Effects of Widespread Fish Introductions on Paedomorphic Newts in Europe

MATHIEU DENOEL[1], GEORG DZUKIC[2], MILOS L. KALEZIC[2,3]

Abstract

As a result of factors such as global warming, habitat destruction, and species introduction, amphibians are declining worldwide. No one, however, has analyzed the status of polymorphic amphibian species at a national or continental scale, although some local reports exist. Our aim was to report on the loss of intraspecific heterochrony as a loss to diversity in determining the consequences of fish stocking on European populations of paedomorphic newts. Paedomorphosis is a polymorphism in which larval traits are retained in the adult stage. We surveyed 39 paedomorphic populations of the alpine (*Triturus alpestris*) and palmate (*T. helveticus*) newts, all but one of which initially occupied fishless ponds and lakes in France, Italy, Slovenia, Bosnia, Montenegro, and Greece. Exotic fishes were found in 44% of the studied aquatic habitats, with a 100% presence in Montenegro. At all sites paedomorphs disappeared and metamorphs declined. Only fish explained these population changes because alternative factors such as drying were not significant. More catastrophically, fish introductions occurred in habitats known to support the largest populations of newts and even some endemic subspecies. If management and legislative measures are not taken to stop fish stocking, protect paedomorphs as conservation units at national and international levels, and restore natural habitats, all the largest paedomorphic populations may disappear in the near future. Their disappearance would represent a loss of one of the rare, fascinating examples of intraspecific heterochrony.

Resumen

Los anfibios están declinando mundialmente debido a causas como calentamiento global, destrucción de hábitat e introducción de especies. Sin embargo, nadie ha analizado el estatus de especies polimórficas de anfibios a una escala nacional o continental, aunque existen algunos reportes locales. Nuestro objetivo fue reportar la pérdida de heterocronía intraespecífica como una pérdida de diversidad al determinar las consecuencias del aprovisionamiento de peces sobre poblaciones europeas de tritones pedomórficos. La pedomórfosis es un polimorfismo en el que se retienen características larvarias en la etapa adulta. Muestreamos 39 poblaciones pedomórficas de los tritones *Triturus alpestris* y *T. helveticus*, que, excepto una, inicialmente habitaban charcas y lagos sin peces en Francia, Italia, Eslovenia, Bosnia, Montenegro y Grecia. Encontramos peces exóticos en 44% de los hábitats acuáticos estudiados, con presencia de 100% en Montenegro. Los pedomorfos desaparecieron y los metamorfos declinaron en todos los sitios. Solo peces explicaron estos cambios poblacionales porque factores alternativos como desecación no fueron significativos. Más catastróficamente, las introducciones de peces ocurrieron en hábitats conocidos por sostener a las mayores poblaciones de tritones incluyendo algunas subespecies endémicas. Todas las poblaciones pedomórficas grandes pueden desaparecer en el futuro cercano si no se toman medidas de gestión y legislativas para detener el aprovisionamiento de peces, proteger a pedomorfos como unidades de conservación a nivel nacional e internacional y restaurar hábitats naturales. Su desaparición representaría la pérdida de uno de los ejemplos fascinantes, raros, de heterocronía intraespecífica.

Keywords: extinction; fish introduction; global amphibian decline; paedomorphosis; polymorphism

Palabras clave: declinación global de anfibios; extinción; introducción de peces; pedomórfosis; polimorfismo

Introduction

Recent reports suggest that amphibian species have suffered population declines worldwide (e.g., Alford & Richards 1999; Houlahan et al. 2000; Young et al. 2001). These declines and extinctions have affected species in both pristine environments (Pounds et al. 1999) and high-risk habitats (Carr & Fahrig 2000). Several proximate causes have been identified: habitat destruction (Herbeck & Larsen 1998), population fragmentation (Bradford et al. 1993; Joly et al. 2001), road traffic (Carr & Fahrig 2000), predator introductions (Hecnar & M'Closkey 1997; Knapp & Matthews 2000), pathogens (Berger et al. 1998), increasing acidification (Whiteman et al. 1995), and chemical contamination (Bridges & Semlitsch 2000). Global warming (Gibbs & Breisch 2001) and increasing levels of ultraviolet (UV) radiation (Middleton et al. 2001) have also been proposed. In addition, synergies among these factors

[1] Chargé de Recherches FNRS, Laboratory of Fish and Amphibian Ethology, Behavioural Biology Unit, Department of Life Sciences, University of Liège, 22 Quai Van Beneden, 4020 Liège, Belgium, email: mathieu.denoel [a] ulg.ac.be ; [2] Institute for Biological Research, 29 Novembra 1942, 11000 Beograd, Serbia and Montenegro, [3] Institute of Zoology, Faculty of Biology, Studentski trg 16, 11000 Beograd, Serbia and Montenegro

may be particularly detrimental to amphibian populations (Pahkala et al. 2002).

