Aquaculture Research

Aquaculture Research, 2012, **43**, 1571–1576 doi: 10.1111/j.1365-2109.2011.02962.x

Survival and avoidance response of the freshwater gastropod *Melanoides tuberculatus* (Muller) to different concentrations of tobacco waste

Erick Ochieng Ogello[1], Reuben Omondi[1], Mary A. Opiyo[1], Harrison Charo-Karisa[2], Jonathan Munguti[2] & Christopher Mulanda Aura[3,4]

[1]Kegati Aquaculture Research Center, Kenya Marine and Fisheries Research Institute, Kisii, Kenya

[2]National Aquaculture Research Development & Training Center – Sagana, Kenya Marine and Fisheries Research Institute, Sagana, Kenya

[3]Mombasa Station, Kenya Marine and Fisheries Research Institute, Mombasa, Kenya

[4]Laboratory of Marine Bioresource & Environment Sensing, Hokkaido University, Hokkaido, Japan

Correspondence: E O Ogello, Kegati Aquaculture Research Center, Kenya Marine and Fisheries Research Institute, PO Box 3259 – 40200 Kisii, Kenya. E-mail: erioch2006@yahoo.com

Abstract

The Gastropod *Melanoides tuberculatus* plays a significant role in hampering fish larval production in earthen ponds. This study investigated use of tobacco waste to assess behavioural and survival responses of *M. tuberculatus* at different concentrations of tobacco waste solution of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2 g L^{-1}. Mean escape time varied significantly among concentrations ($P < 0.05$). Escape time decreased in 1-, 2- and 3-day-old solutions. Percentage survival decreased significantly with increasing concentrations of tobacco waste solution and exposure time ($P < 0.05$). Concentrations of 1.75 g L^{-1} and 2.0 g L^{-1} had high hazard ratios and low survival rates of gastropods and were the most effective in eradication of *M. tuberculatus*, hence recommended dose for preparing ponds for stocking. We conclude that tobacco waste solution can be used for control of *M. tuberculatus.*

Keywords: nicotine, *Melanoides tuberculatus*, escape time, survival, toxicity

Introduction

Production of fish larvae is often hampered by high mortality rates, some of which are attributed to infectious diseases caused by parasites (Bricknell & Roy 2005). Parasitic pathogens infecting cultured fish are well known to cause mortality and significant losses both in culture and capture fisheries (Woo 2006; Costello 2009). The two most abundant genera of gastropod snails in Lake Victoria region are *Melanoides* and *Bellamya* (Okedi 1971, 1990) where they play host to harmful nematodes and trematodes (Aleem 1988; Lockyer, Jones, Noble & Rollinson 2004). Freshwater snails play a key role in the life cycle of trematodes because they provide a niche for reproduction and means of transport, through which the parasite can reach its next host (Zbikowska 2009). According to Corbert (1961), *Melanoides* were very common and almost ubiquitous in Lake Victoria region. A survey in north-western Lake Victoria in 1972 revealed *Melanoides* as the dominant gastropod (Mothersill, Freitag & Barnes 1980). Sommerville (2008) discovered that fry of *Sarotherodon spilurus*, *Sarotherodon mossambicus* and *Sarotherodon galilaea* were infected with cercariae of *Haplorchis pumilio* from *M. tuberculata* at an East African fish farm and that *H. pumilio*, whose first intermediate host is *M. tuberculata*, was common among intensively reared tilapia. Umedevi and Madhavi (2006) found embedded metacercariae of *H. pumilio* in muscles of *Channa punctatus*, *Gambusia affinis*, and fingerlings of *Cyprinus carpio*. They concluded that heavy infections induced mortality in cultured fish.

Several authors have documented the use of tobacco waste in freshwater ponds to control

organisms including predators and pests (Jhingran 1975; Aleem 1988), molluscs (Spector 1956; FAO 1970; FAO 1997; Tangkoonboribun 2009). However, the lethal concentration and time at which 50% of the molluscs die (LC_{50}, LT_{50}) in literature differs significantly. For example, whereas Spector (1956) reports LT_{50} at 1 g L^{-1} after 9 days, Aleem (1988) found LT_{50} at 2 g L^{-1} after 71 h. This study aims at providing lethal concentration and time (LC_{50}, LT_{50}) of tobacco waste solution for application against *M. tuberculatus* in freshwater fish ponds.

