Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(3): 1857-1861

Received: 12-03-2017
Accepted: 13-04-2017

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# Cutlassfish infestation (*Trichiurus lepturus*) by *Anisakis simplex* larvae in Moroccan Atlantic coast

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**Abstract**
The aims of this research were to identify the morphology and determine the population dynamics of *Anisakis simplex* which infects Cutlassfish (*Trichiurus lepturus*). The samples of *T. lepturus* were collected at the fishing port of Mehdia at Atlantic coast, Kenitra city, Morocco, from April 2015 to March 2016. 210 fish specimens were collected then transferred in refrigerated condition to laboratory and immediately examined for *Anisakis* larvae. The parasites larvae were cleared in glycerin, observed in light microscope. The prevalence of *A. simplex* larvae was 59.04%. The infection with A. simplex was significantly higher (p=0,0005) in summer. The infected fish size were significantly larger (p=0.0005) than uninfected fish. The prevalence by locality of infection with the larvae was 59.04% and 10.95% in body cavity and musculature respectively. In conclusion, the populations consuming Cutlassfish are at risk to be infected.

**Keywords:** *Trichiurus lepturus*, *Anisakis simplex*, parasite, infestation, Morocco

## 1. Introduction
Fish are a good source of quality protein, but various diseases including parasitic infections could constitute significant economic losses in fish production, *Trichiurus lepturus* is a demersal-pelagic species with a predominantly piscivorous diet, but high feeding plasticity [1, 2]. This species occupies an intermediate position in the marine food chain, feeding on species that are important fishery resources, and is predated by elasmobranchs and small cetaceans. Therefore, the feeding habits and wide diet spectrum of cutlassfish puts them into contact with several potential intermediate hosts of parasites. This might be increasing the presence of *Anisakis* nematodes in these fishes. Larval stages of Anisakis nematodes (super family: Ascaridioidea, Family: Anisakidae) of genera such as *Anisakis*, Dujardin, 1845, are commonly found in the viscera and musculature of many species of teleost fish [3, 4], and can infect humans causing significant clinical disease (i.e. anisakiasis), in a number of countries from Japan, European Union, United States of America, Canada, New Zealand, Chile and Egypt [5, 6]. The fish act as intermediate or paratenic hosts, whereas amphibians, reptiles, birds and marine mammals (Whales and Dolphins) definitive host, harbor the adult stages [7]. The infection of *T. lepturus* with Anisakis larvae has been reported in Brazil [8, 9]. Notifications of *Anisakis simplex* found in others fish species have been also reported [10, 11]. In Morroco, there are only a few reports about the *Anisakis simplex* of fish [4, 12, 13].
The aim of the present study was to determine the morphology, and the population dynamic (Seasonal variation, host length variation and locality) of *Anisakis simplex* (family: Anisakidae) of Cutlassfish (*Trichiurus lepturus*).

## 2. Material and Methods
### 2.1. Sampling hosts
A total of 210 fish specimens, were collected from fisheries (Fig. 1) in Mehdia port (33° 55' & 6° 40' W), Kenitra, Morocco from April 2015 to March 2016. Samples were then transferred in refrigerated condition to laboratory and immediately examined for *Anisakis* nematodes.