Although declines have been documented for species or groups of species, almost nothing is known about the status of intraspecific biodiversity, particularly in polymorphic species. Facultative paedomorphosis occurs in newts and salamanders, mainly from Europe and North America. It is characterized by the existence of two ontogenetic alternatives in a population: paedomorphs, which are individuals retaining larval traits such as gills and gill slits in the adult stage, and metamorphs, which are metamorphosed adults (Semlitsch & Wilbur 1989; Whiteman 1994). Facultative paedomorphosis results from a gene-environment interaction (Semlitsch 1987; Voss 1995) and has been shown to be adaptive because it allows early maturity (Denoël & Joly 2000), resource partitioning (Whiteman et al. 1996; Denoël et al. 2004), and increased energy intake (Denoël et al. 2002). Unlike metamorphic newts, paedomorphs are present in only some populations, making them particularly vulnerable to the causes of amphibian declines (Whiteman & Howard 1998; Denoël et al. 2001). No one has investigated on a large scale the threats that these rare phenotypes face, but a few reports of local declines or extinctions do exist (Dolmen 1978; Breuil 1985; Collins et al. 1988; Whiteman & Howard 1998).

Among the causes contributing to amphibian declines, species introductions in freshwater habitats play a large part (Kats & Ferrer 2003). Various types of introduced species have been implicated in amphibian decline, mainly in fish (e.g., Hecnar & M'Closkey 1997; Bradford et al. 1998; Knapp & Matthews 2000) but also in amphibians (Lawler et al. 1999) and invertebrates (Gamradt & Kats 1996). In many cases, the introduced species is a predator or competitor of the native amphibian species and has been linked to amphibian declines or extinctions (e.g., Goodsell & Kats 1999; Lawler et al. 1999; Smith et al. 1999; Nyström et al. 2001). In addition, introduced species can negatively affect native amphibian species by serving as pathogen vectors (Kiesecker et al. 2001).

The majority of European paedomorphic populations (i.e., approximately 100) occur in the Mediterranean area: the palmate newt (*Triturus helveticus*) is found in Larzac (southern France; Gabrion et al. 1977) and the alpine newt (*T. alpestris*) is found in southeastern France, Italy, and the Balkans (Denoël et al. 2001). Originally, the aquatic habitats of these newts were devoid of fish (remote ponds and lakes). Because fish stocking in ponds and lakes is common in Europe, however, these habitats may now contain fish. Our aim was to find out (1) whether fish stocking had occurred where newt populations contained paedomorphic newts, (2) which fish and newt species occurred at each site, and (3) the impact of fish introductions on paedomorphs and metamorphs.

Methods

Study Sites and Species

We selected all large paedomorphic populations of the alpine newt (except those in Albania, which were recently discovered) and the palmate newt from Larzac (cf. checklists: Denoël et al. 2001; Gabrion et al. 1977). We did not include in this study the sites in which the presence of paedomorphs was only mentioned or in which only one individual was observed. Literature data and personal observations certified that all these sites, except one (Bukumirsko Lake, where there was only one species), were historically devoid of fish. Because we did not consider poorly studied paedomorphic populations, results from the studies we considered for our analysis (Tables 1 & 2) can be considered reliable. Most of the studies, though, did not give exact population sizes, only the number of individuals caught (in *T. alpestris*) or the percentage of paedomorphs (in *T. helveticus*), because taking this type of data was not usual when these studies were conducted (first descriptions between 1926 and 1997). This was not a major problem because we were mainly interested in showing a pattern of extinction and decline. Indeed, because the sampling pressures were at least as good as they were in the former studies, any lower value of sample size would reveal a decline.

We studied 22 paedomorphic populations of alpine newts (Table 1) and 17 paedomorphic populations of palmate newts (Table 2). Most of the alpine newt populations studied were in Italy ($n= 10$) and Montenegro (6); the remainder were in Greece (3), France (1), Bosnia (1), and Slovenia (1). All the palmate newt populations were in France (17). This sample covers the majority of the known paedomorphic populations of alpine and palmate newts and almost all populations for which we could compare present with former status (Gabrion et al. 1977; Denoël et al. 2001). Paedomorphs were abundant in all these populations at the time of their first description (Tables 1 & 2). Alpine newts inhabited isolated natural alpine lakes and mid-elevation ponds, whereas palmate newts were found only in artificial ponds on a limestone plateau. Deeper lakes (i.e., >2 m depth) were occupied only by alpine newts (Tables 1 & 2).

