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Effect of soil surface salt on the density and distribution of the snail *Bithynia siamensis goniomphalos* in northeast Thailand

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Abstract. *Opisthorchis viverrini* infection is associated with human cholangiocarcinoma and northeast Thailand has the highest incidence of this disease in the world. *Bithynia siamensis goniomphalos* is the major freshwater snail intermediate host of *O. viverrini* in this area and an analysis based on geographical information systems was used to determine the effect of variation in soil surface salt on the density and distribution of this snail. A malacological survey was carried out in 56 water bodies in the Khorat basin, northeast Thailand at locations with various soil surface salt levels. Mollusk samples were collected from 10 ecologically representative water body sites with 10-20 sampling stations in each. The shoreline of clear, shallow water bodies was found to be the preferred *B. s. goniomphalos* habitat. The snails were exclusively found in water with salinity levels ranging between 0.05 and 22.11 parts per thousand (ppt), which supports the notion that *B. s. goniomphalos* prefers water with some saline content over pure, freshwater. The highest snail population densities were in rice fields, ponds, road-side ditches and canals within a water salinity range of 2.5-5.0 ppt. However, the presence of *B. s. goniomphalos* was negatively correlated with water salinity ($P \leq 0.05$), both with regard to density and distribution. The areas with the highest density of *B. s. goniomphalos* were those with less than 1% soil surface salt (potential index = 0.314), while the lowest densities were found in areas exceeding 50% soil surface salt (potential index = 0.015).

Keywords: *Bithynia siamensis goniomphalos, Opisthorchis viverrini,* water salinity, soil surface salt, geographical information system, Thailand.

Introduction

Bithynia (*Digoniostoma*) *siamensis goniomphalos* (Morelet, 1866) is the most important snail intermediate host of *Opisthorchis viverrini* (Poirer, 1886; Stiles and Hassal, 1896) in northeast Thailand. Human liver fluke infections by *O. viverrini* remain a major public health problem in the whole of Southeast Asia, including Thailand, Lao PDR, Cambodia and southern Vietnam (Haswell-Elkins et al., 1994; IARC, 1994; Vatanasapt et al., 2000; Le et al., 2006). The disease has been extensively studied in Thailand where an estimated 6 million people are currently infected (Jongsuksuntigul and Imsomboon, 2003). The parasite has a complex lifecycle which requires a snail for its asexual reproduction, which produces sporocysts, rediae and finally cercariae which infect a second intermediate host (cyprinoid fish species) where they encyst and develop into infective metacercariae after approximately one month (Wykoff et al., 1965). Human infection occurs through consumption of raw or improperly cooked fish containing live metacercariae. *B. s. goniomphalos*, the major first intermediate host in northeast Thailand, is a 5.6-8.5 mm wide and 10.2-14.9 mm long operculate snail with separate sexes. The shell is subovately conic with a wide umbilicus (Brandt, 1974; Chitramvong, 1991, 1992).

The *Bithynia* snail habitats in Thailand are typically red-yellow podzolic soils (Papasarathorn et al., 1990) and mixed sand-mud substrates (Chitramvong, 1992). The snail is generally found in shallow, temporary ponds, marshes, rice fields as

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well as in permanent water bodies. Preferable substrates are water plants or mud, rocks and sandy soils mixed with mud along the edge of water bodies at depths of not more than 30 cm (Papasarathorn et al., 1990).

Sixty-five million years ago, at the beginning of the Paleogene, northeast Thailand was submerged under an enclosed inland sea (Suwanich, 1986). The present landscape is dominated by an extensive outcrop of Mesozoic rock, the Maha Sarakham formation, which covers about one third of Khorat, plateau is divided into two depositional basins by the mountain range of Phu Phan: the Sakon Nakhon basin in the north and the Khorat basin in the south. Due to its geological history, the region contains underground rock salt (NaCl), which plays a major role in the distribution of saline soils scattered throughout the region. The level of soil surface salt is related to the dispersal of dissolved rock salt underground (Sinanuwong and Takaya, 1974), resulting in variable levels of salinity in surface water bodies and in underground water (Rimwanich and Suebsiri, 1984; DEDP, 1996). There are altogether about 2.8 million ha of saline soils in the Khorat basin, which makes up 17% of northeast Thailand (Arunin, 1992).

