Veterinary Parasitology 329 (2024) 110215

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Cumulative assessment of *Diplectanum* spp. occurrence, prevalence, and pathological impact in *Dicentrarchus labrax* from varied Egyptian fish farms

Salah Aly [a], El-Sayed Abou El-gheit [b], Hussien Osman [c], Mona M. Tolba [d], Habiba M. Essameldin [b], Mohamed Fathi [b,*]

[a] *Department of Pathology, College of Vet. Medicine, Suez Canal University, Ismailia, Egypt*
[b] *National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt*
[c] *Department of Hydrobiology, National Research Center, Cairo, Egypt*
[d] *Department of Parasitology, Medical Research Institute, Alexandria University, Egypt*

ARTICLE INFO

Keywords:
Dicentrarchus labrax
Diplectanum spp.
Monogenean trematodes
Prevalence
Clinical manifestations
Molecular identification
Fish farms

ABSTRACT

Monogenean trematodes, particularly those belonging to the Diplectanidae family, are significant metazoan parasites with substantial implications for aquaculture expansion. This study, investigatied the occurrence, prevalence, and pathological impact of *Diplectanum* spp. in European seabass (*Dicentrarchus labrax*) across three distinct Egyptian fish farms. During 2021–2022, we sampled 1800 European seabass (*Dicentrarchus labrax*) from three Egyptian fish farms (600 fish per farm). Farms 1 and 2 used semi-intensive earthen pond systems, while Farm 3 utilized an intensive floating cage system. Employing Clinical, post-mortem, parasitological, and molecular examination technique. Pathological lesions were identified, including skin and gill discoloration, emaciation, and internal organ abnormalities. Seasonal prevalence exhibited significant variations between farms, with highest rates observed in spring and Farm 3 reached an overall peak prevalence of 84.67 %. Parasitological examination distinguished two *Diplectanum* species morphologically, while molecular techniques exhibited limited specificity. Histopathology unveiled damage to gill, liver, spleen, kidney, and intestine, attributed to *Diplectanum* haptors including inflammation and internal bleeding, potentially leading to secondary infections. Molecular identification via PCR targeting ITS and 28SrDNA genes, revealing similar band sizes for the two *Diplectanum* species, indicating limited intraspecific genetic diversity. The study emphasizes investigating parasitic infections' prevalence and impact in aquaculture, necessitating robust molecular techniques for species differentiation. This study underscores the importance of investigating the prevalence and impact of parasitic infections in aquaculture. It highlights the need for robust molecular techniques to differentiate species. By focusing on *Diplectanum* spp. infections in *D. labrax,* the study offers valuable insights into managing parasites in aquaculture effectively.

1. Introduction

The European seabass as *Dicentrarchus labrax* (Linnaeus, 11758), is a key species in Mediterranean aquaculture. Its worldwide production amounted to nearly 300,000 tonnes valued at USD 1.8 million in 2021, according to FAO 2023 data (FAO, 2023). The burgeoning interest in intensive marine aquaculture production has emerged as a significant concern for the well-being of aquatic ecosystems. This surge in aquaculture activity has inadvertently heightened stress levels among fish populations, creating conducive environments for the proliferation of various fish pathogens, with parasitic infections being particularly prevalent (Kousoulaki et al., 2015).

Among the pathogens of concern in aquaculture, monogenean trematodes, specifically those in the Diplectanidae family, are significant metazoan parasites with notable implications for aquaculture expansion (Arzul et al., 2023). Notably, species like *Diplectanum aequans* and *Diplectanum laubieri* within this family have been identified as key causative agents (Alvarez-Pellitero et al., 1993; Aly et al., 2020). This family belongs to the Monopisthocotylean group, recognized as one of the primary groups within the Polyopisthocotylea class (Alvarez-Pellitero et al., 1993; WoRMS, 2018). This parasite, known to induce diseases like Diplectanosis, poses a significant threat to European

* Corresponding author.
E-mail address: aquavet@hotmail.com (M. Fathi).

https://doi.org/10.1016/j.vetpar.2024.110215
Received 25 March 2024; Received in revised form 21 May 2024; Accepted 21 May 2024
Available online 22 May 2024

seabass (*Dicentrarchus labrax*) populations (Whittington and Chisholm, 2008). Dezfuli et al. (2007) reported annual losses in juvenile *D. labrax* stocks due to *D. aequans*, estimating losses between 5 % and 10 % in Italy. Ogut and Uzun (2014) documented adverse effects of *D. aequans* on cultured juvenile seabass in the central Black Sea. The *Diplectanum* parasites follow an oviparous reproduction strategy, laying eggs on seabass gills. Their life cycle includes larval (oncomiracidium), post-larval, II. post-larval, intermediate, and adult stages, as outlined by Silan and Maillard (1989). Importantly, this parasite may undergo a host switch at any stage, with temperature influencing developmental progression (Cecchini et al., 2001). Typically, monogenean parasites manifest their presence on the skin and gills of the host fish, resulting in symptoms such as irritation, skin discoloration, and gill paleness (Noga, 2010). When *D. labrax* becomes infected with *Diplectanum* spp., histopathological analysis reveals severe damage to both primary and secondary gill lamellae, accompanied by mononuclear cell infiltrations (Eissa et al., 2017a). The consequences of such parasitic infections extend beyond individual health, affecting growth rates, overall health, and economic performance of fish populations (Fazio, 2019; Ogut and Uzun, 2014).

In light of these challenges, the primary objective of this research is to undertake: 1) investigate the parasitological and histopathological effects of *Diplectanum* spp. infections in European sea bass (*D. labrax)*. 2) Assess the impact of these parasites on the health and well-being fish, 3) Genetically identify the *Diplectanum* species using molecular PCR techniques. By examining the prevalence, pathological effects, and genetic characteristics of these infections, this study contributes to enhance our understanding of the complex dynamics between parasitic pathogens and fish populations in aquaculture systems.

2. Materials and methods

2.1. Fish sampling and location

Between the years 2020 and 2021, a total of 1800 *Dicentrarchus labrax* individuals, encompassing diverse age groups were sampled. These specimens were sourced from three distinct fish farms dedicated to the cultivation of this species. Each of these farms contributed an equal number of 600 specimens, with 150 specimens gathered during each season (three times per season each 50 specimens). The sampling period spanned 12 months, encompassing the entirety of the seasonal cycle within the specified years (Table 1).

