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DETERMINATION OF THE URVIVAL TEMPERATURE OF FRESH WATERFISH (*Parachanna obscura)* NEMATODES

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ABSTRACT

Parachana obscura is one of the major species of fishes that are most common in the Calabar Great Qua River. It is been consumed by about 70% of people within and without Cross River State almost every day. However, it has been noted from various literatures that this species of fish harbors a great number of nematodes. A total of 33 fishes (16larvae and 17adults) were bought from the Fishermen of Great Qua River Calabar, Cross River State –Nigeria, within the month of October and December 2011 respectively. The temperature range at which nematodes survive in these fishes purchased from fishermen of Great Kwa River Calabar, Cross River state- Nigeria was investigated. One hundred and eighty-seven (187) nematodes were extracted from 33 fishes within the months of October and Dec ember 2011. Fifty-One percent (51%) adults and 49% larvae were observed; and the nematodes were identified as *Contracaecum* and *Camallanus species.* Generally, the larvae survive more than the adult nematode. And it was observed that decrease in temperature below 22^0C, decreases the survival rate and increase in temperature above 60^0C completely eliminates the nematodes. Also, the analysis of variance (ANOVA) of temperature and time carried out were highly significant at P (< 0.05). The public should subject *P.obscura* to a temperature range above 60^0C for 30minutes or more to ensure total elimination of nematodes from the fish before consumption.

Keywords: *Parachana obscura*,nematodes,temperature and time.

INTRODUCTION

Temperature is the degree of hotness or coldness of a body or an environment at a particular time. The survival temperature of fresh water fish nematodes therefore refers to a temperature range especially in a temperature – controlled building, beyond which the nematode of fresh water fish will not survive. And the parameter for its measurement can be degree Celsius (^{0}C) or degree Fahrenheit (^{0}F).The degree Celsius can be converted to Fahrenheit using the formular C = (F - 32). 5/9. *Parachanna obscura* commonly called African snakehead fish (Akinsanya et al. 2010; Edama et al, 2008 and Gunter 1861) is also called in Efik language Obon fish, Ubon in

Ibibio and Alila in Igbo language. *Parachanna obscura* belongs to the class; actinoptergyii, order; performes and family channidae (Edema et al, 2008) and has its origin traced to White Nile, Lake Chad basin, Zaire (Congo), Senegal River basins, Gambela region of Ethiopia, (Akinsaya et al. 2010; Bailey 1994 and Boulenger 1907, 1916). Bonou et al. (1992) also listed P. *Obscura* as a common species in Cameroon, Nigeria and especially Cross River State and Edema et al, (2008) identified this fish in Okhuo River Benin, Benin City Nigeria. And this species has generally been classified as carnivorous predator which aids them all kinds of parasites. Several authors on aquarium condition have shown a lot of helminthes of fresh water fishes as well as those of *Parachanna obscura*. According to Jackson (1978), most notorious larval nematode found in fish are representatives of the families Anisakidae (Heterocheiliade), general *Amplicaecum*, *Contracaecum* and *Porrocaecum*, *Dioctophymidae*; genus, *Eustrongylides* and *Rhobolonidae*, with general *Rhabdochona* and *Spinitectus*. Also Edema et al. (2008) stated that two nematode parasites *procamallanus* species and *cucullanus* were observed to have high prevalence in fishes of Okhuo River, Benin City, Nigeria which is also fresh water, and that *Spinitectus* had 16.7% prevalence in *Parachanna obscura*. Akinsanya et al (2010) cited that in the eastern part of Nigeria, the following gastrointestinal helminthes worms have been documented by Ogbulie et al, (2003), *camallanus*, *capillaria* species, *acetodoxtra* species, and *clinostomum* species; and Levson et al, (2006) also recorded camallanus species to be widely distributed in fishes of fresh water.

