

A discrete model for estimating the development time from egg to infecting larva of *Ostertagia ostertagi* parametrized using a fuzzy rule-based system

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ABSTRACT

Ostertagia ostertagi is a nematode, predominantly affecting cattle in the Pampean region of Argentina. A mathematical model parametrized using fuzzy rule-based systems of the Takagi-Sugeno-Kant type (FTSK) for estimating the development time from egg to infecting larval stage L3 of the gastrointestinal parasite *O. ostertagi* is here proposed. The estimation of development time of *O. ostertagi* is essential for the generation of appropriate control mechanisms, since this provides information about the time when parasites are ready to migrate to pastures. For the purpose of reflecting the natural environmental conditions, the mean daily temperature is taken as the main and only regulator of the development time. Humidity conditions are considered to be sufficient for the normal development of the larvae. Hence the individual's daily growth is a function of its length and the mean temperature recorded on the previous day. It is expressed in terms of a difference equation with fuzzy parameters, which are defined using laboratory data. Model outputs are tested against results of field experiments. Simulation results are very satisfactory, yielding a mean estimation error (MEE) of 0.64 weeks, with variance 0.34, and a determination coefficient $R^2 = 0.74$. The model clearly exhibits an inverse relationship between development time and temperature both in controlled and in field conditions. It also exhibits a very sensitive response both to the order in which the temperature sequence occurs, – reproducing the differences observed between spring and autumn – and to the amplitude of the temperature range.

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1. Introduction

The gastrointestinal nematode parasite *Ostertagia ostertagi* is predominant in the Pampean region (Argentina) (Fiel et al., 1994). From the economic point of view, it is considered the most important nematode affecting cattle in temperate regions (Steffan et al., 1982; Entrocasso, 1981) and as such, much effort has been devoted to investigating this parasite species.

The cycle life of *O. ostertagi* is direct, without an intermediary host. There are two distinct stages: the *free-living stage* (Egg-L1-L2-L3) and the *parasitic stage* (L4-L5-Adult). The free-living stage occurs on the ground, first within the dung-pat and later on the grass. Larvae in the L1 and L2 stages feed on fungi and bacteria. The infective larva (L3) is ensheathed and does not feed. Following

ingestion by cattle, L3 larvae undergo a process of exsheathment in the rumen before the fourth parasitic stage begins (L4). Afterwards larvae quickly develop into the adult stage.

The free-living stage has been studied under both controlled and field conditions (Fiel et al., 2008; Rossanigo and Gruner, 1995; Gibson, 1981; Young et al., 1980a, 1980b; Pandey, 1972; Rose, 1969). Several studies have revealed that there is a direct nonlinear relationship between development time and temperature over the range from 5 °C to 35 °C (Fiel et al., 2008; Williams, 1983; Catto, 1982; Pandey, 1972).

The estimation of development time is essential for the generation of appropriate control mechanisms, since this provides information about the time when parasites are ready to migrate to pastures. The variability of responses to different environmental factors makes the use of modelling tools relevant to help understand the complexity of the dynamics of the life cycle of the parasite. Development times can vary from 5 days for larvae under ideally warm and controlled conditions (Williams et al., 1987) to 36 days for larvae at a constant low temperature of 5 °C (Young et al., 1980b). Beyond these limits the mortality rate is high (Levine, 1978). The ideal temperature is within the range of 20–30 °C (Pandey, 1972; Rose, 1969) while the development

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process is difficult to accomplish at temperatures below 5 °C and above 40 °C (Pandey, 1972). Hence, during the warm months development takes only a few days while in winter the period increases to several weeks, especially if the winter is cold and wet (Catto, 1982; Durie, 1961).

In the literature, the models used for estimating development times are mostly of statistical type, strongly based on data from trials performed under controlled conditions, yielding a good fit to the data. Since these models strongly depend on the data, they are disadvantageous when it becomes necessary to extrapolate.

Here we propose a mathematical model, based on difference equations and a fuzzy rule-based system (FRBS), for estimating the development time from egg to L3 of the gastrointestinal parasite *Ostertagia* which adapts very adequately to the environmental conditions in the Pampean region of Argentina. The model consists of a difference equation and the parameters are functional forms defined through a FRBS which incorporates both quantitative and qualitative information on the processes involved. These fuzzy parameters allow for the flexibility needed when attempting to replicate field conditions, which are fundamental for developing any control strategy. The concept on which this model is based allows it to respond efficiently to wide temperature ranges.

2. Methods

2.1. Model description

The length of the larva is taken as an indicator of development, owing to the parasite’s elongated shape. If the lengths at hatching and at the time of reaching the infective stage (L3) are known, then it is possible to determine how many days an individual larva needs to be able to complete its development under a particular environment (characterized by temperature). Larvae increase their mobility as the time of their development to L3 is nearly over and, therefore their development rate increases as they age.

If $L_t(a)$ is the length of a larva that was born on day t and is now aged a , then its growth is described by the difference equation:

$$L_t(a + 1) = L_t(a) + r(T_{(t+a)})L_t(a) = [1 + rT_{(t+a)}] L_t(a)$$

$$IC \quad L_t(0) = l_0(T_t)$$

where $r(T_{(t+a)})$ is the development rate, which depends on $T_{(t+a)}$ the average temperature of the current day $t + a$, and $l_0(T_t)$ is the hatching length. The difference equation is solved using a daily step. Clearly all the larvae in same cohort (born the same day) grow exactly at the same pace.

