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# Site-occupancy modelling: A new approach to assess sensitivity of indicator species

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ABSTRACT

One of the most challenging aspects of quality indices has been to compile reliable measures of the species' sensitivity to various magnitudes and different kinds of ecosystem attributes. Occupancy modelling has become increasingly useful to ecologists because provides a flexible framework to estimate the habitat use as a function of site information. We modelled occupancy of oligochaete species from physicochemical variables of Pampean streams; and we described the change in occupancy along the gradient of each explanatory physicochemical variable. We proposed three phases (resistance, tolerance and extinction) to describe the sensitivity of the species in terms of occupancy. Seventeen of the 33 taxa of oligochaetes were enough abundant to be modeled. In eight species, we obtained a total of 11 different models including physicochemical covariates. Occupancy was explained by conductivity in four species, by dissolved oxygen in three species, and by nutrients in four species. The analysis of phases (resistance, tolerance and extinction) to describe the sensitivity of the species in terms of occupancy, offers a new methodology to understand how the species behave along a stressor gradient. Detailed descriptions of sensitivity of these local species, will helps ecologists to generate more accurate biotic indices.

## 1. Introduction

There is an urgent need to assess the ecological status of ecosystems and determine how they are being affected by anthropogenic activities (Revenga and Kura, 2003). A worldwide extended approach is the use of biotic indices, which are built from indicator species (Hermoso et al., 2010; Birk et al., 2012). Indicator species are particularly sensitive to stressors related to human disturbance, and the species sensitivity refers to the degree to which an organism can withstand theses stressors (Yuan, 2004). However, one of the most challenging aspects of theses biotic indices has been to compile reliable measures of the sensitivity of indicator species to various magnitudes and different kinds of stressors (Leonardsson et al., 2015). Usually, scientific knowledge about the ecology of species is limited, which makes it hard to assign them sensitivity values based on documented knowledge. Species sensitivity values used so far are either based on literature data combined with expert knowledge (e.g. Borja et al., 2000; Teixeira et al., 2010), or empirical derivation based on presence of species in relation to ecosystem attributes (Pearson and Rosenberg, 1978; Rosenberg et al., 2004; Leonardsson et al., 2009). These approaches frequently result in rudimentary categories (e.g. tolerant/intolerant) or fixed values of sensitivity of species, which radically reduce the diagnostic power of indicator species (Oberdorff et al., 2002; Ferreira et al., 2007). Only in a few cases it is possible to obtain values of the sensitivity of a species along the range of a stressor, and in almost all these cases theses values are obtained under laboratory conditions (e.g. pesticides, heavy metals; Frampton et al., 2006; Malaj et al., 2016).

Occupancy modelling has become increasingly useful to ecologists because provides a flexible framework to investigate ecological questions and processes such as species distribution modelling, habitat relationships, multispecies relationships and community dynamics (Bailey et al., 2014). Various extensions of the original model have been proposed to simultaneous modelling habitat and occupancy dynamics, estimate species occurrence at multiple spatial and temporal scales and modelling occupancy dynamics as a function of the occupancy states of nearby sites (Bled et al., 2011; Miller et al., 2012; Pavlacky et al., 2012). Some salient features of this modeling approach, as the use of occurrence data, which are relatively easy to collect for some taxa, and the availability of free software packages, have contributed to proliferation of the use of occupancy models, especially

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in vertebrates (e.g. Berkunsky et al., 2014, 2015), plants (e.g. Kéry, 2004) and pathogens (e.g. Adams et al., 2010), but rarely invertebrates (e.g. Wisniewski et al., 2013; Snyder et al., 2016).

Theory predicts the occupancy of a species will decrease as the habitat quality decrease (Boyce et al., 2016). In this framework, if we are able to identify the stressors affecting species occupancy, we will be able to described the sensitivity as a change in the occupancy along the range of ecosystem attributes. Here, we modelled occupancy of oligochaete species from four physicochemical variables of Pampean streams (dissolved oxygen, conductivity, dissolved inorganic nitrogen and phosphate); and we described the change in occupancy along the gradient of each explanatory physicochemical variable. We expected a negative relationship between occupancy and conductivity, dissolved inorganic nitrogen and phosphate; and a positive relationship between occupancy and dissolved oxygen. The analysis of occupancy decline could be a useful tool to describe the sensitivity of the species.

