Temperature thresholds and statistical modelling of larval *Wuchereria bancrofti* (Filariidea: Onchocercidae) developmental rates

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(*Received 2 February 1996 ; revised 12 June and 10 August 1996 ; accepted 10 August 1996*)

SUMMARY

Developmental rates for *Wuchereria bancrofti* larvae maturing in the vector *Aedes polynesiensis* were estimated by analysing stage-frequency data consisting of counts of larval stages in mosquitoes reared at 20, 22·5, 25, 27·5, 30 and 32 °C. Base temperatures (i.e. low temperature thresholds) for *W. bancrofti* development were estimated by the x-intercept method and the model of Lactin *et al*. (1995). Resulting values were similar with both methods and were ≈ 12·5 °C for microfilariae (mf) in thorax, ≈ 17 °C for L1, 15·5 °C for L2 and 16·5 °C for L3. Upper thresholds estimated by the Lactin *et al*. model were 29·3 °C for mf, 29·1 °C for L1, 32·2 °C for L2 and 31·5 °C for L3. In addition, an original method was devised for computing the L3 upper threshold, by modelling L3 length shrinkage with temperature. It gave a value of 31·4 °C. At 32 °C, L2 and L3 stages exhibited altered morphology, larvae being shorter and wider than expected. The model of Lactin *et al*. described adequately the non-linear relationship between developmental rate and temperature, but a linear degree-day approach may be applied for temperatures below 27–28 °C.

Key words: *Wuchereria bancrofti*, *Aedes polynesiensis*, developmental rate, temperature thresholds, statistical modelling.

INTRODUCTION

Wuchereria bancrofti, Cobbold, 1877 is the causative agent of Bancroftian filariasis which still affects millions of people in the inter-tropical area. In French Polynesia, the parasite is transmitted by the mosquito *Aedes polynesiensis* Marks, 1951 which has long been recognized as the major vector (Rosen, 1955). Since the 1940s, filariasis control programmes have been implemented in the French Polynesian islands, emphasizing the mass administration of diethylcarbamazine (DEC) (Perolat *et al*. 1986). However, lymphatic filariasis is still of public health importance in this country and, at present, other prophylactic strategies, mainly based on ivermectin and/or DEC-salt treatments, are being tested. In addition, research is carried out on ecological aspects of transmission in order to produce a comprehensive mathematical model which could be used in further attempts to control the disease. Much work has been done on the elaboration of mathematical models of the transmission dynamics of filarial worms (Anderson & May, 1991; Dietz, 1982). *Onchocerca volvulus*, the causative agent of onchocerciasis or river blindness, has received particular attention, leading to the development of ONCHOSIM, a simulation model which is used in planning control strategies (Remme *et al*. 1986; Plaisier *et al*. 1990; Habbema *et al*. 1996). Some attempts have been made to develop models adapted to *W. bancrofti* transmission (Hairston & Jachowski, 1968; Rochet, 1990; Grenfell & Michael, 1992), and LYMPHASIM, a microsimulation model similar to ONCHOSIM is at present under development (Plaisier *et al*. 1995; Remme, Alley & Plaisier, 1995). All these models take into account the 'vector phase' with various degrees of complexity. The simplest assumption is likely to be the one included in the LYMPHASIM model, where a single relationship between the microfilariae (mf)-density in man and the number of resulting L3 larvae describes the 'vector phase'. Such a relationship is more likely to be a crude approximation to all the events involved in the transmission of parasites by the vector. Model predictions are likely to be improved by incorporating processes such as the uptake of mf (Pichon, 1974*a*; Bryan & Southgate, 1988; Basáñez *et al*. 1994), the limitation phenomenon in the vector and the resulting parasite's yield (Pichon, 1974*b*; Prod'hon, Pichon & Rivière, 1980; Southgate & Bryan, 1992; Basáñez *et al*. 1995), the distribution of parasites in their vectors (Pichon, Prod'hon & Rivière, 1980) and other epidemiologically relevant aspects (WHO, 1990). Among the latter, environmental factors, and in particular the role of temperature, are usually considered as important deter-

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minants of local variations in transmission dynamics. Temperature may be a key factor as it influences many of the interactions between the parasite and its vector. For example, developmental rates, mean duration of the parasite's cycle in the vector and vector life-expectancy depend on temperature. If vectors die before the completion of the parasite's cycle, transmission will not occur. This may explain seasonal and regional differences in parasite transmission intensity which have often been noticed in French Polynesia (Rivière, 1988). Not only survival, but other biological phenomena may be affected by temperature (e.g. fecundity, behaviour, transmissibility of parasites etc.) and they should be taken into account within a comprehensive model of parasite transmission.

