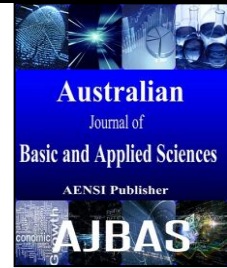




## AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN: 1991-8178  
EISSN: 2309-8414

DOI: 10.22587/ajbas.2017.11.16.10  
Journal home page: www.ajbasweb.com



### Prevalence of Trematode Larval Infections in Some Red Sea Snails

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**ARTICLE INFO**

**Article history:**

Received 12 October 2017

Accepted 22 December 2017

Available online 31 December 2017

**Keywords:**

Marine snails, Prevalence, Trematode larvae, Cercaria

**ABSTRACT**

Marine snails play an important role in the transmission of cercariae to fish as a source of zoonotic diseases. In Saudi Arabia, studies on parasitic infections in marine snails are rare. In the current study, a total of 550 marine snails belonging to the genus, *Nerita* were collected randomly from Obhor bay, Red Sea at Jeddah city, Saudi Arabia. Snails were collected monthly at the period from January to December 2016. They were exposed to light and crushed to assess the presence of larval trematodes. The most prevalent infected snail species was *N. histrio* (31.94%). Nine distinguishable cercariae were identified; *Haematoloechus similis*, Virgulate, Microphalliae, *Ascorhytis charadriiformis*, *litorina saxatilis* VII, *litorina saxatilis* V, *Maritrema lingulla*, *Microphallus similis* and Furcocercous cercariae. Virgulate cercaria was the most prevalent type of recorded cercariae (33.89%). There is a positive correlation between infection prevalence and snails' length. Trematode infection of marine snails is influenced by temperature and varied seasonally. Such studies can assist in collecting data on the ecological relevance of disease distribution in the sympatric fish and the pattern of transmission of digenean trematodes by snails and finally, in prevention and control of the following fish and human diseases.

**INTRODUCTION**

Mollusks are important to humans as food, decoration and materials. Gastropods are one of the most diverse and largest group of mollusks, with more than 62,000 described living species and they comprise about 80% of living mollusks. Most snails can become intermediate hosts for trematode cercariae, which may be transmitted to humans and animals. The life cycle of trematodes is very complex, as they require an intermediate host such as snails or fish for the maturation to the infective stage. Sporocysts and redia are formed inside the first intermediate host and feeds through diffusion across the tegument and develop into cercariae inside the snail. The cercariae are adapted for dispersal outside the snail and exhibit a large variety in morphology to recognize and penetrate the second intermediate host. They have behavioral and physiological adaptations, which are not found in earlier stages. To determine the ability of a snail species or a snail population to act as an intermediate host of a given species of digenea, it is necessary to measure several parameters such as the prevalence and intensity of snail infection (Graczyk & Fried 1999). Until present, only few studies have been carried out on the diversity and abundance of cercarial infection in the Red Sea snails. There is an urgent need to obtain information regarding environmental influences acting on emergence of cercariae in marine system (Koprivnikar & Poulin 2009). Thus, the aim of the present study was to elucidate the monthly and seasonal changes in the rate of cercarial infections of marine *Nerita* snails, as well as the effect of snails' length and temperature on the infection prevalence in the Red Sea, Jeddah coast. Saudi Arabia.

**Open Access Journal**

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**To Cite This Article:** Amaal Hassan, Muslimah Al-solami and Sabah M. Hassan Prevalence of Trmatode Larval Infections in Some Red Sea Snails *Aust. J. Basic & Appl. Sci.*, 11(16): 69-76, 2017

## MATERIALS AND METHODS

### *Collection and Identification of snails:*

A total of 550 marine snails were collected randomly from Obhor bay, which located about 30 km north of Jeddah city on the east coast of the Red Sea, west of Saudi Arabia, with global positioning system (GPS) reading of 21° 32' 36 N and 39° 10' 22 E (Figure 1). Snails were collected using a wooden handle paddle. They were put in glass jars containing fresh seawater and were transferred to the Parasitology laboratory, Department of Biology, Faculty of Science, King Abdul Aziz University, Jeddah. Snails were collected during the period from January to December 2016.

Collected snails were identified according to their shell morphology using identification keys as Rusmore-Villaume (2008) and Knaap & Loker (1990). They were found belonging to the family Neritidae and the genus *Nerita*, which is classified into six species; *Nerita albicilla*, *N. grayana*, *N. polita*, *N. quadricolor*, *N. orbignyana* and *N. histrio* (Figure 2).



