RIESGO DE DEPREDACIÓN DE ANFIBIOS EN AMBIENTES FORESTALES

Tesis
Entregada a la Universidad de Chile
En cumplimiento parcial de los requisitos para optar al grado de

Magíster en Ciencias Biológicas

Facultad de Ciencias

Soledad Pilar Puente Torres

Director de Tesis
Dr. Javier A. Simonetti

Santiago, Marzo 2017

INFORME DE APROBACION

Se informa a la Escuela de Postgrado de la Facultad de Ciencias que la Tesis de Magíster presentada por la candidata.

Soledad Pilar Puente Torres

Ha sido aprobada por la comisión de Evaluación de la tesis como requisito para optar al grado de Magíster en Ciencias Biológicas en el examen de Defensa Privada de Tesis rendido el día Martes 10 de Enero de 2017.

Director de Tesis:

Dr. Javier A. Simonetti

Comisión de Evaluación de la Tesis:

Dr. Ramiro Bustamante

Dr. Rodrigo Vásquez

A mi padres, Omar y Viviana...

RESUMEN BIOGRAFICO

Nací en el sur de Chile (Región de los Lagos) en el año 1988. Soy Licenciada en Ciencias Biológicas de la Universidad Austral de Chile (2012). Llegué al Laboratorio de Conservación Biológica en el año 2014 a comenzar mis estudios de postgrado, donde descubrí nuevas formas de hacer ciencia y fui de a poco definiendo mis intereses de investigación. Durante el transcurso del primer año me di cuenta que la ecología de anfibios era el área en la que me quería desarrollar, dado que previamente ya había trabajado evaluando la diversidad de anfibios en humedales urbanos, por lo que mi investigación de postgrado se enfocó en como varía el riesgo de depredación sobre anfibios en un paisaje que se encuentra en constante transformación. A partir de todo este proceso, logré definir mis intereses donde la ecología de anfibios en ambientes alterados como, áreas urbanas, sistemas forestales y zonas agrícolas son algunos de los escenarios en los que me gustaría desarrollar investigación en pos de contribuir a la conservación de estos organismos.

AGRADECIMIENTOS

A Javier Simonetti, por recibir a este "anfibio" es su laboratorio, por guiarme en el camino de hacer ciencia, por incentivarme a ver más allá de lo que conocía hasta el momento, por apoyarme en mis locuras y por animarme cuando las energías decaían. Nuestro agradable encuentro deja grandes lecciones en mi corazón, tanto para enfrentar la vida académica, como para enfrentar los desafíos de la vida.

A los integrantes del Laboratorio de Conservación Biológica, por hacer que el día a día sea grato, compartiendo sonrisas y conocimientos, demostrando que son un grupo de personas excepcionales, siempre dispuestos a ayudar a través de una explicación de cómo realizar un GLMM o simplemente con un abrazo.

A los integrantes del "Queules Group", por compartir esos intensos y agradables terrenos, por contribuir a mis conocimientos sobre insectos, aves, roedores, entre otros, por colaborar en mis experimentos y por alegrar los días con sus locuras, anécdotas, chistes y comidas ricas. En especial quiero agradecer a Ronny Zúñiga, por ser nuestro guía en terreno, por solucionarnos la vida, por arreglar nuestros desastres, y por hacer que mágicamente lo imposible se vuelva posible. Gracias Ronny por tus enseñanzas, por esas largas conversaciones y por contagiarnos con tu música.

A mis padres, Omar y Viviana, por su incondicional apoyo, por su esfuerzo para lograr que yo sea mejor y pueda realizarme como profesional, por darme las herramientas que he necesitado para llegar hasta donde estoy: perseverancia, optimismo, nobleza y una sonrisa.

A mi Mauro, porque llegaste en el momento preciso para apoyarme en las últimas etapas de esta aventura. Gracias por tanto amor en los momentos que más lo necesité.

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RESUMEN

La transformación de los ecosistemas puede generar cambios en la intensidad de las interacciones biológicas. El efecto que tiene la pérdida y transformación del hábitat sobre el riesgo de depredación de anfibios, ha sido escasamente evaluado. En este contexto, primeramente se evaluó de forma experimental si el riesgo de depredación sobre anfibios a escala de paisaje varía de igual forma como se ha descrito para otros grupos taxonómicos. En el segundo capítulo se evaluó si las glándulas lumbares, que simulan ojos, presentes en *Pleurodema thaul* tendría una función antidepredatoria disminuyendo la probabilidad de ataque y desviando los ataques hacia zonas no vitales del cuerpo. Los principales resultados muestran que 1) existe una variación a escala de paisaje del riesgo de depredación de anfibios, siendo mayor en ambientes perturbados (fragmentos de bosque y plantaciones de pino adultas y jóvenes) en comparación con el bosque nativo. Sin embargo, esta variación en la tasa de depredación no estaría dada por la abundancia de depredadores, sino que varía en función de la cobertura vegetacional. 2) La presencia de glándulas lumbares efectivamente genera una disminución en el riesgo de depredación de *P. thaul,* donde aquellos individuos con glándulas lumbares son 1.5 veces menos atacados que aquellos sin glándulas lumbares, y además, cuando se produce el ataque este es desviado hacia zonas no vitales del cuerpo. Estos hallazgos sugieren que a pesar del aumento en la presión de depredación que se genera producto de la transformación del paisaje, los anfibios se podrían estar adaptando haciendo uso de ciertas estrategias anti depredatorias o utilizando las plantaciones forestales como refugio y fuente de alimento, aumentando la probabilidad de persistencia de estos animales en un paisaje que cambia constantemente producto de actividades productivas.

ABSTRACT

The transformation of ecosystems may generate changes in the intensity of biological interactions. The effect of habitat loss and transformation upon amphibian predation risk has been scarcely evaluated. In this context, for the first chapter we experimentally assessed whether the predation risk upon amphibians at the landscape scale varies according to literature on other taxonomic groups. In the second chapter, we assessed if lumbar glands that mimic eyes in *Pleurodema thaul*, present an antipredatory function by decreasing the probability of being attacked and deflecting attacks to non-vital body parts. Main results show that 1) amphibian predation risk at the landscape scale varies, from highest in disturbed environments (forest fragments, adult pine plantations and young pine plantations) to lowest in native forests. However, this variation in predation risk is not related to the abundance of avian predators, and instead varies according to vegetation cover. 2) The presence of lumbar glands effectively decrease predation risk on *P. thaul*, made explicit when individuals with lumbar glands were attacked 1.5 times less than individuals without lumbar glands, and furthermore, also deflect attacks to non-vital body parts. These findings suggest that despite the increased pressure on predation, a product of landscape transformation, amphibians could be adapting by using certain antipredatory strategies or employing forest plantations as shelter and food source, increasing the probability of persistence of these animals in a constantly changing landscape product of productive activities.