This paper presents a first approach toward understanding the role of temperature on transmission of *W. bancrofti* by *A. polynesiensis*. It describes the influence of temperature on developmental rates of the larval stages of the parasite. The essential assumption implicit in all such studies is that development is related to ambient temperature and time (age). At a basic level, development of organisms depends on the rates of various biochemical reactions which are temperature dependent. Generally speaking, the relationship between developmental rate and temperature in poikilotherm organisms is non-linear. Typically, it increases as a shallow sigmoid (S-shaped) curve from zero development at a low temperature threshold (i.e. the 'base' temperature) to a maximum rate at an optimal temperature (i.e. developmental maximum threshold or upper threshold) and then decreases rapidly to zero at a lethal temperature (Higley, Pedigo & Ostlie, 1986). The base temperature and the upper threshold are two important values in degree-days computations, and their estimation is also the baseline for deriving phenology models. In addition, quantification of the relationship between developmental rate and temperature may contribute towards the formulation of more complex and general models of population dynamics (McDonald *et al*. 1989). Usually, developmental maximum and base temperatures are calculated as single values, but they may vary with the age of the organism (Wang, 1960). *W. bancrofti*, which develops through 3 stages in the mosquito, may have different threshold

tively affect the progression of the parasite cohort. In some filaria–vector combinations, mortality of infected vectors can occur early during the extrinsic incubation period, in particular if mf are ingested in high quantity. In the pair *W. bancrofti*/*A. polynesiensis*, excess mortality of infected mosquitoes due to the ingestion of mf has never been reported in laboratory experiments, as long as the number of ingested mf was less than 100 (Hairston & Jachowski, 1968; Prod'hon *et al*. 1980; Failloux *et al*. 1995). However, vector mortality was always dependent on the parasitic load. As such, in our experiments the only significant phenomenon was parasite-induced vector mortality, which was taken into account as follows.

The mean number of each larval stage found in daily batches of mosquitoes was first computed. This value was corrected for parasite-induced mortality using information from Pichon *et al*. (1980). They found that vector mortality due to parasitism did not occur suddenly once some parasite density threshold had been reached, but that survival decreased exponentially with parasite burden. The mean number (X) of parasites in a vector population was then:

$$X = k \times \theta \times Y/[Y \times (1-\theta)+k], \qquad (1\,a)$$

where k is the parameter of the negative binomial distribution of parasites among vectors, Y the mean number of parasites if no parasite-induced mortality occurs, and θ a pathogenicity coefficient ($0 < \theta < 1$) which tends to 1 if the parasite is not very pathogenic for its vector and to 0 if highly pathogenic. In the pair *W. bancrofti*/*A. polynesiensis*, $k = 2$ (for a zero-truncated negative binomial) and $\theta = 0{\cdot}97$ (Pichon *et al*. 1980).

Thus following equation (1 *a*) to take into account the parasite-induced vector mortality in obtaining stage-frequency data, the mean number of parasites was computed as

$$Y = -k \times X/[X \times (1-\theta)-k \times \theta]. \qquad (1\,b)$$

This value was then multiplied by 100 and rounded to the closest integer to enable computations in the stage-frequency analysis.

Various methods have been proposed for the analysis of stage-frequency data (Manly, 1989). Among them, the procedure of Pontius, Boyer & Deaton (1989) was chosen because of its non-parametric approach which, unlike other methods, has no particular assumption to be fulfilled for the estimation of mean times to stage appearance. The procedure supposes that animals under study can only be observed by sacrificing them or their habitat (i.e. in our experiments, the mosquito host). A cohort of these animals begins in stage 0 (i.e. ingestion of mf) at time t_0 and subsets of the cohort are sampled, by killing, periodically in time until the cohort is in a particular stage (i.e. the L3 stage in the present study). Moreover, this model provides reliable estimates under many survival distributions which is of interest because the survival function of *W. bancrofti* larvae is not known.

Estimation of base temperature

Various methods are available to estimate the minimum temperature threshold (Arnold, 1959; Kirk & Aliniazee, 1981; Yang, Logan & Coffey, 1995). The procedure most often used is the x-intercept method of Arnold (1959) which extrapolates to zero the quasi-linear portion of the relationship between developmental rate and temperature. A least-squares unweighted regression line was computed between rates $r(T)$ and the corresponding temperatures (T) in this linear range (i.e. $r(T) = a+b \times T$). The base temperature is the value of T for which $r(T) = 0$, i.e. $T_{base} = -a/b$. This approach was used to calculate the base temperature of the 4 developmental stages of *W. bancrofti*. These values were compared with the direct estimate given by the model of Lactin *et al*. (1995), which was used to describe the relationship between developmental rate and temperature quantitatively (see equation (2) below). Here, the base temperature is obtained by equating the function to zero and solving for temperature once model parameters are estimated.

