Original Article

Zoonotic helminths and protozoa infesting commercially important marine crustaceans along the Kenyan coast

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Abstract

Marine crustaceans support important small-scale fisheries along the Kenyan coast. Annual catches have been declining, with climate change, pollution, overfishing and parasites proposed as causes. It is unknown whether parasite intensity and diversity change between locations, seasons and host species. The current study aimed to address this by analysing gastro-intestinal (GIT) parasites of two prawn species, *Penaeus indicus* and *Metapenaeus monoceros*, and two lobster species, *Panulirus ornatus* and *P. homarus*. A total of 240 prawns and 240 lobsters were collected from Malindi, Kilifi and Gazi, and intestinal contents microscopically examined for the presence of parasites. Observed parasites included *Schistosoma mansoni* (mean egg per gram (EPG) of 112.33), *Strongyloides* spp. (mean EPG of 94.80), *Hymenolepis diminuta* (mean EPG of 104.55), and *Hymenolepis nana* (mean EPG of 104.85). The Protozoa *Entamoeba coli* (mean EPG of 75.76) was observed. There was a significant difference in parasite intensity among host species (p<0.00) as well as intensity between seasons. There was no significant difference in intensity among landing sites, except in *E. coli* (p=0.05). Parasite diversity was not dependent on sites or seasons but varied with host species. The current study found that all the GIT parasites identified are zoonotic and could present a human health threat.

Keywords: crustaceans, Kenyan coast, gastro-intestinal parasites, zoonotic parasites, parasite intensity

Introduction

Parasites are organisms that live on the outside or inside of another organism (host) and depend on the host for food and shelter (Brazenor *et al.,* 2018). The parasites that live on the outer body of the host are referred to as ectoparasites. On the other hand, endoparasites are parasites that live inside the host's body. This contact weakens the host by generating diseases, leading to death in severe cases.

Coastal and marine waters contain parasites that have severe implications for population dynamics, management and conservation of fisheries resources (Aloo *et al.,* 2004). Kenya is one of the developing nations in the western Indian Ocean region whose coastal people rely on marine fisheries for food and employment (Kimani *et al.,* 2018). Economically important organisms targeted on the Kenyan coast range from fish, lobsters, molluscs and crabs. However, the sustainability of these resources remains uncertain since annual catches from most of these fisheries have indicated declining trends over the past decades (Fulanda *et al.,* 2011).

A decline in catch landings can be attributed to overfishing, recruitment failure or population bottlenecks

through parasite infestations and diseases (Overstreet *et al.*, 2017). In most cases, fisheries managers have used management options such as fishing gear restrictions to prevent the entry of illegal gear among fishers who may want to boost their dwindling catches (Cinner *et al.,* 2008). The sustainability of food production, either originating from capture fisheries or aquaculture ponds, could be greatly hampered by altering the biological, chemical and physical environments either under anthropogenic or climate change perturbations (Petriki *et al.,* 2021). An alteration of the external environment in which these organisms live could easily compromise the organism's immune system, thus leading to disease outbreaks, which might result in heavy mortalities, recruitment failure or population collapse, among which parasites and protozoa play an important role.

Several species of parasites are zoonotic (transmissible between animals and humans), being able to cause health problems in humans (Chaiaa *et al.,* 2005). Adequate knowledge on disease-causing parasites infesting crustaceans in the Kenyan marine waters is generally lacking. The aim of this study was to conduct a comparative assessment of the parasites infesting prawns and lobsters in Kenyan marine waters. The specific objective was to determine whether parasite intensity significantly differed among sampling sites, host species, and seasons.

It is intended that the findings from this study on parasite species composition, temporal and geographic distribution trends, intensity and diversity will provide insight into parasite dispersal patterns, which is crucial for managing and preserving exploitable fisheries resources.

