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Research Article

Aggregation of Nematode, *Hysterothylacium aduncum* in whiting, *Merlangius merlangus*

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ABSTRACT

Although aggregation of macroparasites characterized by unequal distribution of parasites on the host is a known axiom in marine fishes, aggregation of nematode, *Hysterothylacium aduncum* was not previously studied in whiting, *Merlangius merlangus*. Here, we investigated the host-related (fish condition factor) and parasite-related factors (parasite load) as well as the distribution pattern of *H. aduncum* in whiting to determine whether aggregation existed or not. The distribution of *H. aduncum* (third larval stage) in whiting has been shown to be aggregated. Aggregation of *H. aduncum* was assessed by Weibull distribution. The aggregation degree of nematode, *H. aduncum* in whiting was changed by the individual fish. The observed pattern of parasite distribution by the individual fish enabled the recognition of aggregation for the first time in whiting. The prediction of the intensity of *H. aduncum* in whiting improved the understanding of the host-parasite system, particularly for the dynamics of the parasite.

Keywords: Aggregation, *Hysterothylacium aduncum*, nematode, whiting, *Merlangius merlangus*, Weibull

Introduction

Parasites are important elements in fish ecology. Fish morphology, behavior, physiology, and reproduction can be affected by parasites (Timi and Poulin, 2020). The macroparasite, *Hysterothylacium aduncum* (Nematoda: Raphidascarididae) has been categorized as the generalist nematode because it has been registered for about 30 various fish species in marine teleost (Özer et al., 2016). The occurrence of *H. aduncum* has been reported for whiting (*Merlangius merlangus*) in the Black Sea previously (Ismen and Bingel, 1999). The third larval stage of *H. aduncum* is the infective period for fish. Previous stages are found in one or more crustacea as intermediate hosts (Navone et al., 1998). Quantitative descriptors of *H. aduncum* and host-parasite interaction in whiting have been studied in detail (Özer et al., 2016). The molecular description of *H. aduncum* in whiting has been published in recent studies by Pekmezci et al. (2013) and Pekmezci (2019) as well as in some other marine fish species (*Sparus aurata*, *Diplodus vulgaris* and *Solea solea*) by Keskin et al. (2015). The studies on the characteristics of nematode, *H. aduncum* in the whiting revealed its occurrence throughout the year and the increase in infection by the increase in age and length (Ismen and Bingel, 1999; Özer et al., 2016).

Macroparasites are typically aggregated on their host populations (Gaba et al., 2005; McVinish and Lester, 2020). Aggregation defines the uneven distribution of parasites within hosts. It is particularly common for the parasites hence some individuals have lots of parasites while most have very few. Aggregation is of importance in aquatic ecology concerning the factors such as reducing the size of parasite population and parasite fecundity (Wilson et al., 1996; McVinish and Lester, 2020). Fish mortality due to excessive parasite load as reflected by aggregation is also significant (McVinish and Lester, 2020). Macroparasites can be aggregated over their host (fish) populations; some fish have high parasite burdens with the uncountable number of parasites while others have one or a countable number. The distribution pattern of parasites across their host is not quantitatively identical. The aggregated distribution of parasites has been known as a law of parasite ecology (Poulin, 2011). The aggregation degree can affect the balance between host (fish) and parasite (Wilson et al., 1996). The heterogeneity in the counts of parasites per host (fish) is so conspicuous that the mean number of parasites per fish is generally a pointless characteristic for the parasitic infection level (Rózsa et al., 2000). Parasites have a tendency towards aggregation and log-normal distribution does not provide a good fit to parasite data, that's why the classical linear regression analysis can be considered irrelevant. Although there is no universally recognized standard method or mathematical model to measure aggregation (McVinish and Lester, 2020) the Weibull distribution was obviously more convenient for various degrees of aggregation (Gaba et al., 2005; Balard et al., 2020). Weibull distribution has the advantages of fitting the heavily invaded host in relation to its flexibility and low sensitivity to small sample size (Gaba et al., 2005).

