

# A non-destructive morphometric technique to predict *Ligula intestinalis* L. plerocercoid load in roach (*Rutilus rutilus* L.) abdominal cavity

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## Abstract

The aim of the present work was to propose a model for the estimation of the endoparasitic load using morphological descriptors easily accessible without killing the animal i.e. non-destructive method. The study was conducted using plerocercoid forms of *Ligula intestinalis* in its second intermediate host, the roach (*Rutilus rutilus*). The Kohonen Self-Organizing Map (non-supervised neural network) made it possible to present the complex data matrix in a two-dimensional space, with individual clusters visualised by the U-matrix method. The six main descriptors were selected and used to build the predictive model, four lateral and two thickness measures. The generalisation ability of the backpropagation algorithm (supervised neural network) is confirmed by a determination coefficient higher than 0.90 between observed and predicted values. The study for the first partial derivatives of the parasitic load with respect to the six morphological variables is used to identify the factors influencing the parasitic load and the mode of action of each factor. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Artificial neural network; Roach; Parasite burden; Predictive model; Non-destructive method

## 1. Introduction

A wide range of protozoan and metazoan parasites with complex life cycles are able to alter host phenotype in a way that favours the continuation of their life cycle (Combes, 1991; Poulin, 1998). For example, many trophically transmitted parasites increase the vulnerability of the intermediate

host to the predatory definitive hosts (Holmes and Bethel, 1972; Helluy, 1984; Moore, 1984; Hechtel et al., 1993). The extent to which the behaviour of the host is modified is often dependent on the level of infection. However, for fish infected with endoparasites, accurate information regarding the level of infection is obtainable only following dissection. Because ecologists are under pressure to use methods that reduce the suffering caused to animals, non-destructive approaches to the estimation of the parasite load have an ethical, as well as a scientific value.

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*Ligula intestinalis* (L.) is a widespread and common pseudophyllidean cestode whose complex life cycle is completed in three distinct hosts. The first intermediate host, a copepod, is ingested by a cyprinid fish inside which *Ligula* larvae evolve into plerocercoid stages located in the abdominal cavity of the second host. The final host is a fish-eating bird in which *Ligula* reaches its maturity and reproduces in the host's intestine. Parasite eggs are released into the water with bird faeces. From a pathogenic point of view the second host is most important because plerocercoids occupy the body cavity of the fish for several years and produce harmful effects on the fish (Van Dobben, 1952; Dence, 1958; Orr, 1966; Wilson, 1971; Holmes and Bethel, 1972; Harris and Wheeler, 1974; Sweeting, 1976).

The roach (*Rutilus rutilus* L.) acquire the parasites following consumption of infected copepods, and they grow rapidly to a large size in the fish's body cavity, characteristically distending the abdominal region and having significant effects on many aspects of the physiology and behaviour of the host fish. The intensity of the abdominal distension and fish morphology changes depend on the total mass of parasite tissue present.

The present study proposes the use of a non-invasive morphometric technique to predict the mass of *L. intestinalis* tissue in the roach body cavity. The technique aims to separate groups of fish at different levels of infection according to morphometric characters and focus on the use of advanced modelling technique, based on artificial neural networks (i) to select the relevant variables explaining the parasite load, and (ii) to use these variables to build the predictive model to predict and explain the parasite load.

## 2. Methods

### 2.1. Sampling

Samples of *Rutilus rutilus* were collected from the Lavernose-lacasse gravel pit located near Toulouse in south-west France. Fish were collected with a drag seine 30 m long and 10 mm

mesh size during December 1999 and January 2000. A total of 90 individuals were sampled and deep-frozen as soon as possible in the laboratory.

### 2.2. Morphometry

For a sample of 45 infected and 45 uninfected fish, 39 morphological measurements usually used in fish morphological study were taken (Hubbs and Lagler, 1967) (Table 1). A standard protocol for processing each specimen was established. All measurements were made by a single individual (GL) to eliminate differences as a result of technique. All morphometric characters were measured using a digital electronic calliper to the nearest 0.01 mm. Each fish was dissected to determine the parasite load. Plerocercoid larvae present in the abdominal cavity were weighed to the nearest 0.001 g. Since morphological characters strongly depend on size, we decided to correct all parameters for the individual body size of the host.