Parachanna obscura are very important to man as a good source of protein in man's diet and as well, a vector of some human disease pathogens. According to Gonzalez et al, (2006), Anisakiasis is one of the major problems pose to human by fish helminthes. Howgate (1998) also confirmed the hazardous nature of nematode parasites in fishery products to human. Akinsanya et al, (2010) reported3 Laryngo-pharyngitis (acute irritation of the throat) from temporary attachment of worms (clinostomum species) eaten with raw food. And Jasmine (2006) reported that Anisakis are infective to humans, that people who produce immunoglobulin 'E' (IGE) in response to this parasite may subsequently have an allergic reaction, including anaphylaxis, after eating fishes that are infected by Anisakis. Also that Anisakids pose a risk to human health through; intestinal infection with worm from under processed fish and allergic reaction of chemicals left by the worm in the fish flesh. The larvae can invade the mucosa causing severe abdominal pain (may be spasmodic) sometimes accompanied by nausea, vomiting and occasionally fever (helminthological society of Washington, 1995-2011) and according to Oeltmann et al. (2005), these larvae burrows into the stomach or intestinal wall and causes inflammatory responses ranging from localized granulomata to massive, eosinophilia, hemorrhagic, tumor-like growth (neoplasms with larvae at the center of the lesion) consequently swelling of the intestinal wall may cause intestinal obstruction, peritionitis and the development of abscesses. Most cases have been reported from countries where fishes are eating raw like California and Hawaii, Masai east of Africa and can still be obtained here in Nigeria. Temperature range which has been found to be the most suitable and common with *Parachanna obscura* is 22 – 28 degree Celsius (72 – 82^{0}F) and P^H of 6 – 7.5 (Pouder et al. 2005 and Rufa G. 2007). In White Nile, the suitable temperature is during rainy season which is averagely 15 – 26^{0}C and Lake Chad has the maximum range which is 32^{0}C (Alan et al. 2001). All the activities of these nematodes as well as their mortality rate are always influenced by temperature. According to Burton et al, (2005), "at ambient temperature, Anisakis larvae migrates from the intestinal tract of fishes into the flesh, where they present a greater threat of being ingested by humans". When ingested by human, the third stage larvae attach to the stomach and develop to the fourth stage larvae and finally to the adult stage (Deardoff et al., 1986).

Since some people here in Nigeria also eat the so called "half done" which are under cooked, partially roasted or even sun-dried fishes, determination of the ambient survival temperature of freshwater fish nematodes will therefore help in the prevention of Anisakiasis and similar problems associated with these nematodes in human. And will be achieved by roasting, boiling cooking, smoking or frying thenfected fishes beyond the survival temperature which this work will provide.

AIM AND OBJECTIVES

The sole aim and objective of this work include;

- Extraction of nematodes from *Parachanna obscura* obtained from the great Kwa River and identification of the extracted nematodes.
- Determination of the temperature ranges and the space of time at which those nematodes will survive or die.

MATERIAL AND METHODS

STUDY AREA

The area from which the samples (*Parachanna obscura*) were collected is the Great Kwa River. The Great Kwa took rise from Oban hill south-east of Nigeria and meanders southwards through a distance of about 30km before discharging into the Cross River Estuary near Calabar, South-Eastern Nigeria.

The river is geographically located between latitude 4^0 45^0N and longitude 8^0, 20^0E and 8^0 31^0E and has a drainage basin of approximately 1,670km^2 (figure 3.1). It is surrounded by the thick forest belt of southeast, the swamp forest and the mangrove swamp. The other segment of this work will be completed in the laboratory of the department of Zoology and Environmental Biology, Faculty of Science, University of Calabar, Cross River State Nigeria (figure 3.1).

DURATION OF THE STUDY

The study duration is three months (from October to December) 2011.

TYPE OF STUDY

This work is an experimental study which encompasses both field and laboratory work. This, in earnest includes the collection of fish from the river and dissection for extraction of nematodes in the laboratory as well as identification and experimentation.

EXPERIMENTAL PROCEDURE

COLLECTION OF SAMPLES

Parachanna obscura as stated earlier, were purchased from artisanal fishermen at Obufa Esuk, station these men uses gill net of about 5.08cm and 7.61cm stretched mesh sizes, hooks and lines to capture the fishes. And they were transported in a plastic bucket (20 lit with aquarium water) to the laboratory for further study.