The purpose of the present study was to analyze the distribution of the macroparasite, *H. aduncum* in whiting, *M. merlangus* to determine if the aggregation was prevailing form or not. The estimation of parasite intensity and aggregation pattern of *H. aduncum* in whiting described for the first time in this study contribute to our knowledge of the characteristic of parasitic systems and disease ecology.

Material and Methods

Data Sources

The whiting, *M. merlangus* (N=70) were obtained in January of 2019 from the fish market in Ankara (Turkey). The origin of the fish was the city of Sinop in the Black Sea. Fish and their parasites were examined at the Laboratory of Fish Health, the Department of Fisheries and Aquaculture, Ankara University. Fish weight and length were measured. The number of parasites (third larval stage) in the digestive system of whiting, *M. merlangus* were examined and recorded (Figure 1).

Figure 1. Nematode, *Hysterothylacium aduncum* in the digestive system of whiting

The prevalence was determined by Bush et al. (1997). The condition factor (K) was calculated from whiting samples by the equation

K=W/L3 (W: body weight (g), L: total length (cm))

Molecular Identification

Taxonomic identification of the nematode was done by adequate molecular analyses by Keskin et al. (2015). Briefly, nematode samples were preserved in absolute alcohol. DNA was extracted using QIAGEN QIAquick Extraction kit (Germany) following the manufacturer's protocol. Sequencing was carried out on an ABI Prism 310 genetic analyzer. Sequences have been archived in GenBank and accession numbers are OM884008 and OM884009.

Statistical Analysis

The relationship between the condition factor of fish and the parasite counts was tested using regression analysis.

Weibull distribution is adequate for macroparasite distributions (Gaba et al., 2005). For aggregation assessment, Weibull analyses were applied to the parasite counts using SigmaExcel. The Weibull distribution is expressed by its shape and scale parameters. The maximum likelihood method is suitable for the estimation of these parameters in the Weibull distribution (Yang et al., 2019). The parameters related to aggregation are the scale and shape in Weibull analyses (Balard et al., 2020).

Results and Discussion

Weibull distribution displayed the aggregation of the nematode. *H. aduncum* in whiting. The variance in parasite numbers was higher than the mean of *H. aduncum* counts. The model was fit using likelihood maximization (Table 1). The predicted *H. aduncum* intensity (in parasite number) can be compared to the recorded parasite distribution (Figure 2).

The aggregation expresses the case of variance higher than the mean number of parasites. The aggregation of macroparasites has been described in marine fish (Lester, 2012). Aggregation of parasites is known to be related to fish characteristics such as feeding behavior, sex, age, and resistance (Timi and Poulin, 2020). Aggregation provides a better indication for parasite distribution than the mean number of parasite load. In this study, the distribution of nematode (*H. aduncum*) in whiting (*M. merlangus*) showed aggregated pattern and the aggregation degree varied by the individual fish. The primer outcome of the present study is that the actual differences of parasite distribution among the fish is associated with generating an aggregated distribution of nematod in whiting.

Weibull distribution showed the aggregation type of nematode in fish, giving predictions, particularly for the probability of higher parasite intensity in our study. Heterogeneity in parasite loads is well represented in maximum likelihood models as with Weibull distribution (Wilson et al., 1996; Gaba et al., 2005). The variance of the parasite numbers (26.52) is higher than the mean (5.1), showing the aggregated distribution of nematodes in whiting. The shape parameter of Weibull analysis is a valuable descriptor and inversely proportional to aggregation (Gaba et al., 2005). In our study, the low level of shape parameter (1.208) showed the aggregated distribution of the nematode. Scale parameter correlated to aggregation was higher than 1, evidencing aggregation modulated by the mean number of nematode, *H. aduncum.* Weibull distribution fitted the heavily infected fish with *H. aduncum* in relation to the flexibility of the Weibull. Thus, the parasite frequency ranged from 1 to 33 in heavily infected fish. In the evaluation of aggregation degree for our study, it can be concluded that the major determinant of aggregation may be the number of infected intermediate hosts ingested by fish. The life cycle strategy of nematod may play a critical role in this type of aggregation. The findings of Klimpel and Rückert (2005) supported our interpretation of the link between parasite aggregation and fish feeding on crustacea, reporting that Hyperiids (Crustacean) identified as the obligatory intermediate host of *H. aduncum* beared nematode larvae in their haemocoel in high numbers. The resistance of fish in the context of fish immunity can be another factor in the aggregation of nematodes in fish as stated by Lester (2012). However, we have no data on the immune response of fish to support this phenomenon.