### 2.3. Statistical analysis

The data processing was realised using a modelling method based on the principle of neural networks. We used, firstly the Kohonen Self-Organizing Map (SOM) algorithm (Kohonen, 1995) to map the complex database on the two-dimensional plane visualising the parasitic load, and to determine the relevant variables contributing to the model. Secondly, the backpropagation algorithm (Rumelhart et al., 1986) was used to establish the predictive model of the parasitic load and to study the sensitivity of the variables in the model.

The Kohonen neural network includes an input layer connected to the 39 morphological measurements of each fish, and an output layer formed by 100 nodes on a hexagonal lattice.

Each neuron of the output layer stores a virtual fish with morphological measurements to be computed. During the training, the virtual fish are modified in order to approximate the probability density function of the input data. The main characteristic of the SOM classification is the conservation of the topology: close fish (in a

morphological measurement sense) are associated with the same node or to nearby nodes on the map.

Table 1  
List of 39 phenotypic morphological lateral traits and their corresponding codes

Morphological measurements	Code
Postorbital distance	<i>poO</i>
Preorbital distance	<i>prO</i>
Length of head	<i>lc</i>
Upper jaw length	<i>lmx</i>
Head depth (at centre of eye)	<i>hco</i>
Minimum body depth (least depth of caudal peduncle)	<i>h</i>
Distance between dorsal fin base and caudal measuring point	<i>DC</i>
Distance between dorsal fin base and pectoral fin base	<i>DP</i>
Distance between dorsal fin base and ventral fin base	<i>DV</i>
Distance between dorsal fin base and anal fin base	<i>DA</i>
Horizontal diameter of eye	<i>Oh</i>
Vertical diameter of eye	<i>Ov</i>
Predorsal distance	<i>pD</i>
Prepectoral distance	<i>pP</i>
Preventral distance	<i>pV</i>
Preanal distance	<i>pA</i>
Distance between pectoral fin base and ventral fin base	<i>PV</i>
Distance between pectoral fin base and anal fin base	<i>PA</i>
Distance between pectoral fin base and caudal measurement point	<i>PC</i>
Distance between ventral fin base and caudal measurement point	<i>VC</i>
Distance between anal fin base and caudal measurement point	<i>AC</i>
Depth of dorsal fin	<i>hD</i>
Depth of anal fin	<i>hA</i>
Length of pectoral fin	<i>lP</i>
Length of ventral fin	<i>lV</i>
Length of upper lobe of caudal fin	<i>lC1</i>
Length of middle part of caudal fin	<i>lC2</i>
Length of lower lobe of caudal fin	<i>lC3</i>
Length of dorsal fin base	<i>lD</i>
Length of anal fin base	<i>lA</i>
Interorbital distance	<i>io</i>
Body width at the level of pectoral fin insertion	<i>laP</i>
Body width at the level of ventral fin insertion	<i>laV</i>
Body width at the level of anal fin insertion	<i>laA</i>
Body width at the level of dorsal fin insertion	<i>laD</i>
Maximum body width	<i>la</i>
Body height at the level of posterior point of head	<i>HH</i>
Body height at the level of ventral fin insertion	<i>HV</i>
Body height at the level of anal fin insertion	<i>HA</i>

The SOM algorithm is an unsupervised learning procedure which can be summarised as follows:

- The virtual fish are initialised with random samples drawn from the input data set.
- The virtual fish are updated in an iterative way:
  - A sample unit is randomly chosen as an input unit.
  - The Euclidean distance between this sample unit and every virtual fish is computed.
  - The virtual fish closest to the input is selected and called ‘best matching unit’ (BMU).
  - The morphological measurements of the BMU and its neighbours are changed in order that these virtual fish moved a bit towards the input unit.

The training was broken down into two parts previously defined by Giraudel and Lek (2001):

- Ordering phase (the 2000 first steps): when this first phase takes place, the fish are highly modified in a wide neighbourhood of the BMU.
- Tuning phase (50 000 steps: 500 times the number of neurons in the Kohonen map): during this phase, only the virtual fish adjacent to the BMU are lightly modified.