LABORATORY ANALYSIS

To extract the nematode, the sacrificed fishes were dissected starting from the anus through the stomach, to the head with a pair of dissecting scissors. The gastrointestinal tract, the gill and other organ, as well as the muscle were examined for parasites in Petri-dishes and the parasites were collected with the help of either forceps or Pasteur pipette and placed in test tubes from which they were taken to the experimental thermo-flask (Edema et al. 2008) as seen in plate 1. The number of nematode collected from a particular fish were documented, with the length and weight of each fish, all recorded with the use of a meter rule an electronic weighing balance to ensure accuracy. The adult nematodes were not entirely microscopic but can be notice due to their movement in the muscle fillet and intestinal content. Also the larvae were observable with the aid of hand lens.

MICROSCOPY AND PHOTOGRAPHY

The X10 magnifying lens of the microscope were used for the structural identification of the nematodes since they shear common features and some are difficult to locate and identify.

IDENTIFICATION OF NEMATODES

Although Anisakids share common features of all nematodes; the body plan cylindrical, elongated, round in cross section and lacks segmentation, reduced body cavity to a narrow pseudocoel, with mouth located anteriorly (Jansmine et al, 2006). Several other structural and behavioral differences are still obtainable in different species of nematodes of fresh water fishes as given by Yamaguti, (1961). According to Pouder et al. (2005) and Yanong, (2011), there are unique characteristics which can be use to identify each genus. *Camallanus sp.* for instance, are found at the posterior end of the intestinal tract, and other organs, it appears smooth, cylindrical, elongated, reddish, tread-like worm, up to 1cm in length, with serpentine movement. *Capillaria sp.* are common in the gastrointestinal tract they are relatively transparent with male approximately 5.4-7.4mm, and gravid female approximately 9.4-16.5mm in length and shows serpentine movement and the female *capilleria* are easily noticed if it carries the brown barrel-shaped egg with a plug-like structure at each end. *Contracaecum sp.* which are common to the body cavity, liver, muscle, heart, swim bladder and appears grossly visible, often seen coiled (encysted), approximately 20mm in length, and *Eustrongylides* are found outside the internal tissues, it can be found in muscle "free" within the body cavity or encapsulated on the liver and other organ, also grossly visible, coiled, reddish and usually 11-83mm in length, and movement not usually seen in fish (Kanerak, et al 2009). And many other features and keys were used for identification. As for the larvae, they are relatively smaller than the adults of their species, the unique features of the adults are not seen in the larvae for instance the presence of a buccal capsule (mouth structure) that is divided into two lateral values as seen in adult *Camallanus sp.* are not noticeable in their larval stage. Also some of the larvae are encapsulated and are darker when view with magnify hence. Some of the nematodes identified are shown in figure 4.6 to 4.9.

DETERMINATION OF THE EFFECT OF DIFFERENT TEMPERATUER RANGES ON PARASITES WITH TIME

Materials for experiment

Electric heater, thermo-flask, thermoseal, thermometer, weighing balance, meter rule, magnifying lens, dissecting set, had gloves, among others were used for the experiment.

Method of Experiment

Different sets of nematodes (5per sample set) were subjected to different ranges of temperatures; 0^0C, 22^0C, 27^0C, 37^0C, 50^0, 60^0c 65^0C and 1000C with time variations in a controlled temperature system. The number of life and death in each set (flask) were recorded after the expected time like 10mintes, 20minute and 30minute elapsed. And that was done for the larvae and the adult nematodes. The raw data obtained are as found in the table at appendix.

STATISTICAL ANALYSIS

The raw data collected from sampling were carefully analyzed using completely randomized design (CRD) and was subjected to analysis of variance (ANOVA) table to determine the significance of the temperature and time on the survival rate of nematodes of *Parachanna obscura* both adult and larval stages.

RESULT

A total of hundred and eighty-seven (187) nematodes were extracted from the thirty-three fishes, collected from great Kwa River. The adult nematode among them was ninety-six (96), while remaining ninety-one (91) were larvae.

Table 4.1 shows the number of larval nematode that survived at different levels of temperature with time, while figure 4.1 shows how the survival rate of the larvae nematodes increases with increases in temperature within the lower ranges of temperature and this same rate then decreases with increase in temperature within the higher range of temperature.

