Mar. Life Sci. (2024) 6(1): 10-16 **E-ISSN: 2687-5802**

Marine and Life Sciences

Journal Homepage: https://dergipark.org.tr/en/pub/marlife

Predictive model for the nematode (*Hysterothylacium aduncum*) in horse mackerel, *Trachurus trachurus* from the Black Sea

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Please cite this paper as follows:
Yavuzcan Yildiz, H., Genç, E., Kaya, D., & Yilmaz, B. (2024). Predictive model for the nematode (*Hysterothylacium aduncum*) in horse mackerel, *Trachurus trachurus* from the Black Sea. *Marine and Life Sciences*, *6*(1), 10-16. https://doi.org/10.51756/marlife.1424384

Research Article

Article History
Received: 23.01.2024
Accepted: 11.03.2024
Published Online: 05.04.2024

Keywords:

Parasite load
Hysterothylacium aduncum
Weibull
Horse mackerel

ABSTRACT

Hysterothylacium aduncum is a parasitic nematode that infects mainly marine fish. The information on the heterogeneity in the aggregation of macroparasites in fish species is still limited, particularly for the Raphidascarid nematode *H. aduncum* that infects exploitable marine fish species including horse mackerel (*Trachurus trachurus*). This study aimed to investigate the heterogeneity of the distribution of *H. aduncum* to **better** understand macroparasite aggregation in marine fish. Weibull tests comprising Maximum likelihood were used to analyze the frequency distribution of parasites within a fish host population over the three years period. The parasite, *H. aduncum* (third larval stage) prevalence was found to be 88% and the condition factor was 1.30∓0.029 in un-infected fish and 1.12∓0.022 in infected fish. The clustered pattern was observed in *H. aduncum* in horse mackerel. The relation between parasite load and the fish condition factor was found to be significant and conformity of length and weight was affected by parasite load. It is the first time that aggregation has been observed in *H. aduncum* distribution and forecasts of nematode intensity in horse mackerel. The findings related to the large individual variations suggest that this pattern is significant and should be considered for host-parasite dynamics.

INTRODUCTION

Parasites impact biodiversity, the food web, competition between various organisms, and host structure, so parasites are significant in ecology. Due to the close relationships between parasites and their hosts, infections are anticipated to affect hosts across various organizational levels, spanning from gene expression to population dynamics (Barber & Wright, 2005).

Hysterothylacium aduncum (Nematoda: Raphidascarididae), a macroparasite, has a broad host range and has been identified in approximately 30 marine teleost fish species, categorizing it as a generalist nematode (Rohde, 2002; Özer et al., 2016). *H. aduncum* has previously been reported in horse mackerel (*Trachurus trachurus*) from Spain coasts in the Mediterranean Sea (Adroher et al., 1996) and in *Trachurus mediterraneus* from North African coasts (Amor et al., 2011). In terms of the Black Sea's geographical location, the nematode *H. aduncum* has been documented in horse mackerel (Pekmezci et al., 2013) and in whiting (Ismen & Bingel, 1999; Özer et al., 2016; Yavuzcan et al., 2022)

In fish, *H. aduncum* infects during the third larval stage, whereas previous stages are found in one or more crustacean intermediate hosts (Navone et al., 1998). Investigations into *H. aduncum* in whiting have demonstrated its continual presence throughout the entire year although higher incidences of infection were observed in older and larger fish (Ismen & Bingel, 1999; Özer et al., 2016).

The aggregation of macro-parasites in their host populations has been described previously (Gaba et al., 2005; Grear & Hudson, 2011; McVinish & Lester, 2020). Aggregation denotes the uneven distribution of parasites among hosts.