At the end of training, the morphological measurements are known for each virtual fish, the BMU is determined for each fish, and each real fish is set in the corresponding hexagon of the Kohonen map. Fish which are neighbours on the grid are expected to represent neighbouring clusters of fish; consequently, fish having a large distance to each other, (according to morphological measurements), are expected to be distant in the feature space.

In order to detect a clustering structure in the resulting SOM, a new map was built using the U-Matrix method (Ultsch and Siemon, 1990; Giraudel and Lek, in press). When the learning process has been completed, the 39 morphological measurements for each virtual fish were used to compute the Euclidean distance between each node located in two adjacent hexagons of the SOM. High value distances gave an indication of cluster boundaries. Inserting a new hexagon between each adjacent hexagon, the distances were

visualised in each new hexagon as a grey-level display. But distance values are only available for the new hexagons, so in each hexagon including a virtual fish, a distance has been added, computed as the minimum of its adjacent hexagons and visualised as a grey-level display. Dark colours were used for large distances and light colours for short distances, then, a triangle-based cubic interpolation (Watson, 1992) had been applied to the U-matrix and a landscape was formed with light plains separated by dark ravines. When the fish are mapped on the U-matrix, the individuals in the plains are close to each other so they are similar (for morphological measurements).

Then to highlight the contribution of each morphological measurement in the cluster structures, 39 U-matrices were coloured using the Euclidean distance based on each morphological measurement (Kaski et al., 1998). The obtained clusters were compared with those previously obtained using all the morphological measurements. The more similar the patterns, the more relevant the morphological measurements are. By this way, 6 measurements were selected and used for the predictive modelling.

For the predictive modelling, a multilayer feed-forward neural network (MLP) was used. The processing elements in the network, called neurons, are arranged in a layered structure. The first layer, called the input layer, connects with the input variables. In our case, it comprises six neurons corresponding to the six selected morphological variables. The last layer, called the output layer, connects to the output variable. It comprises a single neuron corresponding to the value of the dependent variable to be predicted (parasitic load). The layers between the input and output layers are called the hidden layers. There can be one or more hidden layers and the number of neurons of each layer is an important parameter of the network. The network configuration was approached empirically by testing various possibilities and selecting the one that provides the best compromise between bias and variance (Geman et al., 1992). Each neuron was connected to all neurons of adjacent layers. Neurons received and sent signals through these connections. Signals were transmitted only in one direction:

from input layer to output layer through hidden layers. Connections were given a weight which modulate the intensity of the signal they transmit. To testify the predictive quality of the ANN model, we used the 'leave-one-out' cross-validation procedure (Efron, 1983; Kohavi, 1995) which is adapted in our study where the database is small and each observation was tested using a model trained by all other remaining observations. In fact, we performed 90 training phases with 89 observations followed by 90 testing phases with only one observation each time. The quality of the model was judged through the determination coefficient between measured and predicted values.

One disadvantage of backpropagation algorithm is its lack of explanation power. Classical analyses, such as multiple linear regression (MLR), can identify the contribution each individual input make on the output and can also give some measures of confidence about the estimated coefficients. In mathematical terms, each coefficient of a linear model is the partial derivative of the response of the model with respect to the variable of that coefficient. The MLR partial coefficients therefore generally give an indication of input variable reality, although it is not possible for this type of model to represent a non-linear relationship such as that which probably exists between parasitic load and some influencing morphological factors. On the other hand, currently, there is no theoretical or practical way of accurately interpreting the weights attributed in ANN. For example, weights cannot be interpreted as a regression coefficient nor difficulty used to compute causal impacts or elasticity. But in ecology, it is necessary to know the impact of the explanatory variables. Some authors have proposed methods allowing the determination of the impact of the input variables (Garson, 1991; Goh, 1995; Lek et al., 1996a,b; Dimopoulos et al., 1995, 1999).

