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ORIGINAL PAPER

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The effect of temperature on the development of *Angiostrongylus cantonensis* (Chen 1935) in *Pomacea canaliculata* (Lamarck 1822)

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Abstract Angiostrongyliasis cantonensis, clinically presented as eosinophilic meningitis, is a snail-borne parasitic disease. We studied the effects of different temperatures on the larval development of *Angiostrongylus cantonensis* in the freshwater snail *Pomacea canaliculata*. Six groups of snails were infected and each group was cultured under different temperature conditions. At predefined intervals, four snails from each group were dissected to examine the larval development. The development–time curve of each group was drawn according to the fraction of third-stage larvae present. The developmental time was defined as the time needed until 50% of the first-stage larvae developed into third-stage larvae. A linear regression model was established based on the time (D; in days) and the corresponding temperature (T; in degrees Celsius): $DT = 15.04 \times D + 262.53$. The threshold temperature for larval development was 15.04°C and the thermal constant was 262.53 degree-days. These parameters could be helpful in estimating the number of parasite generations in a year and the impact of climate change on the distribution of *A. cantonensis*.

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Introduction

Angiostrongylus cantonensis was described for the first time in Guangzhou (Canton) in 1933 (Chen 1933). The full life cycle of *A. cantonensis* was described by Mackerras and Sandars (1955). First-stage larvae are excreted in rats' feces and infect intermediate host snails where they develop into third-stage larvae which in turn infect rats and other mammals, including humans. However in humans, the larvae usually do not reach the lung artery and develop into mature worms but rather invade the cerebrospinal tissue. Nomura and Lin (1945) reported the first human infection with *A. cantonensis* in Taiwan. Until 1992, over 2,500 cases of *A. cantonensis*-related meningitis have been reported in approximately 30 countries (Kliks and Palumbo 1992). The number of eosinophilic meningitis cases rapidly increased in recent years, partly due to some outbreaks involving many persons in Jamaica (Slom et al. 2002), Taiwan (Tsai et al. 2001; Tsai et al. 2003; Tsai et al. 2004), and China (Xue et al. 2000; Lin et al. 2003). Today, *A. cantonensis* is regarded as the primary cause of human eosinophilic meningitis in many parts of the Indo-Pacific region (Prociv et al. 2000). In addition, the parasite is expanding its range (Kim et al. 2002; Raccurt et al. 2003), and new intermediate hosts have been identified (el-Shazly et al. 2002; Lin et al. 2005).

Similarly, the number of human infections in mainland China rapidly increased in recent years. The first human infection with *A. cantonensis* was reported in 1984 (He et al. 1984), and only two other cases were reported until 1996 (Lin et al. 2003). However, 84 cases of angiostrongyliasis were documented from 1994 to 2003, including two outbreaks involving 55 patients from Zhejiang and Fujian provinces (Chen et al. 2005). *Pomacea canaliculata* was involved in all these outbreaks.

The freshwater snail *P. canaliculata* is a suitable intermediate host for *A. cantonensis* (Nishimura et al. 1986). It easily becomes established in new environments (Estebenet and Martín 2002; Carlsson and Lacoursière 2005) and since its introduction in Guangdong province in 1981, it has considerably extended its range (State

Environmental Protection Administration of China and Chinese Academy of Sciences 2003). Today, it is found throughout the southeast and south of China and plays a significant role in the epidemiology of angiostrongyliasis (Xue et al. 2000; Lin et al. 2003).

The current climate change (global warming) will affect the distribution and survival rate of parasite vectors and intermediate hosts and also directly influence the reproduction and maturation rate of parasites carried by them (McCarthy et al. 2001). As early as 1990, the Intergovernmental Panel on Climate Change (IPCC) had warned that the climate change could affect the prevalence of vector-borne parasitic and viral diseases (Houghton et al. 1990). The ongoing colonization of new habitats by *P. canaliculata* and the expansion of the potential habitat caused by increasing mean temperatures could impact on the transmission of angiostrongyliasis in China. Therefore, we made an attempt to experimentally study the effect of different temperatures on the development of *A. cantonensis* larvae in *P. canaliculata*.