**Fig. 1:** Site of snails' collection from the Red Sea, Jeddah coast (by Google map)

### *Measurement of shell length:*

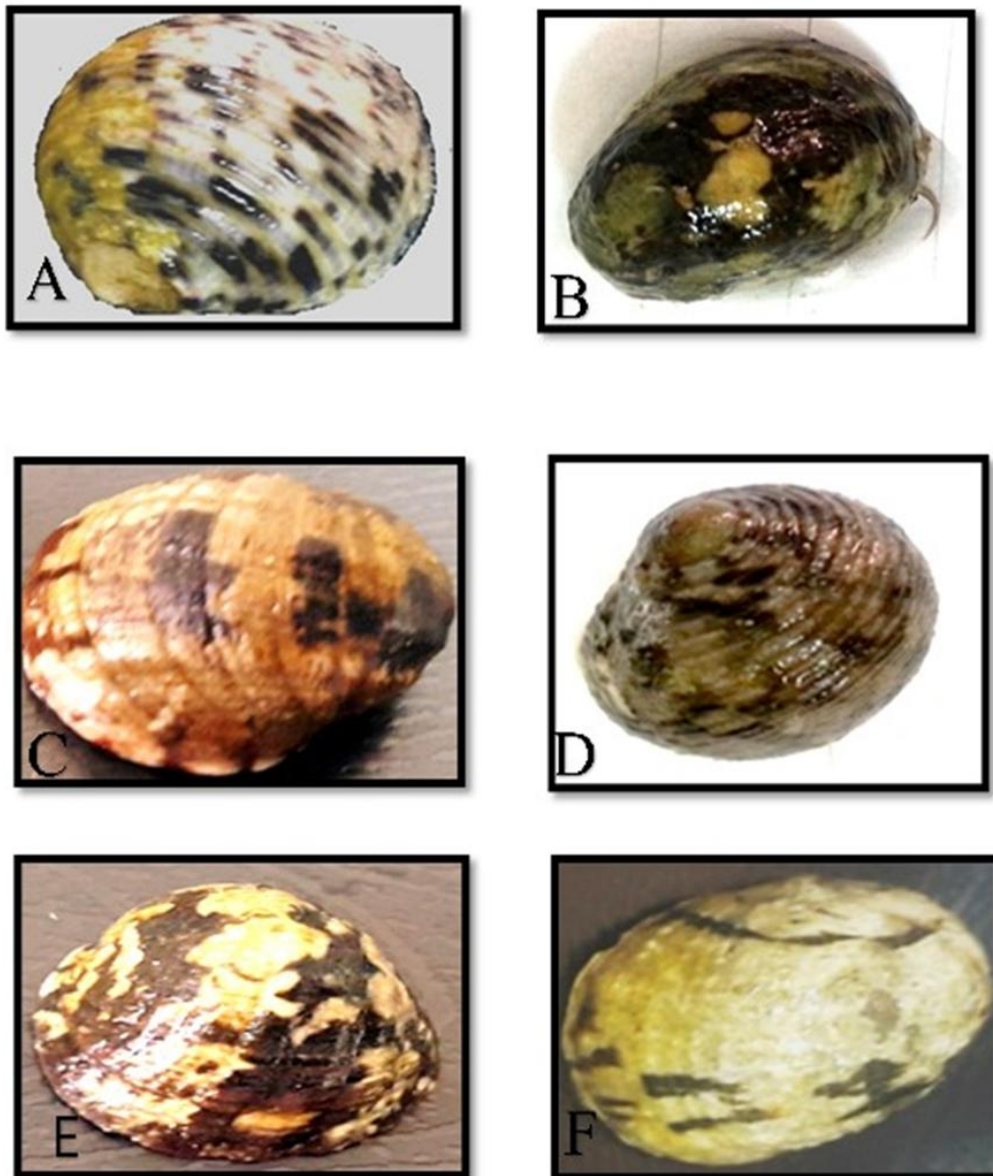
Maximum shell length was measured from the apex to the end of the shell using a Vernier Calipers Series 530-Standard Model. Snails species were classified according to their length into five classes, 2-3.9mm, 4-5.9mm, 6-7.9mm, 8-9.9mm, 10-11.9mm and 12-14mm.

