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Health status of the swim bladder of the European eel *Anguilla anguilla* in northeastern Algeria's Lake Oubeïra

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Abstract
While there have many numerous studies regarding the spread of the nematode *Anguillicoloides crassus* in its host, few of these have addressed the pathology itself. In the present work, we examined the status of the swim bladders of European eels populating Lake Oubeïra, by assessment of their Swim bladder Degenerative Index (SDI). We found that the 450 eels that we captured were aged between 19 and 79 months, and that they exhibited an extremely fast growth rate.
Our assessment of the REPRODUCTIVE capacity of the European EELs (EELREP) [13] revealed that 3.78% had not undergone sexual differentiation, while 95.78% were females, of which more than half were silvered; and only 0.45% were silvered males. The parasitism by *Anguillicoloides crassus* exhibited the following epidemiological parameters: P=50.44%, I=7.04±3.18 parasites per swim bladder, and A=3.74±2.04 parasites per eel (the latter varies significantly with the SDI). Lastly, we noticed that 95% of the examined swim bladders were damaged (the SDI varied between 1 and 5) and that this worm does not spare any age group.

Keywords: eels, swim bladder, SDI, *Anguillicoloides crassus*, otolith, Oubeïra

1. Introduction
Introduction of exotic parasites has been frequently expounded in the scientific literature [8], as is the case for the nematode *Anguillicoloides crassus* [25]. Its introduction in Europe corresponds with the importation of the Japanese eel *Anguilla japonica* to Germany from Taiwan and New Zealand [33, 37]. The recent association between the parasite and its definitive host *Anguilla anguilla* is thought to be the cause of a pronounced pathology [22, 31, 41]. It has consequently also caused a considerable decline in the population of eels in Europe as well as in the North Africa [24, 29]. Thus, the European eel *A. anguilla*, recently considered 'outside safe biological limits' [18] has become red-listed as 'Critically Endangered' [16]. Repeated infections during the continental life of the eel generally lead to profound damage to the swim bladder, and this may impair their spawning migration to the Sargasso Sea [7, 15, 25, 34]. *Anguillicoloides crassus* has a complex life cycle that includes one obligatory intermediate host (copepods), and one non-obligatory paratenic host (small fish) [10, 42, 43] that is the origin for the infestation of the population of eels in Algerian hydro-systems following the introduction of carp fry by importation from Hungary.
Studies of the European eel have been performed at the various hydro-systems in the extreme northeast of Algeria where this fish is typically found [12, 26, 28]. These studies only focused on the inventory of the parasites in lakes, rivers, and lagoons; without consideration of the pathological aspect or the parasitic chronology of the fish. Indeed, the most severe stage of the infection depends on the health status of the swim bladder (e.g. anatomical deformations and physiological dysfunction resulting from the infestation), rather than on the number of parasites in this organ [24].
The objective of the present study was to evaluate the current infection rate (by lumen worms) and past infections (e.g. as evidenced by damage to the swim bladders) of the European eels that populate Lake Oubeïra (Northern Africa).

2. Materials and Methods

2. 1. Study area

Lake Oubeïra is a fresh water lake with a surface area that covers 2,200 hectares, and that has a maximal depth of 4m. It is located approximately 4 km from the shores of the Mediterranean sea, at N36°50, E08°23 (Fig. 1). It is a designated wilderness area (registered with the "RAMSAR" Convention) of the Parc National d'El Kala that has the unique distinction of hosting the most important wetland complex of the Maghreb area [5].