Salinity has a broad ecological impact on water-dwelling flora and fauna, including *B. s. goniomphalos*, whose density, distribution pattern, growth and population dynamics are strongly influenced (Donnelly et al., 1983; Rollinson et al., 2001; Pascual and Drake, 2008). It was decided to determine the effect of soil surface salt on the density and distribution of *B. s. goniomphalos* as this should provide insights into the distribution of liver fluke infection and human cholangiocarcinoma incidence in the region.

Materials and methods

Study area and sampling localities

The Khorat basin occupies a total area of 33,000 km^2 in 12 provinces in northeast Thailand: Khon Kaen, Maha Sarakham, Kalasin, Roi Et, Yasothon, Ubon Ratchathani, Nakhon Ratchasima, Chaiyaphum, Amnart Chareon, Sri Saket, Surin and Buri Rum. Representative sampling localities for snail surveys were selected according to a Thai soil survey map and based on six classes of soil surface salt (none, <1%, 1-10%, 10-50%, >50% and underground rock salt) (Table 1).

Snail sampling

B. s. goniomphalos snails were recovered in a variety of water bodies down to a depth of 3 m, mainly in clear, slow-running water including road-side ditches, ponds, canals and rice fields. They were collected from 56 water bodies in the wetland areas of the Khorat basin. The localities were randomly selected so that the number of localities in each class was proportional to the total surface area of the six classes of soil surface salt used. Sampling was carried out from October 2006 to August 2009 (Fig. 1). All localities investigated were geo-referenced using a global positioning system (GPS) instrument (Garmin model Nuvi-310, Taiwan). The number and species of snails at 10-20 sampling stations were recorded at each locality, with 10 stations at the edge of water bodies and 10 stations in deeper water. In the case of shallow water bodies or rice fields, only 10 stations were selected. The ecological characteristics and presence of aquatic plant species at each sampling station was recorded. The sampling methods used depended on

Table 1. Thai soil survey map of Khorat basin, northeast Thailand (Land Development Department, Ministry of Agriculture and Cooperatives) with the number of sampling localities in each soil surface salt class selected based on area of each class compared to the total surface area.

Soil surface salt class	Percentage of the total area	Sampling locality
Underground rock salt	21.8	12
No surface salt*	45.0	7
<1%	28.7	14
1-10%	4.1	13
10-50%	0.2	6
>50%	0.1	4