### *Shedding and harvesting of cercariae:*

Shedding and harvesting of cercariae were done according to Aboelhadid *et al.* (2016). Different snail species were separated in plastic specimen cups, cleaned and each snail was put in petri dish containing seawater and were kept in the laboratory at 23°C. They were exposed to artificial light for two hours. Water in each dish was checked at short intervals for emerging cercariae under a binocular dissecting microscope. The infected snails were separated and put in 250 ml beaker to facilitate emerging of cercariae. The non-shedding snails were crushed and examined under the binocular dissecting microscope searching for early larval trematode stages. Living cercariae were stained with diluted vital stain, neutral red or methylene blue. The stained cercariae were examined and photographed under a research microscope. They were identified according to their morphological characters using taxonomic keys and recent articles.

### *Statistical analysis:*

The data were analyzed using Chi-square analysis to compare the infection rate capacity between infected and non-infected snails. The differences in snail's species between non-infected and infected during all months and different lengths were tested separately. All statistical analyses were performed using SPSS software program (version 22). Probability of  $P < 0.05$  was regarded as significant at 95% confidence limit.



**Fig. 2:** The collected snails species of the genus *Nerita*, A- *Nerita histrio*, B-*Nerita albicilla*, C- *Nerita orbignyana*, D-*Nerita quadricolor*, E- *Nerita grayana*, F-*Nerita polita*

### **Results:**

#### **Overall prevalence of infection in snail's species:**

A total of 550 marine snails were collected randomly from Obhor region at Jeddah coast. The snails were classified based on their shell morphology and were found belonging to family Neritidae and genus *Nerita*. Six species from the genus *Nerita* were identified and examined; (189 of *Nerita albicilla*, 91 of *N. grayana*, 53 of *N. polita*, 93 of *N. quadricolor*, 52 of *N. orbignyana* and 72 of *N. histrio*. *Nerita albicilla* was found to be infected with three types of cercariae (virgulate cercaria, *maritrema lingulla* and *Litorina saxatilis* V). *Nerita grayana* was found to be infected with two types of cercariae (*litorina saxatilis* V and *litorina saxatilis* VII). *Furcocercus* cercaria infects *N. polita* and cercaria of *Maritrema lingulla* infects *N.quadricolor*. Four types of cercariae were collected and investigated from *N. orbignyana*; *Microphallus similis*, *Haematoloechus similis*, *Maritrema lingulla* and *Ascorhytis charadriiformis*. Two types of cercariae were detected from the snail, *N. histrio*: *Microphalliae* and *Haematoloechus similis*. The mean prevalence of larval trematode infections was 21.45%. The most prevalent infected snail species was *N. histrio* (31.94%) followed by *N. albicilla* (24.34 %) and *N. orbignyana* (23.08 %). The prevalence of infection in the species *N. grayana* was 21.98% and *N. quadricolor* 16.13%, while *N. polita* snails had the lowest infection prevalence of 3.77% (Table 1).

**Table 1:** The overall infection prevalence in different snail species collected from north Obhor

Infection percent	Type of cercaria	Snail species
24.34	virgulate cercaria <i>maritrema lingulla</i> <i>Litorina saxatilis</i> V	<i>Nerita albicilla</i>
21.98	<i>litorina saxatilis</i> V <i>litorina saxatilis</i>	<i>Nerita grayana</i>
3.77	Furcocercus cercaria	<i>Nerita polita</i>
16.13	<i>Maritrema lingulla</i>	<i>Nerita quadricolor</i>
23.08	<i>Microphallus similis</i> , <i>Haematoloechus similis</i> , <i>Maritrema lingulla</i> <i>Ascorhytis charadriiformis</i>	<i>Nerita orbignyana</i>
31.94*	Microphalliae <i>Haematoloechus similis</i>	<i>Nerita histrio</i>

**Overall prevalence of cercariae:**

Nine distinguishable cercariae were collected from *Nerita* spp. The overall prevalence of infection for each cercarial type in all snail species was recorded. Cercaria of *Haematoloechus similis* (5.93%), virgulate cercaria (33.89%), cercaria of Microphalliae (16.95%), cercaria of *Ascorhytis charadriiformis* (1.69%), cercaria of *litorina saxatilis* VII (7.63%), cercaria of *litorina saxatilis* V (11.86%), cercaria of *Maritrema lingulla* (16.95%), cercaria of *Microphallus similis* (3.39%) and furcocercous cercaria (1.69%). The highest percentage of infection was recorded for virgulate cercaria (33.89%), while the lowest was recorded for cercaria of *Ascorhytis charadriiformis* and Furcocercous, 1.69% (Table 2).

