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Measuring the health state of the eel swimbladder following infections by the invasive nematode *Anguillicoloides crassus*: comparison and critical evaluation of two alternative indices

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Running title: anguillicolosis and eel swimbladder indices

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Abstract

In this study our aim was to compare the performance of two alternative indices for quantifying the pathological impact of the nematode *A. crassus* on the swimbladder of eels *Anguilla* spp. Two observers recorded twice the scores obtained by the two indices on a same set of 71 fish (from elver to silver eels, French Mediterranean lagoons). The Length Ratio Index (LRI, Palstra *et al.* 2007) proved to better perform in three out of our four pre-defined criteria of decision. First of all, the LRI tended to better correlate with an estimate of the swimbladder volume reduction, a functional consequence of the infection (representativeness). Also, the LRI was shown to be less prone to subjectivity (inter-observer variability) and more precise (intra-observer variability), although less easy to generate (time needed for measurement/assessment) than the so far prevailing Swimbladder Degenerative Index (SDI, Lefebvre *et al.* 2002). Using a sub-sample of 32 unaffected eels (showing minor if any swimbladder damage and no living worm at dissection), we ascertained a linear relationship between the swimbladder length and the total body length, a pre-requisite condition of isometric growth to definitively accept the new ratio index as a valid alternative to the SDI. Also, because the LRI offers the possibility to be recorded on live specimens with radio-imageries (non-invasive method), we henceforward recommend to start using it extensively wherever desirable and possible, and provide a graph of correspondence between the SDI scores, the LRI scores and the estimated proportion of gas loss in the swimbladder.

Keywords: Anguilla anguilla, helminthiasis, anguillicolosis, invasive species, pathologies, gas bladder, indexing.

Introduction

The need of assessing the health state of the eel swimbladder has long been recognised by those people concerned with anguillicolosis (i.e., fish disease affecting the members of the eel genus *Anguilla* and caused by bloodsucking nematodes of the genera *Anguillicoloides* and *Anguillicola*). First published attempts to score the pathological signs in the infected organ dated back to Liewes & Schaminee-Main (1987), just a few years after the first reports of the Asian parasite *Anguillicoloides crassus* (Kuwahara, Niimi & Itagaki 1974) into European waters (for review, see Kirk 2003). Since then, the invasive species has span its geographical range over the one of its new host *Anguilla anguilla* (L.), and most parasitological

investigations yet integrate some measures of the swimbladder degradation along with other classical epidemiological parameters (i.e., parasite count). Infection-induced pathologies are primarily caused by larvae L3 (entering the eel *via* the ingestion of infected preys, crustaceans and small fishes essentially) that migrate to and within the swimbladder wall, where they feed and grow at the expense of the swimbladder tissues (De Charleroy, Grisez, Thomas, Belpaire & Ollevier 1990; Molnár, Szakolczai & Vetési 1995). Then, after moulting into L4 stages, they enter into the swimbladder lumen where pre-adults and adults actively feed on the capillary system irrigating the swimbladder organ (Würtz & Taraschewski 2000).

The combined action of both larvae and adults results in thickening, inflammation, haemorrhages, fibrosis, and changes in the epithelial cells of the swimbladder wall, as well as alterations of the gas secretion into the swimbladder, which may eventually lead to impaired functioning of the organ (Molnár, Baska, Csaba, Glávits & Székely 1993; Haenen, van Wijngaarden, van der Heijden, Höglund, Cornelissen, van Leengoed, Borgsteede & van Muiswinkel 1996; Würtz, Taraschewski & Pelster 1996; Nimeth, Zwerger, Würtz, Salvenmoser & Pelster 2000). In the most severe cases, after multiple and repetitive infection events, the eel swimbladder may turn into a compact ball of necrotic tissues, with no lumen left (Molnár *et al.* 1993; Würtz & Taraschewski 2000). A similar pathogenic situation is observed in the American eel host *A. rostrata* (Ooi, Wang, Chang, Wu, Lin & Hsieh 1996; Barse, McGuire, Vinores, Eierman & Weeder 2001; Sokolowski & Dove 2006), whereas no such pronounced effects were documented in the native Japanese host *A. japonica* (Egusa 1979; Nagasawa, Kim & Hirose 1994; Knopf & Mahnke 2004). Again, no severe pathological effects have been reported in *Anguilla australis* when infected by their historic nematode *Anguillicoloides novaezealandiae* (Lefebvre, Shuster, Münderle, Hine & Poulin 2004). So, it seems that highly pathogenic effects are only observed in the case of recent allopatric host-parasite interactions, which may explain the yet inappropriate host response and the uncontrolled parasite exploitation (Taraschewski 2006; Kennedy 2007; Sasal, Taraschewski, Valade, Grondin, Wielgoss & Moravec 2008). However, and whatever the exact causes, this "useless virulence" (to quote Combes 2001) creates a singular situation of infection-state dependence, in which the pathological consequences of previous infections limit and eventually prevent the establishment of new infection events (feedback effect) (Van Banning & Haenen 1990; Molnár, Székely & Perényi, 1994; Lefebvre, Contournet & Crivelli 2002). In such a context, the sole use of classical epidemiological parameters (i.e., intensity, abundance, prevalence) constitutes a severe pitfall in assessing the overall impact suffered by eels.

Indeed, the absence of living parasites at the autopsy may correspond to two opposite situations: either a very low or extremely high parasite pressure.

Variations in the observed degrees of pathological damages of the swimbladder were shown to be the best predictor of eel mortality in two independent studies conducted in semi-experimental conditions (Molnár 1993; Lefebvre, Contournet & Crivelli 2007). Also, one may reasonably question the chance of silver eels to reach their spawning site in the Sargasso Sea (6000 km transoceanic migration) when having an impaired swimbladder (Nimeth *et al.* 2000; Lefebvre, Acou, Poizat & Crivelli 2003; Münderle, Sures & Taraschewski 2004). For instance, in a recent mark-recapture study in the Baltic Sea, heavily infected silver eels appeared to cover less distance and to swim in shallower waters, lacking the typical vertical migrations that healthy silver eels normally do (Sjöberg, Petersson, Wickström & Hansson 2009). To that respect, the conclusion of the last international collaborative effort aiming to investigate the reproductive status of the European eel (EELREP project in 2005) was explicit enough: "in case of heavy swimbladder infection and/or damage… [silver eels]… will never reach the spawning grounds and cannot contribute to recruitment" (Palstra, Heppener, van Ginneken, Székely & van den Thillart 2007; Székely, Palstra, Molnár & van den Thillart 2009). For all the above exposed reasons, but also for epidemiological surveys and for comparative purposes between sites, dates and eventually also between eel species (cross-specific comparisons being thus very informative concerning the origin and the evolution of the disease), it turned to be essential to dispose of suitable measures to purposely assess the health state of the eel swimbladder.

Until recently, there existed six published attempts to score the swimbladder damages (Liewes & Schaminee-Main 1987; Csaba, Láng, Sályi, Ramotsa, Glávits & Rátz 1993; Hartmann 1994; Molnár *et al.* 1994; Beregi, Molnár, Békési & Székely 1998; Lefebvre *et al.* 2002), all based upon the severity of the gross pathologies as observed at dissection (see Table 1). In 2005, Palstra and co-workers in the EELREP project introduced an alternative index based on the observed shortening of the swimbladder as a result of infection (thereafter LRI, for Length Ratio Index, also see Palstra *et al.* 2007). This metric is *de facto* promising and offers interesting new perspectives (especially when coupled with non-invasive radio-diagnostic methods), but its biological rationale needs to be validated and its scores compared to the so far prevailing index (i.e., the SDI, for Swimbladder Degenerative Index, see Lefebvre *et al.* 2002).