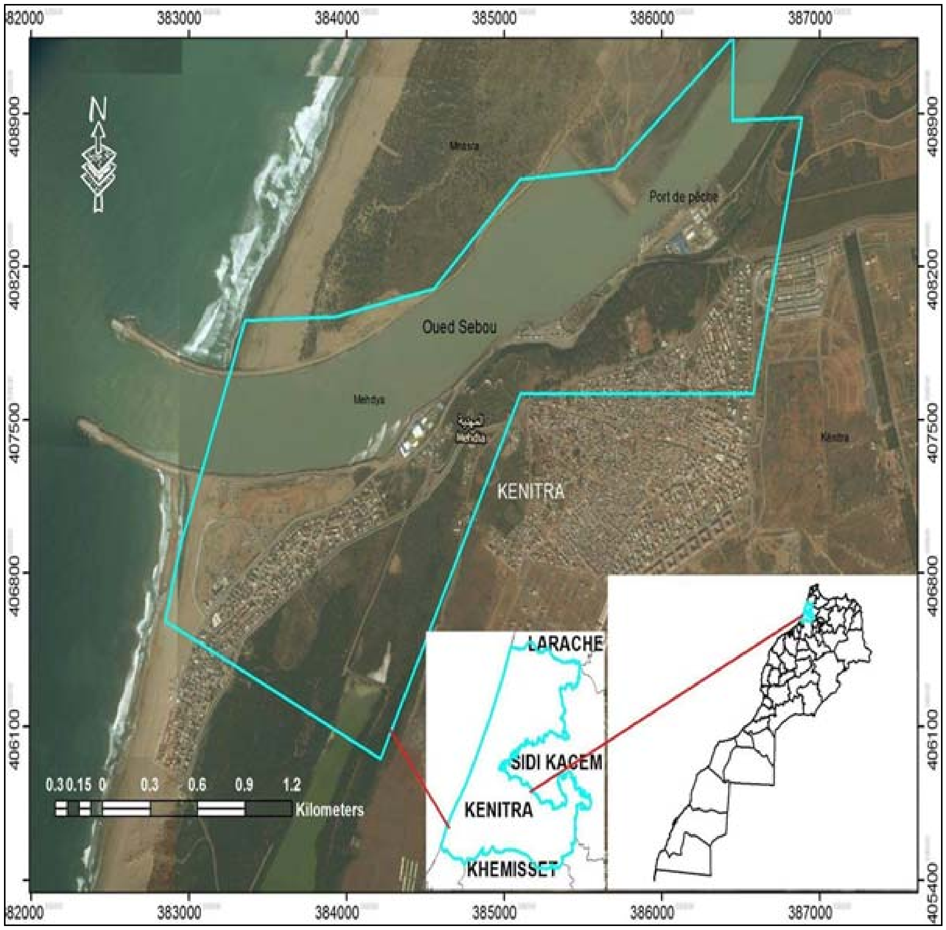


**Fig 1:** Zone of collection at Mehdia port in Atlantic coast, Morocco.

### 2.2. Parasite detection

Fish was measured and weighed, then were dissected carefully and examined thoroughly for *Anisakis simplex* larvae inhabiting the stomach, intestine, visceral organs, abdominal cavity, and muscles. The larvae were removed from the surrounding host tissues and counted. Larvae were relaxed in tap-water, fixed and stored in 70% ethanol. Morphological examinations were carried out on fresh and fixed larvae, using light microscope. The parasites were cleared in Glycerin or Berlese.

### 2.3. Morphological examination

The recovered larvae were cleared in lactophenol and permanently mounted in Glycerin or Berlese. The slides were left to dry for 24 hours and examined microscopically. The larvae were then identified according to their morphological characteristic features following [14].

### 2.4. Ecological parameters

Ecological parameters such as prevalence (percentage of infested hosts in a sample), mean intensity (mean number of parasites per infected host) and abundance (number of parasites per fish examined) were used as recommended by [15].

### 2.5. Statistical analysis

Statistical analyses were performed using XLSTAT. Results were presented as frequencies, percentage, sum, range (minimum, maximum), abundance and mean intensity. The abundance is a number of parasites per fish examined. The mean intensity is mean number of parasites per infected host. The prevalence is presented by the infected fish percentage. P-values for proportions were determined using Binomial test in the case of 2*2 table and Chi-Square test for 2*n tables. A p-value < 0.05 was considered as significant.

