

A multi-scale approach to facultative paedomorphosis of European newts (Salamandridae) in the Montenegrin karst: Distribution pattern, environmental variables, and conservation

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Abstract

Facultative paedomorphosis, a process in which newt larvae can opt for reproduction before or after metamorphosis, is geographically heterogeneous. Despite numerous ecological studies and recent evidence of declines in paedomorphic populations, however, no attempt to model environmental variables that explain the presence of paedomorphs has been made at a multi-scale level. Our aim was to fill this gap in studying three newt species (*Lissotriton vulgaris*, *Mesotriton alpestris*, and *Triturus macedonicus*) of the Montenegrin karst as model species. To this end, we used multivariate analysis on three scales of habitat: the breeding pond, the land use and the climatologic features. Results show that the study area is both an important hotspot for paedomorphosis and where intraspecific diversity is quickly disappearing (20–47% extirpation) because of fish introductions. Other habitat variables (water permanency, pH or the habitat origin) were shown to act on paedomorphosis but not consistently across species, confirming complexity of the evolutionary and ecological processes. This study appeals for more long-term and detailed landscape studies of polyphenisms, a neglected but promising topic, to better understand and protect alternative modes of development. Particularly, measures should be taken to identify hotspots of intraspecific diversity at a global scale and stop fish introductions before we reach a point of no-return.

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1. Introduction

Facultative paedomorphosis occurs in many tailed amphibian species; larvae either metamorphose into terrestrial adults or attain sexual maturity while retaining larval morphology (e.g. external gills and gill slits) (e.g. Semlitsch and Wilbur, 1989; Denoël et al., 2005a). The appearance and maintenance of alternative adult phenotypes is based on genotype–environment interactions experienced by larvae and paedomorphs, which can retain the ability to metamorphose (for the most recent review, see: Denoël et al., 2005a). Particularly, mesocosm experiments showed that paedomorphosis was selected in permanent waters and at low density (Harris, 1987; Semlitsch, 1987).

Several field studies examined habitat characteristics of paedomorphic and metamorphic populations. Among possible determinants of developmental alternatives, streambed structures, and permanency

of water (Semlitsch, 1987; Bennett and Chippindale, 2006) as well as the harshness of the terrestrial environment (Wilbur and Collins, 1973; Healy, 1974; Sprules, 1974a,b) were outlined. Paleontological studies also suggested the importance of global cooling for the success of paedomorphs (Roček, 1995). However, the presence of paedomorphs in a large variety of water bodies indicates a multifactorial nature of the environmental context of the expression of the polyphenism (Sprules, 1974b; Semlitsch, 1987; Breuil, 1992; Whiteman, 1994; Denoël et al., 2001). For instance, Collins (1981) did not find significant habitat variables to distinguish between alternative morphs for elevation or landscape traits and Denoël (2006) found often paedomorphs in semi-permanent waters.

Advancements of statistical tools, specifically multivariate modeling and complex spatial procedures (see e.g. Mazerolle, 2006), have allowed a large number of studies that focused on the lands-

cape and other habitat determinants of amphibians (e.g. Joly et al., 2001; Van Buskirk, 2005; Denoël and Ficetola, 2007) to emerge. Multiple spatial scales can be particularly important to understand the ecological determinants of amphibian distribution (Denoël and Lehmann, 2006; Murray et al., 2008). Despite this, studies have all focused on interspecific diversity. It is thus unknown whether polyphenisms such as facultative paedomorphosis could be affected at various scales by climate, landscape and breeding site features. Such determination is of primary importance to understand the success of alternative developmental pathways in the field.

Understanding distribution of different alternative morphs and their relationship with environmental features is an essential pre-requisite to determine their status and provide efficient conservation measures. Although amphibian conservation has been the target of many studies (Collins and Storfer, 2003; Stuart et al., 2004), paedomorph conservation remains little explored (Whiteman and Howard, 1998; Ćirović, 2005; Denoël et al., 2005b; Denoël, 2007). Much remains to be done, such as identifying hotspots for paedomorphosis, as well as proximate and ultimate causes of this process. The Montenegrin karst area, a restricted part of the Dinaric Alps is already appreciated as a recognizable center of facultative paedomorphosis with populations composed of metamorphs and paedomorphs of three different species (Džukić et al., 1990; Breuil, 1992; Denoël et al., 2001; Ćirović, 2004).

