examine the morphometric features of European eel population in five estuarine systems in Northern and Western Greece, ii) to investigate the prevalence of *A. crassus* in the examined eel population, and iii) to identify the risk factors associate with parasitism.

## MATERIALS AND METHODS

### Study area

European eels were collected from five sampling sites in Greece: i) Vistonis estuarine System (VES) (41,063014 25,110996), ii) Evros estuarine system (40,762811 26,082179), iii) Amvrakikos Gulf [Tsoukalio (39,061730 20,837649) and Mazoma lagoons (39,015624 20,749426)], iv) Mesolonghi lagoons complex consisting of Palaiopotamos (38,306326 21,147821), Tholi (38,320215 21,245855), Schinias (38,329382 21,338881), Vasiladi (38,332902 21,402550), Petalas (38,375333 21,101881), and Kleisova (30,345712 21,438324) lagoons, and v) Prokopos lagoon (38,147646 21,396340) (Fig. 1).

### Eels capture, morphometric data and parasites' collection

*A. anguilla* individuals were collected from 2012 to 2015. All eels were captured with the assistance of local fishing cooperatives using permanent installed traps (barrier traps), a traditional fishing technique for the exploitation of the lagoons in the Mediterranean (Koutrakis et al., 2007). Eels were euthanized according to Algers et al. (2009) and frozen (-20 °C) for later dissection. After eels were defrosted, total length ( $L_t$ ), total weight ( $W_t$ ), the horizontal and the vertical (right and left) eyes diameter, and the length of pectoral (right and left) fins were measured. The total length and total weight of eels were recorded to mm and g, respectively. The eyes diameter and pectoral fin length were measured to 0.01 mm, by means of a digital calliper.

Ocular Index (OI) was calculated according to Pankurst (1982). According to this author, eels with OI value greater than 6.5 are classified as silver eels while those with OI value less than 6.5 are referred to as yellow eels. Pectoral Fin Index was calculated

as the mean of right and left fin length. Gender determination of eels was carried out by macroscopic observation of gonads (Beullens et al., 1997). Condition Factor (K) assumes that the weight of the fish is proportional to the cube of the total length:  $K=100(W_t/L_t^3)*100$  (Richer, 1975; Wootton, 1990).

The  $L_t$ - $W_t$  relationship of eels was estimated using the formula  $W_t=a(L_t)^b$  (Le Cren 1951), which was converted into a linear equation,  $\text{Log}(W_t)=\text{Log } a+b*\text{Log}(L_t)$ , where “a” is a coefficient related to body form and “b” is an exponent describing the fish growth (isometric or not) (Koutrakis and Tsikliras, 2003).

The swimbladder was dissected and all pre-adult stages of parasitic dracunculoid nematodes were removed, counted and identified as *Anguillicola crassus* macroscopically.

### Statistical analysis

Results of descriptive statistical analysis were expressed as mean, minimum (min) and maximum (max) values. Parameters *a* and *b* and the regression coefficients of the sampling sites were calculated by the least square method (Chi-Square test). To test whether the regression coefficients depart significantly from 3, t-test was conducted. The relationship between  $L_t$  and  $W_t$  was assessed using the simple linear regression. The effect of the independent variables (sampling sites, eel length, eel weight, OI, fin index, factor K, year of sampling, stage of maturity and gender) on a sample being infected or not by a parasite was evaluated through the utilization of binary logistic models with forward LR selection. Initially, a

test of the full model against a constant only model was performed, in order to assess whether there was a statistically significant effect of the examined independent predictors on the response variable. Omnibus Tests of Model Coefficients, were performed additionally, which employ the Chi-square test to identify if there is a significant difference between the log-likelihood (-2LL) of the baseline model (constant model) and the model with the predictors. Furthermore, the Hosmer & Lemeshow (H-L) test was carried out to test whether the model provides a good fit to the data. Descriptive statistical analyses, t-test, and binary logistic regression analyses were executed using the SPSS statistics software (version 19.0). Principal component analysis (PCA) was applied for the interpretation of data variability (Reid and Spencer, 2009) and was adopted to visualize the association of various morphometric features, year and sampling sites. PCA based on eigenvalue decomposition of the data correlation matrix was performed to present relationships among sampling sites. PCA was completed using STATISTICA (version 12.0) (Stat Sft, Inc., Tulsa, OK, United States 2013).