The geographical distribution of these farms adds depth to the study's insights, The depth added to this study primarily stems from the geographical distribution of the fish farms under investigation. By including farms located in diverse geographical settings, such as proximity to northern Mediterranean lakes and inland waters like Lake Qarun, we enhance the breadth of our insights into the parasitological and histopathological effects of *Diplectanum* spp. infections. Additionally, the characterization of farming systems, including semi-intensive systems with earthen ponds and intensive systems with floating cages, provides a nuanced understanding of the relationship between farming practices and parasite prevalence and pathology. This multi-faceted approach allows for a more robust analysis of the complex dynamics between parasitic pathogens and fish populations within aquaculture systems. The first two farms strategically situated in proximity to northern Mediterranean lakes, while the third farm occupies the inland waters of Lake Qarun. Farm 1, positioned in Borg El-arab, Alexandria Governorate. On the other hand, Farm 2, located in El-Deeba-triangle, Shata, Damietta Governorate. Both Farm 1 and Farm 2 are characterized by their utilization of a semi-intensive system, employing earthen ponds as the foundation for fish cultivation. In contrast, Farm 3, situated in Wadi-el-Rayan, Fayoum Governorate and adopted an intensive cultivation system, leveraging floating cages to rear the fish population (Fig. 1).

The collected fish were promptly transferred to ice-filled containers to ensure their preservation. Subsequently, these containers were expeditiously transported to the Laboratory of Fish Diseases at the National Institute of Oceanography and Fisheries (NIOF) in Alexandera, Egypt. The examination procedures adhered to the rigorous research guidelines outlined by Jenkins et al. (2014), thereby upholding ethical standards and scientific integrity in the use of fish specimens for investigative purposes.

2.2. Clinical and post mortem (PM) investigation

The evaluation of the fish specimens followed a thorough process guided by established protocols outlined by Dezfuli et al. (2007) and Ogut and Uzun (2014). This methodical approach included a detailed visual examination of the collected fish, which involved a comprehensive assessment for any deviations in behavior and the presence of visible lesions. Both external and internal aspects were carefully inspected, covering the skin, gills, internal organs, and body cavities, to detect any potential abnormalities or clinical signs.Top of Form

Table 1
Temporal and spatial distribution of fish sampling in farms across different seasons and geographic locations in Egypt.

Farm no.			Farm 1	Farm 2	Farm 3
Site Governorate			Borg El-arab, Alexandrea	El-Deeba-triangle, Shata, Damietta	Wadi-el-Rayan, Fayoum
Coordinates of the farms			30° 50′ 56″ N, 29° 36′ 42″ E	31° 24′ 32″ N, 31° 52′ 19″ E	29° 8′ 52″ N, 30° 23′ 33″ E
Fish sampling	Season	Sample No.	Fish No.	Fish No.	Fish No.
	Spring	1st Sample	50	50	50
		2nd Sample	50	50	50
		3rd Sample	50	50	50
		Total	150	150	150
	Summer	1st Sample	50	50	50
		2nd Sample	50	50	50
		3rd Sample	50	50	50
		Total	150	150	150
	Autumn	1st Sample	50	50	50
		2nd Sample	50	50	50
		3rd Sample	50	50	50
		Total	150	150	150
	Winter	1st Sample	50	50	50
		2nd Sample	50	50	50
		3rd Sample	50	50	50
		Total	150	150	150
Total sample			600	600	600

Fig. 1. The geographical distribution of farms (Map was generated using Google Earth Pro, version 7.3.3.7692, https://www.google.com/earth/versions/." (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07058)*.

2.3. Examination for monogeneans

We conducted a detailed examination of monogenean trematodes to understand their presence and distribution. This involved obtaining these trematodes from either the skin or gills of the fish specimens using wet mounts. We carefully prepared whole gills from the fish and placed them on slides, covering them with coverslips for examination under a Stereomicroscope (Optika), allowing us to thoroughly observe the collected monogeneans.

To ensure accurate preservation and analysis, we preserved the acquired monogeneans using two different solutions. For molecular identification, we used a solution containing 70 % ethyl alcohol to preserve the genetic material of the specimens. Additionally, for effective parasitological examination, we employed a solution consisting of 5 % buffered formol saline in a mixture of 70 % alcohol and 5 % glycerin.

We followed well-defined diagnostic criteria outlined by Gonzalez-Lanza et al. (1991) and Whittington (2005) for the identification and classification of the parasitic specimens. This meticulous approach ensured the reliability and accuracy of our parasitological examination, strengthening the validity of our subsequent findings.

2.4. Seasonal and spatial prevalence of infection

To comprehend the prevalence and distribution of infection, fish samples were systematically collected from the three distinct farms situated across geographically disparate locations. This collection was orchestrated on a seasonal basis to capture potential temporal variations. Subsequently, logistic regression analyses were analyzed using SPSS 27.0 (SPSS Inc., USA). The Chi square was applied to determine significant differences among groups. The predetermined threshold for statistical significance was set at $p < 0.05$, ensuring rigorous adherence to accepted scientific standards.

2.5. Histopathological examination

A comprehensive histopathological analysis was conducted to understand the impact of Diplectanum spp. infections on Dicentrarchus labrax. Vital organs, including the gills, liver, kidney, spleen, and intestine, were meticulously examined. The process involved careful excision of organs from fish specimens, followed by preservation in a formalin solution, dehydration, embedding in paraffin wax, and slicing into thin sections (Eissa et al., 2017a).

Each step of the histopathological examination was executed with precision to ensure accurate observations. Transverse slices of the organs were stained with Mayer's hematoxylin and eosin (H&E) stain to enhance visibility and aid in identifying pathological alterations. This rigorous analysis aimed to uncover any abnormalities caused by Diplectanum spp. infections, providing valuable insights into their impact on the European seabass.

