Environmental Parameters and Temporal Dynamics of *Anguillicoides crassus* in Tonga Lake and Mafrag estuary (North-East of Algeria)

1Djebbari Nawel, 1Hamza Imen, 1Ladjama Imane, 2Kaouachi Nouha, 2Barour Choukriand 2Bensouilah Mourad

### ABSTRACT

The presence of the parasitic swimbladder nematode, *Anguillicoides crassus*, in eels of the aquatic ecosystem of the northeast of Algeria, was recorded in 1999. Since we had only results related to epidemiological parameters of parasites, no study of environmental influence in temporal dynamics of *A. crassus* was performed. The aim of this study is firstly, to collect monthly the nematode *A. crassus* from eels caught in Tonga lake and Mafrag estuary to assess the spatial temporal dynamics of this parasite, secondly to identify the influence of some environmental factors on the epidemiological parameters (prevalence, intensity of infestation and abundance) of the nematode. For this, we have proceeded, on a monthly rate, in collecting parasites and measuring some abiotic parameters (temperature, salinity, dissolved oxygen and pH) from the two water dam. Eel samples collected from Tonga lake (360 eels of length ranged from 25 and 75 cm) and Mafrag estuary (360 eels of 19-71 cm length) revealed respectively a parasite prevalence of 48 and 50%, a mean intensity of infestation of 3.6 and 3.7 per infected host, values of abundance of 1.7 and 1.9 per examined host. Our results show that the highest prevalence values are recorded in young specimens; however, the highest values of mean intensity and abundance are noted in large eels. The survey of the evolutionary dynamic of parasitism reveals that *A. crassus* is present throughout the year in both water dam. In both sites, the lowest seasonal infestation rate is recorded in summer. The use of principal component analysis shows that water temperature and salinity are correlated negatively with prevalence of *A. crassus*; this parasite shows the lowest values of infestation rates in summer and autumn when water temperature and salinity present the highest values. The first two major components contain 86.73% and 84.32% of the total inter-month variability of the matrix of biont and abiotic variables in Tonga lake and Mafrag estuary respectively. In Tonga lake, Axis 1 explains 56.46% of the total variation; it is positively correlated with salinity variables \((r = 0.89; \cos^2 = 0.79)\) and negatively correlated with dissolved oxygen \((r = -0.94; \cos^2 = 0.89)\). In Mafrag estuary, Axis 1 explains 59.91% of the total variability; it is positively correlated with temperature variables \((r = 0.95; \cos^2 = 0.91)\), salinity \((r = 0.84; \cos^2 = 0.70)\) and negatively correlated with dissolved oxygen \((r = -0.71; \cos^2 = 0.51)\). The low salinity that characterize the two water dam did not affect life cycle of *A. crassus* and may explain the similarity of the epidemiological parameters values obtained.

**KEY WORDS:** Prevalence; *Anguillicolacrusss*; wetland; Northeast of Algeria; environmental parameters.

### INTRODUCTION

According to Combes (1995), parasites occur in various ecosystems and in all genera, and interact with their host in different ways depending on the stability of the ecosystem. Parasite presence or abundance may be directly influenced by both the host environment and the environmental condition of the ecosystem (Kadlec et al., 2003). Changes in the environment are thus susceptible to modify the host–parasite interactions by either increasing the parasites effect through an increase in host susceptibility (Kadlec et al., 2003) or of contaminant vectors, or disadvantaging the parasites by increasing host mortality or decreasing the abundance of intermediate hosts (Pampoulie et al., 2001).

Because most parasitized organisms are generally in poorer condition than unparasitized ones (Combes, 1995; Flegr, 2008; Thomas et al., 2007) and because parasites interact with natural and anthropogenic stressors...
to increase mortality and reduce animal health in myriad ways (Marcogliese & Pietrock, 2011), parasitism is among the reasons invoked to explain the European eel collapse (Brusle, 1994; Fazio et al., 2005; Haenene et al., 2010; Kennedy, 2007).

