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Potential ectoparasites of the endemic Mediterranean banded killifish (*Aphanius fasciatus*, Valenciennes, 1821) of the northern Sahara (Algeria)

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Introduction

The *Aphanius fasciatus* is a euryhaline and eurythermal fish, naturally present in many brackish water habitats little oxygen, along the Mediterranean coast, rarely in freshwater (Maltagliati, 1999). The species is endemic to the Mediterranean area, where it is found almost all coasts. Recently, Chaibi *et al*., (2015), reported the presence of the species in the northern part of the northern Sahara region, which widens the biogeographic area known distribution of the species, which was previously limited to coastal areas around the Mediterranean to continental habitats, including brackish water systems in the desert region of Algeria. In terms of conservation, *A. fasciatus*, is classified as a "Least Concern" (LC) species by the International Union for Conservation of Nature (IUCN); strictly protected in the Berne Convention (B2), and is not included in the list of protected species in Algeria. This new discovery of populations of *A. fasciatus* living in xeric area encouraged us to study one of the elements that contribute to the enrichment of knowledge about the life history of these special populations evolving in very demanding environmental conditions. Also, to our knowledge, parasites affecting *A. fasciatus*, have never been studied both in Algeria and in the south shore of the Mediterranean. They are much more focused on the parasites of marine fish as those of continental hydrosystems (Argilas, 1931; Brian, 1932; Rose and Vasière 1952; Euzet, 1954; Hamza *et al*., 2007; Ramdane *et al*., 2009; Boualleg *et al*., 2012). The objective of this work is twofold: (i) establish a pioneer list, even preliminary, of ectoparasites affecting *A. fasciatus* living in the northern Algerian Sahara; (ii) highlight the population dynamics of the *A. fasciatus* ectoparasites, according to weather variation, sex, age and size of fish hosts.

Materials and methods

Sampling site

Still Wadi is located in the vast northern Sahara region in the northeastern part of Algeria. It is part of the watershed of Chott Melghigh at an average height of 7 m above the sea level (Fig. 1). The width of the Still Wadi is variable, reaching a maximum of 70 m and one meter deep (Chaibi et *al*., 2015). The climate of the area is typically Saharan Africa, where the dry period extends throughout the year with average annual mean temperatures above 25 °C. The monthly variation of climatic parameters can distinguish a cold period of four months (November to February) with a monthly temperatures mean of 12.5±2.4 °C; and a hot period that spans the rest of the year with 27.0 ± 6.0 °C. Precipitations, very low and erratic with a range of 50 to 100 mm per year, are mainly concentrated in the cold period. (Ghazi and Si Bachir, 2016).

The sampled station (34° 14 '53' 'N 5° 55' 8'' E) is characterized by an average depth of 0.8 m. The substrate is formed of a mixture between the fine sand and sandy rock. The vegetation cover is characterized by the dominance of species with halophytic affinity. Still Wadi is crossed by the National Road N° 3 to about 1 Km from the town of Still.

Fishing and sampling methods

Sampling of fish was carried out monthly, between September 2012 and July 2013, through a hoop nets and trap gully with nylon (mesh: 12/13 mm). The water temperature in °C is measured in situ by a mercury thermometer down in water to 30 cm deep for few minutes. The surveys are carried out in the morning (between 8 am and 9 am). The fish caught then immediately placed in closed transparent boxes, containing water collected on the same site in order to keep them alive. In the laboratory, each individual was measured to the nearest millimeter (LT: total length) using an ichtyometer and weighed to the gram(PT: total weight). Abdominal dissection allowed sex determination through the gonads examination with the naked eye. The scalimetric method was used to determine the age (Leonardos and Sinis, 1999). The collected specimens of *A. fasciatus* are first carefully examined under the microscope to look for ectoparasites that are found on the skin and fins, which can also be seen sometimes with the naked eye, especially where the place is reddened, or where the mucus has an opaque grayish appearance.

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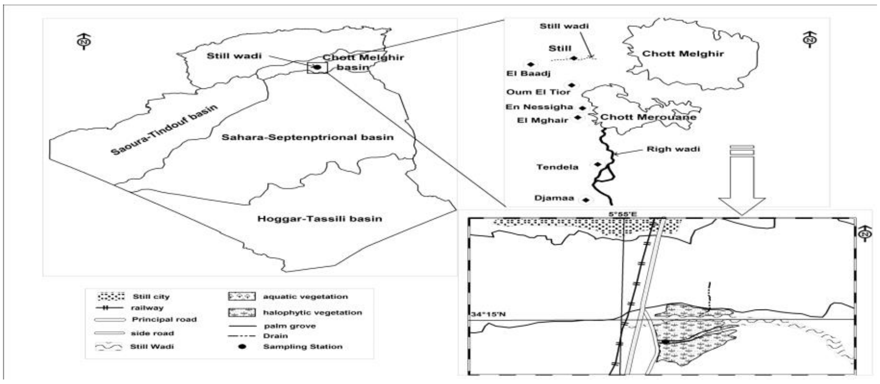

Figure 1: Location of the sampling site on the Wadi still (northern Sahara, Algeria).