Table 4.2 shows the number of adult nematode that survival at different ranges of temperature, also figure 4.2 displays the increase in survival rate with increase in temperature, at lower range of temperature, and decrease in survival rate with increases in temperature at higher ranges of temperature.

Figure 4.3, 4.4 and 4.5 shows the comparison between the survival rate of the adult and larvae for 10, 20 and 30minutes respectively. Finally figure 4.6 show the comparison between the numbers of adult that survival and
that of the larvae that survived.

Table 4.1: Larval survivors at different temperature ranges, per given time

Time/Temp.	10mins	20mins	30Mins
0^0	2	3	1
22^0	5	5	4
27^0	5	4	3
37^0	5	2	1
50^0	3	1	0
60^0	0`	0	0
65^0	0	0	0
100^0	0	0	0

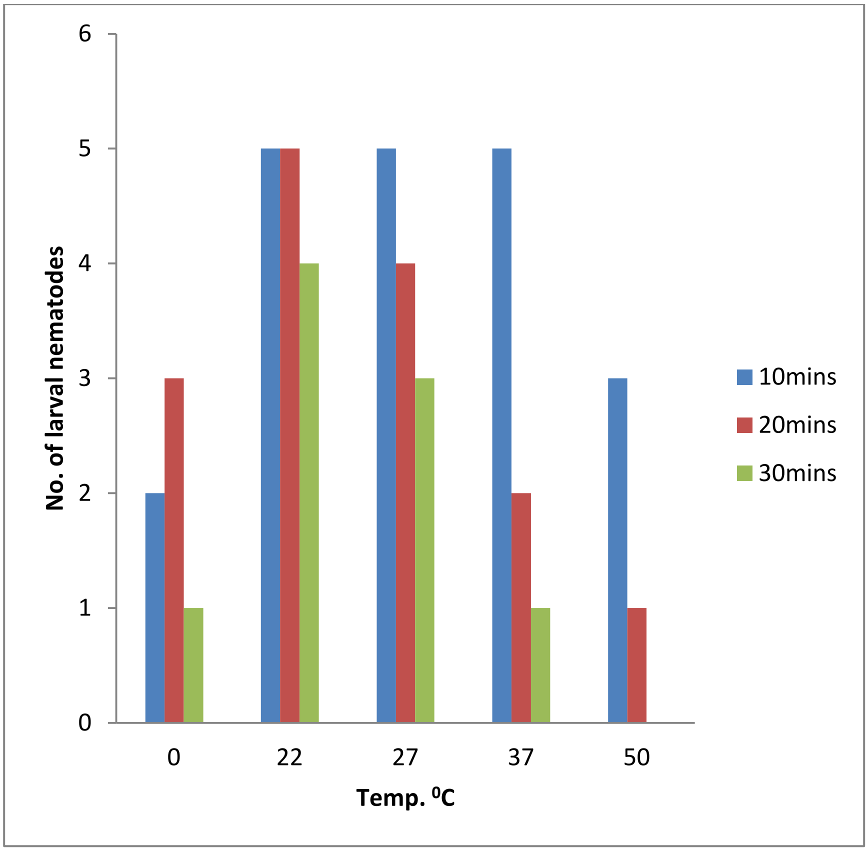

Fig. 4.1: A Composite bar chart showing the rate of survival in larval nematodes at different temperature ranges and at three different times.

Table 4.2: Showing the number of adult nematodes that survived out of every set of 5 nematodes subjected to a particular temperature at a given time

Time/Temp.	10mins	20mins	30Mins
0^0	0	1	0
22^0	5	4	3
27^0	3	5	2
37^0	2	2	1
50^0	3	1	1
60^0	1	0	0
65^0	0	0	0
100^0	0	0	0

