

**The parasitic isopod *Anilocra physodes* (Cymothoidae; Crustacea) on its fish host from the Atlantic coast of Morocco.**

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**Abstract**

This study examined the parasitism of three species of fish, *Pagellus bogaraveo*, (family Sparidae), *Sardina pilchardus* (family Clupeidae) and *Umbrina canariensis* (family Sciaenidae), collected from the Atlantic coast of Morocco. A total of 5419 fish individuals were examined. Among them, 3600 fish individuals of *Sardina pilchardus*, 920 of *Umbrina canariensis* and 899 of *Pagellus bogaraveo*, were found to carry 328 isopod parasites of the species *Anilocra physodes* (family Cymothoidae). Most of these ectoparasitic isopods were primarily located on the body surface and within the gill chambers of their fish hosts. Parasitism of fish species was quantified using various parasitic indices (prevalence, intensity which is the mean number of parasites per infected host and abundance) were calculated for all species. The

evaluation of the parasitic indices revealed that the infestation rates by *A. physodes* were relatively low and do not exceed 11.11% of prevalence. Regarding the parasite loads, the mean infestation intensity is ranged between 1 and 2 parasites per infected fish. On the other hand, the abundance values remain below 0.14 parasites per fish examined in all the host species by *A. physodes*. These results confirm that infestation is strongly correlated with the host fish species studied. This study provides valuable insights into the effects of this ectoparasite crustacea on fishes health, emphasizing the need for fisheries management strategies and marine conservation in the region.

## **Abbreviations**

A. physodes: *Anilocra physodes*

## **1. Introduction**

Morocco has the privilege of having two maritime facades of 3500km long, including more than 500km longer in the Mediterranean and slightly less than 3,000km long in the Atlantic. Cymothoid isopods belong to a group of parasites known to infect numerous fish families and are highly host-specific and renowned ectoparasitic crustaceans of fishes worldwide, mainly in marine waters but also in freshwater [1,2]. There are 43 established genders [2], and hematophagous protandrous hermaphrodites [3], their life cycle involves only one host (holoxenic cycle) [4]. These parasites can be found on the external surface (body surface), envelop itself in a pouch, or burrow inside the flesh, buccal cavity, or gill chamber [5,6]. Infect many families and species of freshwater and marine fish, which can cause substantial economic losses to fisheries and the aquaculture industry [7]. These isopods cause various levels of damage to their hosts, ranging from minor tissue damage at the binding site to differential mortality [8]. Like most isopods, cymothoids are considered to feed principally on blood, but they may consume subcutaneous tissues, the mucus, and the epithelium of their host [4]. They have been reported to cause slow growth rates, tissue damage, anemia and ultimately fish death

[7]. Bacterial or fungal inflammation and contamination have often been found around wounds inspired by these ectoparasites and may facilitate the infection of other pathogenic microorganisms [9,10]. In some field studies, isopod-infested fish have been found to suffer from decreased condition index and growth [11,12], altered reproduction and reduced lifetime [12]. Cymothoid infestation cannot be generalized and standardized treatments cannot be performed for all fish. This is because of the biology of the host and cymothoid such as size, age, life stage, sex and behavior [1,13]. Generally, studies carried out in the field of parasitology are always based on parasitological indices. Indeed, these indices give us a clear idea of the state of infestation of the population studied and even provide information on the degree of affinity of the parasite for its host; high infestation rates would explain the affinity of a parasite to its host.

In Morocco, little information is known about the fish infestation status by *Anilocra physodes*, their distribution, host specificity, and ecological impacts along the Atlantic coast of Morocco. This region supports diverse fisheries critical to local economies, yet no comprehensive studies have assessed how *A. physodes* infestations affect commercially important fish species.

In this study, the main objectives were, to study the infestation rate, and characterize the prevalence and parasitism intensity of *Anilocra physodes* (Cymothoidae; Isopoda) on fish species hosted on the coasts of Morocco. This research will provide valuable insights into the prevalence and intensity *A. physodes* in Moroccan waters and contribute to the development of sustainable management strategies to mitigate parasite impacts on fish populations.

