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METAZOAN PARASITES AND HEALTH STATE OF EUROPEAN EEL, *ANGUILLA ANGUILLA* (ANGUILLIFORMES, ANGUILLIDAE), FROM TONGA LAKE AND EL MELLAH LAGOON IN THE NORTHEAST OF ALGERIA

F. Bakaria^{1*}, S. Belhaoues², N. Djebbari^{1,2}, M. Tahri^{1,2}, I. Ladjama², L. Bensaad²

¹ Faculty of Sciences; Université Chadli Bendjedid El Tarf, B.P. 73, El Tarf 36000, Algeria ²Laboratory of Ecobiology forMarine Environment and Coastlines, Faculty of Science, Badji Mokhtar University-Annaba, BP12 Annaba 23000 Algeria *Corresponding author: E- mail: bakaria_11@yahoo.fr

Metazoan Parasites and Health State of European Eel, Anguilla anguilla (Anguilliformes, Anguillidae), from Tonga Lake and El Mellah Lagoon in the Northeast of Algeria. Bakaria, F., Belhaoues, S., Djebbari, N., Tahri, M., Ladjama, I., Bensaad, L. — The aim of the study was to examine metazoans parasite communities of European eels (*Anguilla anguilla*) in freshwater (Tonga Lake) and brackish water (El Mellah lagoon) in the northeast of Algeria. Six parasite taxa were collected: one monogenean, *Pseudodactylogyrus* sp.; two crustaceans, *Ergasilus* sp. and *Argulus foliaceus*; two nematodes, *Cucullanus* sp. and *Anguillicola crassus*; one cestode, *Bothriocephalus claviceps*. The most prevalent parasite taxa in freshwater were *Pseudodactylogyrus* sp., *A. crassus* and *Bothriocephalus claviceps*; whereas in the brackish water, eels were infected mainly with *A. crassus*. The characteristics of the parasite component community structure revealed low parasite species diversity and high dominance values in eels from the two localities. Both communities were dominated by a single parasite species: Tonga eels by the monogenean *Pseudodactylogyrus* sp. and El Mellah lagoon eels by the nematode *A. crassus*, verified by high Berger–Parker dominance values of 0.76 and 0.87 respectively.

Key words: health state; Anguilla anguilla; metazoan parasites; Tonga Lake; El Mellah lagoon; Algeria.

Introduction

Over the past several decades, the European eel population has shown a sharp decline throughout Europe which continued until 2012 (ICES 2013). This decline was related to a certain number of factors as overfishing (Moriarty and Dekker, 1997), habitat fragmentation (Winter et al., 2006), oceanic conditions (Baltazar-Soares et al., 2014), pollution (Robinet and Feunteun, 2002), parasitism and disease (Kirk, 2000; Ginneken et al., 2005).

According to Jakob et al. (2009), 161 species or taxa have been described in the European eel from fresh, brackish, and marine waters in 30 countries of Europe and North Africa. Among them, the introduced swimbladder nematode *Anguillicola crassus* and the gill monogenean *Pseudodactylogyrus* sp. are considered as an important factor able to induce a stress that probably decreases the host fitness and seriously hampers the recovery of the European eel (Kennedy, 2007).

Pseudodactylogyrus sp. is a gill monogenean specific to eels; at very high intensities, the attachment and feeding on host epithelia and mucus can cause hemorrhage, hyperplasia of tissue and fusion of lamellae, reducing gill surface area and impairing respiration (Chan and Wu, 1984; Abdelmonem et al., 2010). Pathogenic effects of infection are more common in high density farmed conditions (Kennedy, 2007) and severe cases can cause decreased feeding, lethargy, movement to the water surface and areas of low water velocity, and impingement on outlet screens (Buchmann, 2012). Although there is currently little evidence to expect *Pseudodactylogyrus* spp. will affect *Anguilla anguilla* migration success, there is the potential for cumulative energetic effects when *A. crassus* intensity is also high (Køie, 1991).

Anguillicola crassus is an invasive species introduced into Europe with Japanese eels from the Far East (Køie, 1988). A. crassus is swimbladder nematode specific to eels; juvenile A. crassus nematodes migrate from the eels' gut to the swim bladder wall, before entering the lumen as adults where they feed on the host's blood and tissue (Banning and Haenen, 1990). Movement and feeding can lead to inflammation, oedema, fibrosis and haemorrhaging of the swim bladder wall, resulting in a reduced or collapsed (in severe cases) lumen and altered gas composition (Banning and Haenen, 1990; Molnár et al., 1993; Würtz et al., 1996). Reduced swimming performance, in combination with organ damage and the energetic costs of sanguivorous activity, is believed likely to reduce the probability of migratory A. anguilla reaching their spawning grounds in the Sargasso Sea (Palstra et al., 2007; Barry et al., 2014).

