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SEASONAL PATTERNS AND HOST FACTORS INFLUENCING ANGUILLICOLOIDES CRASSUS INFESTATION IN EELS FROM NORTHEASTERN ALGERIA

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Abstract. This study investigates the infestation by *Anguillicoloïde crassus* of eels from Tonga Lake and El Kebir Wadi, with a focus on epidemiological indexes, seasonal trends and evaluates the current infection rate (by lumen worms of the swim bladders) and past infections (e.g., as evidenced by damage to the swim bladders). During this study, A seasonal assessment was conducted by collecting parasites and measuring morphological parameters, including the length and weight of the fish. A total of 213 eels were examined, and the fish length ranged between 336- 740 and 297 – 601 mm from Tonga Lake and El Kebir Wadi respectively. The SDI and the classical epidemiological parameters were computed following dissection. The epidemiological indexes were: A=58.47% and 55.84 %, I=3.41 and 3.267 per infected host and A=2.01 and 1 per examined eel in Tonga Lake and El Kebir wadi respectively. Concerning the seasonal evolution, winter showed the highest values rates of *A. crassus* abundance and infestation intensity. On the other hand, more than a third of the swim bladders eels from El Kebir Wadi were intact, while 63.9% of indicate a moderate damage in Lake Tonga

Key words: *Endangered species, Parasite, invasion, Swim bladder.*

Introduction

The European eel (*Anguilla anguilla*) is a species of ecological and economic importance, widely distributed across European and North African freshwater and coastal habitats. However, Over the past few decades, the European eel population has been facing a drastic decline to the extent that the IUCN red list classed it as critically endangered (Pike et al., 2020). This decline is due to the conjunction of numerous factors, including parasitism (Moriarty & Dekker, 1997).

One of the most studied parasites in European eels is *Anguillicoloïdes crassus,* a swim bladder nematode of Japanese that was introduced to Europe from the East in the mid-1970s, and early 1980s, probably through the aquaculture trade (Koops & Hartmann, 1989). This parasite has multiple impacts on its host causing inflammation of the swim bladder leading to several bacterial infections, stress, and loss of appetite (Kirk, 2003; Lefebvre et al., 2013) and consequently affecting the body condition swimming capacity and survival (EIFAC, 2009; Palstra et al., 2007).

Cross Europe, eel populations at varying stages of growth experienced a significant mortality rate due to this parasite (Dekker et al., 1998; EELREP, 2005; Kirk, 2003; Lefebvre et al., 2003). The first record of this worm's existence in eels from the aquatic habitat in northeastern Algeria dates back to 1999 (Meddour & Meddour-Bouderda, 1999).

In Algeria, except the study conducted by Rouag-Laouirain (2012) at El Kebir wadi (Jijel, Skikda), eels have not yet been the subject of any parasitological research in this area.
This study aims to present new data on the seasonal variation of the European eel population infected by *A. crassus* in North-East of Algeria (Tonga Lake and El Kebir Wadi). Furthermore, it intends to assess the effect of this parasite on the Swim Bladder Degeneration Index (SDI) to better understand the pathological consequences of the infection. This research adds a crucial knowledge for the effective conservation and management of eels by offering new insights into the parasitic pressures that eels face in the investigated zones

Materials and Methods

Study area

Sampling was conducted seasonally between December 2021 and September 2022 from two localities Tonga Lake (36°51.511″ N; 8°30.100″ E) and El Kebir Wadi (N 36° 32′ 16; E 6° 15′ 54) (Fig. 1).

Tonga Lake: is situated in the National Park of El Kala, extreme North East of Algeria. It is a large shallow lake (surface of 2 200 ha, maximum depth = 1.5 m; mean altitude = 2.20 m). It is fed by two tributaries: Oued El Hout from the southeast and Oued El Eurg from the east. On the north part of Tonga Lake, the artificial canal of the Messida represents the connection with the Mediterranean Sea.