## 2. Results

The examination of the gills and external surfaces of these fish, allowed us to collect 328 parasites belonging to the family Cymothoidae, species *Anilocra physodes* (Figure 1) of which 38 individuals were found in *Umbrina canariensis*, 44 individuals in *Pagellus bogaraveo* and a high number of parasites were recorded in *Sardina pilchardus* by 246 individuals. The evaluation of the parasitic indices revealed that the infestation rates by *Anilocra physodes* are globally low and do not exceed 11.11% of prevalence (Figure 2). In regarding the parasite loads, the values of the average intensity of infestation were very low, ranged between 1 and 2 parasites per infested fish. On the other hand, the abundance values remained below 0.14 parasites per fish examined in all the host species.

### 2.1. Enumeration of harvested parasites

The results revealed that the highest number of parasites collected was recorded in *Sardina pilchardus* in November with 21 parasites on the South Atlantic coast and the lowest number was recorded in March on the North Atlantic coast, with the lowest rate equal to 3. For the specie *Umbrina canariensis* was present in January, June and July respectively (with 3 ectoparasitic), at the level of the North Atlantic coast. Finally, for *Pagellus bogaraveo*, it was in October on the South Atlantic coast (with 6 ectoparasitic). In general, the number of parasites collected from *Sardina pilchardus* is more important on the North Atlantic coast than on the South Atlantic coast, in contrast it is greater on the South Atlantic coast than on the North Atlantic coast for the host species *Pagellus bogaraveo*. For *Umbrina canariensis*, we observed an equality between the two study areas.

### 2.2. Distribution of parasitological indices

#### 2.2.1. By host species

The host-parasite relationship constitutes a biological entity that is expressed by parasite specificity [14]. According to Lumbery [15], the specificity of a parasite is the number of hosts.

This is a way of measuring the strength of the relationship between a parasite and its host. Data analysis suggested that infestation rates are globally low and do not exceed 11.11% of prevalence. The highest values were observed in *Sardina pilchardus* and *Pagellus bogaraveo* ( $P = 11.11\%$ ) followed by *Umbrina canariensis* ( $P = 8.33\%$ ) (Figure 2). In terms of parasitic load, the highest average infestation intensity was noted for *Sardina pilchardus*. On the other hand, the abundance values do not exceed 0.14 parasites/fish examined in *Sardina pilchardus* and remain below 0.13 parasites/fish examined in the other species.

### 2.2.2. According to the geographical distribution

On the southern Atlantic coast, *Sardina pilchardus* and *Pagellus bogaraveo* have the highest infestation rates of the ectoparasitic *Anilocra Physodes* ( $P = 11.11\%$ , during November, January and August respectively). However, in *Umbrina canariensis*, the highest prevalence values were recorded in the South Atlantic coastal locality ( $P = 9.09\%$ , during July). For the mean intensity, our results revealed that the parasite load (which varies between 1 and 2 parasites/infested fish), and abundance are very low and are more important at the northern Atlantic coast than at the southern Atlantic coast during the study period.

## 3. Discussion

This study highlights significant variations in infestation rate across different host fish species, with *Sardina pilchardus* supporting the highest infestation, followed by *Pagellus bogaraveo* and *Umbrina canariensis*.

In general, isopods parasitize many fish species worldwide, causing significant economic losses [16]. The species *Anilocra physodes* has been reported in the Atlantic Ocean, Mediterranean Sea, Black Sea and Adriatic Sea [17–22]. This euryxene species has a wide host range. However, in Tunisia, there is a clear predominance of infestations of Sparidae (*D. annularis*, *S. cantharus*, *Dentex vulgaris*, *Pagrus auriga*, *B. boops*) and on the Pomatomidae (*Pomatomus saltator*) [23,24], and in France, on the Sparidae and the Maenidae [19].

Infestations by the cymothoid isopod *Anilocra physodes* can have significant biological and ecological consequences for their host fish, affecting growth, reproduction, and overall health. As an ectoparasite, *A. physodes* attaches to the body surface of fish, often on the head or flank, and feeds on blood and tissue fluids, leading to chronic stress and energy depletion in the host [4]. Sasal et al., [25], reported that the *Acanthocephaloides propinquus* parasite of Marine fish, have negative effects on females reproduction level by reduced gonadosomatic index and egg production. Also Fogelman [26] suggest that *Anilocra apogonae* negatively affects the growth and reproduction of its host. Ecologically, such impacts on individual fish can translate into population-level effects, especially in heavily infested populations, potentially altering community dynamics and predator-prey relationships. Therefore, *A. physodes* infestations represent not only a parasitic burden to individual fish but also a potential threat to the health and sustainability of fish populations in natural ecosystems.