## 2. Methods

### 2.1. Study area and taxa

The study area is located in the Southeastern center of Buenos Aires province, in the area occupied by the Tandilia mountain system. On northern hillside of Tandilia have their headwaters a lot of streams that it drains in direction NE through foothill and plains areas, both with a strong agricultural development. Although this area is considered endangered and of maximum priority due to its great transformation, biological uniqueness, and the absence of protected areas (Bilenca and Miñarro, 2004), currently there is a lack of information on the ecological status of these aquatic systems.

In freshwater systems, the oligochaetes are often the most diverse and/or abundant group of benthic invertebrates. These annelids participate in the trophic networks of the aquatic systems as a feeding resource of numerous taxa including others invertebrates, amphibians, fish, and birds (Ezcurra de Drago et al., 2007). Because their presence in all environment, the oligochaetes are widely utilized as indicators of environmental conditions. However, due to the difficulty of species determination, is common that oligochaetes are included in ecological studies without a fine level of taxonomical resolution (Alves et al., 2006). The sensitivity of oligochaetes to quality habitat is generally referred down to class, family or subfamily levels (Cortelezzi et al., 2011; Linhares Frizzera and da Gama Alves, 2012). Theses higher taxonomic levels show a relatively wide ecological valence as a result of the large number of species, which may yield skewed results when assessing water quality (Verdonschot, 2006; Cortelezzi et al., 2011). However, at the species level, the oligochaetes are sensitive enough to enable their implementation as indicators of water-quality indices (Lin and Yo, 2008).

### 2.2. Surveys

In 2012 and 2015, we conducted surveys in 43 sites distributing in 8 streams of northern hillside of Tandilia Mountain System. The surveyed streams are characterized by the absence of riparian forest vegetation, the lack of a dry season or extreme temperatures and development of dense and rich macrophyte communities (Feijoó et al., 2005). In order to promote independence, the minimum distance among sites was 4 km. In order to avoid seasonal variability, we choose conduct all surveys in one season (i.e. autumn). At each site, we collected three samples of sediment (i.e. 129 sediment samples) with an Ekman grab (100 $cm^2$), we washed each sample over a 500 μm mesh sieve, we separated the oligochaetes under a stereomicroscope (Olympus SZ40), and we identified them through standard keys (Brinkhurst and Marchese, 1992; International Commission on Zoological Nomenclature, 2007). Two taxa (i.e. Enchytraeidae and Megadrili) were not identified at the species level since the appropriate identification keys were not available. We preserved the collected material in 70% (v/v) aqueous ethanol.

At each site we recorded the following water quality variables: dissolved oxygen (YSI 52 dissolved oxygen meter), temperature and pH (Hanna HI 8633), and conductivity (Lutron CD-4303). We also collected one sample of water to analyze oxygen demand ($BOD_5$ and COD), and concentrations of phosphate ($P{-}PO_4^{-3}$), ammonium ($N{-}NH^{+4}$), nitrate ($N{-}NO_3^{-1}$), and nitrite ($N{-}NO_2^{-1}$; Mackereth et al., 1978; APHA, 1998). All these physicochemical variables of water were used as indicators of habitat quality.