Effects of high temperatures and estimation of upper threshold

While counting *W. bancrofti* larvae in *A. polynesiensis*, observations were made on the morphology of stages and larval growth in width and length (unpublished data). It appeared that L3 larvae shortened as temperature increased, and at 32 °C all L3 larvae were abnormal, banana-shaped and much wider and shorter than L3 larvae reared at lower temperatures or than those found in nature and which were supposed to have undergone 'normal' developmental conditions. These observations served as a baseline to calculate the upper threshold for L3 larvae. First, the shrinkage in larval length with temperature was analysed using the growth model of Schnute (1981). This model is of general use and may describe many of the growth patterns observed in nature (see the general formulation of the model in the legend of Fig. 2). Larval length (L) was entered in Schnute's function as $1/L$ because the model only describes growth *per se* as opposed to shrinkage. Because the model is non-linear in its parameters, the simplex method of Nedler & Mead (1965) applied to the least squares minimization function was used to compute parameter estimates. Then, the estimated parameters were used to calculate L3 lengths at temperatures T in steps of 0·2 °C. At each temperature step, length shrinkage (LS_T) was computed as $LS_T = L_{T-0{\cdot}2} - L_T$, where L_T

is length at temperature T. The fraction of shrinkage in relation to larval length may be expressed as $(L_{T-0\cdot2}-L_T)/(L_{T-0\cdot2})$. The graphical representation of the percentage shrinkage against T (see Fig. 2B) enabled identification of a limiting temperature above which shrinkage rapidly tends to 100% (i.e. length tends to 0). This temperature was interpreted as a maximum sustainable temperature (i.e. a thermal maximum for L3 larvae, slightly above their upper threshold). For the remaining larval stages of *W. bancrofti*, upper thresholds were estimated roughly at the point where the developmental rate levelled off or declined on Arrhenius plots (i.e. Log (rate) against $1/T$). As described for the base temperature, upper thresholds were also estimated directly from the equation of Lactin *et al.* (1995) (see equation (4) below).

Statistical modelling of developmental rates

The relationship between developmental rate and temperature may be modelled according to thermodynamical principles (Sharpe & De Michele, 1977) or using empirical functions (Logan, 1988). Numerous existing models describe the developmental rate as a function of temperature (Wagner *et al.* 1984; Higley *et al.* 1986). Most of them are more or less descriptive and sometimes biologically unrealistic, leading to poor estimates when predicting developmental times. The equation of Logan *et al.* (1976), modified by Lactin *et al.* (1995), avoids these weaknesses. It has biologically realistic parameters and, unlike others, can account simultaneously for the non-linear developmental response to temperature and the developmental thresholds.

The model gives the developmental rate $r(T)$ at temperature T as:

$$r(T) = \exp(\rho \times T) - \exp[\rho \times T_{max} - (T_{max} - T)/\Delta] + \lambda, \quad (2)$$

where T_{max} is a thermal maximum, i.e. the 'lethal' temperature at which life processes can no longer be maintained for prolonged periods of time, Δ is the temperature range over which 'thermal breakdown' becomes the overriding influence, ρ can be interpreted as a composite value for critical enzyme-catalysed biochemical reactions. The parameter λ is the value of the rate $r(T_{max})$ (i.e. when $T = T_{max}$) and allows the curve to intersect the abscissa at sub-optimal temperatures, permitting estimation of the base temperature by allowing $r(T) = 0$ to be solved for particular parameter values.

Non-linear regression methods (see previous section) were used to estimate model parameters for developmental stages of *W. bancrofti*.

The upper threshold T_{upper} is the value of T for which $r(T)$ is maximum (i.e. the first derivative, $r'(T)$, is equated to zero and solved for T)

$$r'(T) = dr(T)/dT = \rho \times \exp(\rho T) - (1/\Delta) \times \exp[\rho \times T_{max} - (T_{max} - T)/\Delta], \quad (3)$$

which evaluated at $T = 0$ gives the initial rate, equivalent to parameter b of the x-intercept method. The value of T for which $r'(T) = 0$ is then

$$T_{upper} = [\Delta \times \mathrm{Log_e}(\Delta \times \rho)/(1 - \Delta \times \rho)] + T_{max}, \quad (4)$$

which is equivalent to the expression given by Logan *et al.* (1976).

RESULTS

Temperature-controlled experiments

The high microfilaraemic level in blood meals ensured 100% mf infection of mosquitoes. About 100% still harboured mf in the thorax, and L3 larvae were recovered from 78–85% of mosquitoes in the last samples of each experiment. Experiments carried out at 34 °C were unsuccessful: all mosquitoes died within a few days and *W. bancrofti* larvae were not able to mature in such a short time. At all other lower temperatures the filarial extrinsic cycle was entirely completed. Stage-frequency data were consistent and L3 larvae were observed several days after the first day of appearance of this stage. Infective larvae were observed for 9 days at 20 °C (i.e. from day 26 to day 34), 14 days at 22·5 °C (from day 17 to day 30), 7 days at 25 °C (from day 12 to 18), 11 days at 27·5 °C (from day 12 to day 22), 4 days at 30 °C (from day 9 to day 12) and 3 days at 32 °C (from day 9 to day 11).

Estimation of base temperature

Table 1 gives mean times to appearance of *W. bancrofti* larval stages at each temperature as estimated by the method of Pontius *et al.* (1989). Developmental rates were computed as 1/[mean time] and plotted against temperature. This allowed selection of the number of points inside the quasi-linear range of the curve which were used in the regression analysis to estimate the base temperature by the x-intercept method. Linear regression results (i.e. a and b values in equations $r(T) = a + b \times T$) are given in Table 2, as well as determination coefficients (R^2) and base temperature estimations for each of the *W. bancrofti* larval stages. Estimations of base temperatures by the model of Lactin *et al.* (1995) are given in Table 3.