2.6. Molecular identification

A robust molecular identification method was used to understand the genetic makeup of the identified Diplectanum spp. This process aimed to enhance the accuracy of species differentiation by complementing morphological identification with genetic data. Here's a simplified breakdown of the methodology:

Four representative samples, consisting of two instances each of Diplectanum aequans and Diplectanum laubieri, were meticulously selected based on prior microscopic identification to ensure a comprehensive representation of the targeted species. Genomic DNA extraction was performed using the DNeasy Tissue kit (Qiagen), followed by concentration to a final volume of 15 μL using Microcon-100 columns (Millipore) to facilitate subsequent amplification and analysis. Two sets of primers were chosen for molecular analysis: the first targeting complete ITS cluster genes (ITS1-5.8S-ITS2) with primers BD1 and BD2 (the forward primer BD1 (5'-GTCGTAACAAGGTTTCCGTA-3') and the reverse primer BD2 (5'-TATGCTTAAATTCAGCGGGT-3')), following the methodology outlined by Luton et al. (1992), and the second targeting partial 28SrDNA fragments with primers C1 and D2(the forward primer C1 (5'-ACCCGCTGAATTTAAGCAT-3') and the reverse primer D2 (5'-TGGTCCGTGTTTCAAGAC-3')), as described by Hassouna et al. (1984). The PCR amplification process involved an initial denaturation step at 95 °C for 3 min, followed by 38 amplification cycles with specific temperature and duration parameters for denaturation, annealing, and primer extension. A final extension step at 72 °C for 5 min ensured completion of any partial DNA strands. This molecular identification approach illuminated the genetic profiles of the selected Diplectanum spp. samples, enhancing insights into their differentiation and strengthening the overall accuracy of species determination when combined with morphological identification.By using this molecular identification approach, we shed light on the genetic profiles of the selected Diplectanum spp. samples, providing valuable insights into their differentiation. This method, combined with morphological identification, strengthened the overall accuracy of species determination.

3. Results

3.1. Clinical and post mortem examination

The assessment of D*icentrarchus labrax* (*D. labrax*) fish in most cases appeared apparently normal. However, the severely infected fish with *Diplectanum* spp. revealed a spectrum of clinical indications, including skin and gill discoloration, emaciation, debilitation, surface clustering, and lethargic swimming. Examination of hemibranches unveiled excessive mucus secretion, paleness, congestion, hemorrhagic skin, and alterations in the internal organs. The liver appeared pale, while the spleen and kidney exhibited congestion, as illustrated in Fig. 2.

3.2. Examination for monogeneans

Upon conducting parasitological examination, two distinct monogeneans species were identified within the *Diplectanum* genus, belonging to the *Diplectanidae* family of the Monopisthocotylea order within the Platyhelminthes phylum. Through meticulous microscopic scrutiny, the initial species recognized was *Diplectanum aequans* (Wagener 1857, Diesing 1858), as depicted in Fig. 3. Subsequently, the second species, *Diplectanum laubieri* (Lambert and Maillard 1974), was also identified and is illustrated in Fig. 4 and 5.

3.3. Seasonal and spatial distribution of infection

Logistic regression analyses were conducted to assess associations between site locations, seasons, and the prevalence of *Diplectanum* spp. The outcomes are summarized in Table 2, offering insights into these relationships. Across different seasons, varying parasite prevalence rates were observed at different sites, underscoring the need to consider both factors when evaluating *Diplectanum* spp presence. In the "Borg El-Arab" site, during summer and autumn, the prevalence was 36 %, while in spring and winter, it was 40 % and 20 % respectively, resulting in an

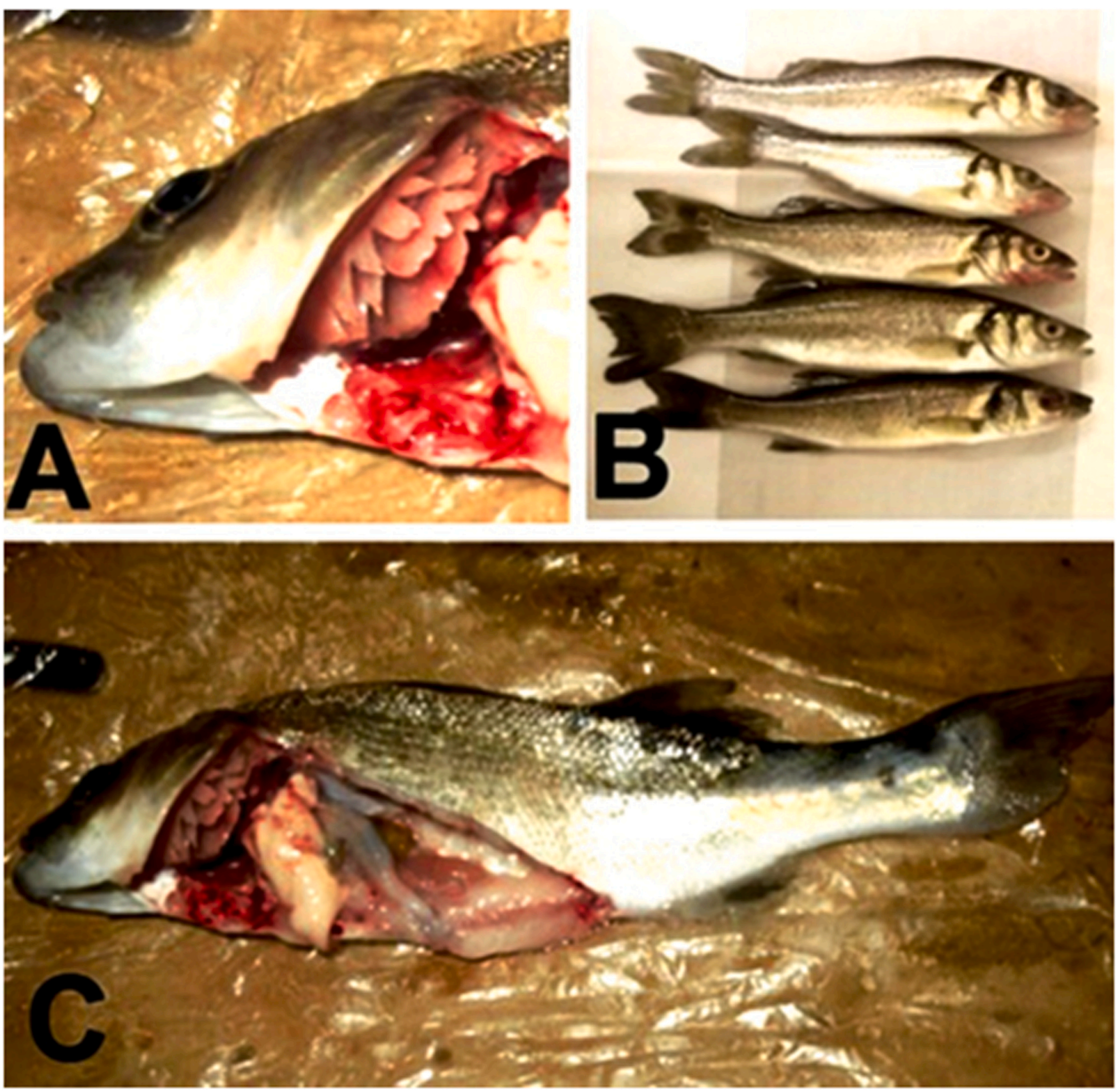

Fig. 2. *D. labrax* infected with *Diplectanum* spp., **(A)** The gill filaments exhibited paleness with hemorrhagic infiltrations and excessive mucus secretions. **(B)** Fingerlings and juveniles showing emaciation. **(C)** The adult *D. labrax* showing pale gills and liver, along with sloughing of scales and eroded caudal fins. (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07059)*.