Methodology

Study Area

Kenya has a coastline of about 640 km stretching from 1°30′S at the Somali border to 5°25′S at the Tanzanian border. The marine waters of Kenya are warm – a characteristic of the tropical climate in the region. Two main rivers, Sabaki and Tana drain their waters into the Indian Ocean, thus creating estuarine habitats suitable for the existence of a diverse fisheries resource. The coastal region is influenced by the South East (SE) monsoon winds between April and October and the North East (NE) monsoon winds between November and March (McClanahan, 1988). Other than the monsoonal winds, the region is also influenced by the northerly flowing East Africa Coastal Current (EACC) and the southerly flowing Somali Current (SC). The Somali current reverses its flow direction

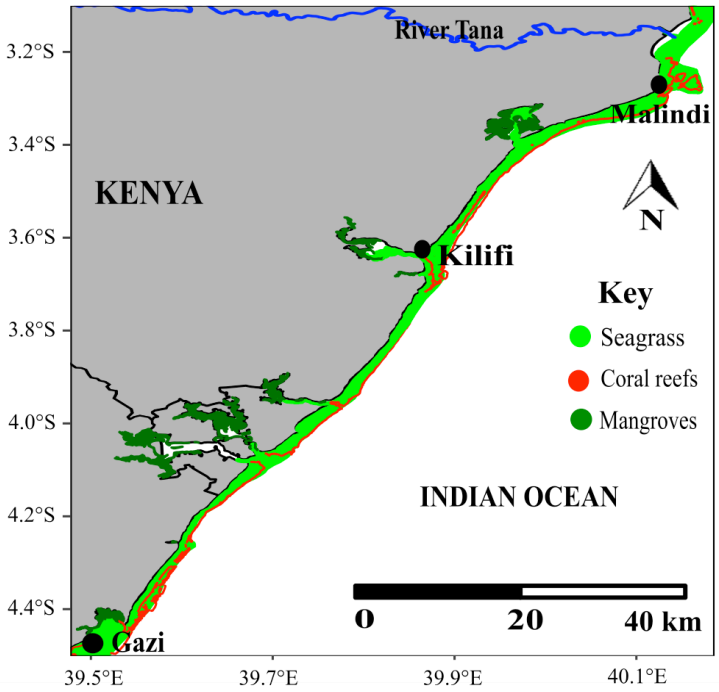

Figure 1. Map of the Kenyan coast showing sampling sites.

between April and October to align itself with the SE monsoonal wind direction (McClanahan, 1988). The area where the SC and EACC converge marks the beginning of an offshore South Equatorial Counter Current (SECC). These oceanographic features of the Kenyan coast presumably facilitate host and parasite larval dispersal and mixing, with implications for distribution patterns and genetic population structure for both parasites and hosts. Samples were collected at Mijikenda landing site in Malindi, Kilifi Central landing site in Kilifi and Gazi landing site (Fig. 1).

Sample collection and preparation

Two hundred and forty whole prawn and 240 lobster samples (total 480 samples) were collected from the three study sites, namely Gazi, Kilifi and Malindi (i.e., 20 individuals ×4 species ×3 sites × 2 seasons=480) between September 2020 and May 2021. Sampling was conducted during the NE and SE monsoon seasons. Each sampling station was sampled once per season, making two temporal sampling events at each site. Fresh prawns and lobsters were bought from 15 artisanal fishers at random, depending on who fished in the sampling area and had the species of lobsters and prawns needed. The samples were stored on ice in cooler boxes for four hours while in the field and frozen at -80 °C in the laboratory prior to parasitological examinations.

Laboratory procedure

During the parasitological examination, each sample specimen was defrosted at room temperature. The eyes, skin, carapace, nostrils and mouth cavity of each specimen was investigated using a hand lens for lesions or any symptoms of disease manifestation. The body cavity was opened using sterilised scissors to examine the liver, stomach, pyloric caeca, intestines and gonads for endoparasites. Stool samples were collected from the intestines and parasites were microscopically observed using the wet mount technique (CDC, 2021a), where a drop of faecal matter was placed on a slide, stained with 10 % Lugol's iodine, covered with a coverslip and observed under a compound microscope at a magnification of 40×10. Parasites were identified up to the species level morphologically by using guides available for faecal parasites (CDC, 2021a). McMaster concentration technique, according to WHO (2019), was used to quantify the parasites, where the number of parasites and eggs found in that portion of stool was be multiplied by 50 for estimation of eggs per gram (EPG).