Material and methods

A. cantonensis larvae and life cycle

We obtained third-stage larvae of *A. cantonensis* from Lianjiang county, Fujian province. To establish the life cycle, we infected Sprague–Dawley (SD) rats by intragastric injection of 100 third-stage larvae. After 42 days, we collected fresh (12 h) feces from the infected animals and isolated the first-stage larvae by the Baermann technique (Barçante et al. 2003). These larvae were then used to infect *P. canaliculata* in which the larvae developed into infective third-stage larvae again.

Intermediate host

Eggs of *P. canaliculata* were obtained from Fuzhou in Fujian province and hatched to get a breeding stock of uninfected snails. All snails used in the experiment were offspring of this initial group.

Experimental procedures

The snails were fasted for 48 h before the start of the experiment. Groups of five snails were exposed to 40,000 first-stage larvae suspended in 200 ml water in dishes. The snails were exposed to the larvae for 12 h at room temperature, randomly allocated to one of six groups, and further bred in an incubator (EYELA KCL-2000A) at constant temperatures of 14, 16, 19, 22, 25, and 28°C, respectively. Every group consisted of 36 snails. All snails were kept at 80% relative humidity with a light period of 8 h per day. They were fed a mixed diet consisting of vegetables and fish food.

At every time point, four snails were randomly selected from each group and dissected. The ctenidia and lung tissues of the four snails were homogenized and digested in artificial gastric juice (0.2% pepsin in 0.7% hydrochloric acid) at 37°C for 2 h. The resulting sediment was transferred into Petri dishes and diluted. The *A. cantonensis* larvae were then categorized according to the morphology proposed by Hata and Kojima (1990) and enumerated to obtain stage-specific counts.

Data analysis

All analyses were done using the statistical software Statistical Package for the Social Sciences v. 11.0.

Development-time curve and determination of developmental time

The developmental rate was estimated based on the percentage of larvae having reached the third stage at specific time points, and a development–time curve was fitted for each temperature. The time needed for 50% of all first-stage larvae to develop into third-stage larvae was considered representative for the developmental time from the first to the third stage. The mathematical function of the fitted development–time curve for each temperature was used to calculate the expected developmental time.

Threshold temperature and thermal constant

A linear regression model was established according to the formula proposed by Ikemoto and Takai (2000). The expected developmental time is considered as the independent variable (D, in days), and the product of the expected developmental time and the corresponding temperature (T, in degrees Celsius) forms the dependent variable (DT). The thermal constant and the threshold temperature for development were calculated subsequently.

Results

Development–time curve

The experiment lasted 14 weeks. Altogether, 11 snails died before their scheduled dissection. The development of larvae from the first to the third stage could be observed in the groups raised at 19, 22, 25, and 28°C, respectively. No third-stage larvae were observed in snails kept at 14 and 16°C at the end of the experiment.

Figure 1 shows the development of the fraction of third-stage larvae over time and the fitted curves. The Verhulst logistic model (Eq. 1) was selected to fit a curve for each group. Its parameters at different temperatures as well as