## 3. Results

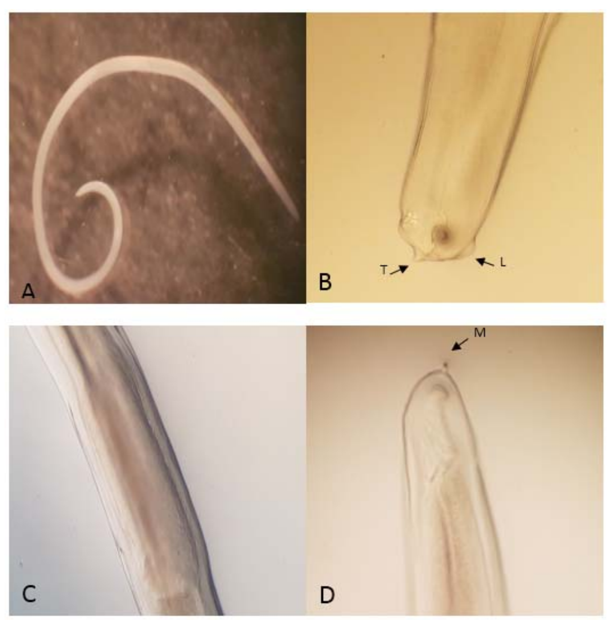


**Fig 2:** Morphological characteristics of Anisakid larvae *A. simplex* parasites from *Trichiurus lepturus.*

The morphological examination revealed that all the specimens larvae examined were identified as third-stage larvae of *Anisakis simplex* (fig. 2). The larvae ($L_3$) are characterized by:
-Length: 9- 36 mm, small worms (Fig. 2 A);
-Anterior view, cuticular Tooth (T), Lips inconspicuous (L) (Fig. 2 B);
-Ventriculus region (Fig. 2 C);
-Posterior view, Mucron (M); (Fig. 2 D).
All specimens in the present study showed these features [14]. The larvae were observed mostly within the body cavity or attached to the organs and rarely in the musculature, its intensity ranged from 1-9 and 2-263 parasites per fish in the musculature and body cavity respectively (Table 1).

**Table 1:** Distribution of *A. simplex* larvae in the body cavity and the musculature of *T. lepturus.*

| Habitat | Non-infected fish n (%) | Infected fish n (%) | p-values | Mean number of larvae (range) |
|---|---|---|---|---|
| Body cavity (n=210) | 86 (40.95) | 124 (59.05) | 0.011 | 3557 (2-263) |
| Musculature (n=210) | 127 (89.05) | 23 (10.95) | 0.0005 | 110 (1-9) |

Ecological parameters of *Anisakis simplex* in cutlassfish (*T. lepturus*) by season are shown in table 2. This table illustrates that 124 fish infected (or harbored the larvae $L_3$ of nematodes) of 210 fish (*Trichiurus lepturus)* examined. Seasonal prevalence of *A. simplex* larvae in *T. lepturus* was significantly higher in summer (83.0%), while it was 75% in winter, 43.8% in autumn and 35,1% in spring. The intensity during spring season was higher (37.9), while the lower intensities was 17.52 in autumn (Table 2).

**Table 2:** Ecological parameters of *A. simplex* in cutlassfish (*T. lepturus*) by season.

| Season | Non-infected fish n (%) | Infected fish n (%) | p-value | Number of larvae | Abundance | Mean Intensity |
|---|---|---|---|---|---|---|
| Winter (n=52) | 13 (25.0) | 39 (75.0) | 0.0005 | 1160 | 22.30 | 29.74 |
| Spring (n=57) | 37 (64.9) | 20 (35.1) | | 758 | 13.29 | 37.9 |
| Summer (n=53) | 9 (17.0) | 44 (83.0) | | 1381 | 26.05 | 31.38 |
| Autumn (n=48) | 27 (56.3) | 21 (43.8) | | 368 | 7.66 | 17.52 |
| Total (n=210) | 86 (41.0) | 124 (59.0) | | 3667 | - | - |

Ecological indices of *A. simplex* larvae by length in cutlassfish (*T. lepturus*) are shown in table 3. A significant difference was found between the host length and the infection. *T. lepturus* (length< à 60 cm) showed no infection by the larvae of *A. simplex*, compared with other length group (≥60), but the higher prevalence (100%) was presented in host length ((≥80) (Table 3).

**Table 3:** Ecological parameters of *A. simplex* larvae by length in cutlassfish (*T. lepturus*).

| Host Size group (cm) | Non-infected fish n (%) | Infected fish n (%) | p-value | Number of larvae | Abundance | Mean intensity |
|---|---|---|---|---|---|---|
| 30-40 (n=12) | 12(100.0) | 0(0.0) | 0.0005 | 0 | 0.00 | 0.00 |
| 40-50 (n=48) | 48(100.0) | 0(0.0) | | 0 | 0.00 | 0.00 |
| 50-60 (n=15) | 15(100.0) | 0(0.0) | | 0 | 0.00 | 0.00 |
| 60-70 (n=10) | 9(90.0) | 1(10.0) | | 1 | 0.10 | 1.00 |
| 70-80 (n=3) | 2(66.7) | 1(33.3) | | 2 | 0.67 | 2.00 |
| 80-90 (n=8) | 0(0.0) | 8(100.0) | | 192 | 24.00 | 24.00 |
| 90-100 (n=7) | 0(0.0) | 7(100.0) | | 127 | 18.14 | 18.14 |
| 100-110 (n=66) | 0(0.0) | 66(100.0) | | 1694 | 25.67 | 25.67 |
| 110-120 (n=33) | 0(0.0) | 33(100.0) | | 1365 | 41.36 | 41.36 |
| 120-130 (n=8) | 0(0.0) | 8(100.0) | | 286 | 35.75 | 35.75 |
| Total (n=210) | 86 | 124 | | 3667 | - | - |