Gill arches were gently detached after removing the cover using a clamp down as close to the eye and mouth. The recovered gills are scraped and rinsed three times in Petri dishes. The rinse water was also recovered in labeled vials with the code for each test article. Using a Pasteur pipette, a few rinse water droplets are spread over the entire surface of a slide and observed under an optical microscope at different magnifications (Gr: x10, x40, x100) until all of the rinse water is examined (Justine *et al*., 2012). According to their sizes, parasites were recovered by various means (pipette, needle, and brush) and stored in 70% ethanol (Gholami *et al*., 2011). Identification and taxonomy of isolated parasites were based on morphological and anatomical characteristics, with reference to several identification keys (Pariselle, 1995; Lom and Dyková, 2006). Identification of parasites is pushed to genus. On each slide prepared, all the parasites are identified and numbered, following the counting of the number of individuals of each observed taxa.

Data analysis

The data on the dynamics of the identified pest populations are exploited by calculating parasite rates set by Bush *et al*. (1997):

Prevalence (Pr%)=

$$\frac{\text{number of infected hosts}}{\text{number of examined hosts}} \times 100\%$$

Mean intensity (IM) =

$$\frac{\text{Total number of parasites}}{\text{number of infected hosts}}$$

Abundance (AB) =

$$\frac{\text{Total number of parasites}}{\text{number of examined hosts}}$$

The variation in parasite rates calculated, over time, sex, age class and size class has been tested by the Chi^2 test (χ^2) Pearson. The effect of weather period, sex, age and total length on the variation of ectoparasites numbers was tested using a generalized linear model (GLM) (Poisson-distributed error and a log link function). Only variables with p<0.05 were interpreted as statistically significant. Statistical tests were performed using the R (R Development Core Team, 2014)

Results and discussion

Taxonomic inventory

On the total of 95 pieces of *A. fasciatus* examined, the overall sex ratio is towards females (0.79). The average total length recorded in females (n = 52) is 44.2 ± 5.24 mm (minimum 29.5 mm;

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53.1 mm maximum). In males (n=43), the average total length is 40.0±5.04 mm (minimum 29.5 mm; maximum 48.3 mm). The longevity of females is higher (maximum of 3.75 years) than in males (maximum of 3.0 years).

The identification of parasites isolated from all the 95 pieces of *A. fasciatus* has allow to identify 116 individuals of at least 6 different genus, belong three Protozoa (*Chilodonella; Trichodina; Myxobolus*) and three Plathelmintha (*Dactylogyrus, Onchobdella, Posthodiplostomum*). The digeneans isolated from the gills are at the metacercariae stage (Table 1). All the parasites identified and recorded are only ectoparasites found on the gills. Indeed, the examination of the skin, fins and mucus revealed the complete absence of parasites. At the Phylum scale, only the protozoa are represented by three classes. Dactylogyridae is the most diverse family with 2 genera. In terms of abundance, the identified parasites are dominated by Digenea 49 individuals (42% of the total), followed and *Dactylogyrus* with 40 individuals (34%) (Table 1).

Table 1: Systematic and numbers (N) of ectoparasites of *Aphanius fasciatus* of northern Algerian Sahara.

Phylum	Class	Order	Family	Genus	N
Protozoa	Oligohymenophorea	Peritrichida	Urceolariidae	*Trichodina*	3
	Phyllopharyngea	Cyrtophorida	Chilodonellidae	*Chilodonella*	10
	Myxozoa	Bivalvulida	Myxobolidae	*Myxobolus*	6
Plathelmintha	Monogenea	Dactylogyridea	Dactylogyridae	*Dactylogyrus*	40
				Onchobdella	8
	Trematoda	Diplostomida	Diplostomidae	*Posthodiplostomum.*	49

Parasitism variation according to climate

The values of the three parasitic indices calculated vary between the two climate periods: the cold period (November to February) and the hot period (March to November). The most important numbers (91 individuals) is noted in the hot period against only 25 in the cold period. The prevalence, are faintly higher in the cold period (Pr = 38% of infected fish) than in the hot period. The average intensity and abundance recorded during the hot period (IM= 3.5 parasites by infested host. AB=1.22 parasites by host); are slightly higher than those observed in the cold period. However, the Chi2 test shows that the variation in function of time are not significant (Ddl=1; $\chi 2$ =0.05; $p<0.05$) (Table 2). The GLM model obtained shows that only the variation of parasite numbers with climatic season is not statistically significant (Table. 3). Generally, the majority of parasites have a greater impact when the temperature of water increases (Gholami et *al*., 2014). Similarly, Yemmen et *al*. (2011) reported that the rate of infestation of *Mugil cephalus* (Mugilidae) by *Trichodina puytoraci* (Urceolariidae) is more important

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during the spring, when the water temperature is above 18 °C.