El Kebir Wadi: is the result of the confluence of two Wadis (Wadi Enndja and Wadi Rhumel) (Zeghmar et al., 2022), which covers an area of around 1110 km^2. In its downstream section, to the east of Jijel, El Kebir Wadi flows into the sea, shaping the entire coastal area. This wadi supports diverse habitats and contributes to the ecological health of associated wetlands, including the Beni Belaid Nature Reserve, which is classified as a wetland under the Ramsar Convention (Bougherira et Ghodbani, s. d.). This area is characterized by a complex of lakes and marshes that depend on the river's flow for their ecological balance.

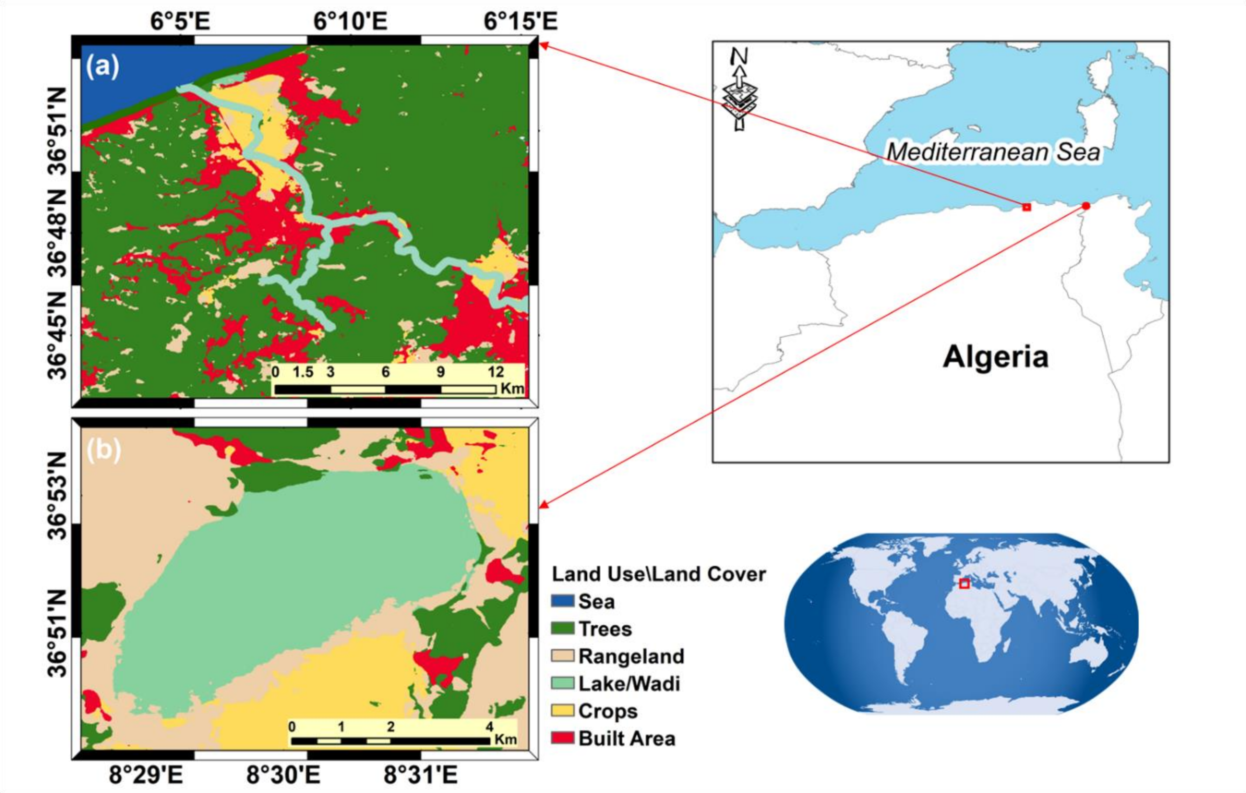

Figure 1: study area (a) El Kebir wadi (b) Tonga Lake

Environmental parameters:

At Tonga Lake, water temperature generally ranged between 15.11 and 17.11 °C in winter and from 20.42 to 29.73 °C in summer (means seasonal temperatures were 16.23 and 25.24

°C, respectively. The mean dissolved oxygen values of surface water ranged between 8.80 and 9.27 mg/L. Salinity ranged from 0.02% to 0.07%. Water pH was relatively neutral to alkaline (Naili et al., 2021); while in El Kebir Wadi the average temperature varied from 14.85 ± 5.42 to 20.16 °C , and the salinity is in general equal to 0.74 ± 0.26 PSU. The average values of dissolved oxygen varied between 3.85 ± 1.51 to 8.55 ± 0.73 mgl^{-1}. The water of the Kebir-Rhumel watershed is alkaline (Saal et al., 2021).

