EVALUATION OF THE CRAYFISH, *PROCAMBARUS CLARKII* AS A BIO-CONTROL AGENT AGAINST SCHISTOSOME-TRANSMITTING SNAILS IN STREAM HABITATS WITHIN THE RIVER ATHI BASIN

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Evaluation of the Crayfish, *Procambarus Clarkii* as a Bio-control Agent Against Schistosome-Transmitting Snails in Stream Habitats within the River Athi Basin

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DECLARATION

This thesis is my original work and it has not been submitted for a degree in any other University.

Signature…… Date……

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my wife Sarah, my sons; Alfred, Mark, Sam and my only daughter Miriam for their humble support.

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ABBREVIATION AND ACRONYMS

AIDS	Acquired Immune-Deficiency Syndrome
CBRD	Centre for Biotechnology Research and Development
ERC	Ethical Review Committee
HIV	Human Immune Deficiency Virus
KEMRI	Kenya Medical Research Institute
NEMA	National Environmental Management Authority
NTD	Neglected Tropical Diseases
P. C.	*Procambarus clarkii*
pH	Potential Hydrogen
PZQ	Praziquantel
SSC	Scientific Steering Committee
S. H.	*Schistosoma haematobium*
S. M.	*Schistosoma mansoni*
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

ABSTRACT

Schistosomiasis is a water-based snail-transmitted parasitic disease. More than 210 million people are currently thought to be infected world-wide. Although several approaches can be applied for schistosome control, chemotherapy is the most commonly used and recommended by WHO. Praziquantel is the only drug available for individual case management and for mass treatment. The main objective of this study was to determine the ability of the crayfish, *Procambarus clarkii* to control schistosomiasis in stream habitats in the Machakos County within the Athi river basin. Planorbids snails were collected at random from the single stretches of the stream sectors using scoops, placed in 24 well culture plates, counted and shed to determine infected snails while crayfish were trapped using onion bag net traps from Lake Naivasha and translocated into 2 experimental streams. Out of 2325 total sampled snails, 161 turned out to be positive for mammalian schistosomes following shedding of cercariae representing a prevalence of 6.9%. The pH, water and temperature did not vary significantly in the different streams (*P-value* = 0.7524 at *P>0.05*). Increase in water temperature showed significant positive correlations with *B. pfeifferi* (r^2 = 0.665; *P < 0.01*) and *B. nasutus* (r^2 = 0.0.665; *P < 0.05*) *Lymnea natalensis* (r^2 = 0.589; *P < 0.010*). The overall mean pH value was 7.8 ± 0.8 with values ranging from 7.34 in Kwa Mutanga River to 8.6 recorded in Kyanguli River. Snail abundance in habitats in which crayfish were introduced rapidly declined within 2 months to a significant level (paired *t test* = 5.524, *p value* = 0.0001), relative to the decline observed in the control habitats (paired *t test* = -7.727, *p value* = 0.082. Crayfish and snail sampling record forms was used to collect the data for the snails and the crayfish respectively. Data analysis was done using SPSS Version 21.0. The findings showed that the relationships between water temperature and snail abundance varied with different species of snails *Biomphalaria pfeifferi* and *Bulinus nastus* (16.7%), *Lymnea natalensis* 6.7%. This study indicates that crayfish can establish and form thriving population; especially where the water velocity was low. Crayfish reduced schisto-transmitting snail *B. pfeifferi* and *B. nastus*. While *P. clarkii* holds much promise as a supplementary schistosomiasis control strategy, the effect of abiotic and biotic factors on the predator should not be ignored when planning biological control interventions.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Schistosomiasis is one of the neglected tropical ry listed 13 neglected tropical diseases; seven helminthes infections (i.e. schistosomiasis alodiseases (NTDs) that are pervasive in Africa and elsewhere in the developing world. An initial inventong with three common soil-transmitted (Ascariasis, Hookworm disease and *Trichuriasis*), *dracunculiasis*, lymphatic filariasis and *onchocerciasis*). Schistosomiasis has been known since antiquity (Davis, 2009) and from a global public health perspective is the most important water-based disease (Steinmann *et al*., 2006). Despite numerous control efforts the estimated world prevalence of schistosomiasis has not changed over the past 50 years (Engels *et al*., 2002; Savioli *et al*., 2004). It is prevalent in tropical and subtropical regions and affects more than 74 countries globally (WHO, 2010). Schistosomiasis affects more than 210 million people worldwide, whereby 92% occurs in African countries contributing to about 200,000 deaths annually (Simões *et al.*, 2015; WHO, 2017).

More than half of the world's population is at risk of NTDs and over 1 billion people are currently infected with one or several NTDs concurrently with helminthes infections showing the highest prevalence rates (Hotez *et al*., 2008). Despite the life-long disabilities the NTDs might cause they are less visible and receive lower priorities compared to; for example the 'big three' that; is- malaria, tuberculosis, and HIV/AIDS (WHO, 2006) because NTDs mainly affect the poorest and marginalized populations in the developing world (King, 2010).

Intestinal schistosomiasis can result in abdominal pain, diarrhea and blood in the stool (King *et al.,* 2005). Liver enlargement is common in advanced cases and is frequently associated with an accumulation of fluid in the peritoneal cavity and hypertension of the

abdominal blood vessels (WHO, 2002). In such cases there may also be enlargement of the spleen (Richter *et al.,* 2000). In children, schistosomiasis can cause anemia, stunting and a reduced ability to learn properly (King *et al.,* 2005). Fibrotic responses to schistosome eggs trapped in the intestines, liver and other organs of the infected person are the cause of the schistosomiasis (Caldas *et al*., 2008). It is associated with retarded cognitive development and those children with the heaviest parasitic load show the greatest impairment because they have harbored the worm burden for a long period of time (Jukes *et al*., 2002). Having a heavy infection of schistosomiasis is associated with a drop-in performance in the digit span forwards and backwards and with increased reaction time in choice reaction time tasks **(**Jukes *et al.,* 2002). It has been established that schistosomiasis infection contributes to high prevalence of malnutrition in children in the developing nations Assis *et al*., 1998). Treatment of light- to moderate-intensity schistosomiasis infections has a positive effect on weight, height, mid upper arm circumference and body mass index in school-age children, thus infection is itself an obstacle to optimal growth (Ana *et al*., 1998).

Schistosomiasis is a neglected tropical parasitic infection that continue to plague the resource limited countries of the tropical and sub-tropical regions of the world and are now being targeted for elimination through a concerted global effort (WHO, 2013). Current schistosomiasis control efforts rely largely on treatment of infected people with the antischistosomal drug, praziquantel (PZQ). However, this approach is difficult to sustain as re-infections frequently occur after successful treatment (Njenga *et al*., 2014). While drug-based control efforts may reduce infection prevalence, morbidity or level of environment contamination and parasite transmission and may also have an impact on the genetic diversity of the parasite (Norton *et al*., 2010), such efforts do not completely stop transmission. Although schistosomiasis control depends on a continual application of PZQ, this is by no means readily available in most sub-Saharan African countries (Hotez *et al*., 2010) and therefore, sustainable control of schistosomiasis may remain problematic for a long time in many of the endemic areas.

Schistosomiasis control has been attempted in several ways: chemotherapy, vector elimination, improved sanitation and health education (WHO, 2010). Although in more recent years the establishment of a number of national control programs offering chemotherapeutic treatment with praziquantel has helped to reduce the burden of schistosomiasis (Kabatereine *et al*., 2007). It is very difficult to halt transmission solely through drug treatment. This is because like with many human helminthic infections individuals remain susceptible to re-infection after treatment. Chemical control by molluscicides is performed by using different compounds (Augusto *et al.,* 2017). However, high costs of chemical molluscicides and the possible built up of snail resistance to molluscicides and their toxicity to non-target organisms has drawn much attention during recent years for the use of plant molluscicides. The successful control of schistosomiasis should be based on an integral approach including the control of intermediate snail host snails (Kenawy & Rizk, 2004; De SLuna *et al*., 2005) hence the advocacy for use of biological control agents such as crayfish. Crayfish, *P. clarkii* does not only eat snails but they also consume and reduce the macrophyte habitats that typically harbor the highest densities of snails (Lodge *et al*., 1994).

1.2 Statement of the Problem

Malocophagous crustaceans especially crayfish *P. clarkii* are effective bio-control tools against schistosomiasis transmitting snails. However, the *P. clarkii* experiments have in the past mostly been confined to laboratory experiments and benthic habitats (Huner and Lindqvist, 1995). There is need to evaluate the effect of this predator in lotic habitats and the abiotic factors favoring its thriving (Oluoch, 1990). Current control programs in Africa focus almost exclusively on treating people with the antischistosomal drug, praziquantel, a strategy that reduces infection prevalence but never brings it to zero. This strategy has not been sustainable partly because of re-infection of treated people. A better understanding of the abiotic factors influencing the establishment of crayfish in lotic habitats will not only provide insights into vector control but it will also provide an overall and augmented measure to step down schistosomiasis transmission through vector control.

1.3 Study Justification

School going children bear the greatest brunt of schistosomiasis. Since it is not feasible to achieve millennium development goals especially Goal No. 2 of universal primary education without adequately addressing Sustainable Development goals (SDG) to combat HIV/AIDS, Malaria and other diseases. There is need to explore other bilharzias control strategies to augment the existing methods. So far schistosomiasis control relies primarily on chemotherapy using the antischistosomal drug praziquantel (PZQ). While PZQ is a rapid and very effective way to reduce morbidity and interrupt transmission re-infections rapidly occur after successful treatment and so re-treatment of re-infected individuals has to be done from time to time. Furthermore, intensive use of PZQ could lead to development of PZQ-resistance or insusceptibility. Alternative approaches to complement chemotherapy are therefore needed for effective and sustainable control of schistosomiasis. The proposed research aims to test the ability of *P. clarkii* to survive in stream habitats in the Machakos-Kitui area and to explore their ability to eliminate *B. pfeifferi* populations. The longterm goal of this work is to provide pragmatic and needed information that can be applied and used to complement existing praziquantel-dependent schistosomiasis control efforts.

1.4 Research Questions

1. Are the seasonal stream habitats in the Machakos-Kitui area major schistosomiasis transmission sites?
2. Can crayfish, *P. clarkii,* establish thriving populations in the seasonal stream habitats in the Machakos-Kitui areas?
3. Will crayfish be able to eliminate schistosome transmitting snails?
4. Do abiotic parameters significantly influence snail distribution and crayfish survival?

1.5 Broad Objective

To determine the ability of crayfish *P. clarkii* to control schistosomiasis in stream habitats in the Machakos-Kitui area within the river Athi basin

1.6 Specific objectives

1. To determine the prevalence of *S. mansoni* and *S. haematobium* in schistosomiasis transmitting snails in Machakos County within the river Athi basin.
2. To determine the ability of ***P.*** *clarkii* to survive and establish thriving populations in stream habitats which serve as transmission sites for *S. mansoni*, causal agent of intestinal schistosomiasis in humans in river Athi basin.
3. To determine the ability of *P. clarkii* to reduce populations of *B. pfeifferi and B. africanus,* snail hosts of *S. mansoni* and *S. haematobium* respectively in the stream habitats and control schistosomiasis in river Athi basin.
4. To elucidate the impact of abiotic factors on snail distribution and crayfish establishment within the study area.

1.7 Hypotheses schistosomiasis.

H0 - Introduction of crayfish in stream habitats will not substantially reduce schistosomiasis transmitting snails leading to sustainable control of

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology and life cycle of *Schistosoma mansoni*

The adult schistosomes are found in the blood stream of their definitive hosts. The parasite eggs produced when male and female schistosomes mate are passed into the environment via excreta (for *S. mansoni* it's passed with feces), and when they end up in freshwater, they hatch into motile larval forms called miracidia, which actively search for and enter a suitable snail (*Biomphalaria spp*) (Mutinga & Ngoka, 1971). Following penetration into the snail host, the miracidium differentiates into a mother sporocyst which transforms into a daughter sporocyst, and in the latter, produce another motile larval form by asexual reproduction called cercariae which are released by the snail into the water. The cercariae are infective to the definitive host (usually humans and other primate mammals), they penetrate the unbroken skin of the mammalian host, and transform into schistosomulae which then migrate in the blood stream to their final destination where they mature into adult worms, and begin reproduction all over again. In the case of *S. mansoni,* the schistosomulae migrate in the bloodstream through the lungs and then to the hepatic portal system and eventually, to the mesenteric veins where they mature into adults and reproduce (Doumenge *et al.,* 1987). The life cycle of human schistosomes is diagrammatically presented in figure 1.1.