Our study revealed a high degree of host specificity for this parasite in the Atlantic coast of Morocco. We note the presence of this ectoparasite in the three species of fish collected, *Pagellus bogaraveo* (family Sparidae), *Sardina pilchardus* (family Clupeidae), and *Umbrina canariensis* (family Sciaenidae). Brusca [27] noted that some species of Cymothoidae prefer a host based on its ecological characteristics over its taxonomic identity.

In the present study, a census of parasites in the host species shows high infestation on the host species *Sardina pilchardus* where we recorded the greatest number of parasite species (N = 246), in contrast to *Pagellus bogaraveo*, which harbors only 44 parasite species and *Umbrina canariensis* only 38 species. According to Zender and Kesting [28], the eutrophic state of an environment would increases the infestation of the host. However, the prevalence of infested fish can vary over time and space [1]. In our study, we found high parasitization of *Anilocra physodes* on *Sardina pilchardus*, followed by *Pagellus bogaraveo*, and *Umbrina canariensis* respectively (Figure 2). The statistically significant effect of parasitism on the host-

parasite relationship was determined via one-way ANOVA for the species studied during the study period, which revealed that the prevalence of parasite species was significantly greater within the intergroups ( $F = 4.432$  and  $p = 0.001$ ), as well as for the intensity ( $F = 5.456$  and  $p = 0.0001$ , Table 1). In Morocco, Dollfus and Trilles [29], provided some figures on the intensity of Cymothoidae and reported values that varied between 1 and 2 parasites per fish, without specifying the number of fish specimens examined. The results obtained from our parasite load assessment are in accordance with those of Dollfus and Trilles [35], which who reported that the intensity of infestation is very low, ranged between 1 and 2 parasites per infested fish. According to Ternengo et al. [30], each species of fish presents a characteristic parasitofauna and particular levels of infestation. Isopod dispersion and abundance are influenced by many environmental factors such as salinity, predators, water temperature, light intensity, and food availability [31]. In our study, the three host species sampled presented abundance values of less than 0.14 parasites per fish examined. The study of parasitism according to the geographical distribution of the fishes examined confirmed that: in *Sardina pilchardus* the highest rate of infestation was recorded on the North coast. In contrast it is more important in the South Atlantic in the host species *Pagellus bogaraveo*. In the host *Umbrina canariensis* there was an equality between the two study areas. This may be related to and due to anthropogenic pressure, which can affect the immune system of fish hosts and host-parasite interactions. Zharikova [32], reported that in response to pollution, the infestation of fish by parasites decreases and that the appearance of dominant species could be considered an adaptive response of the parasite. The multivariate linear model test (Table 2), confirmed that infestation by the ectoparasite *Anilocra physodes* was closely related to the host fish species studied with significant variations ( $p < 0.0001$ ). Comparatively, there was no significant association between time and locality ( $p = 0.121$  and  $I = 0.230$ ) and ( $p = 0.828$  and  $I = 0.833$ ) respectively. The interaction between time-species locality were not significant (see Table 2). The causes of these

variations may be due, according to Combes [33], to the influence of many factors related to biogeography, the environment, ethology, the immune system, the presence of other parasites, genetics, and the age of the host. The results of the distribution of parasite indices of *Anilocra physodes* collected by host species show an inequality in the face of parasitism toward the latter; they vary from one host to another and from one marine area to another along the Moroccan coasts. Although *Anilocra physodes* is a common and widely distributed species, some aspects of its ecology and biology, such as the impact of specific host traits, and environmental conditions on the parasite dynamics and diversity of this ectoparasite have rarely been studied. For that reason, studying the defense mechanisms in host species parasitized by these ectoparasites and completing the present study via a comparative genetic study of the chromosomes of *Anilocra physodes* from different Moroccan marine coasts is recommended.

**Table 1:** Impact of ectoparasite *Anilocra physodes* on host species shown by one-way ANOVA test. Significant values are highlighted in bold:  $P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ .

		Sum of squares	Average of squares	F	Signification
Prevalence	Inter- groups	228,043	45,609	4,432	<b>,001***</b>
	Intra-groups	2160,979	10,290		
	Total	2389,022			
Intensity	Inter- groups	,037	,007	5,456	<b>,000***</b>
	Intra-groups	,286	,001		
	Total	,323			



**Table 2:** Results of the multivariate approach linear model test comparing the prevalence and intensity of *Anilocra physodes* in the three fish species according to factor month, species and locality. Significant values are highlighted in bold,  $P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ .

Source of variation	Dependent variable	F value	P value
Month	Prevalence	1,546	,121
	Intensity	1,301	,230
Species	Prevalence	11,598	<b>,000***</b>
	Intensity	14,254	<b>,000***</b>
Locality	Prevalence	,047	,828
	Intensity	,045	,833
Month * Species	Prevalence	,917	,574
	Intensity	1,153	,300
Month * Locality	Prevalence	1,101	,365
	Intensity	1,348	,204
Species * Locality	Prevalence	,047	,954
	Intensity	,551	,577
Month * Species * Locality	Prevalence	1,267	,204
	Intensity	1,354	,147

## 4. Materials and methods

### 4.1. Study site

The study was conducted during 2022 on the Atlantic coast of Morocco. The study area is subdivided into two zones, as follows: zone 1, the Moroccan North Atlantic coast from Tanger to North West to Essaouira, and zone 2: from Agadir to South West to Dakhla corresponds to the Moroccan South Atlantic coast (Figure 3).

### 4.2. Fish collection

A total of 5419 marine fish specimens belonging to three fish species infested by *Anilocra physodes* (Linnaeus, 1758) (Figure 1), including 3600 individuals of *Sardina pilchardus*, 920 individuals of *Umbrina canariensis* and 899 individuals of *Pagellus bogaraveo*, were collected with the aid of fishers from commercial catches and individual fishermen from January 2022 to December 2022. This temporal coverage allowed the detection of any seasonal variations in infestation prevalence. The choice of sampling sites was based on the previous frequent records

indicating isopod infestations in those areas. Samplings were performed twice a month with three repetitions for each site during our study period to enhance temporal resolution and statistical reliability. The prevalence (which is the percentage ratio of the number of infested hosts/total number of examined hosts  $\times 100$ ), mean intensity (total number of parasites collected/total number of infested hosts), and abundance (total number of parasites collected/total number of examined hosts) of the ectoparasite during the entire study period (12 months), were calculated according to Margolis et al. [34] and Bush et al. [35]. These parameters define the level of parasitic infestation.