## 4. Discussion

The larvae of Anisakis *Anisakis simplex* are common parasites of a wide range of *Trichiurus lepturus* marine fish species. These larvae has a stage which was identified as third-stage larvae ($L_3$) of *Anisakis simplex.* Larvae stages are commonly found in the body cavity and musculature of fishes [3]. However, some authors recorded two nematoda genera of *Anisakis* sp. and *Raphidascaris* sp. already known from *T. lepturus* in Brazil [8, 16]. *T. lepturus* serves for the nematodes as intermediate or paratenic host, where the larval stages accumulate until they reach their final host. Moreover, *T. lepturus* represents an important parasite transmitter, especially for the mammalian and elasmobranch final hosts.
In our study, The distribution and locality of infections by Anisakis simplex showed that the majority of nematodes were found in body cavity and rarely in muscles at a prevalence (59.04%) and (10.95%) respectively. These results are in accordance with the results previously reported by Hassan [17] and El Asley [18]. *A. simplex* was found in body cavity, digestive tract and gonads, although for other authors, the majority of these larvae were found in peritoneum than other organs, for example in *Trichiurus lepturus* [19], some commercial flatfish [20]. Although a low number of the larvae were detected in the musculature, it is an indication that the larvae migrate to muscles after capture [21] and would increase the risk of human anisakidosis, furthermore there is a degree of cross reactivity between *Anisakis* allergens and other *anisakids* which render individuals sensitive to the allergens of other species [22]. Furthermore, Infection prevalence and intensity of *A. simplex* larvae were increasing with host and size, thus larger fish appear as more susceptible to the infection rather than the smaller one. The host size group less than 60 cm showed no infection by the larvae of *Anisakis simplex*, compared to other length group (≥60 cm). From

length host (80 cm), the prevalence was higher (100%) but the highest mean intensity (41.36) was recorded in length group (110-120 cm). The relationship between the prevalence, intensity and the host fish length presented here, agrees well with the other authors results for various hosts species from various areas [3, 23, 24]. This is may be explained by the fact that larger fish provide more internal and external space for the establishment of parasites and have high rates of infection because they feed on a larger number of infected prey and provide a large contact area for the establishment of parasites [25]. According to Tavares [26], the parasitism may not necessarily increase with the size of the fish through a process of accumulation and longer exposure time, but may be related to changes in food items in different age groups of the host population and the population dynamics of intermediate hosts. Moreover, The peak of seasonal prevalence of *A. simplex* larvae was observed in summer (83.01%), nearly the same results was recorded by El-Asely [18] and Margarena [19], this may be attributed to the temperature factor which enhance the life cycle of the parasite [27] and the abundance of natural food specially crustacean which is the main food taken by cutlassfish in summer and play role in Anisakid life cycle. According to Carvalho [8], the reproduction of *T. lepturus* may be associated with local processes of productivity, since the upwelling areas that occur near the coast in summer coincide with the peak breeding of the species. This synchronicity between increased nutrient availability and the reproductive period is a strategy used by marine teleosts to ensure that the larvae have access to a greater concentration of food, thereby preventing their spreading out over a wider area and benefitting their survival [28].

## 5. Conclusion

The occurrence of worms in flesh reduce the marked value of fish, and thus represent some economical loss of the fisheries industry. In addition nematode genera *Anisakis,* found in fish may cause the most severe problems for human health, thus, sanitary regulations covering raw fish food products for human consumption are important to prevent anisakiasis in humans.

## 6. Acknowledgment

The present authors are grateful to the Delegation of Maritime Fisheries, and thank all the artisanal fisherman in Kenitra who helped to make this study successful.

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