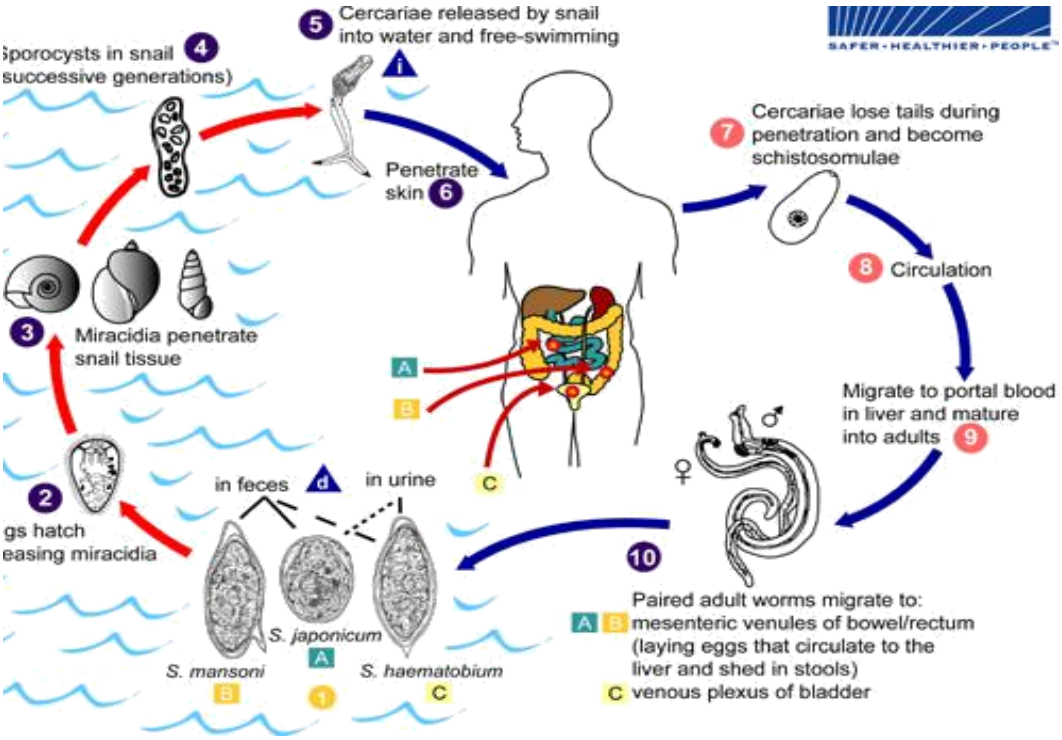


Figure 2.1: Life cycle of Schistosoma mansoni, adapted from www.dpd.cdc.govt/dpdx

2.2 Ecology of the Louisiana red swamp crayfish

P. clarkii has a mean size of 100 mm and large individuals can reach a length of 200 mm (Arrignon *et al.*, 1990; Arrigon, 1996). Sexual maturity is generally reached at 11 months (Oluoch, 1990) and seems to be dependent on water levels (Guttierez- Yurrita & Monts, 1999). Life span at low altitude does not exceed 3 years but can reach 5 years in higher latitudes. *P. clarkii* is extremely tolerant of poor water quality (Arrignon *et al.*, 1990): Oxygen>3 PPM; alkalinity>50 PPM (in CaCo3); pH of 6.-8.5; salinity<15% and temperature of 22-25^{0}C (Crandall & Buhay, 2008). It copes well with alternatively inundated and dry areas through burrowing (Inundation periods allow the proliferation of the macrophyte component of its diet and many of its predators are eliminated during the dry periods) (Cumberlidge, 2009).

2.3 The Kenyan schistosome vector distribution

In Kenya, *B. pfeifferi* is widely distributed, including in the tributaries feeding Lake Victoria, canals in major irrigation schemes in the Kano plains (Western Kenya) or in the Mwea irrigation scheme in central Kenya (Olsson *et al.*, 2009); it is also found in small impoundments and both seasonal and perennial streams throughout the country, except in the tropical lowland belt along the coast of Kenya (Loker *et al.*, 1993). Nonetheless populations of this species and of the schistosome it transmits can be widely separated by regions of aridity in Kenya (Lowery & Mendels, 1977). As a consequence, it is possible that *S. mansoni* exhibits a greater degree of compatibility with its local *B. pfeifferi* population than it does with other populations of the same species further removed geographically. Theory predicts that a parasite should be more adapted to sympatric than to allopatric hosts, and that the superior adaptation of a parasite to local hosts should be more pronounced when the host has a discontinuous rather than continuous distribution (Ebert *et al.*, 1994). A number of factors including high rates of local extinction (such that co-evolutionary associations do not have a chance to develop) high rates of migration of host or parasite populations or time lags in response may break down or obscure patterns of local adaptation (Morand's *et al*., 1996; Prugnote *et al*., 2006). In Kenya as assessed by microsatellite analysis, *S. mansoni* from Mwea (central Kenya) and Kisumu (western Kenya) are genetically diverse (Agola *et al*., 2009). In addition to being genetically diverse *S. mansoni* enjoys relatively rapid rates of migration owing to existence of long-lived adult worms in mobile human hosts. By comparison, *B. pfeifferi* is a strong self-fertilizer (Charbonnel *et al.*, 2005) and its movement is relatively limited owing to its restriction to aquatic habitats. Based on these considerations *S. mansoni* might be expected to exhibit strong local adaptation to *B. pfeifferi* has manifested by shorter pre-patent periods, higher compatibility, or higher levels of cercariae production when exposed to sympatric as opposed to allopatric snails. Conversely *B. pfeifferi* might also be predicted to exhibit local adaptation to schistosomes and consequently show lower compatibility following exposure to sympatric than allopatric schistosomes (Agola *et al*., 2009). These topics have not been

addressed in Africa with a reciprocal cross design approach using field-derived snails and parasites not subjected to the biases resulting from prior laboratory propagation; this approach better represents the conditions in natural transmission sites.

2.4 Schistosomiasis control and challenges

Human schistosomiasis is a common waterborne parasitic disease that is relatively easy to treat but hard to control. Fresh water habitats play an integral role in the life cycle of the parasitic flatworms responsible for causing schistosomiasis one of the most common infections of humanity (Walsh & Warren, 1979). The disease affects more than 249 million people (WHO, 2014) with a global disease burden calculated at 24-56 million disability-adjusted life years lost (King, 2010).

Various strategies for control include chemotherapy to treat infected people, improved sanitation, public health education programs, and snail control. Today public health campaigns in endemic regions in the tropics and subtropics focus on mass drug administration using the oral drug, praziquantel (Pica-Mattocia & Cioli, 2004). Though praziquantel is fairly efficacious against sexually mature forms of the parasite, it is often unable to cure infections due to its inability to kill juvenile schistosomes at 2-4 weeks post infection (Aragon *et al*., 2009). The Artemether is known to be effective against immature schistosomes in the definitive host as demonstrated by Elboby (Egyptian Organization for Biological and Vaccine Production) and (Utzinger *et al*., 2001). Most of the schistosomiasis endemic areas are also endemic for malaria transmission hence reluctance for its use for fear of malaria resistance. Another shortcoming for praziquantel is that re-exposure to cercarial infested water leads to rapid re-infection of treated patients in endemic areas (Webster *et al*., 2013).

Biological control of Schistosomiasis as a supplement to existing control strategies isn't being used in a major way in any control programs. This may be attributed to the debate of nativeness and alien species (Mkoji *et al.,* 1992). A bacterial pathogen of snails *Paenibacillus glabratella* has been tested on *Biomphalari aglabrata*. The bacterium

causes massive mortality and affects both adult and embryonic stages (Duval *et al*., 2015). However, it is unclear at this point whether the bacteria are specific to the snails that are intermediate hosts for schistosomes, or if the bacteria would infect many invertebrate species. Sokolow *et al*. (2014) on river Senegal has suggested that Europe, Africa, Central and South America and South-East Asia, river prawns are voracious predators. The red swamp crayfish reaches maturity in approximately three months, and in warm climates it may produce two generations per year (Dorr *et al*., 002). Some of the life history traits, such as rapid growth rate, high fecundity, polytrophism, resistance to diseases, pollution and extreme environmental conditions, make *P. clarkii* an invincible bio-control species (Barbaresi *et al.*, 2004). It is not known whether crayfish introduction in the Athi River basin was by or by accident. Crayfish apart from predating on snails also feed on leeches (Lynne *et al*., 2009).

2.5 The sub-Saharan Africa perspective and challenges to control

In Sub-Saharan Africa one barrier to achieving long-term control of this disease has been re-infection of treated patients when they manually irrigate their crops using watering cans, swim, bathe, or wade in fresh water infested with snails that harbors and release larval parasites (Aragon *et al.*, 2009). Most planorbid snails are obligate intermediate hosts of schistosome parasites, reducing snail densities may reduce cercaria in water, minimizing infection risk. With the ever-increasing use of praziquantel, there is a possibility of the development of schistosomes to the drug, hence the necessity to explore other 'additive' measures (Savioli *et al.,* 2004). Here, we evaluate the potential for snail control by predatory decapod crustacean crayfish *P. clarkia* which preferentially feed on snails and submerged macrophytes

2.6 Previous Schistosomiasis control efforts using Crayfish

A preliminary study in the 1990 in manmade water impoundments had found a strong negative correlation between the presence of *P. clarkii* and schistosome transmitting snails (Hofkin *et al.*, 1991; Lodge and Lorman, 1987). The crayfish eradicated or greatly

reduced snail populations in boththe laboratory and field enclosures (Hofkin *et al*., 1992; Oluoch, 1990). However no studies have been done in seasonal stream habitats which form a significant focus for schistosome transmitting snails.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This was a baseline descriptive cross-sectional and experimental study design employing quantitative techniques for data collection as indicated in the flow chart below

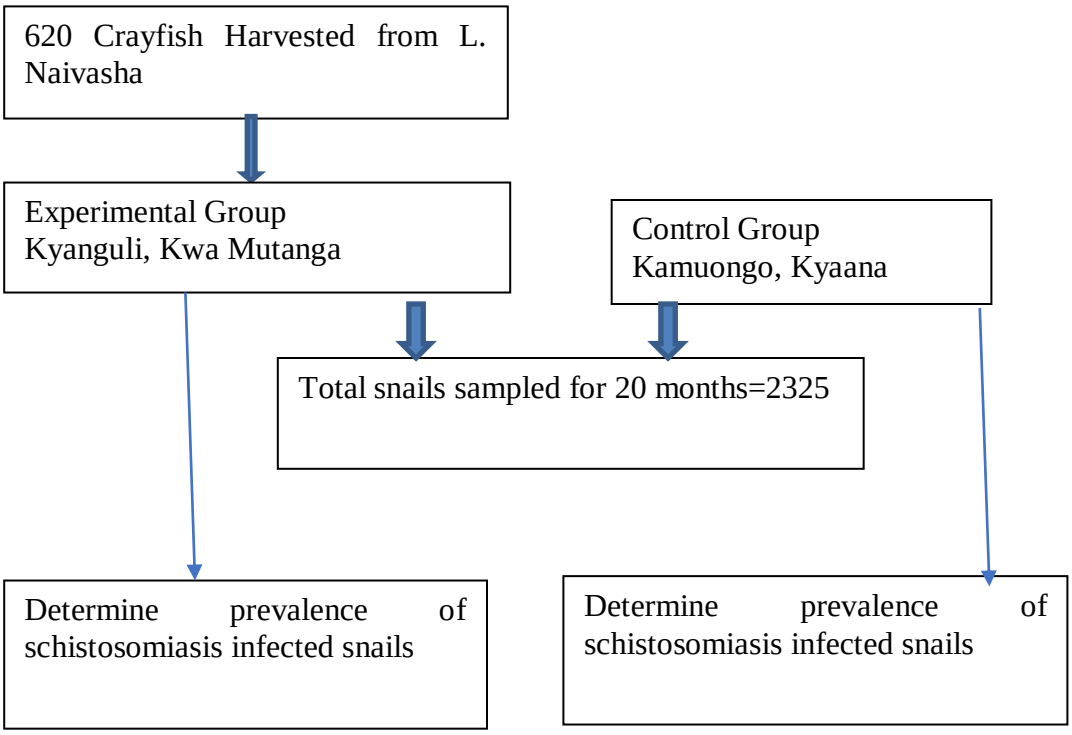


Figure 3.1: Baseline Descriptive Cross-Sectional and Experimental Study Design

3.2. Study Area

The study was conducted in Machakos County, Kenya within the river Athi drainage basin. Machakos, Kitui and Makueni were part of the six counties of Eastern province extending between latitudes38^{0}15'E and 39^{0}30'East as well as 1^{0}N and 3^{0}S. Machakos has a population of 1,120,137, Kitui 603,505. Makueni- 953,227 (CBS Analytical Report on Population projection Vol.VII. P.32). The annual mean temperature was 22.93^{0}C the distribution of rainfall is typically bi-modal with two distinct rainy seasons the first one with its peak in April and the second with its peak in November. The average annual rainfall is 400mm-2200mm.RiverAthi and Thwake Rivers drain these counties Machakos map (Appendix III).