AGRADECIMIENTOS

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The transformation of ecosystems may generate changes in the intensity of biological interactions. The effect of habitat loss and transformation upon amphibian predation risk has been scarcely evaluated. In this context, for the first chapter we experimentally assessed whether the predation risk upon amphibians at the landscape scale varies according to literature on other taxonomic groups. In the second chapter, we assessed if lumbar glands that mimic eyes in *Pleurodema thaul*, present an antipredatory function by decreasing the probability of being attacked and deflecting attacks to non-vital body parts. Main results show that 1) amphibian predation risk at the landscape scale varies, from highest in disturbed environments (forest fragments, adult pine plantations and young pine plantations) to lowest in native forests. However, this variation in predation risk is not related to the abundance of avian predators, and instead varies according to vegetation cover. 2) The presence of lumbar glands effectively decrease predation risk on *P. thaul*, made explicit when individuals with lumbar glands were attacked 1.5 times less than individuals without lumbar glands, and furthermore, also deflect attacks to non-vital body parts. These findings suggest that despite the increased pressure on predation, a product of landscape transformation, amphibians could be adapting by using certain antipredatory strategies or employing forest plantations as shelter and food source, increasing the probability of persistence of these animals in a constantly changing landscape product of productive activities.

INTRODUCIÓN GENERAL

La transformación de los ecosistemas puede generar cambios en la riqueza y abundancia de las especies, lo que se traduce en cambios en la intensidad de la interacciones en las que estas especies están involucradas (Fahrig, 2003; Simonetti et al. 2006).

Interacciones de depredación, particularmente para el caso de los anfibios están restringidos principalmente a estudios de larvas en que se evalúan cambios morfológicos, conductuales y de rasgos de historia de vida (Brito, 2008; Kenison et al. 2016). La depredación de anfibios a escala de paisaje, y particularmente en un ambiente con distintos grados de perturbación jamás ha sido evaluada a pesar de que la perdida y transformación del hábitat es señalada como la principal amenaza que afecta a las poblaciones de anfibios en la actualidad (Vié et al. 2009). Bajo este contexto, en el capítulo I evaluamos de forma experimental si la variación en la abundancia de depredadores a escala de paisaje, la que determinaría los niveles de depredación sobre anfibios, se traduce en diferencias en los niveles de depredación entre las distintas unidades del paisaje.

Cambios en las interacciones de depredación pueden estar determinadas por ciertas estrategias antidepredatorias. Existe evidencia de que la presencia de "eyespots" genera una disminución en el riesgo de depredación, lo que ha sido descrito para distintos grupos animales, (e.g., insectos, peces y mariposas) (Kamoun, 1991; Gagliano, 2008; Kodandaramaiah, 2011). Esta disminución en el riesgo de depredación se generaría a través de dos mecanismos: 1) disminuyendo el riesgo de depredación sobre aquellos anfibios que presentan los "eyespots", y 2) desviando los ataques de los depredadores hacia zonas no vitales del cuerpo (Cei, 1962; Stevens, 2005). A partir de estos antecedentes, y utilizando como modelo a *Pleurodema thaul*, el cual presenta un par de glándulas lumbares que se asemejan a los "eyespots", en el capítulo II se evalúa de forma experimental, si la presencia de estas glándulas lumbares constituirían una estrategia antidepredatoria, generando una menor frecuencia de ataque sobre aquellos modelos que presentan glándulas lumbares respecto de aquellos que no las poseen. Además, se pone a prueba si la presencia de glándulas lumbares efectivamente desvía los ataques hacia zonas no vitales del cuerpo.

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A través de esta investigación se espera tener mayor claridad en el escenario que enfrentan los anfibios en ambientes forestales, teniendo en cuenta que el riesgo de depredación en general no es considerado en estrategias de conservación, a pesar de que esta variable puede estar determinando en cierto grado la ocurrencia y abundancia de anfibios, siendo un factor de amenaza adicional a la pérdida y transformación del hábitat (Creel & Christianson, 2008).

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Chapter I

PREDATION RISK ON AMPHIBIAN IN FOREST ENVIRONMENTS

OF CENTRAL CHILE

ABSTRACT

Human activities transform the landscape, generating changes in the intensity of biological interactions as result of changes in richness and abundance of interacting species. We used clay models that simulate amphibians, to assess differences in predation risk by birds among different habitats in a forest environment of central Chile, with landscape units that differ in structural complexity and abundance of predators. The frequency of attacks was higher in all disturbed environments (forest fragments, adult pine plantations and young pine plantations) compared with the undisturbed habitat (continuous forest), where the habitat with greater predation risk, corresponding to forest fragments, showed a frequency of attacks 3.6 times greater than continuous forest. The vegetation cover would have a greater effect than abundance of birds on predation risk, which acts mediating predation interactions. Our result showed the dynamism of a landscape-scale interaction which depends of the environmental context and suggest that changes in predation risk could be added to the direct effects of habitat loss as threat factors on amphibian population in forest landscapes.

Key words: predation risk, amphibian, forest environments, clay models

INTRODUCTION

Land use changes modify species richness and abundance, which in turn lead to variations in the intensity of the ecological interactions in which species are involved (Fahrig, 2003; Simonetti et al. 2006). Clearing vegetation to enable areas intended for agricultural and forestry activities are the major factor of landscape transformation (Foley et al. 2005, Ellis et al. 2010), changes that modify the intensity of predation risk at the landscape level. Nest and insect predation for instance correlates to variations in

species richness and abundance of predators, particularly generalist ones (e.g., Chalfoun et al. 2002).

Besides predator abundance, predation risk depends of the structural complexity of the habitat (Shmidt, 1999; Seibold et al. 2013), where a greater complexity might convey either a higher number of refuges for preys, or an obstacle for predators, reducing theirs capabilities to detect and capture preys, decreasing predation risk (Hixon & Beets, 1993; Lecomte et al. 2008). However, a greater structural complexity might also increase richness and abundance of predators, raising an increase in predation risk. Indeed, insectivory inside exotic forest plantations varies according to abundance of predators, which are more abundant in complex plantations compared with those less complex, resulting in a highest number of insect larvae preyed upon (Poch & Simonetti, 2013).

Predation risk upon amphibians in forested environment has been scarcely evaluated despite the worldwide habitat loss and transformation are the main threats impinging upon amphibian survival. Up to 61% of the total described amphibians are impacted by habitat changes (Vié et al. 2009; 2003; Brito, 2008; Kenison et al. 2016). Whether changes in predation adds to variations in quality and quantity of resources offered by altered habitat is yet to be assessed.

In Chile the scenario is similar. Up to 36% of the amphibian species are threatened, mainly due to habitat loss and transformation (Soto-Azat & Valenzuela-Sánchez, 2012). In central Chile, particularly along the costal range, landscape transformation is mainly due to the expansion of exotic forestry plantations (Echeverría et al. 2006). Currently the landscape is a mosaic of native forest remnants embedded in large tracts of plantations of Monterrey pine (*Pinus radiata*) (Heilmayr et al. 2016). In this landscape, richness and abundance of predatory birds vary among different landscape units. Forest fragments and pine plantations support increased bird abundance, compared to the original continuous forest (Estades & Temple, 1999; Vergara & Simonetti, 2004; González-Gómez et al. 2006). Coupled to this increase, nest, insect and seed predation are higher in altered environments (Simonetti et al. 2006). In this context we experimentally analyze if predation upon amphibians also vary accordingly, hence

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changes in predation rate could be added to the direct effects of habitat loss as threat factors on amphibian population in forest landscapes.

METHODS

The study was carried out in Trehualemu, coastal range of Región del Maule, central Chile, from February to December 2015. The area consists of forested environment composed by a tract of continuous native forest, a suite of different sized forest fragments inserted in a pine matrix (*Pinus radiata*), with stands of different ages, from mature plantations to 2 yr plantations. Each habitat type was characterized according to the vegetation, measuring vegetation cover using 50m line-intercept transects.