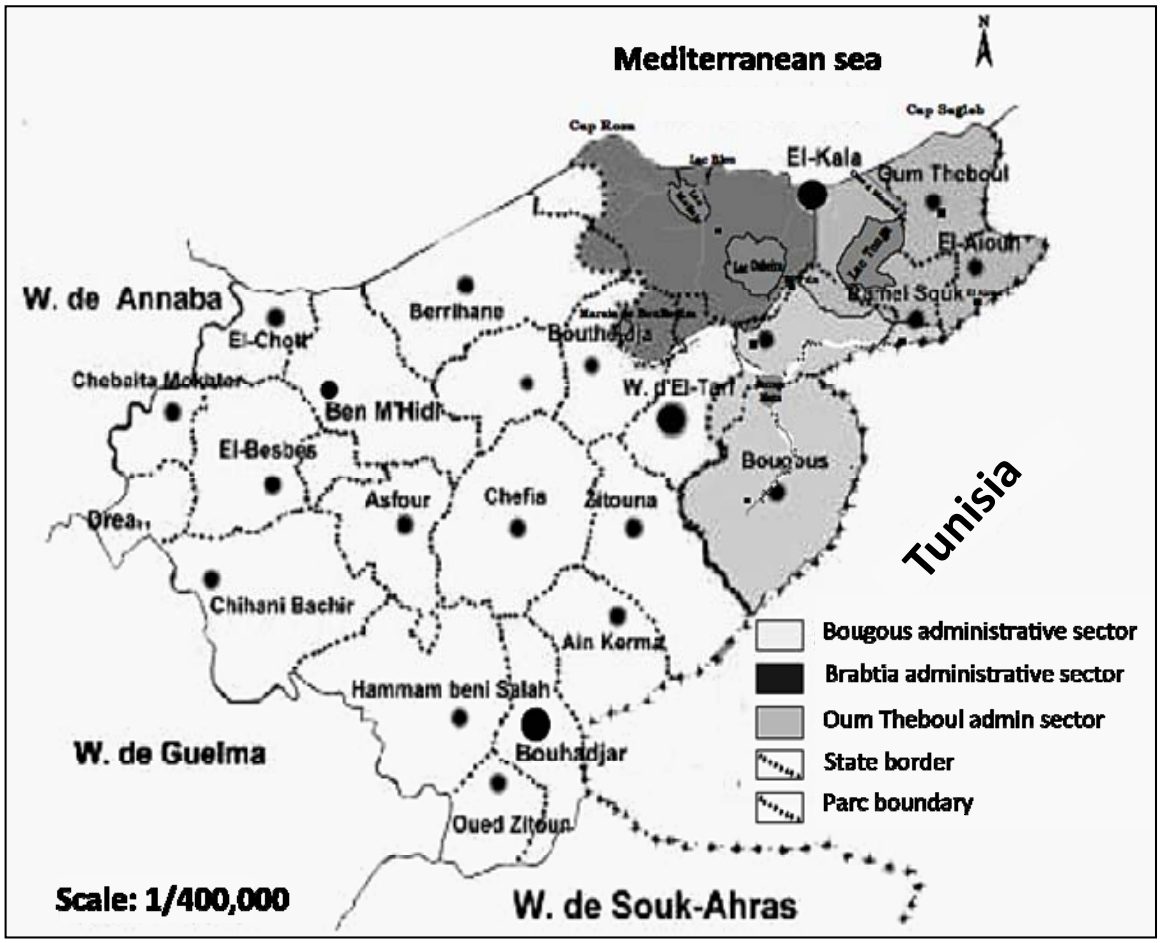

Fig 1: Lake Oubeïra, in northeastern Algeria.

The average monthly temperature of the water ranges from 8.8 °C to 15.2 °C in January. The average air temperature, determined for a period of 28 years from 1968-1969 until 1995-1996, is 17.50 °C. For January (the coldest month) this value is 11.65 °C and for August (the hottest month) it is 25 °C [5].

There is currently no hydraulic management framework for the lake, and recruitment of the populations of eels is done by natural means.

2.2. Capture and treatment of the eels

Sampling was carried out monthly (30 fish) between November 2010 and May 2012. Eels were captured using nets with a 10 mm mesh, they were brought back alive to the laboratory on ice and then weighed (total mass, Wt, to the nearest 0.1 g) and measured (total length, Lt, in mm).

The protocol for assessment of the Swim bladder Degenerative Index (SDI) was adapted from Lefebvre *et al.* [24]. This index is based on macroscopically visible alterations in the swim bladder, and it ranges from 0 to 6. The index comprises 3 criteria (each one being given a score of 0, 1, or 2) which are: the thickness, the opacity, and the pigmentation of the swim bladders.