Data analysis

Data was recorded in Microsoft Excel and imported into the IBM SPSS Statistics software (IBM Corp, Armonk, NY, USA) for analysis. A statistical test was considered significant when the p-value was ≤0.05. Analysis of Variance (ANOVA), which is used to compare means between more than two groups (Hae-Young, 2014), was used to test for any significance difference in the parasite intensity among the three landing sites. ANOVA was also used to determine if there was any significant difference in the parasite intensities among the hosts (two species of

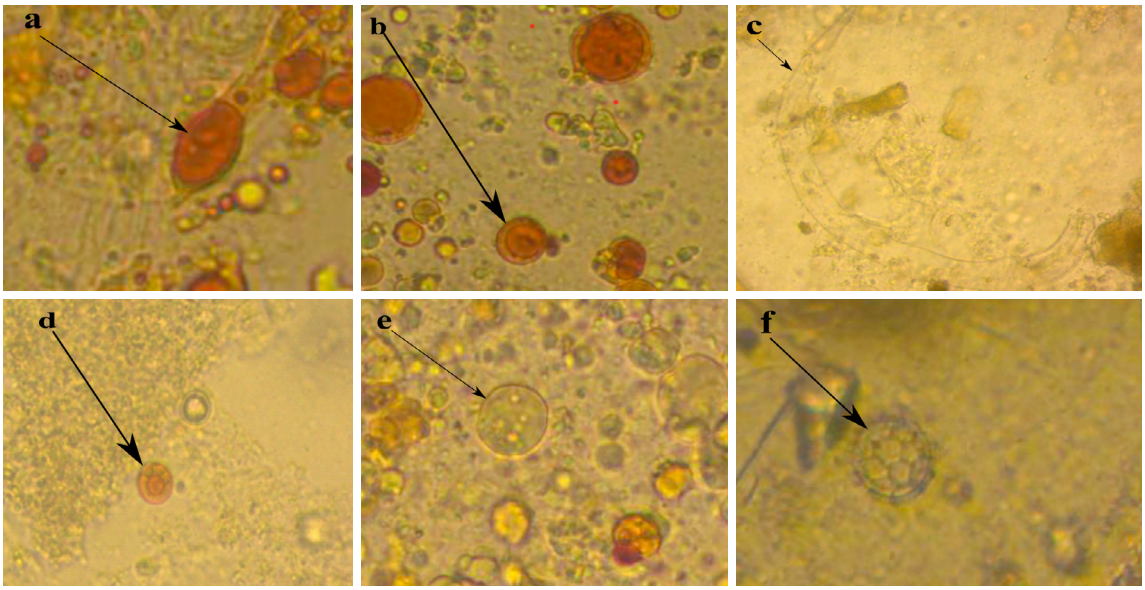

Figure 2. Helminth eggs (a) *Schistosoma mansoni*, (b) *Hymenolepis diminuta*, (c) *Strongyloides spp*, (d) *Hymenolepis nana*, (e) *Entamoeba coli* found in both prawns and lobsters, and (f) an unidentified cyst found in lobsters under x400 magnification.

Table 1. Mean egg per gram (EPG) ± standard deviation (SD) and analysis of variance test results conducted to compare means in parasite load among host species.

Parasite	Mean EPG ± SD *P. indicus* (n=120)	Mean EPG ± SD *M. Monoceros* (n=120)	Mean EPG ± SD *P. homarus* (n=120)	Mean EPG ± SD *P. ornatus* (n=120)	df	F	*p*-value
Strongyloides spp	101.22 ± 54.19	106.06 ± 49.62	78.85 ± 42.83	86.00 ± 33.91	3	12.43	<**0.00**
Schistosoma mansoni	118.97 ± 57.60	104.65 ± 48.57	110.53 ± 42.75	111.67 ± 61.14	3	8.50	<**0.00**
Hymenolepis nana	115.63 ± 57.41	112.50 ± 49.82	97.06 ± 51.45	77.78 ± 30.78	3	19.70	<**0.00**
Entamoeba coli	103.85 ± 55.76	70.00 ± 38.19	76.67 ± 37.16	57.69 ± 18.78	3	2.15	0.09
Hymenolepis diminuta	110.34 ± 52.41	113.79 ± 47.99	89.13 ± 52.13	100.00 ± 51.45	3	3.64	<**0.00**