This equation has two border conditions: an initial condition, $l_0(T_t)$, and a final condition, $l_{L3}(T)$, the length of a larva when reaching the L3 stage. It is worth mentioning that these conditions also vary depending on the environmental temperature.

Once a day t is fixed, the age a at which the larva reaches the L3 stage is such that $L_t(a) < l_{L3}(T) \leq L_t(a + 1)$. Hence, if $\tau(t)$ is the development time of a larva which was born on day t , then $\tau(t) = a + 1$.

The model was implemented using GNU Scilab 4.1. The inputs of the model are the daily average temperatures, which are loaded as a vector. The program runs the simulation with two output options:

1. Creates a graph which shows the time of development for each cohort hatching each day within the range of the temperature vector.
2. Creates a graph which describes the development of one specific cohort starting on a selected Julian day; the temperature vector must start on that day.

The temperature vector should be long enough so that the full development of the larvae can be attained.

Table 1

Definition of all membership functions used in the parameterization of the model. Function types are as defined in Appendix B.

| Name of membership function | Type | Parameter values | | |
|-----------------------------|--------------|------------------|---------|----|
| | | a | b | c |
| Temp10 | $z(x;a,b)$ | 10 | 15 | |
| Temp15 | $T(x;a,b,c)$ | 10 | 15 | 20 |
| Temp20 | $T(x;a,b,c)$ | 15 | 20 | 25 |
| Temp25 | $T(x;a,b,c)$ | 20 | 25 | 30 |
| Temp30 | $T(x;a,b,c)$ | 25 | 30 | 35 |
| Temp35 | $s(x;a,b)$ | 30 | 35 | |
| $f_{[10]}$ | $L(x;a,b)$ | 6 | 323 | |
| $f_{[15]}$ | $L(x;a,b)$ | -13.8 | 620 | |
| $f_{[20]}$ | $L(x;a,b)$ | 1.2 | 320 | |
| $f_{[25]}$ | $L(x;a,b)$ | -4.6 | 465 | |
| $f_{[30]}$ | $L(x;a,b)$ | -2.4 | 399 | |
| $f_{[35]}$ | $L(x;a,b)$ | 9 | | |
| $g_{[10]}$ | $L(x;a,b)$ | 4 | 799 | |
| $g_{[15]}$ | $L(x;a,b)$ | 2.8 | 817 | |
| $g_{[20]}$ | $L(x;a,b)$ | -5.8 | 989 | |
| $g_{[25]}$ | $L(x;a,b)$ | -3.6 | 934 | |
| $g_{[30]}$ | $L(x;a,b)$ | -13.8 | 1240 | |
| $g_{[35]}$ | $L(x;a,b)$ | 21.62 | | |
| $h_{[10]}$ | $L(x;a,b)$ | 0.0036 | | |
| $h_{[15]}$ | $L(x;a,b)$ | 0.0184 | -0.2 | |
| $h_{[20]}$ | $L(x;a,b)$ | 0.0049 | 0.0696 | |
| $h_{[25]}$ | $L(x;a,b)$ | 0.0136 | -0.1485 | |
| $h_{[30]}$ | $L(x;a,b)$ | -0.0031 | 0.3545 | |
| $h_{[35]}$ | $L(x;a,b)$ | 0.007 | | |

Pandey’s data (1972) was used to compute the parameters of each membership function.

2.2. Parametrization of the model

The model has three parameters which are the length of the newly hatched larva (l_0), the length of the L3 larva (l_{L3}) and the development rate (r), all of which depend on the daily average temperature. Each of these parameters is modelled using a fuzzy rule-based system of the Takagi-Sugeno-Kant type (FTSK) (see Appendix A), with the temperature as the input variable.

The construction of the membership functions depending on temperature was based on the work of Pandey (1972), who investigated the effect of temperature (between 4 °C and 40 °C) on the development of larvae in the free-living stage. The “temperature” variable (Temp) is partitioned into six membership functions, named respectively Temp10, Temp15, Temp20, Temp25, Temp30, and Temp35 (i.e. Temp15 corresponding to temperatures within an interval centered at 15 °C). In each case, the maximum value of membership is coincident with that in Pandey’s work. These parameters are detailed in Table 1.

Data on the length of newly hatched larvae, the length of the infective larvae and development times were used to build the consequent function of the FTSK system.

2.2.1. Length of newly hatched larvae $l_0(T_t)$

A wide variation in the size of the newly hatched larvae was observed at different temperatures, the smallest larvae being obtained at 35 °C and the largest at 15 °C (Pandey, 1972). As mentioned earlier, $l_0(T_t)$ is a FTSK system with “temperature” as the input variable. The consequent functions are constructed according to Pandey’s data, which allow the possibility of locally describing the dynamics of the problem in approximate terms.