The parasites in the swim bladders were removed and counted upon dissection. Species identification was performed using a stereoscopic microscope. Lastly, classical epidemiological parameters (e.g. prevalence, mean intensity, and abundance) were calculated [6].

The non-parametric test of Kruskal-Wallis was used in this study.

2.3. Otolith analysis

The neatness of the otolith growth marks of the captured eels allowed us to interpret them directly without prior preparation using a Leica WILD M3Z microscope with reflected light against a dark background. Assessment of their age was done by counting of the winter rings [36].

For modeling of growth of the eel population in question we used the following Von Bertalanffy (FCVB) equation: $Lt = L\infty (1-e^{-K(t-t0)})$

Lt: total length;

L∞, K and t0: parameters to be determined.

3. Results

3.1 The captured eels

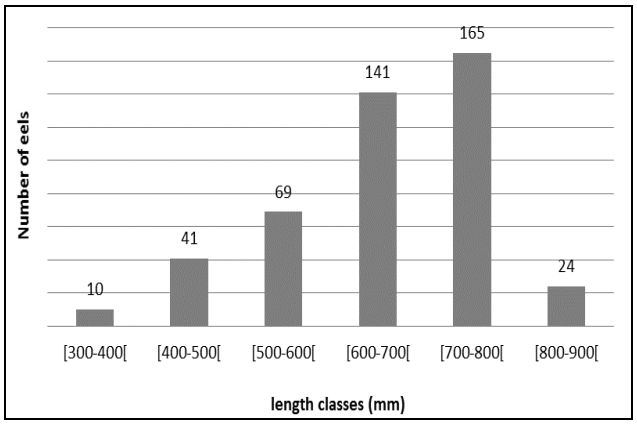

Fig 2: Size distribution ranges for eels captured between 2010 and 2012 (N=)

Over the course of 15 months we captured 450 eels, ranging in size from 300 mm to 895 mm (Fig. 2).

The relationship between the size (Lt) and the total weight (Wt) can be described as follows: $Wt=1E\text{-}07Lt^{3,3973}$

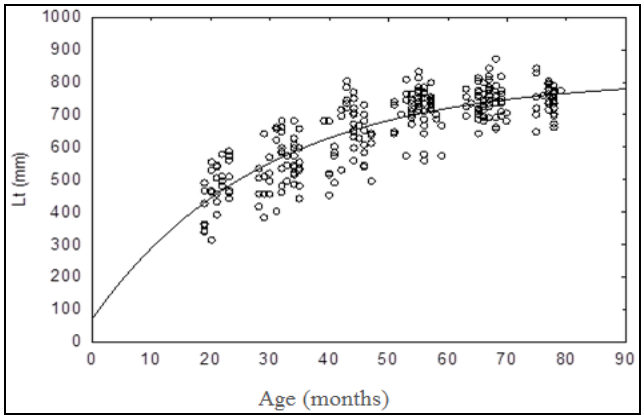

Fig 3: Modeling of the growth of captured eels according to the Von Bertalanffy formula ($L_t=L_\infty*(1\text{-}e(-K*(âge\text{-}t_0)))$) (N =294).

The age of the eels populating Lake Oubeïra ranged from 19 to 79 months, and they exhibited an extremely fast rate of growth (Fig. 3).

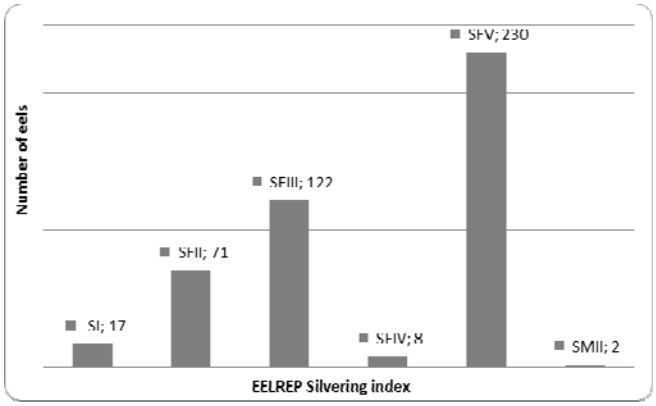

Fig 4: Classification of the EELREP index in terms of the degree of silvering in Lake Oubeïra.