Capture and treatment of the eels*:*

Eels were captured using nets with a 10 mm mesh size and transported alive to the laboratory in ice-cooled containers to minimize stress. Each eel's total length (TL) was measured to the nearest millimeter using an ichthyometer. Using a precision scale, total weight (TW) was recorded to the nearest gram.

External and internal anatomical features:

The horizontal and vertical diameters of both the right and left eyes, as well as the length of the pectoral fins, were measured to the nearest 0.1 mm using a digital caliper to calculate the ocular index according to (Pankhurst, 1982).

After dissection, gonads were excised and weighed (MG), along with the liver (ML). The gut was dissected at the anal region and weighed separately (MGu), to calculate the Gonadosomatic Index (GSI), the Hepatosomatic index (HIS) and the Gut index (IGu) respectively (Durif et al., 2005).

Length-weight relationship

The length-weight relationship was determined using the equation:

W(t)=a·LtbW(t) = a \cdot Lt^b W(t)=a·Ltb ,

where:

The exponent *b* reveals the growth type: isometric when $b = 3.0$, positive allometric when $b > 3$, and negative allometric when $b < 3$.

Silvering Index

The degree of silvering was assessed to estimate the reproductive capacity of European eels (EELREP, 2005), following a multi-criteria analysis that categorizes the stages of silvering, as described by (Durif et al., 2009).

Swim bladder Degenerative Index (SDI)

After dissection, the swim bladders of all eels were removed and examined macroscopically for the presence of *A. crassus*. The number of worms present in the lumen was recorded for each specimen. Epidemiological parameters, including prevalence (P), mean intensity (I), and abundance (A), were calculated following the methodology outlined by (Bush et al., 1997),

The protocol for assessing the Swim Bladder Degenerative Index (SDI) was adapted from (Lefebvre, Contournet, & Crivelli, 2002). This index, which ranges from 0 to 6, is based on macroscopic alterations visible in the swim bladder. It incorporates three distinct criteria, each one being coded 0, 1, or 2, to quantify the degree of degeneration. These criteria include the thickness, the opacity, and the pigmentation of the swim bladders.

Statistical analysis

Several statistical measures were used to assess the difference between the two studied sites, considering morphometric parameters but also the rate of infection, prevalence, intensity, and abundance were calculated. All calculations and graphs were performed by the R statistical language software (version 4.3.2).

Results:

Morphological characteristics of the eels sampled

A total of 213 eels were examined, the fish length ranged between 336 to 740 and 297 to 601 mm from Tonga Lake and El Kebir Wadi respectively.
The most important length recorded were 450 - 500 and 400 - 450 in Tonga Lake and El Kebir Wadi respectively (Tab. 1).

Table.1 Lenth and weight of the sampled eels

Season	**Location**	**N**	**TL (mm**	**W(g)**

			Min	Max	Min	Max
Winter	Tonga Lake	30	385	545	90.10	308.10
	El Kebir Wadi	32	297	455	44.70	191.00
Spring	Tonga Lake	31	420	740	150.00	881.00
	El Kebir Wadi	30	420	601	149.40	487.80
Summer	Tonga Lake	28	336	600	65.00	425.00
	El Kebir Wadi					
Autumn	Tonga Lake	31	370	605	121.00	417.00
	El Kebir Wadi	31	375	600	85.00	385.00