Several subspecies of alpine newts have been identified in southern Europe, although the status of these subspecies is controversial (Breuil & Guillaume 1984; Arano & Arntzen 1987; Herrero et al. 1989; Sotiropoulos et al. 2001). Populations from the Apennines belong to *T. a. apuanus* (*T. a. inexpectatus* in Calabria) and those from Greece to *T. a. veluchiensis* (Arano & Arntzen 1987). In the Dinaric Alps, subspecies have been described that are much more localized, occurring only in a single site. We included most of them here because these subspecies contained large populations of paedomorphic individuals: *T. a. lacustris* from Jezero in Slovenia (Seliskar & Pehani 1935), *T. a. reiseri* from Prokosko Lake in Bosnia (Werner 1902), *T. a. montenegrinus* from Bukumirsko Lake in Montenegro (Radovanovic 1951), *T. a. piperianus* from Manito and Kapetanovo lakes in Montenegro (Radovanovic 1961), and *T. a. serdarus* from Zminicko Lake in Montenegro (Radovanovic 1961).

Sampling Procedure

We conducted surveys between 1991 and 2003, but particularly in 2002. Each site was visited several times (previous observations, such as first description, were also done by one of us in a few cases). We used the last and best data for each site in this study. We caught newts in dip nets and minnow traps from the shore, from a boat, or by scuba diving. Transects in shallow ponds (i.e., <2 m depth) allowed the capture of almost all individuals because we stopped searching only when no more newts were captured (Tables 1 & 2). In deeper lakes, we did captures along transects along the shore (the only habitat where newts were sampled at the first census), and in open waters. Thus, the sampling pressure was similar or higher in our study. In four sites, we used mark and recapture procedures (Drakolimni in Tymphi and

Table 1. Checklist and status of the studied paedomorphic alpine newt populations.[a]

Locality (Country)	Coordi-nates	Depth (m)	Drying prob.	First published description	Fish intro.	Orig. paed. pop.	Last paed. pop.	Meta.	Year of introduction	Introduced species[c]
Paedomorph disappearance										
Jezero (Slovenia)	46°19'N, 13°50'E	11	no	Seliskar & Pehani 1935	yes	>423–71% (1934)	0 (1995)	no	1941–1955	Ssp
Prokosko Lake (Bosnia)	43°57'N, 17°46'E	12	no	Wolterstorff 1938	yes	>5–5% (1926)	0 (1991)	yes	1961–1963	OM-SA-ST
Trnovacko Lake (Montenegro)	43°15'N, 18°43'E	9.2	no	Radovanovic 1961	yes	>17 (1959)	0 (2002)	yes	1961	OM
Zminicko Lake (Montenegro)	43°06'N, 19°14'E	3.8	no	Radovanovic 1961	yes	>37–51%	0 (2002)	no	~1970–1980	CA-CI-ST
Vrazje Lake (Montenegro)	43°05'N, 19°08'E	10.6	no	Pocrnjic & Kosoric 1966	yes	present (1966)	0 (2002)	yes	~1960–1970	OM-SA-ST
Kapetanovo Lake (Montenegro)	42°48'N, 19°14'E	37	no	Radovanovic 1961	yes	many–~75% (1960)	0 (2002)	yes	~1970	OM-SF
Manito Lake (Montenegro)	42°48'N, 19°14'E	13.4	no	Radovanovic 1961	yes	>471–25% (1984)	0 (2002)	yes	~1990	ST-SA
Bukumirsko Lake (Montenegro)	42°36'N, 19°33'E	16.8	no	Radovanovic 1951	yes	>238 – 97%	0 (2002)	yes	since 1912	OM
Murazzano (Italy)	44°28'N, 8°01'E	2	yes	Andreone 1990	yes	>12–>50% (~1990)	0 (1997)	no	>1990	CA
Lago della Bega (Italy)	44°13'N, 10°14'E	0.8	high	Ferracin et al. 1980a,b	yes	~1000–50% (1979)	0 (1997)	yes	>1979	—
Revigliasco (Italy)	45°01'N, 7°43'E	1	high	Andreone & Sindaco 1987	no	>23 (1986)	0 (1997)	yes	—	—
Colorio (Italy)	43°45'N, 12°06'E	0.4	yes	Denoël et al. 2001	no	28–93% (1997)[b]	0 (1999)	no	—	—
Parana (Italy)	44°17'N, 9°51'E	0.7	high	Bovero et al. 1994	no	>21–65% (~1994)	0 (1998)	yes	—	—
Paedomorph maintenance										
La Cabane Lake (France)	44°24'N, 6°24'E	7.5	yes	Breuil 1986	no	500–80% (1981)	811–80% (1997)	yes	—	—
Fontanelle (Italy)	44°38'N, 9°32'E	2	yes	Fasola & Canova 1992	no	89–55% (1997)[b]	1 (1998)	yes	—	—
Mont-Megna (Italy)	44°37'N, 9°32'E	1	low	Fasola & Canova 1992	no	81% (~1990) b	60–58% (1998)	yes	—	—
Pra di Lama (Italy)	44°07'N, 10°25'E	0.1	yes	Denoël 1997	no	6–46% (1994)[b]	1–7% (1997)	yes	—	—
La Pianca (Italy)	43°44'N, 12°08'E	2	no	Denoël et al. 2001	no	>100 (1997)[b]	>100 (1998)	yes	—	—
Lago dei due Uomini (Italy)	39°32'N, 16°01'E	>1	yes	Dubois & Breuil 1983	no	>6–27% (1982)	>1–99% (1997)	yes	—	—
Drakolimni—Smolikas (Greece)	40°05'N, 20°54'E	3.7	no	Breuil & Parent 1987	no	<73–77% (~1984)	19322–74% (1999)	yes	—	—
Drakolimni—Tymphi (Greece)	39°59'N, 20°47'E	4.9	no	Breuil & Parent 1987	no	>51–48% (~1984)	5491–34% (1999)	yes	—	—
Valtos (Greece)	39°59'N, 20°46'E	1	no	Breuil & Parent 1987	no	>20–23% (~1984)	19–34% (1999)	yes	—	—