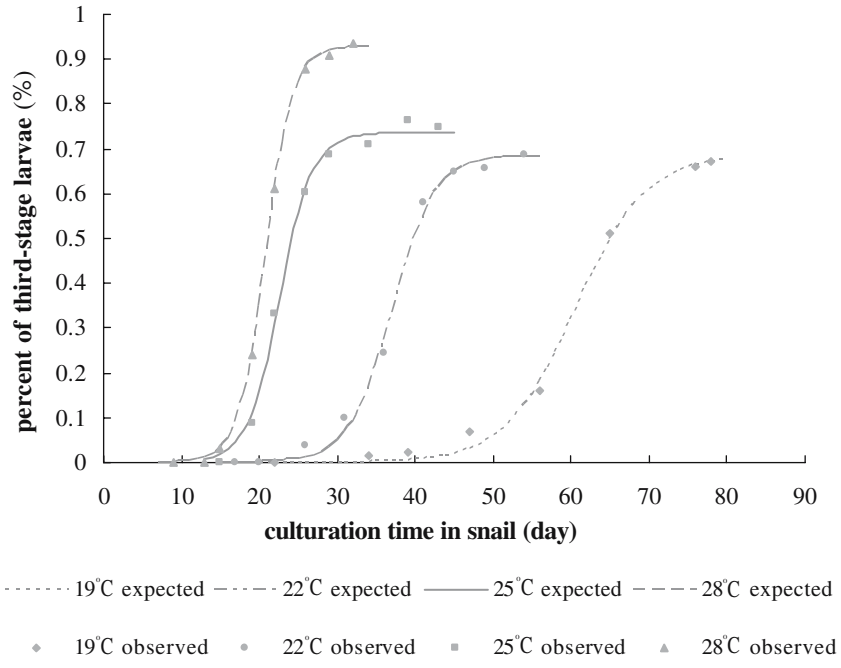

Fig. 1 Fraction of third-stage larvae of *A. cantonensis* in *P. canaliculata* at different temperatures and time points. Temporal evolution of the fraction of third-stage larvae in ctenidia and lung tissues at 19, 22, 25, and 28°Celsius

the value of R^2 (the coefficient of determination which demonstrates the goodness of fit) are presented in Table 1.

$$w(T) = \Omega \frac{\exp(\alpha + \beta * D)}{1 + \exp(\alpha + \beta * D)} \quad (1)$$

$w(T)$: percent of third-stage larvae at a given temperature
D: culture time in snails
Ω: upper limit of $w(T)$ as $D \rightarrow \infty$
α, β: parameters determining the logistic distribution.

Threshold temperature and thermal constant

We estimated the expected time needed by first stage larvae to develop into third-stage larvae at different temperatures by setting $w(T)$=0.5 and solving the equation. The expected developmental times are 65.06, 39.83, 24.21, and 21.13 days at 19, 22, 25, and 28°C, respectively.

Figure 2 shows the linear relationship between the expected developmental times and the required degree-days at different temperatures. The slope of the line represents the threshold temperature for larval development (15.04°C) and the intercept describes the thermal constant (262.53 degree-days).

Table 1 Parameters of the fitted logistic curves at different temperatures

Temperature (°C)	Ω	α	β	R^2
19	0.685	13.459	−0.222	0.997
22	0.684	13.477	−0.363	0.995
25	0.736	10.842	−0.479	0.995
28	0.927	11.871	−0.569	0.999

Ω Upper limit of $w(T)$ as $D \rightarrow \infty$; α, β parameters determining the logistic distribution; R^2 coefficient of determination which demonstrates the goodness of fit

Discussion

Ishii (1984) had studied the relationship between temperature and larval development of *A.cantonensis* in *Biomphalaria glabrata*. He reported a threshold temperature for larval development of 15.7°C and a thermal constant of 123 degree-days. We found a threshold temperature of 15°C and a thermal constant of 262.5 degree-days. According to the results of an experiment reported by

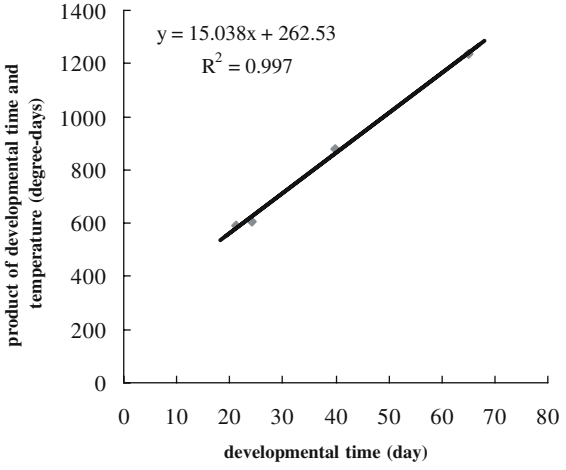

Fig. 2 Relationship between the developmental time needed by *A. cantonensis* larvae to reach third stage and the product of the developmental time and the corresponding temperature (degree-days). *Threshold temperature for larval development* 15.038°C, *thermal constant* 262.53 degree-days