### 2.3. Modelling

We used occupancy models to estimate the influence of physicochemical variables affecting the occupancy of each oligochaetes taxon. The basis of occupancy model is that there are two stochastic processes occurring that affect whether a species is detected at a site. A site may be either occupied or unoccupied by the species; if it is occupied then at each visit there is some probability of detecting the species. For each site we built a detection history of three simultaneous visits, and we excluded from the modelling those species that were detected in less than 5 samples out of a total of 129 sediment samples. We evaluated the baseline model for each species, in which both detection and occupancy probabilities were assumed to be constant across all sites [denoted as $\psi(.)\ p\ (.)$]. Then, we developed a model set that incorporated site covariates through a logit link function. We explored the structure of covariation of physicochemical variables, and then we reduced the variables dimension resulting in four independent covariates globally used to define the water quality: dissolved oxygen (%DO, range from 14 to 160), conductivity (range: 185–1207 μS/cm), dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite, range: 0.3–13.1 mgN/l), and phosphate (range: 0.02–1.29 mgP/l). Under the assumption that the occupancy of species decrease as the water quality decrease, we expected a negative relationship between occupancy and conductivity, dissolved inorganic nitrogen and phosphate; and a positive relationship between occupancy and dissolved oxygen. We evaluated all potential models with 2–4 parameters (including the intercept and probability of detection) to avoid the occurrence of spurious results, and by maintaining an approximate ratio of data to parameters > 10 (n = 43 sites; maximum number of parameters = n/10; Burnham and Anderson, 2002). For each model, we calculated the estimates of parameters (β) and their standard errors for the intercept (βo) and each covariate, considering a valid model if the occupancy of species increases with water quality (i.e. the β sign of conductivity, dissolved inorganic nitrogen and phosphate were negative; and of dissolved oxygen was positive). Also, we excluded those models which covariates had confidence interval containing the zero due to lack of effect. Finally, we ranked models using Akaike's Information Criterion (AIC). We kept all models that were better than constant-occupancy model [i.e., $\psi(.)\ p(.)$] and that were less than two AIC units [$\Delta AIC < 2$] of the best model. For each species, we used the best model to evaluated its sensitivity to physicochemical covariates. We used Unmarked package in R (Fiske and Chandler, 2011) to perform occupation model.

### 2.4. Analysis of sensitivity

Under the assumptions (i) the species's occupancy is maximum when the habitat quality is optimal, and (ii) the species' occupancy decrease as the habitat quality decreases; we proposed that the species's sensitivity could be described by three phases of occupancy decline: resistance, tolerance, and extinction (Fig. 1). The resistance reflects the capacity of species to hold occupancy as habitat quality decreases. In the framework of occupancy modelling, the resistance phase is associated to the intercept of the model (i.e. species with a high resistance will show a higher intercept values) and it can be interpreted according to units of the quality habitat variable (e.g. a species resists up to ***a***). The tolerance phase is the range of the habitat quality variable for which the occupancy shows the highest decline (i.e. the tolerance of a

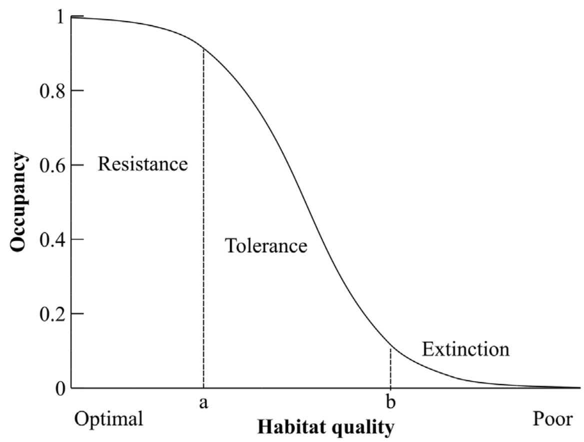


**Fig. 1.** Hypothetical phases of the sensitivity, in terms of occupancy, to habitat quality. a and b represents the limits between phases.

species is between ***a*** and ***b***). The extinction phase is defined from the limit value of the habitat quality variable which the occupancy is very low. In terms of occupancy, we suggest define the extinction phase from the value of the habitat quality variable from which the occupancy is less than 10% (i.e. a species reaches the extinction phase when the habitat quality is under ***b***). The joint analysis of the three proposed phases allows describe and compare the sensitivity of species in terms of occupancy.

## 3. Results

Seventeen of the 33 detected oligochaete taxa were enough abundant to be modeled (i.e. taxa that were detected in more than five samples). We obtained a total of 11 different models including physicochemical covariates and ranking better than the constant model [ψ(.) *p* (.)] in eight species (Table 1). For the remaining nine taxa (*Limnodrilus udekemianus, Aulodrilus pigueti, Chaetogaster diaphanus, Pristina acuminata, Pristina aequiseta, Pristina osborni, Aeolosoma* sp., *Enchytraeidae* sp1., Megadrili sp1.) the addition of physicochemical covariates did not improve the constant model.