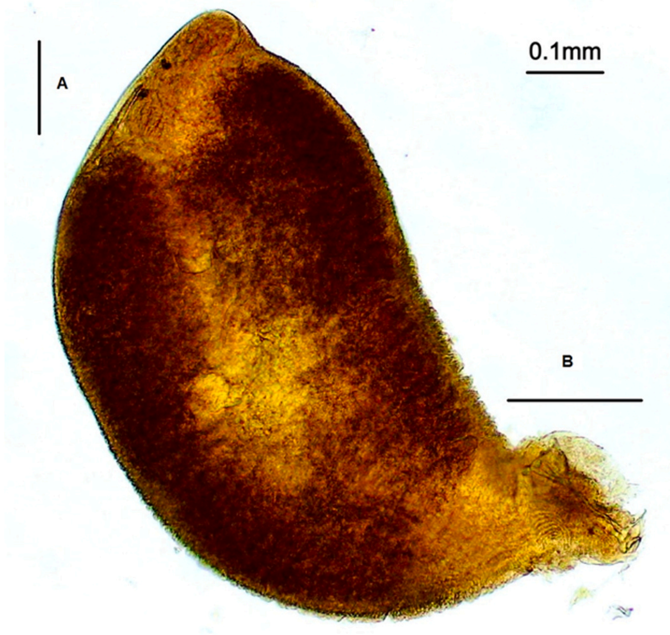

Fig. 3. Showing a *Diplectanum aequans* monogenean in its entirety, with a scale bar measuring 0.1 mm. (A) The anterior prohaptor has two pairs of eyespots and a spherical pharynx. (B) The posterior opisthaptor is depicted with squamodiscs, hamuli, and marginal hooklets forming a transverse bar. (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07060)*.

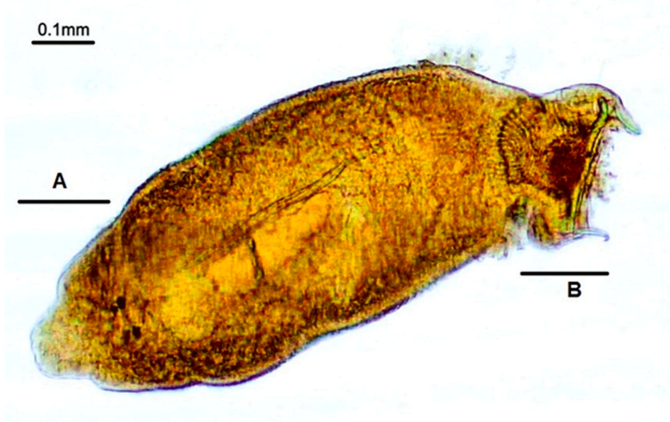

Fig. 4. Showing a *Diplectanum laubieri* monogenean in its entirety, with a scale bar measuring 0.1 mm. (A) The anterior prohaptor features two pairs of eyespots and a rounded spherical pharynx.(B) The posterior opisthaptor is depicted with squamodiscs, hamuli, and marginal hooklets forming a transverse bar. (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07061)*.

overall rate of 33 %. The Chi-Square statistic for this site-season combination was 5.3370, with a p-value of 0.1487, suggesting that while there are seasonal variations, statistical significance may be lacking.

Similarly, at the "Edeeba" site, prevalence rates during summer, spring, autumn, and winter were 32 %, 48 %, 38 %, and 28 %, contributing to an overall rate of 36.5 %. The Chi-Square value was 4.8970, with a p-value of 0.1795, indicating potential seasonal differences in *Diplectanum* spp presence without statistical confirmation. In contrast, the "Rayan" site exhibited significant disparities. During summer and autumn, prevalence rates were 64 % and 62 %, while in spring and winter, they decreased to 42 % and 36 %. The overall prevalence rate across seasons was 61.5 %. The Chi-Square statistic for this site-season combination was notably high at 24.5592, with a p-value of < 0.0001, strongly suggesting significant associations between the "Rayan" site and *Diplectanum* spp prevalence across different seasons.

Combining data from all sites, summer, spring, autumn, and winter prevalence rates were 44.00 %, 86.00 %, 45.33 %, and 28.00 %, respectively, resulting in an overall prevalence rate of 43.67 %. The Chi-Square value for this combination was 39.2836, with a p-value of < 0.0001, highlighting highly significant associations between site, season, and *Diplectanum* spp presence. While the combining data across different seasons., in Borg El-Arab, Edeeba, and Rayan, the respective percentage of cases with parasites were 33 %, 36.5 %, and 61.5 %, respectively. The Chi-Square statistic was 26.5324, with an associated p-value of < 0.0001, highlighting significant associations between site and parasite prevalence across all seasons. These results underscore the need to consider both site and seasonal factors when evaluating the likelihood of *Diplectanum* spp presence.

The prevalence of *Diplectanum* spp. infection in the examined *D. labrax* individuals exhibited a pronounced disparity ($p \leq 0.05$) in both comparisons across different farms and within the same farm. The highest infection rates were detected during the spring season, reaching a prevalence of 57.30 %. In subsequent seasons, infection rates displayed a descending trend, with rates of 45.56 % in summer, 44.44 % in autumn, and 27.78 % in winter. Farm 3 exhibited the most prominent prevalence rate at 61.83 %, followed by Farm 2 with 36.50 %. In contrast, the lowest prevalence rate was noted in Farm 1, measuring 33 % (Table 2 & Fig. 6).

Of significance, the prevalence rates within different farming systems exhibited intriguing patterns. Farm 3, which employed an intensive cage culture system, recorded the highest prevalence rate. This was closely followed by Farm 2, also utilizing an intensive system, and Farm 1, which employed a semi-intensive pond culture system. Moreover, the age of the host organism appeared to exert a notable influence on the fluctuating prevalence of the parasite.

3.4. Histopathological findings

The histopathological analysis unveiled significant lesions primarily affecting the gills. These lesions encompassed the shedding and demise of the delicate, plate-like epithelial tissue, accompanied by the loss of surface secretory cells. Furthermore, discernible degenerative changes manifested in the liver, spleen, kidney, and intestine, resulted intra-tissue hemorrhaging, as visually depicted in Fig. 7.