The primary objective of the present study was thus to compare and critically evaluate the two alternative approaches in using data from a same set of fish. For doing so, we first

defined the expected attribute of a 'good' index, and then investigated the performance of our two indices on the following selected criteria: representativeness, objectivity, precision and easiness. Also, the condition of isometric growth between the swimbladder and the total body size will be checked (in the absence of any infection sign), as a prerequisite to validate the new LRI. Based on the herein obtained results, the pros and cons of the two alternative indices will be drawn and consensual recommendations will be suggested.

Materials and methods

Detailed historic of the swimbladder damage assessments

In the literature, previous attempts to score the swimbladder damages at dissection (see Table 1) all integrated a thickness component for the swimbladder wall, eventually performing a graduated measure. They also stressed on the progressive loss of the natural yellowish transparency of the swimbladder wall that turns into a smoke-like opacity in case of infection. In addition, it was argued that, besides the observed changes in the swimbladder wall, the amount of exudate and remains within the swimbladder lumen was also an important factor for evaluating the severity of the past infections. Probably because of the difficulties in clearly distinguishing between the different grades (some mixing histo-pathological changes with the presence/absence of lumen worms), and also because of the confidentiality of some original publications (in Dutch for Liewes & Schaminee-Main 1987; in Hungarian in a national journal for Csaba *et al.* 1993; PhD thesis in German for Hartmann 1994), these early attempts never entered in the common use. In the continuity of previous workers, Lefebvre *et al.* (2002) proposed a codified metric (SDI, see below), based on the cumulated values in three clearly defined criteria to be scored individually. It became for a time the most popular index at hand (as evaluated, for instance, by the ISI citation reports).

The Swimbladder Degenerative Index

The assessment was based on macroscopically visible alterations, as observed in the swimbladder organ when removed from the rest of the body. Three criteria were used, each one being coded by the values 0, 1 or 2 (increasing degradation). The first criterion focused on the opacity of the swimbladder wall. A value of 0 was assigned to normal-looking swimbladder (i.e., transparent-yellowish coloration, see Clarke & Witcomb 1980). Total opacity (when no reading is possible through the swimbladder wall) was assigned a value of 2, and all intermediate cases a value of 1. The second criterion examined the presence of

pigmentation and exudate instead of gas in the swimbladder lumen (dead worms, erythrocytes, decaying swimbladder tissue, eggs and L2 stage of *A. crassus*). A value of 0 was given to swimbladders with no pigmentation and no exudate inside. A value of 2 was assigned to swimbladders that exhibited both pigmentation and exudate, and value 1 to those that show either signs of pigmentation or exudate. The third criterion concerned the thickness of the swimbladder wall. The codification was adapted from an early proposition by Molnár *et al.* (1994): a value of 0 was assigned to thin walled-swimbladders (less than 1 mm), a value of 2 was given to swimbladders with little if any lumen left (more than 3 mm thick wall), and value 1 to all other intermediate cases. Thus, individual criteria can be conveniently and quite safely scored applying extreme values (0 or 2) to normal-like and severely degradated swimbladders, respectively. In cases of doubt, and for all intermediate situations, a medium value of 1 is applied. The SDI is then computed by adding the scores obtained for the three separated criteria, and so may span over seven discrete values, ranging from 0 to 6.

The Length Ratio Index

In 2005, arguing for subjectivity in the computation of the SDI, the EELREP document warned for possible mis-interpretations in using the above index, and introduced a synthetic but easy metric for capturing overall pathological damages (i.e., LRI, later discussed in Palstra *et al*. 2007). They based their index on the observation that swimbladders thicken and shorten as a result of multiple -repetitive- infection events (also see, for instance, Würtz & Taraschewski 2000), so that the severity of the pathology could be tentatively encapsulated into the linear measure of the infected organ (in relation to body size, by dividing the swimbladder length by the total length). In other terms, the higher the parasite pressure suffered by the eel, the shorter the relative size of the swimbladder, and so the smaller the resulting LRI score.

General considerations on indexing

The process of indexing is closely similar to the process of sampling, in the sense that the otherwise complete operation (measuring the true value through exhaustive enumeration or measurements) is either unsustainable or impracticable, and finally unnecessary if the very nature and properties of the estimators are properly considered. Expected attributes of a good index might thus closely match those expected for an adequate sample or sub-sample. Here is a proposed list of criteria of decision for evaluating any index metric (for similar

considerations, see for instance Bolger & Connolly 1989, in selecting suitable body condition indices):

a. Representativeness (or the degree of capability to reflect the biological characteristics at measure). A good index might provide useful and meaningful information, while being able to accurately discriminate among a continuum of possible biological values. In our case, with the two indices at hand, we aim to score the damages done to the swimbladder, as a mean to ultimately evaluate the functional impact of the infection on the eel hosts. The 'true' biological value to investigate is here tentatively assumed to be close to the loss of internal swimbladder volume. Indeed, the eel swimbladder is an organ involved in many important functions such buoyancy control, gas exchange, absorption and secretion (Zwerger, Nimeth, Würtz, Salvenmoser & Pelster 2002; Tesch 2003), so that any reduction in the lumen gas volume is expected to severely impact on the general metabolism and physiology of the fish (Würtz *et al.* 1996; Palstra *et al.* 2007).

b. Precision (or the degree of achievable reproducibility between repeated measurements). A good index might not vary substantially in its score between separate measures, and be closely reproducible under similar conditions. In our case, the swimbladder is a delicate, flexible and inflatable organ, and all related data (length or pathological signs) are expected to vary to some extent between repeated measures. Here, the precision (or inversely the measurement error) of the indices could be investigated by recording twice the scores of the same observers (i.e., intra-observer variability).

c. Objectivity (or the degree of concordance between multiple observers). A good index might be unequivocally understood and so clearly defined to minimize the inter-observer variability (subjectivity). In our case, the swimbladder in embedded in a conjunctive tissue at both poles so that its delimitation may differ from observers to observers. Also, when the criteria used to assess the pathological damages do not correspond to objective metric measures (as for the SDI), it is *de facto* attached to some subjectivity. Here, the objectivity of the indices could be tested by recording the scores of two independent observers (i.e., inter-observer variability).

d. Easiness (or the degree of simplicity in the measurement, computation or interpretation). A good index might thus be cost- and time-effective enough to ensure a widespread application among possible end-users (i.e., managers, researchers), and to ultimately serve comparative purposes between individuals, or to track changes between years or between areas. In our case, both indices are logistically easy to generate (as compared to the 'true' value, herein approximated by the internal swimbladder volume), and to understand (the LRI decreases and the SDI increases with the severity of the observed damages). Here, the retained criterion

would be the time spent in recording the necessary data for computing each index, i.e., the histo-pathological aspect on one side and the swimbladder length on the other side.

Detailed protocol for comparing the two indices

Eels originated from the brackish waters of the Vaccarès lagoon in the Rhône river delta (Camargue, Southern France). Several fyke nets (6 mm mesh in the funnels) were set on eight consecutive days in the third week of June 2009. On the day of capture, eels were placed in a tank of 10-20 cm water depth, and killed by adding an overdose of anaesthetic (0.5 ml/l Eugenol).

Animals were examined fresh, closely following the protocols as given in the original publications (i.e., Lefebvre *et al.* 2002 for the SDI; Palstra and co-workers in EELREP 2005 for the LRI). The total body length of the eel (L_T) was recorded to the nearest 1 mm (from the tip of the snout to the tip of the tail), and the vertical and horizontal diameters of both eyes (D_V and D_H) were measured to the nearest 0.1 mm with a Vernier calliper. Eels were then ventrally opened, sexed whenever possible (for individuals longer than 300 mm), and the swimbladder together with the *ductus pneumaticus* (in functional connection with the swimbladder in physostome fishes, see Pelster 1998) were carefully detached from the rest of the body. The naturally gas-filled organ was immerged in a water-filled graduated tube, and the volume of water displaced (to the nearest 0.1 cm^3) was used to approximate the total inflated volume of the swimbladder (including the *ductus pneumaticus*).