This means that, for example, if the lengths of newly hatched larvae given by Pandey are $l_0(10^\circ) = 383$ at 10 °C and $l_0(15^\circ) = 413$ at 15 °C, then for intermediate temperature values a linear functional relationship is assumed, the function being $f_{[10]}(x_1) = 6x_1 + 323$. Then, the fuzzy rule for $x_1 \in \text{Temp10}$ is:

$$\text{If } x_1 \text{ is Temp10} \Rightarrow l_0(x_1) \text{ is } f_{[10]}(x_1) = 6x_1 + 323$$

Table 2
Summary of the list of rules that link temperature ranges to consequent functions $f_{[x]}$ (length of larva at hatching given temperature x). The resulting parameter function $l_0(T)$ is displayed in Fig. 1(a).

| | | | |
|-----------|--------|------------------|------------|
| IF x is | Temp10 | THEN $l_0(x)$ is | $f_{[10]}$ |
| | Temp15 | | $f_{[15]}$ |
| | Temp20 | | $f_{[20]}$ |
| | Temp25 | | $f_{[25]}$ |
| | Temp30 | | $f_{[30]}$ |
| | Temp35 | | $f_{[35]}$ |

The same methodology is applied to temperatures in the intermediate ranges 15–20 °C, 20–25 °C, 25–30 °C, 30–35 °C and 35–40 °C, yielding the corresponding consequents and rules. The functions, $f_{[10]}$, $f_{[15]}$, $f_{[20]}$, $f_{[25]}$, $f_{[30]}$ and $f_{[35]}$ and their parameters are detailed in Table 1 and the complete list of rules is summarized in Table 2. The resulting parameter function $l_0(T)$ is displayed in Fig. 1(a).

2.2.2. Length of infective larvae $l_{L3}(T_t)$

Infective larvae grown at different temperatures also exhibit a size variation in the initial L3 length. The infective larva is the largest when the development takes place at 20 °C. Above or below 20 °C, its length decreases gradually, the smallest being at 35 °C (Pandey, 1972).

As before, a linear relationship between different temperatures is used for the construction of the consequents. For example, given $l_{L3}(25^\circ) = 844$ and $l_{L3}(30^\circ) = 826$, the linear function for intermediate temperature values x_1 is $g_{[25]}(x_1) = -3.6x_1 + 934$. Then a fuzzy rule for $x_1 \in \text{Temp25}$ is:

If x_1 is Temp25 $\Rightarrow l_{L3}(x_1)$ is $g_{[25]}(x_1) = -3.6x_1 + 934$

As before, this methodology is used for all the other functions. The membership functions and their parameters are detailed in Table 1 and the complete list of rules is summarized in Table 3. Fig. 1(b) shows the parameter function $l_{L3}(T_t)$.

2.2.3. Estimation of the daily development rate $r(T_{(t+a)})$

As mentioned earlier, the length of a larva born at day t now aged a , depends on its length on the previous day $t + a - 1$ as well as on that day's mean temperature. The model assumes that at age a the larva will grow proportionately to the length attained at age $a - 1$. This proportion has values in the interval $[0, 1]$ and is also modelled by a FTSK system.

Under controlled conditions, the temperature is kept constant and so is the moisture in the dung-pat which is adequate for development. Hatching and infective lengths of larvae were calculated by Pandey (1972) under these conditions. Hence, it is assumed that when the temperature remains constant the larval development rate will be constant.

The values that $r(T_{(t+a)})$ take at different temperatures are then calculated. For these estimations, it is assumed that the development rate and temperature are kept constant over the appropriate time interval so that the development of a larva can be described

Table 3
Summary of the list of rules that link temperature ranges to consequent functions $g_{[x]}$ (length of larva when stage L3 is reached given temperature x). The resulting parameter function $l_{L3}(T_t)$ is displayed in Fig. 1(b).

| | | | |
|-----------|--------|---------------------|------------|
| IF x is | Temp10 | THEN $l_{L3}(x)$ is | $g_{[10]}$ |
| | Temp15 | | $g_{[15]}$ |
| | Temp20 | | $g_{[20]}$ |
| | Temp25 | | $g_{[25]}$ |
| | Temp30 | | $g_{[30]}$ |
| | Temp35 | | $g_{[35]}$ |