prawns and two species of lobsters). A student's t-test, used to compare means between two groups (Hae-Young, 2014), was used to determine if there was any significant difference in parasite intensity between the two monsoon seasons. R programming environment (R Core Team, 2021) was used to generate diversity box plots. Mean EPGs were generated using Microsoft Excel.

Results

A total of 240 specimens of prawns (i.e., 120 *Penaeus indicus* and 120 *Metapenaeus monoceros*) and 240 lobsters (i.e., 120 *Panulirus ornatus* and 120 *Panulirus homarus*) were collected and observed for parasites. The specimens were healthy externally showing no lesions or any symptoms of disease manifestation. Five species of intestinal parasites were found to infest prawns and lobsters in the study sites. These included both helminth and intestinal protozoa. Helminth eggs identified include *Schistosoma mansoni*, *Hymenolepis diminuta*, *Strongyloides spp, Hymenolepis nana* and one protozoan cyst *Entamoeba coli* (Fig. 2). These parasites were observed under the magnification of ×400. One cyst found in lobsters under the magnification of ×400 was unidentified (Fig. 2).

Parasite intensity and diversity between prawn and lobster host species

There was no significant difference in *E. coli* intensity among the different host species analysed (p=0.09), but there were significant differences in the other parasite intensities (p<0.001) (Table 1). Parasite diversity also varied in prawns and lobsters, with a diversity index of 0.75 and 0.00 respectively (Fig. 3). Mean EPGs indicated that prawns were more parasitized than lobsters.

Parasite intensity and diversity among landing sites

There was no significant difference in parasite intensities among landing sites (p>0.05) (Table 2). A significant difference in means among the landing sites was noted in *E. coli* (p =0.05). The diversity of parasites was not dependent on landing sites, since all sites had a diversity index of 0.00 (Fig. 4).

Parasite intensity and diversity between seasons

The student t-test showed a significant difference in parasite intensity between the two seasons in all the parasites (p<0.05) and no significance in the protozoa *E. coli* (p=0.59) (Table 3). The diversity of parasites does not depend on the seasons since both seasons had a diversity index of 0.00 (Fig. 5).

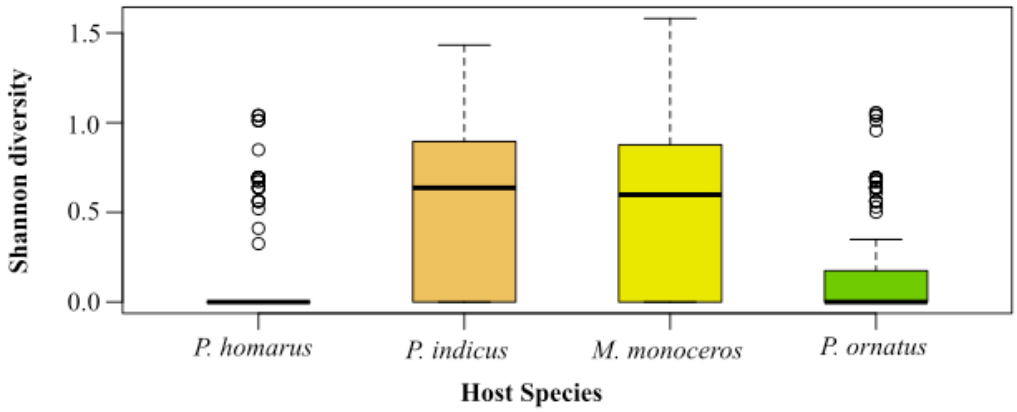

Figure 3. A boxplot showing parasite diversity among the host species of prawns and lobsters.

