PRINT ISSN 1119-8362
ELECTRONIC ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 22 (11) 1761–1767 November 2018

Prevalence of Parasitic Infestation in Freshwater Crab, *Sudanonautes africanus* (A. Milne-Edwards, 1869) (Brachyura: Potamonautidae) from selected Rivers in Edo and Delta States of Nigeria

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ABSTRACT: Freshwater crabs support fishery resources and serve as intermediate host to the major groups of animal parasites, hence, this study investigates the prevalence and intensity of parasitic infestation in freshwater crab *Sudanonautes africanus*, and its relationship to carapace width (CW) by collecting 350 specimens between March, 2007 and January, 2010 from selected rivers in Edo and Delta States. The results showed that a total of 174 (49.71%) individuals were infected with immature stages of nematode and digenean parasites in the 4 study sites. The prevalence of 86.54% was recorded in Iyi-Ekwu River, 65.74% in Oke River and 30.36% in Ikpoba River. However, the least prevalence of 18.18% was recorded in Orogodo River. Data analysis by Kruskal-Wallis, one-way ANOVA reveals there was no statistically significant difference ($p>0.05$) in prevalence among the four sampling sites. A total of 166 (47.43%) individuals from the four study sites were infected with larval nematodes, while 8 (2.29%) were infected with juvenile trematodes from both Oke and Orogodo Rivers. Result obtained from unpaired t-test indicates that crabs measuring 4 to 6 cm CW had a significantly higher prevalence ($p<0.05$) of parasitic infections. Furthermore, the intensity of nematode larvae isolated from each crab ranges from 1-25 (mean 5), 1-3 (mean 2), 1-4 (mean 2.3) and 1-6 (mean 2.8) nematodes per crab in Rivers Oke, Ikpoba, Iyi-Ukwu and Orogodo, respectively. The length of nematode larvae ranged from 0.7mm – 6mm long with a mean length of 2.4mm. Studies are on-going to experimentally obtain adults of the nematode larvae and juvenile trematodes for species identification.

DOI: https://dx.doi.org/10.4314/jasem.v22i11.9

Dates: Received: 12 October 2018; Revised: 06 November 2018; Accepted 26 November 2018

Keywords: Carapace width, Freshwater crab, Intensity, Nematode, Prevalence, Trematode

West African freshwater bodies are inhabited by decapod crustaceans that are common to its inland water bodies. In West and Central Africa, freshwater crab of the genus *Sudanonautes* consists of ten species distributed within the region (Cumberlidge, 1999; Awodiran *et. al.,* 2016). In many tropical countries, crab hunting remains one of the valued components of small-scale coastal fisheries (Chande and Mgaya, 2003). In America, the blue crab *Callinectes spidus* encourage regional fisheries along the eastern seaboard and Gulf of Mexico in the United States. Landings for the fisheries averaged 213 million pounds from 1989–1993 with a dockside value of US$137 million in 1994 (Lohan *et al*., 2012). Otter, mongoose, fishes and many other bird species depend on freshwater crabs as a source of food making it a major constituent of their food (Purves *et al*., 1994). Crab meat is a good source of protein, vitamins, essential minerals and substances (Emmanuel, 2008). Crabs contain chromium which acts with insulin in the metabolism of sugar helping the body to maintain normal blood glucose level. Hence, crabs are exploited by man and other animals for food (Vogan *et al.,* 2001). These crabs utilize energy from diverse trophic levels and contribute to energy and resource recycling within the river ecosystem. The crabs are important detritivores reducing the particles sizes of leaf litter and organic debris, presenting a source of nutrition to collectors and fills feeding fauna and enabling microbial activity (Hill and O'keeffe, 1992). Crabs are subject to the basic forms of infection that attack aquatic creatures and serve as intermediate host to the major groups of animal parasites. Parasitic infections of these crabs reduce their nutritional value, marketability, and abundance because these infections especially high parasitic infections cause loss of colour, the appearance of dots, making the crabs unattractive as well as cause death. Parasitic infection of crabs also causes a reduction in their protein content, destruction of reproductive organs, deformation of the nervous system, increased juvenile mortality and infection of the gills reduces the rate of respiration (Xianle and Yamping, 2003; Siddeek *et al*., 2010). In the most extensive review on *Hematodinium*-like infections, Stentiford and Shields (2005) noted that factors such as circulation,