* Highland and mountainous areas

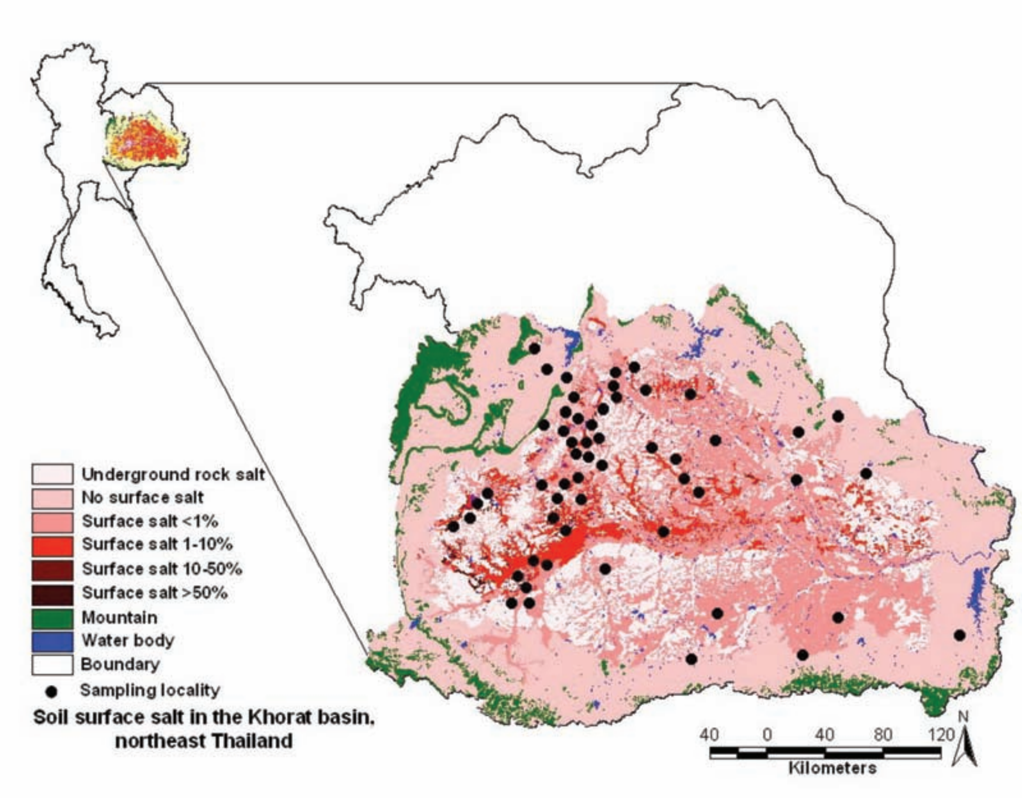

Fig. 1. Map of northeast Thailand showing 56 sampling localities.

the ecological characteristics of the stations. The snail sampling was performed manually by two persons for 5 min at stations with soil, sand, mud or rock strata, while a scoop net collection method was used (repeated five times) to collect snails at stations with aquatic vegetation cover. At stations with deep water, two samples were taken using an Ekman dredge (Tesana, 2002). Snail density at each collection locality was estimated by calculation of the total number of snails found at all sampling stations divided by the number of stations.

The physicochemical characteristics of water were determined at each water body and each collection date at a depth of 25 cm. The focus was on salinity, but the measurements included also pH, dissolved oxygen and conductivity as these values were simultaneously provided by the portable electrochemical analyzer used (model TPS 90-FL, TPS Pty Ltd, Australia). Turbidity was assessed by a HACH instrument (model 2100P, USA). The presence or absence of aquatic plants and their identity were also recorded.

Statistical analysis

The relationship of the density of *Bithynia* snails to the physicochemical factors of the water at the various sampling points was analysed. The SAS® (Version 9.2) GENMOD procedure was used to conduct a negative binomial regression of the data, with density of *B. s. goniomphalos* (rounded to highest integer value) as the dependent variable and salinity as the predictor variable.

Geographical information system (GIS) data analysis

The spatial analysis extension of ArcView 3.3 (ESRI, Bangkok, Thailand) was used for the application of the prediction model of the density and distribution of *B. s. goniomphalos*. The potential migratory ability of the snails was estimated by extracting values from buffer zones with a radius of 500 m centered on the 56 collection localities. The extent of the area of each of the six surface salt classes was calculated for each extraction area. For each soil surface salt class, a "potential index" was obtained by utilising the following formula, first described by Herbreteau et al. (2005):

$$\text{BSG-index} = \Sigma[(S_i/S_t)*(BSG_i/BSG_t)]$$

where S_i is the surface area of the given salt surface class inside the locality i, S_t is the total surface area per locality, BSG_i is the average number of *B. s. goniomphalos* collected inside the locality *i* and BSG_t is the total average number of *B. s. goniomphalos* collected. This potential index was used to create a prediction map of potential distribution and density of *B. s. goniomphalos* in the Khorat basin weighted by the density of *B. s. goniomphalos* found in each class of soil surface salt. This was then validated by a second random field survey of 30 additional localities randomly selected from the risk surface area.

Results

Water temperatures ranged from 21.9°C to 38.6°C with dissolved oxygen varying between 0.01 and 6.47 ppm. Conductivity varied between 0.12 and 63.4 mS/cm, salinity between 0.05 and 32.00 ppt and pH between 6.02 and 8.07. The turbidity varied from 3.2 to 420.3 NTU.