In this study we use a simple method based on the use of the partial derivatives of the network response with respect to each descriptor. The link between the modification of inputs,  $x_j$ , and the variation of outputs,  $y_j = f(x_j)$ , is the Jacobian matrix  $dy/dx^t = [\partial y/\partial x]_{m \times n}$ . It represents the sensitivity of the network outputs according to small input perturbations. For a network with  $n$  inputs,

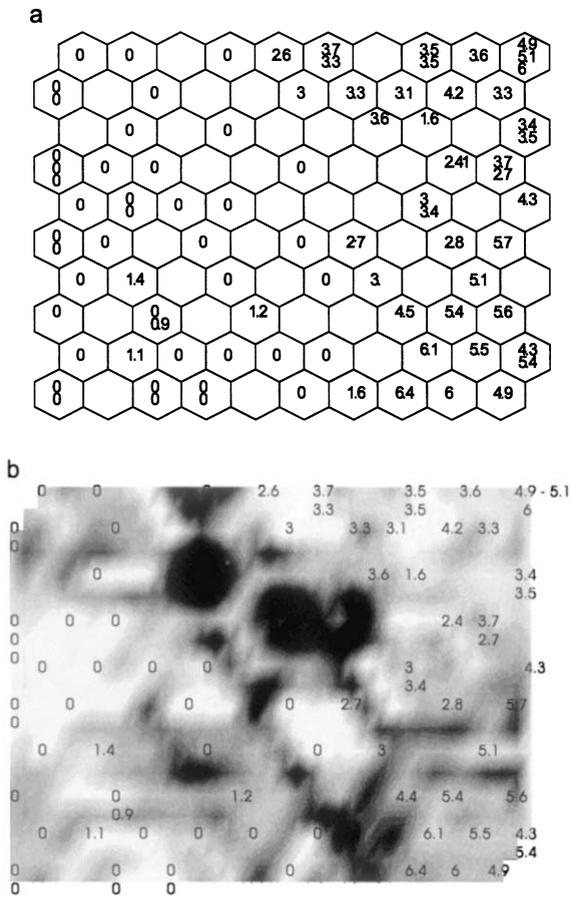


Fig. 1. (a) The output layer-grid of the SOM model with all the parasitic load values, (b) U-matrix showing plains (light grey) separated by several ravines (dark grey).

one hidden layer with  $n_i$  nodes, and one output (i.e.  $m = 1$ ), the gradient vector of  $y_j$  with respect to  $x_j$  is  $d_j = [d_{j1}, \dots, d_{je}, \dots, d_{jm}]^T$  (Dimopoulos et al., 1995, 1999), with:

$$d_{je} = \sum_{i=1}^{n_i} w_{is} I_{ij} (1 - I_{ij}) w_{ei} \quad (1)$$

(under the assumption that a logistic sigmoid function is used for the activation). When  $s_j$  is the derivative of the output node with respect to its input,  $I_{ij}$  is the output of the  $i$ th hidden node for the input  $x_j$ , the scalars  $w_{is}$  and  $w_{ei}$  are the weights between the output node and the  $i$ th hidden node, and between the  $e$ th input node and the  $i$ th hidden node).

The sensitivity of the ANN output for the data set with respect to input  $x_e$  is:

$$SSD_e = \sum_{j=1}^N (d_{je})^2 \quad (2)$$

and the derivative can be efficiently computed as a minor extension to the backpropagation algorithm used for training.

All computational programmes were realised in a Matlab<sup>®</sup> environment and computed with an Intel Pentium<sup>®</sup> processor.

### 3. Results

#### 3.1. Self-organizing map

After the training of the SOM using the 39 measured morphological characters, the parasitic load value was displayed on the map for each fish (Fig. 1a). Even though the SOM was built without the parasitic load having been taken into account, a gradient had been observed on the map. The uncontaminated fish were localised in the left part of the map, the contaminated fish were observed in the right part of the map: the most contaminated in the lower part, the least contaminated in the upper and only three contaminated fish with low parasitic load were observed among the uncontaminated ones. On the U-matrix (Fig. 1b), the observation of the dark ravines in dark shades strengthens the ability of the SOM to reveal a structure in the fish population. The map was divided by ravines in the three clusters mentioned above: the uncontaminated fish area, the less contaminated and the most contaminated. It is worth noting that the parasitic load values had been plotted, a posteriori, on the U-matrix and had not been used in the construction of the U-matrix.