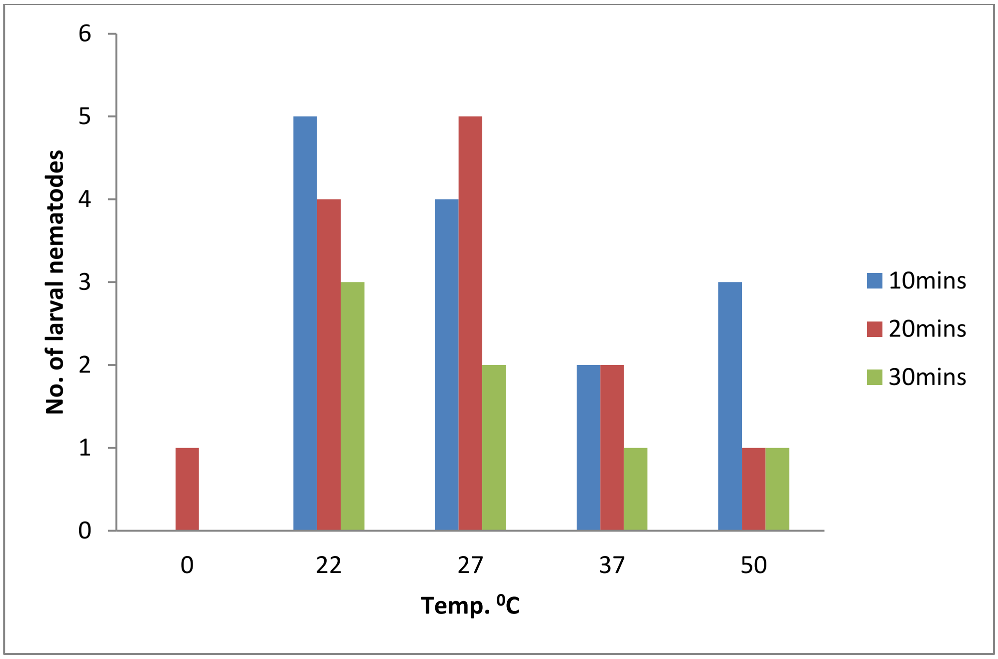

Fig. 4.2: A Composite bar chart showing the rate of survival in adult nematodes at different temperature ranges and at three different times

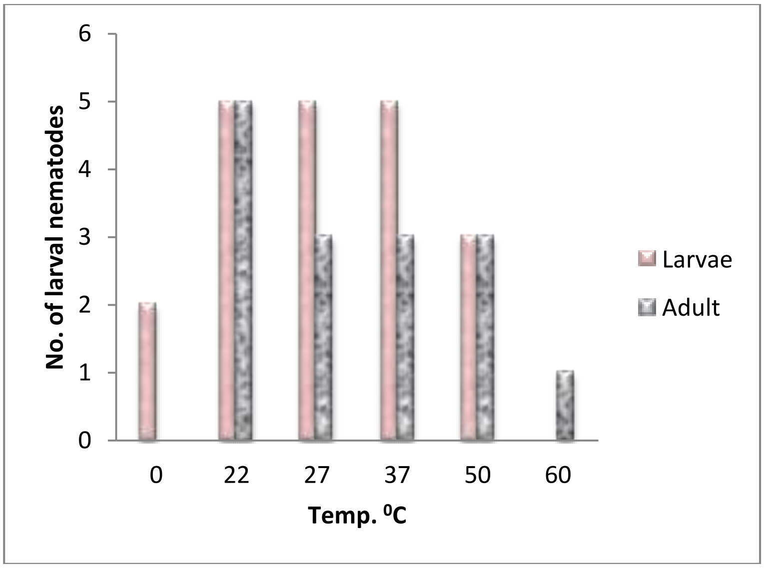

Fig. 4.3: **Composite bar chart showing the survival rate of larval and adult nematodes at different temperature range within 10 minutes.**