**Table 2:** Overall prevalence (%) of trematode cercariae in marine water snails collected from north Obhor, Jeddah (\* referred to most prevalent cercaria)

Types of Trematode cercariae	Prevalence (%)
<i>Haematoloechus similis</i> cercariae	5.93
Virgulate cercariae	33.89*
Microphalliae cercariae	16.95
<i>Ascorhytis charadriiformis</i> cercariae	1.69
<i>litorina saxatilis</i> VII Cercaria	7.63
<i>litorina saxatilis</i> V Cercaria	11.86
<i>Maritrema lingulla</i> Cercaria	16.95
<i>Microphallus similis</i> Cercaria	3.39
Furcocercous cercariae	1.69

**Monthly changes in infection of *Nerita* spp:**

Monthly changes in the infection prevalence in each snail species was studied in table (3). Infection of *N. albicilla*, *N. quadricolor* and *N. histrio* were the highest in November with percentages of 86.36%, 77.78% and 71.42%, respectively. On the other hand, in *N. grayana* the highest infection prevalence was in June (71.42%). *N. polita* was infected during April and May only with infection rate of 50% for each month. The highest rate of infection in *N. orbignyana* was recorded in May (75%).

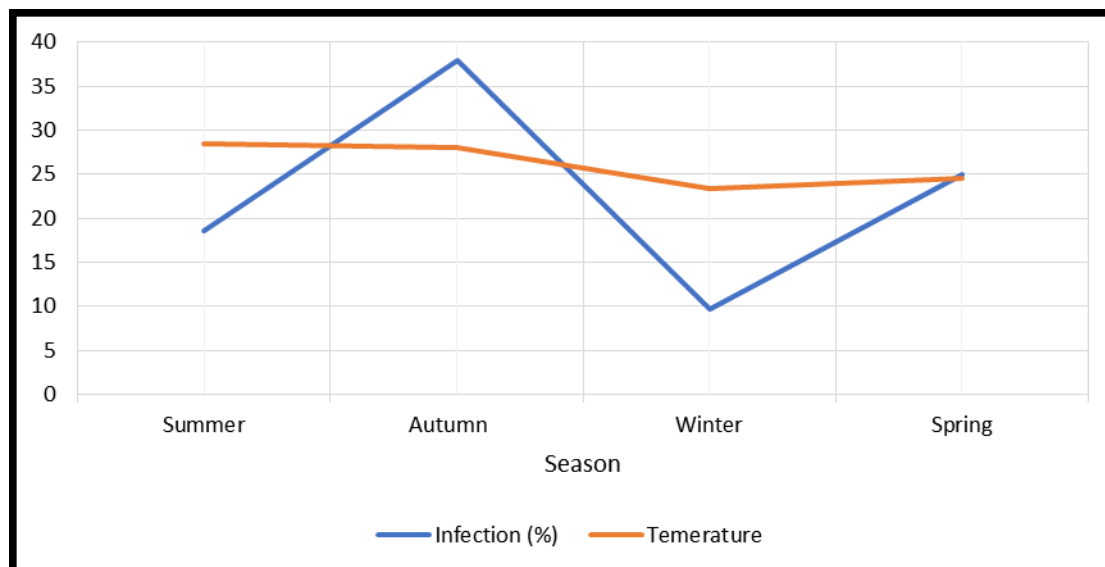
**Table 3:** Monthly prevalence of cercarial infection in *Nerita* snails (\* referred to highest infection percent)

<i>N. histrio</i>	<i>N. orbignyana</i>	<i>N. quadricolor</i>	<i>N. polita</i>	<i>N. grayana</i>	<i>N. albicilla</i>	Types of snails
						Months (snail examined /Snail shedding) %
-	20	-	-	16.67	4.34	January
-	-	-	-	42.10	5.56	February
-	-	-	-	-	-	March
-	12.5	-	50	33.33	-	April
50	75*	50	50	50	77.78	May
63.63	-	9.09	-	71.42*	41.67	June
-	-	-	-	18.18	-	July
-	33.33	-	-	-	-	August
-	50	50	-	14.29	12.5	September
-	-	14.29	-	12.5	16.67	October
73.68*	25	77.78*	-	42.86	86.36*	November
12.5	33.33	-	-	25	24.13	December



### Seasonal changes in the infection prevalence:

As shown in Table (4) and Figure (3), the results showed significantly different prevalence of infection percentages, with no correlation between seasonal prevalence and mean temperature. The highest prevalence of cercarial infection in snails was recorded during autumn (September, October and November) with 38% prevalence, whilst it was lowest during winter (December, January and February) with 9.67% prevalence. Mean temperature was highest in summer (29°C) and lowest in winter (23°C). In autumn, the mean temperature was (28°C) and in spring (25°C).