Table 2. Mean egg per gram (EPG) ± standard deviation (SD) results and analysis of variance results for a test conducted to compare means in parasite intensity among landing sites.

Parasite	Mean EPG ± SD in Mijikenda (n=160)	Mean EPG ± SD Kilifi Central (n=160)	Mean EPG ± SD in Gazi (n=160)	df	F	*p*-value
Strongyloides spp	93.33 ± 50.68	103.57 ± 48.63	86.84 ± 43.00	2	1.31	0.27
Schistosoma mansoni	113.33 ± 54.77	107.41 ± 50.85	116.67 ± 57.15	2	2.01	0.14
Hymenolepis nana	97.06 ± 53.57	108.11 ± 52.06	109.38 ± 48.26	2	0.35	0.70
Entamoeba coli	79.55 ± 49.96	75.00 ± 41.00	71.43 ± 32.31	2	3.13	**0.05**
Hymenolepis diminuta	113.46 ± 52.07	100.00 ± 50.00	102.63 ± 51.92	2	1.30	0.27

Discussion

Gastro-intestinal parasites found were zoonotic helminths, which cause diseases in humans and animals. An increase in helminth burden causes manifestation of the disease, leading to abdominal pain, nausea, weight loss, general body weakness, diarrhoea, blood in stool, loss of appetite and sometimes lymphedema, in extreme cases in humans (Bogitsh *et al.,* 2013). Presence of these parasites in prawns and lobsters could indicate their possible roles as reservoir hosts and faecal contamination of Kenyan marine waters.

Hymenolepis nana and *H. diminuta* are species of rodent tapeworms that cause Hymenolepiasis, using grain eating arthropods as intermediate hosts (CDC, 2021b). Human infections occur on ingestion of infected arthropod or insects (Panti-May *et al.,* 2020). Cysticercoids develop in intermediate hosts upon ingestion of mature eggs in faeces, passed by infected rodents or humans, and infect humans and rodents when the intermediate host is eaten, then develop into adults in the small intestines (CDC, 2021b). Eggs are released by gravid proglottids when they disintegrate in the ileum. In *H. nana*, autoinfection occurs when the eggs release their oncosphere embryo that penetrate the villus (Muehlenbachs, 2015). Infected insects could be eaten by amphibians, reptiles and humans, who then release eggs through faecal matter that finds its way into the ocean, infecting the prawns and lobsters.

Schistosoma mansoni is a parasitic flatworm that causes *Schistosomiasis*, also known as Bilharzia in humans, which has been categorised as a neglected tropical disease. More than 250 million people are infected, with 85 % occurring in Sub-Saharan Africa (Rinaldo *et al.,* 2021). Their presence in lobsters and prawns could also be a contributing factor for the highly prevalent parasitic infection. When eggs are released in faecal matter, they hatch into miracidia which penetrate snail tissues. In the snail, multiple generations of sporocysts and cercariae are produced, and upon release, the free swimming cercariae penetrates the skin of the human, shedding their tail to become schistosomulae and migrate to the lungs (CDC, 2021c). Adult worms migrate to blood vessels where females lay eggs that are released in stool or urine (WHO, 2021), depending on the parasite species. Infestation in prawns and lobsters could be by penetration of free swimming cercariae through the skin as observed in humans or when snails, which miracidia develops in, are eaten by prawns (Sokolow *et al.,* 2017). Symptoms of Schistosomiasis in humans include abdominal pain, blood in stool, hepatomegaly and splenomegaly (Rinaldo *et al.,* 2021). Lobsters infested with microsporidian parasites