Swimbladders were then measured to the nearest 1 mm with a Vernier calliper (L_S, natural extended length) (see text in page 70 and Fig. 4, in EELREP 2005). Observed pathologies were recorded using the SDI by attributing individual scores (from 0 to 2, increasing damages) to the following three criteria of opacity, abundance of pigmentation/exudate, thickness of the swimbladder wall (see above). To estimate objectivity and to quantify the precision in the measurements, the L_S and the SDI scores were taken twice by two different observers. So, for each eel, after a first set of measures by observer 1, measurements were taken by observer 2, then repeated again by observer 1, and finally one last time by observer 2. Throughout, the duration of each of these operations (metric measurements and damages assessments) was recorded to the nearest 1 s, so that the elapsed time could be used to estimate the easiness in scoring the two indices.

At this stage, the swimbladder (and the *ductus pneumaticus*) was fully cut-opened and the number of living lumen worms was recorded (pre-adults and adults). The volume of parasites (in case of current infection) and the deflated volume of the swimbladder (after total removal

of the internal gas, and eventually exudate and other dead worms remains) were estimated using again the volume of water displaced after full immersion in a water-filled graduated tube.

Analyses and statistical treatments

The stage of maturation of the eels was evaluated using the Ocular Index (based on the relationship between the mean dimensions of both the right and left eyes and the total body size), according to the Pankhurst's formula: $OI = ((D_V + D_H) / 4)^2 \times (\pi / L_T) \times 100$. Only those eels that met the threshold value of $OI \geq 6.5$ were considered in the silvering process (i.e., onset of sexual maturation, see Pankhurst 1982). The internal volume of the swimbladder (V_S in cm^3, including the *ductus pneumaticus*) was calculated by removing the deflated volume (open swimbladder), and eventually the volume of parasites (in case of infection), to the full inflated volume (as measured immediately after dissection). Normality was studied with the Shapiro & Wilk (*W*) test because of its good power properties for small to medium sized samples (StatSoft Inc. 2001). Central tendencies were expressed by arithmetic means ± standard deviations (SD), or by the median values ± 25th percentiles (0.25-0.75 quartiles interval) in case of severe departure from normality. The amount of variability around the two indices (either between the two observations of the same observers or between the two observers for the same set of observations) was compared using the coefficient of variation (CV) which is the SD expressed as a percentage of the mean (and calculated from the average of each pairwise CV). It is an absolute measure of dispersion in the sense that it is independent of the unit employed, and of the magnitude of the mean (Sokal & Rohlf 1995). Apart from their use in estimating variability, the scores obtained in the four observations of the same swimbladder were pooled as arithmetic means (and approximated back to the closest integral in the case of the SDI), so that $n = 71$ for all variables except otherwise mentioned. Non-parametric Spearman rank (*Rs*) correlations, Mann & Whitney (*U*) tests and Wilcoxon matched-pair (*T*) tests were applied wherever conditions for parametric analyses were violated. All statistical treatments and graphics were performed using Statistica 6 (StatSoft Inc.).

Results

A sample of 71 eels was collected in the Vaccarès lagoon over the fishing week. Unfavourable wind conditions (limiting the movements of the eels) and an occasional bloom of jellyfishes (clogging up the meshes of the nets) may explained the low amount of capture

(as compared to previous records in the same area, unpublished data of Alain J. Crivelli and the Tour du Valat station). The L_T ranged from 188 to 761 mm, for a mean value of 428.07 ± 135.46 mm. The overall length frequency was normally distributed (Shapiro & Wilk test: W = 0.98, P = 0.21), and at the very exception of the youngest elver eels (too small to be caught by the 6 mm mesh of the nets) all size classes were represented in the sample. Nearly 1/4th of the eels were under silvering process according to the criterion of Pankhurst (IO ≥ 6.5, n = 17), and the sex-ratio was strongly female-biased (≈ 8% males). The number of adults and pre-adults *A. crassus* in the eel swimbladder (and *ductus pneumaticus*) ranged from 0 to 21, for an overall mean abundance of 2.17 ± 4.04 worms per host (median abundance: 0.00 ± 0.00-2.00; prevalence: 41%, n = 29; mean and median intensities: 5.31 ± 4.85 and 3.00 ± 1.00-8.00). The parasite distribution strongly deviated from normality (Shapiro & Wilk test: W = 0.61, P < 0.001), and best fit with a negative binomial function (i.e., aggregated distribution with few eels harbouring many parasites while most having just a few if any).

The two indices and the measure of swimbladder volume

a. SDI

The obtained SDI scores extended over the seven possible values of the index (i.e., from 0 to 6). However, the frequency distribution of the SDI was skewed to the right (i.e., right tailed; see Fig. 1a), and significantly deviated from normality (Shapiro & Wilk test: W = 0.90, P < 0.001). More than half of the sample (43/71 = 61%) showed a SDI ≤ 2 (slightly or not degradated swimbladders) for an overall median value of 2.00 ± 1.00-3.00 (Fig. 1a). Compared to previous years in the same area (see for instance Lefebvre *et al*. 2002), the swimbladders of the 71 investigated eels can be considered quite healthy, with only a minor fraction (10/71 = 14%) showing SDI values ≥ 5 (severely degradated swimbladders). Multiple Spearman correlations revealed that the two SDI criteria of opacity and thickness were highly auto-correlated (Rs = +0.84, P < 0.001), whereas the presence of pigmentation/exudate was relatively independent of the other two (*vs* opacity: Rs = +0.50, P < 0.001; *vs* thickness: Rs = +0.63, P < 0.001). Overall, the criteria of thickness best fit with the final SDI value (thickness *vs* SDI: Rs = +0.92, P < 0.001; opacity *vs* SDI: Rs = +0.87, P < 0.001; pigmentation/exudate *vs* SDI: Rs = +0.78, P < 0.001). Throughout the sample, the SDI score increased with the size of the eel (Rs = +0.31, P < 0.01) and with the parasite abundance (Rs = +0.30, P < 0.05), but there was also a trend for long eels to harbour more parasites (L_T *vs* abundance: Rs = +0.23, P = 0.06). Overall, silver eels showed a significantly higher SDI score than the rest of the

sample (median values: 4.00 ± 2.00-5.00 *vs* 2.00 ± 1.00-3.00; Mann & Whitney test: U = 206.50, $P < 0.001$).

b. LRI

The obtained LRI scores ranged from 0.04 for the most severely degradated swimbladders to 0.20 for healthy ones (Fig. 1b). The frequency distribution did not deviate from normality (Shapiro & Wilk test: $W = 0.97$, $P = 0.14$) with a mean value of 0.13 ± 0.02. For comparison purpose, in Palstra *et al.* 2007, the LRI values of silver eels ranged from 0.03 to 0.19 for a mean of 0.11 ± 0.03. So, based on the yet two available studies, it seems that the swimbladder length can vary from 1/5th or 20% of the total body length (upper relative size in normal condition) to 1/33th or 3% when severely damaged. Within the sub-sample of healthy swimbladders (harbouring no parasite and having a SDI value ≤ 2, $n = 32$), the swimbladder length L_S (numerator of the LRI) was almost normally distributed (Shapiro & Wilk test: W = 0.93, $P = 0.03$; Kolmogorov & Smirnov test: $D = 0.13$, $P > 0.20$; mean: 51.15 ± 15.75 mm), as well as the total length of the eel L_T (Shapiro & Wilk test: $W = 0.95$, $P = 0.18$; mean: 386.09 ± 108.52 mm). For these healthy eels, the relationship between the two components of the LRI significantly fitted with a straight line (Fig. 2a; $R = +0.83$, $P < 0.001$). Attempts to transform one or the two of the variables did improve the R value (i.e., natural logarithm of both variables: $R = +0.86$; inverse function of both variables: $R = +0.88$), while the same transformations did not work on the whole sample ($R = +0.79$ on natural scaled values; R = +0.81 on Log transformed values, $R = +0.80$ on inverse transformed values). In other terms, in the absence of any infection sign, the eel body length may account for more than 75% of the swimbladder length variability ($R^2 = 0.77$ for the inverse function, $n = 32$), and so the condition of isometric growth between the two variables of the ratio index was here ascertained. Throughout the whole sample, there was a trend for the LRI to decrease with L_T ($Rs = -0.21$, $P = 0.08$) and to increase with parasite abundance at dissection ($Rs = +0.21$, P = 0.08), but there was also a trend for long eels to harbour more parasites (L_T *vs* abundance: Rs = +0.23, $P = 0.06$). Overall, silver eels showed a significantly lower LRI score than the rest of the sample (median values: 0.12 ± 0.11-0.13 *vs* 0.14 ± 0.12-0.15; Mann & Whitney test: U = 281.00, $P < 0.05$).