The objective of this study was to survey the water bodies of Montenegro to determine the occurrence and distribution of alternative developmental morphs and to seek environmental characteristics related to paedomorphosis in three newt species at three scales: the breeding aquatic site, the land use and the local climatic characteristics, using analytical tools allowing to take into account the complex spatial structure of data. Moreover, distribution of paedomorphic populations have changed in time (i.e., some populations disappeared). We therefore evaluated environmental features affecting changes in distribution of paedomorphic populations. This allowed testing the effect of environmental change on the distribution of morphs.

2. Materials and Methods

2.1. Sampling procedure

The research was carried out between 1970 and 2005 (mainly 2000–2005) at 145 breeding sites in the Montenegrin karst (Fig. 1). Our surveys covered an area of about 10080 km². Most of the breeding sites (90%) were visited for at least two breeding seasons. Visual Encounter Survey (VES; Crump & Scott, 1994) was the most frequently used technique throughout the study. VES are effective in most habitats and for the study species, which breed in lentic water. Each aquatic site was surveyed for at least 45 min. Most newts were identified by sight (*Triturus macedonicus*, *Lissotriton vulgaris*, and *Mesotriton alpestris*; these newts were previously named *T. carnifex macedonicus*, *T. vulgaris*, and *T. alpestris*), but dip nets were used in areas with vegetation or where water clarity was poor. Newts were classed according to morph (paedomorphs: adult gilled newts; metamorphs: adult metamorphosed newts). Ponds with paedomorphosis were surveyed several times during the study period to determine changes in morph expression, i.e. potential extirpation of paedomorphosis.

For each site, we collected three geographic and eight environmental variables. First, at the pond scale, we determined habitat origin (natural vs. artificial), hydroperiod (from permanent to temporary), pH, and presence of fish. Data were gathered from direct observations and communications from owners of the sites. Moreover, for the 31 ponds with fish and newts, we obtained information on the composition of fish community by looking in data of the fisheries management program for Montenegro, netting the ponds, and interviewing owners and fishermen. Second, we used GIS data to determine the geographic coordinates (longitude, latitude, and altitude) and to class terrestrial habitat 50 m around each site (Corine land cover data: forest, agriculture, other open landscapes, see Kati et al., 2007 for applications in amphibians). We determined climatological features using Worldclim 1.4 at 30 arc-seconds resolution (Hijmans et al., 2007). In the study area, a resolution of 30'' corresponds to cells

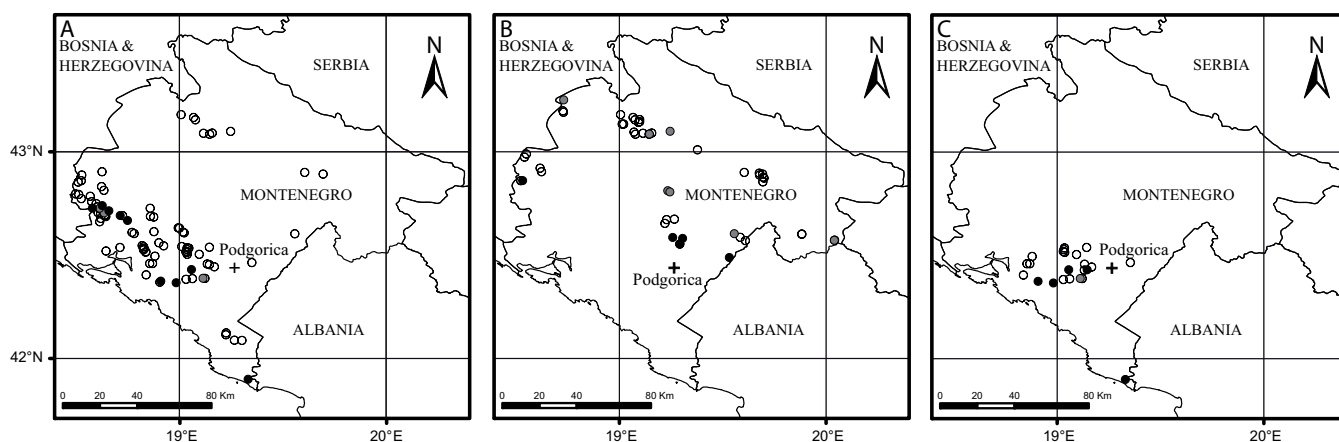


Fig. 1. Distribution of populations with facultative paedomorphosis and only metamorphosis in the Montenegrin karst in (a) *Lissotriton vulgaris*, (b) *Mesotriton alpestris*, (c) *Triturus macedonicus*. Full circles: presence of paedomorphs; grey circles: extirpation of paedomorphs after fish introduction; open circles: absence of paedomorphs, presence of metamorphs.