[a]Column heading abbreviations: Drying prob., probability of pond drying; Fish intro., fish introduction; Orig. paed. pop., size and proportion of the paedomorphic population at the time of the first description (year); Last. paed. pop., size and proportion of the paedomorphic population at the last census (this study); Meta, presence of metamorphs at the last census (this study).
[b]Refers to personal observation (M.D.) because the original description did not mention accurate sample size.
[c]Species abbreviations: BP, *Barbus peloponnesius*; CA, *Carassius auratus*; CI, *Ctenopharyngodon idella*; OM, *Onchorhynchus mykis*; SA, *Salvelinus alpinus*; SF, *Salvelinus fontinalis*; ST, *Salmo trutta*; Ssp, *Salmo* species.

Smolikas, La Cabane Lake, and Parana) to obtain accurate estimates of population size (see Denoël & Joly 2001; Denoël & Šchabetsberger 2003 for more details). Because we did not find paedomorphs in former-Yugoslavian lakes, we did not use marking techniques. We kept newts in containers throughout the sampling session and released them at the end of the census.

We determined fish presence and identified fish species by direct observations, dip netting, fishing, and interviewing local anglers, as well as from the literature. Both paedomorphic and metamorphic

Table 2. Checklist and status of the studied paedomorphic palmate newt populations in Larzac (France).[a]

Locality	Coordinates	Depth (m)	Drying prob.	Fish intro.	Orig. paed. pop. (%)	Last paed. pop. (year)	Meta.	Introduced species[b]
Paedomorph disappearance								
Aire de la Blaquerie	43°57'N, 3°15'E	0.8	low	yes	6	0 (2002)	no	CA
La Rigalderie	43°55'N, 3°28'E	1.5	low	yes	20	0 (2002)	no	CA
Château de Sorbs	43°53'N, 3°24'E	1	low	yes	37	0 (2002)	no	CA
Les Rives nord	43°50'N, 3°16'E	0.9	high	yes	26	0 (2002)	yes	PP
Puits de la cave	43°50'N, 3°26'E	0.5	low	yes	67	0 (2002)	no	CA
Soulages (Mas de Jourdes)	43°48'N, 3°28'E	1.5	high	yes	74	0 (2002)	no	CA-GA
Saint Pierre de la Fage nord	43°47'N, 3°25'E	1.3	low	yes	37	0 (2002)	no	CA-LG
Blandas nord ouest Falque-nette	43°55'N, 3°30'E	0.03	yes	no	91	0 (2002)	no	—
Combefère	43°52'N, 3°16'E	0.8	high	no	12	0 (2002)	yes	—
Les Rives est “Bartasse”	43°51'N, 3°16'E	0.7	yes	no	9	0 (2002)	yes	—
Saint Michel sud - Le Laquet	43°50'N, 3°23'E	1.1	high	no	62	0 (2002)	yes	—
La Roque est	43°47'N, 3°23'E	0.8	yes	no	65	0 (2002)	yes	—
La Canourgue (ferme)	43°48'N, 3°22'E	0.5	yes	no	11	0 (2002)	yes	—
Paedomorph maintenance								
Combe Redonde	43°57'N, 3°19'E	0.8 m	low	no	42	>6–10% (2002)	yes	—
Bergerie de l'hôpital	43°51'N, 3°22'E	1.5 m	low	no	45	1–8% (2001)	yes	—
La Prade nord	43°50'N, 3°23'E	0.5 m	yes	no	46	6–27% (2002)	yes	—
Devois la Trivalle	43°45'N, 3°28'E	1 m	low	no	79	20– 66% (2002)	yes	—

[a]Column abbreviations: Drying prob., probability of pond drying; Fish intro., fish introduction (occurring between 1976 and 2002); Orig. paed. pop., proportion of the paedomorphic population at the time of the first description (Gabrion et al. 1977); Last. paed. pop., size and proportion of the paedomorphic population at the last census (this study); Meta, presence of metamorphs at the last census (this study); Depth, maximum water depth in 2002.
[b]Species abbreviations: CA, *Carassius auratus*; GA, *Gambusia affinis*; LG, *Lepomis gibbosus*; PP, *Phoxinus phoxinus*.

newts reach sexual maturity (swollen cloacae), but only paedomorphs retain gills and gill slits (Denoël 2002).