Eels of the genus Anguilla exhibit a complex amphihaline life cycle, with a reproductive phase in the ocean and a growing phase in coastal, estuarine and inland waters. Partly because of this long-run life cycle, eels are exposed to many risk factors, including oceanic changes, migration barriers, habitat loss, pollution, food depletion, etc. Since the 1970–1980s, both scientific monitoring and fishery statistics have recorded a drastic decline in recruitment, yield and stock of eels (Dekker 2003; Casselman & Cairns, 2009). The European eel A. anguilla, now considered “outside safe biological limits” (ICES, 2007) and red-listed as Critically Endangered (Freyhof & Kottelat, 2008). Since the 1980–1990s, the eel Anguilla Anguilla is facing a new threat; The invasive nematode Anguillicoloides (formerly Anguillicola) crassus has been expanding over the geographic range of its new host (Jakobet al., 2009a; Rockwell et al., 2009; Lefebvre et al., 2012). It is considered one of the most pathogenic parasites of eels, with possible negative effects on body condition, swimming capacity and survival (Kennedy 2007; Palstra et al., 2007; ICES, 2009a; Haenene et al., 2010). Moreover, repeated infections during the continental life of the eel lead to profound damages of the swimbladder, which may impair the spawning migration to the Sargasso Sea (Palstra et al., 2007; Clevestam et al., 2011; Lefebvre et al., 2012).

The commercial fishing of eel Anguilla anguilla has begun for already three decades in the various water bodies of El Kala wetland complex national park. The presence of the parasitic swimbladder nematode, Anguillicoloides crassus, in eels of the aquatic ecosystem of the northeast of Algeria, was recorded for the first time in 1999 (Meddour et al., 1999). In 2005, it was noted that the largest number of parasites was observed in eels collected from Tonga and Oubeira freshwater lakes, where their number was 4-5 times those collected from El Mellah brackish water lagoon (Djebbari et al., 2005). In 2007-2008, the study of evolutionary dynamics of A. crassus reveals that the prevalence, the intensity and the abundance of this nematode show monthly variations (Boudjadi et al., 2008). The aim of this study is firstly, to assess the evolutionary dynamics of the nematode A. crassus throughout the year from one month to the next, and on the size of the eels in the Tonga lake and Mafrag estuary of the Northeast of Algeria, and secondly to identify the influence of some environmental factors on the dynamics of the parasite on using the principal component analysis.

MATERIAL AND METHODS

The sampling of eel was conducted in two localities: the Tonga lake (36°51.511’N; 8°30.100’E) and The Mafragestuary (36°50.18.26’N; 7°57.27.81’E) situated in Algeria North-East (fig.1). A total of 720 eels (30 specimens per month and per localities) were collected between January 2008 and December 2008 by local fishermen. Eels were transferred on ice to the lab where they were measured (total length, to the nearest millimeter). Afterwards, they were killed with anesthetic MS222 (Sigma chemical Co. St Louis, MO, USA). The eels were opened by a longitudinal incision along the belly. The intestinal tract was cut at each end and lifted out to reveal the swimbladder. The swimbladder was dissected out, placed in a Petri dish, split longitudinally and carefully examined under a binocular microscope. Nematodes were collected, counted, washed in physiological saline (0.9%), fixed in hot 70% ethanol and stored in ethanol.

The nomenclature adopted in reporting the prevalence the mean intensity and abundance values of Anguillicoloides crassus by months and the size of eels is that customarily used (Bush et al., 1997). After each monthly sampling, temperature, pH, salinity and dissolved oxygen of water were measured "in situ", using amultiparameter(Consort 535).

Fig. 1: Northeastern of Algeria; stars show localization of sampling sites of eels.
Statistical Analysis:

Statistical analysis of the data was performed under R (R Development Core Team, 2014 Version 3.1.2) developed by Ross Ihaka (1996). The normality condition of the distributions was checked beforehand by applying the Shapiro-Wilk (not shown). Distributions, being usually of asymmetric time, forced us to choose non-parametric alternatives for the statistical analysis.