of about 700 x 900 m, and is therefore appropriate to describe environmental factors that may influence newts at the spatial scale of this study. We extracted four environmental layers describing climate: minimum temperature in January (T min); maximum temperature in July (T max), average annual temperature (T mean), and summed annual precipitation (precipitation).

2.2. Statistical analysis

Elevation, T max, T min, T mean, and precipitation were strongly correlated: high elevation ponds had lower temperature and less precipitation (Pearson correlation, all $|r| > 0.87$). The inclusion of strongly intercorrelated variables in the same regression analysis can bias the result of regressions (Bowerman and O'Connell, 1990). Therefore, we used a principal component analysis (PCA) to reduce correlated variables. PCA was performed on untransformed variables, using the correlation matrix and variable scaling. PCA extracted a single component with an eigenvalue > 1 (PCA-1), explaining 96% of the variance and describing a gradient from high elevation ponds where temperature is cold and precipitation low, to warmer ponds with higher annual precipitation. The correlations of PCA-1 with the environmental variables were: PCA-1/altitude: $r = -0.992$; PCA-1/precipitation: $r = 0.937$; PCA-1/T min: $r = 0.980$; PCA-1/T mean: $r = 0.996$; PCA-1/T max: $r = 0.994$ (all $P < 0.001$). In subsequent analyses, we used PCA-1 as measure of climatic variability.

We used Moran's I to evaluate whether the distribution of populations with paedomorphosis was spatially autocorrelated; significance of Moran's I was evaluated using 10000 Monte Carlo simulations. For each species, we repeated this test two times: first, we evaluated the spatial autocorrelation of historical populations (i.e. including populations before extirpation), and then we evaluated the spatial autocorrelation of present-day populations. This comparison provided insights on spatial patterns of change in the different species.

We used generalized linear models (GLM) assuming binomial error (i.e., logistic regression) to evaluate the relationship between environmental variables and the developmental trait of newt populations (facultative paedomorphosis vs. metamorphosis). To evaluate whether our results are robust to the elimination of the ponds not surveyed recently, we repeated the analysis without the ponds not surveyed after 2000 (17 ponds). The results of this analysis are qualitatively very similar to the analysis including all data. In the present paper, we report the analysis including all data to maximize power.

We repeated the analysis two times. First, using "historical" populations with paedomorphosis as a dependent variable, we evaluated the factors determining the past distribution of paedomorphs. Subsequently, we evaluated the factors that determined if a population with paedomorphosis persisted over time or went extinct. This second analysis was performed only on the subset of localities where paedomorphosis was historically found.

Because presence of spatial autocorrelation may bias results of traditional regressions (such as logistic regression), we evaluated whether the residuals of our best models were spatially autocorrelated, following the Monte Carlo procedure detailed in Lichstein et al. (2002), using 99 permutations and the default $\alpha = 0.05$. If residuals were spatially autocorrelated, we used Spatial Eigenvector Map-

ping (SEVM) instead of standard GLM. SEVM is a recently developed method, allowing translation of the spatial arrangement of data points into explanatory variables capturing the spatial effects (Dormann et al., 2007). In SEVM, eigenvectors reducing spatial autocorrelation of residuals are computed and then included as spatial predictors into generalized linear models. SEVM is a technique appropriate for ecological analyses because, unlike other methods, it aims to remove residual spatial autocorrelation (Dormann et al., 2007). We extracted eigenvectors following the procedure of Griffith and Peres-Neto (2006). We also calculated Nagelkerke's $R^2(R^2_N)$ as a measure of the proportion of variance explained by each model.