Predation risk was experimentally assessed relying on the frequency of attacks upon clay models. Studies of predation in nature is often difficult to assess, because the rarity of observing an event of predation. The use of clay models is a reliable alternative, and ethical one (Shepard, 2007) previously used in predation studies of snakes, lizards and frogs (Brodie, 1993; Watson et al. 2012; Flores et al. 2015).

We constructed frog models mimicking *Pleurodema thaul*, one of the most common amphibians in the area. Each model was approximately 35 mm of length snouth-vent. We used 500 frog models placed in 50 sites, distributed in continuous forest (n=10 sites), forest fragments (n=10), adult pine plantations (n=10) and young pine plantations (n=20). In each site we place 10 frog models separately by 3 m disposed towards the four cardinal points from a central tree. After 48 hours, we collected all models and from each site and recorded the number of attacks (Saporito et al. 2007). Recognition of the marks on frog models, and the identity of predator was made based on the description by Low et al. (2014) which show marks by insects, mammals, birds and reptile on clay models.

Predators were assessed through bird richness and abundance as some birds species preyed on amphibians (Vergara & Simonetti, 2003; Biondi et al. 2005; Jimenez,1995). Richness and abundance were estimated surveying birds in circular sampling plots of 50 m radius during 5 minutes. All observed and heard birds were recorded, as described by González-Gomez et al. (2006). Fifty sampling plots were established, distributed in continuous forest (n=10), forest fragments (n=10), adult pine plantations

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(n=10) and young pine plantations (n=20). Species richness was considered as the total number of bird species recorded in each site and the abundance as the average number of individuals observed among different survey events (Vergara & Simonetti, 2004).

We used a zero-inflated model (Poisson with log-link) to assess differences in the relative frequency of attacked models between habitats, and the contribution of bird abundance and vegetation cover on frequency of predation attacks. This approach is for data set with excess of zeros and allows the use fixed and random factors. In the analysis we included frog models which exhbitied marks attributed to birds and the number of missing model, as in some cases we found frog models displaced a few meters from the original position, with clear marks of attacks (Hegna et al. 2011).

RESULTS

The vegetation cover was significant different among habitats, were forest fragments have the highest vegetation cover, while the lowest vegetation cover were in young pine plantations (ANOVA, $F_{(3,N=46)}$= 63.88; p<0.001; Figure 1).

We recorded a total of 31 bird species (Table 1). Birds were mainly carnivorous birds (71% of total species), followed by granivorous birds (23%), while nectarivorous and herbivorous birds were the least represented. Species richness of birds that prey on amphibians were not significantly different among habitats (Figure 2) (F(3,346)=1.49; p=0.2).

The abundance of birds that prey on varies among habitats (F(3,346)= 6.63; p=0.01)(Figure 2). Bird abundance was higher in adult pine plantation compared with young pine plantations (p=0.001) and continuous forest (p=0.007).

A total of 612 frog models were attacked by birds. The best zero-inflated model was chosen based on Akaike Information Critera (AIC) values (Table 2), and due to scarce difference in AIC value of model 1 and 2, we perform a Vuong test, which indicate that model 1 is better than model 2 (Vuong-statistic= -2,37; p= 0,008).

Table 2. Akaike Information Criteria (AIC) values for 5 zero-inflated poisson models of the predation rate on amphibian clay models.

Model	Variables	AIC
1	habitat type x vegetation cover x bird abundance	1146,41
2	habitat type x vegetation cover + bird abundance	1149,88
3	habitat type + vegetation cover x bird abundance	1153,26
4	habitat type + vegetation cover + bird abundance	1153,27
5	habitat type	1808,99

The frequency of bird attacks on frog models was significantly different among habitats. The highest predation level occurred in forest fragments (coef=0.98; SD=0.22; z value= 4.38; p<0.001), frog models being 3.6 times more attacked than continuous forest (coef=-3.6; SD=0.8; z value= -4.48; p<0.001), while predation levels in pine adult pine plantations (coef=0.84; SD=0.02; z value= 5.85; p<0.001) and young pine plantations (coef=3.02; SD=0.02; z value=5.85; p<0.001) are two times more than in continuous forest (Figure 4). The model also showed that only vegetation cover contribute to explain the frequency of attacked models (coef=0.11; SD=0.02; z value=4.8; p<0.001) (Figure 4), while bird abundance do not contribute to explain the frequency of preyed models (coef=-0.02; SD=0.01; z value=-1.77; p=0.07)(Figure 4).
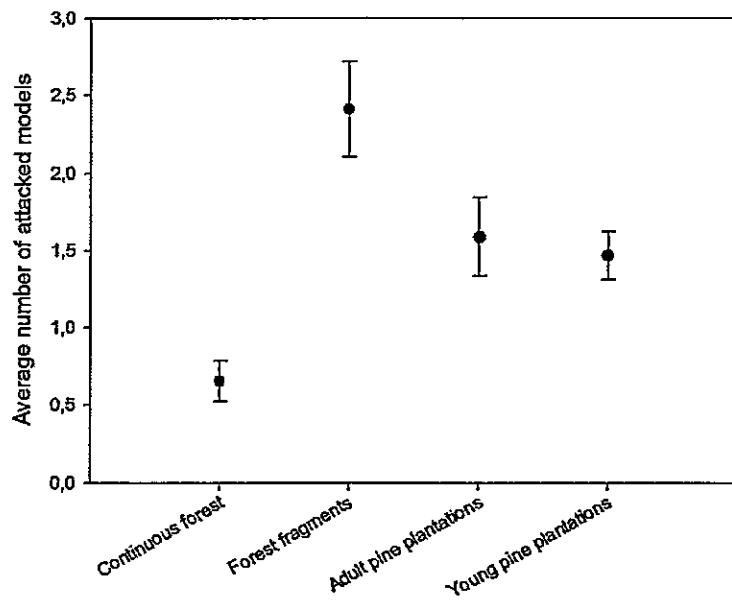
Figure 3. Bird predation upon frog models in the assessed habitats. Different letters

represent significant differences.
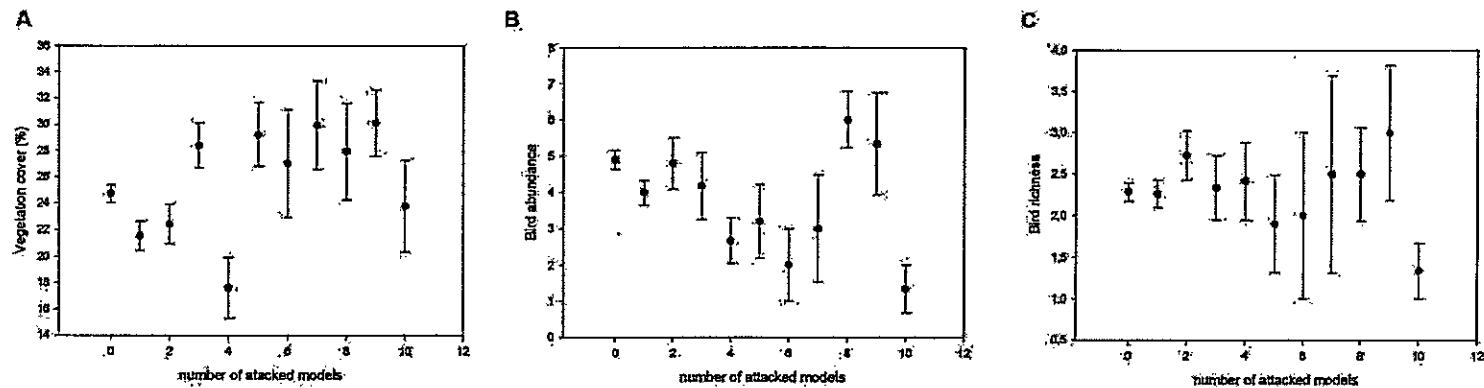
Figure 4. Relation among number of attacked models and a) vegetation cover, b) bird abundance and c) bird richness