**Fig. 3:** Seasonal changes in temperature and infection percent in *Nerita* snails

### Correlation between snail's length and infection prevalence:

As shown in Table (5), there is a positive correlation between infection prevalence and snail's length. The infection of the snail species, *N. albicilla* with the length of 12-14 mm was the highest (78.26%). The length 10-11.9 mm had the highest infection prevalence (71.42%) in *N. grayana* snails. In the snail species *N. polita*, infection was found only in the length 8-9.9 mm (22.22%). In *N. quadricolor* snails, the infection percentage was the highest in the length 8-9.9 mm (37.5%). As for *N. orbignyana* the highest percentage of infection was recorded in the snail length 10-11.9 mm (50%). The percentage of infection in *N. histrio* with length 10-11.9 mm was the highest (80%). The lowest rate of infection was recorded from snails of length 2-3.9mm.

**Table 4:** Seasonal prevalence of trematode larval infection in some marine snails (\* referred to highest infection percent)

Season	Total collected	(%)
Summer	113	18.58
Autumn	150	38*
Winter	207	9.67
Spring	80	25

**Table 5:** The infection prevalence in relation to *Nerita* snails length (\* referred to highest infection percent)

Size of snails/ infected (%)						Number of examined snails	Snail species
12-14 mm	10-11.9 mm	8-9.9 mm	6-7.9 mm	4-5.9 mm	2-3.9 mm		
78.26*	42.42	27.78	10	13.33	7.35	207	<i>Nerita albicilla</i>
40	71.42*	20	12.5	21.73	2.86	101	<i>Nerita grayana</i>
-	-	22.22*	-	-	-	63	<i>Nerita polita</i>
20	-	37.5*	33.33	13.04	1.67	112	<i>Nerita quadricolor</i>
25	*50	14.29	40	33.33	8	62	<i>Nerita orbignyana</i>
36.84	80*	50	40	33.33	18.18	62	<i>Nerita histrio</i>

### Discussion:

One of the most important factors that influence the occurrence of trematode infection in marine water is the availability of suitable snail host. Snails play a very important role in the transmission of trematode species as they act as intermediate hosts in which three larval stages of the trematodes life cycle are completed

(Sporocysts, rediae and cercariae). Several snail species of marine snails were found in the Red Sea that act as intermediate hosts for different trematodes that affect our life stock and birds.

In the present study, cercariae of nine species of digenetic trematodes belonging to six morphotypes were collected from marine *Nerita* snails. Total prevalence of larval trematodes in all *Nerita* species was 21.45%. The most prevalent infected snail species was *N. histrio* (31.94%) while, *N. polita* snails had the lowest infection prevalence (3.77%). Few studies concerned with the prevalence in marine snails' cercarial infection such as Byers *et al.* (2008). On the other hand, many studies interested in the study of infection rate of fresh water snails were done all over the world such as, Hasanat *et al.* (2017) who recorded infection rate of 11.07 % for *Lymnaea* snails in Pakistan. Jayawardena *et al.* (2011) also found 16% infection rate for the snails, *Thiara scabra*, *T. tuberculata*, *Paludomus sherica* and *Gyrulus saigonensis* in Srilanka. In addition, Ahmed *et al.* (2006) recorded 14.9% infection rate in the snail, *Bulinus truncates* in Sudan. Differences in the overall prevalence of trematode infections may involve many factors, including different collection time, locality, season, type and number of parasite and intermediate host, availability of infected definitive or reservoir hosts. The prevalence of snails in different locations of the country was different. This was due to difference in the environmental and managerial conditions (Kulsantiwong *et al.* 2015 & Pfukenyi *et al.* 2005). The most prevalent month of cercarial infection in Iran is May to September (Sharif *et al.* 2010).

Seasonality that is mirrored by changes in environmental variables can intervene in snail's ecology and influence the larval development of a trematode inside its host snail. It may also affect cercarial shedding (the release of cercariae from the host snail in nature). However, the influence of environmental elements on cercarial shedding is trematode-specific (Abrous *et al.* 1999).