According to Terech-Majewska et al. (2015), *A. crassus* infestation in European eels could be responsible for a decreased immune response, which would result in higher susceptibility to other pathogenic conditions. Because a greater pathogenicity is often observed for recent (vs. long-term coevolved) host-parasite associations as for *Pseudodactylogyrus* sp. and *A. crassus* in *A. anguilla* (Moravec, 1992; Kennedy, 1994; Kania et al., 2010), we suppose that the least parasitized silver eels by these introduced species could be the most susceptible to reach the spawning grounds and to reproduce in the Sargasso Sea compared to the most heavily infected ones.

In the extreme North East of Algeria, studies of the European eel only focused on the inventory of parasites (Loucif et al., 2009) or on the spatio-temporal dynamic of *A. crassus* (Boudjadi et al., 2008; Djebbari et al., 2009, 2015).

Our objectives were (i) to describe the assemblage of metazoan parasite of eel subpopulations collected from freshwater and brackishwater bodies, and (ii) to evaluate the health state of swim bladder and the potential parasitic constraint for eel subpopulation of each site.

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 El Mellah lagoon (36°53.565′ N; 8°19,560′ E) situated in the northeast of Algeria (fig. 1) within the El-Kala National Park (PNEK), a protected natural reserve established since the adherence of Algeria to the Ramsar Convention in 1982.

The Mellah lagoon has a surface of 865 ha, a mean water depth of about 3.5 m and communicates with the sea by a long (900 m) and narrow (10–20 m) channel, which has undertaken substantial widening and deepening in 1988 (FAO 1987) and is periodically dredged due to its recurrent filling with silt. This lagoon is fed by three tributaries: Oued Soug R'guibet from the West, Oued Belaroug from the South East and Oued El Mellah from the South. Salinity ranges from 26 to 35g/l.

Lake Tonga 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 represent the connection with the Mediterranean Sea.

All samples were caught in fyke nets or traps by local fishermen. 360 fishes perlocality were brought back alive to the laboratory, measured, weighed and dissected. Stomach, intestine, liver, swim bladder, and gills were examined for helminth and crustacean parasites. The swim bladder was examined macroscopically for the presence of pre-adult and adult *A. c rassus* in the lumen, and for pathological alterations of the tissue. All metazoan parasites were fixed and preserved in 70 % ethanol.

The terms prevalence (P, percentage of eels infected), mean intensity (mean number of parasite individuals per infected hosts), intensity (I, number of parasites per infected host) and mean abundance (A, mean number of parasite individual per all examined hosts) were used as defined by Bush et al. (1997).

Analyses of parasite community structure were carried out at a component level (Holmes and Price, 1986). Measures used to describe component community structure of the metazoan parasites were species richness (s), Shannon's diversity index (H') and evenness (E), and the Berger–Parker dominance index (d). All indices were defined as in Magurran (1988) using natural logs (ln) where appropriate.

The protocol for assessment of the Swim bladder Degenerative Index (SDI) was adapted from Lefebvre (Lefebvre et al., 2002). 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 bladder. The swim bladder was then assigned to one of these two categories: normal (transparent and thin wall), or damaged (smoke-like opacity and thick wall).

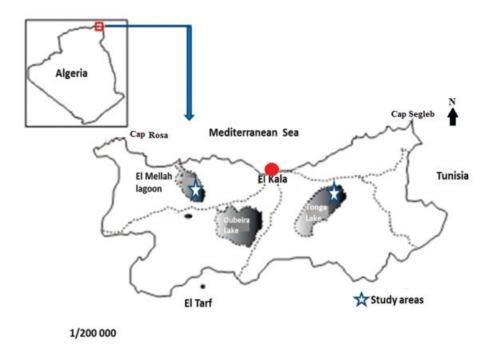


Fig. 1. Northeast of Algeria; stars show localization of sampling sites of eels.

Results

Parasite community composition

A total of 4164 parasites were detected in the 720 studied eels, belonging to six taxa (table 1): one monogenean, *Pseudodactylogyrus* sp.; two crustaceans, *Ergasilus* sp. and *Argulus foliaceus*; two nematodes, *Cucullanus* sp. and *A. crassus*; one cestode, *Bothriocephalus claviceps*.