Live snails were present in water conditions covering the full range of temperatures, dissolved oxygen, pH and turbidity described above, but the ranges regarding conductivity (0.12-40.2 mS/cm) and salinity (0.05-22.11 ppt) were more limited. The highest number of *B. s. goniomphalos* snails was found in areas with a paucity of aquatic plants and only a few macrophytes (such as *Typha angustifolia, Ipomoea aquatic, Neptunia oleracea* and *Nymphaea lotus*). *B. s. goniomphalos* snails were observed crawling on these aquatic plants or on the surface of the soil, but no snails were found in areas with a high density of water plants or in rapidly running water. The highest density was found in a water salinity range of 2.51-5.00 ppt within four classes of soil surface salt, i.e. areas with underground rock salt, areas with less than 1% salt, with 1-10% salt and with 10-50% salt (Table 2).

B. s. goniomphalos was found to have a preference for rice fields, where snails were seen at high densities and generally more widely distributed than in other habitats. However, the highest densities were found in canals during the dry season when the snails clump together while moving with the flow of water draining from the rice fields into canals (although only three canals were sampled out of the total 56 localities). Although no *Bithynia* snails were found in sampling areas with many water plants, they thrived in water of transient, high turbidity after heavy rains, where the water normally clears within a few days.

The negative binomial regression calculations all produced significant results with deviance ratios near 1, indicating a relatively good fit. Indeed, goodness-of-fit statistics showed this model to be superior to the Poisson regression. The dispersion parameter estimate, being greater than zero, confirmed an over-dispersed density (response variable). The *B. s. goniomphalos* density was negatively correlated with salinity ($P \leq 0.05$) (Fig. 2), while no other physicochemical factor reached a statistically significant correlation. No snails were found in salty ponds, i.e. water bodies with the salinity exceeding 18 ppt (Table 3).

The GIS data analysis showed a very high potential density and distribution of BSG (potential index = 0.314) in areas where the soil surface salt was less than 1%, while high potential densities and distributions were found in areas with surface salt values of 1-10% (potential index = 0.226). Medium potential density and distribution were found in areas with underground rock salt (potential index = 0.200) and low potential densities and distributions were found in areas with surface salt measurements of 10-50% (potential index = 0.072) or in areas with no surface salt (potential index = 0.120). The lowest potential density and distribution was found in areas where the surface salt exceeded 50% (potential index = 0.015)

Table 2. Average density (average number of snails/station at each locality) of *B. s. goniomphalos* at 56 localities as compared to water salinity and the six soil surface salt classes in the Khorat basin, northeast Thailand.

Water salinity (ppt)	Sampling localities in each salt class						Total sampling locality	Locality with *B. s. goniomphalos*	Average density of *B. s. goniomphalos*
	Underground rock salt	No surface salt	<1%	1-10%	10-50%	>50%			
0.0-2.5	6	7	7	3	-	-	23	23	46.1
2.5-5.0	3	-	4	4	3	-	14	14	69.2
5.1-7.5	1	-	1	3	3	-	8	8	50.7
>7.5	2 *	-	2	3 **	-	4 ***	11	5	11.4
Total	12	7	14	13	6	4	56	50	-

* *B. s. goniomphalos* was found in 1 of the 2 localities; ** *B. s. goniomphalos* was found in 1 of the 3 localities; *** *B. s. goniomphalos* was found in 1 of the 4 localities

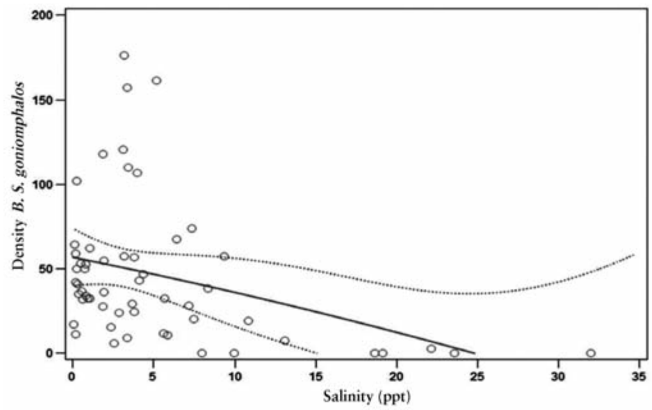

Fig. 2. Relationship between density of *B. s. goniomphalos* and salinity with 95% confidence limits about the mean (n = 56).