Yousif and Lammler (1975), larvae of identical parasite strains of *A.cantonensis* do not need the same developmental time from the first to the third stage in different intermediate hosts. The same observation was made in studies with different other nematodes in gastropods, e.g., *Umingmakstrongylus pallikuukensis* and *Elaphostrongylus rangiferi*, where the threshold temperature and thermal constant may vary from one intermediate host to another (Halvorsen and Skorping 1982; Kutz et al. 2001). Therefore, the different intermediate host may be the main reason for the higher thermal constant observed in our experiment. The calculated thermal threshold for larval development of 15°C and the absence of any third-stage larvae after 14 weeks probably suggest a very slow larval development at temperatures close to the threshold temperature.

The comparison of our results with those from earlier studies is further complicated by different definitions and endpoints used. We considered the developmental stage of larvae recovered from ctenidia and lung tissues as representative for the average development of *A. cantonensis* larvae in *P. canaliculata*. However, other researchers determined the developmental time by assessing all larvae recovered from the intermediate host (Ishii 1984), while still others considered the first appearance of a specific larval stage (Yousif and Lammler 1975). Our choice to concentrate on larvae from ctenidia and lung tissues only was based on the following reasoning. First, there is no study that indicates a slower developmental rate of larvae in ctenidia and lung tissues as compared to larvae from other tissues. The developmental rate in the lung could even be higher due to the elevated oxygen concentration and the higher number of larvae in the lungs (He 1982; Xing et al. 1998). Second, these tissues are thinner than other organs and the nodules in which the larvae are embedded are easier to identify under the microscope, making them suitable for rapid identification of snail infection and determination of the larval developmental state. Therefore, selecting larvae from ctenidia and lung tissues leads to higher accuracy.

In our study, we determined the developmental time of *A. cantonensis* larvae by calculating the time until 50% of the first-stage larvae reached the third stage. A whole range of different growth curves have been developed to model general biological growth. They are all based on variations of the classical logistic growth equation (Tsoularis and Wallace 2002; Cramer 2004). We used the Verhulst logistic growth equation for curve fitting due to its simplicity and ease of use.

We determined the threshold temperature for larval development and the thermal constant by linear regression (Yang et al. 1995; Ikemoto and Takai 2000). Another widely used method to determine the threshold temperature and which is similar to the regression method is the *x*-intercept or development rate method (Arnold 1959). However, this method has serious limitations that preclude its use in this research (Ikemoto and Takai 2000). First, the method should be applied in an optimum temperature range which is usually difficult to detect. Second, it assumes a constant variance of the developmental rate at different temperatures, a prerequisite that cannot be taken for granted without prior investigation. Third, it neglects errors in temperature, leading to underestimations of the thermal constant. Ikemoto and Takai (2000) proposed an improved linear regression model which was used in the present study.

Other researchers had studied the effect of temperature on *P. canaliculata* and pointed out that although it was still confined to a few southeast provinces in China, the large areas of suitable habitats could allow the snail to expand its range northward (Hu and Hu 1991; Zhou et al. 2003). We are concerned whether *A. cantonensis* could be carried accidentally by the snail to new habitats in neighboring provinces with suitable climate where it could become established in new locations and create new foci of transmission. Therefore, we suggest surveying all places where *P. canaliculata* is already established for the presence of the parasite and monitoring adjacent suitable habitats for the appearance of the snail. This will allow to detect new foci of snails and potential transmission sites in an early stage and to set up preventive measures.

Other environmental and biological factors may impact on the reliability of the presented degree-day model under natural conditions, and further research is needed in such areas as the highest and lowest lethal temperature for snails and larvae, the actual number of degree-days needed for larval development under variable temperatures, and the possible expansion of the snail habitat due to global warming, e.g., through increased rainfall. Only the sum of all these results will allow to reliably determine the critical environmental conditions required by the parasite and its intermediate host to survive and to estimate the expansion of the potential endemic area due to the ongoing climate change.