In order to make explicit the contribution of each morphological measurement in the organisation of the SOM, a U-matrix was coloured for each morphological measurement. Each U-matrix has been observed and those with ravines relevant to the discrimination between uncontamination, low contamination and high contamination (Fig. 2a–f) were selected as contributing to the determi-

nation of the clusters. In that way, six relevant variables were determined and used for the prediction of the parasitic load. More precisely,

(*HH*) the body height at the level of the posterior point of the head and (*DV*) the distance between the dorsal fin base and the ventral fin base, are

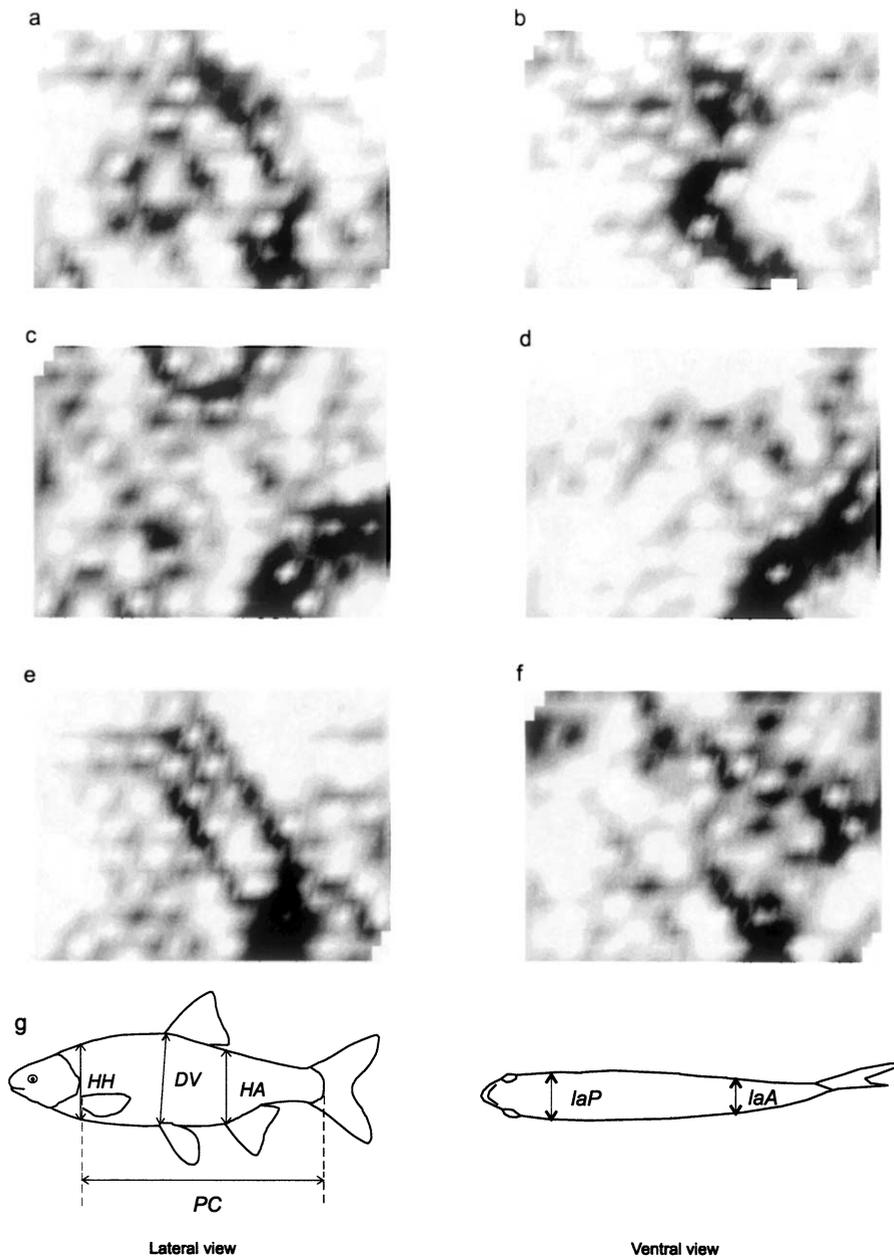


Fig. 2. Contribution of the six relevant variables in the local cluster structures. (a) *HH*, the body height at the level of the extreme anterior point of the head; (b) *DV*, the distance between the dorsal fin base and ventral fin base; (c) *HA*, the body height at the level of the anal fin insertion; (d) *laA*, the body width at the level of the anal fin insertion; (e) *laP*, the body width at the level of the pectoral fin insertion; (f) *PC*, the distance between the pectoral fin base and the caudal measurement point; (g) the location of the 6 measurements in the lateral and ventral views.

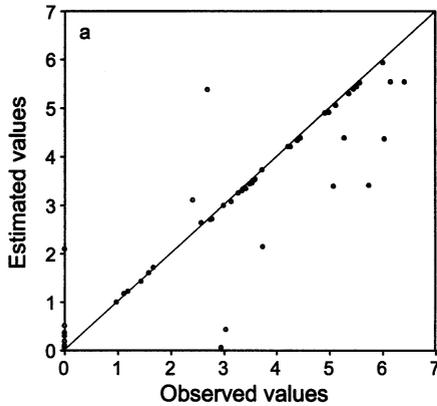


Fig. 3. Predictive quality of the multilayer perception neural network models, using the leave-one-out cross-validation procedure: the relationship between the observed and the estimated values of the parasitic load.

relevant for the discrimination between infected and uninfected fish, (*HA*) the body height at the level of the anal fin insertion and (*laA*) the body width at the level of the anal fin insertion, discriminate the most heavily infected fish from the others and the two variables: (*laP*) the body width at the level of the pectoral fin insertion and (*PC*) the distance between the pectoral fin base and the caudal measurement point, are relevant for the discrimination between all levels of fish infestation.

### 3.2. Prediction of parasitic load

With the six morphological variables selected and using the 'leave-one-out' cross-validation approach, a good predictive model can be obtained using a MLP with three neurons in the hidden layer and sigmoid as the activation function. The high value of the determination coefficient demonstrates the great predictive power of the model ( $R^2 = 0.90$ ). Most of the points in the whole range of the parasitic load values