According to the EELREP classification of the degree of silvering, we encountered

17 eels that were not sexually differentiated (SI); 2 males; and 431 female eels, of which 238 were silvers (Fig. 4).

3.2. Parasitism by *Anguillicoloides crassus*

The extent of eel infestation by *Anguillicoloides crassus* in Lake Oubeïra was 50.44%.

The mean intensity was 7.04±3.18 parasites per swim bladder, and the mean abundance was 3.74±2.04 parasites per eel.

During the study period, we counted up to 55 parasites in a single swim bladder.

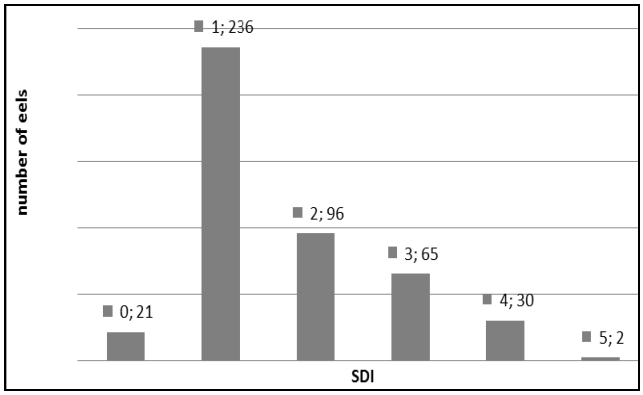

Fig 5: Distribution of the Swim bladder Degenerative Index (SDI) in Lake Oubeïra (SDI; number of eels)

The above graph (Fig. 5) shows the frequency distribution of the SDI. A value of 0 corresponds with an intact swim bladder, values from 1 to 3 correspond with a moderately damaged swim bladder, while values from 4 to 6 correspond with a severely damaged swim bladders (at a value of 6, no lumen is left).

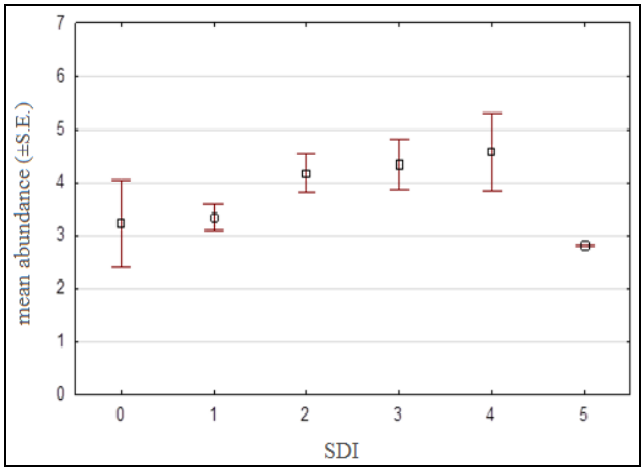

Fig 6: Mean abundance (±S.E.) of *Anguillicoloides crassus* by SDI.

The mean abundance of *Anguillicoloides crassus* (Fig. 6) varied significantly as a function of the values of the Swim bladder Degenerative Index (SDI) (Kruskal-Wallis test $H(4,450)=14,6213$; $P<0,0005$).

4. Discussion

Of the 450 eels captured during the study period (November 2010-May 2012), more than half of the population were migrant silver females. Their size ranged from 300 mm to 895

mm, with a high proportion of the eels falling into the 600-800 mm size range. The mean size of females silvering increased with the size of the watershed, indicating that the physical context of the watershed exercised a degree of control over the sexual differentiation of the eels [1], and thus also over the reproductive potential of the watershed. This preponderance of large female eels was also a reflection of the use of nets with a 10 mm mesh. Due to the selectivity of the fishing gear, the distribution of the observed sizes does not fully reflect the size distribution of the whole eel population in Lake Oubeïra.

This size distribution is similar to the one observed by other investigators at the same study site (330-380 mm); on the other hand, eels were found to be much smaller (230-630) in the El Mellah lagoon [12].