We used an information-theoretic approach to find the best models describing the relationship between environmental variables, distribution of paedomorphs, and loss of paedomorphosis (Burnham and Anderson, 2002; Mazerolle, 2006). First, we built generalized linear models or SEVMs including all possible combinations of environmental variables (excluding interactions). To avoid the issue of collinearity, we did not consider models with variance inflation factors > 5 (Bowerman and O'Connell, 1990). For each model, we calculated Akaike's Information Criterion (AIC), and Δ -AIC, which equals the difference in AIC values between the candidate model and model with the lowest AIC (i.e., the best model). We did not correct AIC for small sample size, since effectiveness of such correction remains unclear (Richards, 2005). As an automated model selection using AIC can have drawbacks (Ellison, 2004), we used two additional criteria for the definition of candidate models. We considered only models performing significantly better than the null model (Maggini et al., 2006), following a likelihood ratio test. Moreover, AIC may select overly complex models (Richards, 2008). Therefore, we considered a complex model only if it had a Δ -AIC less than the Δ -AIC of all its simpler nested models (Richards, 2008).

We performed PCA in SPSS 13 (©SPSS, 2004) and all other statistical analyses in R 2.5, using `sdep` for spatial analyses (Bivand, 2007).

3. Results

We found 91 populations of *L. vulgaris*, 51 populations of *M. alpestris*, and 15 populations of *T. macedonicus* (Fig. 1). Out of these, 12 populations of *L. vulgaris*, 15 populations of *M. alpestris*, and five populations of *T. macedonicus* were inhabited by paedomorphic individuals. The most recent surveys (2000–2005) indicated that there remain nine populations with paedomorphs in *L. vulgaris*, eight in *M. alpestris*, and four in *T. macedonicus*. This means that *M. alpestris*, *L. vulgaris*, and *T. macedonicus*, respectively, lost 47%, 25%, and 20% of their historical presence of paedomorphosis.

Thirteen species of introduced fish have been found in the 31 water bodies that were inhabited by newts (Table 1). The most abundant fish species were the brown trout (*Salmo trutta*), the rainbow trout (*Oncorhynchus mykiss*), and the Arctic charr (*Salvelinus alpinus*) in sites with metamorphs but with differences among newt species (Table 1). The same pattern was found for sites with paedomorphs for the two first fish species, with the Arctic charr and the European chub (*Squalius cephalus*), coming next. On average (\pm SE), 2.3 ± 0.2 species of fish were present in stocked water bodies.

Table 1. Occurrence of introduced fish (percentage of ponds occupied) in water bodies of Montenegro that were also inhabited by newts: paedomorphs (Paedo.), metamorphs (Meta.), *Mesotriton alpestris* (*M. a.*), *Lissotriton vulgaris* (*L. v.*), and *Triturus macedonicus* (*T. m.*). Only ponds with fish are described in this table.

Fish species	Family	Occurrence (%)				
Scientific name		Paedo. (n=11)	Meta. (n=31)	<i>M. a.</i> (n=25)	<i>L. v.</i> (n=15)	<i>T. m.</i> (n=1)
<i>Oncorhynchus mykiss</i>	Salmonidae	45	35	44	27	0
<i>Salvelinus alpinus</i>	Salmonidae	18	58	68	33	0
<i>Salvelinus fontinalis</i>	Salmonidae	9	3	4	0	0
<i>Salmo trutta</i>	Salmonidae	27	71	88	67	0
<i>Thymallus thymallus</i>	Salmonidae	0	6	0	7	100
<i>Barbus barbus</i>	Cyprinidae	0	3	4	0	0
<i>Barbus meridionalis</i>	Cyprinidae	0	3	0	7	100
<i>Carassius auratus</i>	Cyprinidae	9	13	12	20	100
<i>Ctenopharyngodon idella</i>	Cyprinidae	9	3	4	7	0
<i>Phoxinus phoxinus</i>	Cyprinidae	9	16	12	20	100
<i>Squalius cephalus</i>	Cyprinidae	18	10	0	20	100
<i>Cottus gobio</i>	Cottidae	0	3	0	7	100
<i>Ameiurus nebulosus</i>	Ictaluridae	9	6	0	13	0

Table 2. Candidate regression models explaining the distribution of newt populations with paedomorphosis on the basis of environmental features. All models reported here perform significantly better than the null-model. For *Lissotriton vulgaris*, we used generalized linear models, for *Mesotriton alpestris* we used Spatial Eigenvector Mapping (SEVM). Models are ranked according to their Δ -AIC; the model with the lowest Δ -AIC is the best AIC model. K: number of parameters in the model; Δ -AIC: difference between the AIC of each model and the AIC of the best model. R^2_N = Nagelkerke's R^2 .