The correlations between the sets of parameters are evaluated by the non-parametric Spearman correlation coefficient (r) to analyze the intensity of relations between our parameters. Furthermore, the inter-sites comparisons of median of the intensity and the abundance of parasite were performed using the nonparametric Mann Whitney test. Moreover, Principal component analysis (PCA) was carried out using the package FactoMineR (Husson et al., 2014) on the normalized data. The principal component analysis (PCA) was used as a descriptive and exploratory method aiming to characterize, through a multivariate approach, the structuring of our inter-months variations and to highlight the contribution of environmental parameters measured on prevalence, mean intensity and abundance of A. crassus parasite. All packages used were downloaded from the official website of CRAN (The Comprehensive R Archive Network): http://cran.r-project.org/web/packages/.

Results:

Eels length ranged from 25 to 75cm in Tonga lake and from 19 to 72 in Mafrag estuary.

1. Evolutionary dynamics of A. crassus:

Prévalence:

The monthly infestation rate vary from 20% (in July) to 63.33% (in December) in Tonga lake and from 23% (in November) to 70% (in January) in Mafrag estuary. In both sites, the highest seasonal infestation rate is recorded in spring and the lowest in summer (fig.2).

Fig. 2: Prevalence of A. crassus as a function of the months.

Mean intensity:

In Tonga lake, the monthly mean intensity of infestation show variation between 2.4 and 8.7 worms per infested eel in Tonga lake and between 1.77 and 7.32 in Mafrag estuary. The lowest seasonal value of mean intensity of infestation is recorded in spring in both sites (fig.3).

Fig. 3: Mean intensity of A. crassus as a function of the months.

Abundance:

The monthly value of the abundance of A. crassus vary from 0.8 (in September) to 2.6 (February) per eel examined in Tonga lake and from 0.5 (in May) to 3.9 (from August to November) in Mafrag estuary. The lowest seasonal value is recorded in autumn and winter respectively in Tonga lake and Mafrag estuary (fig.4).
The application of Mann-Whitney non parametric test in comparison of abundance and intensity median between Mafrag estuary and Tonga lake reveals the absence of significative differences.

2. Environmental parameters:

The temperatures of water vary from 12.4°C to 31.5°C in Tonga lake, and from 10.2°C to 30°C in Mafrag estuary. It appears from figure 5 that temperatures of more than 20°C are recorded from May to September in the two sites.

In Tonga lake, the values of salinity range between 0.04 g/l and 0.24 g/l but in Mafrag estuary salinity contents vary from 0.32 g/l to 18.06 g/l; The highest values of water salinity are recorded in summer (fig.5).

The dissolved oxygen contents vary from 6.02 mg/l to 14.03 mg/l in Tonga lake and from 6.3 mg/l to 12.5 mg/l in Mafrag estuary. The dissolved oxygen contents of more than 7 mg/l are often recorded in Mafrag estuary (except months of March and June); however, in Tonga lake, contents under 7mg/l are denoted from June to December (fig.5).

In both sites pH is alkaline; however, in Mafrag estuary the pH values are highest than those noted in Tonga lake (fig.5).

Fig. 5: Spatial temporal variations of physical-chemical water parameters.
3. Epidemiological parameters of *A. crassus* related to eel size:

- The study of parasitism evolution from a consideration of the size of the eel collected from Tonga lake shows that the highest prevalence values are recorded in young specimens; however, the highest values of mean intensity and abundance are noted in large eels (fig.6).

![Fig. 6: Epidemiological parameters of *A. crassus* as a function of the size of eels collected from Tonga lake.](image)

- In the Mafrag estuary the highest values of infestation was observed in small sized eels (25-35 cm) and large sized specimen (67-73 cm). The maximum number of parasites per host infested and host examined was recorded respectively in large sized specimens (61-67cm) and specimens of size range between 49 and 55cm (fig.7).