Effects of high temperatures and estimation of upper threshold

As temperature increased, L3 lengths decreased significantly. Mean length was 1·714 mm (standard error = 0·183) at 20 °C; 1·793 mm (0·154) at 22·5 °C; 1·608 mm (0·130) at 25 °C; 1·616 mm (0·088) at 27·5 °C; 1·411 mm (0·153) at 30 °C, and 0·816 mm (0·093) at 32 °C. In addition, mean width at 32 °C

Table 1. Estimates of mean time (in days) and standard deviation (in parentheses) to appearance of *Wuchereria bancrofti* developmental stages in laboratory reared *Aedes polynesiensis* after experimental infection upon a local carrier of mf

	Temperature (°C)					
Larval stage	20·0	22·5	25·0	27·5	30·0	32·0
Thoracic mf	2·65	1·87	1·57	1·50	1·58	1·50
	(1·60)	(0·56)	(0·38)	(0·29)	(0·40)	(0·28)
L1	6·00	3·65	2·28	2·50	1·99	2·50
	(2·25)	(0·46)	(0·50)	(0·29)	(0·58)	(0·28)
L2	16·18	9·50	7·69	6·50	6·50	5·70
	(0·58)	(0·29)	(0·49)	(0·29)	(0·29)	(0·54)
L3	28·27	17·79	12·11	11·68	9·53	9·89
	(2·04)	(1·10)	(0·80)	(1·19)	(0·80)	(0·62)

Table 2. Parameters of regressions (standard error in parentheses) and low temperature thresholds (i.e. base temperature) in °C for *Wuchereria bancrofti* larval stages estimated by the x-intercept method of Arnold (1959)

	x-intercept method				
Larval stage	No. of points	*a*	*b*	R^2	Base temperature
Thoracic mf	3	−0·652	0·052	0·99	12·7
		(0·127)	(0·005)		(1·1)
L1	3	−0·934	0·055	0·98	17·2
		(0·158)	(0·007)		(0·7)
L2	4	−0·173	0·012	0·97	15·4
		(0·050)	(0·002)		(1·2)
L3	3	−0·154	0·009	0·99	16·4
		(0·015)	(0·0006)		(0·4)

Table 3. Estimates of parameters for the Lactin *et al*. (1995) model (standard errors within parentheses) and computation of base temperatures and upper thresholds (°C) for *Wuchereria bancrofti* developmental stages

	Model parameters				Base	Upper
Larval stage	rho	T_{max}	Delta	Lambda	temperature	threshold
Thoracic mf	0·0515	43·38	10·53	−1·385	12·1	29·3
	(0·0017)	(0·31)	(0·46)	(0·127)		
L1	0·0410	41·96	7·63	−1·790	16·9	29·1
	(0·0053)	(0·31)	(3·02)	(0·305)		
L2	0·0265	57·13	17·34	−1·098	15·6	32·2
	(0·0024)	(0·42)	(2·78)	(0·234)		
L3	0·0126	56·72	10·98	−1·179	16·5	31·5
	(0·0005)	(0·51)	(1·28)	(0·189)		

was significantly larger (0·035 mm (0·005), $n = 21$ measurements) than that of infective larvae found in mosquitoes captured in the field (0·023 mm (0·002), $n = 181$, unpublished data) (Student's $t = 10·9$, D.F. $= 200$, $P < 0·0001$). As a result, L3 larvae had a strange appearance at 32 °C (Fig. 1A) with unusual overall shape and proportions (a short length and a large width), as compared to the usual shape of larvae developing in wild mosquitoes (Fig. 1B). These morphological alterations affected all L3 larvae only at 32 °C, but did not seem to influence their motility within the vector as some altered L3 larvae were recovered from mosquito heads, thoraces and abdomens. *W. bancrofti* L2 larvae appeared