These pathological changes are likely attributed to the presence of *Diplectanum haptors*, which utilize their marginal hooks to attach to the gills, which serve as the parasite's target organ. This infestation led to desquamation and necrosis in the primary lamellar epithelium of the gills. Subsequently, there was destruction of the secondary gill lamellae, with fusion occurring for most or part of their length. Additionally, the edge of the affected filaments sometimes exhibited numerous surface secretory cells, this attachment triggers an inflammatory response, disrupting the integrity of blood and immune barriers. Consequence, the affected fish becomes susceptible to secondary infections from bacteria, fungi, or viruses. This secondary infection cascade ultimately exacerbates the harm inflicted on internal organs. Furthermore, degenerative changes were observed in other organs such as the liver, spleen, kidney, and intestine. These changes included cellular degeneration accompanied by hemorrhagic infiltration between the tissues,

3.5. Molecular characterization

The analysis revealed minimal distinguishable disparity in molecular dimensions between the two *Diplectanum* species. The *D. aequans*, exhibited bands with molecular size of 975 and 960 base pairs (bp) when using the first primer. For the second set of primers, the sizes 770 and 765 bp. On the othe hand, the *D. laubrei* displayed smaller sizes with the

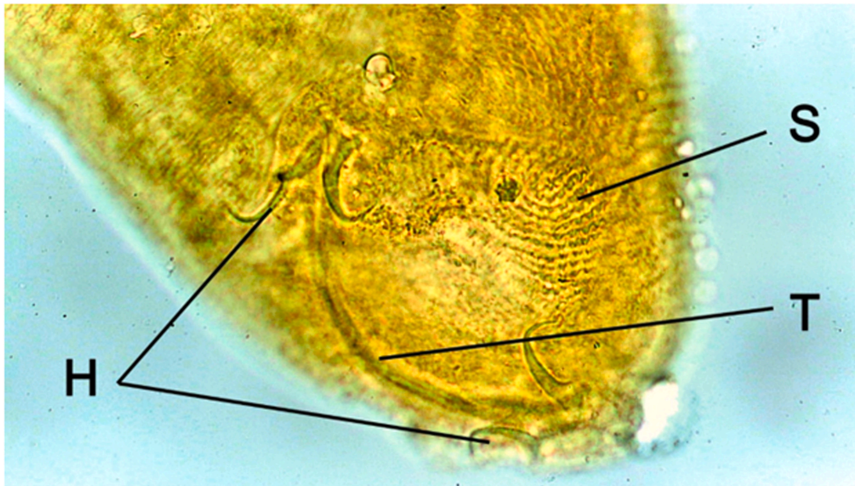

Fig. 5. The posterior opisthaptor of both *Diplectanum* species. The letter (S) referred to the squamodiscs, the letter (H) referred to the marginal hooklets, and the letter (T) refereed to the transverse bar. (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07062)*.

Table 2
The logistic regression analyses to examine the association within each site, within each season and the outcome variable presence of *Diplectanum* spp (P ≤ 0.05).

		Site				
Season		**Borg El-Arab**	**Edeeba**	**Rayan**	**Chi-Square**	**p**
Summer	(%)	36	32	64	8.4469	0.0146
Spring	(%)	40	48	84	22.4564	< .0001
Autumn	(%)	36	38	62	12.3377	0.0021
Winter	(%)	20	28	36	3.1746	0.2045
All seasons	(%)	33 %	36.5	61.5	26.5324	< .0001
Chi-Square		5.337	4.897	24.5592		
p		0.1487	0.1795	< .0001		

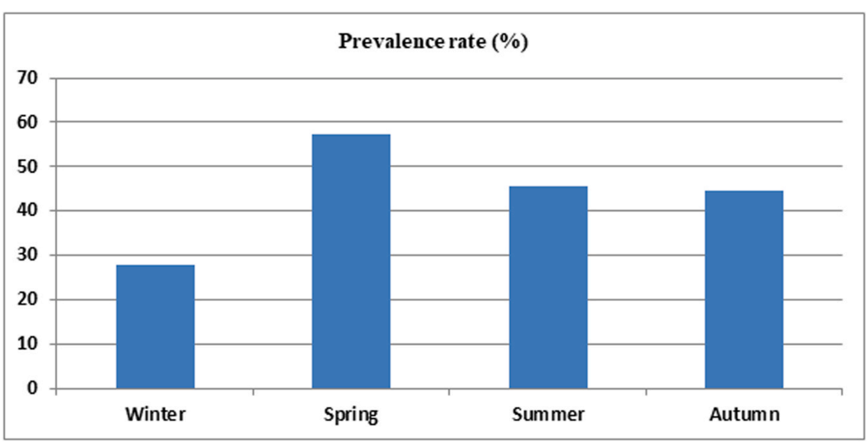

Fig. 6. Showing the seasonal prevalence differences of *Diplectanum* spp in *D. labrax*. (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07063)*.

first primer set, measuring 950 and 940 bp. However, both samples shared a common 750 bp product when subjected to the second primer set (Fig. 8 and 9).

4. Discussion

The comprehensive examination of clinical and post-mortem findings in the present study unveiled a range of clinical manifestations in *Dicentrarchus labrax* fish, including skin and gill discoloration, emaciation, debilitation, surface clustering, and lethargic swimming. consistent with prior studies in Adriatic Sea (Mladineo, 2005), Italy (Fioravanti et al., 2006), and Egypt (Abdel-Mawla and El-Ekiaby, 2012; Eissa, et al., 2017b).

Notably, the identification of two distinct *Diplectanum* species, *Diplectanum aequans* and *Diplectanum laubieri*, both belonging to the *Diplectanidae* family. This identification corroborates the findings of previous studies by Aydin and Pekmezci (2023) and Derbel et al. (2022), thus strengthening the body of knowledge surrounding the taxonomy of these monogenean parasites. While these species exhibited a similar body shape, variations in size were evident, with *D. aequans* being larger than *D. laubieri* (Vanhove et al., 2022; Li et al., 2022).