![Fig. 7: Epidemiological parameters of *A. crassus* as a function of the size of eels collected from Mafrag estuary.](image)

4. Statistical analysis:

The use of principal component analysis (PCA) as a preliminary and exploratory descriptive approach helped visualizing the structure of the temporal variation in Mafrag estuary and Tonga lake according to seven measured variables: water temperature, pH, Dissolved Oxygen, Salinity, prevalence, mean intensity and abundance.

The PCA has been used as a tool for modeling linear relationships between *A. crassus* and abiotic variables. It is noteworthy that the nematode variable was used as an additional quantitative variable to achieve the PCA by the FactoMineR package. Moreover, PCA was performed on the reduced-centric data (standard PCA), whose results are summarized in figures (8 and 9).
4.1 Monthly change in epidemiological parameters of A. crassus and abiotic parameters in Tonga lake:

The first two major components contain 86.73% of the total inter-month variability of the matrix of biotic and abiotic variables in Tonga lake (fig.8). Axis 1 explains 56.46% of the total variation; it is positively correlated with salinity variables (\( r = 0.89; \cos^2 = 0.79 \)) and negatively correlated with dissolved oxygen (\( r = -0.94; \cos^2 = 0.88 \)). This axis is of a clear difference between the group of the warmest months (July, August, September, October) and the cold months (January, February, March). The epidemiological parameters (abundance and prevalence) seem to be negatively correlated with salinity and positively correlated with dissolved oxygen. The second axis explains only 30.28% of the total variation; this axis is built mainly by the pH variables (\( r = 0.80; \cos^2 = 0.64 \)) and temperature (\( r = 0.74; \cos^2 = 0.56 \)); these variables seems to be positively correlated with mean intensity of A. crassus.

Fig. 8: Principal component analysis based on inter-month variations in Tonga lake. Plan factorial (1, 2) axis a1: 56.46%, axis 2: 30.28 %. Correlation circle of biotic and abiotic variables with the first two principal axes. Projection of months on the first two principal axes.

4.2 Monthly change in epidemiological parameters of A. crassus and abiotic parameters in Mafraf estuary:

The two first main components (plan 1-2, fig. 9) of the PCA based on the four environmental variables provided approximately 84.32% of the information (inter-months variability). Different epidemiological parameters of parasite counts were used as additional quantitative variables.

Axis 1 explains 59.91% of the total variability; it is positively correlated with temperature variables (\( r = 0.95; \cos^2 = 0.91 \)) and salinity (\( r = 0.84; \cos^2 = 0.70 \)) negatively correlated with dissolved oxygen (\( r = -0.71; \cos^2 = 0.51 \)). This axis shows the difference between the group of the cold months (January, February, November and December) and the warmest months (July, August and September). Prevalence of A. crassus seems to be negatively correlated with salinity. Axis 2, which explains 24.41% of the total variability, is essentially built by the pH variable (\( r = 0.77; \cos^2 = 0.59 \)), this variable seems to be positively correlated with abundance of A. crassus.
Fig. 9: Principal component analysis based on inter-month variations in Mafrag estuary. Plan factorial (1, 2) axis a1: 59.91%, axis a2: 24.41%. Correlation circle of biotic and abiotic variables with the first two principal axes. Projection of months on the first two principal axes.

Discussion:
Eel samples collected from Tonga lake and Mafrag estuary revealed respectively a parasite prevalence of 48 and 50%, a mean intensity of infestation of 3.6 and 3.7 per infected host, values of abundance of 1.7 and 1.9 per examined host. In Tonga lake, Loucif et al., (2009) and Djebbari et al. (2009) denoted infestation rates of 68 and 34% respectively. In Mafrag estuary, the epidemiological parameters reported by Boudjadi et al. (2008), were close to those revealed by our investigation (P = 51%, I = 3.9, A = 2.02).