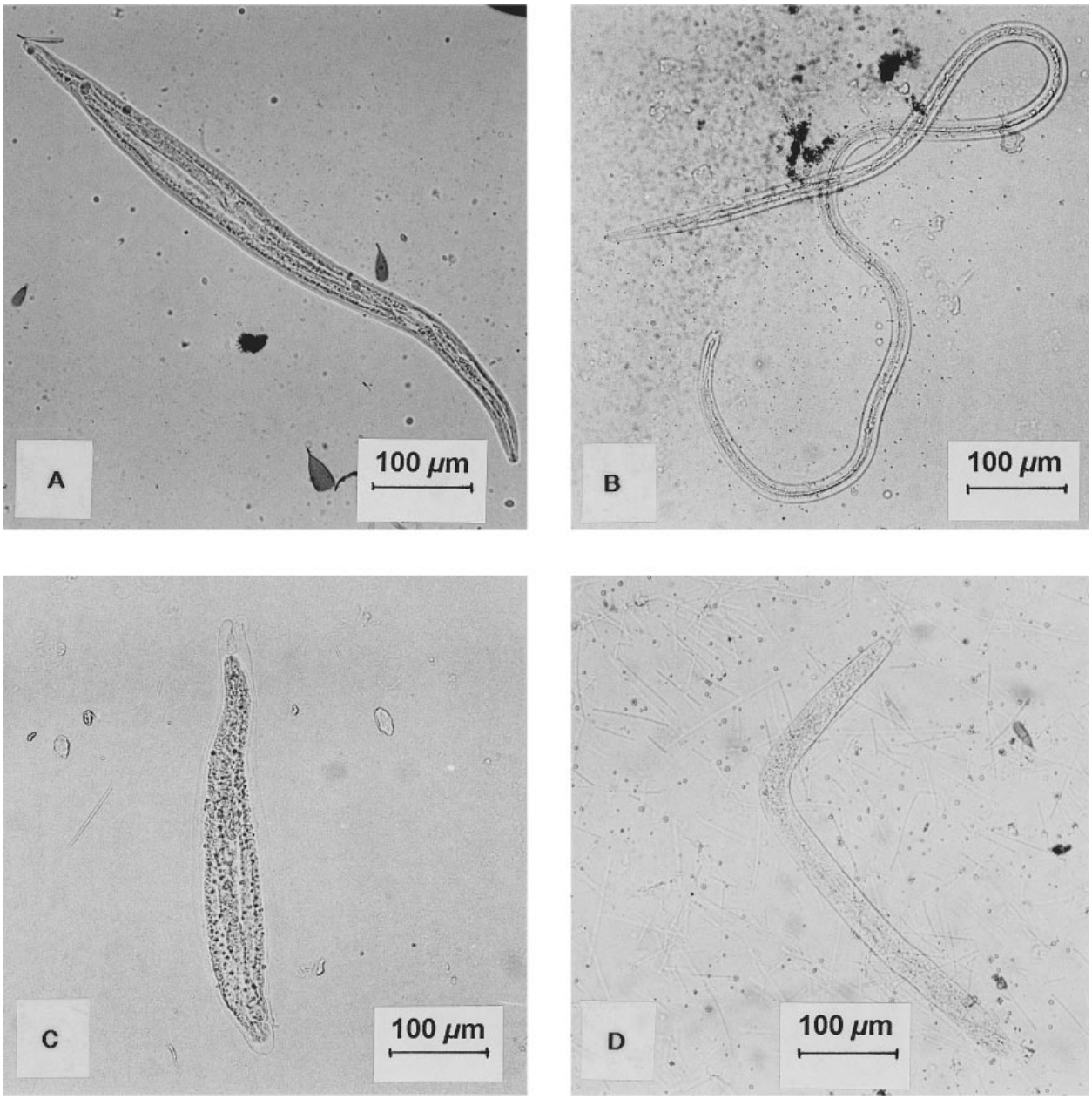

Fig. 1. Photomicrographs of altered L2 and L3 *Wuchereria bancrofti* larvae developing in *Aedes polynesiensis* reared at 32 °C compared to normal specimens developing in naturally infected mosquitoes. (A) Altered L3 larvae in head of vector. (B) Normal-shaped L3 larvae in head of vector. (C) Altered L2 larvae in thorax. (D) Normal L2 larvae in thorax of vector.

normally shaped at the beginning of their development at 32 °C, but at the end, more than 90% exhibited a length shrinkage and a width increase (Fig. 1C, D), indicating that the pre-infective stage was also sensitive to this temperature. This was not the case for *W. bancrofti* L1 stage whose shapes at 32 °C were apparently similar to those at lower temperatures.

The relationship between L3 length and temperature is given in Fig. 2A, along with Schnute's (1981) fitted curve. Two asymptotes were computed. The first one corresponded to an hypothetical temperature $T_{\infty} = 32{\cdot}2$ °C at which L3 larvae would have null length. The second one indicated an hypothetical mean length at low temperature of $L_0 = 1{\cdot}778$ mm. Using the equation of the fitted curve, percentiles of L3 length shrinkage were computed and plotted against temperature (Fig. 2B). For a 10% shrinkage, the plot indicated a limiting temperature of 31·6 °C while a 5 and 15% shrinkage gave $T = 31{\cdot}3$ and 31·7 °C respectively. The shape of the curve suggested that 10% shrinkage may be a limiting value and may correspond approximately to the upper threshold for L3 larvae.

Approximate values of upper thresholds estimated on Arrhenius plots were 29·0 °C for mf and 30·0 °C for L1, L2 and L3. Estimates given by the model of Lactin *et al.* (1995) are presented in Table 3.