Materials and methods

The study was conducted in a laboratory at Kenya Marine and Fisheries Research Institute, Kegati Aquaculture Research Station, Kisii, Kenya (00° 42″S; 034°47″E). The *M. tuberculatus* were scrapped off from sides and bottom of hapa nets in ponds by use of a plastic plate. *Melanoides tuberculatus* with a shell length of 25–30 mm were transferred to the laboratory in plastic basins containing pond water. They were washed thoroughly and left in a basin of pond water, with aeration at a temperature of 24.00 ± 2°C, for an hour. Twenty *M. tuberculatus* were selected at random for each experiment.

Tobacco is an agricultural product processed from the leaves of plants in the genus *Nicotiana*. It is most commonly used as a recreational drug. Finely ground powder of dry tobacco waste was used to make solutions of concentrations: 0.00 (control), 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 g L^{-1} using fresh pond water and poured into 250 mL beakers.

The tobacco waste dissolved readily and completely in all tested concentrations at an average water temperature of 23 ± 2°C. Each concentration was in triplicate. Twenty actively crawling *M. tuberculatus* were placed in each beaker containing 200 mL of tobacco solution. Similar treatment was performed in 1-day, 2-day and 3-day-old tobacco waste solutions. The behaviour of the *M. tuberculatus* was monitored along with the time taken to crawl out of the beaker for all the treatments for 24 h.

For survival studies, only freshly prepared solutions were used. Twenty snails were placed in sealed 250 mL conical flasks (three replicate flasks per treatment) and survival determined at 2-day intervals for 12 days. The concentrations tested were: 0.00 (control), 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 g L^{-1} at an average temperature of 24 ± 2°C.

Data were recorded and analyzed statistically for means, standard errors and significant differences at a value of $P < 0.05$ using ANOVA tests. The median lethal time and concentration were determined following Fry (1971). The survival analysis was performed using SAS 9.1 (SAS Institute, Cary, NC, USA). Cox regression model (PROC PHREG in SAS) was used to estimate survival. The survival time of each member in a population was assumed to follow its own hazard function h_i (t) expressed as: h_i (t) = h_o(t) exp $z_i\beta$; Where h_o(t) is an arbitrary and unspecified hazard function, z_i is the vector of measured explanatory variables for the i-th individual and p is the vector of unknown regression parameters associated with the explanatory variables. For the data presented herein β will contain two parameters, one relating to initial percentage survival and the other to tobacco waste concentration. The model assumes that for a given initial proportion, the hazards at different concentrations of tobacco waste are proportional to each other. The analysis included all gastropods till time of death in the beaker. The survival rates among beakers was compared using multiple comparisons with a Bonferroni-corrected alpha (significance considered at a value of $P < 0.008$) to minimize the possibility of Type I statistical error. The chi-square test was used in the determination of variations in variables at a value of $P < 0.05$.

Results

Behaviourally, the gastropods reacted strongly to the tobacco waste solutions in two ways: they would either withdraw into the shell and close their operculum, or attempt to climb out of the beaker. The mean escape time increased significantly ($P < 0.05$) with increasing concentrations but decreased significantly ($P < 0.05$) with the age of the solutions. Mean escape times are shown in Table 1. *Melanoides tuberculatus* did not move out of the beaker in the control experiment (0.0 g L^{-1}). Therefore, no time results were recorded for the control experiment. At 1.5 g L^{-1} and 1.75 g L^{-1} *M. tuberculatus* remained with their operculum closed for a longer time before attempting to climb out of the beaker. However, at 2.0 g L^{-1}, no *M. tuberculatus* was able to climb out of the beaker and the animals would release a

Table 1 Mean ± SE Gastropod Escape Time (min) in different concentrations of tobacco waste solution during the study period