were distributed along a perfect predicted diagonal line (Fig. 3). Only a few points were slightly isolated to the perfect fit line, i.e. slightly under or over estimated by the model. The residuals of the model were weak (mean = 0.07, S.D. = 0.69), the study of the relationship between residuals and values estimated by the model showed complete independence ( $r = -0.03$ ,  $P = 0.79$ ).

### 3.3. Influence of factors

The sensitivity study of the MLP model, according to the partial derivative, led to the layout of Fig. 4. The relative contribution allows the classification of the morphological variables according to their decreasing influence on the parasitic load: (*LaP*; 41.49%), (*PC*; 18.62%), (*DV*; 13.88%), (*HA*; 10.91%), (*LaA*; 10.85%), (*HH*; 4.25%).

The study of partial derivatives for each morphological variable (Fig. 4) leads to the following remarks:

- The influence of *HH* on the parasitic load is rather complicated and non-linear (Fig. 4a). The positive values of partial derivatives ( $dHH$ , in  $y$  axis) for the high values of *HH* ( $x$  axis) show that the increase of the *HH* contributes to the increasing of the parasitic load. Moreover, the very strong values of *HH* seem to have negative values of  $dHH$ , confirming the negative contribution to the parasitic load of this parameter.
- The increase of *DV* supports the increase of parasitic load (Fig. 4b). However, we can observe two tendencies: (i) for the values of  $DV < 0.28$ , the derivative is negative, (ii) for the *DV* values over 0.28, we obtain the positive values of the derivative, certifying that these values of *DV* contribute to the increasing of the parasitic load.
- The profile of the influence of *HA* on the parasitic load (Fig. 4c) is in inverse order to the two previous parameters. The derivative values,  $dHA$ , negative over all the range of the *HA* value, testify to the negative contribution of this variable to the parasitic load. One can notice only few points with positive or nil derivative values.
- The parasitic load decrease with the increasing values (*LaA*), i.e. negative derivative values (Fig. 4d), for the whole range of *LaA*. The strong negative values of the derivative for high values of *LaA* ( $> 0.09$ ) demonstrate the strong negative impact of *LaA* on the parasitic load.
- The positive derivatives  $dLaP$  with *LaP* testify that the parasitic load increases with *LaP* (Fig. 4e). Most of the points have the positive

derivatives, especially for the values of  $Lap$  between 0.12 and 0.14.