Occupancy was explained by conductivity in four species, by dissolved oxygen in three species, and by nutrients (phosphate and DIN) in four species. Two species of Tubificinae (*Aulodrilus limnobius* and *Limnodrilus hoffmeisteri*) included more than one physicochemical covariate in their models (Table 1).

We describe the occupancy of the eight species across the range of each physicochemical covariate (Fig. 2). *Limnodrilus hoffmeisteri* showed a high occupancy throughout a very long resistance phase for dissolved oxygen and conductivity; while, in the case of phosphate, the resistance phase was shorter than the tolerant phase (Fig. 2). *L. hoffmeisteri* did not show extinction phases for conductivity and phosphate. *Paranais frici* showed a short resistance phase with an abrupt decline in occupancy, reaching the extinction phase at 80% of dissolved oxygen (Fig. 2a). *Nais variabilis* and *Dero pectinata* showed a smoothed decline in occupancy reaching values of 1200 and 900 μS/cm of conductivity, respectively, at the beginning of their extinction phases (Fig. 2b). *Slavina isochaeta* showed a rapid decline in occupancy reaching the extinction phase at 750 μS/cm of conductivity. *Slavina appendiculata* showed an instantaneous decline in occupancy reaching the extinction phase at very low concentrations of phosphate (Fig. 2d). The occupancy of *Aulodrilus limnobius* and *Dero digitata* were low without resistance phase.

## 4. Discussion

Half of modelled oligochaete species of Pampean streams included at least one physicochemical covariates in their top models, and we found sensitive species to all covariates. Eight species would have the potential to be assessed as indicator species of water quality of streams in this region. Most of these sensitive oligochaetes responded by varying their occupancy to changes in conductivity and dissolved oxygen, rather than nutrients (i.e. phosphate and dissolved inorganic nitrogen).

In most cases, we were able to identify the three phases of the theoretical model to describe the sensitivity to physicochemical covariates. Modelling worked for both, low tolerant (e.g. *Slavina isochaeta*) and high resistant (*Limnodrilus hoffmeisteri*) species. *Limnodrilus hoffmeisteri* is used as indicator of low quality habitat because of it dominant presence in polluted sites (Alves et al., 2006; Jabłońska, 2014). In concordance, in Pampean streams, we found a high occupancy of *L. hoffmeisteri* throughout a very long resistance phase for dissolved oxygen and conductivity. However, the sensitivity to physicochemical covariates was different, showing a low resistance to phosphate.

**Table 1**
Top performing site occupancy models (ΔAIC < 2) and coefficients of covariates (β) for eight oligochaetes species in Pampean streams, Argentina. The best model for each species is showing the estimated probability of detection (*p*). DO = Dissolved Oxygen; DIN = Dissolved Inorganic Nitrogen; P-PO4 = Phosphate.

| Taxon | Model | *n* | ΔAIC | *p* | Intercept | Dissolved Oxygen | Conductivity | DIN | P-PO4 |
|---|---|---|---|---|---|---|---|---|---|
| **Tubificinae** | | | | | | | | | |
| *Aulodrilus limnobius* | Ψ (DO + DIN) p (.) | 4 | 0.00 | 0.63 | −3.92 ± 1.96 | 1.54 ± 1.3 | | -3.53 ± 2.34 | |
| | Ψ (DIN) p (.) | 3 | 0.02 | | −2.58 ± 0.97 | | | -1.88 ± 1.17 | |
| | Ψ (.) p (.) | 2 | 3.93 | | | | | | |
| *Limnodrilus hoffmeisteri* | Ψ (DO) p (.) | 3 | 0.00 | 0.79 | 13.18 ± 12.29 | 6.46 ± 5.9 | | | |
| | Ψ (Conductivity) p (.) | 3 | 0.61 | | 3.95 ± 1.46 | | −1.84 ± 0.89 | | |
| | Ψ (P-PO4) p (.) | 3 | 1.50 | | 3.95 ± 2.52 | | | | −1.37 ± 1.05 |
| | Ψ (.) p (.) | 2 | 5.40 | | | | | | |
| **Naidinae** | | | | | | | | | |
| *Dero digitata* | Ψ (DIN) p (.) | 3 | 0.00 | 0.72 | −3.33 ± 1.53 | | | −2.5 ± 1.81 | |
| | Ψ (.) p (.) | 2 | 4.08 | | | | | | |
| *Dero pectinata* | Ψ (Conductivity) p (.) | 3 | 0.00 | 0.64 | −1.18 ± 0.45 | | −1.83 ± 0.83 | | |
| | Ψ (.) p (.) | 2 | 9.30 | | | | | | |
| *Nais variabilis* | Ψ (Conductivity) p (.) | 3 | 0.00 | 0.41 | −0.01 ± 0.48 | | −1.04 ± 0.56 | | |
| | Ψ (.) p (.) | 2 | 3.50 | | | | | | |
| *Paranais frici* | Ψ (DO) p (.) | 3 | 0.00 | 0.38 | −1.11 ± 0.76 | 3.61 ± 1.53 | | | |
| | Ψ (.) p (.) | 2 | 15.25 | | | | | | |
| *Slavina appendiculata* | Ψ (P-PO4) p (.) | 3 | 0.00 | 0.25 | −8.61 ± 5.54 | | | | −15.96 ± 11.3 |
| | Ψ (.) p (.) | 2 | 7.21 | | | | | | |
| *Slavina isochaeta* | Ψ (Conductivity) p (.) | 3 | 0.00 | 0.31 | −3.02 ± 1.21 | | −4.2 ± 2.25 | | |
| | Ψ (.) p (.) | 2 | 8.56 | | | | | | |