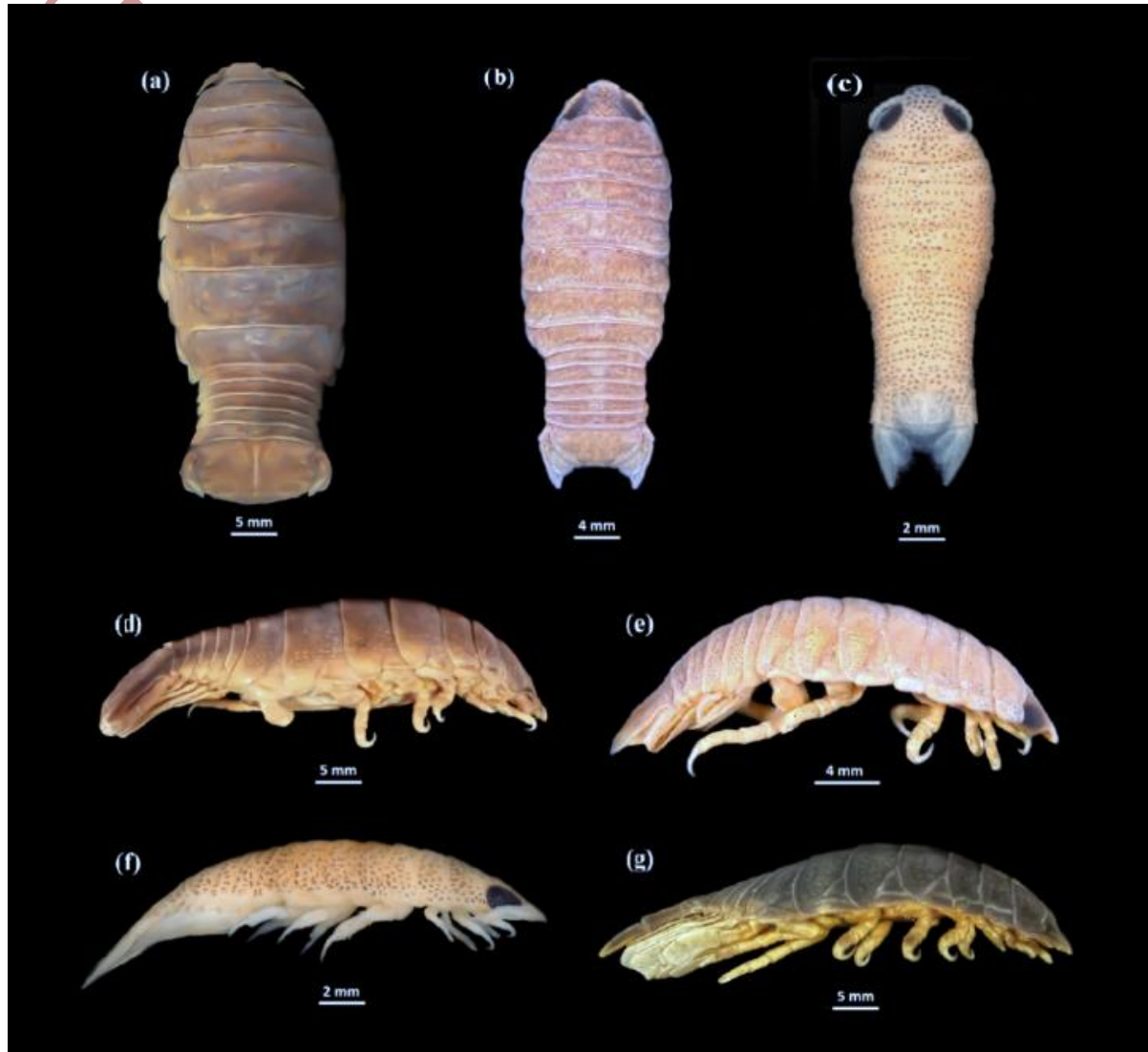
#### **4.3. Treatment and identification of parasites**

Isopods were removed from the host and immediately fixed in 70% ethanol. The sampling locality, date and host fish were noted. In the laboratory, specimens were identified. Identification was carried out using stereoscopic microscopes in collaboration with specialists and reference materials. We use some keys which are [19,36–38]. Host nomenclature and fish taxonomy are performed according to the guide of identification of the marine resources of Morocco [39], and the FishBase database [40–42]. This process was conducted at the Laboratory of Agricultural Production Improvement, Biotechnology and Environment, Faculty of Sciences, Mohammed First University.