The survey of the evolutionary dynamic of parasitism reveals that A. crassus present throughout the year in both water dam; which are characterized by a low salinity (0.02–18 g/l). The monthly infestation rates vary from 20% (in July) to 63.33% (in December) in Tonga lake and from 23% (in November) to 70% (in January) in Mafrag estuary. In both sites, the highest seasonal infestation rate is recorded in spring and the lowest in summer. In Mafrag estuary, monthly infestation rates recorded in 2009 were slightly higher; varying from 30 to 76% (Boudjadi et al., 2008); In the Rhone River Delta, Lefebvre et al (2002) found prevalence ranging between 40 and 72%. In Moulouya estuary (Morocco), values of monthly prevalence of A. crassus show a great amplitude; they are ranging between 0 and 70% (Rahhou et al., 2001). The amplitudes of variability of the mean intensity in eel samples of both sites are similar. Lefebvre et al. (2002) noted similar values in the Rhone River Delta. In Mafrag estuary, Boudjadi et al. (2008) found lowest values (3 and 5.7 worms/eel infected) than those of the present study.

In both sites, the lowest seasonal infestation rate is recorded in summer. The statistical analysis revealed that water temperature is positively correlated with salinity and negatively with dissolved oxygen. The use of principal component analysis shows relationship between water temperature and salinity and evolutionary dynamics of the nematode A. crassus. In both sites, these two variables are correlated negatively with prevalence of A. crassus; this parasite shows the lowest prevalence of infestation rates in summer and autumn when water temperature and salinity present the highest values.

The monthly epidemiological variation may be related to the thermal changes. High summer (30–31°C) and lower winter temperature (10–12°C) in the sites studied would prevent the progress of the life cycle of this nematode. Petter & Fontaine, (1989) show experimentally that the larval development of this parasite at the intermediate host cyclopidea is inhibited at a temperature varying from 1 to 13°C. Under such conditions, the larvae did not grow in cyclopidea and died after about one month. However, temperature from 20 to 22°C causes rapid growth of larvae in this host’s haemocoel. Other authors have demonstrated that the increase of the temperature could be lethal for larvae of this parasite (De Charleroy et al., 1989). According to Lefebvre et al., (2002) the seasonal decrease in the prevalence of A. crassus in summer may be attributed to the death of the more severely affected eels during the warmest seasons.

The seasonal fluctuation of the rate of parasitism may also be related to changes in the period of eel feeding. The eels undergo two critical periods during which they do not feed: once in the winter, when the temperature is lower than 10°C, and again in the summer, when the temperature may rise above 30°C (Lecomte-Finiger, 1983). Abstinence from food could limit the arrival of the infective parasite stage at the eel during these periods.

Correlation between salinity and epidemiological parameters of A. crassus is also reported by many authors; In wetlands of the Northeast of Algeria, Djebbariet al., (2009) found that the rates of infestation observed in eels collected in the lake Tonga are 3 times those of the eels of the El Mellah lagoon. Kirk et al. (2000a) show that infestation levels decrease with increasing salinity; Lefebvre et al. (2003) found a negative correlation between epidemiological parameters and salinity.

Many authors noted that eel farms using seawater were often found free of the parasite (Kamstra, 1990; Kieft, 1991), and eels living in brackish and coastal waters most often showed a lower infection rate than those taken in neighboring freshwater areas (Dekker & van Willigen, 1989; Nielsen, 1997; Morrison & Secor, 2003). High salinities can create unsuitable or non-optimal eco-physiological conditions for the parasite to complete its life cycle. First, high salinity may narrow the range of available/compatible hosts (whether intermediate or paratenic), and the presence of vector hosts in the medium is of primary importance, especially in the context of parasitic colonization (Taraschewski 2006; Anderson & Sukhdeo, 2010). Second, experimental studies clearly demonstrated that high salinity concentrations adversely affect egg hatching, larval survival and infectivity of Anguillicoloides crassus(De Charleroy et al., 1989; Kirk et al., 2000a), likely via a deleterious osmotic stress operating at all the free-living stages of the parasite cycle (Scholz & Zerbst-Boroffka, 1994).