Parasites collected from gills were *Pseudodactylogyrus* sp. (3155 individuals), *Ergasilus* sp. (5 individuals) and *A. foliaceus* (6 individuals); those from digestive tract were *B. claviceps* (343 individuals) and *Cucullanus* sp. (1 individual) and from the swimbladder *A. crassus* (654 individuals).

The number of non-infected eels was 17 and 338 in Tonga and El Mellah respectively. In freshwater pond, prevalence was 95.27 %, abundance 11.48 parasites per analysed eels and mean intensity 12.04 parasites per infected eels; in brackish water, prevalence was 6.11 %, intensity was 1.3 parasites per infected eels, abundance was 0.08 parasites per analysed eels.

The most prevalent parasite taxa in freshwater were monogeneans and nematodes, while in the brackish water, eels were infected mainly with nematodes.

In Tonga Lake, the higher records were for *Pseudodactylogyrus* sp., with a prevalence of 85.8 %, a mean abundance of 8.7 individuals per analysed eels and mean intensity of 10.2 individuals per infected eel. The most prevalent and abundant species in the lagoon is the swimbladder nematode *A. crassus*. Both prevalence and abundance of *A. crassus* are lower than in the freshwater (5 vs. 40 % and 0.07 vs. 1.74). In Tonga Lake, prevalence of *B. claviceps* was 26.38 %, intensity was 3.6 per infected eel and abundance 0.95 individuals per analysed eel (table 1).

Parasites	Infeo Fis			ected iduals	Preva 9		Mea		Inter Rar			dance ± SE
Locality	Т	М	Т	М	Т	М	Т	М	Т	М	Т	М
Pseudodactylogyrus sp.	309	2	3153	2	85.8	0.55	10.2 ± 3.84	1 ± 0.39	4.6– 17.8	1	8.7 ± 3.42	0.005 ± 0.1
<i>Ergasilus</i> sp.	4	1	4	1	1.1	0.27	1 ± 0.45	1 ± 0.28	1	1	0.01 ± 0.02	$\begin{array}{c} 0.002 \pm \\ 0.008 \end{array}$
Argulusfoliaceus	6	-	6	-	1.6	-	1 ± 0.49	-	1	-	0.02 ± 0.03	-
Bothriocephalus claviceps	95	1	342	1	26.38	0.27	3.6 ± 1.49	1 ± 0.29	1– 5.7	1	0.95 ± 0.8	$\begin{array}{c} 0.002 \pm \\ 0.008 \end{array}$
<i>Cuculanus</i> sp.	1	-	1	-	0.3	-	1	-	-	-	$\begin{array}{c} 0.002 \pm \\ 0.0008 \end{array}$	-
Anguillicola crassus	144	18	628	26	40	5	4.4 ± 1.73	1.4– 0.79	2.4- 8.7	1–1.7	1.7 ± 0.53	0.07 ± 0.07

Table 1. Composition of parasite communities with information on prevalence, abundance of infection in eels from the two localities (T = Tonga Lake; M = El Mellah lagoon)

Parasite component community structure

The characteristics of the parasite component community structure revealed low parasite species diversity and low dominance values in eels from the two localities (table 2). The most diverse community was detected in the eels from Tonga Lake (H' = 1.03), with lowest dominance (d = 0.76) values. Both communities were dominated by a single parasite species: Tonga eels by the monogenean *Pseudodactylogyrus* sp., and El Mellah logoon eels by the nematode *A. crassus*, verified by high Berger–Parker dominance values of 0.76 and 0.87, respectively.

In freshwater pond, the total species richness was six and the maximum infracommunity richness (number of species per fish) was four, found only in one eel (0.29 % of infected eels), while 46.64 % of the sampled eels harboured only one parasites species (140, 14 and 6 eels harboured *Pseudodactylogyrus* sp., *A. crassus* and *B. claviceps* respectively) and 40.23 % two different parasite species (*A. crassus*, *Pseudodactylogyrus* sp. and *B. claviceps* were present in more than 95 % of infected eels of this group). Only 12.82 % of the infected eels presented three different species (table 3).

Swim bladder health state

The figure 2 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).