Table 3. Average density of *B. s. goniomphalos* according to habitat type and salinity level.

Habitat	No of localities	Average snail density (SD)*	Salinity (ppt)
Rice field	30	55.2 (42.4)	0.05-13.06
Road-side ditch	16	28.6 (25.9)	0.16-22.11
Pond	5	36.7 (33.8)	0.18-9.33
Canal	3	78.2 (39.6)	1.89-3.17
Salty pond	2	0.00 (0.00)	18.66-23.60

* Standard deviation

Table 4. Total area (in ha) of the six classes of soil surface salt within 0.5 km radius buffer extraction zones as compared to potential index, potential presence and total density of *B. s. goniomphalos* at the 56 localities in the Khorat basin.

	Underground rock salt	Amount of soil surface salt					Sum (ha)	Total density of *B. s. goniomphalos*
		No surface salt	<1%	1-10%	10-50%	>50%		
Total buffer zone area (ha)	2,959	1,843	5,749	4,670	1,312	564	17,097	2,560
Potential index	0.200	0.120	0.314	0.226	0.072	0.015	1	
Potential presence in prediction model	Medium	Low	Very high	High	Low	Very low		

(Table 4).

Discussion

GIS technology has been used to describe the ecology, distribution and abundance of medically important snail intermediate host-parasite systems, including *Schistosoma japonicum-Oncomelania hupensis* (Maszle et al., 1998; Zheng et al., 1998; Zhou et al., 1999; Yang et al., 2005), *S. japonicum-O. nosophora* (Nihei et al., 2006), *S. mansoni-Biomphalaria pfeifferi* (Kristensen et al., 2001), *S. haematobium-Bulinus truncatus* (Yousif et al., 1998), *O. viverrini-B. funiculata* (Ngern-klun et al., 2006) and *Fasciola-Lymnaea* spp. (Zukowski et al., 1991, 1993; Yilma and Malone, 1998). However, the research presented here is the first application of GIS technology for the investigation of density and distribution of *Bithynia* snails in northeast Thailand.

In the rainy season, very low numbers of snails were found in the canals due to rapidly moving water. Other authors have also noted that the density of snails in different localities in relation to the physical and biological characteristics do not only vary according to the habitat, but are also influenced by other factors such as level and velocity of the water (Seto et al., 2008) as well as substrate type (Haynes, 1988). Rather