In the case of natural fish populations, macroparasites are often aggregated, meaning that the majority of hosts have no parasites while a minority harbor a large number of them. Poulin (2011) describes aggregation as "a defining feature of metazoan parasite populations". Aquatic ecology emphasizes the importance of aggregation, which can reduce parasite population size and fecundity and decrease fish mortality resulting from excessive parasite loads (Wilson et al., 1996; McVinish & Lester, 2020). Parasites may exhibit an uneven distribution within their host populations with certain fish harboring a high number of parasites, while others have only a few.

This aggregation pattern is a fundamental law of parasite ecology (Poulin, 2011) and can influence the equilibrium between host and parasite (Wilson et al., 1996; Rabajante et al., 2020). The mean number of parasites per fish is often inadequate to measure parasitic infection levels due to the heterogeneity of parasite counts per host (Rózsa et al., 2000). The log-normal distribution is unsuitable for parasite data due to the tendency of parasites to exhibit aggregation and classical linear regression analysis may not be appropriate. Although there is no universal method or mathematical model to measure aggregation, The Weibull distribution provides a practical choice for modelling different levels of aggregation (Gaba et al., 2005; Balard et al., 2020). The Weibull distribution is a versatile method used in reliability analysis and life data analysis due to its ability to model a variety of life behaviors (Vijayalakshmi and Pushpanjali, 2022). Weibull analysis has been applied in various fields including nematode distribution in sheep (Gaba et al., 2005). Weibull analysis is used to analyze data sets containing values greater than zero (Vijayalakshmi and Pushpanjali, 2022) thus, the Weibull analyses may provide an appropriate approximation of the extensive data set of nematode numbers in fish. The Weibull distribution is advantageous because it can fit the heavily infected host while remaining flexible and less sensitive to small sample sizes (Gaba et al., 2005). The study aimed to examine the distribution of the macroparasite *H. aduncum* in horse mackerel (*T. trachurus*) and assess whether aggregation predominated. It also presents initial findings on parasite intensity and aggregation patterns, contributing to our understanding of macroparasite ecology.

MATERIALS AND METHODS

Parasite Identification

The visceral cavity and digestive system of the fish were examined for the existence of L3 of *H. aduncum*. The identification of the parasite was carried out by Adroher-Auroux & Benítez-Rodríguez (2021) using the morphological criteria of the parasite.

Data Collection

The horse mackerel (N_{total}=358; $N_{infected}$=168; $N_{non\text{-}infected}$=190) was obtained from a fish market for three consecutive years from 2019 to 2021 in Ankara (Turkey) from a fish market. The fish originated from the central Black Sea, Turkey. Subsequently, they underwent examination, and the quantification of their parasites took place at the Laboratory of Fish Health at Ankara University.

The condition factor (K) of fish was calculated by using the fish weight and length in the equation below.

$$K = \frac{W}{L^3}$$

where, W is body weight (g), L is total length (cm).

The number of parasites (3rd larval stage) in the digestive system of horse mackerel was examined and recorded. The prevalence of the parasites was calculated the method suggested by Bush et al. (1997).

Statistical Analysis

The correlation between the fish's condition factor and parasite counts and between the length and weight in infected and un-infected individuals was tested using regression analysis. Gaba et al. (2005) state that the Weibull analyses is adequate for macroparasite distributions. The Weibull distribution is suitable for assessing random effects between individual variations for macroparasites. Thus, for the aggregation assessment, Weibull analyses were employed to analyze parasite counts through SigmaExcel. The Weibull distribution is defined by its shape and scale parameters, which were estimated using the maximum likelihood method (Yang et al., 2019).

RESULTS

The variance in parasite numbers exceeded the mean count of *H. aduncum*. Therefore, likelihood maximization was utilized to fit the model, as shown in Table 1.

Table 1. Weibull distribution (Maximum likelihood estimates with the following goodness-of-fit metrics: confidence level 95%, log-likelihood -155.210, and an Anderson-Darling *P*-value < 0.01).