The parasite prevalence was 85% (60/70). The median values for the nematode and condition factor were 4 (min 1 and max 33) and 0.73 (min 0.013 and max 1.38), respectively. The relation between the parasite number and the condition factor of fish was not significant ($p>0.01$) as assessed by linear regression analysis (Table 2). The multiple R was 0.46, indicating a fairly weak linear relationship between the condition factor and parasite counts.

Concerning quantitative characteristics of *H. aduncum*, we found similar prevalence values in the whiting obtained from Sinop (South Black Sea), however, higher than the Balaklava Bay (North Black Sea) samples (Özer et al., 2016). In general, increasing parasite aggregation has been considered to be linked to the host's age or body size and higher parasitism has been expected in larger fish (Wilber et al., 2017). Nematode (*H. aduncum*) load has been reported to be more in the larger or longer whiting (Ismen and Bingel, 1999; Özer et al., 2016). Conversely, in the present study, the relation between the condition factor and mean parasite load was not significant, showing the condition factor as a measure of fish length and fish weight is not important in host-parasite distribution. The possible explanation can be related to the life cycle of *H. aduncum* because fish can feed an unknown number of infected intermediate hosts. This approach in relation to the feeding intake of fish was also reported to explain the seasonal infection pattern of *H. aduncum* in whiting stocks in the Black Sea, interpreting the seasonally changing amount of infected intermediate host (Özer et al., 2016). We also elucidated the aggregation of *H. aduncum* with a similar argument as explained above. Consequently, the parasite aggregation can be interconnected to the heterogeneity in the process of fish-infected intermediate host encounters, resulting in varying aggregation degrees by the individual fish. In this respect, the infected intermediate host (fed by fish) might be essential in considering interrelations between the aggregations and *H. aduncum* infections.

Table 1. Weibull values based on maximum likelihood estimates (Goodness-of-Fit: Log-likelihood -155.210, confidence level 95%, Anderson-Darling 1.548, AD *P*-value < 0.01).

Parameter	Estimate	SE Estimate	Lower 95.0% CI	Upper 95.0% CI
Shape	1.208	0.10868337	1.01281112	1.441
Scale	5.432	0.616627	4.348	6.785
Mean (MTTF)	5.101	0.550829	4.128	6.303
Standard Deviation	4.241	0.542502	3.301	5.450
Variance	26.522			
Variance/mean	5.2			
Percentage	**Percentile (Time)**	**SE Percentile**	**Lower 95.0% CI**	**Upper 95.0% CI**
0.1	0.01786015	0.010053541	0.005925715	0.053830629
0.135	0.022899764	0.012388002	0.007931579	0.066115365
0.5	0.067791268	0.030212635	0.028302024	0.162379
1	0.120574	0.04769606	0.055531545	0.261799
5	0.464726	0.130418	0.268114	0.805515
10	0.843264	0.195612	0.535193	1.329
25	1.937	0.327546	1.390	2.698
50	4.010	0.502877	3.136	5.128
75	7.118	0.767136	5.763	8.792
90	10.833	1.186	8.741	13.426
95	13.470	1.554	10.744	16.886
99	19.228	2.515	14.880	24.846
99.5	21.594	2.960	16.507	28.250
99.865	25.925	3.835	19.400	34.645
99.9	26.896	4.041	20.036	36.105