## RESULTS

### Morphometric features and $L_t$ - $W_t$ relationship

A total of 1,343 European eels were undergone morphometric assessment. Morphometric measurements were determined for each sampling site (Table 1). The  $L_t$  of the samples ranged from 320 mm to 991 mm and the  $W_t$  from 46 g to 2,760 g. The max  $L_t$  (991 mm) and  $W_t$  (2760 g) were recorded in eels from VES, while the mean  $L_t$  and  $W_t$  in VES were 795.19 mm and

**Table 1:** Prevalence (%), mean abundance, and the number of *Anguillicola crassus* per infected eel in European eels in Greece.

Sampling sites	N	n	Prevalence (%) (95% CI)	Number of <i>A. crassus</i> per infected eel	Mean abundance (95% CI)	Mean abundance (95% CI)	
						Silver eels	Yellow eels
i) Vistonis estuary system	282	179	63.5 (57.8-68.8)	0-71	4.29 (3.35-5.23)	5.04 (3.85-6.23)	1.78 (1.12-2.45)
ii) Evros estuary system	459	93	20.3 (16.6-24.2)	0-35	0.69 (0.46-0.93)	0.23 (0.06-0.39)	0.84 (0.53-1.15)
iii) Amvrakikos Gulf (all):	53	13	24.5 (12.6-36.5)	0-8	0.49 (0.13-0.85)	0.71 (0.02-1.41)	0.46 (0.05-0.86)
Tsoukalio lagoon	20	8	40.0 (20.0-65.0)	0-8	0.90 (0.04-1.76)	-	0.25 (-0.02-0.52)
Mazoma lagoon	33	5	15.2 (6.1-27.3)	0-4	0.24 (0.00-0.51)	0.83 (0.04-1.62)	0.93 (-0.32-2.18)
iv) Mesolongi lagoon complex (all):	488	81	16.7 (13.4-20.0)	0-40	0.70 (0.47-0.93)	0.75 (0.46-1.04)	1.60 (0.41-2.79)
Palaiopotamos lagoon	135	2	1.5 (0-37.0)	0-10	0.08 (0.00-0.23)	0.03 (-0.03-0.08)	-
Tholi lagoon	45	16	35.6 (22.2-51.2)	0-40	2.31 (0.33-4.29)	3.23 (-0.69-7.15)	0.50 (-5.85-6.85)
Vasiladi lagoon	26	0	0	-	-	-	-
Schoinias lagoon	37	0	0	-	-	-	-
Petalas lagoon	133	22	16.5 (1.1-23.3)	0-10	0.72 (0.38-1.06)	0.58 (0.27-0.89)	2.17 (-0.11-4.45)
Kleisova lagoon	112	41	36.6 (27.7-44.6)	0-11	1.14 (0.74-1.54)	1.12 (0.71-1.53)	1.60 (-1.64-4.84)
v) Prokopos (Peloponnese) lagoon	61	11	18.0 (9.8-27.9)	0-25	1.36 (0.29-2.43)	1.50 (0.25-2.75)	0.56 (-0.73-1.84)
Total	1343	377	27.1 (25.7-30.5)	0-71	1.47 (1.23-1.72)	1.99 (1.58-2.41)	0.96 (0.71-1.20)

N: Total number of examined eel; n: Number of infected eels; CI: confidence interval

**Table 2:** Gender and classification of maturity stage of sampled *Anguilla anguilla*.

Sampling sites	Gender		Maturity classification	
	Female (N)	Male (N)	Silver (N)	Yellow (N)
Vistonis estuary system	282	-	217	65
Evros estuary system	459	-	110	349
Tsoukalio lagoon	18	2	6	14
Mazoma lagoon	28	5	1	32
Palaiopotamos lagoon	134	1	38	2
Tholi lagoon	35	10	22	2
Vasiladi lagoon	16	13	25	1
Schoinias lagoon	28	9	35	2
Petalas lagoon	16	117	121	12
Kleisova lagoon	40	67	107	5
Prokopos lagoon	44	17	52	9
Total	1100	241	734	493