	Mean ± SE gastropod escape time (min)				ANOVA	
Concentration (g L^{-1})	Freshly prepared	1 day old	2 days old	3 days old	F	*P*-value
0.25	22.0 ± 1.73	23.0 ± 0.58	16.67 ± 0.33	15.0 ± 0.58	26.6667	0.06
0.5	27.0 ± 0.58	24.67 ± 0.33	16.0 ± 0.0	16.0 ± 0.58	23.3333	0.07
0.75	37.33 ± 1.2	26.0 ± 0.0	19.67 ± 1.2	16.0 ± 0.0	25.31	0.06
1	59.0 ± 0.58	40.33 ± 0.33	28.0 ± 0.58	17.33 ± 0.88	43.3333	0.00
1.25	201.0 ± 1.0	67.33 ± 1.45	53.0 ± 1.53	26.0 ± 1.15	101.0000	0.00
1.5	249.67 ± 0.88	178.0 ± 1.53	138.0 ± 1.15	59.33 ± 0.88	133.3333	0.01
1.75	252.67 ± 1.45	241.33 ± 0.88	199.33 ± 1.20	162.67 ± 1.45	150.0000	0.00

Vertical comparisons are at $P < 0.05$.

mucous like substance and fall back at the bottom of the beaker.

For the freshly prepared tobacco solutions, *M. tuberculatus* attempted to climb out of the beaker much faster compared to 1-day, 2-day and 3-day-old solutions. The experiment, however, recorded much less escape time for all the gastropods in the 3 days old solutions (Table 1).

Percentage survival of the gastropods at different concentrations of tobacco waste solutions decreased significantly with increasing concentrations of tobacco ($P < 0.05$) and time of exposure. All the gastropods (100%) survived at 0.0 g L^{-1}. Survival was 90% after 48 h for 0.75 g L^{-1} but decreased to 15% after 240 h (Fig. 1). The experiment recorded LT_{50} of 219, 188, 168, 150, 110, and 66 h for concentrations of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 g L^{-1} respectively (Fig. 1). However, after 48 h 1.75 g L^{-1} and 2.0 g L^{-1} were the most effective toxic concentrations as they both recorded high mortality rates (Fig. 1).

The mean survival time was 136.50 ± 6.8 h (range 48–288 h). Gastropods with large and small body sizes experienced equal survival probabilities ($P > 0.05$). The parameter estimates and standard errors for the Cox proportional hazards model are given in Table 2. The presence of both initial proportions of snails and tobacco waste concentration were found to be insignificant (change in −2 log likelihood from model without covariates = 92.11 on 2 d.f., $P = 0.00$). No interaction between initial proportions and tobacco waste concentration or quadratic effects could be detected. The model for the i-th individual is h_i (t) = exp* (3.23 × initial proportion + 1.26 × tobacco waste concentration) h_o (t).

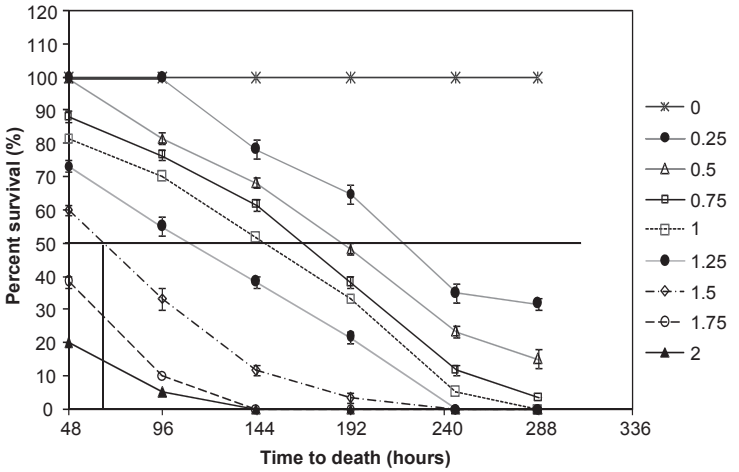

Figure 1 Time survival curves of *Melanoides tuberculatus* exposed to various concentrations of freshly prepared tobacco waste solutions during the study period.