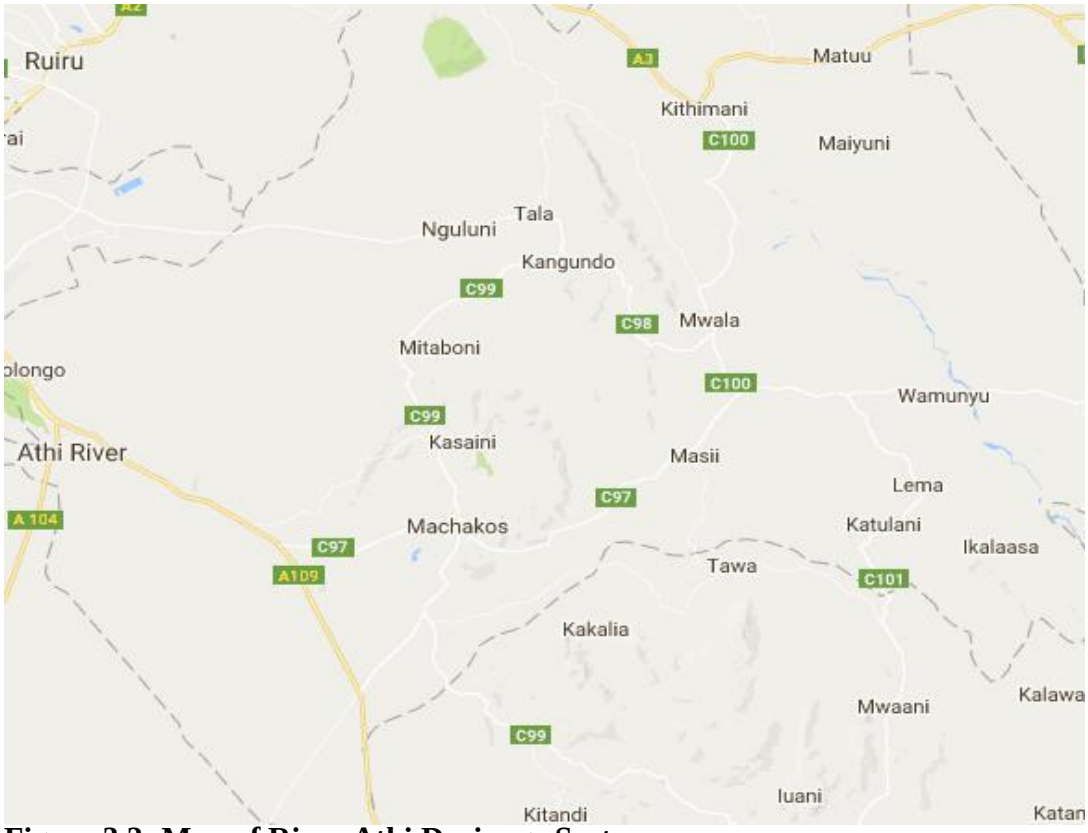


Figure 3.2: Map of River Athi Drainage System.

3.3 Study population

Study population involved 15 streams during baseline and finally, 6 streams chosen for further study, 3 designated control and the other 3, experimental, however, in the course of the study, 2 additional streams which had initially been included in the study became dry, the data were not considered further, ending up with 4 streams, Kamuongo and Kyaana for the control and Kwa Mutanga and Kyanguli for the experimental groups.

3.3.1 Inclusion Criteria

Streams with ease of accessibility, marked human-water contact, presence of schistosomiasis transmitting plarnobidsand absence of crayfish were included in study.

3.3.2 Exclusion Criteria

Inaccessible streams, rapid water velocity and presence of *P. clarkii* were not included in the study.

3.4 Research variables

3.4.1 Dependent variable

Crayfish, *P. clarkii*, were collected from L. Naivasha and introduced to the study streams. The ability to survive and predate on the snails was determined.

3.4.2 Independent variables

Stream Abiotic factors including temperature, pH, turbidity.

This number was based on the Fisher Equation (Fisher RA, 1935) used for calculation of a proportion in a finite population:

$$n = \frac{N * X}{X + N - 1\ e^2} \text{ where } X = \{Z^2 * P\ (1-P)\}$$

Z= value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% C.I.)

N= Total population number of crayfishes=620 (*P. clakii* collected from Lake Naivasha)

P is expected true proportion (The true prevalence is not known therefore we use standard of 50%) e is desired precision (half desired C.I. width) = 0.05X = 1.96^2 x 0.50(1-0.50) =0.806736/0.0025=384.16 n = 620 x 384/ 384+620-1 = 238080/780 =204.59 n=205 crayfish

But 10% was increased attrition rate 205/100*10=25 therefore 205+25=230 crayfish.

3.6 Sampling design

In this study two methods were employed in sampling. Simple random sampling and stratified sampling methods were used to recruit study subjects.

3.6.1 Sampling/Selection of Streams

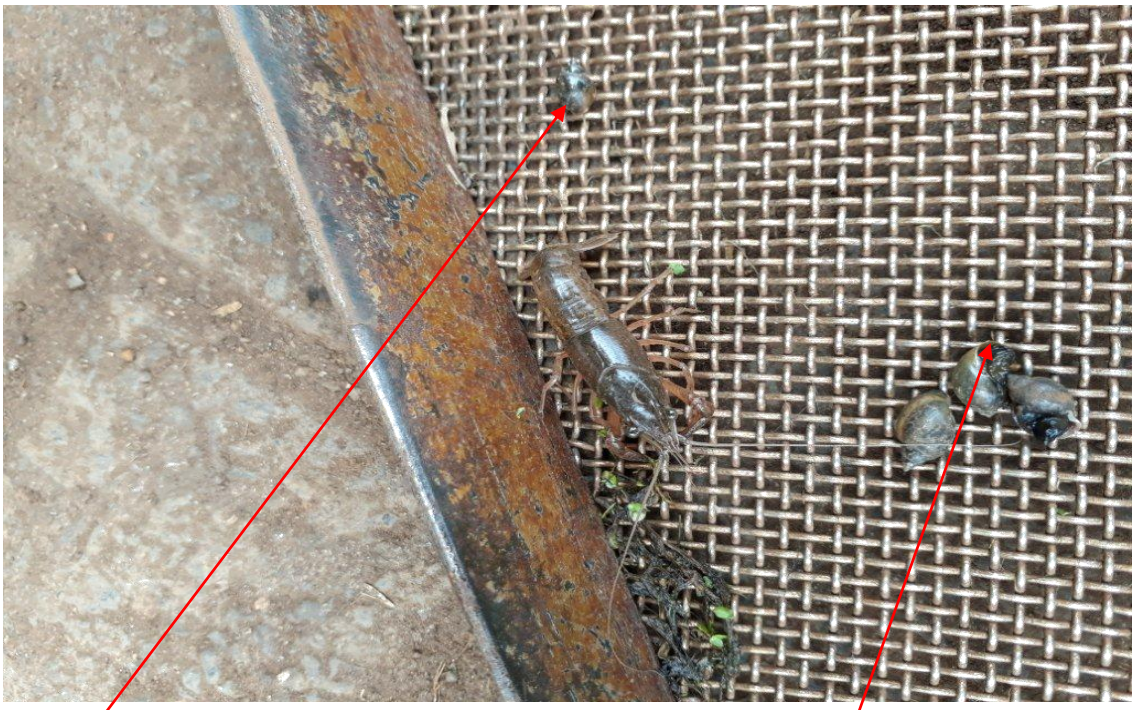
Simple random sampling technique was used to select the 15 streams within the Sub-County for presence/absence of crayfish and snail abundance in Athi Rivers**.** The streams were randomly divided into sectors where Kalaala, Chaana, Kamuongo were serving as controls while Kakulutuine, Kyanguli and Kwa Mutanga were serving as experimental Rivers. The study streams were stratified into experimental or control treatments on a pairwise basis based as far apart as possible `on similar initial. Each habitat had 3 sampling stations, measuring approximately 20 meters running length and

spaced 10 meters apart. All sites of sampling were considered representative of the water bodies.

3.6.2 Snail survey and sampling

3.6.2.1 Snail collection

Snails were collected at random from the single stretches of the stream sectors using scoops made from stainless steel sieves with a mesh size of 2×2 mm, supported on an iron frame and mounted on a 1.5 m long wooden handle Plate (3.1). Snails were randomly sampled for 15 minutes by two trained field collectors per sector along the littoral zones, total, 45 minutes per stream.



juvenile crayfish planorbid snails.

Plate 3.1: Snail scoop mesh frame with juvenile crayfish and planorbid snails.

Sampling time was fixed, between 9.00 Am and 12.30 PM. Snails were transported to the regional labs in plastic bowls provided with stream water and lined with vegetation. In the lab, they were sorted out into species based on shell characteristics, using standard taxonomic identification keys (Gow *et al.,* 2005; Douris *et al.,* 1998). The planorbids were counted in respect to species and placed individually in wells of 24- well plastic culture plates containing 1 ml de-chlorinated water, and left on the bench for 2 hours in indirect sunlight to induce shedding of cercariae plate (3.2). The wells of the plates were then examined using a dissecting microscope for presence of cercaria. All non-shedders were returned to their respective habitats to maintain ecological stability while Positive snails were taken back to KEMRI, Nairobi, schistosomiasis laboratory for parasite cycle maintenance and academic demonstration.

Plate 3.2: Snails being screened in the field using 24 wells culture plate to isolate shedders from non-shedders

3.6.2.2 Capture and Selection of crayfish

Procambarus clarkii (n=620; standard for carapace length 45-60mm and no exterior damage i.e. missing or broken limbs) were collected from L. Naivasha (Kamere beach and hippo point, see map) in January 2015 using 30 meat baited traps. Lake Naivasha is situated at the eastern arm of the great rift Valley at 0^0 45' S: $36^0$21'E, and approximately 1890 m.a.s.l, situated about 100 KM north-west of Nairobi. This period was chosen to avoid early mid-March-early April flooding events which could easily wash away the predators before establishment. All crayfish individuals were sexed as previously described (Yue *et al*., 2009).

3.6.2.3 Holding, Translocation and follow up survey of crayfish

Crayfish were temporarily held in perforated buckets with dump gunny bags at the bottom and top of the bucket (H: 90 centimeters, top radius: 60 centimeters, bottom radius: 45 centimeters). Each bucket held approximately 120 crayfish. The crayfish were transferred the same day using an enclosed double cab pickup to experimental sites. Crayfish collected from L. Naivasha were introduced into the snail habitats of localities designated as experimental sites. No crayfish were released into control snail habitats. Stocking density was a low-end density for standard aqua cultural stocking between 1 and 2 crayfish per meter square. After stocking experimental habitats were sampled bi-monthly for crayfish until the study ended in March 2016. During each sampling session 15 meat baited traps were tethered with a nylon string and immersed in water for 1 hr. The traps were constructed of wire and covered with nylon mesh (onion bag type, mesh size 1×1 cm), were 45 cm long with a diameter of 20 cm (Plate 3.3). Traps were checked after 30 min and 60 minutes. Captured crayfish were sized, sexed and counted and recorded. Crayfish could also be spotted on the edges of the river banks, others were inadvertently caught on snail scoops. Trapped crayfish were returned to the habitat. *Procambarus Clarkii* Onion bag Crayfish trap Plate 3.3 Onion bag crayfish trap with several *Procambarus clarkii* caught after 1 hr.

3.7 Study Tools and Data Collection

3.7.1 Crayfish traps

The traps were made up of plain wire which was cut into several 45 cm pieces. Where 3 pieces were folded to form circles and welded at the joints. Three straight wires were running the length of the three circles at equal distances and riveted at each point of contact with the circular wires (Plate 4.2). The traps were checked after 30 minutes interval. Captured crayfish were sized sexed counted and returned into the water. Only male catches were analyzed because they provide the best index of crayfish abundance (Lodge *et al*., 1999).