The observed temporal and spatial distribution of infection within

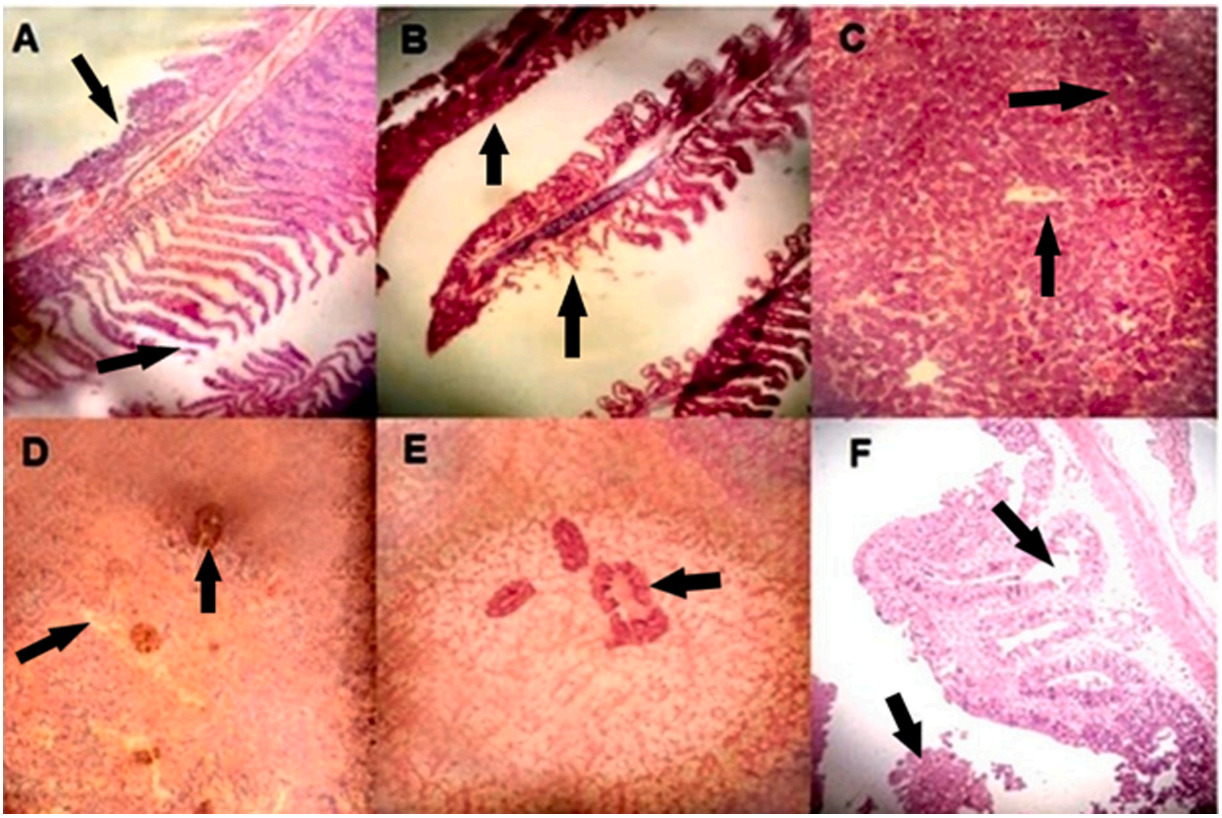

Fig. 7. Images A and B depict the gills of *D. labrax* that are infested with *Diplectanum* spp., highlighting shedding (SH) and necrosis (NC) of the primary and secondary lamellar epithelium. Meanwhile, images C, D, E, and F demonstrate degenerative changes and hemorrhages between the tissues in the liver, spleen, kidney, and intestinal mucosa, respectively. (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07064*).

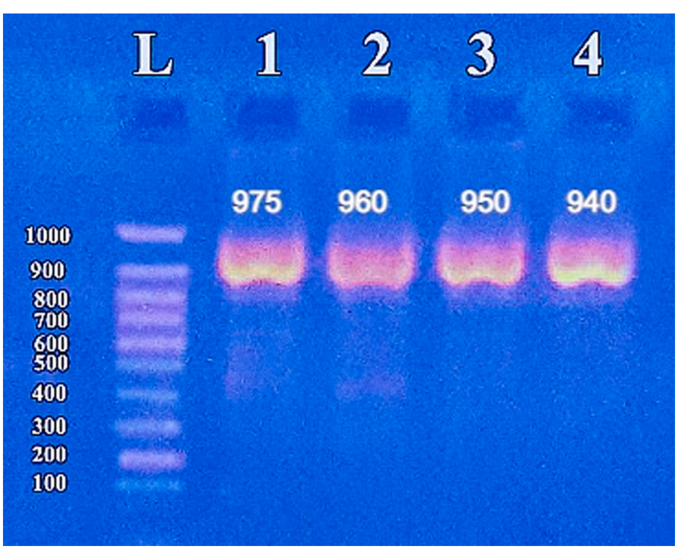

Fig. 8. Using the complete ITS cluster primer, PCR amplification was performed for all four *Diplectanum* species. The DNA ladder, which ranged from 100 to 1000 bp, is represented by "L". For *D. aequans*, the resulting molecular bands ranged from approximately 960 to 975 bp and are depicted in lanes 1 and 2. In comparison, *D. laubrei* produced molecular bands sized around 940–950 bp, as illustrated in lanes 3 and 4. (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07065*).

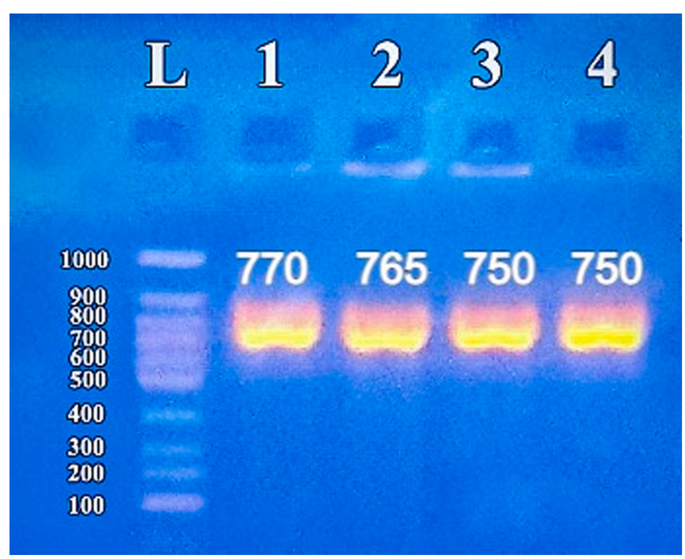

Fig. 9. PCR amplification was performed using the Partial 28SrDNA fragments primer for all four *Diplectanum* species. The DNA ladder ranging from 100 to 1000 bp is represented by "L". The molecular bands for *D. aequans* were approximately 765–770 bp, as depicted in lanes 1 and 2. For *D. laubrei*, both samples produced molecular bands of 750 bp, shown in lanes 3 and 4. (*A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07066*).

the examined D. labrax specimens necessitates careful examination. The heightened prevalence rates observed during the spring season are consistent with findings reported by Li et al. (2022) and Özer et al. (2019), suggesting a potential seasonal pattern in Diplectanum spp. infection prevalence. This phenomenon may be influenced by environmental variables, such as temperature and water quality, known to impact parasite lifecycle dynamics. Moreover, the variability in prevalence rates among different farms underscores the influence of farm-specific conditions on parasite dynamics. The intensive cage culture system implemented by Farm 3 is associated with a higher prevalence rate, likely due to increased host density and elevated stress levels (Jerônimo et al., 2022; Anshary, 2020). These observations align with the findings of Negreiros et al. (2021), emphasizing the significance of considering farming practices in comprehending parasite transmission dynamics.