DISCUSION

Landscape transformation alters predator-prey interactions, since it generates changes in the abundances of the interacting species. Total bird abundance and abundance of bird that preyed on amphibians, varies among different units of landscape as several taxa also varies in the fragmented forest of central Chile (Simonetti, 2006). That bird abundance does not explain the frequency of attacks reveals that habitat structure might be the underlying factor in predation risk in this landscape. Comparing predation rate, bird abundance and vegetation cover among two contrasting scenarios as the continuous forest and young pine plantations, reveals that despite bird abundance was similar in both environments, predation rate varied mainly according to vegetation cover.

In forest, vegetation cover would generate a decrease in encounter rate because it constitutes a physical impediment in search efforts of predators (Janssen et al. 2007; Chalfoun and Martin, 2009). Moreover, the scarce vegetation present in young pine plantations, make amphibians are more exposed to predators, possibly generating this high predation rate (Denno et al. 2005; Shepard, 2007). These results corroborate the fact that vegetation cover would be mediating predation interactions (Crowder & Cooper, 1982), and besides that some landscape variables may be more important than bird abundance to explain the predation levels (Morris & Conner, 2016).

Forestry plantations reduces species richness and abundance (Allen et al. 1995; Estades et al. 2012), however if they are structurally complex at the understory level, such a feature mitigates its impact on native species offering alternative habitats even for endangered species (Brockerhoff et al. 2008; Cerda et al. 2015). Clearly young plantations following clear felled stands are unsuitable for frogs on the basis of its predation risks. Interestingly, increasing vegetation complexity could also mitigate this risk, where structural complexity acts as a mediator of the interaction predator-prey suggesting that changes in predation risk could be added to the direct effects of habitat loss as threat factors on amphibian population in forest landscapes.

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Chapter II

ANTIPREDATOR EFFECT OF LUMBAR GLANDS: AN EXPERIMENTAL TEST WITH

PLEURODEMA THAUL

ABSTRACT

Antipredator strategies as the presence of eyespots, which simulate eyes, are present in several species of animals. It has been postulate that eyespots intimidates predators enabling the escape of the prey, and deflects the attacks to non-vital regions of the body. The Four-eyed frog (*Pleurodema thaul*) is characterized by the presence of a pair of lumbar glands that mimic eyes. By using clay models it was evaluated if the presence of the lumbar glands would have an effect on predation risk of *P. thaul*, and if this trait deflects the attack to non-vital regions. The results showed that clay models with lumbar glands were 1.5 times less preyed than those models without lumbar glands, and were less attacked in vital zones of the body as head and torso, than those models without lumbar glands, suggesting that lumbar glands effectively have an antipredator function. Besides, the effectiveness of the eyespots varies depending of predation risk associated to each habitat. Our investigation is the first experimental approximation which tests the antipredator function of this morphological trait in amphibians.

Key words: amphibians, lumbar glands, eyespots, predation risk

INTRODUCTION

Antipredator strategies among amphibians are commonly related with aposematism, either through coloration or the presence of a particular trait such as eyespots (Toledo & Haddad, 2009). Eyespots, located in the lumbar region, are expected to intimidate predators enabling the prey to escape or its presence is supposed to deflect the predator attack to non-vital body parts, enhancing survival (Stevens, 2005; Mukherjee &

Kodandaramaiah, 2015; Prudic et al. 2015). Described for several amphibian species such as *Physalaemus nattereri*, *P. deimaticus*, *Edalorhina perezi*, and at least nine of the 15 *Pleurodema* species (Wells, 2007; Toledo et al. 2011; Flaivovich et al. 2012), lumbar glands are supposed to play a defensive role based on the presumed toxic secretion contained by the granular alveoli in them, along with the disposition of chromatophores in the skin mimicking eyes (de Toledo & Jared, 1989; Ferraro et al. 2013). Further, species like the four-eyed frog (*Pleurodema thaul*), who exhibits elongated and protuberant lumbar glands, when disturbed might rise the lumbar region lowering the head clearly exhibiting the eyespots, attitude regarded as well as a defensive posture (Cei, 1962).

Here we experimentally test the hypothesis that eyesposts serve as antipredatory defense in *P. thaul*. If the presence of lumbar glands is an antipredator strategy, individuals of *P. thaul* exhibiting lumbar glands ought to have lower probabilities of being preyed upon than individuals without lumbar glands. Further, if the presence of lumbar glands effectively deflects attacks toward non-vitals body parts, individuals with lumbar glands would be less attacked in vital regions compared with those without such glands. Furthermore, predation risk varies at the landscape level, pending on habitat type and abundance of predators (Laundré et al. 2010). Hence, in riskier habitats, individuals exhibiting lumbar glands ought to have higher survival probabilities than individuals without lumbar glands compared to less riskier ones.

METHODS

The effect of lumbar glands as antipredator strategy was experimentally evaluated using clay models under field conditions (Figure 1). The use of clay models has been extensively used in predation experiments with amphibians among other vertebrates (e.g., Paluh et al. 2013; Farallo & Forstener, 2012; Bateman et al. 2014).

The study was conducted in Trehualemu, central Chile. The site corresponds to a forest landscape composed by native forest and forest fragments inserted in a matrix of pine plantations (*Pinus radiata*) of different ages including both mature and recently clear-felled stands. We placed clay models, five models with and five without lumbar glands in native forests (10 sites), forest fragments (10 sites), adult pine plantations (10 sites)

and young pine plantations (20 sites). Within a given site, models were interspersed, at least 3 m apart (Saporito et al. 2007).

Recognition of predator marks on amphibian models follows Low et al. (2014) identifying predation marks made by insects, mammals, birds and reptiles. To assess differences in the frequency of attacked models depending of the presence or absence of lumbar glands, was used a GLM with Poisson distribution, considering only clay models attacked by birds, as they are known frog predators (e.g., Jimenez, 1995; Biondi et al. 2005). Also we assess the effect of lumbar glands depending of the habitat type using an ANOVA.

To estimate differences in the frequency of attacks in the different body regions, we assessed differences of attacks upon head, forelimbs, torso, gland zone and hind limbs (Kutcha, 2005) testing differences with an ANOVA.

Figure 1. Photograph of *P. thaul* and clay models with lumbar glands and without lumbar glands.

RESULTS

A 4% of the models were attacked (160 out of 4000 clay model deployed in all repetitions) (Figure 2). Overall, models of *P. thaul* with lumbar glands were 1.5 times less attacked than those without lumbar glands (GLM, Wald=6.66; p=0.009; Figure 3).