3.7.2 Abiotic parameters

Water velocity (V) was measured with a Schiltknecht (Switzerland) MiniAir2 type flow meter fitted with a 22 mm propeller (Plate 3.4). Water velocity was measured by immersing the propeller against water flow. Turbidity was measured by drawing water into a bowl and immersing the turbidometer for 5 minutes. Depth and velocity were taken at 4 different spots within a stretch of 30 meters and averaged.

Schiltknecht apparatus

Plate 3.3: Schiltknecht apparatus, used for measuring water velocity.

3.8 Data Storage, Management and analysis

All data were entered into a field note book and later transferred into an excel spread sheet and statistically analyzed using SPSS version 21.0 software. Absolute counts of snails both cercaria shedding and non-shedders were done. A two-dimensional Kolmogorov-Smirnov test was carried out to compare the joint distribution of predator and prey to the distribution of predator and prey were independent (Fason & Franceschini, 1997) (A two-dimensional Kolmogorov-Smirnov is a non-parametric test used to test goodness of fit test.)

All graphical representations were carried out using Sigma Plot 9 and graph pad. The sampling sites were mapped in the previous study using a global positioning system (GPS). New sites were mapped using a similar method and the reading imported into an ArcView version 3.3 and ArcGis version 8.3 (Environmental Systems Research Institute, Redlands, CA). Soft copies of the data were stored on flash disks. This was to

serve as a link between the field, laboratory reports and data to be entered in the computer. Data generated was kept in files which were secured in locations in KEMRI, CBRD where the principal investigator (PI) and his co-investigators were the custodian of the working documents. Generated information was stored in a computer and secured with passwords known only to the PI and his colleagues. Hard disks and removable disks were used for safe storage and backup.

3.9 Ethical considerations

Before introduction of the crayfish into the study habitats, permission was sought from the local community through local community leaders and opinion leaders via (public meetings). Permission was also sought from other stake holders like The National Environment Management Authority (NEMA/10/22/VOL.1) and fisheries departments. Study approvals were obtained from KEMRI Scientific Steering Committee and Ethical Review Committee (SERU) approved the study (SSC PROTOCAL NO. 2798). The necessary permits were obtained for the described field studies from; The Kenya Wild Life Services (KWS/BRM/5001) about handling snails and crayfish. Care was taken to ensure that field assistants were not accidentally exposed to schistosome infected snails. Heavy duty leather gloves were used to handle crayfish. All animal work was conducted in accordance to institutional and national guidelines to minimize discomfort to animals. All farmers who were bordering the streams where crayfish was released were informed of the study in the national language, Swahili, and the local language Kikamba. Plarnobids potentially harbor infectious organisms. Latex rubber gloves were worn during snail sampling procedures while heavy duty leather gloves were worn to militate against crayfish bites.

CHAPTER FOUR

RESULTS

4.1 Overview of the baseline study prevalence of mammalian schistosomes in river Athi Basin in snails

Result indicated that out of 2325 schistosome transmitting snails were sampled during the September 2014-March 2016 in the 4 study streams. No crayfish was found during the baseline survey. Out of the total number of snails sampled 161 on shedding turned out to be positive for mammalian schistosomes representing 6.9% (Figure 1 and 2). The month of January 2015 had the highest number of infected snails while March 2016 had no schistosome shedders. Prevalence schistosomiasis in snails between March 2015 and March 2016 there was a strong statistical difference between the control and the experimental groups (Fig 4.1 & 4.2).

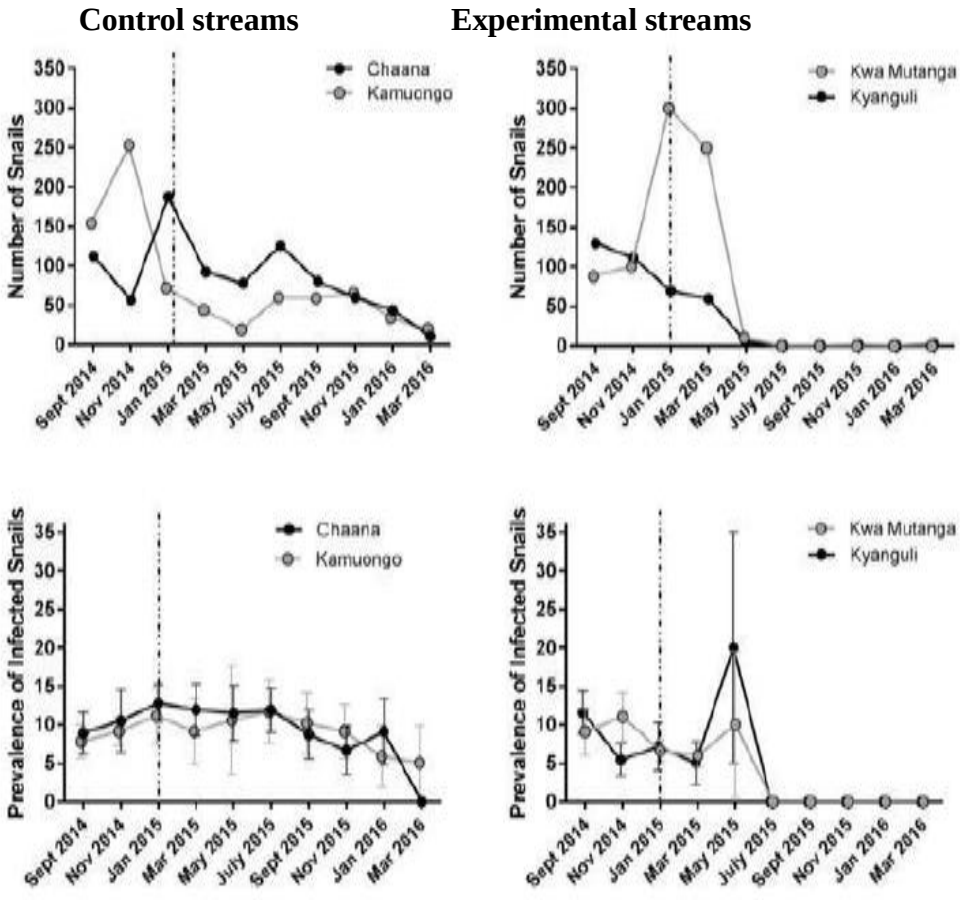


Figure 4.1: Prevalence of schistosomiasis in snails between March 2015 – March 2016 in control/experimental rivers. Dotted lines indicating the time point when crayfish were introduced.

Experimental streams

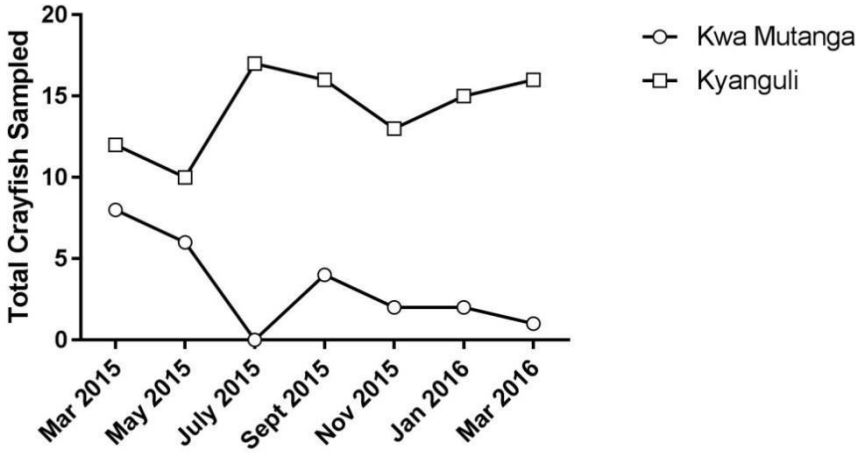
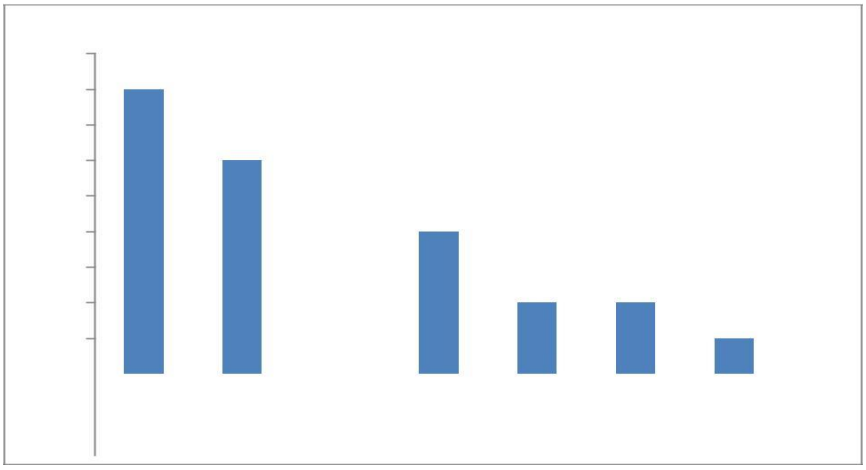


Figure 4.2: Total crayfish sampled between March 2015 – March 2016 in control/experimental rivers

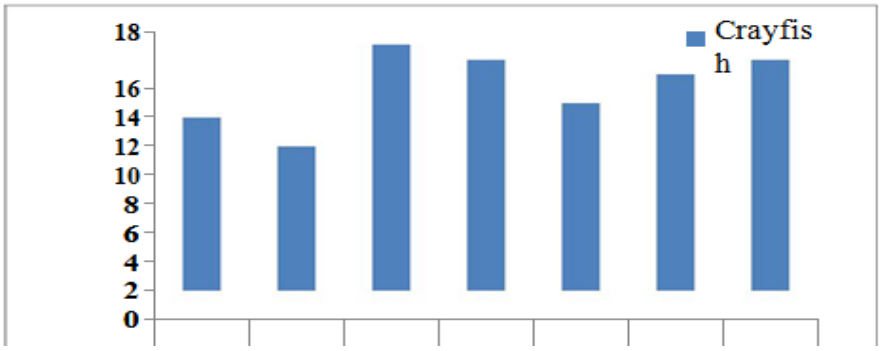
4.2 Ability of crayfish to survive and establish thriving populations in stream habitats after translocation from L. Naivasha

During the 20 months period of the survey 6 juvenile crayfish, an indication of breeding, were spotted on the edges of the experimental streams. Seventy-seven adult predators were captured in Kyanguli stream over the entire study duration while 28 adult crayfish in Kwa Mutanga within the first 3 months post introduction representing a *P-value* of 0.002. No crayfish was neither spotted nor trapped in the control streams. The number of snails across the study duration at Kyanguli stream was high at the start then went down and eventually pattered out in May, 2015 while the number of crayfishes went up and over time and eventually stabilized as the study went by (Figure 4.3 and 4.4).



MAR MAY JULY SEP NOV JAN MAR
2015 2016 Year and Month

Figure 4.3: No. of crayfish by year and months for the treatment group (Kwa Mutanga in the month of March 2015 to March 2016.



MAR MAY JULY SEP NOV JAN MAR
2015 2016 Year and Month

Figure 4.4: No. of crayfish by year and months for the treatment group (Kyanguli) in the month of March 2015 to March 2016

Plate 4.1: A photograph of crayfish caught in a trap at Kyanguli stream where the predator was able to establish Retrospective comparison of the relative snail densities in experimental streams before crayfish introduction and after crayfish introduction reflects a dramatic decrease in snail populations.