Table 2. Component community structure of parasites and their diversity characteristics in eels from the two localities

Locality	Tonga Lake (Freshwater)	El Mellah lagoon (Brackish water)
Number of parasites species, s	6	4
Shannon's diversity index, H'	1.03	0.77
Shannon's evenness, E	0.40	0.39
Berger–Parker dominance index, d	0.76	0.87
Dominant species	Pseudodactylogyrus sp.	Anguillicola crassus

(Proportion of eels = 0.29 %)

Number of species	Species	Infected eels
1 species	Anguillicola crassus	14
(Proportion of eels = 46.64 %)	Pseudodactylogyrus sp.	140
	Bothriocephalus sp.	6
	Ac + Pseudo	87
2 species (Proportion of eels = 40.23 %)	Ac + Bothrio	4
	Pseudo + Bothrio	42
	Pseudo + Ergasilus	2
	Pseudo + Argulus	1
	Bothrio + Ergasilus	2
	Ac +Pseudo + Bothrio	40
3 species	Ac +Pseudo + Argulus	2
(Proportion of eels = 12.82%)	Ac +Pseudo+cucullanus	1
	Ac + Argulus + Bothrio	1
4 species	Ac +Pseudo + Argulus + Bothrio	1

Table 3. Composition of parasite communities with information on number of species per infected eel from Tonga Lake (Ac = Anguillicola crassus; Pseudo = Pseudodactylogyrus sp.; Bothrio = Bothriocephalus claviceps)

Proportion of intact swim bladder was three times higher in brackish water; eels from freshwater showed higher proportion of severely damaged organs (fig. 2). Among those eels having no parasite at the autopsy, the proportion of individuals with a damaged swim bladder was relatively high: 58.4 % and 93 % respectively in freshwater and brackish water. When adding currently and previously infected individuals, it appeared that the proportion of eels affected by anguillicolosis was high whatever the site (Tonga Lake = 93% and El Mellah lagoon = 78 %).

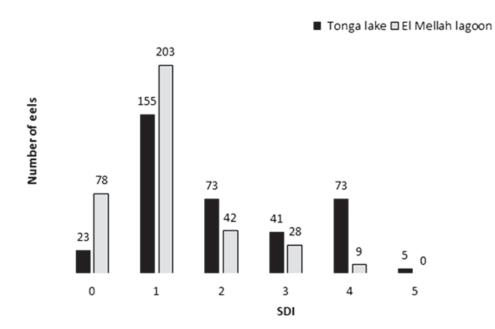


Fig. 2. Distribution of the swimbladder degenerative index (SDI) in eels from the two localities.

Discussion

Parasite community composition

Many authors noted that eel farms using seawater were often found free of the parasite and eels living in brackish and coastal waters most often showed a lower infection rate than those taken in neighbouring freshwater areas (Køie, 1991; Nielsen, 1997; Djebbari et al., 2009, 2015).

In Tonga Lake, the higher records were for *Pseudodactylogyrus* sp., while the most prevalent and abundant species in the lagoon was the swimbladder nematode *A. crassus*. Both prevalence and abundance of *A. crassus* are lower than in the freshwater. Similar results were reported by Mayo-Hernandez et al. (2015) in Mar Menor lagoon (Spain) where *A. crassus* infected only 3 % of eels, and showed very low mean abundance with 1 ± 0.75 individuals per analysed eel and low mean intensity with 3 ± 0.44 individuals per infected eel. Dezfuli et al. (2014) reported that prevalence and abundance of *A. crassus* are higher in the river than in the saline lagoons of Comacchio (28.0 vs. 11.9 % and 1.12 vs. 0.1 max). According to Lefebvre et al. (2012) high salinities limit, if not preclude, high infection rates by *A. crassus*. As a result, hatching rate, larval survival and the period of infectivity decline with salinity (Kirk et al., 2000), may explain the lower infection levels in lagoons.

No parasitic pathology probably occurred in the gills of El Mellah lagoon eels because of the rarity of *Pseudodactylogyrus* sp. (only two infected eels by one individual monogenean) and *Ergasilus* sp. (one individual) and the absence of *A. foliaceus*. The absence of these species in El Mellah lagoon can be related to various factors. The abiotic variables like the salinity or the water temperature influence the installation of certain parasites. According to Raibaut and Altunel (1976), *Ergasilus* sp. is a copepod specific of the environment with low salinity and high water temperature.

Characteristics of community structure

The characteristics of the parasite component community structure revealed low parasite species diversity and high dominance values in eels from the two localities. The more diverse community was detected in the eels from Tonga Lake (H' = 1.03) and lower dominance (d = 0.76) values. Both communities were dominated by a single parasite species: Tonga eels by the monogenean *Pseudodactylogyrus* sp. and El Mellah lagoon eels by the nematode *A.crassus*, verified by high Berger–Parker dominance values of 0.76 and 0.87 respectively. According to Kristmundsson and Helgason (2007), the component community of marine and freshwater eels was characterised by low diversity and high dominance of a single species. These results support the hypothesis of Kennedy et al. (1997) that parasite communities of the European eel are characterized by low species diversity and high dominance of a single parasite species, although the dominant species can vary.