**Table 3:** Total eel length-weight relationship in Greece.

Sampling sites	Total length-weight relationship		
	$a$	$b$ (p-value)	$r^2$
Victonis estuary system	7.416	3.624 (p=0.076)	0.878*
Evros estuary system	5.992	3.120 (p=0.393)	0.899*
Amvrakikos Gulf (all):	4.833	2.645 (p=0.669)	0.590
Tsoukalio lagoon	4.320	2.468 (p=0.498)	0.826*
Mazoma lagoon	5.002	2.700 (p=0.787)	0.578
Mesolongi lagoons (all):	5.845	3.032 (p=0.612)	0.971*
Palaiopotamos lagoon	5.977	3.078 (p=0.748)	0.893*
Tholi lagoon	5.800	3.023 (p=0.0919)	0.965*
Vasiladi lagoon	4.632	2.573 (p=0.219)	0.964*
Schoinias lagoon	6.258	3.089 (p=0.5250)	0.963*
Petalas lagoon	5.155	2.761 (p=0.360)	0.880*
Kleisova lagoon	5.845	3.035 (p=0.018)	0.960*
Prokopos (Peloponnese) lagoon	5.416	2.886 (p=0.585)	0.960*

a, b= regression coefficients (constant term and slope);  $r^2$  = coefficient of determination; \*Significant relationship on length-weight relationship (p<0.01)

1,338.18 g, respectively. The min  $L_t$  (320 mm) was found in the eels from Petalas lagoon and the min  $W_t$  (46 g) in Mazoma lagoon.

Condition factor (K) ranged from 0.02 (Mazoma lagoon) to 0.44 (VES and Evros estuarine system). OI ranged from 0.12 to 13.45, and the captured eels were classified as yellow (493 individuals) and silver (734 individuals) eels. The min and max OI were found in Mazoma lagoon and in Prokopos lagoon, respectively. The mean pectoral Fin Index was 4.64 (95%CI: 4.57-4.75), while the min and max values were 2.07 and 7.62, respectively. A total of 1100 individuals were identified as female, and 246 as male in all the sampling sites (Table 2).

From the regression results on  $L_t$ - $W_t$  relationship,

the coefficient of determination ( $r^2$ ) showed a strong correlation between  $L_t$ - $W_t$ . The coefficient of determination ( $r^2$ ) for  $L_t$ - $W_t$  revealed positive and statistically significant (p<0.01), ranging from 0.826 to 0.971 among the sampling sites (Table 3).

The values of the regression coefficient “b” were retrieved to be between 2.468 and 3.624 in the sampling sites. The highest “b” value (3.624) was discovered in the eels from VES and the lowest (2.468) from Tsoukalio lagoon (Amvrakikos Gulf). According to the t-test, the “b” value in all sampling sites, demonstrated that the regression coefficients were not significantly different from 3, indicating an isometric growth in eels.



### Modelling *A. crassus* infection in European eels

The overall prevalence of *A. crassus* (Fig. 2) was 27.1% (CI: 26.0-31.0). The results are displayed in Table 1. Among the different sampling sites, eels collected from VES had the highest prevalence [63.5% (CI: 57.8-68.8)] followed by the two lagoons from Messo-longhi lagoon complex: Kleisova lagoon (36.6%) and Tholi lagoon (35.6%). No infection was observed in two lagoons in Mesolonghi lagoon complex. Furthermore, the highest parasitic burden was recorded in VES (71 parasites), followed by Tholi lagoon and Evros estuarine system. In the Figure 3, it is showcased the percentage of the number of individual *A. crassus* in eels collected in different areas. VES, Tholi and Prokopos lagoons observed the highest percentage of eels that were infected with more than ten parasites.

The multivariate binary logistic model analysis revealed that the significant factors associated with the infection of *A. crassus* into the Greek eel population, were the condition factor K, the year of sampling, gender (female), life stage (yellow eels) and eels origin from VES, and Kleisova lagoons. Females had 2 times greater odds of being infected than males. Eels from VES and Kleisova lagoon were more likely to be infected than the eels from Petalas lagoon, 4.45 and 2.45 times, respectively. Yellow eels had 2.3 times increase of being infected than the silver eels. Condition factor K was also a significant factor in the model. For a 0.1 unit of increase in K we expected to see 294

times increase of eels being infected by the parasite. Eels captured during 2013 and 2014 had more than 3 times greater odds to have parasites in comparison with the year 2015 (Table 4).