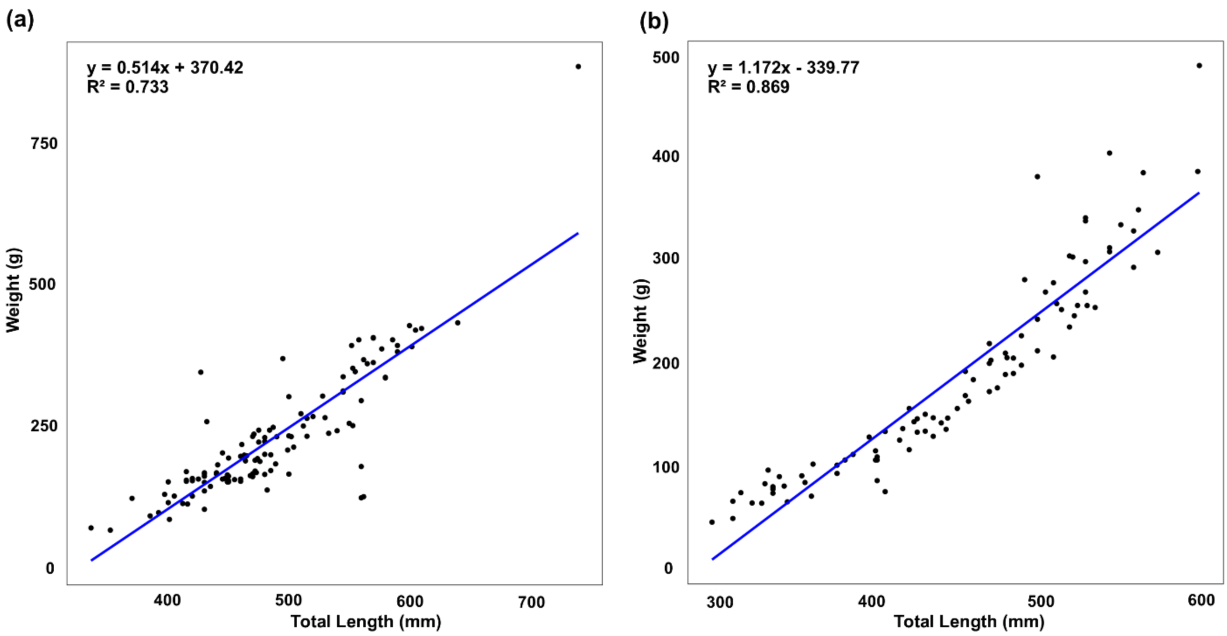

Figure 2: Length-weight relationship of A. anguilla, (a) in Tonga Lake, (b) In El Kebir Wadi

Both El Kebir Wadi and Tonga Lake exhibit strong positive correlations, with Pearson correlation coefficients of $r = 0.93$ and $r = 0.86$, respectively. The extremely low p-values ($< 2.2e-16$) for both sites indicate that these correlations are statistically significant, providing robust evidence of a strong association at both locations (Fig. 2).

The dynamic evolution of A. crassus:

The seasonal infestation rates demonstrated fluctuations ranging from 48.15 to 66.67(%) in Tonga Lake and from 41.9 - 65.62 (%) in El Kebir Wadi. Winter was the season with the highest values of *A. crassus* abundance and infestation intensity across all sites. Smaller eels (30–40 cm) show a 16.7% prevalence, but it jumps to 100% in the largest size class (70–80 cm) in Tonga Lake however in El Kebir Wadi; smaller eels (20–30 cm) have a 100% infection rate, but it declines in medium-length eels and were completely absent in the largest length class (70–80 cm).

The highest intensity and abundance observed in eel ranged between 500-600 mm in Tonga Lake and between 300 - 400 mm in El Kebir Wadi (Tab. 2).

Table 2. Epidemiological parameters of A. crassus during the sampling seasons

Season	**Location**	**N of parasites**	**Prevalence (%)**	**Intensity**	**Abundance**	**SDI**
Winter	Tonga Lake	98	66.67	4.90	3.27	1.33
	El Kebir Wadi	118	65.62	5.62	3.69	1.19
Spring	Tonga Lake	51	60	2.83	1.70	1.77
	El Kebir Wadi	32	60	1.78	1.07	1.90
Summer	Tonga Lake	49	48.15	3.77	1.81	1.18
	El Kebir Wadi	–	–	–	–	–
Autumn	Tonga Lake	39	58.06	2.17	1.26	1.71
	El Kebir Wadi	31	41.9	2.38	1.00	1.87

Table 3: Epidemiological parameters of A. crassus as function as length classes of the eels sampled