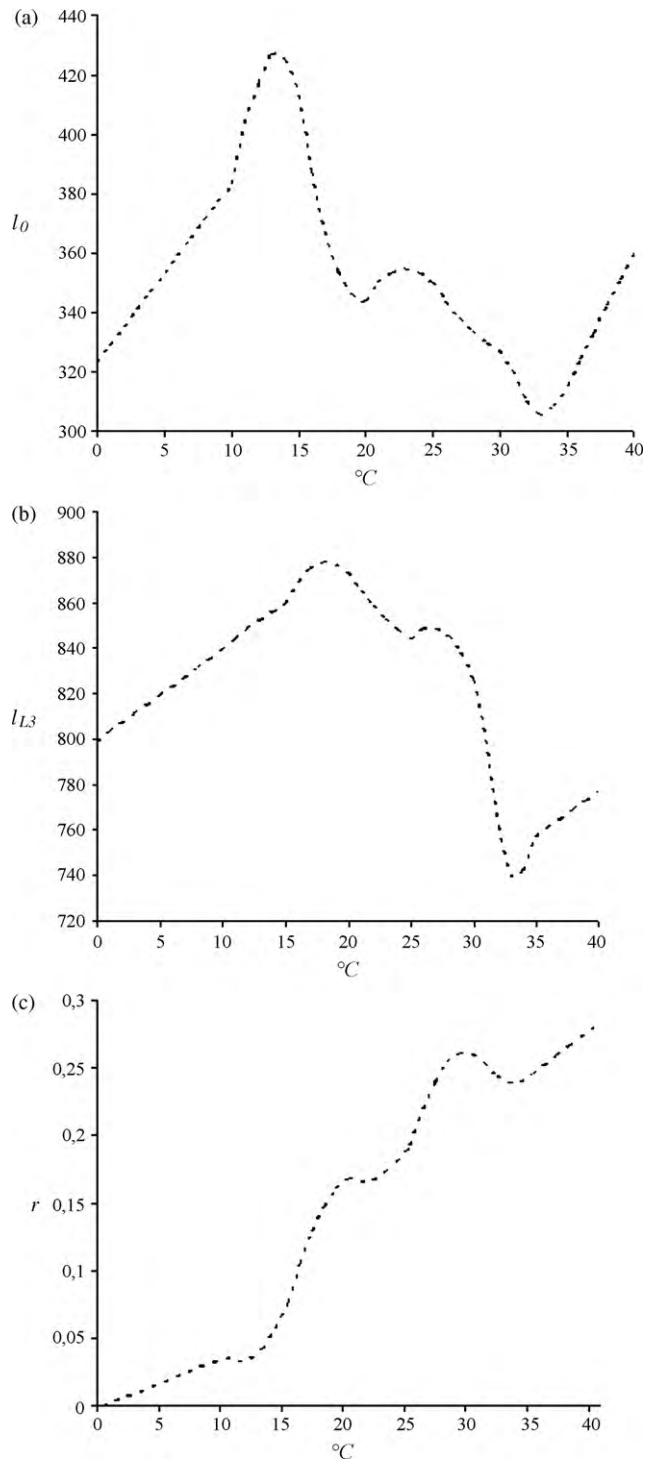


Fig. 1. Parameter functions resulting from the application of a fuzzy rule-based system of the Takagi-Sugeno-Kant type (FTSK) with the temperature as the input variable: (a) $l_0(T_t)$ length of larva at hatching given temperature T_t ; (b) $l_{L3}(T_t)$ length of larva when stage L3 is reached given temperature T_t ; (c) $r(T_t)$ development rate given temperature T_t .

by the equation

$$L_t(a + 1) = [1 + r(T_{(t+a)})] L_t(a)$$

Observe that if temperature is kept constant equal to T , then time t is irrelevant. So that for $r(T_{(t+a)}) = r(T)$ we obtain.

$$L(a + 1) = [1 + r(T)] L(a)$$

Table 4

Summary of the list of rules that link temperature ranges to consequent functions $h_{i(t)}$ (development rate given temperature x). The resulting parameter function $r(T_i)$ is displayed in Fig. 1(c).

| | | | |
|-----------|--------|----------------|-------------|
| IF x is | Temp10 | THEN $r(x)$ is | $h_{i(10)}$ |
| | Temp15 | | $h_{i(15)}$ |
| | Temp20 | | $h_{i(20)}$ |
| | Temp25 | | $h_{i(25)}$ |
| | Temp30 | | $h_{i(30)}$ |
| | Temp35 | | $h_{i(35)}$ |

and we can express

$$L(a + 1) = [1 + r(T)]^{1+\tau}L(0)$$

Also note that $L(0) = l_0(T)$, $L(\tau + 1) = l_{L3}(T)$, and τ are obtained from Pandey's data. Then, the solutions of a very simple equation yield the values of $r(T)$ for each temperature value T so that these values can be used for the construction of the consequent. The functions for the FTSK system are built similarly to those corresponding to the functions above.

For example, a fuzzy rule for this FTSK system is:

$$\text{If } x_1 \in \text{Temp15} \Rightarrow r(x_1) \text{ is } h_{i(15)}(x_1) = 0.02x_1 - 0.19$$

The membership functions and their parameters are detailed in Table 1 and the complete list of rules is summarized in Table 4. Fig. 1(c) shows the parameter function $r(T_i)$.

2.3. Model validation using field data

In order to validate the model, two indicators were used:

1. Mean estimation error (MEE) between the observed T_i and the estimated \hat{T}_i times which is defined by $MEE = (1/n)\sum_{i=1}^n (T_i - \hat{T}_i)$.
2. Coefficient of determination R^2 , which is defined by $R^2 = 1 - (\sum_{i=1}^n (\hat{T}_i - T_i)^2 / \sum_{i=1}^n (T_i - \bar{T})^2)$, where \bar{T} is the average observed development time. As the value of R^2 is closer to 1, it indicates a better fit.

Model outputs were obtained through various simulations that included conditions similar to those present from July 1994 to June 1998, when field data was retrieved, particularly the corresponding daily mean temperatures. Using the output data on the development time for each daily cohort, the monthly average development times were calculated for all months. This was done because the temporal resolution of the field experiments (weekly) was low compared to the daily output of the model.

The field data used to compare to the output of our model were provided by Fiel et al. (2008). As mentioned above, field experiments were carried out from July 1994 till June 1998 on a 0.96 ha. paddock located on the University Campus (UNCPBA) at Tandil (37°19'08"S 59°08'05"W). The climate in the region is temperate and humid, with an annual average temperature of 13.7 °C (Source: National Meteorological Service, 1911–1991).

In order to estimate the development time under field conditions, weekly samples of 5 cm² of dung were taken from the paddock. Whenever the sample contained at least 25% of larvae in the L3 stage, the period from deposition until the moment of sampling was taken to be the development time (Fiel et al., 2008). From these samples, development times were estimated to be within the ranges of 1–2 weeks in summer, 3–5 weeks in autumn, 4–6 weeks in winter, and 1–4 weeks in spring, depending on meteorological conditions. The estimated monthly average development times in the field from July 1994 to June 1998 are summarized in Fig. 2.

2.4. Model's response to variation in temperature sequences

One interesting observation on the results of field trials is that, even if spring and autumn mean temperatures are very similar, development times in spring are shorter than in autumn (Fiel et al., 2008). We used the model to assess to what degree does the temperature time series affect the development times.