The risk analysis of all the other factors which were evaluated in this study, such as the  $L_t$ ,  $W_t$ ,  $OL$ , and fin index showed no statistically significant correlation with the parasitic infection.

PCA produced two principal components (PC) and a linear combination of variables to consider a high amount of variance in the data set. The component 1 and component 2 both represent 33.80% of the total data variability. Scatterplot indicated that the presence of *A. crassus* was positively related to the following factors with the potential effect on parasitism: K, female eels, eels from VES, and the years: 2012, 2013 and 2015. However, it was negatively correlated with the male eels and with the sampling sites: Petalas, Palaipotamos, Vasiladi, Shoinias and Prokopos lagoons (Fig. 4).

### DISCUSSION

In the investigated sites, *A. anguilla* had a commercial value, protected status by fishing rules and high pressure on the population caused by unidentified reasons. The morpho-anatomical features and the  $L_t$ - $W_t$  relationship parameters of *A. anguilla* coming from five different sampling sites have been measured. The

**Table 4:** Binary logistic regression model related to *Anguillicola crassus* infection presence in European eel.

Variables	B	SE	Wald	Df	p	OR	95%CI	
							Lower	Upper
Date			11.564	2	0.003			
2013	1.157	0.393	8.654	1	0.003	3.180	1.471	6.874
2014	1.355	0.423	10.267	1	0.001	3.878	1.693	8.884
Female eels	0.702	0.304	5.312	1	0.021	2.017	1.111	3.663
Yellow eels	0.835	0.230	13.182	1	0.000	2.304	1.468	3.615
Sampling Sites (SS)			81.945	8	0.000			
SS: Vistonis estuary system	1.494	0.482	9.606	1	0.002	4.453	1.732	11.452
SS: Evros estuary system	-1.114	0.419	7.086	1	0.008	0.328	0.145	0.745
SS: Palaipotamos lagoon	-2.604	1.070	5.921	1	0.015	0.074	0.009	0.603
SS: Tholi lagoon	0.903	0.506	3.183	1	0.074	2.468	0.915	6.657
SS: Vasiladi lagoon	-19.800	7822.563	0.000	1	0.998	0.000	0.000	-
SS: Prokopos lagoon	-0.509	0.460	1.225	1	0.268	0.601	0.244	1.480
SS: Kleisova lagoon	0.897	0.323	7.737	1	0.005	2.453	1.304	4.617
SS: Shoinias lagoon	-20.078	6525.108	0.000	1	0.998	0.000	0.000	-
Condition factor (K)	5.684	2.504	5.150	1	0.023	293.991	2.171	39815.051
Constant	-4.143	0.575	51.908	1	0.000	0.016		

$R^2$  (Nagelkerke): 29.6%

Omnibus Tests of Model Coefficients:  $\chi^2$  (13)=251.663, p=0.000

H-L test:  $\chi^2$  (8)=8.760, p=0.363

nematode parasite *A. crassus* was detected in all the examined sampling sites in Greece of this study and a number of risk factors, associated with the infection, were identified.

Even if the eel population in the four estuarine systems denoted an isometric growth in eels, the exponential value “b” suggested that eels from VES gained weight at a faster rate concerning the length than in the other sampling sites. It is generally considered that “b” values for most of the fishes in aquatic ecosystems may differ among the species and could be affected by factors that were not examined in the present study; like quality and/or quantity of food, and gonadal development (Amin et al., 2005). The  $L_t$ - $W_t$  relationship of *A. anguilla* implied a positive allometric pattern of growth in VES, Evros estuarine system, while in Amvrakikos Gulf, Vasiladi and Prokopos lagoons, the growth was found to be negative allometric. The “b” values were very close to the value of “3” for four lagoons in Mesolonghi complex. This betrayed that *A. anguilla* manifested an isometric growth in Palaiopotamos, Tholi, Schoinias and Kleisova lagoons. Hence, it can be concluded that the biological conditions in each sampling site varied in the availability of food and the growth of eels. Even though in an open lagoon complex (Mesolonghi), the pattern of growth diverged in each lagoon as “b” ranged from 2.53-3.089.