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substratum, depth, temperature, and salinity, as well as host sex, size, and density, could exhibit differing influences over transmission and infection across species and regions. Usually, parasitic transmission appears to be associated with host moulting (Eaton *et al*., 1991), although transmission may also take place via cannibalism2009) or physical contact (Stentiford and Shields, 2005). Crabs of the genus *Sudanonautes* serve as intermediate host for some trematodes in Nigeria and Cameroon (Voelker and Sachs, 1977). They also serve as intermediate host of *Paragonimus westermani*. Some of the major parasites of crabs and shrimps which have been recorded in Nigeria include acanthocephalans, cestodes, nematodes and trematodes (Mordvinova, 1978). Parasites of crab have been widely reported for many crabs, e.g. Snow crabs, *Chionoecetes opilio*, Tanner crab, *C. bairdi* and Blue crab, *Callinectes amnicola* (Stentiford and Shields, 2005; Ekanem *et al.,* 2013). In view of the dearth of information on parasites infecting freshwater crabs in Nigeria, the authors undertook a preliminary investigation on prevalence and intensity of parasites occurring in the freshwater crab (*S. africanus)* from Edo and Delta States, south-south Nigeria, as part of an on-going research on the biology of freshwater crabs in inland water bodies.

MATERIALS AND METHODS

*Study site***:** The study was conducted at four selected sites over a period of four years, between March, 2007 and January, 2010 in Edo and Delta States of Nigeria. In this study, crabs were collected from Oke River at Ugbogui, the downstream of Ikpoba River, Iyi-Ukwu River and Orogodo River in Agbor. Site 1, Oke River has a substratum that consists of silt, fine and coarse sand. Site 2, Ikpoba River is a fourth order stream that rises from the Ishan Plateau in the northern part and flowing in south western direction in a steeply incised valley and through sandy areas before passing through Benin City and joining the Ossiomo River. Ikpoba River is highly disturbed while passing through Benin City due to the high population density and the dependence on the river. Site 3, Iyi-Ukwu River gets its source from a rock from which it forms a river at lowland. Site 4, Orogodo River is located along the Old Agbor-Warri Road. These sites have riparian vegetation and canopy cover. Anthropogenic influences on these sites are mainly crop cultivation, livestock farming, bathing, domestic washing, fishing and logging.

Collection and identification of specimens: Freshwater crabs (n=350) were collected from the four selected rivers. The crabs were caught using a trap with baited basket and hand-picked with protective rubber gloves. The specimens were transported to the laboratory in a bucket with moist plants. Identification of crabs was done using the keys provided in Cumberlidge (1999) and confirmed by Professor Clarkson Edema of Ecology and Systematics of Arthropods, Department of Animal and Environmental Biology, University of Benin, Benin City.

Examination of freshwater crab for parasites and its preservation: The crabs were pinned down ventrally on a dissecting board, measured and sex identified using the method described by Sachs and Cumberlidge (1991). Using a pair of scissors, the dorsal carapace was removed to expose the internal organs. The internal organs were sieved, using a small sieve in water. The resulting filtrate was left to settle. The supernatant was discarded and more saline added to the sediment and left to settle. The process was repeated until a clear supernatant was obtained, then the supernatant was discarded and the sediments obtained were kept aside for examination. A little quantity of the sediment was then poured into a Petri dish and normal saline was added. This sample was then viewed under a compound microscope. The section of Petri dish under the field of the compound microscope was critically searched and any parasite seen was extracted using a Pasteur pipette into a clean Petri dish containing normal saline. The extracted parasite was washed and mounted on a glass slide for close examination under the microscope. Parasites were counted and some were fixed and preserved in 3% formal saline. Some of the parasites were photographed using a Sony DSC-W800/B 20.1MPdigital camera.

Statistical analysis: The data were analyzed using the Microsoft Excel Statistical Analysis tool pack and SigmaPlot version 14.0 from Systat Software, Inc., San Jose California USA. The mean, sample size and standard deviation of prevalence (%) of parasites in the study sites were determined by descriptive statistics. Sites prevalence (%) was compared using Kruskal-Wallis One-way analysis of variance (ANOVA) on ranks at $p<0.05$ and the host class size's prevalence were compared using unpaired t-test at $p < 0.05$.

RESULTS AND DISCSSION

In a sample of 350 freshwater crabs, *Sudanonautes africanus* (A. Milne-Edwards, 1869) (Brachyura: Potamonautidae) collected, 92 (26.28%) were from Oke River; 56 (16.00%) from Ikpoba River; 52 (14.86%) from Iyi-Ukwu River and 99 (28.27%) from Orogodo River.