	Tonga Lake			El Kebir Wadi		
Length Class (cm)	P	I	A	P	I	A
[20, 30]	0	0	0	100	4	4
[30, 40]	16.7	2	0.333	68	5.76	3.92
[40, 50]	50.7	2.71	1.38	43.2	2.44	1.05
[50, 60]	73.7	4.29	3.16	60.7	2.29	1.39
[60, 70]	3.5	3.5	2.8	50	1	0.5
[70, 80]	100	6	6	0	0	0

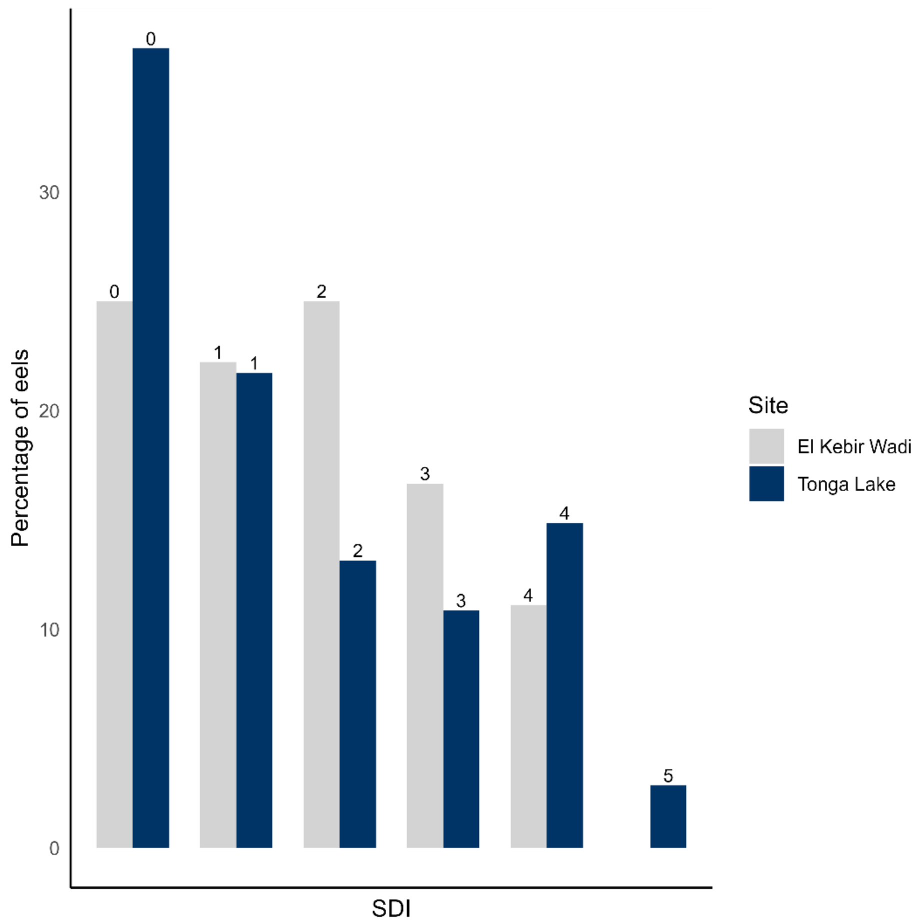

Figure.3. The distribution of the Swim bladder Degenerative Index (SDI) in Tonga Lake and El Kebir Wadi

A higher percentage of intact swim bladders eel was find in El Kebir Wadi (36.6% vs. 25.0% in Lake Tonga) while the moderate damage in the swim bladders was found in eels captured at Tonga lake (63.9% vs. 45.7% in El Kebir Wadi) (Fig. 3).