Parameter	**Estimate**	**SE of Estimate**	**Lower (95% CI)**	**Upper (95% CI)**
Shape	1.465	0.154348	1.192	1.801
Scale	10.990	1.124	8.993	13.429
Mean (MTTF)	9.951	0.980560	8.203	12.071
Standard Deviation	6.905	0.855236	5.417	8.802
Variance	270.35			
Variance/mean	13.16			
Percentage	**Time (Percentile)**	**SE (Percentile)**	**Lower (95% CI)**	**Upper (95% CI)**
0.1	0.098479031	0.053104515	0.034224408	0.283369
0.135	0.120882	0.06261861	0.043795115	0.333654
0.5	0.295837	0.125948	0.128430	0.681458
1	0.475641	0.179402	0.227103	0.996175
5	1.447	0.384277	0.859859	2.435
10	2.365	0.515968	1.542	3.627
25	4.695	0.735554	3.454	6.383
50	8.557	0.973295	6.847	10.694
75	13.734	1.333	11.355	16.613
90	19.419	1.942	15.962	23.625
95	23.240	2.476	18.860	28.638
99	31.168	3.830	24.497	39.656
99.5	34.299	4.437	26.617	44.197
99.865	39.879	5.601	30.283	52.515
99.9	41.106	5.869	31.073	54.381

Note: SE: Standard error; CI: Confidence interval.

Table 2. Linear regression statistics for condition factor (K) of horse mackerel and nematode, *H. aduncum* numbers

Regression Statistics		**ANOVA**					
Multiple R	0.30363632		*df*	*SS*	*MS*	*F*	*Significance F (p)*
R Square	0.09219501	Regression	1	0.00452908	0.00452908	16.8586564	6.3057E-05
Adjusted R Square	0.08672631	Residual	166	0.04459594	0.00026865		
Standard Error	0.01639055	Total	167	0.04912502			
Observations	168						

The parasite intensity of *H. aduncum* was predicted based on the actual parasite distribution using Weibull Ln probability test (Figure 1). The black line represents the best-fitting model or curve to the data and the most likely scenario, while the red lines indicate the range of variation that is statistically plausible at a 95% confidence level. The confidence interval is illustrated by the red lines. The interval is constructed such that there is a 95% probability that the true values of the parameters lie within this range.

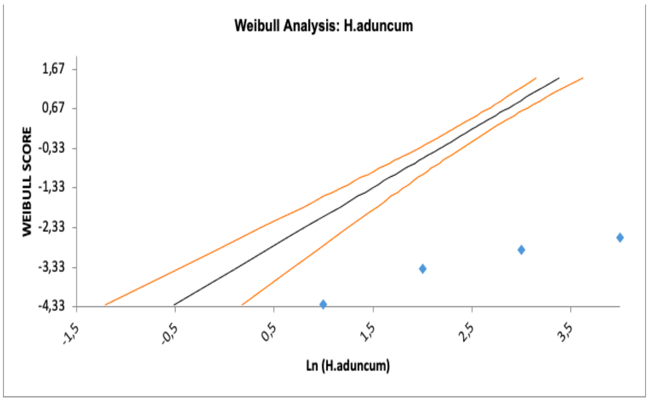

Figure 1. Weibull probability plots depicting *H. aduncum* intensity, accompanied by 95% two-sided confidence bounds. The optimized fit is represented by a black line, while the 95% confidence interval (CI) of the fit, accounting for all parameters varying within their 95% CI, is illustrated by the red lines.