Table 2 Estimated values of the coefficients of the covariates on fitting a proportional hazards model

Covariate	Estimate of β	SE of β	Wald chi-square	*P*-value
Proportions	3.23	0.63	12.04	0.0000
Concentration of tobacco waste	1.26	0.94	42.45	0.00052

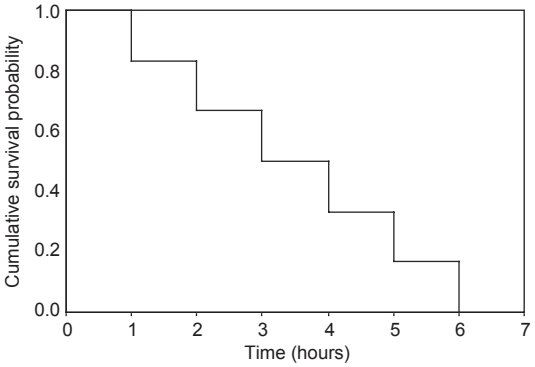

Figure 2 Overall cumulative survival probability of gastropods when exposed to different concentrations of tobacco solutions. For the *x*-axis: 0 = 48 h, 2 = 96 h, 3 = 144 h, 4 = 192 h, 5 = 246 h and 6 = 288 h.

Hazard of death increased with increasing concentration of tobacco waste and time of exposure. For every 48 h increment, the hazard ratio increases by a factor of exp (0.48 × 3.23) = 1.15 (95% confidence interval 3.13 to 5.58) which is equivalent to reducing by a factor of 1/1.205 = 0.83. Similarly, for every 0.25 g L^{-1} increase in tobacco waste concentration the hazard ratio increases by exp (0.25 × 1.26) = 0.32 (95% confident interval 0.30 to 0.35). To clarify these results, a plot of the survivor function estimates for the eight doses of tobacco waste concentration is shown in Fig. 2. Survival rates for treatment with 2 g L^{-1} and 1.75 g L^{-1} were the lowest and the survival rates in the other six treatments differed significantly (Bonferroni-corrected probability, all $P < 0.008$). On the other hand, cumulative survival probability decreased significantly ($P < 0.05$) with increase in time of exposure of the gastropods to the solution under different concentrations (Fig. 2). When compared with 2.0 g L^{-1}, the hazard ratios for treatments

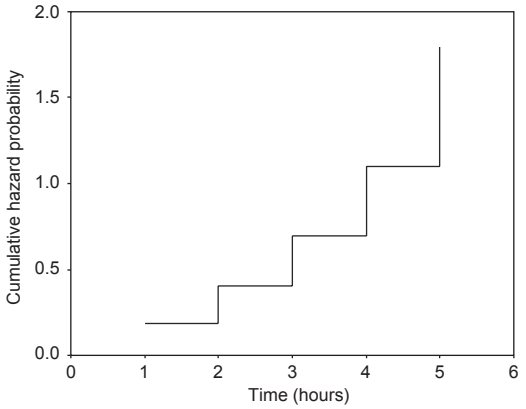

Figure 3 Overall cumulative hazard probability of gastropods when exposed to different concentrations of tobacco solutions. For the *x*-axis, 0 = 48 h, 2 = 96 h, 3 = 144 h, 4 = 192 h, 5 = 246 h and 6 = 288 h.

0.5 g L^{-1}, 0.75 g L^{-1}, 1.0 g L^{-1}, 1.25 g L^{-1} and 1.50 g L^{-1} were 0.14, 0.16, 0.19, 0.28 and 0.31 respectively. The hazard probabilities for the gastropods increased with increased time of exposure to the different concentrations of tobacco solution (Fig. 3).

Discussion

This study reported differences in behavioural changes and survival in *M. tuberculatus* exposed to different concentrations of tobacco waste solutions. The mean escape time increased with increasing concentrations of tobacco waste probably due to irritation from increased nicotine content (Aleem 1988). The mucous like substance released was perhaps a mechanism to neutralize effects of nicotine. The movements of *M. tuberculatus* were lower in freshly prepared solutions as compared to 1-day, 2-day and 3-day-old solutions. This indicated that the gastropods were less irritated by the older solution compared with freshly prepared solution as was found by Aleem (1988) who indicated that older solutions had lost an appreciable amount of nicotine toxicity.