In the present study, the highest cercarial infection rates in snails were observed during autumn (38%), while it was lowest during winter. Infection rate of snails in different seasons are fluctuated. Hasanat *et al.* (2017) recorded seasonal data with high prevalence rate during autumn (19.6%) and low in spring (4.2%). Aboelhadid (2004) detected that, the prevalence of *Schistosoma mansoni* cercariae was high in spring (8.3 %) and Imani-Baran *et al.* (2011) found high infection rate in early summer (June- July) in Iran. Seasonal data of Tanveer & Khan (1989), Maqbool *et al.* (1998), Khan *et al.* (2008) showed significantly higher number of snails in summer. Almost all snails' genera had highest prevalence in summer season. Different results for seasonally infection rate might be due to low levels of water, slow-flowing in the stream during hot non-rainy season, also it could be due to high contamination by the faecal matter of livestock, birds and other animals. It is probably that snail abundance is low in the summer and miracidia enter most available snails. Thus, it can be anticipated that, both snail's propagation and their infection with trematodes are correlated with seasonal variations. Various ecological factors such as season and water temperature, pH and dissolved oxygen influence the emergence of cercariae from the snails and their release inside the water resources. snails can reach their maximum incidence during summer and autumn providing optimum temperature required for their breeding and reproduction (Farahnak *et al.* 2011).

Environmental conditions in different habitats, in different climatic zones may support the trematode life cycle differently. Trematodes show distinct and direct relationship with the temperature in their transmission process. Cercarial output is directly influenced by the temperature due to both stimulating effect of temperature increasing the emergence from the snail and the acceleration of cercarial production within the snail host. However, under high temperature conditions some cercariae encyst within snails. Even though, snail populations grant higher trematode diversity (Smyth 1962, Poulin 2006). The cercarial shedding is related to temperature, humidity and rainfall; temperature between 25-30 °C is favorable for shedding of cercariae (Alim 1997, Rahman *et al.* 1997). These conditions favor the growth and development of vector snails and hasten the development and shedding of cercariae from snails by rapid development of miracidium to sporocyst, redia and finally cercariae (Alim 1997).

Transmission of digenetic trematodes from the snail to the next host in the life cycle depends largely on the proportion of snails that release cercariae, as well as the number of cercariae released from each infected snail (Anderson & May 1991). These factors may be quantified, respectively, in terms of the prevalence and intensity of patent infection (Margolis *et al.* 1982, Bush *et al.* 1997). In field populations, both these factors tend to increase with increasing snail size. In the present study, infection prevalence of *Nerita* spp is positively related to their length. Among freshwater snails, for example, a positive correlation between snail size and the prevalence of infection was reported in 73% of the field studies reviewed by Sorensen & Minchella (2001). Size may also be positively correlated with intensity of infection (Smith, 1984). For both mammalian and avian schistosomes, the prevalence and intensity of infection tend to be highest among the largest snails (Sturrock 1973, Kulesa *et al.* 1982, Loker 1983, Woolhouse 1989, Niemann & Lewis 1990). Large snails are older, on average, than small snails, within a given population (Minchella *et al.* 1985). This age variation translates into differential exposure to and duration of infection among snails of differing sizes because larger-older snails may have been exposed to more miracidia or may have been infected for a longer period and thus have patent, highly productive infections. A complete coupling between age and size would require further experimental work. Trematode induced gigantism can disrupt the correlation between age and size of snails by accelerating the

growth of infected snails. Thus, infected snails would always be in the largest size classes. On the other hand, most authors who have reported such gigantism found that the growth acceleration is overcome on the time scale of these experiments. They concluded that gigantism does not underline the increases in prevalence and intensity with increasing size in this field system (McClelland & Bourns 1969, Loker 1979, Minchella *et al.* 1985, Joosse & van Elk, 1983).

#### **Conclusion And Recommendations:**

We have concluded that there is a positive correlation between infection percent and snail's length. The highest infection prevalence was recorded in autumn. Studying both the infection prevalence of larval trematodes as well as the most prevalent infected snail length in marine snails are of great importance. These trematodes can be transmitted to the surrounding fish, birds and other vertebrates in marine habitat. Such studies can assist in collecting data on the ecological relevance of disease distribution in the sympatric fish and the pattern of transmission of digenean trematodes by snails and finally, in prevention and control of the following fish and human diseases.

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