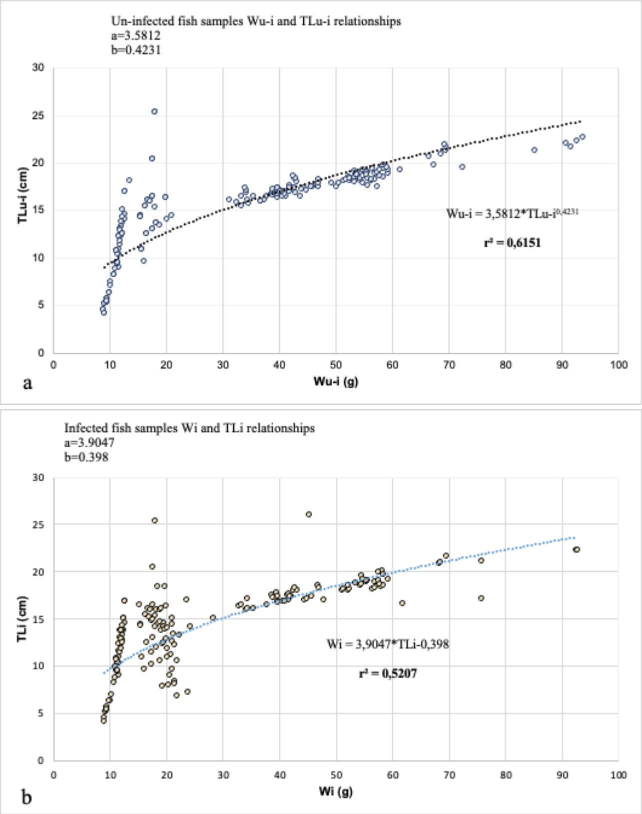

Figure 2. Relationship between fish length and fish weight in uninfected (a) and infected fish (b).

The parasite prevalence was 88% (168/190). The condition factor was 1.30∓0.029 in un-infected fish and 1.12∓0.022 in infected fish. The difference in the condition factor between infected and uninfected individuals was significant ($p<0.01$).

Linear regression analysis indicated a significant relationship ($p<0.01$) between the number of parasites and the condition factor of fish (Table 2). In addition, the multiple R value of 0.30 provided evidence of a significant linear correlation between parasite counts and the condition factor.

There were significant differences between infected and uninfected horse mackerel in their total lengths and body weight ($p<0.05$). The regression degree of relation between fish weight and length was 0.61 in uninfected horse mackerel and 0.52 in infected fish (Figure 2a, b). In un-infected fish $r^2=0.61$ showed that there was a significant and positive association between fish weight and length. In infected fish, $r^2=0.52$ indicated a moderate, positive linear correlation between the two variables (fish length and weight).

DISCUSSION

The aggregation of *H. aduncum* nematodes in horse mackerel was evident from the Weibull distribution. Aggregation manifests when the variance of parasite count surpasses the mean number of parasites, a phenomenon noted in marine fish, as documented by Lester (2012). Parasite aggregation is influenced by various fish characteristics, including host size, host specificity, feeding behavior, sex, age, resistance and schooling behavior (Amarante et al., 2015; Timi & Poulin, 2020). Therefore, aggregation provides a more accurate representation of parasite distribution than the mean parasite load. The present study found that the distribution of the nematode *H. aduncum* in horse mackerel exhibited an aggregated pattern that varied among individual fish. Our primary finding indicates that variations in parasite distribution among fish contribute to the aggregated distribution of nematodes in horse mackerel.

This study utilized the Weibull distribution to determine the aggregation pattern of nematodes in fish, allowing us to predict the probability of higher parasite intensity. Maximum likelihood models, such as the Weibull distribution, effectively capture the heterogeneity of parasite loads (Wilson et al., 1996; Gaba et al., 2005). The observed variance of parasite counts (270.35) was more significant than the mean (13.16), indicating an aggregated distribution of nematodes in horse mackerel. In Weibull analyses, parameters associated with aggregation are the scale and shape (Balard et al., 2020). Our findings are consistent with previous research suggesting that the shape parameter of the Weibull distribution exhibits an inverse relationship with aggregation, with a low shape parameter of 1.208 indicating an aggregated distribution of nematodes (Gaba et al., 2005). Additionally, we found that the scale parameter, which is correlated with aggregation, was more significant than 1, suggesting that the mean number of *H. aduncum* modulates the level of aggregation. The flexibility of the Weibull distribution made it an appropriate fit for heavily infected fish with *H. aduncum*, with parasite frequencies ranging from 1 to 76 in these fish. According to our results, the key factor influencing aggregation is the quantity of infected intermediate hosts consumed by fish. Supportively, Amarante et al. (2015) reported that the degree of parasite aggregation, especially for endoparasites typically transmitted at a trophic level, may be linked to the feeding habits of hosts.