We used a chi-square test in 2 × 2 contingency tables to compare the proportions of sites with and without paedomorphs or metamorphs in fish and fishless ponds and to test for the relative sensitivity of paedomorphs and metamorphs to fish. To test for an effect of drying or water depth, we computed a logistic regression for both metamorphs and paedomorphs. It was not possible to include fish in this logistic regression model because we had a quasicomplete separation, where the maximum likelihood estimates did not exist (Albert & Anderson 1984).

Results

We detected fish introductions in 17 (44%) of the studied sites: 7 in France, 2 in Italy, 1 in Bosnia, 1 in Slovenia, and 6 in Montenegro (Tables 1 & 2). Fish introductions occurred in all of the studied countries except Greece.

In the Dinaric Alps, some introductions were of “native” species (*Salmo trutta*), but all lakes contained alien specimens (i.e., non-European species of fish such as *Oncorhynchus mykiss*, *Salvelinus alpinus*, and *S. fontinalis*) (Table 1). Fish stocking of ponds in Italy and France mainly involved small and ornamental fish species: *Carassius auratus*, *Gambusia affinis*, *Lepomis gibbosus*, and *Phoxinus phoxinus* (Table 2). Contrary to the situation in the Dinaric Alps, fish introductions were not an organized stocking effort but occasional introductions by local people.

Fish introductions were always followed by the extirpation of paedomorphic individuals of both *T. alpestris* (Table 1) and *T. helveticus* (Table 2). Paedomorphs disappeared in 44% of the studied populations. There was a significant difference in the proportion of sites with paedomorphs in ponds with fish (0 out of 17) versus in ponds without fish (13 out 22; $\chi 2= 15.07$, 1 df, $p < 0.001$). All of the ponds were initially devoid of fish. There was also a significant difference in the proportion of sites with metamorphs in ponds with fish (8 out of 17) versus in ponds without fish (19 out of 22; $\chi 2= 6.95$, 1 df, $p < 0.01$). More lakes with fish had metamorphs than had paedomorphs ($\chi 2= 6.16$, 1 df, $p < 0.05$). Drying and water depth did not significantly affect the presence of paedomorphs (drying: Wald test = 2.187, $p= 0.14$; water depth: Wald test = 2.779, $p= 0.1$) or metamorphs (drying: Wald test = 0.173, $p= 0.68$; water depth: Wald test = 0.4, $p= 0.5$).

There were no paedomorphic populations in sites in the Dinaric Alps. We found no *T. a. lacustris* in Jezero and only metamorphs of *T. a. montenegrinus* in Bukumirsko Lake, *T. a. piperianus* in Kapetanovo and Manito lakes, and *T. a. serdarus* in Zminicko Lake. If paedomorphs are still present, their numbers are very low because none were found in the last censuses in 2002 (Table 1).

Discussion

Paedomorphic newts have disappeared from all sites in which fish were introduced. Because our study was based on almost all the main European paedomorphic populations of palmate and alpine newts, this observed decline means that paedomorphosis in the study species is an endangered process.

The only other known large paedomorphic populations occur in Albania ($n= 7$) and France ($n= 2$), but they have only recently been discovered and thus have not been censused (Denoël et al. 2001). If we include these populations in the list of known large paedomorphic populations, one-third of these populations disappeared following fish introductions. The introduction of fish in the Dinaric Alps dates back to the second half of the nineteenth century. Introductions reached their peak during the 1970s and 1980s and continue today (Kosoric & Pocrnic 1966; G.D. & M.K., personal observation). At the start of fish introductions, paedomorphs were present, at least at some sites (Zminicko Lake, Vrazje Lake, and Bukumirsko Lake; G.D. & M.K., personal observation), but disappeared after several years of fish stocking (2002 censuses). The first reported extinction was from Jezero (Breuil 1985). In 1934 Jezero had hundreds of paedomorphs (Seliskar & Pehani 1935). In Larzac and Italy, fish introductions have been ongoing since the late 1970s. Because ornamental exotic species of fishes are not naturally present in European ponds (e.g., arid plateau of Larzac) and salmonids are unable to naturally colonize the remote alpine lakes we studied, their presence can only be attributed to introductions.

Fish introductions have altered both paedomorphic and metamorphic newt populations. But because paedomorphs are present in considerably fewer places (*T. alpestris*: 87 populations in Europe, Denoël et al. 2001; *T. helveticus*: 21 populations in Larzac, Gabrion et al. 1977) than their metamorphic counterparts (thousands of populations in Europe, Zuiderwijk 1997a,b), paedomorphs are more vulnerable to extinction. American populations of paedomorphic newts and salamanders may be at the same risk of disappearance because they are rather rare (Petranka 1998) and because fish stocking is a common practice, already known to have had large effects on amphibian populations (e.g., Knapp et al. 2001). Although no global pattern has been determined, a few local cases of paedomorph disappearance have been noticed after fish introductions (Collins et al. 1988; Whiteman & Howard 1998; M.D., personal observation), which indicates that facultative paedomorphosis is an endangered process on a worldwide scale.