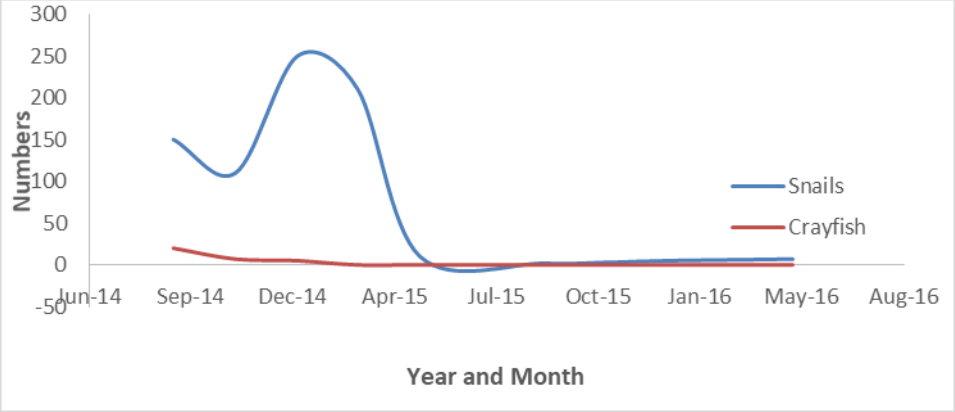


Figure 4.5: Predation potential of *P. clarkii* against schistosome transmitting snails in lotic in Kwa Mutanga

Table 4.1: Snail population pre and post introduction of Crayfish

Streams	**Pre-Crayfish Introduction**	**Post-Crayfish Introduction**	**Paired *t test***	***P value***
Experimental streams				
Kyanguli	411	0	5.524	0.0001
KwaMutanga	300	15	7.88	0.0001
Control Streams				
Chaana	343	487	-7.727	0.082
Kamuongo	245	356	-6.28	0.443

The crayfish temporarily established within the first 3 months post introduction and almost wiped out the snails but the crayfish population fettered out by the 4th month leading to a resurgent of snails, this may have been due to stream pollution occasioned by washing of motorcycle (Bodaboda) washing Plate 3.6. In Kyanguli stream, the crayfish were able to establish thriving populations leading to complete decline of snails

Plate 4.2: Kwa Mutanga stream, experimental stream where crayfish failed to establish due to effluent and oil from Bodaboda (Motorcycle washing)

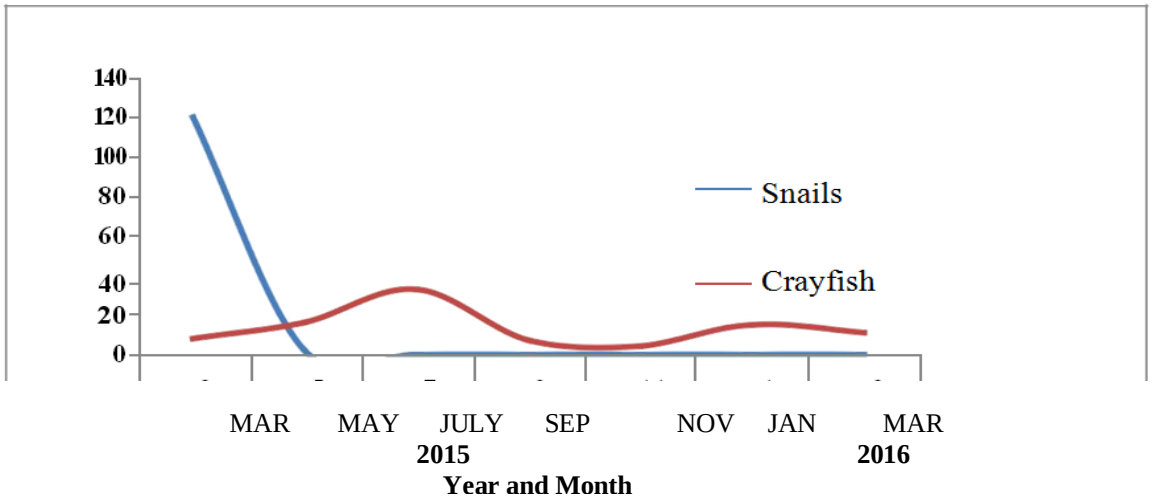


Figure 4.6: *Biomphalaria pfeifferi* and *Procambarus clarkii* numbers between March 2015- March 2016 (Kyanguli)

The population of both the predator and prey decreased during the study period. A linear regression analysis to establish the kind of relationship showed a positive association (r^2 = 0. 502). However, the positive association was not significant (*p value* = 0.115).

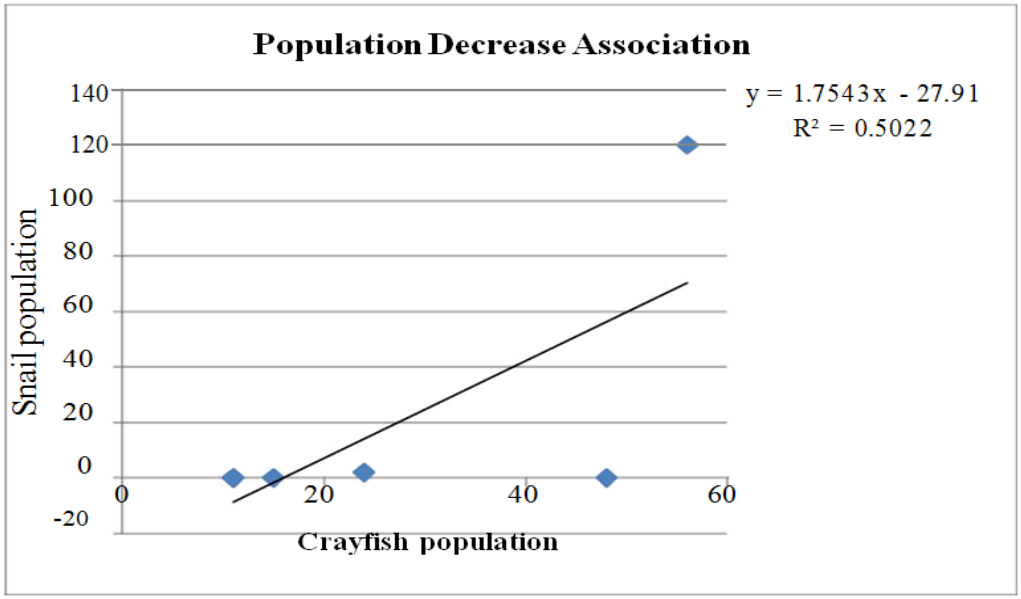


Figure 4.7: Crayfish vs. Snail population decrease association

4.1.4 Seasonal variation in Abiotic parameters and relationship to prey and predator density

Several physico-chemical parameters were considered in this study which included temperature, turbidity, velocity and pH. The pH, water and temperature did not vary significantly in the different streams (*P-value*=0.7524 at *P 0.05*) respectively. However, the mean water temperature values in the dry and wet seasons were 16.8±2.1^{0}C and 25.8 ± 1.1^{0}C respectively.

The relationships between water temperature and snail abundance varied with different species of snails: *B. pfeifferi*, *B. nastus* and *Lymnea natalensis*. Increasing in water

temperature showed significant positive correlations with *B. pfeifferi* (r = 0.665; P<0.01) and *B. nasutus* (r = 0.0.665; P<0.05) *Lymnea natalensis* (r = 0.589; P<0.010). The overall mean pH value was 7.8 ± 0.8 with values ranging from the minimum value 7.34 recorded in Kwa Mutanga River (March, 2016) to 8.6 recorded in Kyanguli River March, 2016 (Table1.2). There was significant correlation in various pH values of the river bodies. The pH of the water bodies showed significant positive correlation with the abundance of *B. pfeifferi* (r = 0.665; P<0.05), however, the positive relationship was not significant with the abundance of *Ceratophalus.* Negative relationships were observed between pH of the following aquatic snails; *B. ceratophalus, B. forskalii* during September 2015. The overall mean value of air velocity was 1.41 ± 4.6 m/s.

4.1.5 The different physico-chemical attributes for the two study treatment streams Kyanguli and Kwa Mutanga

The result indicated that there was no significant relationship between any of the physico-chemical attributes and the snail density in the two treatment streams (Kyanguli and Kwa Mutanga) in the study (Table 4.2)

Table 4.2: Different attributes for the two treatment streams (Kyanguli and Kwa Mutanga with regard to quality of Physico- chemical parameters

Physico-Chemical Attribute	Rho (ρ)	P-Value
PH	-0.2002	0.6669
Air Velocity	0.0692	0.8707
Water Velocity	-0.0560	0.9051
Air Temperature	0.4962	0.2110
Water Temperature	0.6264	0.1323
Water Turbidity	-0.2838	0.5374

4.1.6 Different physico-chemical attributes for the two study control streams Chaana and Kwa Muongo

Result indicated that there was a significant relationship (P = 0.9525, P=0.0003) between Water Turbidity and Snail Density. The results showed that when all the two control streams in the study are considered then water turbidity and snail density have a positive relationship. This showed that there was a significant positive relationship (P = 0.9525, P=0.0003) observed between the water turbidity and snail density (Table 4.3).

Table 4.3: Different attributes of the two study control streams Chaana and Kwa Muongo with regard to quality of Physico-chemical parameters

Physico-Chemical Attribute	Rho (ρ)	P-Value
PH	0.3842	0.3474
Air Velocity	-0.5052	0.2016
Water Velocity	-0.3433	0.4051
Air Temperature	-0.5874	0.1257
Water Temperature	-0.6822	0.0623
Water Turbidity	0.9525	0.0003*

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Spartial heterogeneity in streams is a complex and evident across multiple spatial scales (Schlosser, 1991). Stream ecosystems have very variable structure because materials are constantly moved downstream and organisms including snails and crayfish often must recolonize disturbed areas from refugial habitats (Osborne & Wiley, 1992). In this study, this phenomenon was observed in Kyanguli stream where despite visible establishment of the crayfish, the population kept fluctuating with respect to rainfall intensity. Shortly after heavy down power, the crayfish catches dwindled, but during seasons of delayed rainfall, water velocity reduced resulting in pools of water which seemed to support re-establishment of crayfish resulting in increased catches and visible spotting of the predator. However, in control streams, Kamuongo and Kivaani, during heavy and shortly after heavy down pour, the snails would be washed away as evidenced by reduced numbers of snails collected from scooping. One and a half months post heavy rains; the snails would re-establish and thrive uncontrollably in control habitats, unlike in Kyanguli stream (Experimental) where the crayfish thrived at the expense of snails.

The result indicated that during March 2015-March 2016 there was a strong statistical difference between the control (Paired *t test* = 2.143, *p value* = 0.278) and experimental groups (paired *t test*

= 1.651, *p value* = 0.0001). The crayfish was able to establish a good colony that wiped out the snail population that transmits schistosomes. In the Kwa Mutanga stream, the number of crayfishes after introduction went up and pattered out in September 2015. The number of snails across the study duration at Kyanguli stream was high at the start then went down and eventually pattered out in May 2015 while the number of crayfishes went up and over time and stabilized eventually as the study duration went by. This

observation is in agreement with previous at study that showed the total number of snails would be significantly lower in areas where crayfish successfully established though in lentic set ups (Mkoji *et al*., 1999). Another study showed that using *Oncomelania hupensis* to control *Schistoma japanicum* is potential biocontrol measure Suleiman *et al*., 2013). Crayfish is able to use snail as food while establishing its colony which makes it a potential biocontrol of schistosomiasis. Biocontrol measures are not only environmentally friendly but also more stable and longer lasting (Sharma *et al*., 2013). Schistosomiasis originates from water sources which makes it had to use chemicals due to pollution that may adversely affect water ecosystem as well as negatively affecting people and animals that use the same water. As the snail population decreased there was a decrease of crayfish population suggesting that snail was a major food sources for the crayfish population growth. However, human factors were also a threat to the crayfish colonization. This was observed in Kwa Mutanga stream when people washing their motorcycles, hence polluting water, led to rapid decline of crayfish and reemergence of snails. The balance between human factors and biocontrol measures is necessary to ensure the success of this method.

Streams are particularly pertinent systems for examining issues about scaling because their structure poses some clear spartial gradient (Downes *et al*., 2000). First, rivers are linear systems that change relatively predictably in discharge, water temperature, substrate size and channel size between river sections. Collectively, these changes are thought to cause large differences in biotic composition between locations along rivers (Vannote *et al*. 1980). Second, because rivers can have particular and distinct flow regimes. Discharge and its associated measures of water velocities, depths and turbulence have strong influences on stream communities (Hynes, 1970; Allan, 1995). Consequently, the geomorphological and hydrological features of catchments (and channel morphology) are often assumed to set most of the spartial scales that affect stream biota (Frissel *et al*., 1986). Several physico-chemical parameters were considered in this study which included temperature, turbidity, velocity and pH. The pH and water temperature varied significantly in the different streams ($P = 0.7524$, $P>0.05$)

respectively (Table 1). The mean water temperature values in the dry and wet seasons were 16.8 ± 2.1^0 C and 25.8 ± 1.1^0C respectively.