Attacks varied among different body regions (ANOVA, $F_{(4,N=50)}=5.46$, $p<0.001$) (Figure 4). While models without lumbar glands were more attacked in the head (33.3%) with no attacks in the lumbar zone, models with lumbar glands, received more attack on hind limbs (39.2%), the torso being the body region less attacked (5.8%).Comparatively, clay models exhibiting lumbar glands were 1.5 times less attacked in the head (p=0.03) and 3.6 times less attacked in the torso (p=0.01) than replicas without lumbar glands.
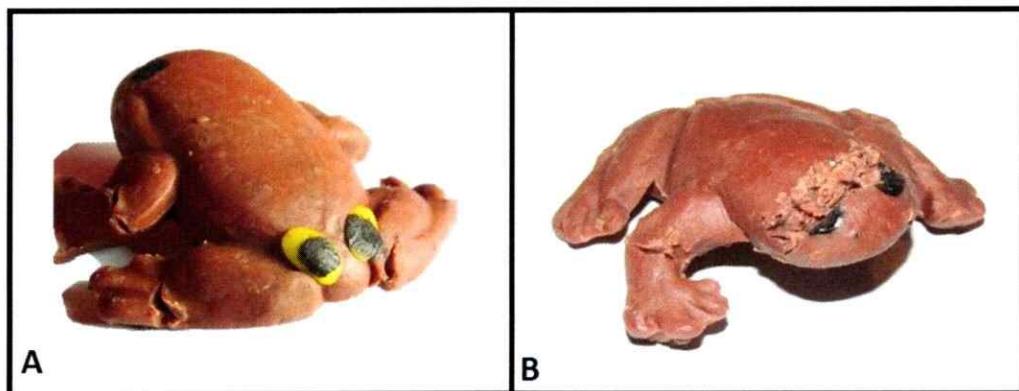
Figure 2. Models of *P. thaul* (A) with and (B) without lumbar glands preyed by birds.

The frequency of attacks varied spatially (GLM, Wald=24.59; p<0.001), being lowest in continuous forests, and highest in young pine plantations (Figure 3). In continuous forest and adult pine plantations, models with lumbar glands were 1.7 times less preyed than models without lumbar glands, while in forest fragments the replicas with lumbar glands were 1.2 times less attacked than replicas without lumbar glands (Figure 3).
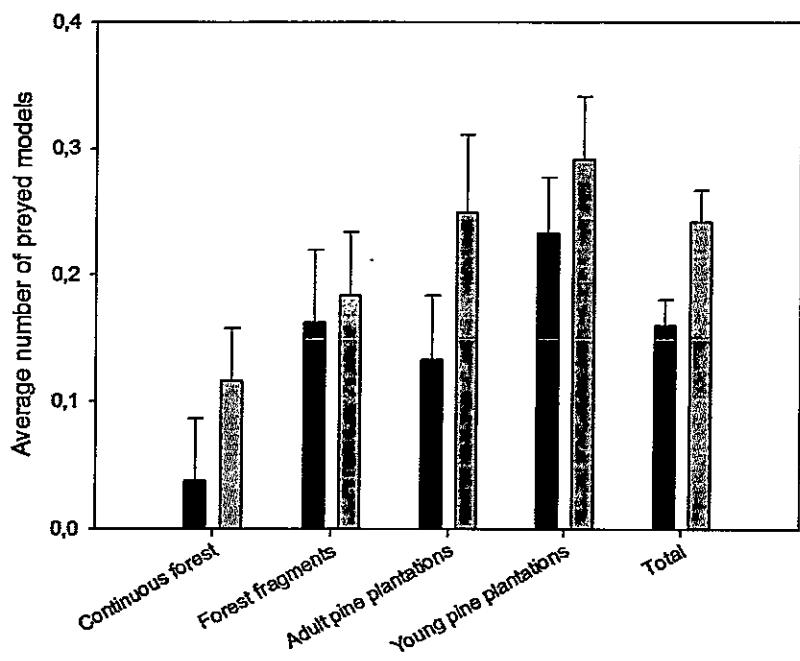
Figure 3. Average number of attacked models with and without lumbar glands in each habitat type and total. Black bars correspond to models with lumbar glands, and grey bars to models without glands.
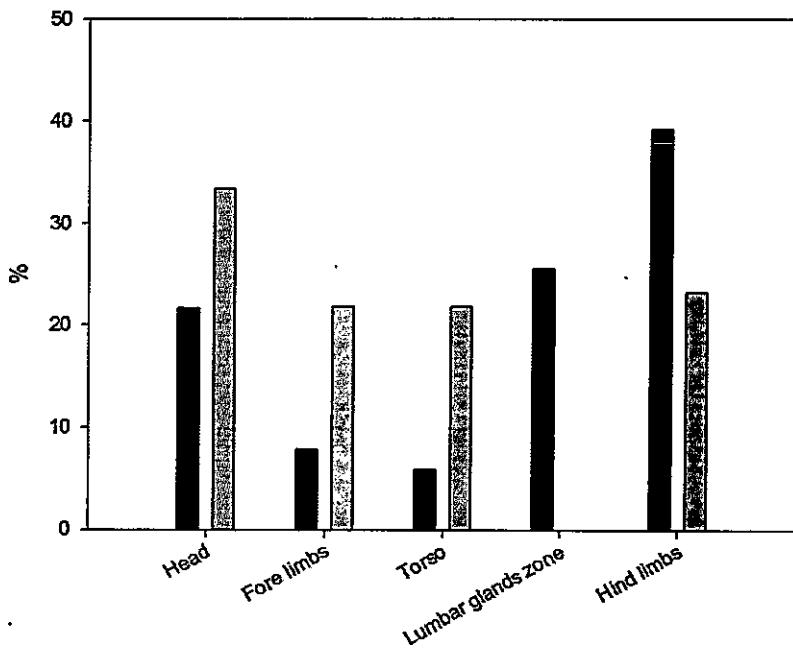
Figure 4. Proportion of models of *P. thaul* attacked (with and without glands) in different parts of the body. Black bars correspond to models with lumbar glands and grey bars to models without glands.

DISCUSSION

The role of amphibian coloration upon predation rates well established but the evidence of the antipredator effect of particular morphological traits such as eyespots is scarse (Dreher et al. 2015, Hegna et al. 2013; Kuchta, 2005; Noonan & Corneault, 2009; Stuart et al. 2012).

The presence of lumbar glands mimicking eyes reduces the odds of being preyed upon. Further, if attacked, predators hit non-lethal body regions potentially enabling the prey to escape as assessed in *P. thaul*. These experimental results agree with observations in butterflies, where eyespots located on the wings decrease mortality by predation, reflected in a higher percentage of escape events compared with butterflies without eyespots (Mukherjee & Kodandaramaiah, 2015; Prudic et al. 2015). Deflecting attacks,

eyespots attacks are concentrated in the posterior zone (hind limbs and lumbar glands) in *P. thaul* exhibit the same antipredator effect than on butterflies, where those with eyespots are more attacked in the fore wings (68.4% of the attacks), while butterflies without eyespots were more attacked in the head, thorax and abdomen (81.5%) (Olofsson et al. 2010; Prudic et al. 2014).