c. swimbladder volume

The internal swimbladder volume, V_S, ranged from 0 to 13.4 cm^3. The frequency distribution strongly deviated from normally (Shapiro & Wilk test: $W = 0.80$, $P < 0.001$) and was highly

right-skewed (best fit by a Log-normal distribution) with a median value of 1.00 ± 0.40-3.80 cm^3 (Fig. 1c). For the sub-sample of healthy swimbladders (harbouring no parasite and having SDI values ≤ 2, n = 32), V_S positively and significantly correlated with L_T (Rs = +0.91, P < 0.001). Multiple transformations were applied to best relate the two variables (including polynomial, power, square root, logarithmic functions). The relationship between L_T and V_S was found to best fit with the power 3 function: $V_S = -0.468 + 0.320.10^{-7}*L_T^3$ (R = +0.89, P < 0.001, see Fig. 2b). Then, using the above formula, an expected V_S (i.e., swimbladder volume in the absence of degradation and parasite) can be inferred for all eels (Fig. 1d), so that it became possible to estimate the gas loss due to the infection (% gas loss = (Vexp. - Vobs.)/Vobs.*100).

Comparison between the two indices

There was a significant negative relationship between the SDI and the LRI (Rs = -0.38, n = 71, P < 0.005). In other terms, the higher the SDI score, the smaller the LRI value, and the more severe the corresponding damages in the swimbladder (Fig. 3 and Fig. 4).

a. Representativeness

There was a significant positive relationship between the estimated percentage of gas loss and the SDI (Rs = +0.27, P = 0.03), so that the higher the SDI score the smaller the relative remaining swimbladder volume (Fig. 4). In contrast, there was a significant but negative relationship between the LRI and the estimated percentage of gas loss (Rs = -0.33, P < 0.01). So, the higher the swimbladder volume reduction, the smaller the LRI value, and the higher the SDI score (Fig. 4). Assuming that the percentage of gas loss was properly estimated and closely represents a functional measure of the parasite impact (relative swimbladder volume reduction), then the LRI slightly better perform (although still poorly) in term of biological significance.

b. Precision

The intra-observer variability between the two sets of repeated measures on each of the 71 investigated eels is given in Table 2 for the SDI and the L_S. The SDI showed a much higher coefficient of variation than the L_S (23.97% *vs* 5.08%, combined data for observer 1 & 2, n = 142) which indicated that the swimbladder length was taken with higher repeatability (and so better precision). However, it must be emphasized here that the LRI may suffer additional measurement errors from measuring the total length of the eel (i.e., L_T, the denominator of the

index). From simple calculation, setting a CV on L_T of similar amplitude as for L_S (which actually corresponds to a relatively large measurement error of 5.08*428.07/100 = 22 mm) and applying the approximating formula $CV(X/Y) = [CV(X)^2 + CV(Y)^2]^{1/2}$ (see for instance Oyejola & Mead 1989), the overall coefficient of variation for the LRI would be of about 7%, which is still much smaller than the 24% obtained for the SDI. The intra-observer variability on scoring the SDI slightly co-varied with the index value, whether measured by the SDI score (mean *vs* SD: $Rs = +0.18$, $n = 142$, $P = 0.04$) or by the LRI (mean *vs* SD: $Rs = -0.12$, $n = 142$, $P = 0.17$), whereas the intra-observer variability on measuring the L_S strongly co-varied with the index value (mean *vs* SD for LRI: $Rs = -0.22$, $n = 142$, $P < 0.01$; mean *vs* SD for SDI: +0.20, $n = 142$, $P = 0.02$). Since the two indices inversely varied in scoring the observed damages (see above section), this would indicate that the intra-observer error of measure on both indices tended to increase for severely degradated swimbladders (i.e., better precision for healthy swimbladders).

c. Objectivity

The inter-observer variability over the two sets of independent measures on each of the 71 investigated eels is given in Table 3 for the SDI and the L_S. The SDI showed a much higher coefficient of variation between the two observers (33.38% *vs* 4.23%, combined data for the two sets of observations, $n = 142$) which may reflect a higher subjectivity on scoring the observed pathological signs. Again, if setting a CV on L_T of similar amplitude as for L_S (i.e., 4.23%) and applying the approximating formula $CV(X/Y) = [CV(X)^2 + CV(Y)^2]^{1/2}$, then the overall CV for the LRI would be of about 6%, still very much smaller than the 33% obtained for the SDI. For the linear measure of the swimbladder (L_S), the parameter of variability significantly co-varied with the index value, whether measured by the LRI ($Rs = -0.20$, $n = 142$, $P = 0.02$) or by the SDI ($Rs = +0.33$, $n = 142$, $P < 0.001$), which would indicate that the subjectivity over the swimbladder limit was higher for severely degradated swimbladder (i.e., better objectivity for healthy swimbladders). In contrast, the inter-observer variabilities on the SDI appeared relatively independent of the observed pathologies (mean *vs* SD for SDI: $Rs = -0.03$, $n = 142$, $P = 0.73$; for LRI: $Rs = -0.1$, $n = 142$, $P = 0.94$). In the details however, the subjectivity was maximal for intermediate SDI values and minimal for extreme scores (i.e., better objectivity for slightly and severely degradated swimbladders).