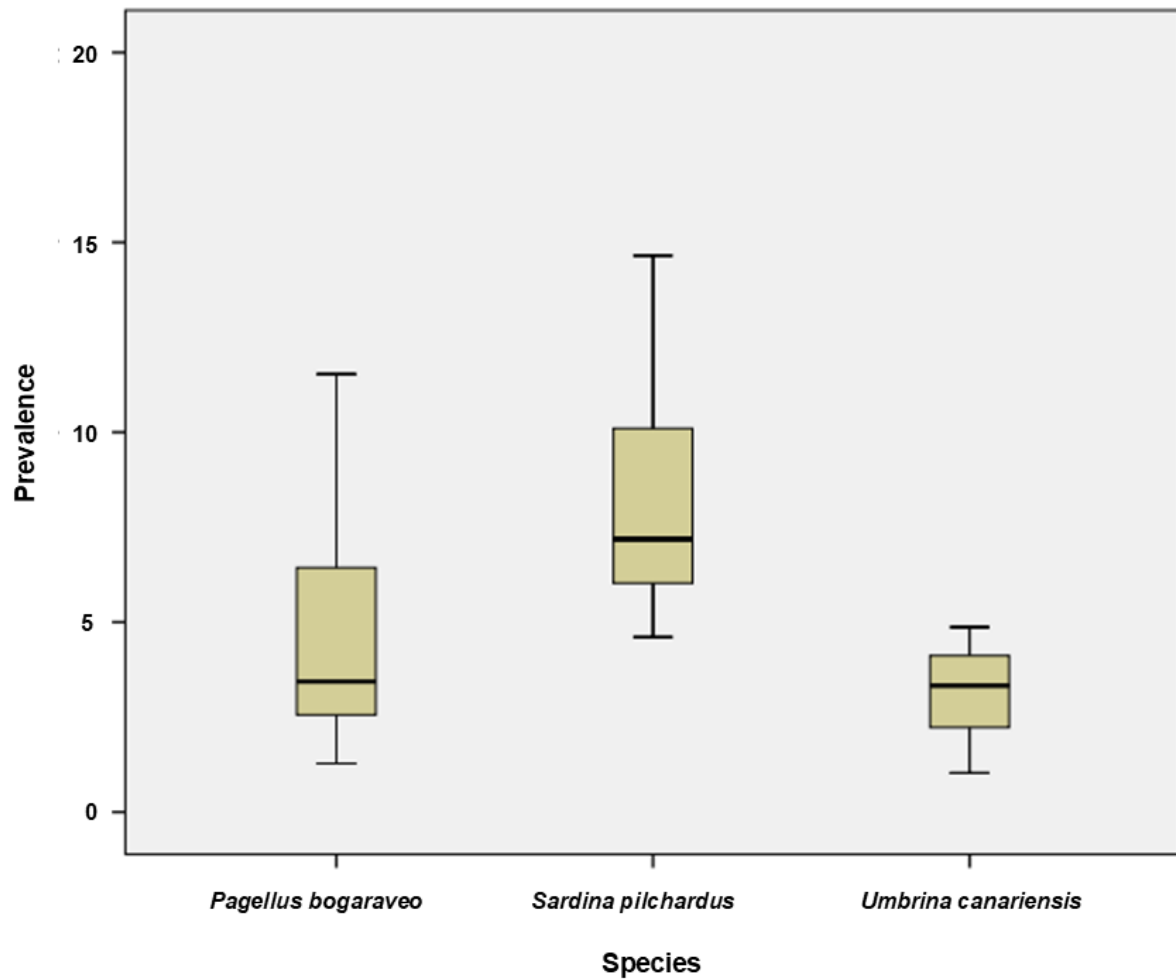
#### **4.4. Statistical analyses**

The raw data obtained from the field and laboratory examinations were entered into Microsoft Excel to create a database. In this study, we present the means of the analyses performed in triplicate with the corresponding standard deviations of our results for each month (for each parameter: 3 determinations  $\times$  3 samples = 9). One-way ANOVA and Tukey's test revealed significant differences between the sources of variation. Significant differences between the 12-month infestation results were determined via the t-student test. The threshold for a significant