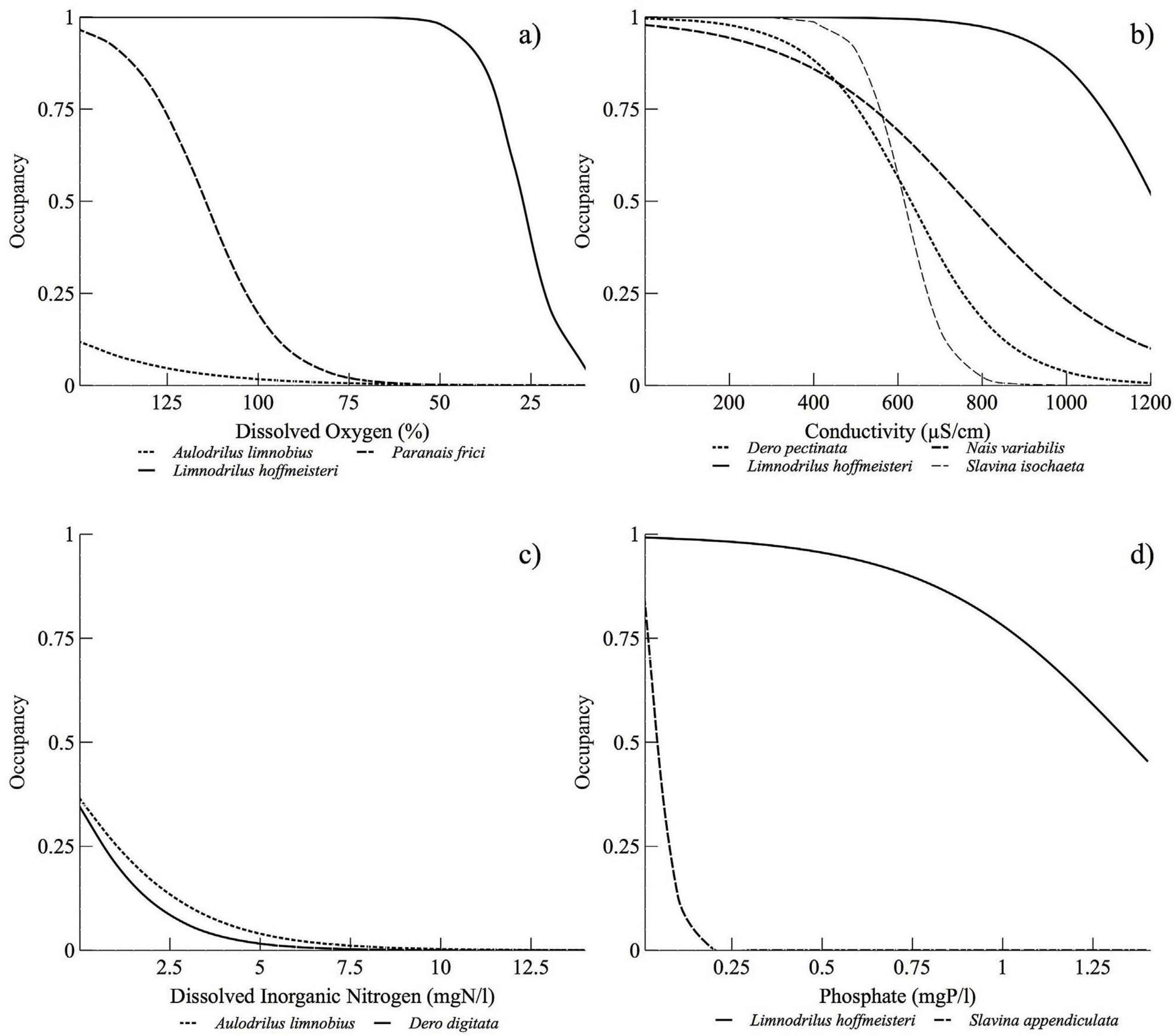


**Fig. 2.** Relationship between occupancy and physicochemical variables of eigth oligochaetes species in Pampean streams, Argentina.

In some cases, we identified two phases of the theoretical model. Here we found two different situations. In those cases where the extinction phase is missing, we hypothesized that the gradient of the variable is incomplete. For example, our sampled phosphate gradient was not enough long to describe the extinction phase of *Limnodrilus hoffmeisteri* (Fig. 2d). To confirm our hypothesis, we need surveys in sites with phosphate concentration higher than 1.25 mgP/l. In those cases where the resistance phase is missing, we found several explanations. For example, the lack of a resistance phase in *Slavina appendiculata* could be associated to a low tolerance to phosphate concentration (Fig. 2d). Meanwhile, the lack of a resistance phase in *Aulodrilus limnobius* and *Dero digitata* could be related to low occupation of these species in the studied sites.

Until now limnologists usually report sensitivity, at family or subfamily levels, based on empirical derivation of presence of taxa in relation to ecosystem attributes (López van Oosterom et al., 2015). Our occupancy modelling approach allowed us to describe the differential contribution of each species to the sensitivity of the family or subfamily level to ecosystem attributes. For example, if we analyze the sensitivity of the Naidinae (as whole subfamily) to conductivity, we can say that it is very tolerant and we could find it in sites with values up to 1200 µS/cm. However, if we analyze the response to conductivity of each one of the three Nadinae species of our study, we will realize that only *Nais vairabilis* would occupy sites with conductivity over 1200 µS/cm, while for *Slavina isochaeta* and *Dero pectinata*, the tolerance phase barely reaches 800 and 1000 µS/cm respectively.