In addition, the life cycle strategy of the nematode may also be crucial, as evidenced by Klimpel & Rückert's (2005) study, which showed that Hyperiids (a type of crustacean) served as the obligatory intermediate host for *H. aduncum* and carried large numbers of nematode larvae in their hemocoel. While we lack data on fish immunity, Lester (2012) suggests that fish resistance may also be linked to nematode aggregation in fish. Future studies should be conducted to reveal the relationship between parasitized and non-parasitic fish, blood, and liver enzymes, and immunity-related gene expressions and parasite aggregation/presence from other perspectives.

In this study, overall fish of 88% were infected with third-stage larvae (L3s), higher than the reported prevalence (31.1%) for horse mackerel on the Mediterranean coasts of South and Southeast Spain (Adroher et al., 1996). Increasing parasite aggregation has been associated with the host's age or body size, and larger fish are generally expected to have a higher parasitism rate (Wilber et al., 2017). The anticipated correlation between the extent of aggregation and body length arises from the expectation that hosts with larger lengths would be capable of harboring a greater number of parasites. This expectation is based on the premise that body size serves as an effective indicator of the total nutrients or energy available in a host to sustain a specific parasite species (Luque et al., 2004). Hence, previous research has documented an increased nematode (*H. aduncum*) burden in larger or lengthier whiting, as indicated by studies conducted by Ismen & Bingel (1999) and Özer et al. (2016).

Similarly, in our study, the relationship between the condition factor and mean parasite load was significant. On the contrary, a recent investigation by Yavuzcan et al. (2022) demonstrated that the distribution of *H. aduncum* exhibited aggregation, and the parasite load did not show a positive correlation with the fish condition factor. However, it should be noted that, according to the r^2 of the length-weight relationship in infected fish is lower compared to the non-infected ones. This indicates that the presence of parasites has an effect on the length-weight relationship of the fish.

Hence, it can be concluded that aggregation is related to the number of infected intermediate hosts consumed by fish (host). The condition factor of fish may change due to the parasite load, whether or not it is dependent on aggregation. Incorporating the varying levels of infected intermediate hosts throughout the different seasons, researchers have elucidated the seasonal infection pattern of *H. aduncum* in whiting populations in the Black Sea (Özer et al., 2016).

Furthermore, the aggregation of parasites may be linked to the heterogeneity of fish-infected intermediate host encounters, leading to varying degrees of aggregation among individual fish. In this regard, infected intermediate hosts (consumed by fish) may play a crucial role in understanding the relationship between aggregation and *H. aduncum* infections. Another important point is related to the drawbacks linked to excessive aggregation: as the number of parasites per host rises, factors like heightened host immune response, increased virulence, and intraspecific competition for energy or space will work to decrease intensity per host by raising the parasite death rate, as stated by Grear & Hudson (2011). Thus, excessive aggregation of the macroparasites in fish populations necessitates further research.

To summarize, parasite aggregation in marine fish is a complex phenomenon influenced by several factors, including host specificity, life cycle, and spatial distribution.

CONCLUSION

A typical aggregation pattern was observed among individuals of horse mackerel infected with the nematode *H. aduncum*. The degree of aggregation varied among individual fish. The Weibull distribution provided an appropriate match for the probability of *H. aduncum* and reasonable forecasts. The Weibull distribution can be applied in the field of fish parasitology to model the distribution of macroparasites. The results pertaining to aggregation and projected parasite intensity could indicate the likelihood of ecological ramifications such as alterations in the population size of either the nematode or the fish. For the first time, this research characterizes the aggregated model of *H. aduncum* in horse mackerel, furthering our comprehension of the dynamics between marine fish and nematodes within host-parasite systems. Understanding the heterogeneity in macroparasite aggregation can be important for studying parasite transmission dynamics, disease ecology, and host-parasite interactions.