A high degree of correlation between  $L_t$ - $W_t$  has been displayed by the regression coefficient ( $r^2$ ). Fish growth is influenced by environmental and physical parameters (Bagenal and Tesch, 1978). Those parameters determine the amount, the composition, the availability of food, as well as the behavior of fishes when they migrate or reproduce (Bond, 1996; Gkanasos et al., 2019); or even when they are under stress (Jobling, 1995; Rubin et al., 2019). It can be assumed that eel habitats in Eastern Macedonia - Thrace are rich in food both in quality and quantity and especially in VES.

The nematode *A. crassus*, which was detected in the estuarine areas of the study, revealed 27.1% prevalence in the European eel. The prevalence was not equally distributed within the sampling sites of the present study (0 - 63.5 %). Similarly to the prevalence, the mean abundance was ranged among estuarine areas. The prevalence occurred in silver eels in Sweden, Denmark, Ireland was 56% (Gérard et al., 2013), 52.7% in France (Lefebvre et al., 2013) and 14.7% in Spain (Martinez-Carrasco et al., 2011). The

prevalence of the exemplified eels from a river that flows into the Mediterranean Sea in the Middle East, was 61.1% (Genc et al., 2007).

The highest prevalence (63.5%) and highest density (71 nematodes) detected in Greece, were observed in VES. Except VES, the prevalence of the remaining sites ranged from 0-40% and the number of parasites in examined eels ranged from 0-40. The prevalence of *A. crassus* on the Evros estuarine system (20.3%) was lower than in VES even though both systems placed in Northern Greece. Many exports from VES have been made for many years, during which the transmission of the parasite (using the same contaminated tools from different ecosystems) may have been easier compared to other areas where exports are either absent or started later (time). This may enable the parasite to be successfully established in the eel populations tracked down in VES over the years, resulting in the presence of a greater parasitic burden on eel population. This quite high prevalence (63.5%) in VES can be justified not only by the long duration of its water rejuvenation, but also by the low salinity of the system, which although it communicates with the sea and has brackish waters in its southern part, it also receives large amounts of freshwater from the three rivers that they flow into it, resulting in very low salinity in its northern part. The salinity of VES fluctuated from 0.6-14 (Markou et al., 2007) all year around. The parasitic L3 infectious larva loses its virulence in salinity waters (Lefevre and Crivelli, 2012) and the outcomes of the present study were in line with the research carried out in Tunisian lagoons where high prevalence was observed in lagoons with low salinity (1 psu) (Abdallah and Maamouri, 2006).

In Mesolonghi lagoon complex, no infected eels were surveilled in the two lagoons (namely Vasiladi and Schoinias), while a highest prevalence was uncovered in Kleisova (36.6%) and Tholi lagoon (35.6%). Regarding these lagoons, the abundance was higher in the yellow eel from Kleisova and in the silver eels from Tholi, respectively. The Mesolonghi lagoon complex is the largest in Greece and the geomorphology of the area has been modified due to human intervention. Nowadays, the area consists of a system of several separate lagoons that present diverse natural and physico-chemical characteristics (Katselis et al., 2003; Alexakis, 2011). Concerning the Amvrakikos Gulf, a quite high prevalence was brought to life in the Tsoukalio lagoon (Amvrakikos Gulf) (40.0%), while in the Mazoma lagoon the prevalence was low-

er (15.2%). The River Louros flows at the western side of the Tsoukalio Lagoon, so its characteristics deviate from those of the neighboring Mazoma Lagoon. It should be highlighted that an additional number of eels from various sites of Amvrakikos Gulf need to be examined as to have an effectuated record of the situation in that area. It appeared not only in different habitats but also in the same lagoon system, such as the Messolonghi complex or the Amvrakikos Gulf, where the infection of *A. crassus* ranged. The mean abundance between silver and yellow eels varied among the sampling sites. Higher abundance was spotted in the silver eels of VES, of Tholi, and of Prokopos lagoons and in the yellow eels of Petalas and of Kleisova lagoons. Furthermore, in several lagoons (VES, Evros, Tholi, Kleisova and Prokopos), a considerable number of eels were infected with a great number of nematodes. In the case of a considerable parasitic burden, the damages in swimbladder are evident, whereas, in a smaller amount any inflammation was not observed (Dezfuli et al., 2021). Additionally, it seems, in the samplings sites of the present study, the mean abundance does not outline with the results of the north European countries where eels from the coastal waters tend to be less infected with *A. crassus* (Simon et al., 2023; Unger et al., 2024).