Rank	K ^a	Δ -AIC	variables	R^2_N
<i>Lissotriton vulgaris</i>				
1	2	0	pH (+); habitat origin (+)	0.138
2	1	0.551	pH (+)	0.089
3	1	0.74	habitat origin (+)	0.085
<i>Mesotriton alpestris</i>				
1	4	0	fish (-); water permanence (+); PCA-1 (+)	0.522
2	3	0.473	water permanence (+); PCA-1 (+)	0.478
3	3	1.208	fish (-); PCA-1 (+)	0.464
4	2	1.231	PCA-1 (+)	0.425
5	3	1.606	habitat origin (+); fish (-)	0.456
6	2	1.745	habitat origin (+)	0.415
7	3	2.610	fish (-); water permanence (+)	0.437

^aFor *T. alpestris*, we used SEVM, therefore K equals the number of environmental variables plus one, because all models included the SEVM eigenvector. For habitat origin, + means a trend toward artificial habitats; for PCA-1, this means lower elevations and warmer climate.

3.1. *Lissotriton vulgaris*

Historical populations of *L. vulgaris* were significantly spatially autocorrelated (Moran's $I = 0.118$; Monte Carlo permutation test: $P = 0.03$) (Fig. 1a). However, present-day distribution of populations with paedomorphosis is not spatially autocorrelated ($I = 0.059$, $P = 0.118$).

The best AIC model shows that the historical distribution of paedomorphosis in *L. vulgaris* was significantly associated with artificial wetlands with high pH (Table 2; Fig. 2). Simpler models, including pH only or habitat origin only had slightly lower

support. The variables had the same sign they had in the best model.

Fish have been introduced in three wetlands with presence of paedomorphic *L. vulgaris*. All these paedomorphs are now extinct. The best AIC model showed that populations with paedomorphic *L. vulgaris* are lost in ponds where fish have been introduced. This model perfectly explained the variation in distribution of populations with paedomorphosis (GLM: $X^2_1 = 13.496$, $P < 0.001$, $r^2_N = 1$). This was the only candidate model performing significantly better than the null model.

3.2. *Mesotriton alpestris*

Historical populations of *M. alpestris* were significantly spatially autocorrelated (Moran's $I = 0.287$; Monte Carlo permutation test: $P < 0.001$) (Fig. 1b). Spatial autocorrelation is still significant in present-day populations ($I = 0.626$, $P < 0.001$).

For *M. alpestris*, residuals of GLM were spatially autocorrelated (Monte Carlo permutation test, $P < 0.05$). For this reason, we used Spatial Eigenvector Mapping instead of GLM. AIC did not select a single best model, but seven models had similar AIC values. The best model suggests that paedomorphosis of *M. alpestris* was significantly associated with wetlands with permanent water, absence of fish and high values of PCA-1 (i.e., low elevation, warm climate) (Table 2; Fig. 2). Furthermore, two models with low Δ -AIC (i.e., with support similar to the best model) suggested that paedomorphic *M. alpestris* were also associated with artificial wetlands (Table 1, models 5 and 6).

For *M. alpestris*, fish introduction occurred in localities with the lowest values of PCA-1 (i.e., high elevation and cold climate; GLM: $R^2_N = 0.9$, $X^2_1 = 16.444$, $P < 0.001$), therefore PCA-1 cannot be considered together with fish introduction as a potential predictor for loss of paedomorphosis. The best AIC model showed that populations with paedomorphic *M. alpestris* are lost in ponds where fish have been introduced (GLM, $X^2_1 = 16.444$, $P < 0.001$, $R^2_N = 0.826$). This was the only candidate model performing significantly better than the null model.

3.3. *Triturus macedonicus*

We did not detect significant spatial autocorrelation for the distribution of paedomorphic historical or present-day populations (all $P > 0.1$). GLM did not find any model performing significantly better than the null model. However, it should be noted that we included in our analysis 23 populations of *T. macedonicus* and only five were inhabited by paedomorphs. Therefore, the power of this analysis was low.

Only one population of paedomorphic *T. macedonicus* is now extinct (Fig. 1). Even if the number of lost populations is too low to perform statistical analyses, it should be noted that this was the only population with past presence of paedomorphosis in which fish were introduced.