Invasive parasites often have higher virulence and greater pathogenic effects in a novel host (Britton et al., 2011; Meeus et al., 2011; Lymbery et al., 2014), which can lead to behavioural changes as a by-product of infection (Taraschewski, 2006; Fang et al., 2008). Adult *A. anguilla*with an *A. crassus* intensity > 10 were observed to have a 19 % reduction in maximum swimming speed (Sprengel and Lüchtenberg, 1991). Gill damage from *Pseudodactylogyrus* spp. in aquaculture conditions can cause reduced activity levels (Buchmann, 2012). Study performed by Newbold et al. (2015) supports a more passive, energy saving behaviour in wild *A. anguilla* heavily infected with *Pseudodactylogyrus* spp., shown by the interaction effect between *Pseudodactylogyrus* spp. and *A. crassus* abundance. As the influence of *Pseudodactylogyrus* spp. on *A. anguilla* behaviour was only apparent when *A. crassus* abundance was high, this suggests that when combined with an additional stressor, infection by *Pseudodactylogyrus* spp. could affect *A. anguilla* behaviour.

There is general agreement that *A. crassus* has deleterious effects upon its eel host and that it can be considered a serious pathogen of eels at high abundance levels (Kennedy,

2007). Apart from histopathological damage to the swim bladder (Knopf et al., 2008), it can reduce the ability of the swim bladder to function as a hydrostatic organ during migration (Lefebvre et al., 2011). It can also impair the swimming performance of infected eels and trial simulations confirmed that eels with high levels of infection could show migration failure (Palstra et al., 2007; Lefebvre et al., 2011).

Swim bladder health state

A. crassus and *Pseudodactylogyrus* sp. were found at all investigated freshwater eels at high prevalence. Although observed pathological alterations of the swim bladders of eels infected with *A. crassus* were moderate to severe, a negative effect on the fitness of eels cannot be excluded. Experimental investigations performed by Würtz and Taraschewski (2000) have shown that macroscopic changes in the swim bladder wall can only be observed after multiple infection events. According to Lefebvre et al. (2002) the most severe stage of the infection depends on the anatomical deformations and physiological dysfunction resulting from the infestation (health status of the swim bladder), rather than on the number of parasites in this organ.

Our results show, among those eels having no parasite at the autopsy, the proportion of individuals with a damaged swim bladder was relatively high 58.4 % and 93 % respectively in both freshwater and brackishwater locality. When adding currently and previously infected individuals, it appeared that the proportion of eels affected by anguillicolosis was high whatever the site (Tonga Lake = 93 % and El Mellah lagoon = 78 %). In Oubeira lake, Tahri et al. (2016) noticed that more than 95 % of the examined swimbladders were degraded (the SDIs ranged from 1 to 5), and that all eel age groups were infested. In Ghar El Melh lagoon (Northeast of Tunisia) Dhaouadi et al. (2014) 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. The unique spawning migration, a distance of almost 5000 km to the Sargasso Sea, requires maximum fitness and health.

Component communities of metazoan parasites of eels widely varied between sites, suggesting differences in host species occurrence and environmental constraints or stressors (including parasitism), with probable consequences on the spawning migration and reproduction success. Indeed, only 18 of the 360 El Mellah lagoon eels were infected and always with a very low parasite intensity. Moreover, they harbored almost no parasites on their gills (only two individuals) and had both low *A. crassus* intensity (1.4 nematodes per swim bladder) and prevalence (5 %) suggesting low parasite pathogeny. The parasite constraint appeared more substantial for the silver eels from Tonga Lake having both damaged swim bladder and gills than for those from El Mellah lagoon since *Pseudodactylogyrus* sp. was almost absent.

Finally, based on the potential fitness loss induced by parasitism, we suppose that the migrant silver eels from El Mellah lagoon are able to contribute to the recruitment of *A. anguilla* population, whereas those from Tonga Lake a lower probability to reach the spawning grounds in Sargasso Sea.

Numerous aspects remain to be explored to explain the decline of the European eel population, probably induced by various interacting abiotic and biotic factors (habitat loss or fragmentation, changing hydrology, overfishing, pollution, and pathogens (Bruslé, 1994; Feunteun, 2002; Robinet and Feunteun, 2002; Fazio et al., 2005; Haenen et al., 2010; Kettle et al., 2011) and resulting in difficult and complex measures of preservation of this species.

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