Of the 350 freshwater crabs examined in this study, 174 (49.71%) were infected with immature parasitic stages of nematode larvae and juvenile digenean

trematode in the four sampling sites. Infections were found in 94 individuals of the 143 freshwater crabs examined from Oke River; in 17 individuals of the 56 freshwater crabs from Ikpoba River; in 45 individuals of 52 freshwater crabs from Iyi-Ukwu River and in 18 individuals of 99 freshwater crabs from Orogodo River. Parasites isolated include nematode larvae and juvenile trematode. Out of 174 freshwater crabs found to be infected, 166 (47.7%) were infected with nematode larvae in all the Rivers, while 8 (2.3%) from both Oke and Orogodo Rivers were observed to be infected with juvenile trematodes (Table 1).

Table 1. Prevalence of nematode larvae and juvenile trematodes in freshwater crab, *Sudanonautes africanus* (A. Milne-Edwards, 1869) (Brachyura: Potamonautidae) in the study sites

		Prevalence (%)	
Sampling site	Host species	Nematode larvae	Juvenile trematode
Oke River	*S. africanus*	87/143(60.84)	7/143(4.90)
Ikpoba River	*S. africanus*	17/56(30.36)	-
Iyi-Ukwu River	*S. africanus*	45/52(86.54)	-
Orogodo River	*S. africanus*	17/99(17.17)	1/99(1.01)
Total		166/350(47.43)	8/350(2.29)

Monthly sampling of the selected sites showed that prevalence and intensity of parasites infections followed a seasonal trend, with a peak in dry and wet seasons, (Figure 1). Although infection prevalence was irregular throughout the wet season, they peaked in March, August and September in site 3 and September in site 1. Dry season peak was in the December (Figure 1). The highest number of freshwater crabs caught was recorded in July in site 1. The result of this study indicates that the freshwater crab, *S. africanus* showed a monthly variation in prevalence and intensity.

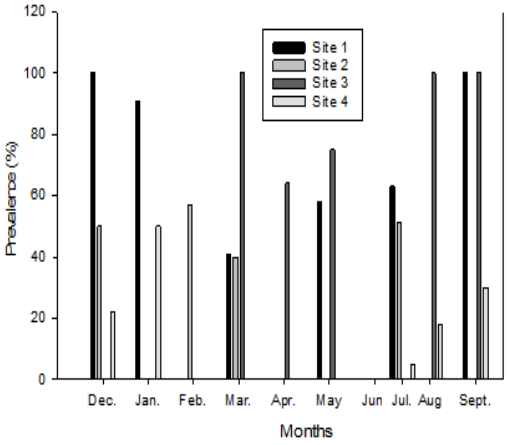

Fig 1. Prevalence of parasitic infection in freshwater crabs *S. africanus* (A. Milne-Edwards, 1869) (Brachyura: Potamonautidae) collected from the study sites across the month

The overall prevalence of infection was 49.71%. The highest prevalence of 86.54% was recorded in Iyi-Ekwu River followed by 65.74% in Oke River (Figure 2.). The least prevalence of 18.18% was recorded in Orogodo River and 30.36% in Ikpoba River. The box plot and the Kruskal–Wallis test showed that prevalence (%) did not differ (P>0.05) between the four sites (Figure 2). The prevalence of nematode larvae infections in freshwater crabs collected from Iyi-Ukwu River varied significantly between the 2-host class sizes during the period of study. Prevalence was significantly higher (P<0.05) in larger freshwater crab measuring 4-6cm CW than those measuring 2-4cm CW (Figure 3). A total of 762 nematode larvae were extracted from the infected freshwater crabs collected from the four study sites. The intensity of nematode larvae isolated from each crab ranged from 1-25, with a mean intensity of 5 larval nematodes per crab in Oke River; 1-3, with a mean intensity of 2 per crab in Ikpoba River; 1-4, with a mean intensity of 2.3 per crab and 1-6, with a mean intensity 2.8 per crab in Orogodo River, Figure 4. The length of the nematode larvae ranged from 0.7mm-6mm with a mean length of 2.4mm. There was no form of metacercaria infection recorded in the crabs examined.

Description of parasites isolated from crabs: Nematode larvae: The nematode parasites isolated from crabs were recognised by their elongated body, pointed anterior and posterior ends (Figure 5A-C).