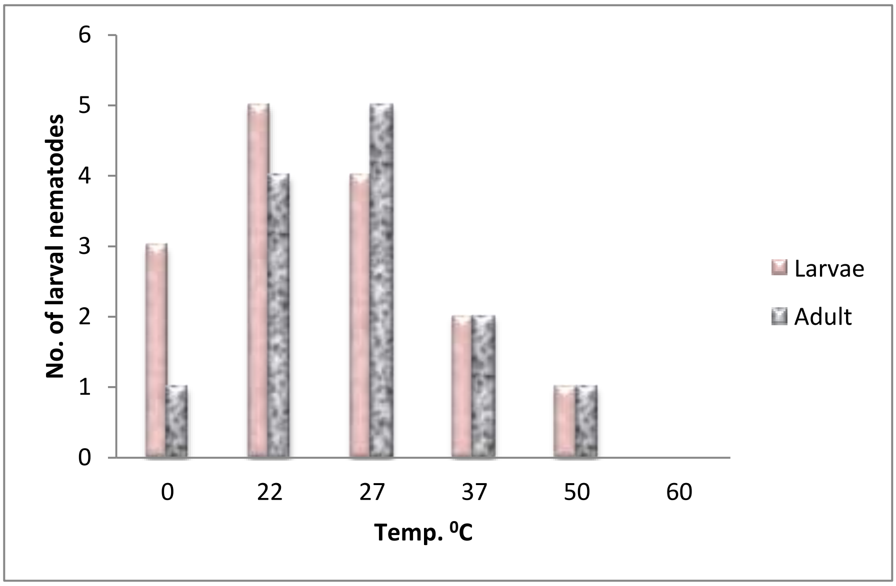

Fig. 4.4: **Composite bar chart showing the survival rate of larval and adult nematodes at different temperature range within 20 minutes.**

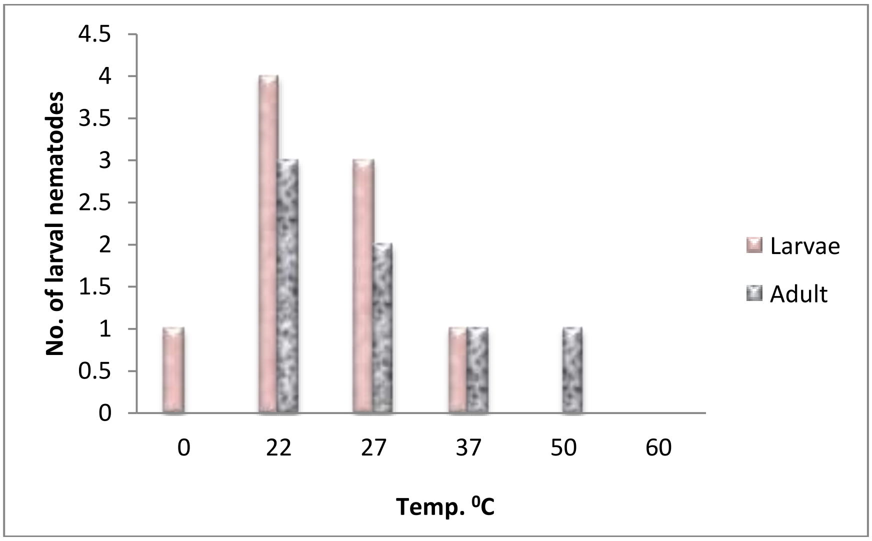

Fig. 4.5: Composite bar chart showing the survival rate of larval and adult nematodes at different temperature range within 30 minutes.