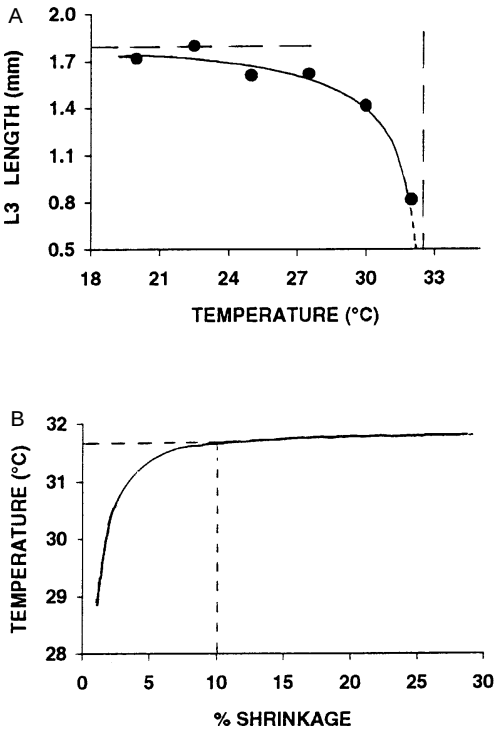

Fig. 2. Estimation of an upper threshold for L3 larvae using growth in length dynamics. (A) Relationship between length and temperature, and the fitted curve (Schnute's model) with the 2 asymptotes at $T_\infty = 32{\cdot}2$ °C and $L_0 = 1{\cdot}778$ mm. (B) Percentage length shrinkage with temperature and estimation of temperature threshold at 10 %. In its general formulation (i.e. $a \neq 0$ and $b \neq 0$), Schnute's (1981) model is:

$$Y(t) = [y_1{}^b + (y_2{}^b - y_1{}^b)(1 - \exp(-a(t-t_1))/(1 - \exp(-a(t_2-t_1)))]^{(1/b)}$$

where $Y(t)$ is the size at 'age' t, y_1 and y_2 represent the sizes at ages t_1 and t_2 respectively (t_1 and t_2 are chosen by the biologist, typically as young and old 'ages'), a is the constant relative rate of relative growth rate and b is the incremental relative rate of relative growth rate.

Statistical modelling of developmental rates

Results of the non-linear estimation of parameters (and their standard errors) in the Lactin *et al.* (1995) model are given in Table 3. Plots of the data and fitted curves for each *W. bancrofti* larval stage are presented in Fig. 3. The percentage of variance explained by the model was 92·2 % for mf in thorax, 90·3 % for L1 larvae, 97·4 % for L2 larvae and 97·5 % for L3 larvae indicating, along with small standard error values, a good fit of the model to the data. Estimates of λ were < 0 for each stage, indicating that base temperatures can be estimated. They appear in Fig. 3 at the intersection of the fitted curves with the x-axis and values are given in Table 3. These base temperatures are in agreement with those computed using the x-intercept method of Arnold (1959) (Table 2). Values for upper thresholds computed according to equation (4) are given in Table 3. The upper threshold of 31·5 °C for L3 larvae is similar to the value of 31·6 °C estimated by analysing alteration of growth in length with temperature (Fig. 2).

DISCUSSION

Estimation of developmental rates

In Tahiti, the mean annual temperature is 25·5 °C. The mean maximum temperature of the hottest month (i.e. March) calculated over the period 1957–88 is 29·5 °C while the mean minimum temperature of the coldest month (i.e. August) is 21·5

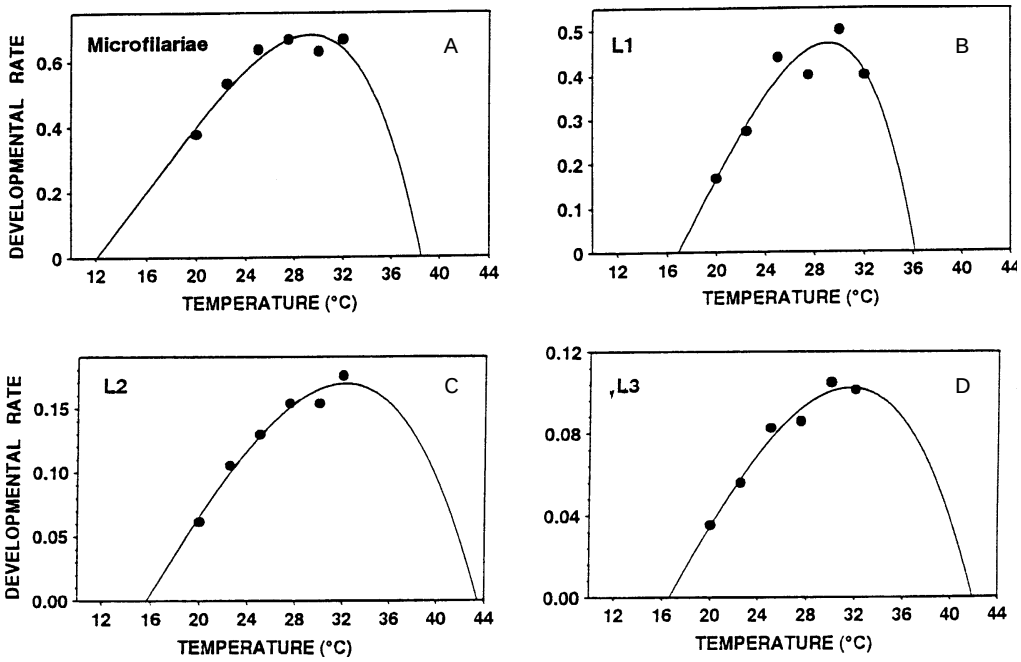

Fig. 3. Temperature-dependent developmental rates (1/days) of *Wuchereria bancrofti* stages in vector. (A) Thoracic microfilariae. (B) Stage 1. (C) Stage 2. (D) Stage 3. Fitted curves follow the Lactin *et al.* (1995) model (equation (2)).

the proportion of parasites developing from one stage to another will remain the same and time to appearance of stages will not be modified. Because no dramatic event occurred during our various experiments (in environment-controlled rearing chambers), it is likely that natural vector mortality may be assumed to be constant.