Parasitism variation by sex, age classes and size classes of host

Over 44% of females of the *A. fasciatus* are infested (92 parasites per 52 females) with a mean intensity of 4 parasites per infested host and an abundance of 1.76 parasites per examined host. In males, the number of infected fish accounts for only 25% of the total number of examined males, and the number of identified parasites is lower (24 parasites per 43 males). The mean intensity in males is 2.18 host infested, whereas the parasite abundance is 0.55 parasite per host examined. The Chi2 test, indicates that the host sex has no significant effect on the variation in parasite prevalence of A. fasciatus (Ddl=1; $\chi2=1.22$, $p>0.05$) (Table 2). The variation in parasite rates between males and females described in our work broadly in line with findings made on *Aphanius* genus, where females are always more infested than males (Öztürk and Özer, 2007; Gholami et *al*., 2011; Rahimi et *al*., 2013). All age classes of *A. fasciatus* are infested by ectoparasites. The highest values of parasitic indices are noted in the fish whose age is between 2 and 4 years, where the parasitic prevalence is more than 48%. The juveniles (less than one year) had prevalence below 5%. The parasite prevalence variation as a function of host age class is statistically significant (Ddl=3; $\chi2=10.14$; $p=0.017$) As for the mean intensity and the parasite abundance, the maximum values are mentioned in fish whose age class is between 3 and 4 years, respectively 9.75 parasites per infested host and 5.57 parasites per examined host (Table 2). This finding is probably due to changing diets (Gjurčević et *al*., 2012), but also to the parasites accumulation period that increases with age (Özer and Erdem, 1998). Parasite prevalence observed in small individuals of *A. fasciatus* (total length less than 3.5 cm) does not exceed 10%. Meanwhile, it is always greater than 35% in tall ones (total length higher than or equal to 3.5 cm). This variation is statistically not significant (Ddl=2; $\chi2=1.97$; $p>0.05$). The parasite load follows the same pattern as the parasite prevalence. The highest mean intensity and abundance were noted in individuals whose size is between [4.5 - 5.5 cm]. The calculated values are respectively of 6 parasites per infested host for the mean intensity; and 2.75 parasites per examined host for the abundance (Table 2). Several mechanisms may be responsible for the increase of parasitism according to the fish size, including the size of the gill surface, which increases with the body size to accommodate more parasites (Tazerouti *et al*., 2009). Also, the larvae of parasites have a greater chance of invading fish, when the water volume that passes through the gills is important (Buchmann 1988). Indeed, the other parameters considered (sex, age, total length) show very highly significant correlation with the numbers of ectoparasites (Table 3).

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Table 2: Change in parasitic indices of ectoparasites of *Aphanius fasciatus* of the northern Algerian Sahara, as a function to climatic periods, sex, age class, and size classes of host (N: number of fish examined).

Variables	N	Pr%	IM	AB	χ^2	Ddl	*P*
Climatic period							
Cold period	21	38%	3.12	1.1			
Hot period	74	35%	3,5	1.2	0.05	1	0.945
Over all	95	36%	3.4	1.2			
Host sex							
Male	43	26	2.18	0.55	1.22	1	0.268
Female	52	44	4	1.76			
Table 2 continued:							
Age classes (years)							
[0–1]	24	Pr%	IM	AB			
[1–2]	33	4	2	0.082	10,14	3	0.017
[2–3]	31	36	1.66	0,60			
[3–4]	7	61	2.89	1.77			
Size classes							
[2.5– 3.5]	10	10	1	0.1			
[3.5 –4.5]	57	37	2.04	0.75	1,97	2	0.375
[4.5–5.5]	28	43	6	2.57			

Table 3: Results GLM testing the effect of climate, sex, age and total length, on the change in the number of ectoparasites identified in *Aphanius fasciatus* of northern Algerian Sahara. (SE: standard error, *p*: *p*-value AIC: Akaike information criterion).

Variables	Estimate	*SE*	Z value	*p*
(Intercept)	0.55	0.89	0.62	0.54
Sex	0.87	0.21	4.19	<0.0001
Age	1.14	0.17	6.89	< 0.0001
LT	-0.08	0.024	-3.4	0.0001
Climate season	0.78	0.54	1.46	0.15
Null deviance	418.36			
Residual deviance	305.42			
Dispersion	2.306			
AIC	434.44			

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