Two questions arise:

1. How important is the order in which daily average temperatures occur? In other words, if we take any given temperature vector and rearrange its components, is the development time the same?

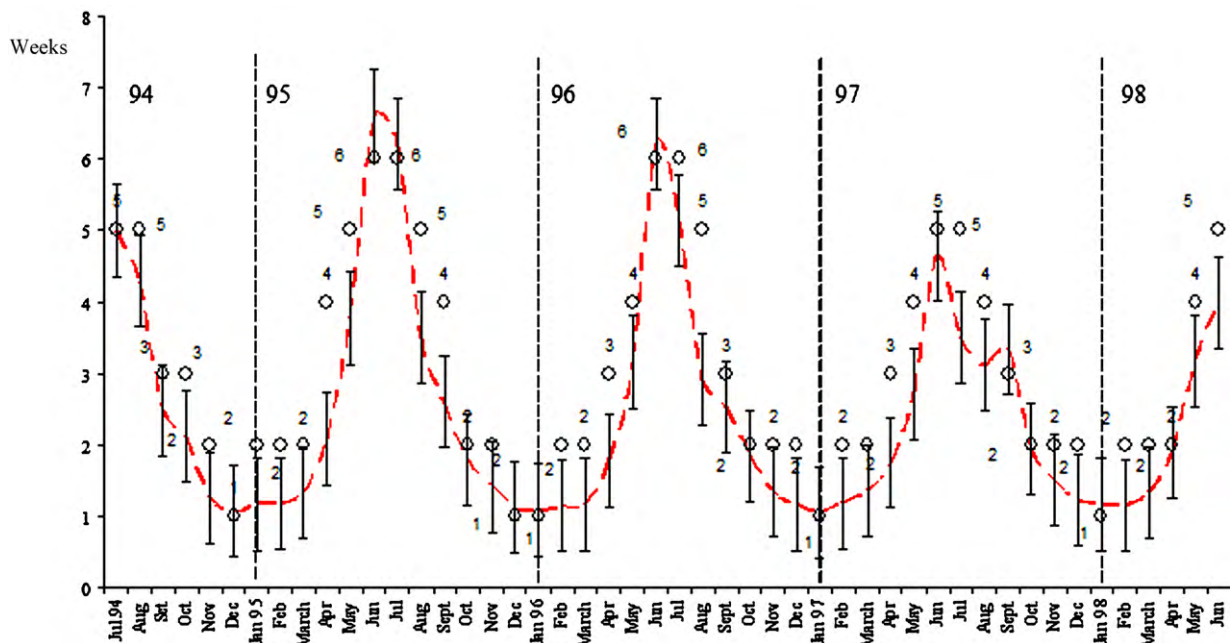


Fig. 2. Estimated monthly average development times from July 1994 till June 1998 are shown: obtained from model outputs (line) and from field experiments (circles) performed by Fiel et al. (2008). Model outputs (daily) were averaged in order to facilitate the comparison with field data.

2. If we take two vectors with mean temperature μ , is there a significant difference between the outputs of the model if the ranges of temperature are different? In other words, if the components of the temperature vector are within the interval $[\mu - \sigma, \mu + \sigma]$ or the interval $[\mu - 2\sigma, \mu + 2\sigma]$, is there any significant difference?

In order to find an answer to these questions the mean μ was selected to be 5 °C, 10 °C, 15 °C, and 20 °C, while the standard deviation $\sigma = 5$ °C was kept unchanged. For each μ , two vectors were generated randomly with uniform distribution, one over the interval $[\mu - \sigma, \mu + \sigma]$ called $T\mu\sigma$ and the other over the interval $[\mu - 2\sigma, \mu + 2\sigma]$ called $T\mu 2\sigma$. Each one of the eight vectors thus generated underwent a process of rearrangement, without replacement, of its components 10,000 times, and therefore 80,000 different vectors were obtained. Each group of 10,000 vectors generated by rearrangement of $T\mu\sigma$ was called vector pool $PV\mu\sigma$ associated to mean temperature μ and standard deviation σ (i.e. $PV20\sigma$ corresponding to $T20\sigma$). These vectors were used as inputs for the simulations and the corresponding output sets were statistically analyzed.

Descriptive statistics were used in order to analyze and describe the effect of the order of temperatures. The Kolmogorov–Smirnov test (K–S) (Rohatgi, 1984) was chosen to compare the result of simulations with same mean and different variance. The Kolmogorov–Smirnov test is a nonparametric test used to compare two samples. It has the advantage of making no assumptions about the data distribution. The Shapiro test was used to check the normality of the samples and the Levene test was used to assess the equality of variance in different samples.

3. Results

3.1. Model simulations and field data

The development times estimated by the model and daily average temperatures are displayed in Fig. 3. The Pearson coefficient of correlation (r^2) was calculated, and its value was 0.78 ($p < 0.01$). The value shows that there is an inverse linear relationship between temperature and development time. It is worth mentioning that

this relationship has been observed in the literature as relevant (Fiel et al., 2008; Williams, 1983; Catto, 1982).

Development times (expressed in terms of weeks) estimated by the model were compared to field data and satisfactory results were obtained. The experimental field data and the model outputs are displayed in Fig. 2. As mentioned earlier, the model outputs were averaged in order to facilitate the comparison with data reported by Fiel (2008). The MEE value was 0.64 weeks with a variance of 0.34 weeks and the corresponding determination coefficient was $R^2 = 0.744$ ($p < 0.05$). These values indicate a very good performance of the model, as compared to the weekly sampling process, given that the MEE is smaller than the sampling error, which is possibly greater than 1 week due to the particular experimental design.