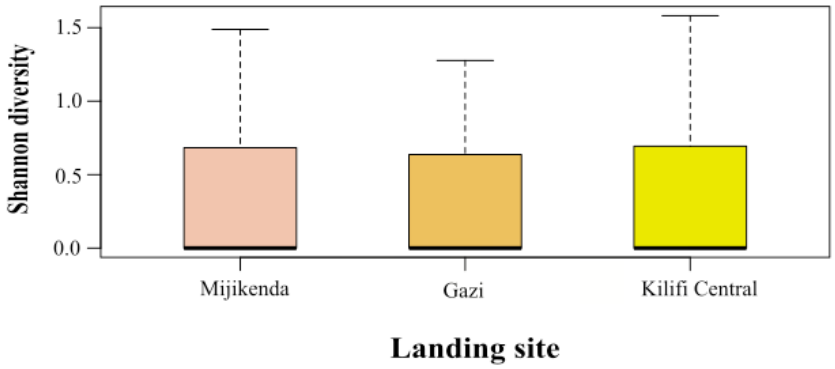

Figure 4. A boxplot showing the diversity of parasites among the landing sites.

Table 3. Mean egg per gram (EPG) ± standard deviation (SD) results and Student t-test results for a test conducted to compare means in parasite intensity between North East (NE) monsoon season and South East (SE) monsoon seasons.

Parasite	Mean EPG ± SD NE monsoon (n=240)	Mean EPG ± SD SE monsoon (n=240)	df	F	*p*-value
Strongyloides spp	96.58 ± 52.92	92.31 ± 40.08	478	7.28	**0.01**
Schistosoma mansoni	114.38 ± 55.58	110.39 ± 52.78	478	6.54	**0.01**
Hymenolepis nana	100.00 ± 51.51	109.43 ± 51.01	478	15.90	**<0.00**
Entamoeba coli	72.73 ± 39.71	78.79 ± 43.36	478	0.29	0.59
Hymenolepis diminuta	111.00 ± 52.77	97.94 ± 48.90	478	30.64	**<0.00**

have been found to be lethargic, with atypical external appearances like altered coloration and increased carapace opacity (Stentiford *et al.,* 2010). Gregarine parasites have been found in the prawn *Macrobrachium rosenbergii* (Zakariah *et al*., 2022).

Strongyloides spp are parasitic nematodes that can be found in animals, insects, water, soil, fruits and vegetables (White *et al*., 2019). There are over 50 species of these parasites affecting millions of people worldwide (Viney and Lok, 2015). These parasites live in the host's gut, where the females lay eggs that are passed through stool, where the eggs are eaten or larvae penetrate the skin to infect a new host (Viney and Lok, 2015). Presence of *Strongyloised spp* in prawns and lobsters indicate fecal contamination in this part of the Indian Ocean. The nematode *Strongyloides strercoralis* is the main source of infection in humans while *S. fuellerboni*, found in African primates, can also be transmitted to human hosts (Viney and Lok, 2015). Autoinfection also occurs in some species of *Strongyloides,* where eggs hatch in the host's gut, releasing larvae. The presence of a large number of primates living in mangroves along the Kenyan coast may explain the presence of *Strongyloides spp* in prawns and lobsters.

Entamoeba coli is a non-pathogenic protozoa transmitted though faecal exposure (Fotedar *et al.,* 2007), indicating that the Kenyan Indian Ocean contains human faecal contamination. Encystation occurs in the small intestine and the cystic eggs are passed through the large intestine into the environment, where transmission occurs by consumption of contaminated food or water (Haidar and De Jesus, 2021). Although non-pathogenic and asymptomatic in humans (Haidar and De Jesus, 2021), the effect of the protozoa on prawns and lobsters is yet to be studied. Most of the microscopic parasites found are zoonotic faecal parasites, suggesting faecal contamination in these coastal waters. Polyparasitism observed is likely to accelerate the decline in host health, reproductive ability and lifespan.