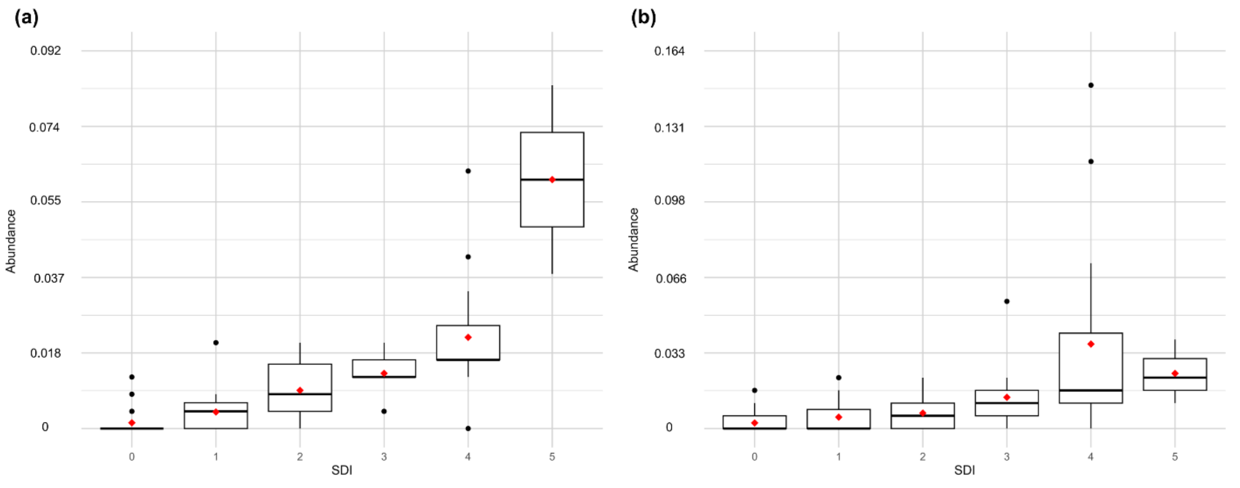

Figure 4: Abundance of A. crassus by SDI, (a) in Tonga Lake and (b) in El Kebir Wadi

The mean abundance of *A. crassus* varied significantly as function of the values of the swim bladder degenerative index (SDI) in both sites (Fig. 4).

Statistical analysis highlighted a very high significant relationship (p-value < 0.001) between abundance of *A. crassus* and SDI for eel populating both sites.

Table 4: Epidemiological parameters of A. crassus based on the eel life stages

	Tonga Lake					**El Kebir Wadi**				
Life stage	P (%)	I	A	N	Infected eels/total	P (%)	I	A	N	Infected eels/total
SI	40	2	0.8	4	02-mai	73.68	6.93	5.11	97	14/19
SFII	-	-	-	0	0	42.86	2.33	1.00	7	03-juil
SFIII	59.459	3.470	2.063	229	68/111	60.00	2.11	1.27	57	27/45

SFV	0	0	0	0	0	33.33	1.00	0.33	1	01-mars
SMII	50	4	2	6	02-mai	36.84	2.71	1.00	19	juil-19

At Tonga Lake, the highest infestation rates and intensity were observed for the premigrant females SFIII (59.46% and 3.47 parasites per infected eel respectively). In contrast, SFII and SFV eels were not infected by *A. crassus*. Meanwhile, SI (resident) and SMII (male migrant) eels exhibited moderate prevalence rates of 40% and 50%, respectively (Tab. 4).

In El Kebir Wadi, the SI eels displayed the highest prevalence (73.68%) with a mean intensity of 6.93 parasites per infested eel. SFIII eels also had a high prevalence (60%), similar to Tonga Lake. SFII and SFV eels showed lower infection rates, with SFII at 42.86% and SFV at 33.33% (Tab. 4).