Histopathological findings provide further insight into the impact of *Diplectanum* spp. on the host's health. The significant lesions observed in the gills, liver, spleen, kidney, and intestine are indicative of the destructive consequences of monogenean infection. These findings resonate with the work of Saraiva et al. (2015) and Chotchaphan et al. (2019), who reported similar histopathological alterations in fish infected with monogenean parasites. The disruption of blood and immune barriers due to the attachment of haptors and subsequent secondary infections aligns with the theoretical framework proposed by Mladineo et al. (2023), highlighting the cascade of pathological events triggered by monogenean infections.

Molecular characterization studies shed light on the genetic dimensions of the studied *Diplectanum* species. The slight differences in molecular sizes between the two species, particularly in the PCR fragments generated by the different primer sets, echo the findings of genetic studies conducted by Desdevises et al. (2000) and Villar-Torres et al. (2019). These subtle variations in molecular dimensions further support the distinctiveness of the identified species and could potentially serve as genetic markers for their discrimination.

To sum up, this study's results offer a thorough summary of the parasitological, histological, clinical, and molecular aspects of *Diplectanum* spp. infection in *D. labrax*. The observed clinical signs, differences in occurrence rates, growth stage vulnerability, and histological changes all highlight how important it is to comprehend how monogenean parasites affect *D. labrax* health and aquaculture. These findings have profound implications for *D. labrax* management and sustainable production as the aquaculture sector develops. In the future, studies could focus on clarifying the molecular pathways that underlie the pathogenic effects that have been identified and exploring possible approaches to intervention to lessen the negative impacts of monogenean infections on fish health and aquaculture productivity.

Ethical Statements

The trial was performed following the Universal Directive on the protection of animals used for scientific purposes according to ethical guidelines approved by the ethics of scientific research committee, Faculty of NIOF Committee for Ethical Care of Marine Organisms and Experimental Animals (NIOF-IACUC), Cairo, Egypt with approved code No. (**NIOF- AQ5- F22-D-030**).

Funding statement

This work received no specific grant from any funding agency

Consent to participate and publish

Not applicable.

CRediT authorship contribution statement

Hussien Osman: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Mona M. Tolba:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Salah Aly:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **El-Sayed Abou El-gheit:** Investigation, Formal analysis, Data curation, Conceptualization. **Habiba M. Essameldin:** Writing – original draft, Resources, Methodology, Investigation, Formal analysis. **Mohamed Fathi:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

Abdel-Mawla, H.I., El-Ekiaby, W.T., 2012. Some studies on parasitic infection among Morone Labrax (sea bass fish) as bio indicator of environmental conditions. Egypt. J. Aquac. 2 (3).
Alvarez-Pellitero, P., Sitja-Bobadilla, A., Franco-Sierra, A., 1993. Protozoan parasites of wild and cultured sea bass, Dicentrarchus labrax (L.), from the Mediterranean area. Aquac. Fish. Manag. 24 (1), 101–108.
Aly, S.M., El-Gheit, A., Fadel, A., Essam El-Din, H., 2020. Digentetic trematodes in Dicentrarchus labrax cultured in egypt: prevalence, clinical features, body condition, and histopathology. Egypt. J. Aquat. Biol. Fish. 24 (3), 463–480.
Anshary, H., 2020. Survey on ectoparasite occurrence of fish groupers sent to Fish Quarantine Agency for diseases inspection. In: IOP Conference Series: Earth and Environmental Science, 564. IOP Publishing, p. 012061 (No. 1).
Arzul, I., Canier, L., Thieltges, D., Wegner, M., de Montaudouin, X., 2023. International Symposium on Ecology and Evolution of Marine Parasites & Diseases. 15th–18th of November 2022, La Rochelle, France. Book of Abstracts.
Aydin, C., Pekmezci, G.Z., 2023. An integrative study of morphological and molecular characterization of Diplectanum aequans (Diplectanidae: Monogenea) infecting European sea bass Dicentrarchus labrax (L.) from Turkish coasts. Ank. Üniv. Vet. Fak. ültesi Derg. 1–20.
Cecchini, S., Saroglia, M., Cognetti-Varriale, A.M., Terova, G., Sabino, G., 2001. Effect of low environmental temperature on embryonic development and egg hatching of Diplectanum aequans (Monogenea, Diplectanidae) infecting European sea bass, Dicentrarchus labrax. Fish Pathol. 36, 33–34.
Chotchaphan, P., Wongkham, W., Wattanachot, J., Sonthi, M., 2019. Gill histopathology of cage cultured Asian sea bass (Lates calcarifer) infected by Lernanthropus sp. and Diplectanum sp. Kaen Kaset = Khon Kaen Agric. J. 47 (Suppl. 2), S509–S514.
Derbel, H., Châari, M., Neifar, L., 2022. Checklist of the Monogenea (Platyhelminthes) parasitic in Tunisian aquatic vertebrates. Helminthologia 59 (2), 179–199.
Desdevises, Y., Jovelin, R., Jousson, O., Morand, S., 2000. Comparison of ribosomal DNA sequences of Lamellodiscus spp. (Monogenea, Diplectanidae) parasitising Pagellus (Sparidae, Teleostei) in the North Mediterranean Sea: species divergence and coevolutionary interactions1Note: Nucleotide sequence data reported i. Int. J. Parasitol. 30 (6), 741–746. https://doi.org/10.1016/S0020-7519(00)00051-5.
Dezfuli, B.S., Giari, L., Simoni, E., Menegatti, R., Shinn, A.P., Manera, M., 2007. Gill histopathology of cultured European sea bass, Dicentrarchus labrax (L.), infected with Diplectanum aequans (Wagener 1857) Diesing 1958 (Diplectanidae: Monogenea). Parasitol. Res. 100 (4), 707–713. https://doi.org/10.1007/s00436-006-0343-4.
Eissa, I., El-lamie, M., Aly, S., Sallam, N., 2017b. Studies on the prevailing parasitic diseases in some marine fishes. Egypt. Vet. Med. Soc. Parasitol. J. (EVMSPJ) 13 (6), 64–77. https://doi.org/10.21608/evmspj.2017.37769.
Eissa, I.A., Ismail, M.M., Aly, S.M., Ahmed, M.M., 2017a. Studies on prevailing parasitic trematodiasis affecting some cultured marine fishes in Ismailia Governorate. Suez Canal Univ. Med. J. 22 (2), 165–183.
FAO, 2023. —Food and Agriculture Organization of the United Nations. Available online: ⟨https://www.fao.org/fishery/statisticsquery/en/aquaculture/aquaculture_quantity⟩ (Accessed 1 November 2023).
Fazio, F., 2019. Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture 500, 237–242. https://doi.org/10.1016/j.aquaculture.2018.10.030.
Fioravanti, M.L., Caffara, M., Florio, D., Gustinelli, A., Marcer, F., 2006. A parasitological survey of European Sea bass Dicentrarchus labrax and Gilthead Seabream Sparus aurata Cultured in Italy. Vet. Res. Commun. 30 (S1), 249–252. https://doi.org/10.1007/s11259-006-0053-5.
Gonzalez-Lanza, C., Alvarez-Pellitero, P., Sitja-Bobadilla, A., 1991. Diplectanidae (Monogenea) infections of sea bass, Dicentrarchus labrax (L.), from the Spanish Mediterranean area. Parasitol. Res. 77 (4), 307–314. https://doi.org/10.1007/BF00930906.
Hassouna, N., Mithot, B., Bachellerie, J.-P., 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA In higher eukaryotes. Nucleic Acids Res. 12 (8), 3563–3583. https://doi.org/10.1093/nar/12.8.3563.