ACKNOWLEDGMENTS

Authors would like to thank undergraduate students of Ankara University, Department of the Fisheries and Aquaculture for assisting the necropsy of the horse mackerel.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

HYY: Conceptualization, Writing – review & editing

EG: Supervision, Writing – review & editing

DK: Investigation, Writing – review & editing

BY: Methodology, Investigation

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

As the fish used in the study were already dead and obtained from a fish market, animal ethical permission was not required.

Funding

Not applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Adroher, F. J., Valero, A., Ruiz-Valero, J., & Iglesias, L. (1996). Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada (Spain). *Parasitology Research*, *82*, 253-256. https://doi.org/10.1007/s004360050105

Adroher-Auroux, F. J., & Benítez-Rodríguez, R. (2021). *Hysterothylacium aduncum*. In: Sitjà-Bobadilla, A., Bron, J. E., Wiegertjes, G., & Piazzon, M. C. (Eds.), Fish parasites: A handbook of protocols for their isolation, culture and transmission (pp. 311-329). 5m Books Ltd. https://doi.org/10.52517/9781789181531.021

Amarante, C. F. D., Tassinari, W. D. S., Luque, J. L., & Pereira, M. J. S. (2015). Factors associated with parasite aggregation levels in fishes from Brazil. *Revista Brasileira de Parasitologia Veterinária*, *24*, 174-182. https://doi.org/10.1590/S1984-29612015031

Amor, N., Farjallah, S., Merella, P., Said, K., & Ben Slimane, B. (2011). Molecular characterization of *Hysterothylacium aduncum* (Nematoda: Raphidascaridae) from different fish caught off the Tunisian coast based on nuclear ribosomal DNA sequences. *Parasitology Research*, *109*, 1429-1437. https://doi.org/10.1007/s00436-011-2391-7

Balard, A., Jarquín-Díaz, V. H., Jost, J., Martincová, I., Ďureje, Ľ., Piálek, J., Macholán, M., Goüy de Bellocq, J., Baird, S. J., & Heitlinger, E. (2020). Intensity of infection with intracellular *Eimeria* spp. and pinworms is reduced in hybrid mice compared to parental subspecies. *Journal of Evolutionary Biology*, *33*(4), 435-448. https://doi.org/10.1111/jeb.13578

Barber, I., & Wright, H. A. (2005). Effects of parasites on fish behaviour: Interactions with host physiology. *Fish Physiology*, *24*, 109-149.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, *83*(4), 575–583. https://doi.org/10.2307/3284227

Gaba, S., Ginot, V., & Cabaret, J. (2005). Modelling macroparasite aggregation using a nematode-sheep system: the Weibull distribution as an alternative to the negative binomial distribution? *Parasitology*, *131*(3), 393-401. https://doi.org/10.1017/S003118200500764X

Grear, D. A., & Hudson, P. (2011). The dynamics of macroparasite host-self-infection: a study of the patterns and processes of pinworm (Oxyuridae) aggregation. *Parasitology*, *138*(5), 619-627. https://doi:10.1017/S0031182011000096

Ismen, A., & Bingel, F. (1999). Nematode infection in the whiting *Merlangius merlangus euxinus* off Turkish Coast of the Black Sea. *Fisheries Research*, *42*(1-2), 183-189. https://doi.org/10.1016/S0165-7836(99)00022-3

Klimpel, S., & Rückert, S. (2005). Life cycle strategy of *Hysterothylacium aduncum* to become the most abundant anisakid fish nematode in the North Sea. *Parasitology Research*, *97*, 141-149. https://doi.org/10.1007/s00436-005-1407-6