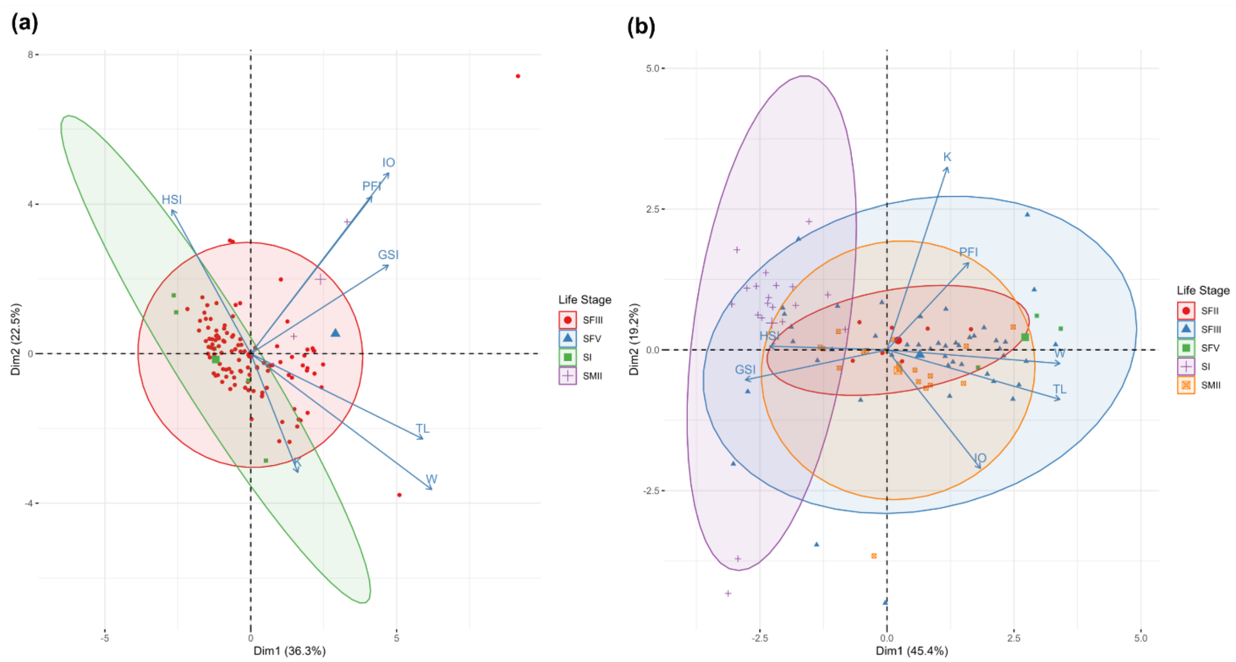

Figure.6. Principal Component Analysis (PCA) of morphophysiological parameters and life stages of A. anguilla in Tonga Lake (a) and El Kebir Wadi (b).

The PCA depicts in Figure.6 highlights that El Kebir Wadi (Fig. 6b) exhibits a higher proportion of variance explained by the first component (45.44% vs 36.27% for Tonga Lake), suggesting a more pronounced structure in the collected data.

At El Kebir Wadi, there is a clearer opposition between growth (TL, W) and gonad and liver development (GSI, HSI), while at Tonga Lake (Figure. 6a), these relationships appear more complex.

On the other hand, a t-test was performed to compare the number of parasites found in eels sampled at the two sites. The results show that there are no significant difference between both sites (t = 0.76011, p = 0.4489). Although the mean parasite count appears to be higher at El Kebir Wadi (3.65) than at Tonga Lake (2.23), this difference is not large enough to be considered significant. The 95% confidence interval [-2.28, 5.11] includes zero, confirming this lack of significance. Thus, on the basis of our data, it cannot be stated that the number of parasites in eels differs significantly between Tonga Lake and El Kebir Wadi, despite a slight trend observed.

Discussion

The purpose of this investigation is the assessment of the infestation rates by *A. crassus* of European eel populating two different ecosystems (Tonga Lake and El Kebir Wadi) and the evaluation of their swim bladder state.

Eel samples collected from Tonga Lake and El Kebir Wadi reveal respectively a mean prevalence of 58.47%±6.63 and 55.84%±10.12 a mean intensity of infestation of 3.41±1.02 and 3.267±1.69 per infected host, values of abundance of 2.01and 1.92 per examined host. Infestation rates of 68% and 34% were reported in Algeria by Nabil et al. (2009) and Dbbari et al. (2009) respectively. The epidemiological values found previously in a close area (the Mafrag Estuary: P = 51%, I = 3.9, A = 2.02) (Boudjadi et al., 2009) were similar to the current study. Compared to Europe, where prevalences of 90 and 100% were often noted, such as in England (Kelly et al., 2000) and Hungary (Csaba et al., 1993), Algeria records lower infection rates.

The highest infection rates were reported in fresh waters 68% by Loucif et al. (2009) in Tonga Lake and 50.44% and by Tahri et al. (2016) in Obeira Lake; however, the lowest were 12.61% by Djebbari et al. (2009) in El Mellah lagoon, and 9.72% by Rouag-Laouira (2012) in El Kebir Rive.