- Fig. 4f shows the complicated and non-linear impact ( $PC$ ) on the parasitic load. It changes according to the change of the  $PC$ -values. Firstly, the derivative is nil for  $PC < 0.78$ ; secondly, the derivative is strongly positive for  $PC$  between 0.78 and 0.8; and thirdly, the derivative is notably negative for  $PC > 0.8$ .

#### 4. Discussion

*L. intestinalis* plerocercoids have been shown by many researchers to induce severe pathological effects on fish (Van Dobben, 1952; Dence, 1958; Dogiel et al., 1961; Wilson, 1971; Holmes and

Bethel, 1972; Harris and Wheeler, 1974; Sweeting, 1976; Taylor and Hoole, 1989). The roach (*Rutilus rutilus* L.) acquire the parasites following consumption of infected copepods, and they grow rapidly over several months. During their development, these parasites grow from a microscopic proceroid to a large plerocercoid worm in the fish body cavity characteristically distending the abdominal region (represented by the variation of  $HH$ ,  $DV$  and  $laP$ ). A displacement of the pectoral fin which moves forward closer to the head is associated with abdominal distension (represented by the variation of  $PC$ ). These morphological changes occur for the two level of infestation (parasite burden  $< 5$  g and  $> 5$  g). The morphological modifications of the host, e.g. body wall distension and fin displacements, are the result of