d. Easiness

The individual time necessary for assessing the SDI ranged from 6.17 to 37.46 s for a mean value of 15.49 ± 5.68 s (n = 284, median: 14.30 ± 11.48-18.10 s). The necessary time for taking the length of the swimbladder (L_S) ranged from 7.40 to 71.74 s for a mean value of 22.67 ± 7.70 s (n = 284, median: 21.48 ± 18.12-26.70 s). Both measures of time strongly deviated from normality (Shapiro & Wilk tests: $W < 0.94$, $P < 0.001$). No significant difference was observed between the duration of the first and the second measurements when measuring L_S (Wilcoxon matched-pair tests: $T = 1120.00$, $n = 71$, $P = 0.37$, for observer 1; for observer 2: $T = 1124.50$, $n = 71$, $P = 0.38$). When considering the SDI, a significant difference was revealed for one of the observers (observer 1: $T = 674.00$, $n = 71$, $P < 0.001$; observer 2: $T = 1086.00$, $n = 71$, $P = 0.27$), but not in the sense of a shorter time for the second measurement (median time: 16.04 ± 11.89-18.35 s for observation 1 *vs* 18.64 ± 13.66-23.35 s for observation 2) which could have indicated a non-independence between the two set of measures (e.g., imprinting of the first score). No significant difference was observed between the time needed by the two observers when measuring L_S (observation 1: $T = 1078.50$, $n = 71$, $P = 0.25$; observation 2: $T = 993.50$, $n = 71$, $P = 0.10$). When scoring the SDI, a significant difference in time was consistently revealed between the two observers (observation 1: $T = 869.00$, $n = 71$, $P < 0.05$; observation 2: $T = 286.00$, $n = 71$, $P < 0.001$), but not in the sense of a shorter time for the observer (FL) who was the most familiar with that index (median time over the two set of observations: 16.56 ± 12.95-21.97 s for observer 1 *vs* 12.90 ± 10.78-15.44 s for observer 2). Overall, when comparing the time needed for each of the index (average of the 4 individual measures per eel, $n = 71$), a Wilcoxon matched-pair test revealed that the SDI was significantly shorter to compute (median time: 15.51 ± 13.64-17.36 s for the SDI *vs* 22.02 ± 18.78-25.24 s for L_S; $T = 60.00$, $P < 0.001$). Since the computation of the LRI also requires a measure of the total length of the eel (L_T), the SDI is obviously faster to generate. There was no significant correlation between the necessary time for assessing the SDI and the severity of the corresponding damages (SDI time *vs* SDI score: $Rs = -0.20$, $n = 71$, $P = 0.09$; SDI time *vs* LRI value: $Rs = +0.09$, $n = 71$, $P = 0.46$). Similarly, no significant correlation was revealed between the time needed for taking the swimbladder length (L_S) and the severity of the pathological damages whether assessed by the LRI or the SDI (L_S time *vs* LRI value: $Rs = -0.21$, $n = 71$, $P = 0.08$; L_S time *vs* SDI score: $Rs = +0.05$, $n = 71$, $P = 0.66$). Although none of the above correlations was statistically significant they tend however to indicate that the relationship between the scoring time and the swimbladder degradation differed for the two indices; for the SDI the more severe the degradation the shorter the necessary time to attribute

an index value, whereas for the L_S the more severe the pathologies the longer the time needed to measure the organ.

Discussion

This work aimed to compare the performance of two alternative tools for measuring the eel swimbladder degradation caused by the invasive nematode *A. crassus*. Since the present study was co-written by the main authors of the two prevailing indices (FL and AJC on the one side, APP and CS on the other side), it represents a joined collaborative effort to critically evaluate the tools at hand, and to address consensual but clear-cut recommendations for all possible end-users.

Pros & cons of the Swimbladder Degenerative Index

Assessing the SDI score necessarily refers to normal -unaffected- swimbladders, and so requires having a visual imprinting of the look-alike of a healthy organ (that would score 0 in its three histo-pathological criteria). To that purpose, illustrations of normal-looking and degradated swimbladders are herein provided on Fig. 5, or can be found alternatively on the internet, for instance, at the following URL: http://cbetm.univ-perp.fr/pages/francais/biblio/publis/THESE-Fazio.pdf (PhD thesis of GF, 2007, Fig. 23, p. 50). The SDI also implies a kind of previous training period in order to get used with the different severity grades it may take. In the present investigation, one of the observer (GF) was not familiar with the SDI scoring (in comparison to FL) but finally proved to report closely similar SDI values (overall mean: 2.29 *vs* 2.39; median value of 2 in both cases), in similar (even shorter) time. Actually, the difficulties inherent to any categorization process were here partly overcome thanks to the use of three clearly defined criteria, each being scored separately with three possible values. Moreover, because the auto-correlation between the three criteria proved to be strong but not total ($+0.50 \leq Rs \leq +0.84$, $P < 0.001$), the use of multiple criteria allows encapsulating additive information, while partial redundancy tends to inflate extreme values, and so the discrimination power of the index. So designed, the SDI is quite meaningful as a degradation index because directly based on the severity of observational pathologies, and easy to work out because bounded within constant -pre-defined- upper and lower limits.

Nonetheless, the computation of the SDI involves making categories instead of reporting observed metric of underlying continuous variables, and so is intuitively more prone to subjectivity. Subjectivity (or inter-observer variability) was one the main critics of the SDI (EELREP 2005). We showed here that the subjectivity in scoring the SDI was indeed much higher than the subjectivity in properly defining the limit of the swimbladder organ for the need of the LRI (coefficient of variation: 33% *vs* 4%, respectively).

Also, categorization can affect the magnitude and the outcome of statistical tests (by changing for instance the response distribution, see Bollen & Barb 1981; Lemon 2009), and the cruder the categories, the greater the discrepancy between the obtained score and the true value. And, because the SDI is entirely based on the pathological status of the swimbladder (and not a weighted-based index as for the LRI), it comes with some inherent statistical problems such as the non-normality of the response distribution (e.g., asymmetry with regard to small and large values). Let imagine a sample of eels entirely and intensively affected by the parasite, then the distribution of the SDI scores will tend to cluster around the upper limit value of 6, which may impose applying specific but limited (i.e., non-parametric) tests in studying the SDI variable in relation to other host or environmental parameters.

Pros & cons of the Length Ratio Index

We showed here that the swimbladder length increased almost linearly with the body length, a way to ascertain that the swimbladder organ follows an isometric growth with the rest of the body. This is a crucial verification in the absence of which the LRI values could not be comparable between eel size classes. Under this assumption, a small LRI value indicates a shortened swimbladder (as a result of infection) relatively but independently to the size of the eel.

The LRI is intuitively a meaningful and easy measure because based on the ratio of two linear -metric- dimensions (swimbladder length divided by total eel length). On practice, the LRI better performed in comparison to the SDI concerning the pre-defined criteria of representativeness, objectivity and precision. In contrast, and quite surprisingly, the LRI proved to be longer to generate than the SDI. It is mainly due to the difficulties in properly extending the swimbladder for the linear measurement and assessing the physical limit of the organ (embedded in connective tissues), especially for severely degradated swimbladder (see Fig. 5b). To the best of our experience, this can be partly overcome in placing the organ on absorbing paper so that the swimbladder sticks on the support and remains extended for the time of the measurement.

The main advantage and most interesting property of the LRI lies in the fact it can be computed using radio-diagnostic imageries so that the histo-pathological status of the swimbladder (and eventually the presence of swimbladder worms) can be assessed without causing damage to the fish (non-invasive method). Indeed, Székely and co-workers repeatedly demonstrated that the diagnostic obtained by X-ray radiography on live -anaesthetised- eels showed good agreement with naked-eye examinations of the dissected organ (Beregi *et al.* 1998; Székely, Molnár, Müller, Szabó, Romvári, Hancz & Bercsényi 2004; Székely, Molnár & Rácz 2005).

As for the SDI, the computation of the LRI (i.e., a weighted ratio of two correlated variables) comes with statistical and practical problems. It mainly revolves around the complex response distribution of a ratio (Creasy 1954), and the inherent difficulties in calculating a standard error or other measures of dispersion (Oyejola & Mead 1989). This was illustrated here with the call for Taylor's approximations to actually be able of generating a coefficient of variation around the measures of the LRI.

Another possible pitfall of the LRI, more biologically oriented this time, could concern the yet unclear interaction between the nematode infection and the growth of the eel. Indeed, in the eventually of interaction with body growth what would be then the rationale of using the eel length as a scaling factor for estimating the swimbladder degradation? So far, most studies on that aspect did not detect any significant impact, but some recent results tend to indicate that infected eels could actually grow faster (Fazio, Moné, Lecomte-Finiger & Sasal 2008; upcoming papers by FL, GF and AJC), while other suggested that the infection may inversely decrease the fish growth (Liewes & Schaminee-Main 1987; van Banning & Haenen 1990; Gollock, Kennedy, Quabius & Brown 2004). Obviously, the actual impact of the infection on the host life history traits remains to be better explored, but for now, in the absence of clearly demonstrated (and published) effect, one might stick on the position that the eel length is not, at least severely, affected by the nematode infection.