Parasite diversity and means in prawn species were higher than those in lobsters. This may be due to the ability of prawns to feed on some intermediate hosts of like snails that are known to carry numerous parasites like *Schistosoma* spp (Sokolow *et al,* 2017). This phenomenon has led to the introduction of prawns and crayfish in water bodies to reduce the prevalence of bilharzia (Swartz *et al,* 2015). The prawn's softer exoskeleton, compared to the lobster's, could also be

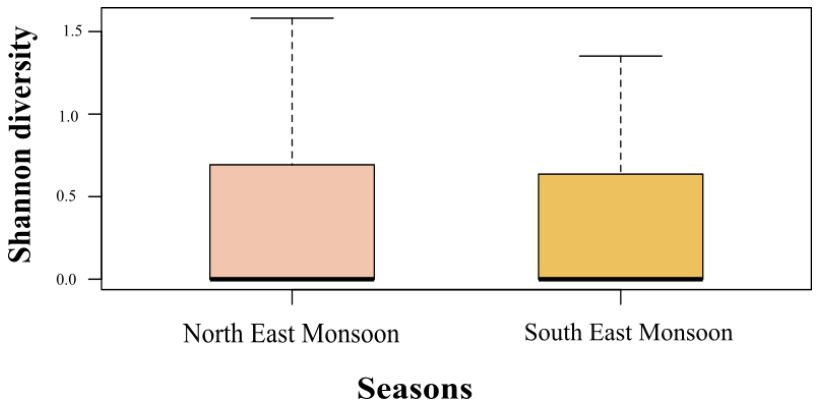

Figure 5. A boxplot showing the diversity of parasites between seasons.

a factor, making it easier for the cercariae to penetrate their bodies in the same way they penetrate the skin of a human host.

While Malindi is a densely populated town in the north coast of Kenya and Kilifi is moderately populated, Gazi is a small, sparsely populated fishing village on the south coast of Kenya. Sewage and garbage from the more populated towns of Malindi and Kilifi easily gets into the ocean, causing introduction of human parasites, like *H. nana, E. coli* and *H. diminuta.* Presence of the same parasites in the fishing village of Gazi could be due to the tendencies of the locals, who use mangroves as lavatories during low tides, and during high tides, the faecal matter is washed into the ocean and parasites introduced into the benthic prawns and lobsters. Monkeys and other primates, which have been recorded to be hosts to several parasites (Teklemariam *et al.,* 2018), are present in abundance, especially in mangroves along the coast of Kenya, increasing the chances of faecal contamination of the ocean.

Parasites found in the prawns and lobsters of Kenya marine waters are zoonotic, causing infections in humans. Early infections in humans are asymptomatic, making it hard to diagnose infected individuals while allowing the parasites to multiply. Measures taken to control these parasites include public health awareness campaigns, where individuals are educated on the risks they face and roles they play in the spread of schistosomiasis. Avoiding contact with contaminated water (CDC, 2021c) is also recommended but impractical for some populations like fishermen. Therefore, preventive chemotherapy using repeated mass drug administrations of antihelminths like praziquantel, administered at a dosage of 40 mg per kg of body weight, for at-risk individuals like fishermen, children and women, who may get infected when carrying out domestic chores (Inobaya *et al*., 2014), has been introduced. Praziquantel, however, only targets the adult worm and does not protect against reinfection (Li *et al.,* 2019), and therefore drinking boiled water in endemic areas and proper washing and cooking of food (CDC, 2021c) is recommended alongside the other control strategies.

Significant differences in parasite intensity between seasons, with more parasite mean EPGs in samples collected during the NE monsoon than those collected during the SE monsoon, could be due to the high productivity associated with the high chlorophyll content of the water during the NE monsoon season due to river runoff after the long rains (Heip *et al.,*1995). High productivity may also lead to increased parasite populations in other marine organisms.

Conclusions

All parasites infesting the prawns and lobsters in this study are zoonotic and this is likely to impact on their commercial value as well as causing infection to human consumers. The parasites are also likely to affect the growth rate of the crustaceans hence also reduce the commercial value. This situation calls for the Ministry of Health to put in place control measures to reduce transmission from these hosts, especially during NE monsoon season since this is when the parasite abundance is highest in the most affected sites. Since very few studies have been done on prawn and lobster parasites, especially the intestinal ones, parasites found were identified using guides available. Metabarcoding should done to check for species integrity and to find out if there are new parasites in prawn and lobster faecal samples collected during this study and preserved at -80 ˚C for future analysis.

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