Moreover, paedomorphic individuals are under higher predatory pressure (by large predatory fishes) than metamorphic newts because they live mainly in the water column, whereas metamorphs are more abundant along the shoreline (Denoël & Joly 2001), and because they cannot escape fish predation by hibernating in terrestrial habitats as metamorphs do (M.D., personal observation). Our results confirm our hypothesis that paedomorphs are more vulnerable to fish introduction than metamorphs because the disappearance of paedomorphs occurred in more lakes than the disappearance of metamorphs. The presence of metamorphs does not mean that they were native of the lake because, unlike paedomorphs, they can migrate from neighboring aquatic sites. In addition, results from laboratory experiments show that larvae are less likely to follow an ontogenetic pathway leading to paedomorphosis in the presence of fish than when fish are absent (Jackson & Semlitsch 1993).

All fish species had detrimental effects on the studied paedomorphic newt populations. Salmonids are active predators of vertebrates (Tyler et al. 1998), including paedomorphic newts (G.D., personal observation). Although smaller fish species such as mosquitofish (*Gambusia affinis*) and goldfish (*Carassius auratus*) cannot consume adult newts, they can consume egg and larval stages of newts (Gamradt & Kats 1996; Monello & Wright 2001). The effects of small fishes were particularly strong because—unlike salmonids—they were introduced to small water bodies that lacked shelters.

Loss of suitable habitats—as a result of fish stocking—decreases the network of habitats from which recolonization can occur. In Larzac, for example, even though newts were observed in a large number of ponds (Gabrion et al. 1977), any decrease in aquatic habitats in such a dry terrestrial environment (limestone plateau) will certainly affect the chance of pond colonization by dispersing newts. Similarly, these effects are the suspected reason for a decline of mountain yellow-legged frogs (*Rana mucosa*) in California (Bradford et al. 1993).

Pond drying is another factor that can directly contribute to paedomorph disappearance (Semlitsch 1987; Denoël 2003). This process is particularly common in the artificially made concrete-lined ponds of the Larzac plateau (Table 2). Regardless of the causes, when a pond dries, metamorphs are able to leave and live on land or colonize other ponds. Paedomorphs, however, have an aquatic morphophysiology, relying on the presence of water. Terrestrial migration of paedomorphs is possible, but only over a few meters (Denoël 2003). In most cases, paedomorphs will either die or metamorphose into the dispersing morph—the metamorph (Denoël 2003). In addition, pond drying may change the ontogenetic pathway toward metamorphosis, as it does in ambystomatid larvae (Semlitsch 1987). The decrease in paedomorphs from the four sites that were unaffected by fish introduction in Larzac is most probably a result of pond drying (M.D., personal observation).

The effect of fish is not an artifact resulting from water drying because drying and water depth had no significant effect on the current absence of paedomorphs (deep lakes where paedomorphs disappear never dry). In contrast to fish introductions, drying is usually a short, catastrophic event because the pond may be filled again with water a few months after drying. Thus, with respect to the age at first reproduction (Denoël & Joly 2000), recovery (i.e., the degree to which and speed at which a lake community returns to its previous state once the perturbation is removed) could be very fast for metamorphs (1 year) and could be relatively fast for paedomorphs (a few years). Paedomorphs were observed in temporary ponds and are thus suspected to be adapted to this event (Kalezic & Dzukic 1985; Breuil 1992; Denoël et al. 2001; this study). Because amphibian populations often fluctuate naturally (Semlitsch et al. 1996; Meyer et al. 1998), population fluctuations are particularly expected in temporary sites (Kalezic & Dzukic 1985; Denoël & Joly 2000). This is the case with the disappearance of newts following drying. In permanent ponds and lakes devoid of fish, though, populations remain stable across years (Breuil 1986; Denoël & Joly 2001; Denoël & Schabetsberger 2003).

Predators other than fish may have altered newt communities (Gamradt & Kats 1996), but the number of these predators in the studied sites was low (only one crayfish was caught in St-Pierre de la Fage Pond) so they cannot be the basis for the extinction of hundreds of newts. Although habitat destruction would explain newt extinction, we focused on habitat that was intact at the time of our observations. Because most of the studied sites are remote, the terrestrial landscape had not been affected.