Jenkins, J.A., Bart Jr, H.L., Bowker, J.D., Bowser, P.R., MacMillan, J.R., Nickum, J.G., Warkentine, B.E., 2014. Guidelines for the Use of Fishes in Research. American Fisheries Society, Bethesda.
Jerônimo, G.T., da Cruz, M.G., Bertaglia, E.D.A., Furtado, W.E., Martins, M.L., 2022. Fish parasites can reflect environmental quality in fish farms. Rev. Aquac. 14 (3), 1558–1571.
Kousoulaki, K., Sæther, B., Albrektsen, S., Noble, C., 2015. Review on European sea bass (Dicentrarchus labrax, Linnaeus, 1758) nutrition and feed management: a practical guide for optimizing feed formulation and farming protocols. Aquac. Nutr. 21 (2), 129–151.
Li, W., Yang, B., Cheng, J., Zou, H., Li, M., Wang, G., 2022. Seasonal dynamics of Dactylogyrus species (Monogenea: Dactylogyridae) on wild and farmed goldfish (Carassius auratus): implication for prevention of dactylogyriasis. Aquac. Rep. 26, 101327.
Luton, K., Walker, D., Blair, D., 1992. Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). Mol. Biochem. Parasitol. 56 (2), 323–327. https://doi.org/10.1016/0166-6851(92)90181-I.
Mladineo, I., 2005. Parasite communities of Adriatic cage-reared fish. Dis. Aquat. Org. 64, 77–83. https://doi.org/10.3354/dao064077.
Mladineo, I., Volpatti, D., Beraldo, P., Rigos, G., Katharios, P., Padros, F., 2023. Monogenean Sparicotyle chrysophrii: the major pathogen of the Mediterranean gilthead seabream aquaculture. Rev. Aquac.
Negreiros, L.P., Neves, L.R., Tavares-Dias, M., 2021. Parasites in Leporinus macrocephalus (Anostomidae) of four fish farms from the western Amazon (Brazil). An. Acad. Bras. Ciênc. 93, e20190988.
Noga, E.J., 2010. Fish Disease: Diagnosis and Treatment, Second Edi. Wiley-Blackwell.
Ogut, H., Uzun, E., 2014. Incidence and prevalence of Diplectanum aequans and its influence on the fitness of juvenile sea bass Dicentrarchus labrax in the Black Sea. Aquac. Res. 45 (4), 742–748. https://doi.org/10.1111/are.12015.
Özer, A., Okkay, S., Öztürk, T., Baki, B., Acar, G., Güven, A., Öztürk, D.K., 2019. Comparative Diplectanum aequans (Monogenea) infections in cultured European seabass (Dicentrarchus labrax) in the Black Sea and the Aegean Sea. Ege J. Fish. Aquat. Sci. (EgeJFAS)/Su ÜR. ünleri Derg. 36 (2).
Saraiva, A., Costa, J., Serrão, J., Eiras, J.C., Cruz, C., 2015. Study of the gill health status of farmed sea bass (Dicentrarchus labrax L., 1758) using different tools. Aquaculture 441, 16–20.
Silan, P., Maillard, C., 1989. Biologie comparee du developement et discrimination des Diplectanidae ectoparasites du Bar (Teleostei). Ann. Des. Sci. Nat. Zool. 13, 31–45.
Vanhove, M.P., Giakoumi, S., Zogaris, D., Kovačić, M., Huyse, T., 2022. First Eastern Mediterranean record of Xenoligophoroides cobitis, the only dactylogyrid Monogenean infecting Mediterranean gobies: just arrived or missed the boat? Diversity 14 (8), 580.
Villar-Torres, M., Repullés-Albelda, A., Montero, F.E., Raga, J.A., Blasco-Costa, I., 2019. Neither Diplectanum nor specific: a dramatic twist to the taxonomic framework of Diplectanum (Monogenea: Diplectanidae). Int. J. Parasitol. 49 (5), 365–374.
Whittington, I.D., 2005. Monogenea Monopisthocotylea (Ectoparasitic flukes). In: Marine Parasitology. CSIRO Publishing, Melbourne, Australia.
Whittington, D.I., Chisholm, L.A., 2008. Diseases caused by Monogenea. In: Eiras, J.C., Segner, H., Wahli, T., Kapoor, B.G. (Eds.), Fish Diseases, 2. Science Publishers, USA.
WoRMS, 2018. World Register of Marine Species. Diplectanum Diesing, 1858. Accessed at: ⟨http://www.marinespecies.org/aphia.php?p=taxdetails&id=119291⟩ on 29 October 2018.