Lester, R. J. G. (2012). Overdispersion in marine fish parasites. *Journal of Parasitology*, *98*(4), 718-721. https://doi.org/10.1645/GE-3017.1

Luque, J. L., Mouillot, D., & Poulin, R. (2004). Parasite biodiversity and its determinants in coastal marine teleost fishes of Brazil. *Parasitology*, *128*(6), 671-682. https://doi.org/10.1017/S0031182004005050

McVinish, R., & Lester, R. J. (2020). Measuring aggregation in parasite populations. *Journal of the Royal Society Interface*, *17*(165), 20190886. https://doi.org/10.1098/rsif.2019.0886

Navone, G. T., Sardella, N. H., & Timi, J. T. (1998). Larvae and adults of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda: Anisakidae) in fishes and crustaceans in the South West Atlantic. *Parasite*, *5*(2), 127-136. https://doi.org/10.1051/parasite/1998052127

Özer, A., Kornyychuk, Y. M., Yurakhno, V., & Öztürk, T. (2016). Seasonality and host-parasite interrelationship of *Hysterothylacium aduncum* (Nematoda) in whiting *Merlangius merlangus* off the southern and northern coasts of the Black Sea. *Helminthologia*, *53*(3), 248-256. https://doi.org/10.1515/helmin-2016-0025

Pekmezci, G. Z., Bolukbas, C. S., Gurler, A. T., & Onuk, E. E. (2013). Occurrence and molecular characterization of *Hysterothylacium aduncum* (Nematoda: Anisakidae) from *Merlangius merlangus euxinus* and *Trachurus trachurus* off the Turkish coast of Black Sea. *Parasitology Research*, *112*, 1031-1037. https://doi.org/10.1007/s00436-012-3227-9

Poulin, R. (2011). *Evolutionary ecology of parasites*. Princeton University Press. https://doi.org/10.1515/9781400840809

Rabajante, J. F., Anzia, E. L., & Gokhale, C. S. (2020). On the mechanistic roots of an ecological law: Parasite aggregation. *bioRxiv*, 680041.

Rohde, K. (2002). Ecology and biogeography of marine parasites. *Advances in Marine Biology*, *43*, 1-86. https://doi.org/10.1016/s0065-2881(02)43002-7

Rózsa, L., Reiczigel, J., & Majoros, G. (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology*, *86*(2), 228-232. https://doi.org/10.2307/3284760

Timi, J. T., & Poulin, R. (2020). Why ignoring parasites in fish ecology is a mistake. *International Journal for Parasitology*, *50*(10-11), 755-761. https://doi.org/10.1016/j.ijpara.2020.04.007

Vijayalakshmi, G., & Pushpanjali, K. (2022). Reliability for weibull distribution using MS-Excel. *International Journal of Statistics and Applied Mathematics*, *7*(5), 27-30. https://doi.org/10.22271/maths.2022.v7.i5a.878

Wilber, M. Q., Johnson, P. T., & Briggs, C. J. (2017). When can we infer mechanism from parasite aggregation? A constraint-based approach to disease ecology. *Ecology*, *98*(3), 688-702. https://doi.org/10.1002/ecy.1675

Wilson, K., Grenfell, B. T., & Shaw, D. J. (1996). Analysis of aggregated parasite distributions: A comparison of methods. *Functional Ecology*, *10*(5), 592-601.

Yang, F., Ren, H., & Hu, Z. (2019). Maximum likelihood estimation for three-parameter Weibull distribution using evolutionary strategy. *Mathematical Problems in Engineering*. *2019*, 6281781. https://doi.org/10.1155/2019/6281781

Yavuzcan, H., Demir, M., & Secer, F. S. (2022). Aggregation of Nematode, *Hysterothylacium aduncum* in whiting, *Merlangius merlangus*. *Aquatic Research*, *5*(4), 268-274. https://doi.org/10.3153/AR22026