The survival rates of *M. tuberculatus* decreased with increase in concentrations of tobacco waste and the time of exposure to the solutions. This could be due to the occurrence of lethal ratios only in concentrations of 1.5 g L^{-1}, 1.75 g L^{-1} and 2.0 g L^{-1}. This was similar to findings by Aleem (1988) who found that the higher the concentration of the toxicant, the shorter the resistance

time. The variables included in the analysis were survival proportions (%) and time (hours) to death. Survival data analysis was used to model the times to death for each of the eight groups of tobacco concentrations (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2 g L^{-1}). The two key functions of survival data analysis are the survival and hazard functions. The survival function is the probability that an individual survives longer than a certain time, whereas the hazard function is defined as the probability that an individual experiences death in the next small time interval given that the individual is alive at the beginning of this time interval. When compared with 2.0 g L^{-1}, the hazard ratios for treatments of 0.5 g L^{-1} to 1.50 g L^{-1} were between 14% and 31.0%, which can each be interpreted as the ratio of the estimated hazard for gastropods at that lowest and highest treatment to the estimated hazard for gastropods at concentration 2.0 g L^{-1} (Allison 1995). Simply put, the likelihood of mortality for gastropods at other concentrations is 14.0–31.0% of the likelihood of mortality for gastropods at concentration 2.0 g L^{-1}. In addition, the probability of death increased with increased time of exposure of the gastropods to increased concentration levels (Fig. 3) due to increased deteriorations of the gastropods because of the toxic influence of the solution. The survival findings were in agreement with studies of Konar (1970), who argued that increasing nicotine concentration reduces the respiratory activity of the snail and hence explains low survival. However, Fry (1971) observed that with all toxicants, a threshold is reached above which there is a drastic change in the survival of an animal. Below this threshold, the animal is in the tolerance zone, but above it the toxicant kills the animal (Aleem 1988). As percentage survival in 1.75 g L^{-1} and 2.0 g L^{-1} were 40% and 20%, respectively, they did not qualify to be considered for LT_{50}. However, they were the most effective toxic concentrations. This could imply that lethal concentrations (LC_{50}) could be found at or slightly above the threshold of 2.0 g L^{-1} and might cause mortality of all gastropods to occur. Tangkoonboribun (2009) applied 625 kg ha^{-1}, 1562.5 kg ha^{-1}, and 3125 kg ha^{-1} of tobacco waste in field trials and obtained 20%, 36% and 56% Golden apple snail (*Pomacea diffusa*) mortality at first day and 100% in 2 days. He reported that tobacco waste increased some water quality parameters such as electrical conductivity, biological oxygen demand and chemical oxygen demand in water and this caused early deaths to the Golden apple snail. On the other hand, pH and dissolved oxygen in water were decreased. Even though his findings concur with our study results, his experiment was performed in the field whereas ours was conducted in the laboratory. Furthermore, Tangkoonboribun (2009) used the golden apple snail whereas we used *M. tuberculatus*. This study agrees with earlier studies (Aleem 1988; Adamu 2009; Tangkoonboribun 2009) indicating that tobacco waste is a cheap and effective control measure to kill *M. tuberculatus* because of short exposure time.

In conclusion, the results revealed that tobacco waste at the tested concentrations had significant effect on behaviour and survival of *M. tuberculatus*. Concentrations of 1.75 g L^{-1} and 2.0 g L^{-1} had high hazard ratios and low survivals rates and were the most effective in eradication of *M. tuberculatus* and are the recommended dose for preparing ponds for stocking. Although this study tested survival of adult *M. tuberculatus* exposed to tobacco waste solutions it can be assumed that the toxin would as well be effective in eliminating *M. tuberculatus* larvae. Further studies are recommended to investigate the impacts of different concentrations of tobacco waste solutions on younger stages of *M. tuberculatus*, fish, plankton and water quality parameters.