Consensual recommendations and conclusion

The two indices have both either actual or potential intrinsic limitations. Based on the herein results however, we recommend to start applying extensively the LRI. For comparative purposes at least, we can only suggest to keep scoring the historic index (SDI) when having dead eels at hand, but otherwise, and for obvious conservative reasons, to adopt the LRI in combination with radiographic methods. Indeed, the SDI cannot be estimated without dissecting the eel since the pathological criteria of opacity and thickness cannot be properly

scored on radiographies. The LRI is thus to become a new alternative tool in all those situations where non-invasive methods are *de facto* needed (e.g., over-years monitoring). Also, a crucial question to be investigated that way: can damaged swimbladder recover in the absence of re-infection? Using radio-diagnostic methods, Székely *et al.* (2005) did not remark any significant improvement after three months in the absence of re-infection, but longer term monitoring would be welcome.

Based on the herein data, and to facilitate the shift, we provide a graph of correspondence between the scores of the two indices and the estimated loss in swimbladder volume (see Fig. 4). Thus, it now becomes possible to say that, for instance, a SDI score of 3 approximately corresponds to a LRI value of 0.12, and that significant gas loss start to occur at about SDI = 2 or LRI = 0.13.

For both indices, we noted that the time between dissection and swimbladder assessment tend to overestimate the actual damages (increased opacity, thickness and overall shrinkage of the organ). So, when working with dead specimens, we strongly advise to measure the swimbladder length, or alternatively to assess the SDI, immediately after dissection of the fish (conservation at open air, in closed tubes or in tap water proved to be inefficient).

More work would be welcome to precisely assess the factors that may interact with the swimbladder measures. For instance, we know that the silvering process induces some morphological, physiological and histological changes in the swimbladder organ (Durif, Van Ginneken, Dufour, Müller & Elie 2009; also see Kleckner 1980 in *A. rostrata* and Yamada, Zhang, Okamura, Tanara, Horie, Mikawa, Utoh & Oka 2001 in *A japonica*), but what is the actual outcome on the SDI and LRI measurements? In our sample, silver eels had their swimbladder significantly more degradated than the rest of the sample, and the difference was particularly marked looking at the SDI scores, but more work is clearly needed (especially taking into account the eel size effect). We encourage people working on silver eels and having fresh material at hand for other research purposes to test the effect of silvering on the two indices, and to communicate their findings.

In conclusion, we now dispose of two alternative and workable tools to follow the epidemiology of the infection and its consequences. We hope this will ultimately help estimating the net losses due to anguillicolosis. Eventually this parameter may yield to calibrate populational models to help propose appropriated management measures for saving this emblematic but endangered fish species.

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Table 1 Severity grades of damage in the first 5 published attempts to score the swimbladder health of eels *Anguilla anguilla* as a result of infections by the nematode *Anguillicoloides crassus*

Liewes & Schaminee-Main 1987	Csaba *et al.* 1993	Hartmann 1994	Molnár *et al.* 1994	Beregi *et al.* 1998
grade 1: normal swimbladder, no worm **grade 2:** normal swimbladder, a few worms **grade 3:** enlarged swimbladder, partly filled with red-brown fluid; wall can be inflated **grade 4:** enlarged swimbladder, filled with red-brown fluid; actively moving nematode larvae can be noticed **grade 5:** rupture of the swimbladder wall or *ductus pneumaticus* which is highly irritated. Secondary infections of surrounding tissues externally visible as a swollen and inflamed abdomen **grade 6:** swimbladder wall replaced by a thick layer of connective; remainders of the nematodes can be found **grade 7:** swimbladder replaced by a hard brown-black mass in which remainders of the nematodes can be found	**grade 1:** normal swimbladder, no worm **grade 2:** normal swimbladder, containing a number of worms **grade 3:** enlarged swimbladder, partly filled with brownish liquid, wall with inflammatory processes **grade 4:** greatly thickened wall, lumen filled with brownish liquid containing larvae L2 actively moving **grade 5:** wall of the *ductus pneumaticus* burst, secondary infections arise **grade 6:** thick wall, remains of lumen worms **grade 7:** hardened tissues, remains of worms, liver often of pale color	**grade 1:** swimbladder without histological changes. Wall transparent, 0.6 to about 1.0 mm thick (depending on the size of the individual) **grade 2:** weak histological changes in the swimbladder wall. Swimbladder with slightly thickened wall and little opacity **grade 3:** strong histological changes in the swimbladder. Wall opaque and less elastic. Swimbladder lumen reduced, but filled with gas. **grade 4:** histological changes and other pathological effects. Swimbladder as in grade 3, plus haemorrhage, inflammation, necrosis, and exudates. Lumina filled with strong pigment deposits, lumen narrowed by the remains of nematode tissues. Swimbladder severely damaged, but still partly functional **grade 5:** advanced histological changes in the swimbladder wall and loss of function. Wall up to 5 mm thick, lumen narrow, gasless and constricted	**grade 1:** swimbladder wall less than 1 mm, although exhibiting signs of passed-off infections (smoke-like opacity, parasitic nodules, pigmentation, minor haemorrhages, etc.) **grade 2:** swimbladder with a 1-3 mm thick wall **grade 3:** swimbladder wall thickness exceeding 3 mm	**grade 1:** swimbladder transparent and thin-walled, with the wall thickness not exceeding 0.3 mm. Pneumatic duct not containing air. Swimbladder collapsing after opening **grade 2:** swimbladder normally filled with air; wall slightly opacified, sometimes containing haemorrhages but not exceeding 1 mm in thickness. Lumen usually containing only a few worms of small size. In some cases, worms also in the lumen of the air-filled pneumatic duct **grade 3:** the dilated, air-filled lumen of the swimbladder containing a small amount of exudates and numerous worms (hollows in the wall). Dilated lumen of the pneumatic duct often containing worms. Wall opaque and slightly thickened (up to 2 mm but only near the two ends of the sac) **grade 4:** one or both ends of the swimbladder atrophied, and sometimes one of its halves completely devoid of air. Lumen containing more or less exudates and few small dead worms. Wall markedly thickened: 2 or 3 mm in the middle third, and even 3 to 5 mm at the ends. Pneumatic duct looking like an airless bundle **grade 5:**

	swimbladder markedly shrunken. Rigid wall containing neither air nor any other material (exudates, worms), uniformly and markedly thickened, reaching 2 to 5 mm. Pneumatic duct thin-walled, containing no air

Table 2 Central tendencies and intra-observer variabilities on the measures of swimbladder length (L_S) and Swimbladder Degenerative Index (SDI), for the 71 investigated eels

	Observer 1 (FL)		Observer 2 (GF)		Observers 1 & 2	
	L_S (mm)	SDI	L_S (mm)	SDI	L_S (mm)	SDI
mean ± SD[a]	54.67 ± 18.37	2.39 ± 1.65	55.06 ± 18.46	2.29 ± 1.74	54.87 ± 18.38	2.34 ± 1.69
median ± 25th percentiles[b]	55.00 ± 39.00-69.00	2.00 ± 1.00-3.00	54.00 ± 39.00-71.00	2.00 ± 1.00-3.00	55.00 ± 39.00-69.00	2.00 ± 1.00-3.00
average pairwise mean[c]	54.67	2.39	55.06	2.29	54.87	2.34
average pairwise SD[d]	± 2.52	± 0.51	± 2.76	± 0.33	± 2.64	± 0.42
average pairwise CV[e]	4.86%	28.82%	5.31%	19.12%	5.08%	23.97%

[a] mean and measure of dispersion over the two sets of repeated measures (n = 142 observations for observer 1, n = 142 for observer 2, n = 284 for observers 1 & 2).
[b] median and measure of dispersion over the two sets of repeated measures (n = 142 observations for observer 1, n = 142 for observer 2, n = 284 for observers 1 & 2).
[c] average of the within-observer means, i.e., between the two repeated measures per eel (n = 71 pairs of observations for observer 1, n = 71 for observer 2, n = 142 for observers 1 & 2).
[d] average of the within-observer Standard Deviations, i.e., between the two repeated measures per eel (n = 71 pairs of observations for observer 1, n = 71 for observer 2, n = 142 for observers 1 & 2).
[e] average of the within-observer Coefficients of Variations, i.e., between the two repeated measures per eel (n = 71 pairs of observations for observer 1, n = 71 for observer 2, n = 142 for observers 1 & 2).