Besides the effect of the presence or absence of the lumbar glands, also might exist a variation in predation risk associated to size of the glands. Exist a geographic variation in size of lumbar glands, where north populations have lumbar gland of 1/9-1/10 related to snout-vent length, while in southern populations it is observed a higher development of the glands (1/4-1/5), suggesting this trait could have an adaptive significance (Cei, 1962). In our study site we observe that average size of lumbar glands is 1/5 in relation to snout-vent length, existing individuals with lumbar sizes of 1/4 until 1/8, recorded similar variability that observed to geographic scale. This variation could generate differences in predation risk, since larger eyespots would be more effective to intimidate predators (Kodandaramaiah et al. 2013). Coloration patterns of eyespots also might mediate predation risk, since some yellow-black combinations are more effective to avoid predator attacks (Barnet, 2016).

The effect of eyespots as antipredator defense is significant in altered environments, who appears as the riskier. In fact, birds are more abundant in disturbed habitats such as plantations (Chapter 1; González-Gómez et al. 2006). Facing increasing habitat changes, eyespots might enable *P. thaul* to thrive in altered environments, exhibiting an advantage compared to syntopic amphibians who does not hold this morphological trait.

This advantage could be reflected in abundances of *Pleurodema thaul* in more exposed habitats, as young pine plantations, where 90% of individuals correspond to *P. thaul*, while 10% is constitute by individuals of *Eupsophus septentrionalis* and *Alsodes vanzolinii*, amphibians that do not have this morphological trait.

Whether landscape level changes are increasing selective pressure upon eyespots is yet to be determined (Schlaepfer et al. 2007; Palumbi, 2001), but certainly the presence of lumbar glands is an effective antipredatory mechanisms, avoiding attacks by predators and deflecting them to non-vital regions, enabling them to thrive in disturbed environments.

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Anexo I

BATRACHYLA TAENIATA (GIRARD, 1855): FILLING THE DISTRIBUTION GAP AND A NEW HABITAT FOR THIS SPECIES IN CENTRAL CHILE

ABSTRACT

We report a new locality for *Batrachyla taeniata*, which fills a 290 km gap in its geographic distribution. The records add a new type of habitat for *B. taeniata*, which was recorded in commercial plantations of *Pinus radiata*.

Key words: Maulino Forest; Amphibians; distribution; pine plantations; conservation

INTRODUCTION

Batrachyla taeniata (Girard, 1855) exhibits the widest geographic range of the four *Batrachyla* species, spanning approximately 1.600 km. Its northern limit is Zapallar (32°33' S, 71°27' W), and its southernmost known record is Río Mosco (48°48' S, 72°58' W) (Correa et al. 2014). Despite its wide distribution, *B. taeniata* exhibits a hiatus in the coastal region of central Chile. In Maule Region, there exist only two records of this species, one in the Andean Range and the other in the Intermediate Depression but none in the Coastal Range of this region (Figure 1). Hence, there is a 290 km gap in the coastal area between the southernmost record in Libertador General Bernardo O'Higgins Region and the northernmost record in Bio-Bio Region (Figure 1). This discontinuous distribution is thought to represent an artifact of incomplete knowledge (Sallaberry et al. 1981). In fact, the herpetofauna of this region has received scarce attention (Simonetti et al. 1995). A recent record supports this assertion and increases the range of environments known for *B. taeniata*.
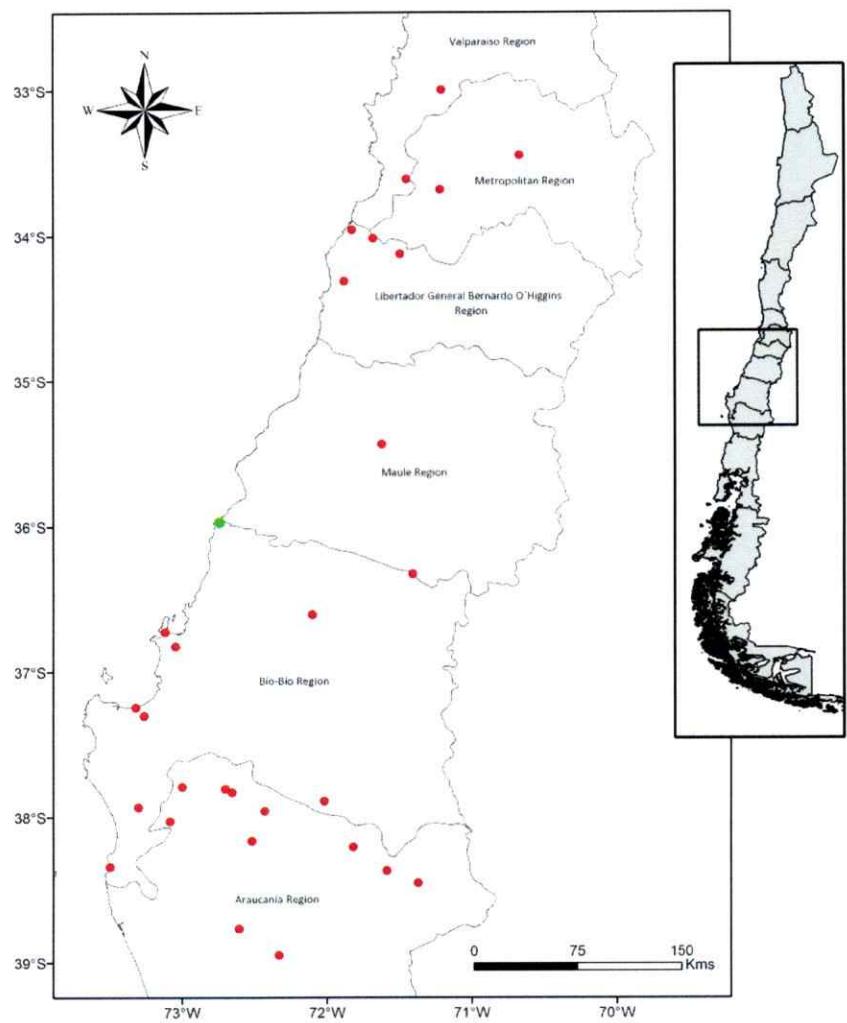
Figure 1. Distribution map of *Batrachyla taeniata*. Red dots represent historic localities (records obtained from Sallaberry et al. 1981; Brieva and Formas 2001; Cuevas and Cifuentes 2010; Correa et al. 2014). The green dot corresponds to the new record.

METHODS

During sampling of amphibians at Trehualemu (35°58' S, 72°44′ W), *B. taeniata* were recorded within a mature *Pinus radiata* plantation (Figure 2). This stand exhibits dense understory vegetation and is located 5.3 km from Reserva Nacional Los Queules (35°59′ S, 72°41′ W). Specimens were not collected but photographed for species determination (Figure 3). Sampling was carried out on four days per month from August to November 2015. Surveying was performed through active search in four transects of 20 x 2 m, in each site of five sites, of four different habitat types: a) native forest, b) fragments of forest, c) mature pine plantations and d) young pine plantations.

Figure 2. Environment in which *Batrachyla taeniata* was found, a plantation of *Pinus radiata* with mature understory.

Figure 3. Individual of *Batrachyla taeniata* (not collected).