The relationships between water temperature and snail abundance varied with different species of snails' *B. pfeifferi* and *B. nasutus* (16.7%), *L. natalensis* 6.7%. Increasing in water temperature showed significant positive correlations with *B. pfeifferi* ($r = 0.665$; $P<0.01$) and *B. nasutus* ($r = 0.0.665$; $P<0.05$) *L. natalensis* ($r = 0.589$; $P<0.010$). The overall mean pH value was 7.8 ± 0.8 with values ranging from the minimum value 7.34 recorded in Kwa Mutunga River (March, 2016) to 8.6 recorded in Kyanguli River (March, 2016). There was significant correlation in various pH values of the river bodies. The pH of the water bodies showed significant positive correlation with the abundance of *B. pfeifferi* ($r = 0.665$; $P<0.05$).The positive relationship was not significant with the abundance of *Ceratophalus.* Negative relationships were observed between pH of the following aquatic snails; *B. ceratophalus, B. forskalii* during September 2015. The overall mean value of air velocity was 1.41 ± 4.6 m/s. The ecology of the snail was influenced by the physicochemical parameters of the river and its role in *snail* abundance was indicated in the study streams. This was shown in positive significances of water, temperature, the pH of water, air velocity/ water respectively. The higher density of snails recorded in the dry season could have been due to the indirect impacts of flourishing Microflora (food supply) and aquatic macrophytes during the season.

The mean pH value in all the water bodies in the present study was within favorable limits for aquatic snail development (Boelee & Laamrani, 2004). The higher mean pH value recorded during the dry season could be due to higher transparency of the water bodies resulting in active removal of carbon (IV) oxide and consequently production of oxygen through photosynthesis. The concentration of hydrogen ions is rarely a factor conditioning the presence and distribution of the snails (Madsen, 2005). This was in agreement with the findings in this study that showed insignificant relationships between abundance of snails and pH values.

CHAPTER SIX

CONCLUSIONS

6.1 Conclusions

This study indicated that the treatment outcomes in treatment streams was quite different and were likely to be driven by the crayfish which the findings showed that the total number of snails was significantly lower in areas where crayfish successfully established. Crayfish can establish and form thriving population especially where velocity is low, like the Kyanguli stream. Schistosome-transmitting snail elimination is the key to sustainable controlling of schistosomiasis. Schistosomiasis control using crayfish is a robust strategy that can easily be adopted in both lotic and benthic habitats to reduce snail vectors in river Athi basin.

6.2 Recommendations

a) Further studies of crayfish are important not only in Africa but worldwide are warranted to elucidate their economic importance as feed supplements in aqua cultural and poultry farms.
b) There is need to have independent measure of schistosomiasis infection in streams like use of sentinel mice as a snail sample of 0 doesn't necessarily mean that there are no snails present (although it could be).
c) Future studies should seek to uniquely tag the introduced predators to differentiate them from F1 and subsequent generations.

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APPENDICES

Appendix I: Malacology Data Record Sheet

NAME OF HABITAT……

DATE	GENUS OF SNAILS SCOOPED	NUMBER OF SNAILS

APPENDICES

Appendix II: Crayfish Sampling Record Form

NAME OF HABITAT…….

DATE	NO.OF CRAYFISH	SEX	REMARKS

Appendix III: Map of River Athi Drainage System

Appendix IV: Ethical Research Committee Approval Letter

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

RECEIVED 25 JUL 2014 P.O. Box 54840 NAIROBI

KEMRI/RES/7/3/1 **July 23, 2014**

TO: **GEOFFREY MAINA,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. KIMANI GACHUHI,**
THE DIRECTOR, CBRD,
NAIROBI

Forwarded 28/7/14

Dear Sir,

RE: **SSC PROTOCOL NO. 2798 (*RESUBMISSION*): EVALUATION OF THE CRAYFISH, *PROCAMBARUS CLARKII* AS A BIO-CONTROL AGENT AGAINST SCHISTOSOME-TRANSMITTING SNAILS IN STREAM HABITATS, WITHIN THE ATHI RIVER BASIN (*VERSION 1.0 DATED JULY 15, 2014*)**

Reference is made to your letter dated July 15, 2014. The ERC Secretariat acknowledges receipt of the revised protocol on July 18, 2014.

This is to inform you that the Ethics Review Committee (ERC) reviewed the documents submitted and is satisfied that the issues raised at the 228th meeting of the KEMRI ERC on 18th February, 2014 have been adequately addressed.

The study is granted approval for implementation effective this **23rd July, 2014**. Please note that authorization to conduct this study will automatically expire on **July 22, 2015.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **June 10, 2015**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the SSC and ERC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

EAB

PROF. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

In Search of Better Health

Appendix V: CBRD Approval Letter

KENYA MEDICAL RESEARCH INSTITUTE

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E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/SSC/102791 13th May, 2014

Geoffrey Maina

Thro'
Director, CBRD
NAIROBI

15/5/14

CENTRE FOR BIOTECHNOLOGY RESEARCH AND DEVELOPMENT
RECEIVED
15 MAY 2014
P.O. Box 54840
NAIROBI

REF: SSC No. 2798 (Revised) – Evaluation of the crayfish, *procambarus clarkii* as a bio-control agent against schistosome-transmitting snails in stream habitats, within the Athi River basin

Thank you for your letter dated 12th May, 2014 responding to the comments raised by the KEMRI SSC.

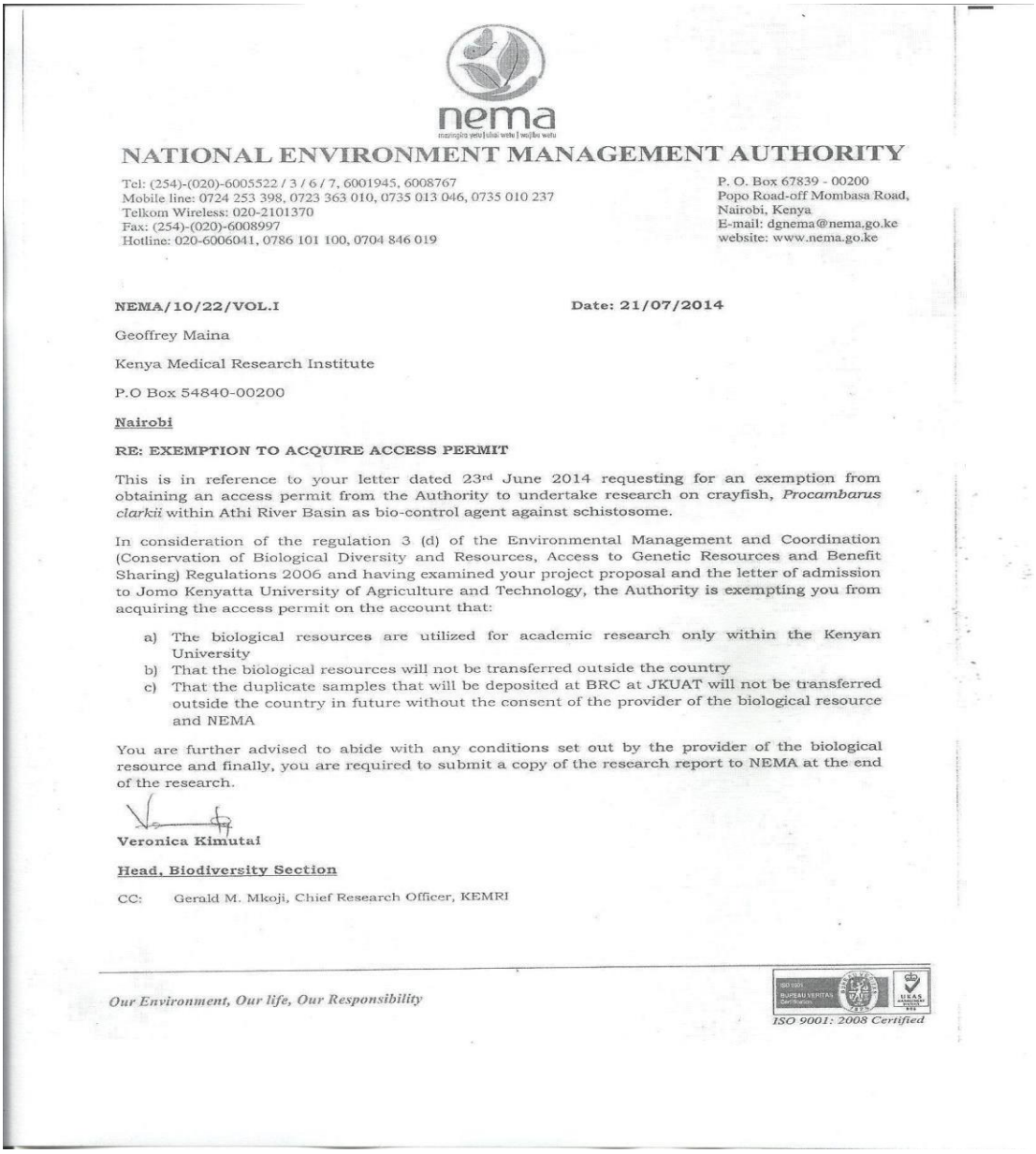
I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

Sammy Njenga, PhD
FOR SECRETARY, SSC

In Search of Better Health

Appendix VI: National Environmental Management Authority (NEMA)



nema
maringira yetu | uhai wetu | wajibu wetu

NATIONAL ENVIRONMENT MANAGEMENT AUTHORITY

Tel: (254)-(020)-6005522 / 3 / 6 / 7, 6001945, 6008767
Mobile line: 0724 253 398, 0723 363 010, 0735 013 046, 0735 010 237
Telkom Wireless: 020-2101370
Fax: (254)-(020)-6008997
Hotline: 020-6006041, 0786 101 100, 0704 846 019

P. O. Box 67839 - 00200
Popo Road-off Mombasa Road,
Nairobi, Kenya
E-mail: dgnema@nema.go.ke
website: www.nema.go.ke

NEMA/10/22/VOL.I **Date: 21/07/2014**

Geoffrey Maina

Kenya Medical Research Institute

P.O Box 54840-00200

Nairobi

RE: EXEMPTION TO ACQUIRE ACCESS PERMIT

This is in reference to your letter dated 23rd June 2014 requesting for an exemption from obtaining an access permit from the Authority to undertake research on crayfish, *Procambarus clarkii* within Athi River Basin as bio-control agent against schistosome.

In consideration of the regulation 3 (d) of the Environmental Management and Coordination (Conservation of Biological Diversity and Resources, Access to Genetic Resources and Benefit Sharing) Regulations 2006 and having examined your project proposal and the letter of admission to Jomo Kenyatta University of Agriculture and Technology, the Authority is exempting you from acquiring the access permit on the account that:

a) The biological resources are utilized for academic research only within the Kenyan University
b) That the biological resources will not be transferred outside the country
c) That the duplicate samples that will be deposited at BRC at JKUAT will not be transferred outside the country in future without the consent of the provider of the biological resource and NEMA

You are further advised to abide with any conditions set out by the provider of the biological resource and finally, you are required to submit a copy of the research report to NEMA at the end of the research.