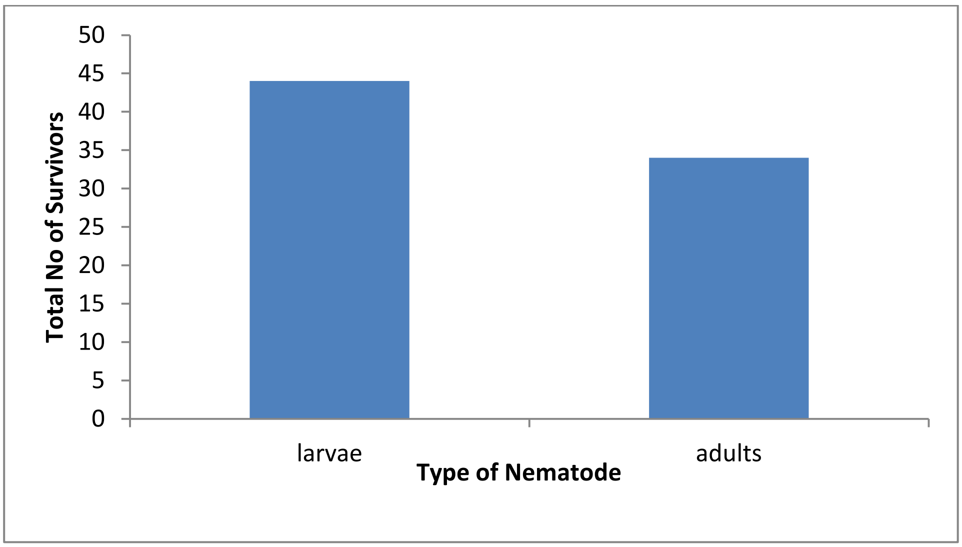

Fig. 4.6. Comparison, between the total number of adult and larval survivors.