4. Discussion

In the present study, integration of field and GIS data with spatial statistics and an information-theoretic approach allowed us to identify several variables that can explain presence of paedomorphic newts and their extirpation. Although variables describing both wetland and climatic features were significant, the most effective variable was presence of fish. These results extend those obtained from the modeling studies on metamorphic populations (Joly et al., 2001; Guerry and Hunter, 2002; Van Buskirk, 2005; Denoël and Lehmann, 2006). They also give support to specific studies on the distribution of paedomorphs (see Denoël et al., 2005a for a review), which have not yet been modeled at regional scale in pond breeding species (see Bennett and Chippindale, 2006 for an analysis of a stream species).

Environmental variables associated with presence of paedomorphs differed between species. Paedomorphosis in *L. vulgaris* was associated with

artificial wetlands and high pH-values waters. It is not known how high pH values could favour paedomorphosis; also none of the ponds had acidic pH (Fig. 2). We cannot exclude that some factors covary with pH and offer good conditions for paedomorphosis. For instance, presence of vegetation could be dependent on habitat type and in turn affect pH through CO₂ intake. Moreover, pH can be related to the geologic substrate, or to the presence of ions in solutions. Montenegro is characterized by large karst areas and thus calcium is expected to covary positively with pH. On the studied localities, ponds on carbonates had indeed higher pH values (t -test, $t_{143} = 5.95$, $P < 0.0001$). Because calcium was evidenced as a necessary element for gill or skin-breathing animals (Rosseland and Staurnes, 1994) and was positively correlated to newt occurrence (*L. vulgaris* and *Triturus cristatus*: Skei et al., 2006), the Montenegrin karst could be considered as a favourable area for paedomorphs that keep this respiratory system at the adult stage. Future experimental works on this topic would be promising to test this hypothesis. Due to fish tolerance to high pH in Montenegro (P. Simonović, pers. comm.), this is not a confounding factor (but see herein for a direct fish effect). The situation was more complex in *M. alpestris*, with paedomorphs present in both artificial and natural wetlands, but mainly in permanent waters. Such a range of habitats was previously outlined by Denoël et al. (2001) on an ecological synthesis of cases of paedomorphosis in this species. This is not surprising, as paedomorphosis is a complex process resulting from a genotype x environment interaction (Semlitsch et al., 1990) that can be elicited by contrasting cues, notably advantageous vs. detrimental proximate factors (Whiteman, 1994). The highest presence of paedomorphic *M. alpestris* in permanent waters conforms with mesocosm experiments that showed that larvae more often become paedomorphic and paedomorphs metamorphose less frequently in permanent (than in drying) ponds (Semlitsch, 1987; Denoël, 2003). Climate appears to also influence the distribution of paedomorphosis in this species, with a trend to lower elevated sites with a warmer climate. However, the range remains large, with paedomorphosis found from 955 to 1971 m elevation a.s.l. and being absent in the highest elevation lakes. This shows that paedomorphosis is not constrained to the highest elevated sites as initially thought (Sprules, 1974b).

In addition to these variables, fish appear as a significant factor explaining the absence of paedomorphs in both *L. vulgaris* and *M. alpestris*. Moreover, the extirpation of paedomorphs from several localities was explained by fish introduction in these ponds. These long-term observations support previous studies that showed detrimental effects of alien species on metamorphosed tailed amphibians (Gamradt and Kats, 1996; Joly et al., 2001; Kats and Ferrer, 2003; Knapp, 2005; Denoël and Lehmann, 2006; Orizaola and Braña, 2006; Welsh et al., 2006) and particularly on paedomorphs (Breuil, 1985; Denoël et al., 2005b). Paedomorphs are more affected than metamorphs by presence of fish, because they stay in water longer than metamorphs (Denoël et al., 2005b), they are sometimes smaller and thus preyed upon by gape-limited predators (Denoël and Joly, 2000), and they often forage in open waters, becoming easy prey for fish (Denoël and Joly, 2001). The size and dietary habits of fish can be expected to affect newt persistence. In Montenegro, the most frequent introduced fish in water bodies known to be inhabited newts were Salmonids, i.e. large predatory fish known to prey on newt adult stages (Tyler et al., 1998; G. Džukić and Kalezić, pers. obs.). However,