Table 3 Central tendencies and inter-observer variabilities on the measures of swimbladder length (L_S) and Swimbladder Degenerative Index (SDI), for the 71 investigated eels

	Observation 1		Observation 2		Observations 1 & 2	
	L_S (mm)	SDI	L_S (mm)	SDI	L_S (mm)	SDI
mean ± SD[a]	55.34 ± 18.59	2.27 ± 1.68	54.39 ± 18.23	2.40 ± 1.71	54.87 ± 18.38	2.33 ± 1.69
median ± 25th percentiles[b]	56.00 ± 40.00-70.00	2.00 ± 1.00-3.00	54.00 ± 39.00-68.00	2.00 ± 1.00-3.00	55.00 ± 39.00-69.00	2.00 ± 1.00-3.00
average pairwise mean[c]	55.34	2.27	54.39	2.40	54.87	2.33
average pairwise SD[d]	± 2.19	± 0.55	± 2.19	± 0.47	± 2.19	± 0.51
average pairwise CV[e]	4.39%	32.04%	4.07%	34.72%	4.23%	33.38%

[a] mean and measure of dispersion over the two observers (n = 142 observations for the first set of measures, n = 142 for the second set, n = 284 for the two sets of measures).
[b] median and measure of dispersion over the two observers (n = 142 observations for the first set of measures, n = 142 for the second set, n = 284 for the two sets of measures).
[c] average of the between-observer means, i.e., between the two independent measures per eel (n = 71 pairs of observations for the first set of measures, n = 71 for the second set, n = 142 for the two set of measures).
[d] average of the between-observer Standard Deviations, i.e., between the two independent measures per eel (n = 71 pairs of observations for the first set of measures, n = 71 for the second set, n = 142 for the two set of measures).
[e] average of the between-observer Coefficients of Variations, i.e., between the two independent measures per eel (n = 71 pairs of observations for the first set of measures, n = 71 for the second set, n = 142 for the two set of measures).

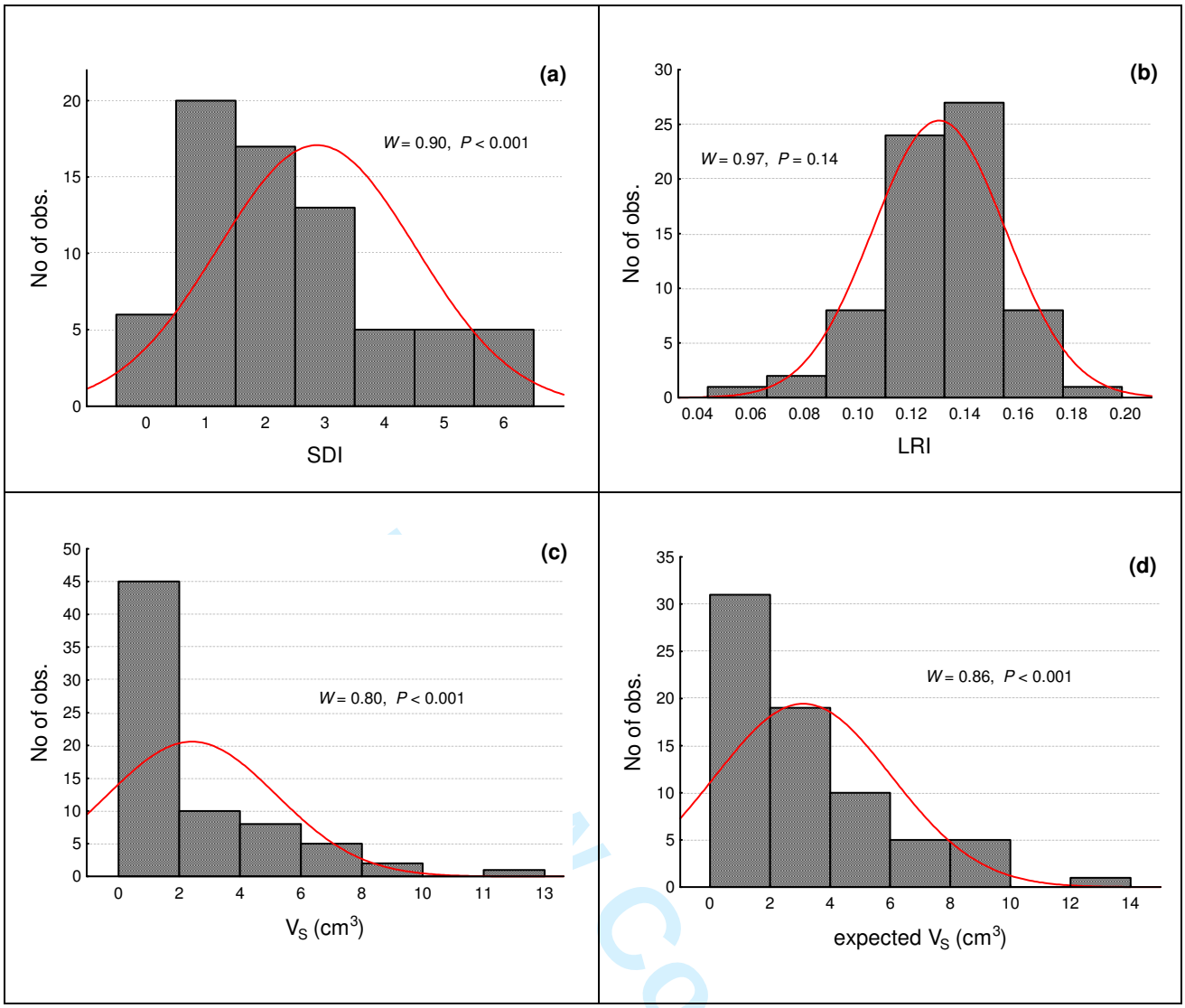

Figure 1 Frequency distribution of the Swimbladder Degenerative Index (a), the Length Ratio Index (b), the observed internal swimbladder volume (c), the expected internal swimbladder volume (d), for the 71 investigated eels. The red curve outlines the bell shape of a normal distribution. The Shapiro & Wilk parameter *W* measures the closeness to normality (in which case its value approaches the unity). Small *W* and *P* values indicate severe departure from a normal distribution.