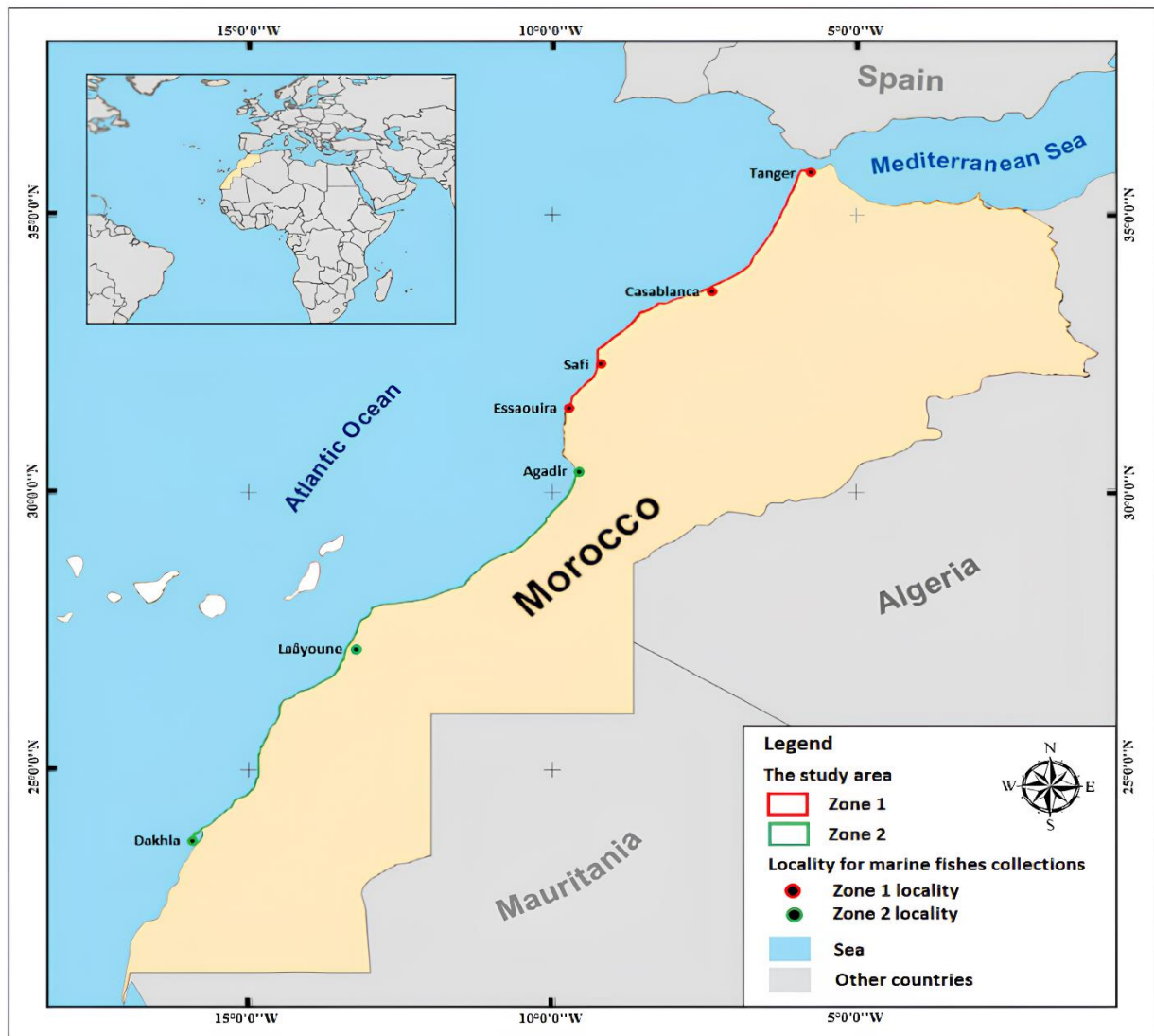
difference was set at 5%. Applications of these statistical analyses were analyzed using IBM SPSS Statistics (version 21 software program).



**Figure 1.** *Anilocra physodes* (Linnaeus, 1758) were collected in this study. (a) Female. (b) Male. (c) Immature stage 3. (d) Gravid female, lateral view. (e) Male, lateral view. (f) Immature stage 3, lateral view. (g) Non-gravid female, lateral view.



**Figure 2:** Prevalence of parasite effects on fish species. The samples were carried out for 12 months: the data were recorded for a total of 3 samples per species per location, repeated 3 times. The boxplot represents averages and error lines of one standard deviation \*\*\* indicates the significant differences at  $p < 0.01$  based on the SNK test.



**Figure 3:** Map showing the sampling areas. The study area is subdivided into two zones, which are, zone 1: the Moroccan North Atlantic coast from Tanger to Essaouira (indicated by red circles), and zone 2: the Moroccan South Atlantic coast from Agadir to Dakhla.

## Conclusions

This study highlights the significant impact of the parasitic isopod *Anilocra physodes* on fish host from the Atlantic coast, Morocco. The evolution of the parasite population of *A. physode* varied from one host species to another and from one locality to another. In addition, the results confirmed that infestation is closely related to the host fish species studied. This is probably due to the intrinsic characteristics of each host species. The *Sardina pilchardus* species is the most infested by *A. physodes* individuals followed by *Pagellus bogaraveo* and *Umbrina canariensis* respectively. The infestation rates were relatively low and did not exceed 11.11% of prevalence. The values of the main intensity of infestation, show a very low average infestation intensity, which ranged between 1 and 2 parasites per infested fish. On the other hand, the abundance values remain below 0.14 parasites per fish examined in all the host species by *A. physodes*. This study provides data on parasitic infections caused by this ectoparasite crustacea of fishes on the Moroccan coast in particular and worldwide in general. These findings stress the need for further studies on defense mechanisms in host species, environmental influences on parasitism and better understand the ecology and biology of this ectoparasite to improve fisheries management strategies and marine conservation.

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