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The execution of the experiments described in this article complied with all applicable laws of the People's Republic of China.

References

Arnold CY (1959) The development and significance of the base temperature in a linear heat unit system. Proc Am Soc Hortic Sci 74:430–445

Barçante JM, Barçante TA, Dias SR, Vieira LQ, Lima WS, Negrão-Corrêa D (2003) A method to obtain axenic *Angiostrongylus vasorum* first-stage larvae from dog feces. Parasitol Res 89:89–93

Carlsson NOL, Lacoursière JO (2005) Herbivory on aquatic vascular plants by the introduced golden apple snail (*Pomacea canaliculata*) in Lao PDR. Biol Invasions 7:233–241

Chen HT (1933) A preliminary report on a survey of animal parasites of Canton China rats. Lingnan Sci J 12:65–74

Chen XG, Li H, Lun ZR (2005) Angiostrongyliasis, mainland China. Emerg Infect Dis 11:1645–1647

Cramer JS (2004) The early origins of the logit model. Stud Hist Philos Biol Biomed Sci 35:613–626

el-Shazly AM, el-Hamshary EM, el-Shewy KM, Rifaat MM, el-Sharkawy IM (2002) Incidence of *Parastrongylus cantonensis* larvae in different fresh water snails in Dakahlia Governorate. J Egypt Soc Parasitol 32:579–588

Estebenet AL, Martín PR (2002) Pomacea canaliculata (Gastropoda: Ampullariidae): life-history traits and their plasticity. Biocell 26:83–89

Halvorsen O, Skorping A (1982) The influence of temperature on growth and development of the nematode *Elaphostrongylus rangiferi* in the gastropods *Arianta arbustorum* and *Euconulus fulvus*. Oikos 38:285–290

Hata H, Kojima S (1990) *Angiostrongylus cantonensis*: in vitro cultivation from the first-stage to infective third-stage larvae. Exp Parasitol 70:476–482

He JZ (1982) *Angiostrongylus* spp. related to human diseases. J Guangzhou Med Coll 10:73–81 (in Chinese)

He JZ, Zhu SH, Yang SQ, Yu BW, Chen YS, Hu GX, Wang XB, Wang L (1984) Discovery of the first human angiostrongyliasis case in mainland China by detecting worms in the cerebrospinal fluid. J Guangzhou Med Coll 12:1–4 (in Chinese)

Houghton JT, Jenkins GJ, Ephraums JJ (1990) Scientific assessment of climate change. Report of Working Group I of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, UK

Hu ZQ, Hu QJ (1991) The morphology and structure of *Ampullaria gigas*. Chin J Zool 26:4–6

Ikemoto T, Takai K (2000) A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. Environ Entomol 29:671–682

Ishii AI (1984) Effects of temperature on the larval development of *Angiostrongylus cantonensis* in the intermediate host, *Biomphalaria glabrata*. Z Parasitenkd 70:375–379

Kim DY, Stewart TB, Bauer RW, Mitchell M (2002) *Parastrongylus* (=*Angiostrongylus*) *cantonensis* now endemic in Louisiana wildlife. J Parasitol 88:1024–1026

Kliks MM, Palumbo NE (1992) Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis caused by *Angiostrongylus cantonensis*, the nematode lungworm of rats. Soc Sci Med 34:199–212

Kutz SJ, Hoberg EP, Polley L (2001) *Umingmakstrongylus pallikuukensis* (Nematoda: Protostrongylidae) in gastropods: larval morphology, morphometrics, and development rates. J Parasitol 87:527–535

Lin JX, Li YS, Zhu K, Chen BJ, Cheng YZ, Lin JC, Cao Y, Chen RZ (2003) Epidemiological study on group infection of *Angiostrongylus cantonensis* in Changle City. Chin J Parasitol Parasitic Dis 21:110–112 (in Chinese)