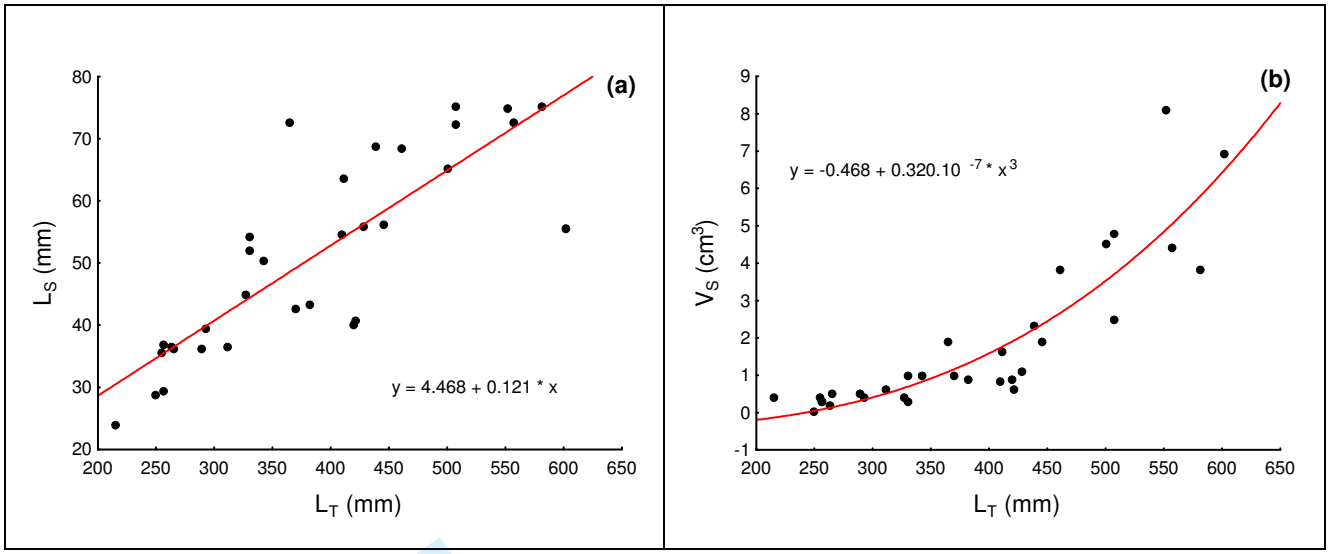

Figure 2 Relationships between the eel length and the length of the swimbladder (a), and the eel length and the internal swimbladder volume (b), in the absence of parasite and degradation in the swimbladder (sub-sample n = 32).

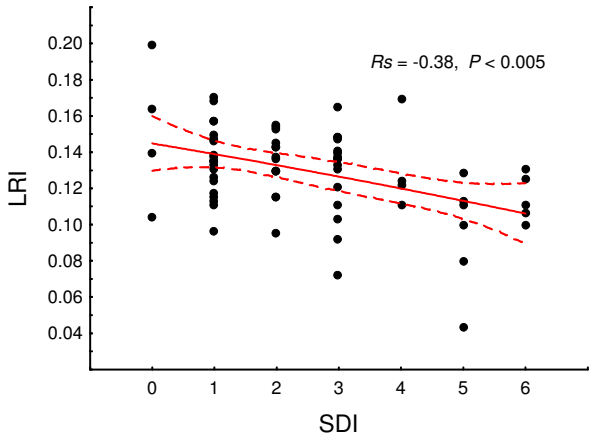

Figure 3 Relationship between the Swimbladder Degenerative Index and the Length Ratio Index, for the 71 investigated eels (± 0.95 confidence limit).

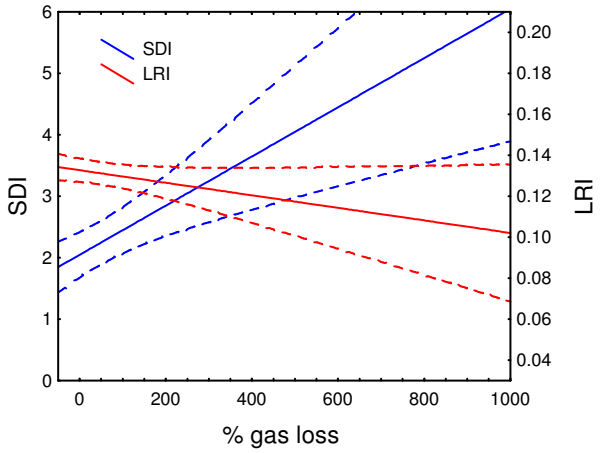

Figure 4 Relationship between the estimated percentage of gas loss (based on the relationship between the V_S and L_T in the absence of infection sign, x-axis) and the corresponding scores in the Swimbladder Degenerative Index (left axis, blue line ± 0.95 confidence limits), and in the Length Ratio Index (right axis, red line ± 0.95 confidence limit).

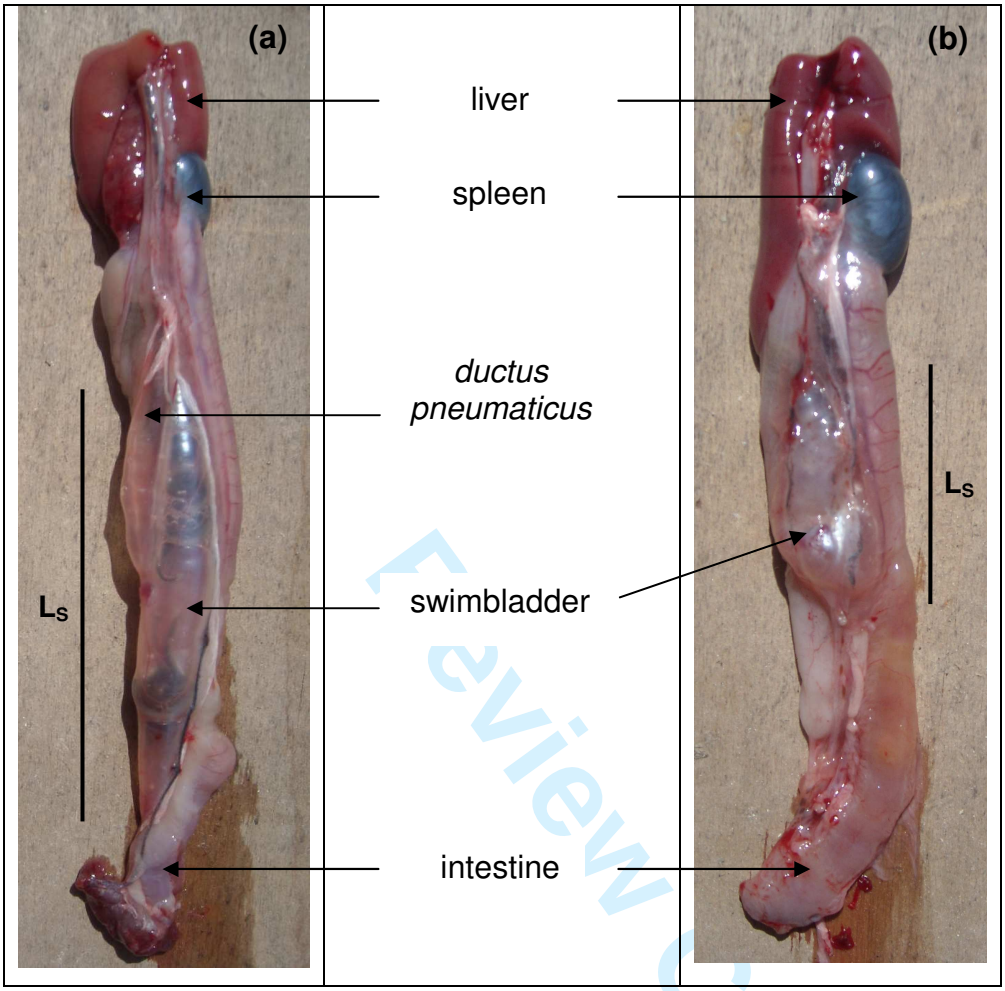

Figure 5 Ventral views of *in situ* swimbladders. (a) Healthy organ with 9 parasites inside (390 mm long male: SDI = 1, L_s = 58 mm, LRI = 0.15). (b) Degradated organ with no parasite inside (406 mm long female: SDI = 6, L_s = 40 mm, LRI = 0.10). On picture (b), because of multiple infection events in the past, the swimbladder has turned into a compact ball with few lumen left and no visible *ductus pneumaticus*, thus offering little space for further parasite establishment. Altogether, these two pictures well illustrate the fact that the simple count of living worm(s) is solely not a suitable measure of the parasite pressure suffered by eels, and so the development of swimbladder degradation indices.