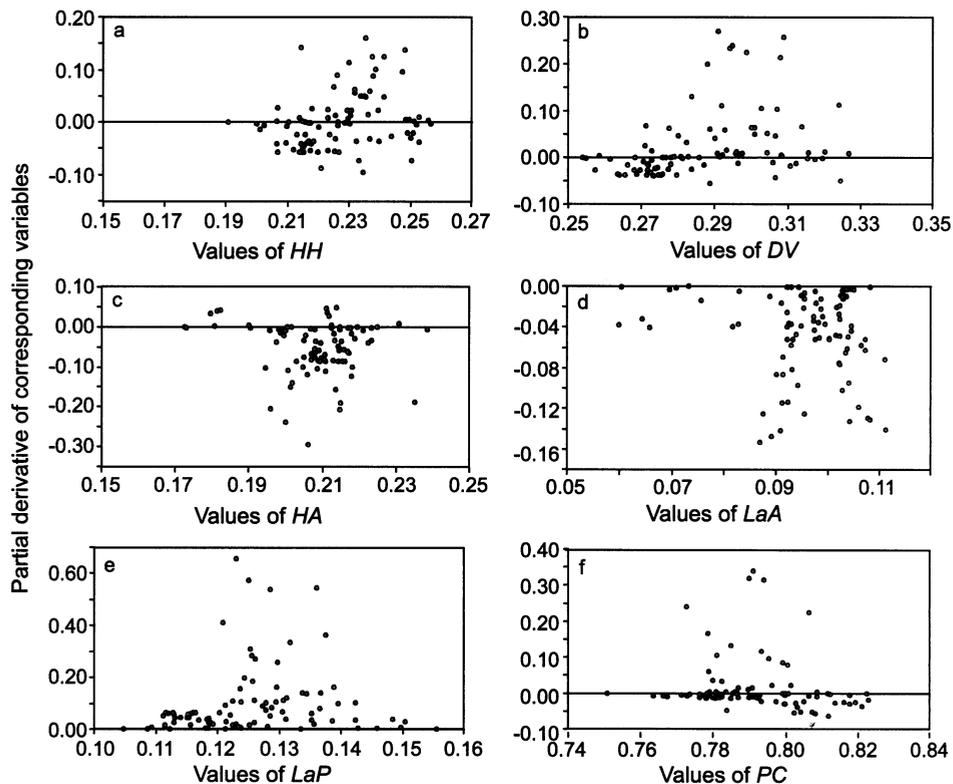


Fig. 4. Partial derivatives of the multilayer perceptron neural network model response with respect to each descriptor. (a)  $HH$ , the body height at the level of the extreme anterior point of the head; (b)  $DV$ , the distance between the dorsal fin base and the ventral fin base; (c)  $HA$ , the body height at the level of the anal fin insertion; (d)  $LaA$ , the body width at the level of the anal fin insertion; (e)  $LaP$ , the body width at the level of the pectoral fin insertion; (f)  $PC$ , the distance between the pectoral fin base and the caudal measurement point.

the accumulation of plerocercoids in the fish i.e. mechanical side-effect. The larger infected roach which possess parasite burdens that approached 5 g were suffering from a deformation at the level of the tail (represented by the variation of *HA* and *laA*). Deformations at the level of the tail are not simply the response to abdomen distension but they may be explained by direct physiological effects of plerocercoids on the host. As pointed out by many authors, physiological mechanisms through which such phenotype modification can be achieved have only recently started to be understood (Wedekind and Milinski, 1996). Generally, pseudophyllidean worms are known to divert energy away and cause a considerable energy drain from the non-vital organs of the fish (Kuris, 1997; Phares, 1996), for instance muscle atrophy at the level of the host's tail.

From this study, three kinds of results emerge: (i) the artificial neural networks with the Kohonen SOM are a good tool to display high-dimensional data in a two-dimensional space. Moreover, with the U-matrix method, the SOM allows cluster analysis. As already mentioned by some authors (e.g. Kaski et al., 1998) we demonstrate in this study that it is possible to extract the relevant variables in the cluster structures. (ii) The MLP with a backpropagation algorithm allows us to predict the parasitic load with very good predictive quality, such as demonstrated by several authors for several ecological areas (e.g. Scardi, 1996; Culverhouse et al., 1996; Recknagel, 1997; Recknagel et al., 1997; Yabunaka et al., 1997; Lek and Guégan, 2000). This method gave much higher correlation coefficients than other methods, due to the ability of MLP to take directly into account any non-linear relationships between the input (morphological variables) and the output (parasitic load) variables (Lek et al., 1996b). These results are in agreement with literature data, where performances of artificial neural network have been repeatedly reported to exceed those of more traditional methods (see complete references in Lek and Guégan, 2000). (iii) The MLP, was known as a 'black-box model'. In this work, we demonstrate that it could be used as explanatory principle, i.e. to determine the contribution of variables at the input to explain the

variations of the output. Many algorithms have been put forward by several authors to determine the impact of input variables on the output, using ecological data (e.g. Garson, 1991; Goh, 1995; Lek et al., 1996a,b; Maier and Dandy, 1996; Balls et al., 1996; Seginer, 1997; Dimopoulos et al., 1999). The profile and percentage contributions of the input variables determined by the Lek and Dimopoulos algorithms provide an explanation of the impact of a set of input variables on parasitic load modelling.

This technique is not biased by the gonad development which increases the body size. The gonad development is inhibited by the parasite and among uninfested fish analysed, 35% had matured females with a gonad weight of  $1.448 \pm 1.184$  g. The intensity of fish morphology changes depends on the total mass of parasite tissue present which exerts significant effects on many aspects of the biological features such as the physiology, the behaviour and the ecology of the host. The availability of this non-destructive morphometric technique allows a pattern of experiments that could lead to a greater understanding of how *L. intestinalis* affects the biology, and particularly the behaviour of the fish hosts, during the course of the infection. The technique is most accurate for studying parasite-induced alterations on the host predator-prey within interactions, coloration, foraging and shoaling behaviour (Combes, 1991; Thomas and Poulin, 1998; Poulin et al., 1998). Such a study has a growing interest not only in contributing to our knowledge of freshwater ecosystems, but also to our understanding of ecological communities in general.

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