Lin JX, Zhou XN, Li LS, Zhang Y, Cheng YZ, Zhang RY, Li YY, Li CX, Chen BJ, Li YS, Dai MY (2005) *Bellamya aeruginosa* acts as the intermediate host for *Angiostrongylus cantonensis*. Chin J Zoonoses 21:24–26 (in Chinese)

Mackerras MJ, Sandars DF (1955) The life history of the rat lungworm, *Angiostrongylus cantonensis* (Chen) (Nematoda: Metastrongylidae). Aust J Zool 3:1–21

McCarthy JJ, Canziani OF, Leary NA, Dokken DJ, White KS (2001) Climate change 2001: impacts, adaptation & vulnerability. Contribution of Working Group II to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, UK

Nishimura K, Mogi M, Okazawa T, Sato Y, Toma H, Wakibe H (1986) *Angiostrongylus cantonensis* infection in *Ampullarius canaliculatus* (Lamarck) in Kyushu, Japan. Southeast Asian J Trop Med Public Health 17:595–600

Nomura S, Lin PH (1945) First case report of human infection with *Haemostrongylus ratti* Yokogawa. Taiwan No Ikai 3:589–592

Prociv P, Spratt DM, Carlisle MS (2000) Neuro-angiostrongyliasis: unresolved issues. Int J Parasitol 30:1295–1303

Raccurt CP, Blaise J, Durette-Desset MC (2003) Presence of *Angiostrongylus cantonensis* in Haiti. Trop Med Int Health 8:423–426

Slom TJ, Cortese MM, Gerber SI, Jones RC, Holtz TH, Lopez AS, Zambrano CH, Sufit RL, Sakolvaree Y, Chaicumpa W, Herwaldt BL, Johnson S (2002) An outbreak of eosinophilic meningitis caused by *Angiostrongylus cantonensis* in travelers returning from the Caribbean. N Engl J Med 346:668–675

State Environmental Protection Administration of China and Chinese Academy of Sciences (2003) Circular on publishing the list of the first batch of alien invasive species in China. (Issue 23)

Tsai TH, Liu YC, Wann SR, Lin WR, Lee SJ, Lin HH, Chen YS, Yen MY, Yen CM (2001) An outbreak of meningitis caused by *Angiostrongylus cantonensis* in Kaohsiung. J Microbiol Immunol Infect 34:50–56

Tsai HC, Liu YC, Kunin CM, Lai PH, Lee SS, Chen YS, Wann SR, Lin WR, Huang CK, Ger LP, Lin HH, Yen MY (2003) Eosinophilic meningitis caused by *Angiostrongylus cantonensis* associated with eating raw snails. Am J Trop Med Hyg 68:281–285

Tsai HC, Lee SS, Huang CK, Yen CM, Chen ER, Liu YC (2004) Outbreak of eosinophilic meningitis associated with drinking raw vegetable juice in southern Taiwan. Am J Trop Med Hyg 71:222–226

Tsoularis A, Wallace J (2002) Analysis of logistic growth models. Math Biosci 179:21–55

Xing WL, Pan CW, Liang SH, Lin HB, Huang HC, Yi WP, Lin BC, Wang KH, Lin DX, Zhou ZL (1998) Distribution of *Angiostrongylus cantonensis* larvae in *Pomacea canaliculata* in Wenzhou City. J Wenzhou Med Coll 28:297–298 (in Chinese)

Xue DY, Ruan YZ, Lin BC, Zheng RY, Fang JQ, Zhao QX, Li MF, Pan CW (2000) Epidemiological investigation on an outbreak of angiostrongyliasis cantonensis in Wenzhou. Chin J Parasitol Parasitic Dis 18:176–178

Yang S, Logan J, Coffey DL (1995) Mathematical formulae for calculating the base temperature for growing degree days. Agric Meteorol 74:61–74

Yousif F, Lammler G (1975) The suitability of several aquatic snails as intermediate hosts for *Angiostrongylus cantonensis*. Z Parasitenkd 47:203–210

Zhou WC, Wu YF, Yang JQ (2003) Viability of the *Ampullaria* snail in China. Fujian J Agric Sci 18:25–28