Acknowledgments

The authors thank Kenya Marine and Fisheries Research Institute (KMFRI) for providing necessary funds and materials required during the study.

References

Adamu K.M. (2009) Sublethal effects of tobacco (*Nicotiana tobaccum*) leaf dust on enzymatic activities of heteroclarias (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). *Jordan Journal of Biological Sciences* **2**, 151–158.

Aleem S.O. (1988) An assessment of tobacco waste for control of the gastropod *Thympanotonus fuscata* (Linnaeus) in brackish water fish ponds. *Aquaculture* **73**, 19–25.

Allison P.D. (1995) *Survival Analysis Using SAS: A Practical Guide*. SAS Institute, Cary, NC, USA.

Bricknell I. & Roy A.D. (2005) The use of immunostimulants in fish larval aquaculture. *Fish and Shell Fish Immunology* **19**, 457–472.

Corbert P.S. (1961) The food of non-cichlid fishes in the Lake Victoria basin, with remarks on their evolution and adaptation to lacustrine conditions. *Proceedings of the Zoological Society of London* **136**, 1–101.

Costello M.J. (2009) The global economic cost of sea lice to the salmonid farming industry. *Journal of Fish Diseases* **32**, 115–118.

FAO (1970) *Reclamation of Ponds, Lakes and Streams with Fish Toxicants: A Review*. FAO Technical paper No. 100. Food and Agriculture Organization, Rome

FAO (1997) *Towards Safe and Effective Use of Chemicals in Coastal Aquaculture*. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Reports and Studies, GESAMP. No. 65. Food and Agriculture Organization, Rome, 40pp.

Fry F.E.J. (1971) The effects of environmental factors on the physiology of fish. In: *Fish Physiology*, Vol. **VI** (ed. by W.S. Hoar & D.J. Randall), pp 1–98. Academic Press, London.

Jhingran V.G. (1975) *Fish and Fisheries of India*. Hindustan Publishing Corporation, India, 371pp.

Konar S.K. (1970) Nicotine as a fish poison. *Progressive Fish Culturist* **32**, 103–104.

Lockyer A.E., Jones C.S., Noble L.R. & Rollinson D. (2004) Trematodes and snails: an intimate association. *Canadian Journal of Zoology* **82**, 251–269.

Mothersill J.S., Freitag R. & Barnes B. (1980) Benthic macroinvertebrates of northwestern Lake Victoria, East Africa: abundance, distribution, intraphylectic relationships between taxa and selected concentrations in the lake bottom sediments. *Hydrobiologia* **74**, 215–224.

Okedi J. (1971) The food and feeding habits of the small mormyrid fishes of Lake Victoria, East Africa. *African Journal of Tropical Hydrobiology and Fisheries* **1**, 1–12.

Okedi J. (1990) Observations on the benthos of Murchison Bay, Lake Victoria, East Africa. *African Journal of Ecology* **28**, 111–122.

Sommerville C. (2008) The pathology of *Haplorchis pulimo* (Loss 1896) infections in cultured tilapia. *Journal of Fish Diseases* **5**, 243–250.

Spector W.S. (1956) (ed.) Handbook of toxicology: acute toxicities of solids, liquids and gases to laboratory animals. In: *Handbook of Biological Data*, Vol.1 (3rd edn), 408 pp. W. S. Saunders Company, Philadelphia.

Tangkoonboribun R. (2009) Molluscicide from tobacco waste. *Journal of Agricultural Science* **1**, 76–78.

Umedevi K. & Madhavi R. (2006) The life cycle of *Haplorchis pumilio* (Trematoda: heterophyidae) from the Indian region. *Journal of Helminthology* **80**, 327–332.

Woo P.T.K. (2006) Fish Diseases and Disorders. In: *Protozoan and Metazoan Infections*, Vol. 1 (2nd edn) (ed. by P.T.K. Woo), pp. 46–114. CABI, Oxfordshire, UK.

Zbikowska E. (2009) One hundred years of research on the natural infection of freshwater snails by trematode larvae in Europe. *Parasitology research* **105**, 301–311.