Veronica Kimutai

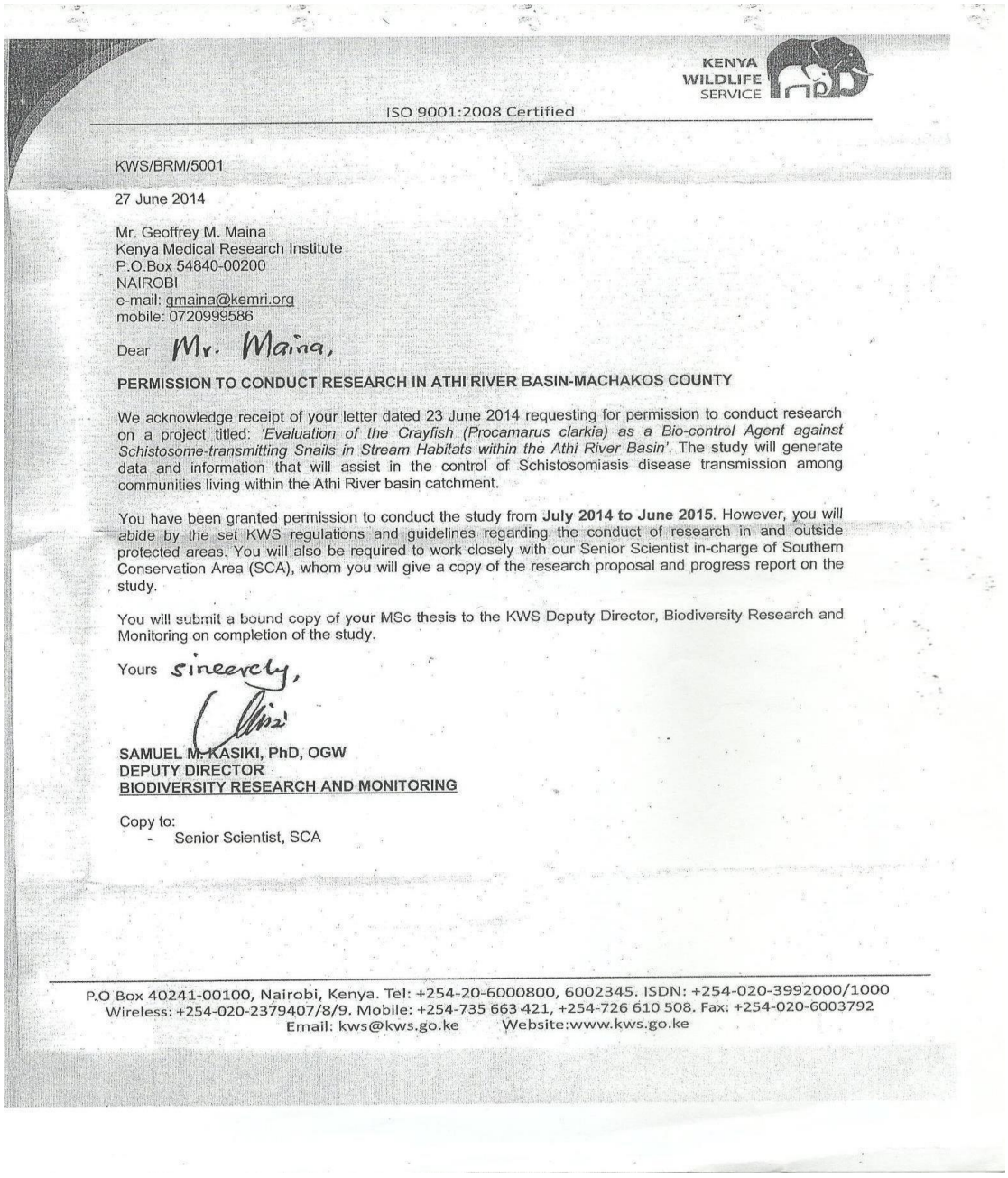
Head, Biodiversity Section

CC: Gerald M. Mkoji, Chief Research Officer, KEMRI

Our Environment, Our life, Our Responsibility

ISO 9001: 2008 Certified

Appendix VII: Kenya Wildlife Service Approval Letter



KENYA WILDLIFE SERVICE

ISO 9001:2008 Certified

KWS/BRM/5001

27 June 2014

Mr. Geoffrey M. Maina
Kenya Medical Research Institute
P.O.Box 54840-00200
NAIROBI
e-mail: gmaina@kemri.org
mobile: 0720999586

Dear Mr. Maina,

PERMISSION TO CONDUCT RESEARCH IN ATHI RIVER BASIN-MACHAKOS COUNTY

We acknowledge receipt of your letter dated 23 June 2014 requesting for permission to conduct research on a project titled: *'Evaluation of the Crayfish (Procamarus clarkia) as a Bio-control Agent against Schistosome-transmitting Snails in Stream Habitats within the Athi River Basin'*. The study will generate data and information that will assist in the control of Schistosomiasis disease transmission among communities living within the Athi River basin catchment.

You have been granted permission to conduct the study from **July 2014 to June 2015**. However, you will abide by the set KWS regulations and guidelines regarding the conduct of research in and outside protected areas. You will also be required to work closely with our Senior Scientist in-charge of Southern Conservation Area (SCA), whom you will give a copy of the research proposal and progress report on the study.

You will submit a bound copy of your MSc thesis to the KWS Deputy Director, Biodiversity Research and Monitoring on completion of the study.

Yours sincerely,

SAMUEL M. KASIKI, PhD, OGW
DEPUTY DIRECTOR
BIODIVERSITY RESEARCH AND MONITORING

Copy to:
- Senior Scientist, SCA

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Appendix VIII: Manuscript

Symbiosis

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Research Article | International Journal of Marine Biology and Research | Open Access

Regulatory Influence of Procambarusclarkii, Girad (Decapoda: Cambaridae) On Schistosome-Transmitting Snails in Lotic Habitats within the River Athi Basin, Kenya

Maina GM[1,2*], Kinuthia JM[1], Mutuku MW[1], Mwangi IN[2], Agola EL[1], Kutima HL[3] and Mkoji GM[1]

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Received: September 21, 2017; Accepted: October 05, 2017; Published: October 11, 2017

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Abstract

Background & Objective: Control of schistosomiasis, a neglected tropical disease has for a long time overly relied on praziquantel. Crayfish, though voracious snail eaters have been tested in small man-made impoundments but not in lotic habitats. The present study aimed to determine the ability of the crayfish, *Procambarus clarkii* to reduce populations of schistosome transmitting snails in lotic habitats.

Methods: Data was collected bi-monthly on the presence or absence of snails and crayfish in 4 stream habitats, over a period of 10 months, and these were identified from a baseline survey to be habitats for *Biomphalaria* snails, transmitters of intestinal schistosomiasis, and were located in the Machakos County within the Athi River basin in south-eastern Kenya. Subsequently, 2 of the habitats were selected for introduction of crayfish (and were designated "experimental sites") and the other 2 habitats were designated "control sites." Each of the "experimental sites" received 110 crayfish. The study sites were sampled for snails using standard snail scoops and for crayfish using meat-baited crayfish traps. The bi-monthly sampling of the habitats was done to determine snail abundance, crayfish survival, and obtain information on biotic and abiotic parameters.

Results: Snail abundance in the habitats that received crayfish rapidly declined within 2 months to a significant level compared with the initial abundance (paired t test = 5.524, *p* value = 0.0001), relative to the decline observed in the control habitats (paired t test = 7.727, *p* value = 0.082).

Interpretation & Conclusion: While *P. clarkii* holds much promise as a complimentary schistosomiasis control strategy to chemotherapy, restocking of habitats should be considered when habitats dry up during extreme weather conditions, for effectiveness of this approach.

Keywords: Crayfish; Predation; Planorbid snails; *Biomphalaria pfeifferi*; Seasonal streams; *Schistosoma mansoni*

Introduction

Schistosomiasis is one of the several so-called neglected tropical diseases (NTDs) that are pervasive in sub-Saharan Africa and elsewhere in the developing world [1]. Various strategies for control include chemotherapy to treat infected people, improved sanitation, public health education programs, and snail control [2]. Today, public health campaigns in endemic regions focus on mass drug administration using the oral drug, praziquantel (PZQ) [3]. Although PZQ is fairly efficacious against the sexually mature forms of the parasite, it is often unable to kill juvenile schistosomes present in the human body [4]. Additionally, there is concern that with the intensive use of PZQ drug resistance could render PZQ ineffective, given that drug resistant strains can easily be selected in the laboratory [5] and field derived strains with reduced susceptibility have been described [6]. Although artemether is known to be effective against immature schistosomes in the definitive host,and since most of the schistosomiasis endemic areas are also endemic for malaria transmission, there is reluctance to use it in such areas for fear of emergence artemether resistance [7]. Efforts to control the snail populations in the past through the use of chemicals or through alteration of snail habitats have resulted in environmental pollution and damage [8]. Another shortcoming associated with chemotherapy is that continued re-exposure to cercarial infested water leads to rapid re-infections of successfully treated patients in the endemic areas [9,10,11].

Biological control of schistosomiasis as a complimentary strategy to existing control strategies is not commonly used in

control programs primarily because it may involve use of exotic species which may negatively impact the local environment, possibly leading to extinction of the native bio-diversity [12]. However, studies carried out on fresh water snails in the Neotropical area have shown that the invading species may have a positive influence from the human health point of view, if their introduction leads to displacement of snail species that are responsible for the transmission of schistosomiasis [13]. The case of Martinique Island is especially relevant as a successful bio-control strategy where *Biomphalaria glabrata* and *Biomphalaria straminea* were displaced by the thiariid, *Melanoides tuberculata* after its accidental introduction into this island [14, 15]. A bacterial pathogen of snails, *Paenibacillus glabratella*, has also, been tested on *B. glabrata*. The bacterium causes massive mortality and affects both the adult and neonate snails [16]. However, it is unclear at this point; if the bacterium is specifically infective to the snails that serve as intermediate hosts for schistosomes, or it can also, infect other invertebrate species. A river prawn,*Macrobrachium vollenhovenii*, has also, been tested with varying success in River Senegal, Diama Dam, using the male prawns [17].

The red swamp crayfish, *Procambarus clarkii*, α native of South-Central United States and North-Eastern Mexico, has been introduced to Europe, Africa, Central and South America and South-East Asia [18]. It was introduced into Lake Naivasha in Rift Valley, Kenya in the 1970's, and has since spread throughout several parts of Kenya. Previous studies indicated that *P. Clarkiiis* an effective predator of snails [20], rapidly eliminated snail populations in small man-made ponds [19], and, effectively reduced schistosomiasis transmission in such habitats [20]. However, no studies have been done in seasonal stream habitats which also, form significant foci for schistosomiasis transmission in endemic areas such as Kenya. In the present study, P. clarkii was tested for its ability to eliminate *Biomphalaria pfeiferi*, the freshwater snails involved in the transmission of *S. mansoniin* stream habitats located in the Machakos County, within the River Athi Basin.

Materials and Methods

Study Approvals

This study received approval from the Kenya Medical Research Institute (KEMRI) through its Scientific and Ethical Review Unit (SERU) and is referenced SSC No. 2798. Permission to undertake this study was also, obtained from the Kenya Wildlife Services (in a letter referenced KWS/BRM/5001) and the National Environment Management Authority (NEMA/10/22/VOL.1). The purpose of the study was explained to the local community in both the Kiswahili language and in the local Kikamba language, particularly to the people that lived near or frequently utilized the study habitats.

Biosafety Matters

Latex rubber gloves were worn by the project staff during snail sampling to prevent accidental exposure to schistosomecercariae, while heavy duty leather gloves were worn to prevent accidental crayfish bites during sampling.

Study Area

The study was undertaken in the Machakos County of Kenya, in localities within the River Athi Basin, in seasonal streams, identified through an initial survey as habitats for *Biomphalaria pfeifferi*, a prominent snail host of *Schistosoma mansoni*, the causal agent of intestinal schistosomiasis.

Baseline Survey and Data Collection

Out of the 15 stream habitats surveyed in the area, 4 were selected as the study habitats, and were sampled bi-monthly between June 2014 and March 2016. Two of the 4 selected study stream habitats (namely Kyanguli and KwaMutanga streams) were designated "Experimental" and received crayfish after an initial 6-months baseline sampling. The other 2 (Kyaana and Kamuongo) were designated as "Control" and no crayfish were introduced into the habitats. All the 4 study habitats selected had thriving populations of *B. Pfeifferisnails* which persisted for the 6 months all the habitats were sampled, prior to the introduction of crayfish into the "Experimental" sites. In each study habitat, 3 sampling stations were identified and marked, and were sampled during each visit to the habitats. The study habitats were sampled to determine presence or absence of crayfish, snail and crayfish abundance, and to determine schistosome infection prevalence in the snail populations.

Snail Sampling and Screening

Snails were sampled in all the 4 study habitats at designated sampling stations using standard snail scoops made of stainless steel sieves with a mesh size of 2×2 mm, supported on an iron frame, and mounted on a 1.5 m long wooden handle. Snails were sampled for 15 min at each sampling station along the littoral zones. Sampling was done between 0900 hrs and 1230 hrs. The snails collected were sorted into species, counted, and screened for mammalian schistosome infections. Screening for schistosome infections was done using the "shedding" method in which the snails are isolated individually into wells of 24-well culture plates in 1 ml of mineral or aerated water, and left under natural or artificial light for 1.5-2hr to induce release of the schistosome larval forms known as cercariae, which develop in the snails and are infective to humans and other susceptible mammalian hosts.

Crayfish Sampling

Crayfish were sampled using locally made traps of plain wire and onion bags as shown in (Figure 1). The wire was cut into several 45 cm pieces. Where 3 pieces were folded to form circles and welded at the joints. Three straight wires were running the length of the three circles at equal distances and riveted at each point of contact with the circular wires. The traps were randomly placed in the water, and left for a maximum of 1hr, but were checked at 15 min intervals. Any crayfish caught were removed from the traps and placed in a lidded plastic bucket. The captured crayfish were then sized, sexed, counted, and then returned into the water after sampling. Only male catches were analyzed because they provided the best index of crayfish abundance [21].