High salinity levels limit if not entirely prevent, high infestation rates by *A. crassus*; indeed, elevated salinities create unsuitable or suboptimal eco-physiological conditions for the parasite to complete its life cycle (Tahri, 2023). Infection rates of *A. crassus* are influenced by latitude, with lower prevalences typically found at higher latitudes. Despite this general trend, the parasite has been documented in the northernmost regions, including above 60°N in Scandinavia for *Anguilla Anguilla* (Jakob et al., 2016) and Canada for *Anguilla rostrata*

(Aieta & Oliveira, 2009). This can, in turn, reduce the range of available and compatible hosts (whether intermediate or paratenic), while the presence of vector hosts in the environment remains crucial, particularly in the context of parasitic colonization (Taraschewski, 2006).

The survey of the evolutionary dynamics of parasitism reveals that *A. crassus* is present year-round at both sites, where salinity ranges from 0.02% to 0.07% in Toga Lake and 1, 0.74 ± 0.26 PSU in El Kebir Wadi. In both sites, the lowest seasonal infestation rates are recorded in summer for Tonga Lake and in autumn for El Kebir Wadi. These monthly variations in infestation are linked to temperature changes. High summer temperatures and lower winter temperatures at the study sites prevent the progression of the nematode's life cycle. The increase in temperature could be lethal for the larvae of this parasite (Charleroy et al., 1989). According to Lefebvre et al. (2002), the seasonal decrease in the prevalence of *A. crassus* during summer may be attributed to the death of most severely affected eels during the warmer seasons.

It might also be influenced by seasonal variation patterns in the eels' feeding patterns. Eels experience two critical periods of feeding cessation: one in the winter when temperatures fall below 10°C, and another in the summer when temperatures can exceed 30°C (Lecomte-Finiger, 1984).

Seasonal variations in epidemiological parameters revealed that *A. crassus* infections are more common in smaller eels compared to larger ones, whereas larger eels tend to harbor a greater number of parasites than their smaller counterparts. This prevalence increases with the size of the host. Similar findings have been reported by Lefebvre et al. (2002) suggesting that the higher prevalence in larger eels may be attributed to a longer exposure time to parasites. However, other studies have demonstrated a negative relationship between parasite abundance and eel size (Abdallah & Maamouri, 2006; Fazio et al., 2008).

Furthermore, this study revealed also that the highest prevalence was observed in SFIII (female premigrant) eels, with 59.46% living in Tonga Lake compared with the immature eels (SI) and male (SMII), it seems to be due to space and time effects, larger and older eels provide a larger habitat for the parasite to establish. Several authors reported the same results (Thomas & Ollevier, 1993). This relationship occurs as eels grow and develop, likely because they consume more food and have longer exposure to the parasite in the environment in El Kebir Wadi. The significant predatory behavior of undifferentiated eels facilitates rapid growth and enhances their probability of encountering paratenic hosts, thereby increasing their exposure to the parasite this explains the highest prevalence (73.68%) observed for the immature eels (SI) resident.

All eels from the sampling areas exhibit pathological signs of swim bladder infection, with those from Tonga Lake being the most severely affected, followed closely by the eels from El Kebir Wadi. the revealed results of this study are close to those reported by Neto et al. (2014) in Portugal’s brackish water systems, where 67% of the swim bladders were found to be damaged (SDI = 1.31±1.23). More severe damage was recorded in French lagoons, where 92% of the eels showed pathological signs of infection, with severely damaged swim bladders containing very few live nematodes (Lefebvre et al., 2002).

Conclusion

This study offers a detailed examination of *A. crassus* eel infestation from Tonga Lake and El Kebir Wadi (Northeastern of Algeria). It reveals significant insights into seasonal variations and site-specific differences. The mean prevalence of infestation was 58.47% in Tonga Lake and 55.84% in El Kebir Wadi. Infestation rates were lowest in summer at Tonga Lake and in autumn at El Kebir Wadi. Larger eels showed higher prevalence rates, with notable differences observed across eel life stages. These findings highlight the impact of environmental factors on *A. crassus* dynamics and underscore the need for ongoing monitoring and management strategies.

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