3.2. Model's response to variations in the temperature sequence

3.2.1. Effects of the order in the temperature sequence

The set of results of the simulations for each input set $PV\mu\sigma$ is being called $SPV\mu\sigma$. The statistics for each $SPV\mu\sigma$ are detailed in Table 5.

For $SPV20\sigma$, the set of outputs for sequences with mean temperature 20 °C and standard deviation σ , the resulting developing times had a mean of 7.6 days with a standard deviation (SD) of 0.55 and a variation coefficient (CV) of 7.13%. The central fifty percent of simulations fell between 7 and 8 days, and this upper limit was coincident with the median. The minimum value obtained was 6 days, and the maximum value was 9 days. The Pearson's skewness index (PS) was -1.7 , therefore the output data was skewed to the left.

For $SPV15\sigma$, the set of outputs for sequences with mean temperature 15 °C, the mean and median were very similar, 11.2 and 11 days respectively, while the SD was 1.32 days and the CV was 11.8%. The range of values of simulations varied from 8 to 16 and the central fifty percent of simulations fell between 10 and 12 days. The PS index suggested that it was a symmetric population ($PS = 0.44$).

In simulations for $SPV10\sigma$ (mean temperature 10 °C) the mean time was 23.2 days with a SD of 1.66 days and a CV of 7.17%. The range of values varied from 18 to 28 days and the central fifty

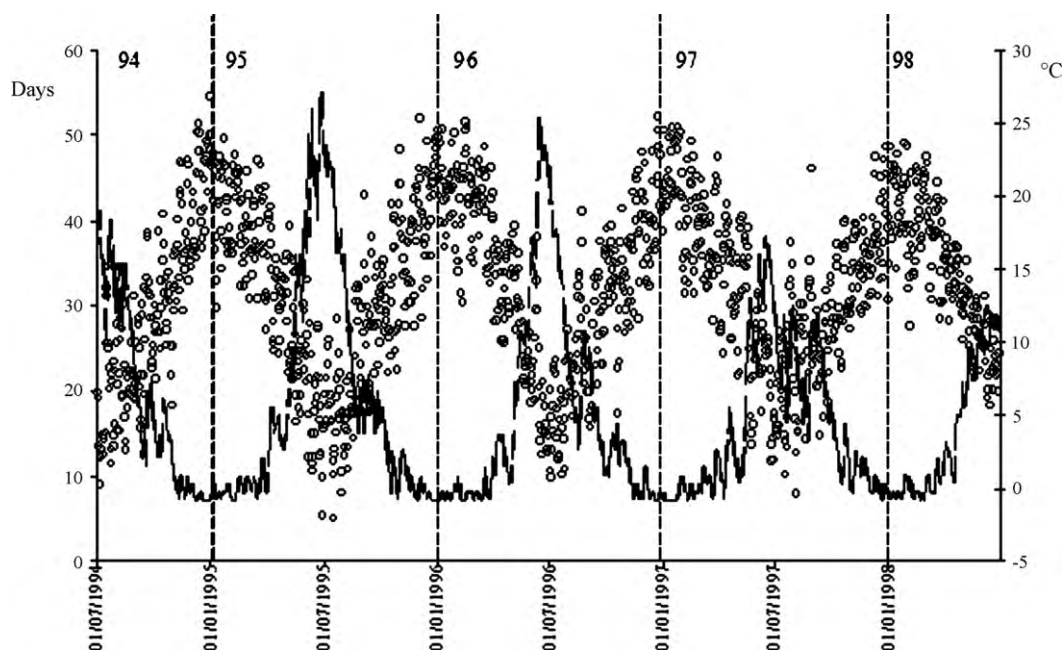


Fig. 3. The development times estimated by the model (line) and daily average temperatures (dots) recorded from July 1994 till June 1998. Inverse relationship between development times and mean daily temperatures is clearly seen.

Table 5

Statistics of the set of simulation outputs for each input set $PV\mu\sigma$ where μ is the mean of the temperature sequence and σ is its standard deviation. Each input set or vector pool $PV\mu\sigma$ consists of 10,000 temperature sequences. Mean, minimum, median, maximum, Q1, Q3, and standard deviation refer to development times in days.

| | $\mu = 20^\circ\text{C}$ | | $\mu = 15^\circ\text{C}$ | | $\mu = 10^\circ\text{C}$ | | $\mu = 5^\circ\text{C}$ | |
|-----------------------------|--------------------------|-------------------|--------------------------|-------------------|--------------------------|-------------------|-------------------------|-------------------|
| | ($\sigma = 5$) | ($\sigma = 10$) | ($\sigma = 5$) | ($\sigma = 10$) | ($\sigma = 5$) | ($\sigma = 10$) | ($\sigma = 5$) | ($\sigma = 10$) |
| Mean | 7.69 | 7.72 | 11.20 | 11.26 | 23.20 | 18.02 | 48.81 | 48.10 |
| Minimum | 6 | 6 | 8 | 7 | 18 | 8 | 37 | 32 |
| Q1 | 7 | 7 | 10 | 10 | 22 | 16 | 47 | 46 |
| Median | 8 | 8 | 11 | 11 | 23 | 18 | 49 | 48 |
| Q3 | 8 | 8 | 12 | 12 | 24 | 20 | 51 | 51 |
| Maximum | 9 | 11 | 16 | 16 | 28 | 28 | 59 | 61 |
| Standard deviation | 0.55 | 0.96 | 1.32 | 1.39 | 1.66 | 2.89 | 3.08 | 4.10 |
| Pearson's index of skewness | -1.70 | -0.86 | 0.45 | 0.57 | 0.36 | 0.02 | -0.18 | 0.07 |
| Coefficient of variation | 7.13% | 12.43% | 11.79% | 12.31% | 7.17% | 16.03% | 6.32% | 8.53% |
| K-S test (<i>p</i> -value) | 1 (<0.01) | | 1 (<0.01) | | 1 (<0.01) | | 1 (<0.01) | |

percent of the populations fell between 22 and 24. The PS index suggested that this was a symmetric population (PS = 0.019).