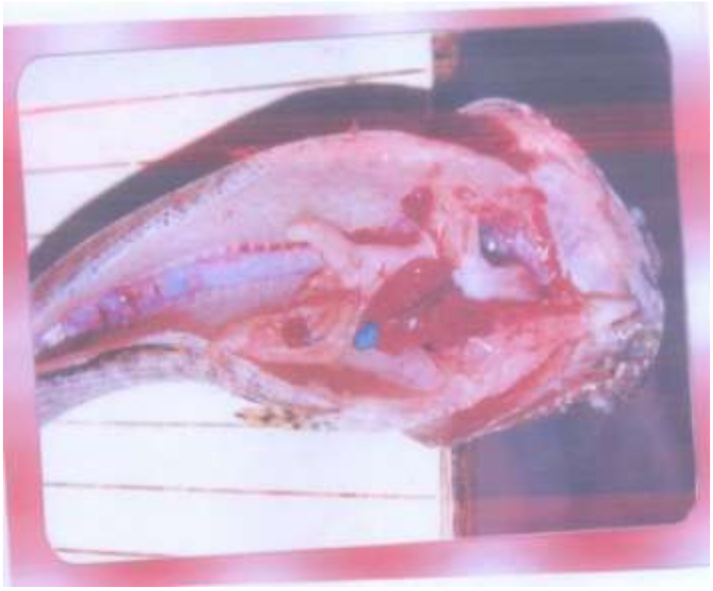

Plate 1: Dissected *Parachanna obscura*

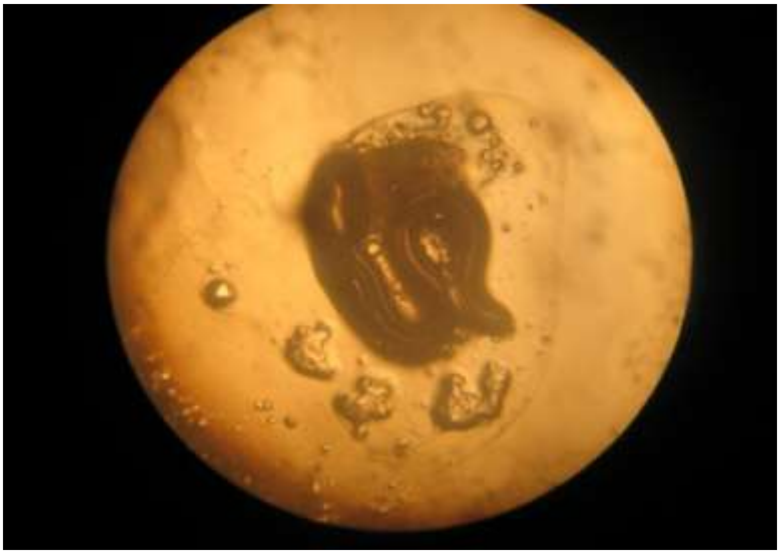

Fig. 4.7: Encapsulated larva

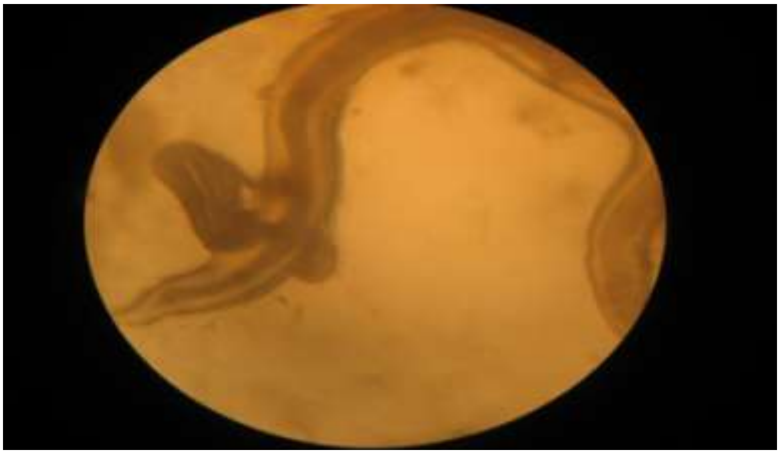

Fig. 4.8: Excysted Larva; carrying the remnant of its cyst.

Fig. 4.9: Adult Contracaecum; uncoiling from its normal coiled form.

Fig. 4.10: Adult Camallanus; showing its characteristics serpentine movement and red coloration.

DISCUSSION

To determine the survival temperature of fresh water fish nematodes and the temperature at which they will all die off, this work has been able to compare different temperature ranges which are significant in the life of these parasites and the temperature range to which the consumer normally subject the fishes that may be infested before eating. And the reasons for using the following temperature ranges was that firstly, studies has shown that Anisakiasis or Contraceaciasis (utilizing the generic name) are common in Scandinavia, Japan, Netherland, and along the Pacific Coast of South America as well as United States (Jasmine 2006); where meals are prepared with raw fishes, hence the need to consider 0^0 Celsius, which is the temperature of raw and unfrozen fish. Also, studies has shown that the habitat of *Parachanna obscura* is within the temperature range of 18-28^{0}C and more importantly the average temperature of the Great Kwa River at the time of this study was approximately 22 ^{0}C, while 27^{0}C is the temperature range suitable for developing larvae (Pouder et al. 2005). 37^{0}C was used because, it is the room temperature of human intestinal tract, 50, 60 and 65^{0}C are the temperature ranges for partly cooked fish while 100^0 is for well cooked fish. The result has shown that the larval nematode can survive at 0^{0}C for 10, 20 minutes and up to 20% of the set sampled for 30min survived. The larvae does well at 22^{0}C, 27^0 and up to 20% of the larvae survived at 50^{0}C for 30min. hence larvae can survive within the temperature range of 0^0 to 50^0.

For the adult nematode, 20% of the sampled set survived at 0^{0}C for 10minutes and non at 30minutes. At 22, 27 and 37^{0}C the adult nematode showed persistence, even at 60^{0}C for about 10minutes 20% of a sample survived. But as from the temperature of 60^0 for 20minute to 100^0 temperature none of the adult nematode survived. (Table, 4.1 and 4.2). Also figure 4, 5 and 6 shows the comparison between the survival rate of larval and adult nematodes. It was observed that generally, the larvae survive more than the adult, but at higher temperature, the adult nematodes strives to survive more than the larvae. Out of 187 nematodes sampled, only 49% survived, from which 23% were larvae while approximately 18% were adults. Finally, the ANOVA table, to which the data were subjected, proved that the temperature and time are highly significant on the survival rate of the
Nematodes.

CONCLUSION

Nematodes of *Parachanna Obscura* can survive at 0^{0}C both larvae and adult. And in accordance with Pouder et al. (2005), adult and larvae nematodes of *Parachanna* are successful within temperature range of 22-27^{0}C. In line with Jasmine 2006 and U.S. food and drug administration, this work has been able to show that nematode can survive par-adventure they gain entrance into human body since they survived at 37^{0}C. Also this work has been able to show that not all "par-boiling or half-done" of fish can get rid of the parasitic nematodes which are harbored by the fish.

RECOMMENDATION

Raw fishes are not recommendable since they can transmit these parasitic nematodes especially their larval stages. Also cooking of *P. obscura* should be done to exceed 100^{0}C and for a longer time since these fishes has scales which can as well limit the amount of heat penetrating to the nematodes. With this, the risk of Anisakiasis and similar disease conditions can be avoided in Nigeria, and more importantly, in other countries where fishes are eaten raw.