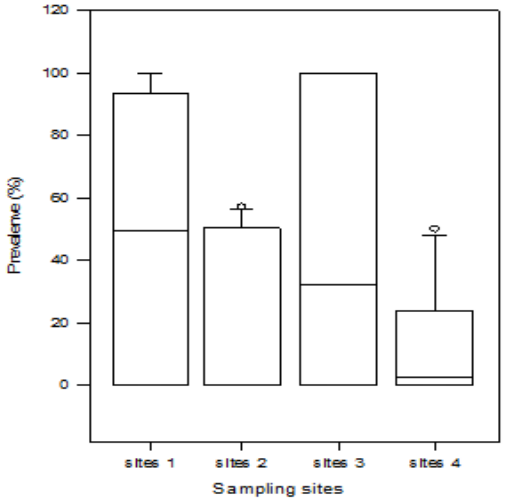

Fig 2. Box plot showing the percentage prevalence of the parasites at the four sampling sites during the period of study

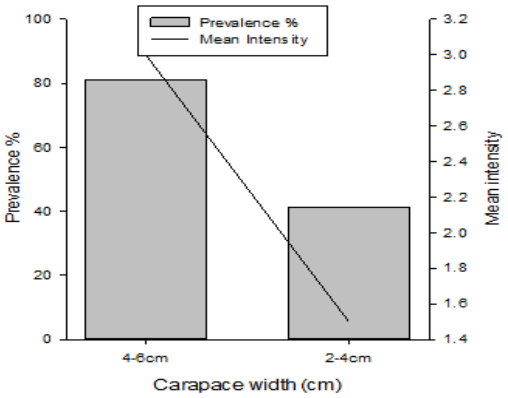

Fig 3. Comparison of prevalence (%) and mean intensity of nematode larvae among freshwater crabs of the two host class sizes collected from Iyi-Ukwu River during the study period

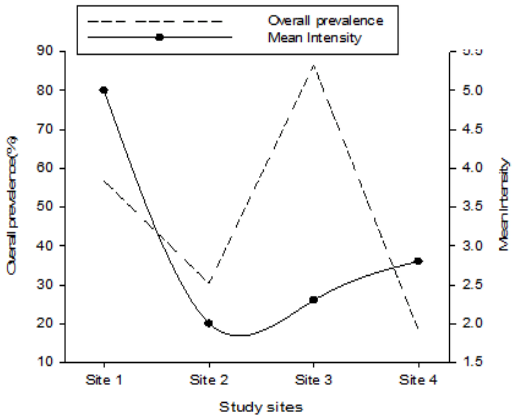

Fig 4. Comparison of overall prevalence (%) and mean intensity of larval nematodes among freshwater crabs from the study sites

*Juvenile trematode***:** The immature stages of digenetic trematode isolated from crabs were recognized by their oral and ventral suckers. Additionally, the presence of excretory bladder and intestinal caeca around the middle of the body (Figure 5D). The edible freshwater crab, *Sudanonautes africanus* was infected with larval nematode parasites, hence serves as an intermediate host to a yet to be identified nematode parasite. 47.43% of nematode infection recorded in this study is comparable with 40% reported by Childer *et al*. (1996), higher than 10% of two nematode larvae *Ascarophis* sp. and spirudae found in the black sea crab, *Pachygrapsus marmoratus* (Fabricius, 1793) but less than 90.2% of larval spirurida from *Macrophthalmus hirtipes* (Heller) (Ocypodidae) recorded in New Zealand (Moravec *et al*., 2003). Furthermore, 2.29% juvenile digenean infection in this study is less than 17.5% infection of *Paragonimus mexicanus* in freshwater crabs, *Hypolobocera chilensis eigenmanni* (Cornejo *et al*., 2000).

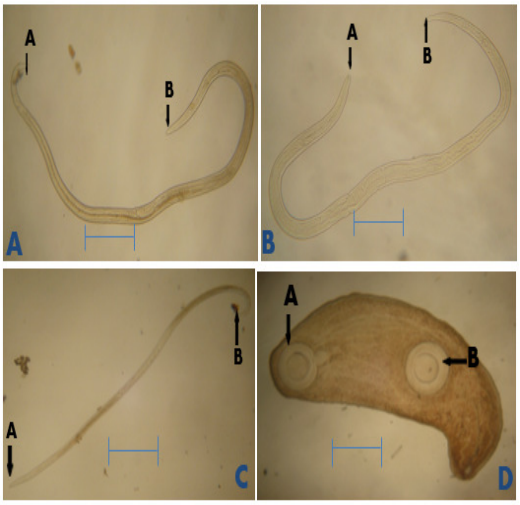

Fig 5. Photomicrographs show parasitic stages isolated from freshwater crab *S. africanus* in south-south Nigeria. A-C. Unidentified nematode larvae; note anterior and posterior end. D. Juvenile of unknown trematode; oral and ventral sucker are clearly discernible. Scale bar = 0.5mm