For $SPV5\sigma$ (mean temperature 5°C) the mean time was 48.8 days with a SD of 3.8 days and a CV of 6.32%. The range of development times was very wide, since it varied from 37 to 59 days, however the central fifty percent fell between 46 and 51 days. The PS index suggested that it was a symmetric population (PS = -0.1844).

3.2.2. Effects of the amplitude of the range

The K-S test was performed on the output of “populations” with the same mean value $T\mu\sigma$ and $T\mu2\sigma$. The null hypothesis was that $SPV\mu\sigma$ and $SPV\mu2\sigma$ had the same distribution; the alternative hypothesis is that they had different distributions. In all cases the null hypothesis was rejected with *p*-value significant at the 5% level. This means that the model is indeed sensitive to the amplitude of the range on mean temperatures recorded during the larval development.

4. Discussion

The model presented here estimates the average development time for every cohort. It is a good tool for estimating when the L3 larvae are ready to migrate to pastures. The estimation of development times depending on the weather is a very important topic for initiating the setup of new strategies for gastrointestinal parasite control in Argentina.

It is worth mentioning that not only developing times are important, but also the behavior of dung-pats as L3 reservoirs which ensure the availability of larvae to pastures. Dung-pats are natural reservoirs hosting cohorts during their full development, from egg to L3 larvae, anywhere between a few weeks and 9 months, depending on the time of the year of their deposition and the weather. Strong precipitations can degrade dung very rapidly (C. Fiel, pers. comm.).

It is believed that the larvae that develop closer to the dung-pat surface have higher oxygen availability and thus would need a shorter development time than those found deeper in the pat where oxygen levels could be lower. In such a case, one cohort would be developing in a sequential form, depending not only on the mean temperature but also on the distance of the larvae from the surface. In other words, the same cohort would exhibit different maturation times under the same weather conditions. A model that would consider a sequential development of a cohort could put more realism into the simulation. However, there is no field data available at this time which may allow its construction.

The model presented here is consistent with the experiments that have been performed both in the field and in the laboratory, and can be considered a first step towards the generation of control strategies through padlock management.

It is also pertinent to take a look at the methodology used for the construction of this model. The sensitivity of the model was analyzed taking into account different real situations, looking at the model's response to different environments, permutations in the temperature data sequences, and changes in the amplitude of the temperature ranges.

It could be argued that the choice of the membership functions may condition the results. Indeed, this is plausible because, just as in any model, the choices of functional relationships affect the response. What could be interesting in this case is to analyze the results obtained with different partitions of the discursive universe or, in other words, by changing the number and type of membership functions used. It would be useful to analyze how the degree of precision used in the construction of the parameter functions can be considered adequate by identifying statistically significant differences in the models' outputs and linking them to different levels of information. However, an analysis of the response of any model to changes in the type and number of membership functions used in its parameterization seems to be of a more theoretical nature and out of the scope of this work. It certainly is an attractive subject for continuing our research in this direction.

5. Conclusions

The model is strongly based on the observation of the biological processes involved in the development of *O. ostertagi* larvae from egg through the larval stages up to L3 with a minimal requirement of experimental data. Laboratory data from Pandey (1972) were used in the construction of the functional relationship between environmental temperature and development times used to parametrize the model. Input to the model are local temperature time series obtained from The National Meteorological Service. Totally independent field data from Fiel et al. (2008) was used for comparison to the output of the simulations thus corroborating the model.

The model is simple, expressed in terms of one difference equation and three FTSK systems, one for each parameter: the initial larval length at hatching (l_0), the larval length at the beginning of stage L3 (l_{L3}), and the larval development rate (r), all depending on the daily mean temperature.

In spite of its simplicity, the model describes very adequately the complexities of the problem. The goodness of the representation obtained through the model is reflected in the accuracy of the output relative to data reported by Fiel et al. (2008). The average estimation error between experimental field data and the output of this model is less than 1 week (0.64 week).

The model exhibits a high sensitivity to the daily variation in temperature and the amplitude of the range of temperatures along a given development period. This is reflected in the outcome of the Kolmogorov–Smirnov analysis and the variability in

the development times obtained from simulations using each set of temperature vectors $PV\mu\sigma$. Because of their particular experimental design, it is not possible to find this variability in the field trials that may allow corroborating this interesting feature. However, this is consistent with the differences observed in the field among development times estimated from samples retrieved in spring and in autumn, when the same mean temperatures yield differences in development times.

The advantageous characteristics of the model result from the use of FTSK systems for its parameterization. This introduces the possibility of describing in a simple way the dynamics of each component that has an effect on individual growth and permits to focus on the biological aspects instead of trying to fit the model to particular data. The description of each factor can be formulated as a composition of simple piecewise linear functions. Then, these combine to convey into the parameter functions the expert's non-quantifiable knowledge on *O. ostertagi* that otherwise could not be introduced, for instance through the use of classic ODE methods.