In Europe, the size of the silver females that were captured at 38 different sites ranged from 450 to 863 mm [46]. Regarding the other species of eels; their American neighbor *Anguilla rostrata* has been reported to have a mean size of 853 mm [45], while female Japanese *Anguilla japonica* are 614±40.5 mm in size [44], and the mean size of Australian *Anguilla australis* is 945 mm [40].

The sampled eels exhibited a very rapid rate of growth. This may be explained by the effect of the environment's temperature on the specimens (the average temperature of the lake being close to 17 °C). The growth rate (GR) is higher in habitats that are in proximity to the sea and that are at greater depths [9].

Furthermore, a high degree of variation in size for a given age was observed for a population, and substantial differences in size ranges have been reported for the various age groups of *Anguilla anguilla* [2, 39, 30, 35]. These disparities could be partially due to interpretation of their age, while the large diversity in environments that the eels occupy may also contribute to the observed variation in growth, as a result of the productivity of the environment (e.g. availability of food) and the density of eels (e.g. competition). Lastly, eel size has been shown to increases as the distance from the sea increases [4].

Assessment of the nematode *Anguillicoloides crassus* infestation rates highlights that it infests essentially half (50.44%) of the sub-population that we studied. This infestation amounted to 7.04±3.18 and 3.74±2.04 parasites per swim bladder and per eel, respectively. Very similar findings have been reported for eels in the Mafrag estuary (P=46%, A=2.02 worms per fish) [3] and in the fresh water bodies of the Parc National d'El Kala (e.g. lakes Tonga and Oubeïra), for which the author reports that there are 4-5 times more worms in these sites than in the El Mellah lagoon [12].

Others authors [25] have confirmed that high salinities limit, if not preclude, high infestation rates by *Anguillicoloides crassus.* In light of this, they have proposed using salinity as a natural mean for disease control. Such a measure has in fact already received backing from many ichthyoparasitologists and eel specialists [19-21, 27, 38].

In the Sebou estuary in Morocco, the infestation exhibits seasonal variations, fluctuating from 12.79% to 55.36%. The prevalence and abundance are relatively high, and they have been positively correlated with the length and weight of the fish [14]. In the Bizerte and Ghar El Melh lagoons of Tunisia, where the salinity approaches that of seawater (33-34‰), the nematode is present for just one to three months of the year, while also exhibiting low epidemiological values [17]. Lastly, a 2 year survey of four habitats in the Camargue area (Rhône delta, South of France) revealed a negative relationships between parasitic parameters (e.g. prevalence, mean intensity, and abundance) and salinity values of (i.e. a prevalence of 52% in brackish waters to 77% in fresh waters) [23].

To date, all of the studies of anguillicolosis in North Africa have been limited to the determination of conventional parasitic parameters, and no studies have been carried out regarding the Swim bladder Degenerative Index (with the exception of the recent study in 2014 by Dhaouadi *et al.*). Yet as of 2005, EELREP has recommended reliance on the SDI, as described by Lefebvre *et al.* [24], to obtain an indication of prior swim bladder infestation, as well as to gain information in regard to their functional status.

During this study, we noticed that more than 95% of the examined swim bladders were degraded (the SDIs ranged from 1 to 5), and that all eel age groups were infested. A Tunisian study [11] has reported an average swim bladder degenerative index of 0.28; and that 6.09% of the eels exhibited signs of previous infection, while 9.75% appeared to have either prior or current infections. More extensive damage has been observed in French lagoons, where 92% of the eels exhibited pathological signs of infection, and the severely damaged swim bladders harbored very few live nematodes [23]. Lastly, *A. crassus* infection has been determined to be widespread in Portugal's brackish water systems, where 67% of the swim bladders were found to be damaged; SDI=1.31±1.23 [32].

5. Conclusion

This study highlights the following points:

*The eels populating Lake Oubeïra exhibit an extremely fast rate of growth;

*Determination of the EELREP silvering index revealed that 3.78% were not sexually differentiated (SI); 95.78% were females (of which more than the half were silvered) and 0.45% were silvered males;

*The abundance of *Anguillicoloides crassus* varied significantly according to the SDI;

*The SDI varies according to the size and age of the captured eels.

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