The composition of aquatic communities appears to recover within 10–20 years after fish disappearance (Knapp et al. 2001), but this pattern is not uniformly widespread because unsuccessful restoration has also been observed (Drake & Naiman 2000). Alpine newt populations in high-altitude lakes also show a fairly high degree of resilience once fish are removed (M.D. & G.D., personal observation). Newt recolonization of lakes that have been returned to a fishless condition may be strongly influenced by the proximity of stable neighboring newt-breeding populations. Initially, a stable population of individuals that follow a metamorphic life history should be expected. For example, Manito Lake (Montenegro) has an abundant newt population that reappeared after fish disappeared from the lake. Only metamorphs are now present in this lake (>500 metamorphs; G.D. & M.K., personal observation), in stark contrast to the preintroduction situation, in which paedomorphic newts were numerous and comprised 25% of the population (>100 paedomorphs; Kalezic et al. 1989). A similar situation occurred in Lago della Bega (Italy), where before 1980, 50% of the population was paedomorphs (around 1000 metamorphs; Ferracin et al. 1980a,b). Although fish were then introduced, by 1997 only metamorphic newts were present (>70 metamorphs; M.D., personal observation) and fish were no longer present. A different pattern has been observed in Bukumirsko, Kapetanovo, and Zabojsko lakes. These lakes have been repeatedly stocked to maintain fish populations. Although metamorphs are still found in these lakes, they are less numerous than in Manito Lake and may be immigrants from neighboring fishless water bodies. From these observations, it appears that fish disappearance can be followed by an increase in metamorphic newts that may reconstitute a large population, but no location has maintained paedomorphosis, although fish disappeared from these places years ago. Because long periods of selection against paedomorphosis can decrease the expression of paedomorphosis in populations (Semlitsch & Wilbur 1989), all alleles permitting paedomorphosis may have been eliminated. For short-time catastrophes, however, such as pond drying during summer, the reappearance of paedomorphs is possible (M.D., personal observation). The only way to determine whether paedomorphosis may still be expressed is to monitor populations on a long-term basis, but preliminary data suggest that major catastrophes—such as several years of fish stocking—have more detrimental effects than a one-summer drying in terms of paedomorph resilience.

Conclusions and perspectives

Although newt species as a whole are not directly threatened by fish introductions, such is not the case for the paedomorphs, which represent a unique living example of intraspecific heterochrony. Our results show that repeated fish introductions have led to widespread extinctions in Europe. If urgent measures are not taken soon, an important kind of intraspecific biodiversity will disappear. Some sites still contain large paedomorphic populations and should be closely protected and surveyed to assure their persistence (particularly in Greece and Albania). Legislation should take into account heterochronic morphs as conservation units (Fraser & Bernatchez 2001) and stopping non-native fish introductions should be considered. Management measures should also be taken to restore disturbed habitats by removing introduced fishes. Similar measures should also take place outside in Europe because preliminary data indicate that this loss of biodiversity is also in process in North America. Large monitoring surveys are needed in North America to find out whether the situation is already as catastrophic as it is in Europe.

Acknowledgments

We are grateful to L. Kats, M. Leigh, G. K. Meffe, R. Schabetsberger, H. H. Whiteman, and two anonymous reviewers for helpful comments on the manuscript; to A. Albert for statistical advice; to F. Andreone, S. Bovero, G. Hauseder, F. Hourlay, P. Joly, P. Petitfrère, and U. Schiftner for their help during field work; and to the numerous landowners for allowing access to their ponds. This research was authorized by the French and Greek Ministries of environment (specifically the Prefectures of Aveyron, Gard, and Herault in France) and the Region of Piedmont in Italy. M. Denoël is a postdoctoral researcher (chargé de recherches) at the Fonds National de la Recherche Scientifique (FNRS). This research was supported by an FNRS grant (crédit aux chercheurs) 2002-2003 1.5.011.03, the Fondation Lefranc, the Concours des Bourses de Voyage, the Patrimoine of the University of Liège, a Serbian Ministry of Science and Development grant (grant 1623), and a Declining Amphibian Population Task Force seed grant (2002).

References

Albert, A., and J.A. Anderson. 1984. On the existence of maximum likelihood estimates in logistic regression models. Biometrica 71: 1-10.

Alford, R. A., and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. Annual Review of Ecology and Systematics 30:133-165.

Andreone, F. 1990. Variabilita morphologica e riproduttiva in popolazioni di *Triturus alpestris* (Laurenti, 1768) (Amphibia, Urodela, Salamandridae). PhD thesis. Universita di Bologna, Italy.

Andreone, F., and R. Sindaco. 1987. Sulla presenza e la neotenia di *Triturus alpestris* (Bonaparte 1839) nella Collina di Torino (Amphibia, Urodela, Salamandridae). Bolletino del Museo regionale di Scienze Naturali, Torino 5:103-112.

Arano, B., and J. W. Arntzen. 1987. Genetic differentiation in the Alpine newt, *Triturus alpestris*. Pages 21-24 in J. J. van Gelder, H. Strijbosch, and P. J. M. Bergers, editors. 4th ordinary general meeting of the Societas Europaea Herpetologica. Faculty of Sciences, Nijmegen, The Netherlands.