The range of 1-25 with mean of 5 larval nematodes per crab recorded in Oke River is similar to 1-18 with mean of 5 larval nematodes per crab of *Macrophthalmus hirtipes* recorded in New Zealand (Moravec *et al.*, 2003). However, it was higher than the range 1-3, with a mean intensity of 2 per crab in Ikpoba River; 1-4, with a mean intensity of 2.3 per crab and 1-6, with a mean intensity 2.8 per crab in Orogodo River recorded in this study (Figure 4). In the present study, prevalence was significantly higher in larger freshwater crabs measuring 4-6cm CW than those measuring 2-4cm. This is contrary to the findings of Messick and Shields (2000) who reported that prevalence was significantly higher in crabs measuring 3-30mm than in those measuring 31-60, 61-90, 91-120,121-150 or >150mm CW ($P<0.005$). Nevertheless, in <47 mm CW crabs, prevalence was inversely related to size in White Bay, but directly related to size in Notre Dame Bay. These differences may be an artefact of the small sample sizes (Mullowney *et al*., 2011). Trematodes infection reported in this study agrees with the previous account of Hashiguchi *et al.* (1974), having diagnostic features like suckers and movement of the juvenile trematode. The absence of the metacercaria infection in this study is similar to the research of Moravec *et al*. (2003). This finding also confirms the observation of Aka *et al.* (2008) who reported a gradual decline in the prevalence of crab infection for the past five decades after successive surveys conducted in Africa, and this

finding is difficult to interpret. The current change of eating habits of local people with giving up on crab consumption by the youngest classes and the health promotion policy might be the foremost justification so that the decrease of prevalence noted in crabs would be an indirect consequence. However, another hypothesis involving the extension of surveys on crabs in zones other than those in which infected patients were detected cannot be excluded.

In addition, 18.18% parasites prevalence recorded at Orogodo River in this study is similar to 18.75% *Paragonimus africanus* infections recorded in freshwater crabs *S. africanus* in Teke village, south-west province of Cameroon including the Kupe Mountain. Moreover, 2.3% prevalence of juvenile trematode in this study is contrary to 70.5% trematode infection of crabs and 18.1% trematode infection of shrimp recorded by Namso (Unpublished data 1985) from three locations, Ikpoba River, Mayuku Creek, Ugbekoko near Sapele and Ase River.

These variations in rates of infection can be attributed to differences in location (geographical areas) and other physico-chemical properties of the water bodies, availability of infective parasitic agents, host susceptibility and seasonality. Differences in rates of infection can also be related to the rate at which infectious elements like faeces and domestic waste are dispersed into the water bodies. Water properties like salinity, temperature, the population density of crabs, differences in rates of transmission and frequency of successful contact between infective agents and host can also lead to variation in the rate of infection (Chapman 2005). The outbreak of *Heamotidinium* sp. has been reported to affect species of crabs at high salinity (Messick and Shields, 2000). The study carried out by Stromberg *et al*. (1977) showed that infection increased with age and size because they show much higher prevalence of infection. This is probably related to the fact that different ages and sizes exhibit different time sequence of exposure and different behavioural patterns that result in greater likelihood of acquiring different parasites. In contrast to the above statement, Ahmed and Khan (1978) and Mayazka (1979) reported that prevalence of infection is not attributed to age and size. It is possible that the conflicting results reflect differences in ecology and geographical conditions. Similar to the observation of Cousin and Browler (1973), examination of crabs in this study did not show any detrimental effect on the freshwater crabs by the parasites. Contrarily, Couch (1983) recorded cases of obvious detrimental effects in heavily parasitized individuals. On the other hand, it was concluded that blue crab (*Callinectes amnicola*) from the Cross-River Estuary, Nigeria is not infested with parasites of zoonotic importance and could be recommended for human consumption (Ekanem *et al.,* 2013).

*Conclusion***:** The results obtained from the present study serves as the starting point for future epidemiological studies, and further investigation is recommended using suitable experimental hosts to ensure species identification of the nematode and trematode parasites to ascertain their status as parasite of man and other animals.

Acknowledgement: We thank Oriaran Lindsey, Odibei Valour and Onoriede Oberhiri for collecting crabs in the field. Also, we appreciate Professor Clarkson Edema of Laboratory of Systematics and Ecology of Fishes and Arthropods, Department of Animal and Environmental Biology, University of Benin, for confirming the identification of the freshwater crabs.

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