The binary logistic regression model disclosed that the sampling site was a risk factor. Eels from all sampling sites except from Amvrakikos Gulf, were significant variables on the model. This output might be related to the habitats, where the environmental elements are dissimilar among the sampling sites. Food availability, salinity, intermediate and paratenic hosts are some of the factors that vary in each ecosystem (Marcogliese, 2002). The model also related with *A. crassus* infection of the European eel signaled that K was a significant to the model. The factor K of a fish reflects the physical and biological circumstances and fluctuations caused by the interaction among feeding conditions, parasitic infections, physiological factors (Le Cren, 1951) and pollutant accumulation (Lortholary et al., 2021). K also connotes the changes in food reserves and therefore is an indicator of the general fish condition and identifies the variation in the physical state of the same species in divers environments (Richer, 1975). The K factor was significantly diverged among sampling sites in Spain (Costa-Dias, 2010) and its value was higher in all the examined sites compared to our study. On the other hand, K was ranged from 0.085 to 0.259 in silver stage eels in Belgium. In the current study, K shifted from 0.01

(Mazoma lagoon) to 0.44 (VES and Evros estuarine system). According to the model, eels had more than 293 chances to be infected when K increased by 0.1 degrees. This finding points out that eels with an overall global health are more likely to be parasitized.

Female individuals were 2 times more likely to have an *A. crassus* infection than male eels according to the model. These findings may be related to the dominance of eel gender which was observed in the study. All captured eels from Evros and Vistonis estuarine systems were females. In these two sampling sites the prevalence fluctuated at 20-63.5 %. The factors influencing the gender determination are associated with the selective mortality, the population density, the feeding conditions (Beullens et al., 1997), the environmental parameters such as water temperature, salt concentration, and habitats type (Andrew et al., 2005). A drop of the proportion of male eels had also been documented in northern Europe ecosystems (Poole et al., 2018; Nzau Matondo et al., 2022). Furthermore, younger eels had greater odds than the mature eels to be infected. This finding is not in accordance with other studies in Europe (Becerra-Jurado et al., 2014; Silva et al., 2018). On the contrary, in a lake in Austria, the mean value of the number of nematodes was more significant in yellow eels, but the infection with *A. crassus* had no effect in yellow eels. However, the silver eels' capacity to cope with ROS (reactive oxygen species) was significantly impaired (Schneebauer et al., 2016).

One of the most significant consequences of the model has been proved to be Vistonis estuary system which is one of the largest ecosystems of the Aegean Sea. VES is illustrated to be an estuarine area rich in food as eels, which originate from there, were found to be larger and heavier in comparison with four other estuarine areas and were more likely to be exposed to *A. crassus*. Large eels consume mostly benthic invertebrates such as arthropods and mollusks, which may pose as an important cause of *A. crassus* infection (Moravec and Skorikova, 1998). Furthermore, large size eels with high parasitic burden have been also commented in a study in France (Lefebvre et al., 2013). The high growth rate and the factor K of eels may be explained by the rich food resources of this estuarine system, which was also regarded in the same species by Golani et al. (1988) in the Lake Kinneret (Lake Tiberias), Israel. In fact, an effect of their food choice differentiates the parasitic burden by *A. crassus* (Barry et al., 2017). Most of the females from



VES were captured during their spawning migration. It seems that parasitism does not affect the eel growth in VES or on their gonads (MacNamara et al., 2014) even though several tissue damages were unearthed on the swimbladders (Kantzoura et al., 2021), since their spawning migration.

## CONCLUSION

We evaluated the body condition of *A. anguilla* and we identified the factors associated with the *A. crassus* infection. Findings of the present study are vital for environmental evaluation studies in the future. A high level of *A. crassus* parasitism in various sampling sites in the study area was retrieved. This proves that *A. crassus* is abundant in the *A. anguilla* habitats in Greece. Thus, according to the results

of the present study, further research is needed to be done in European eel populations, so as geographical distribution models to be developed. These models will monitor the *A. anguilla* population dynamics and the *A. crassus* infection in Greece and other Mediterranean regions for us to better understand the connection of the eel population reduction and the epidemiology of *A. crassus*.

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## CONFLICT OF INTEREST

None declared.

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