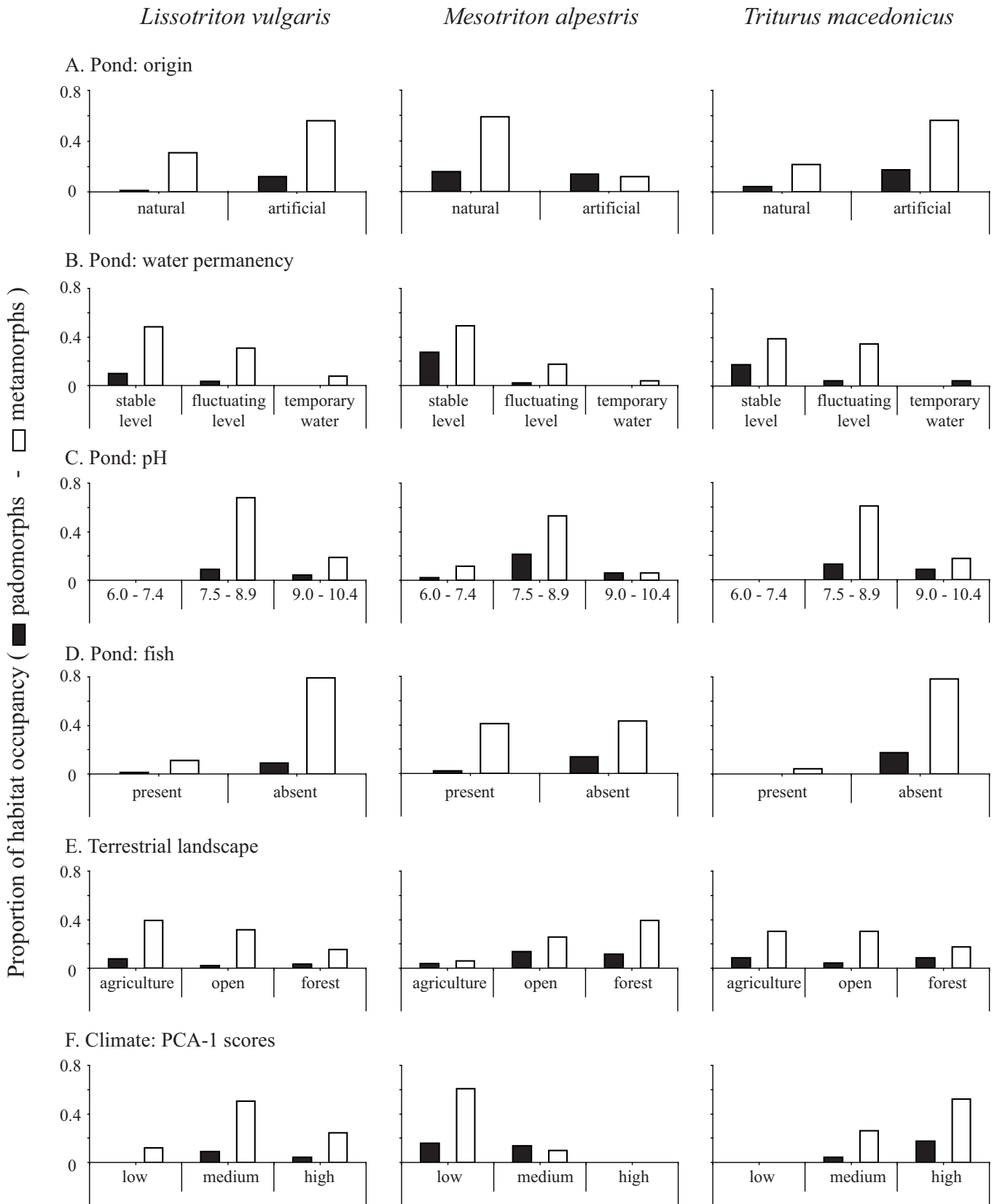


Fig. 2. Proportion of habitat occupancy with facultative paedomorphosis and only metamorphosis in newts of the Montenegrin karst at three scales: pond (A - D), landscape (E), and climate (F). See Table 2 for regression models and text for details on PCA-1 scores.

smaller fish species such as minnows (Schabetsberger et al., 2006) or goldfish (Monello and Wright, 2001) can also negatively affect newt populations. Consequently, all introductions in natural habitats of Montenegro should be seen as alarming.