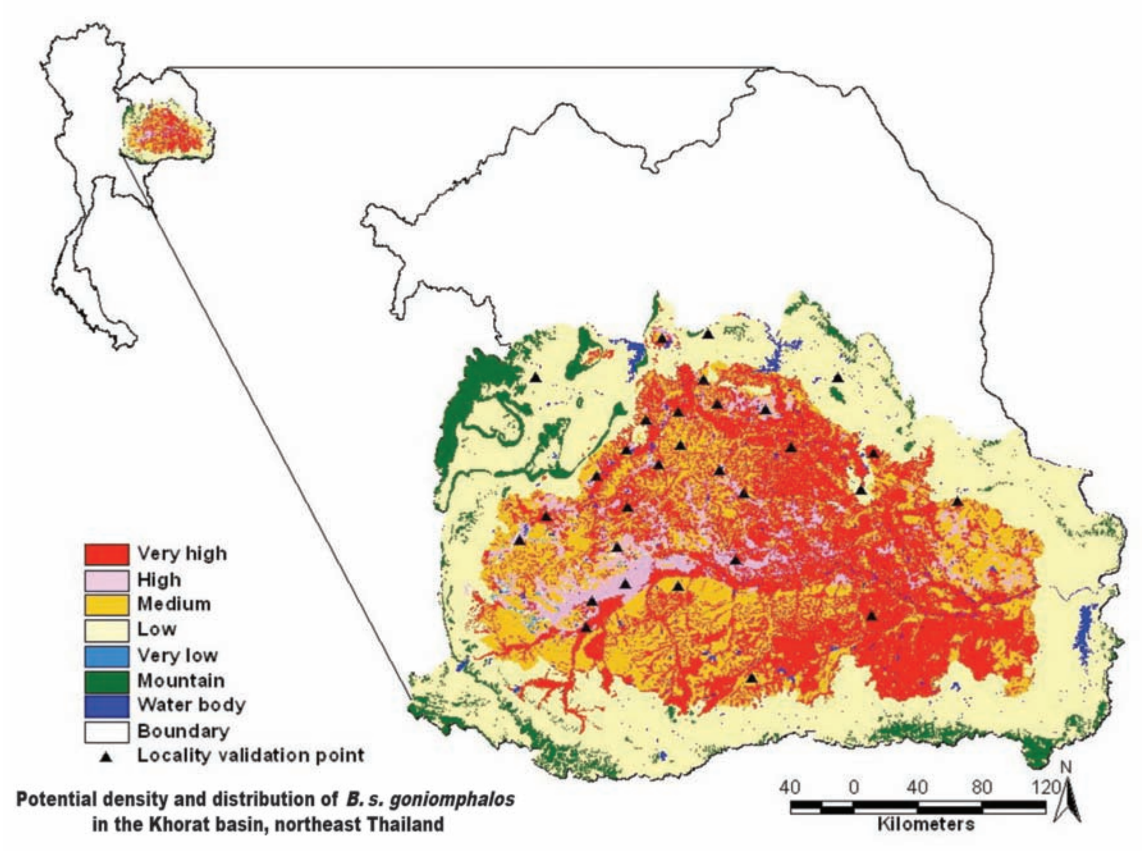

Fig. 3. Prediction model of *B. s. goniomphalos* density and distribution based on the prediction index in the Khorat basin, northeast Thailand. The triangles mark the location of 30 model validation localities.

than being exclusively distributed at depths of 30 cm or less, as reported by Papasarathorn et al. (1990), *B. s. goniomphalos* was found also in deeper water (at least down to 3 m). The difference might be explained by seasonal differences between sampling times. Admittedly, the density of snails in deep water was much lower than that at the edge of the water bodies investigated by us.

Salinity is a main factor influencing the distribution of aquatic organisms (Remane and Schlieper, 1971), including freshwater snail species, since it affects many of their physiological functions (Gainey and Greenberge, 1977; Jacobsen and Forbes, 1997). By contributing to increased metabolism it leads to physiological malfunction and, at high levels, mortality (Cheung and Lam, 1995). The soft tissues of snails have relatively high body water content, which leaves the tissues when exposed to high salinity due to osmotic pressure. This leads to dehydration and alteration of the composition of the body fluids that adversely affects normal metabolism in the snail. Low salinity tolerance (400 milli-osmole) is often reported as the cause of death in many freshwater snails such as apple snails (*Pomacea bridgesi*) and clams (*Lampsilis teres*) (Jordan and Deaton, 1999). The present field data shows that *B. s. goniomphalos* prefer aquatic habitats with <7.5 ppt salinity, while it can also obviously survive at salinity levels of up to 22.1 ppt, i.e. a higher concentration of salt than many other freshwater snail species can tolerate.

Salinity appears to be the most important environmental variable affecting the density and distribution of *Bithynia* intermediate host snails of *O. viverrini* in the Khorat basin. GIS and statistical analysis by negative binomial regression indicate that, although slight salt concentrations seem to be attractive to the snail, the presence is generally negatively correlated with water salinity ($P \leq 0.05$). Further work is planned to analyse greater sample sizes and type of water bodies and to examine the influence of other environmental factors such as slope, soil type, rainfall and satellite-derived temperatures and vegetation indexes as well as imagery of the land cover. Studies are currently underway to determine if salinity-related differences in the snail intermediate host distribution and abundance translate to differences in human infection prevalence of *O. viverrini* and the historical cholangiocarcinoma incidence records in northeast Thailand.

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