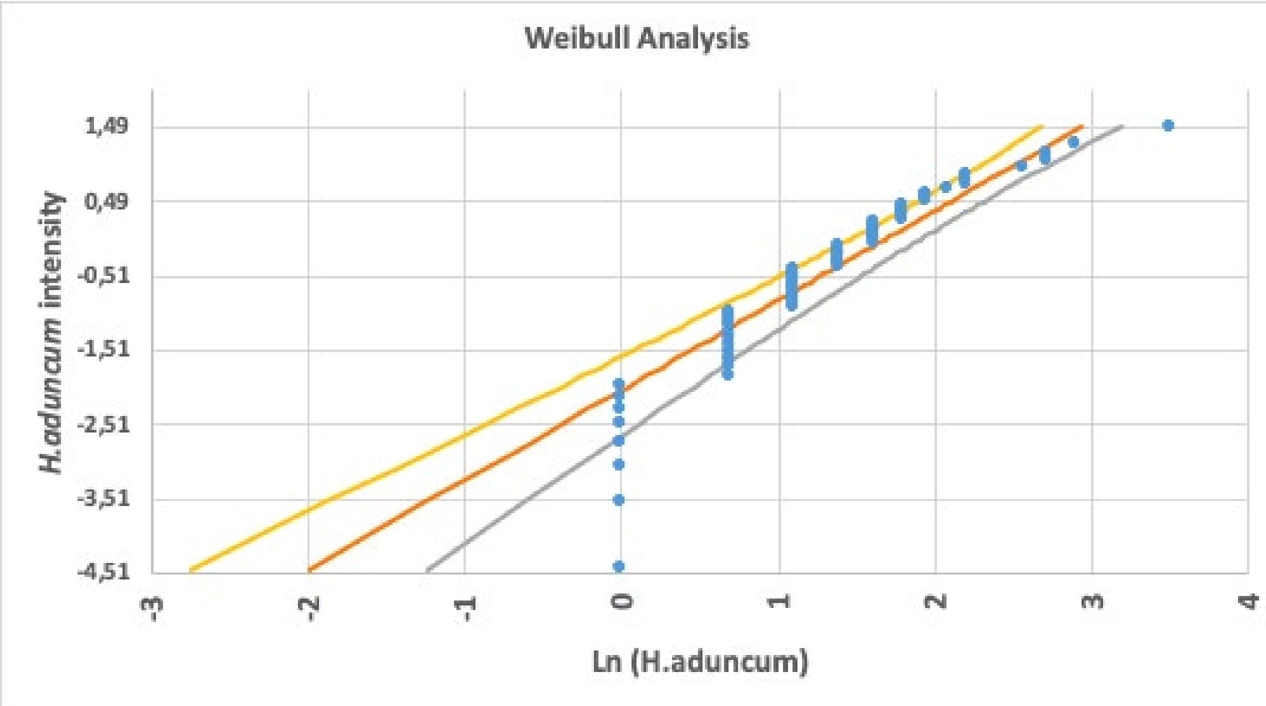

Figure 2. Weibull probability plots of *H. aduncum* intensity with 95% two-sided confidence bounds (The optimized fit is shown by a red line, the 95% CI of the fit as all parameters varying in their 95%CI, is plotted as a yellow and gray line).

Table 2. Parameters of linear regressions concerning nematode, *H. aduncum* counts and condition factor (K) of whiting

Regression statistics		ANOVA					
Multiple R	0.04696849		*df*	*SS*	*MS*	*F*	*Significance F (p)*
R Square	0.00220604	Regression	1	0.00813924	0.00813924	0.12823318	0.72157104
Adjusted R Square	-0.0149973	Residual	58	3.68138694	0.06347219		
Standard Error	0.25193687	Total	59	3.68952618			
Observations	60						

Conclusion

The aggregation of nematode, *H. aduncum* occurs in whiting and its degree varies by the individual fish, showing clear heterogeneity. The Weibull distribution gave suitable fits for the probability of *H. aduncum*. The aggregation and predicted intensity results may provide evidence for the potential for the alteration in the size of the nematode or fish population from the ecological perspective. The aggregation of *H. aduncum* in whiting is described for the first time in this study, adding to current knowledge of the interaction of marine fish and nematode in host-parasite systems.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: The research was not subjected to animal ethical permission because fish were dead and obtained from the fish market.

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Disclosure: -

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