During this study, out of 145 surveyed wetlands, paedomorphic specimens were found in 28 sites (19%). Such high paedomorphosis density nominates the Montenegrin karst region as second in incidence rate (i.e. in terms of occurrence) for facultative paedomorphosis in European newts, after the karst plateau of Larzac (southern France) with a rate of 26% in *T. helveticus* (Denoël, 2007) and 42% considering only the sites where the species was present (M. Denoël, pers. obs.). In Montenegro, the propensity to undergo paedomorphosis differs among the species, but was for each lower than in Larzac: 13%, 29%, and 33% of studied localities of *L. vulgaris*, *M. alpestris*, and *T. macedonicus*, respectively. Despite that only five cases of paedomorphosis were found in *T. macedonicus*, this remains a high incidence rate for the large-bodied *Triturus* newts, for which paedomorphosis is extremely rare (e.g. Džukić et al., 1990). Moreover, coexistence of alternative morphs in two different species (*L. vulgaris* and *T. macedonicus*) was found in five sites, which is also a very rare pattern. This finding alone supports the importance of environment in the appearance of paedomorphosis (Breuil, 1992; Denoël et al., 2005a). On the other hand, detection of spatial autocorrelation in distribution of populations with facultative paedomorphosis suggests that this process is more likely to occur in restricted areas. Although directly dependent on environmental cues, developmental pathways can be favored after generations of selection in specific directions (Semlitsch and Wilbur, 1989; Harris et al., 1990; Semlitsch et al., 1990; Voss and Shaffer, 1997). This may result in the existence of a genetic underpinning which could locally allow individuals to opt for alternative developmental pathways. In the Montenegrin karst, the most striking clustering of populations with paedomorphic populations appears in *M. alpestris*, but was also significant in *L. vulgaris*.

5. Conclusions: towards more in-depth studies for the conservation of alternative morphs

This study appeals for more large-scale and long-term field studies to complement laboratory or mesocosm experiments (Harris, 1987; Semlitsch, 1987; Ryan and Semlitsch, 2003) to determine environmental variables associated with expression of polyphenism in development. Terrestrial and aquatic habitats often need to be oversimplified to fit the power criteria of multivariate statistical analyses. It is thus important to focus on small geographic areas where paedomorphosis is prevalent at a high rate and analyze in-depth micro-components of terrestrial and aquatic habitats. From one side, absence of significant effects of the general Corine categories of terrestrial landscape may mean that they are not suitable to understand morph expression. From another side, in Montenegro, terrestrial habitats remain usually well preserved and thus favorable to newts. More detailed studies of the landscape and large sampling designs would be particularly suitable because of the complexity of developmental pattern variations.

Following Denoël (2007), Montenegro appears to be a hotspot for paedomorphosis because of its high density of populations with paedomorphosis and mainly because all three species present this

alternative life-history trait. Spatial autocorrelation of populations with paedomorphs shows that some areas were particularly favorable to expression of this polyphenism. However, these populations and the hotspot as a whole have critical conservation issues. First, paedomorphs were extirpated from a third of the sites in which this polyphenism was recorded. The current absence of spatial correlation in the distribution of paedomorphs in *L. vulgaris* indicates that the decrease of density of populations strongly modified the spatial pattern of paedomorphs. Second, extirpations affected all populations that were known to be inhabited by the largest numbers of paedomorphs. Indeed, thousands of paedomorphs used to be seen in both ponds with *L. vulgaris* and alpine lakes with *M. alpestris*. From a conservation point of view, it may be too late to restore intraspecific biodiversity in some populations, particularly after a long time of selection against paedomorphosis (see e.g. Semlitsch and Wilbur, 1989). However, recent works have showed that amphibian populations can show resilience after fish removal (Knapp et al., 2007). There is thus potential of recovery in ponds where fish were introduced recently or where paedomorphosis remained in nearby aquatic sites, but this remains to be determined. Netting or drying could help remove current stocks (Knapp et al., 2007) but this is not an easy task particularly for small fish in large Alpine lakes (Knapp and Matthews, 1998). However, because fish cannot always sustain themselves in introduced sites, the interdiction of future stockings may be the best management recommendation to favour native amphibian communities. Because of the specificities of alternative phenotypes, this study appeals for special considerations before management of areas in which polyphenisms are exhibited.

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