IDENTIFICATION

Following Rabanal and Nuñez (2008), recorded individuals were ascribed to *B. taeniata* due to the presence of diagnostic features such as the dark stripe from nares to tympanic ring (Figure 3). The sympatric *B. leptopus* (Cuevas and Cifuentes 2010) can be differentiated from *B. taeniata* by its rounded head and mouth, a highly variable dorsal coloration with irregular spots over a lighter skin as well as the presence of dark pigmented bracelets in the legs. Species identification was further corroborated by José J. Nuñez from Universidad Austral de Chile.

Eighty-two amphibians were recorded with an overall sampling effort of 26.3 person-hours (native forest = 7.5 person-hours; forest fragments=5.6 person-hours; mature pine plantations = 6.2 person-hours; young pine plantations = 7 person-hours). Despite this scant sampling effort, five species were recorded: *Eupsophus septentrionalis* (76 out of 82 individuals), *Pleurodema thaul* (one out 82), *Alsodes vanzolinii* (one out of 82), *Telmatobufo bullocki* (1 out of 82) and *Batrachyla taeniata* (3 out of 82). All *B. taeniata* were recorded in mature plantations (Figure 2).

DISCUSSION

The occurrence of *B. taeniata* at Trehualemu fills the distributional 290 km north-south gap along the coast (Figure 1), reinforcing the contention that this species has a broad continuous distribution (Correa et al. 2014; see also Cei 1962). The absence of records might represent lack of adequate sampling in central Chile or local extirpation due to intense habitat modification (Cuevas et al. 2014). While *B. taeniata* is regarded a common species, populations in central Chile are considered to be declining due to deforestation and wood plantations (IUCN SSC Amphibian Specialist Group 2015). However, its presence in a commercial plantation of an exotic conifer suggest that its habitat breadth might be larger than hitherto recognized. Similarly, *Alsodes vanzolinii* (Rabanal and Alarcón 2010), *Eupsophus septentrionalis*, *Calyptocephalella gayi* and *Telmatobufo bullocki* (Escobar et al. 2005) have also been recorded within *P. radiata* plantations. In all cases, plantations exhibit a mature understory, a structural component that mitigates the impact brought about by forestry plantations and might provide a surrogate habitat, contributing to the conservation of the native fauna, amphibians included (Simonetti et al. 2013, Cerda et al. 2015). Records at Trehualemu confirm the continuous distribution and the capacity of *B. taeniata* to thrive in disturbed habitats such as forestry plantations.

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Anexo II

ECOLOGÍA TROFICA DE PLEURODEMA THAUL EN PLANTACIONES FORESTALES DE LA REGIÓN DEL MAULE, CHILE CENTRAL

RESUMEN

Se analizó la dieta a partir de individuos de *Pleurodema thaul* obtenidos de plantaciones de *Pinus radiata* en la Región del Maule. Se determinó un alto número de ítems presa (35 taxa), donde las presas del orden Coleóptera fueron las más comunes, sugiriendo una dieta principalmente terrestre. Se logró determinar 10 presas hasta nivel de especies, donde *Notocoxelus angustatus* resultó ser la más abundante y frecuente. Este trabajo constituye una primera aproximación sobre el aporte de recursos alimentarios a los anfibios presentes en estos ambientes perturbados.

INTRODUCCIÓN

La información obtenida a partir de los hábitos alimentarios puede ser relevante para identificar condiciones del hábitat y determinar la influencia de la disponibilidad de presas en la distribución de las especies (Gunzburger, 1999; Parker & Goldstein, 2004). La presencia de ciertas especies de anfibios en plantaciones forestales se ha asociado a la presencia de abundante sotobosque, el que podría proveerle de refugio (Rabanal & Alarcón 2010; Escobar et al. 2005), sin embargo, antecedentes sobre los recursos alimentarios que podrían estar aportando estos ambientes a las poblaciones de anfibios es desconocido.

Los aspectos dietarios de los anfibios chilenos es un tema poco estudiado (Vidal & Labra, 2008), existiendo sólo algunos datos en trabajos de descripción de especies o algunos análisis tróficos de especies como *Pleurodema bufoninum, Pleurodema thaul, Batrachyla taeniata, Rhinella spinulosa y Alsodes coppingueri* (Pincheira-Donoso, 2002a, 2002b, Díaz-Páez & Ortiz, 2003; Gutierrez et al. 2008; Nuñez et al. 1982; Alveal et al. 2015).

El sapito de cuatro ojos (*Pleurodema thaul*) se caracteriza por ser un anfibio que se adapta muy bien a los ambientes antropogenizados (Rabanal & Nuñez, 2009), pudiendo ser encontrado también en plantaciones de *Pinus radiata* (Puente-Torres & Simonetti, 2016). Sobre los aspectos dietarios de *P. thaul* de una población de Hualpén, se describe que estos animales consumen principalmente artrópodos, donde los ítems presa más comunes son dípteros y arácnidos (Díaz-Páez & Ortiz, 2003).

El objetivo de este trabajo es describir la composición dietaria de individuos de *Pleurodema thaul*, provenientes de plantaciones de *Pinus radiata*, determinando la abundancia de cada ítem presa y la frecuencia de ocurrencia.

METODOLOGÍA

El análisis de dieta se realizó a partir de 20 individuos de *P. thaul* capturados accidentalmente por trampas para insectos. Los individuos fueron colectados en plantaciones jóvenes de *Pinus radiata* en Trehualemu, Región del Maule, entre los meses de enero y mayo de 2015. Posteriormente los individuos fueron conservados en alcohol de 70°. Se realizó una disección de los individuos para la extracción de los tractos digestivos desde el cardias hasta la base de la cloaca, los que fueron conservados en alcohol de 70° (Pincheira-Donoso, 2002a). Las muestras fueron analizadas en laboratorio bajo una lupa estereoscópica, donde cada presa fue determinada hasta la menor categoría taxonómica posible.

RESULTADOS

El análisis de composición dietaria se realizó a partir de 20 ejemplares de *Pleurodema thaul*, de los que 19 presentaban contenidos estomacal. Se obtuvo un total de 124 presas repartidos en 35 taxa (Tabla 1), destacándose los ordenes Coleoptera, Diptera y Hemiptera, los que en conjunto constituyen el 67,5% de la dieta total de esta especie (Figura 1). Además de la presencia de presas animales, se registró la presencia de restos vegetales en el 70% de los ejemplares analizados.

Tabla 1. Datos dietarios para *Pleurodema thaul*. Valores representan las abundancias de las presas (N y %N) y la frecuencia de ocurrencia de cada ítem presa (F y %F).