Figure 1: A photograph of trapped crayfish taken at Kyanguli stream (Experimental) where the crustaceans established thriving populations leading to decimation of snails.

Figure 2: Juvenile crayfish

Results

The Study Habitats

The streams discharge is highly seasonal with peak flow in April-May when flooding is sometime observed and scanty flow in December-February. The streams bed is very variable ranging from rocky bed, large blocks (> 1m), stones (5-25cm), coarse and fine gravel (2-50mm), and sand (<2 mm) with fine organic matter in small pools. Both emergent and sub-mergent aquatic macrophytes occur. Most of the streams habitats are still kept in natural status save for sand harvesting and small scale irrigation. Anthropogenic activities in these streams include but not limited to; clothes washing, bathing, watering domestic animals, fetching water for domestic use, manual and mechanized small scale irrigation.

Snail Species Present in the Study Habitats

Several snail species inhabit the streams including *B. pfeifferi, B. nasutus, B. forskalii, Ceratophalus* and *Lymnea natalensis*. A total of 2325 schistosome transmitting snails (*B. pfeifferi and B. nasutus*) were sampled during the September 2014-March 2016 in the 4 study streams. The number of the other species sampled was small with *B. forskalii at* 17, *Lymneanatalensis, 125* and *Ceratophalusat* 23.

Crayfish Performance in the New Habitats

Following crayfish introduction in Kyanguli and KwaMutanga streams, the crayfish were able to establish thriving populations at Kyanguli. During the 20 month period of the survey, 6 juvenile crayfish, an indication of breeding, were spotted on the edges of the experimental streams. 77 adult predators were captured in Kyanguli stream over the entire study duration while 28 adult crayfish in Kwamutanga within the first 3 months post introduction. (Figure 3) shows juvenile crayfish captured at Kyanguli stream.

Figure 3: KwaMutanga stream, a motor cycle taxi (Bodaboda) washing site, and where crayfish failed to survive or establish a population, after introduction

Snail Abundance in the Study Habitats

After introduction of the crayfish in the "Experimental" habitats, a rapid decline in snail abundance was observed. For instance, within 3 months of crayfish introduction, snails completely disappeared at Kyanguli stream, never came back during the next 12 months of sampling at this habitat. At KwaMutanga stream, the snail abundance declined rapidly as well within 2 months of crayfish introduction (Figure 6). However, crayfish had rarely established at this site due to human activities specifically cleaning of motor bikes (Figure 3). On the other hand, snails remained relatively abundant in the "Control" habitats (Kyaana and Kamuongo streams), even though the numbers fluctuated over time, during the observation period, snails never completely disappeared from these habitats (Figure 7).

The population of snails sampled was more in experimental streams than in control stream. This could be attributed to the accuracy of the sampling method or even natural variation of the population density due to breeding or even habitat suitability. However, the population in control streams grew to almost same levels as in experimental streams by January 2015 just before crayfish was introduced. This provides a basis to evaluate the effect of crayfish on snail population density that would be used as potential biological control for schistosomiasis.

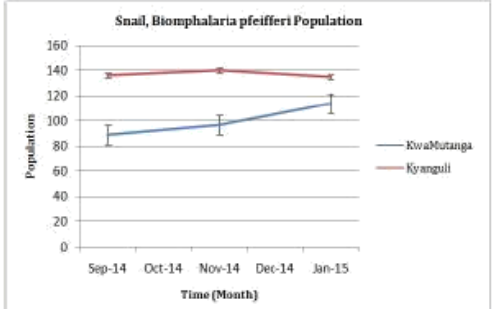

Figure 4: Snail abundance in experimental streams before Crayfish introduction

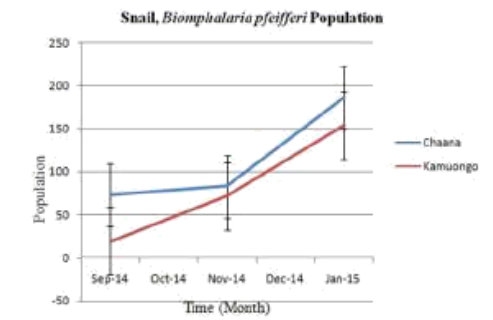

Figure 5: Snail abundance in Control streams before crayfish was introduced in experimental streams

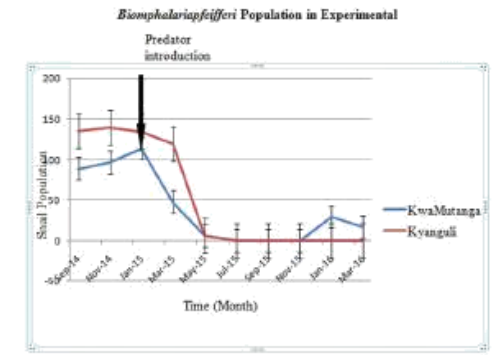

Figure 6: Snail abundance after crayfish introduction (Experimental)

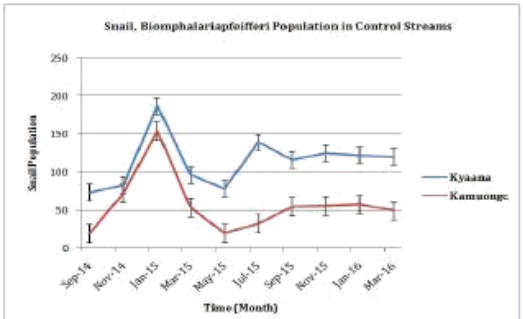

Figure 7: Snail abundance in control streams over the study period

The snail population in experimental streams was significantly reduced (paired *t test* = 5.524, *p value* = 0.0001) compared to control streams (paired *t test* = 7.727, *p value* = 0.082) after the crayfish was introduced. This indicates that the crayfish was able to establish in the habitat and feed on snail as one of their source if nutrients for survival.

Comparison of Experimental and Control snail abundance after crayfish introduction

Kwamutanga stream (Figure 8) the crayfish temporarily established within the first 3 months post introduction, almost wiped out the snails, but the crayfish pettered out by the 4th month leading to a resurgent of snails, may be due to the stream being used for motorcycle taxi washing. However, in Kyaana stream (control) snail population declined from January 2015 till May due to drying of the river and then increased during the months of June and July 2015 (Figure 9). A slight decrease was observed during August as water levels declined and then a steady population reached from November 2015 through March 2016 in Kyaana stream. A comparison using one sample t test showed a significant difference (t = 10.575, p = 0.0001). This shows despite environmental changes such as flooding, drying and human activity, crayfish fed on snail leading to a significant drop in their population.

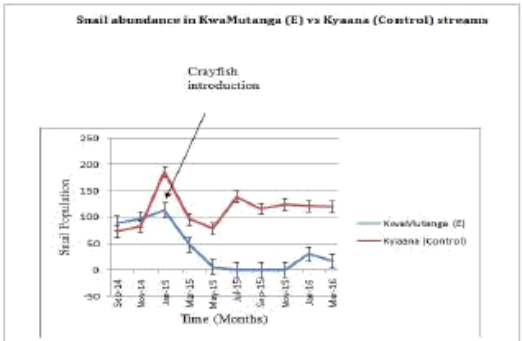

Figure 8: Snail population decline in KwaMutanga after crayfish introduction compared to Kyaana control stream

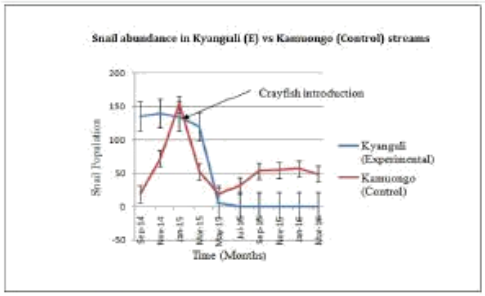

Figure 9: Snail population in Kyanguli after crayfish introduction compared to Kamuongo control stream

A comparison using one sample t test shows significant difference (t = 4.651, p = 0.001) of snail population between the two streams after crayfish was introduced in Kyanguli stream. Even though snail population was affected by environmental factors in Kamuongo stream, the same was also experienced in Kyanguli but the total decline was attributed to the predators (crayfish) introduction. The snail population in Kyanguli stream declined drastically from March 2015 and was totally eliminated by July 2015 through March 2016.

Table 1: Snail population pre and post introduction of Crayfish

Experimental Streams	Snail Abundance Before Crayfish Introduction	Snail Abundance After Crayfish Introduction	Statistical Analysis by Paired *t-Test*	*P value*
Kyanguli	411	0	5.524	0.0001
KwaMutanga	300	17		
Control Streams				
Kyaana	343	487	7.727	0.082
Kamuongo	245	356		

Discussion

This study is the first attempt to determine the effect of crayfish on snail populations in stream habitats. Previous studies in small man-made ponds located within the Athi river Basin in Kenya showed that crayfish rapidly reduced snail numbers in such habitats, and indeed, in one stable habitat, they established self-sustaining populations and prevented re-establishment of snails for several months [25]. In the present study the effect of crayfish on pulmonate snail populations was determined in stream habitats. While crayfish are known to occur in stream habitats in Kenya, their ability to eliminate pulmonate snails of medical and veterinary significance in the country has not been determined. The fact that crayfish were able to persist at Kyanguli over several months and eliminated the snail populations in this habitat suggest that *P. clarkii* could play a role in control of snails of medical or veterinary significance in endemic areas such as Kenya. Although KwaMutanga stream seemed an ideal habitat for crayfish establishment, we experienced difficulties getting the crayfish to survive or establish in this habitat. While we do not have an explanation why crayfish could not establish in this habitat, we suspect pollutants that could potential kill the crayfish may have present in the stream. Apparently, the site at which we released the crayfish into the habitat is frequently used as a motorcycle taxis (locally popularly known *bodaboda*) washing site, and it is possible that petroleum products from the motor cycles such as oil or petrol may be responsible for failure of crayfish to survive and establish at this site.

Bio-control means for control of snails of medical or veterinary importance is preferred to chemical mollusciding, as it is not only considered environment friendly but also offers sustainability [25]. As schistosomiasis is transmitted from water sources frequent use of chemical molluscicides may have long-term adverse affects on water ecosystem as well as negatively affect people and animals that use the same water sources. In the search, for effective biological control agents against schistosome-transmitting snails, the crayfish appears to be particularly promising given that it is an efficient predator of pulmonate snails responsible for transmitting disease causing parasites in both humans and livestock [26]. However, it is envisaged that the widespread use of this crustacean for snail control will most likely be based on continual or repeated restocking of the predator in the transmission sites analogous to the use of molluscicides as transmission sites of such parasites include seasonal streams which are subject to frequent flooding during heavy rains, which end up washing away the crayfish, or to drying out completely in the dry season which may lead to the disappearance of the crayfish [27].

In the current study, though adult crayfish were recovered in Kyanguli stream on subsequent bi-monthly sampling, it was not possible to tell whether the catches represented the originally introduced crayfish or their progenies, future studies should seek to uniquely tag the released crayfish to differentiate them from F1 and subsequent generations. The researchers recommend further work to establish the feasibility of the use of crayfish as biocontrol of schistosomiasis.

Although this study was limited by the number of study habitats, study duration, and also, experienced setbacks with flooding and in one habitat, possible human-related interference which, overall may have influenced the outcome of this study, the data obtained demonstrates the potential of the decapod crustacean, *Procambarus clarkii*, as a biological control agents against aquatic snails responsible for transmission of trematode parasites that cause disease in humans and domestic livestock, such as schistosomiasis (bilharzias) and fascioliasis (liver fluke disease) in stream habitats which may sometimes experience flooding or long dry season, and therefore, may require re-stocking or re-introductions, once in a while. Further studies on this crustacean should therefore, be encouraged. The study limitation included environmental factors such as floods, drought and human activity affected survival of the snails and thus

limits generalization of the results. The snail survival in both experimental and control streams were negatively affected by flooding and human activity such as washing of motorbikes which would reduce the impact of the intervention strategy applied in this study. However, there is a clear indication that crayfish had significant impact to the decrease of the snail population in the experimental streams. This environmental impact would be reduced by replicating the study in artificial setting such as a swimming pool where most variables can be controlled.

Conflict Of Interest

Authors declared no conflict of interest

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