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Appendix A. Concepts and definitions about fuzzy rule-based systems (FRBS)

Lately, several authors have used the fuzzy set theory in different modelling problems (Krivan and Colombo, 1998; Chen et al., 2000; Barros et al., 2003; Jafelice et al., 2002, 2004; Ortega et al., 2003). In every case the results seem appropriate and the models mimic accurately the situations being analyzed. Kosko (1993) showed that fuzzy logic systems are universal approximators to general nonlinear functional relationships, to any desired degree of accuracy. This makes fuzzy logic modelling a powerful tool for exploring complex, nonlinear biological problems.

A fuzzy logic model is also known as a fuzzy inference system or a fuzzy rule-based system. The essence of fuzzy logic rests on the truism that all things admit degrees of vagueness. Black and white cases are the exception in a world of gray (Mackinson et al., 1999). For example, the distinction between a *normal rain* and a *drizzle* is vague. The categories overlap and may also shift in different contexts. When we learn that rainfall is 10 mm/h, then the truth or, more appropriately, the compatibility of '10' with "normal rain" or "pouring rain" is a matter of definition. It depends on our understanding of the concepts "normal or pouring". In set theory, each of these linguistic values is a set of the fuzzy variable rainfall. There is no one point where we can say some rainfall is *normal* or *not normal*, it is a matter of degree. We perceive the precise in a fuzzy way and it is this ability to summarize information into classes (fuzzy sets) that separates human intelligence from machine intelligence (Zadeh, 1965).

In very formal terms a fuzzy set *A* defined in a discursive universe *X* is a set of pairs (*x*, $\mu_A(x)$) where *x* belongs to *X* and $\mu_A(x)$ is a number in the interval [0, 1] representing the degree of membership of *x* in *A*. Expert knowledge is represented by a set of fuzzy rules, they are of the form "IF this THEN that". Rules made associa-

tions between input and output fuzzy sets. They relate one event or process to another event or process, for example: "IF Temperature is *high* THEN Size is *small*"

Basically, any fuzzy logical model is formed by four parts: (a) the *input processor*, which translates nonquantifiable or quantifiable input into fuzzy sets of their respective universes; (b) the *fuzzy rule base*, consisting of a collection of fuzzy IF-THEN rules aggregated by the disjunction or the conjunction, which is a key knowledge-encoding component of fuzzy rule-based systems; (c) the *fuzzy inference engine*, performing approximate reasoning by using the compositional rule of inference, so that a fuzzy set answer or global conclusion will be calculated by aggregation of the partial solutions contributed by each rule; (d) the *defuzzifier*, which assigns a real number that is representative of the corresponding fuzzy set answer. This last process is called *defuzzification*.

In this work, the fuzzy logic models are of the Takagi-Sugeno-Kant type (Nguyen and Walker, 1997) (FTKS). This approach is essentially based on the possibility of describing the local dynamics of a problem in approximate terms. This is the case, for example, when for each member of a fuzzy partition of the input space of *X*, the difference equation of the problem is linear to some degree. This suggests forming rules as follows

$$R_j : \text{"IF } x_1 \text{ is } A_j^1 \text{ and } x_2 \text{ is } A_j^2 \dots \text{ and } x_N \text{ is } A_j^N \text{ THEN } y = f_j(x_1, x_2, \dots, x_N), j = 1, 2, \dots, r$$

where x_i are the actual observed values of input variables, and $f_j(\cdot)$ is some specific linear function, such as

$$f_j(x_1, x_2, \dots, x_N) = \sum_{i=1}^N \alpha_{i,j} x_i$$

For FTSK systems, the consequent in each R_j is expressed by a constant value. The rule R_j will produce a *crisp output* given by:

$$y_j = \tau_j f_j(x_1, x_2, \dots, x_N)$$

where τ_j is the degree of applicability or weight of the rule R_j . Then the overall output value is taken to be a weighted average:

$$y(x_1, x_2, \dots, x_N) = \frac{\sum_{j=1}^r \tau_j f_j(x_1, x_2, \dots, x_N)}{\sum_{j=1}^r \tau_j}$$

Appendix B. Frequent membership functions

Membership function forms used in this work are of the following types:

Z-function

$$z(x; a; b) = \begin{cases} 1 & \text{if } x \leq a \\ 1 - 2\left(\frac{x-a}{b-a}\right)^2 & \text{if } a \leq x \leq \frac{a+b}{2} \\ 2\left(\frac{b-x}{b-a}\right)^2 & \text{if } \frac{a+b}{2} \leq x \leq b \\ 0 & \text{if } x \geq b \end{cases}$$

S-function

$$s(x; a; b) = 1 - z(x; a; b) = \begin{cases} 0 & \text{if } x \leq a \\ 2\left(\frac{x-a}{b-a}\right)^2 & \text{if } a \leq x \leq \frac{a+b}{2} \\ 1 - 2\left(\frac{b-x}{b-a}\right)^2 & \text{if } \frac{a+b}{2} \leq x \leq b \\ 1 & \text{if } x \geq b \end{cases}$$

Triangular function

$$T(x; a, b, c) = \max\left(\min\left(\frac{x-a}{b-a}, \frac{c-x}{c-b}\right), 0\right)$$

Linear function

$$L(x; a, b) = ax + b$$

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