In the other half of modelled species, occupancy was not explained by physicochemical covariates. In those cases, occupancy could be affected by other ecosystem attributes such as organic matter, and/or type of sediment. For example, Tubificinae species (e.g. *Aulodrilus pigueti* and *Limnodrilus udekemianus*) are generally tolerant to organic matter and prefer soft sediments (Othman et al., 2002; Nijboer et al., 2004; Alves et al., 2008); while Nadinae species (e.g. *Chaetogaster diaphanus*) are more often registered in coarse substrates (medium-sized grains of sand, Cortelezzi et al., 2011).

Our modelling approach provides researchers a tool for obtain reliable measures of the species' sensitivity to various magnitudes and different kinds of ecosystem attributes. Commonly, the sensitivity of ecological indicators is indirectly described as the association of the taxa to ecosystem attributes under multiparametric approaches (i.e. Canonical Correspondence Analysis, Principal Component Analysis, etc., Armendáriz et al., 2011); and sensitivity values are frequently presented as fixed values (or range) of each ecosystem attribute. The analysis of phases (resistance, tolerance and extinction) to describe the sensitivity of the species in terms of occupancy, offers a new methodology to understand how the species behave along a stressor gradient. If occupancy modelling assumption are met, then this method can be adapted to deal with data from monitoring programs of any species. Further studies about sensitive of local species to different types of stressors will be required in order to validate the applicability of this methodology. Detailed descriptions of sensitivity of local species, will helps ecologists to better understand the effect of stressors.

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