Ítem de presa	N	F	%N	%F
CRUSTACEA				
Amphipoda	1	1	0,81	5
ARACHNIDA				
Aranae	3	3	2,44	15
Aranae (RSD)	1	1	0,81	5
COLEOPTERA				
Carabidae	15	12	12,2	60
Carabidae (larva)	1	1	0,81	5
Chrysomelidae	1	1	0,81	5
Cryptophagidae	3	2	2,44	10
Curculionidae	1	1	0,81	5
Elateridae	1	1	0,81	5
Elateridae (larva)	1	1	0,81	5
Latrididae	2	2	1,63	10
Leioidae	3	3	2,44	15
Staphylinidae	4	4	3,25	20
Staphylinidae (RSD)	3	2	2,44	10
Zopheridae	16	8	13	40
Coleoptera (RSD)	2	2	1,63	10
COLLEMBOLA	2	2	1,63	10
DERMAPTERA	8	4	6,5	20
DIPTERA				
Ceratopongonidae	2	2	1,63	10
Mycetophilidae	6	2	4,88	10
Simuliidae	4	3	3,25	15
Stratiomydae	1	1	0,81	5
Tipulidae (larva)	3	2	2,44	10
HEMIPTERA			0	0
Aphididae	2	2	1,63	10
Lygaeidae	3	2	2,44	10
Miiridae	1	1	0,81	5
Nabidae	6	3	4,88	15
Typhleocybinae	1	1	0,81	5
Hemiptera (RSD)	1	1	0,81	5
HYMENOPTERA				

|				
Braconidae	1	1	0,81	5
Formicidae	2	2	1,63	10
LEPIDOPTERA (larva)	12	7	9,76	35
ORIBATIDA	1	1	0,81	5
ORTHOPTERA				
Grillidae	7	4	5,69	20
SCOLOPENDROMORPHA				
Scolopendridae	3	3	2,44	15
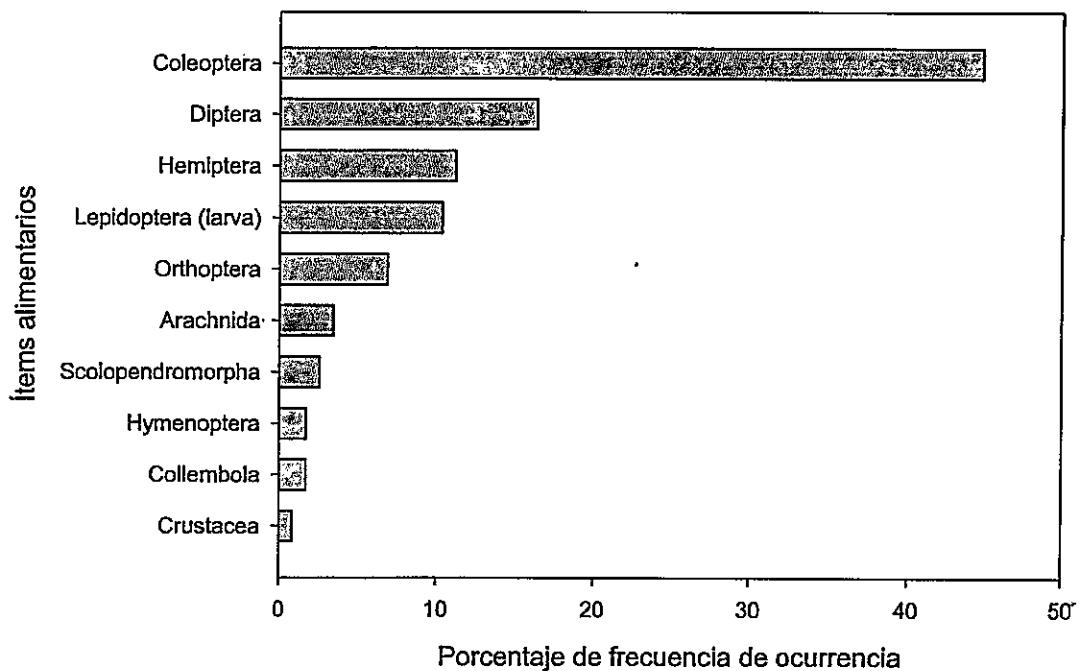
Figura 1. Porcentaje de frecuencia de ocurrencia de los ítems encontrados en los tractos digestivos de *Pleurodema thaul*

Del total de presas obtenidas, el 37% (n=46) pudo ser determinadas hasta el nivel de especie, siendo *Notocoxelus angustatus* la especie con mayor abundancia y frecuencia de ocurrencia (Tabla 2).

Tabla 2. Abundancia y frecuencia de las presas determinadas hasta nivel de especie consumidas por *P. thaul*

Orden	Familia	Especie	N	F	%N	%F
Coleoptera	Carabidae	*Metius femoratus*	1	1	2,17	5
	Chrysomelidae	*Hornius grandis*	1	1	2,17	5
	Cryptophagidae	*Chiliotis formosa*	3	2	6,52	10
	Leioidae	*Eupelates transversestrigosus*	3	2	6,52	10
	Zopheridae	*Notocoxelus angustatus*	15	7	32,61	35
Dermáptera	Forficulidae	*Forfícula auricularis*	8	4	17,39	20
Hemiptera	Lygaeidae	*Bergidia polycroma*	3	2	6,52	10
Hymenoptera	Formicidae	*Solenopsis latastei*	2	2	4,35	10
Orthoptera	Grillidae	*Gryllus fulvipennis*	7	5	15,23	25
Scoloprendromorpha	Scolopendridae	*Akymnopellis chilensis*	3	3	6,52	15

DISCUSION

La predominancia del orden Coleóptera en las presas consumidas por los individuos de *P. thaul*, indica que estos animales se están alimentando principalmente de insectos terrestres, a diferencia de lo reportado para los ejemplares de *P. thaul* de Hualpén, donde se alimentaban tanto de insectos terrestres como acuáticos (Díaz-Páez & Ortiz, 2003). La presencia de restos vegetales en la dieta de anfibios es un tema que aún no tiene una respuesta concreta, sugiriéndose que podría tener un rol nutricional y/o constituir una reserva de agua adicional para evitar la desecación (Anderson et al. 1999).

Destacamos que a pesar del número de ejemplares analizados en este trabajo (n=20) en comparación con el número de ejemplare evaluados en Hualpén (n=160), tanto el número de presas como el número de taxa identificados fue alto, lo que indirectamente podría estar indicando que la disponibilidad de presas en plantaciones forestales es alta. Otro antecedente que podría estar indicando una preferencia por insectos presentes en plantaciones forestales, es la gran abundancia y frecuencia de ocurrencia

de *Notocoxelus angustatus*, coleóptero nativo muy abundante en la matriz de pino (Moreno, 2001).

Información sobre la disponibilidad de presas es relevante en los estudios de dieta (Maneyro & da Roaa, 2004), sin embargo, estudios que incorporen esta información son escasos, particularmente en ecosistemas templados (Hirai & Matsui, 1999), escenario que habitualmente se observa en los estudios de dieta de anfibios chilenos.

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CONCLUSIONES GENERALES

La transformación del paisaje genera variación en las interacciones depredador-presa dado que genera cambios en la riqueza y abundancia de las especies interactuantes, por lo que el riesgo de depredación de anfibios varía a través del paisaje. Nuestros resultados muestran que los niveles de depredación son mayores en los ambientes perturbados (e.g. fragmentos de bosque y plantaciones de pino adultas y jóvenes) respecto del ambiente no perturbado, lo que estaría mediado por la presencia d e vegetación más que por la variación en las abundancias de depredadores.

Sobre el efecto de las glándulas lumbares en *Pleurodema thaul* como estrategia antidepredatoria, se pudo corroborar que efectivamente disminuye el riesgo de depredación y además, que en aquellos casos en que se produce el ataque, éste e s desviado hacia zonas no vitales. Esto sugiere que este anfibio tendría una estrategia que le confiere una ventaja en comparación a otros anfibios que se encuentran en el área, dado que podrían sortear de mejor forma los mayores niveles de depredación asociados a los ambientes más perturbados, lo que concuerda con la típica descripción de esta especie en que se señala que se encuentra bien adaptada a ambientes antropogenizados (Rabanal & Nuñez, 2008).

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