Lefebvre et al. (2012), in their analysis, found a clear negative correlation between salinity and Anguillicoloides crassus prevalence values across all sites; but they also showed that infection rates are influenced by latitude. Thus, low prevalences are more often found associated with high latitudes. Because latitude is a reasonable proxy for mean water temperature, this tends to give support to the ‘temperature limit’
hypothesis, according to which the parasite’s geographic expansion would stop under high latitudes (Knopf et al., 1998). However, this trend observed at a global scale does not preclude the colonization of the northernmost range of the eel hosts, as recently documented by the occurrence of A. crassus above 60° N in Scandinavian countries, for Anguilla anguilla (Jakobet al., 2009a) and in Canada, for Anguilla rostrata (Aïeta & Oliveira, 2009).

According to the results of the analysis performed by Lefebvre & Crivelli (2012), it appears that there is a threshold salinity value (around 15‰) above which Anguillicoloidescrassus development and/or life cycle completion is severely impaired. This threshold salinity value could explain the similarity of the epidemiological parameters values obtained in Tonga lake and Mafrag estuary; according to Khelifi-Touhamiet et al. (2006), the hydrologic cycle functioning of the Mafrag was found as an original state, comprising three phases “river, estuarine and lagoonal”. In this case, eel samples of the Mafrag should be collected from the river phase where life cycle of A. crassus is not impaired.

The study of the parasitism evolution from a consideration of the size of the host shows that the highest prevalence values are recorded in young eels. The value decreases with the increase of the size of the host. However, the values of mean intensity increase when the size of the eel increases. The same result has been reported from the Rhone River Delta (Lefebvre et al., 2002), from Moulouya estuary (Rahlhouet et al., 2001), from northeast Tunisian lagoons (Gargouri & Maamouri, 2006), from Delta region in Egypt (Hussien et al., 2012). This result might be due to the eel diet. In fact, the selection of food by eel is influenced by the size of the prey. Thus, young eels consume shellfish rather than fish (Benajiba et al., 1994). Eels are believed to become more piscivorous as their size increases (Moriarty, 1973).

Subsequently, we think, in agreement with Kirk (2003), that crustaceans as an intermediate host serve as the source of infection for smaller eels, whilst larger eels are mainly infected by preying on paratenic hosts, such as fish, which form an important part of their diet. The low mean intensity values in large eels might be explained by inhibition of juveniles migrating into the swim-bladder lumen when adults are already present there (Barse et al., 2001). The ability of larvae recorded in water of high salinity perhaps contributes to the dissemination and transmission of this parasite in brackish water.

Conclusion:

This study reveals that A. crassus is present in Tonga lake and Mafarg estuary throughout the year. However, it also highlights the importance of sampling by month. Seasonal differences in epidemiological parameters are pronounced. A. crassus infection more prevalent in small sized eels than larger ones, while larger eels harbored more parasites than smaller ones.

The use of principal component analysis shows that water temperature and salinity are correlated negatively with prevalence of A. crassus; this parasite shows the lowest values of infestation rates in summer and autumn when water temperature and salinity present the highest values.

The similarity of the epidemiological parameters values obtained in Tonga lake and Mafrag estuary may be related to the fact that life cycle of A. crassus is not impaired in Mafrag estuary because eel samples should be collected from the river phase of this site which is characterized by low salinity.

Acknowledgements:

This work was carried out with funds allocated by the General Directorate for Scientific Research and Technological Development (DGRSDT).

REFERENCES


rns: Implications of once on successful.

s not the way it is, Transport of inorganic nitrogen and phosphorus to the adjacent coast. (Algeria)

frag estuary (Algeria) conditions.

Computational& Data, eds., Greenacre and Blasius, Chapman and Hall. 9865).

special Refaey Anguillicolacrassus Available at Prague: Charles

situé dans le Nord Est Netherlands. Anguillicolacrassus


Flegr, J., 2008. Frozen Evolution: Or, that’s not the way it is, Mr. Darwin – Farewell to selfish gene. Prague: Charles University, Faculty of Science.


Husson, F. and J. Josse, 2014. Multiple Correspondence Analysis. In The Visualization and Verbalization of Data, eds., Greenacre and Blasius, Chapman and Hall.


http://cran.r-project.org/web/packages/