High microfilarial intakes can be detrimental to the survival of vectors (Ellrott, 1987; Bain & Petit, 1989). This may not always be true for the pair *W. bancrofti*/*A. polynesiensis* where such a phenomenon has been observed only for densities $\geqslant 100$ ingested mf/mosquito (Prod'hon *et al.* 1980). Hairston & Jachowski (1968) also reported that unless high numbers of mf are ingested (i.e. > 100), the proportion of *A. polynesiensis* surviving to development of L3 larvae will not be affected. In the present study, the mean number of ingested mf per mosquito was not different among the various experiments ($F = 1{\cdot}52$; D.F. $= 4, 79$; $P = 0{\cdot}20$), with an overa

The number of mosquitoes killed and dissected daily resulted from a compromise between ethical and rearing constraints on the one hand (i.e. limited numbers of mosquitoes taking a blood-meal on the volunteer), and the aggregated distribution of parasites per mosquito on the other hand. This aggregated distribution suggested that a large number of mosquitoes should be dissected in order to obtain a small standard error of the mean number of parasites per mosquito. The number of parasites among vectors followed a negative binomial distribution, which, for the pair *W. bancrofti*/*A. polynesiensis*, had a k coefficient of 2, whatever the larval stage once mf have reached the thorax (Pichon *et al*. 1980). If D is the relative error in terms of percentage confidence limits of the mean (X), then $D^2 = (t^2/n) \times (1/X + 1/k)$, where t is found in the Student's t-distribution and n is the sample size. In the present study, with a general mean of ≈ 7 parasites per mosquito, as estimated with samples of ≈ 15 mosquitoes, the precision of the mean was $D \approx 0{\cdot}44$ ($D \approx 0{\cdot}43$ with 20 mosquitoes) (i.e. the estimate of the population mean was within $\pm 44\,\%$ [43 %] of the true value), which is tolerable in ecological quantitative studies (Elliott, 1971).

Estimation of base temperature

The estimation of a base temperature (i.e. a developmental lower temperature threshold) is an important step for deriving a degree-days or a phenology model. In a degree-days approach, computations are not straightforward since the starting point for summation of degrees is often problematic. A common approach is to begin summations once temperatures exceed the base temperature, but development may not occur until later (Arnold, 1959). Other approaches, based on phenological events, may be used (Higley *et al*. 1986; Tauber, Tauber & Masaki, 1986). However, for *W. bancrofti* larvae, the use of the base temperature as a starting point may be employed because minimum temperatures recorded in French Polynesia (≈ 19 °C), are close to the lowest temperature studied (i.e. 20 °C), when parasite development hardly occurred. Values estimated by the x-intercept method and by applying the Lactin *et al*. (1995) function were similar for each *W. bancrofti* stage. As such, the curvilinear relationship of Lactin *et al*. and the regression method may be equivalent descriptive tools in a range of temperatures below the upper threshold. If a linear approximation can be used to describe developmental rates of *W. bancrofti*, a degree-day approach may then be applied in the linear portion of the development curve. The lowest base temperature was computed for mf in thorax, the highest for L1 larvae, with a significant difference of ≈ 5 °C as their 95 % confidence intervals did not overlap. However, the estimated base temperature for mf may be biased because the transition from mf in the abdomen to mf in the thorax is fast, even at low temperatures, and observations were made at 1-day intervals which are too large to compute an accurate mf base value. Base temperatures for L1, L2 and L3 were close to each other and ranged from $\approx 15{\cdot}5$ to ≈ 17 °C when estimated by the x-intercept method or by the Lactin *et al*. equation.

Effects of high temperatures and estimation of upper threshold

Estimating a developmental maximum is difficult because variability in developmental rates is usually greater at higher temperatures (Higley *et al*. 1986), and also because mortality is high. The mortality of cage-kept *A. polynesiensis* increased with temperature and at 34 °C, all mosquitoes died within a few days of the infected feed. *W. bancrofti* larvae may probably withstand such temperatures but gross morphological changes usually appear above 32 °C (Basu & Rao, 1939; Nakamura, 1964; Rozeboom, Bhattacharya & Gilotra, 1968; Bruhnes, 1969*a*, *b*; El Dine & Habib, 1969). Such altered parasites may lose their ability to be transmitted by the vector (Bruhnes, 1975), an observation consistent with ours. Despite the reported motility of altered L3 larvae in *A. polynesiensis*, one would doubt their infectivity. High vector mortality rates at high temperatures limit the recording of points above the maximum threshold, and increase the variability of computed upper threshold values. As a result, maximum threshold values computed for *W. bancrofti* must be regarded as approximate estimates only. Because the usual techniques for calculating developmental maxima are not precise, these values are often not determined. However, upper temperature threshold estimates for *W. bancrofti* seem relevant and biologically sensible. Values for mf in thorax and L1 larvae are close to 29 °C whilst those for L2 and L3 larvae are higher (i.e. $\approx 31{\cdot}5$–32 °C). The latter are in agreement with the maximum 'morphological' value computed from L3 length shrinkage, which is more likely to represent a long-term unsustainable temperature. Without a developmental maximum, no upper bound is placed on daily temperatures used to calculate degree-days and a bias may be introduced in computations of development predictions (Higley *et al*. 1986). However, many poikilotherm organisms, and in particular insects, may indirectly regulate their internal temperature by using behavioural and physiological mechanisms (May, 1979). *A. polynesiensis* activity is known to be bimodal, with peaks early in the morning and late in the afternoon, avoiding the hottest hours of the day (Jachowski, 1954). When temperatures are lower, under tree canopy or during cloudy days for example, this mosquito may be active all day long, the peaks being less marked (Lardeux *et al*. 1992). *A. poly-*

nesiensis is able to seek thermally favoured microhabitats, usually cooler than the ambient air temperature, as suggested by higher mosquito capture rates in scrubland than in open fields (Lardeux, 1987). It is likely that *A. polynesiensis* will avoid long exposures to high temperatures such as those recorded in full sunlight. Because daily maximum air temperatures are sometimes above the upper thresholds, one must be aware of bias introduced when calculating degree-days for use in phenological models if thresholds are not taken into account. However, the error introduced may be not too great as the daily maximum temperatures are usually below the developmental threshold of *W. bancrofti* in *A. polynesiensis*, and the mean maximum temperature of the hottest month in Tahiti is below the upper thresholds estimated for *W. bancrofti* larvae. Moreover, air temperature oscillates during a 24 h cycle according to sine-wave functions (Allen, 1976; Parton & Logan, 1981), limiting the duration of high temperatures. In French Polynesia, temperatures above upper thresholds probably do not last long enough to produce abnormal *W. bancrofti* development. Even during the hottest days, it is likely that *A. polynesiensis* will behave so as to remain under the developmental maxima for *W. bancrofti* and permit the entire parasite cycle to be completed without alteration.

Modelling developmental rates

The major advantage of the Lactin *et al.* (1995) model over others is that it can account simultaneously for the developmental threshold and the non-linear response to temperature. The models of Harcourt & Yee (1982) and Hilbert & Logan (1983) may behave similarly but are biologically unrealistic since rates are unbounded as temperatures fall below the base temperature. Due to the lack of data at high temperatures, fitted curves were more skewed than expected in the descending phase. L2 and L3 developmental rates may be less sensitive to high temperature. This is suggested by higher T_{max} values, above the 42 °C limit which may correspond to irreversible protein denaturation, indicating that the decrease of the fitted curve was slower. The curvilinear model of Lactin *et al.* describes developmental rates of *W. bancrofti* larvae adequately. It is a good predictor in the temperature range where the curve is non-linear, but below the upper thresholds, in the quasi-linear portion of the curves, a simpler degree-day approach may be applied. Plots in Fig. 3 indicate that the portion in which a linear degree-day approach may be used ranges from base temperature to 27–28 °C approximately.

In conclusion, the developmental rates of *W. bancrofti* larvae are well described under laboratory conditions. The equation of Lactin *et al.* (1995) fits data gathered under controlled temperature environment adequately. In field conditions, the temperature is not constant but is a function of time $f(t)$ (usually a sine-wave function), and sometimes, the degree-day concept with its linear temperature–rate relationship may not be apt under variable temperature conditons (Stinner, Gutierrez & Butler, 1974). For complete development of each larval stage, by definition, $r(T) \times \Gamma_T = 1$, where $\Gamma_T =$ developmental time at temperature T. If T varies as $f(t)$, d (the developmental time) may be computed by solving $\sum_{t=0}^{d} r(f(t)) \times \Delta t = 1$, where Δt is a time interval where $r(f(t))$ is supposed to remain approximately constant. The function of Lactin *et al.* should then be validated for *W. bancrofti* in varying temperature conditions and the results compared to those obtained here ($\approx$ 115 degree-days for larval *W. bancrofti* to reach the infective stage). Estimates of *W. bancrofti* development thus obtained should then be compared with vector survival under field conditions to assess the probability of successful larval development of *W. bancrofti* conditional to the vector having survived to infectiousness. This will be examined in subsequent publications.

We thank the mf carrier for providing the infective bloodmeals and M. Chebret for his technical assistance in producing the mosquitoes for experimentation. Thanks to Y. H. Le Mouchon for providing encouragement throughout this study. We also thank the anonymous reviewers whose comments greatly improved the earlier version of the manuscript, especially in its statistical aspect. This study received financial support from the Ministère des Départements et Territoires d'Outre Mer (CORDET no. 94TS14-16).

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