Berger, L. et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences of the United States of America 95:9031-9036.

Bovero, S., F. Andreone, and C. Giacoma. 1994. Aspetti comportamentali in esemplari pedogenetici e metamorfosati di *Triturus alpestris apuanus*. Studi Trentini di Scienze Naturali - Acta Biologica 71:151-155.

Bradford, D. F., F. Tabatabai, and D. M. Graber. 1993. Isolation of remaining populations of the native frog, *Rana mucosa*, by introduced fishes in Sequoia and Kings Canyon National Parks, California. Conservation Biology 7:882-888.

Bradford, D. F., S. D. Cooper, T. M. J. Jenkins, K. Kratz, O. Sarnelle, and A. D. Brown. 1998. Influences of natural acidity and introduced fish on faunal assemblages in California alpine lakes. Canadian Journal of Fisheries and Aquatic Sciences 55:2478-2491.

Breuil, M. 1985. Etude des mesures possibles pour assurer la protection des biotopes de tritons alpestres *Triturus alpestris* du Parc National des Ecrins. Rapport du Marché d'étude 69/84; Parc National des Ecrins, France.

Breuil, M. 1986. Biologie et différenciation génétique des populations du triton alpestre (*Triturus alpestris*) (Amphibia Caudata) dans le sud-est de la France et en Italie. PhD thesis. Paris-Sud University, Paris, France.

Breuil, M. 1992. La néoténie dans le genre *Triturus*: mythes et réalités. Bulletin de la Société Herpétologique de France 61:11-44.

Breuil, M., and C.-P. Guillaume. 1984. Etude electrophorétique de quelques populations de tritons alpestres néoténiques (*Triturus alpestris*, Amphibia, Caudata, Salamandridae), du sud de la Yougoslavie. Bulletin de la Société Zoologique de France 109:377-388.

Breuil, M., and G. H. Parent. 1987. Essai de caractérisation du Triton alpestre hellénique *Triturus alpestris veluchiensis*. I. Historique et présentation de nouvelles données. Alytes 6:131-151.

Bridges, C. M., and R. D. Semlitsch. 2000. Variation in pesticide tolerance among and within species of Ranidae and patterns of amphibian decline. Conservation Biology 14:1490-1499.

Carr, L. W., and L. Fahrig. 2000. Effect of road traffic on two amphibian species of differing vagility. Conservation Biology 15:1071-1078.

Collins, J. P., T. R. Jones, and H. J. Berna. 1988. Conserving genetically distinctive populations: the case of the Huachuca tiger salamander (*Ambystoma stebbinsi* Lowe). Pages 45-53 in R. C. Szaro, K. E. Severson, and D. R. Patton, editors. Management of amphibians, reptiles, and small mammals in North America. U.S.D.A. Forest Service, Flagstaff, Arizona.

Denoël, M. 1997. Un cas de néoténie, dans un bâtiment désaffecté, chez le triton alpestre, *Triturus alpestris apuanus* (Salamandridae). Alytes 15:99-103.

Denoël, M. 2002. Paedomorphosis in the Alpine newt (*Triturus alpestris*): decoupling behavioural and morphological change. Behavioral Ecology and Sociobiology 52:394-399.

Denoël, M. 2003. How do paedomorphic newts cope with lake drying? Ecography 26:405-410.

Denoël, M., and P. Joly. 2000. Neoteny and progenesis as two heterochronic processes involved in paedomorphosis in *Triturus alpestris* (Amphibia: Caudata). Proceedings of the Royal Society of London B 267:1481-1485.

Denoël, M., and P. Joly. 2001. Adaptive significance of facultative paedomorphosis in *Triturus alpestris* (Amphibia, Caudata): resource partitioning in an alpine lake. Freshwater Biology 46:1387-1396.

Denoël, M., and R. Schabetsberger. 2003. Resource use in two heterochronic populations of Greek Alpine newts, *Triturus alpestris veluchiensis* (Wolterstorff, 1935). Acta Oecologica 24:55-64.

Denoël, M., R. Duguet, G. Dzukic, M. Kalezic, and S. Mazzotti. 2001. Biogeography and ecology of paedomorphosis in *Triturus alpestris* (Amphibia, Caudata). Journal of Biogeography 28:1271-1280.

Denoël, M., F. Hervant, R. Schabetsberger, and P. Joly. 2002. Short- and long-term advantages of an alternative ontogenetic pathway. Biological Journal of the Linnean Society 77:105-112.

Denoël, M., R. Schabetsberger, and P. Joly. 2004. Trophic specializations in alternative heterochronic morphs. Naturwissenschaften 91:81-84.

Dolmen, D. 1978. De neotene salamanderne ("skrattabborrene") ved Stensele. Fauna og Flora 4:171-177.

Drake, D. C., and R. J. Naiman. 2000. An evaluation of restoration efforts in fishless lakes stocked with exotic trout. Conservation Biology 14:1807-1820.

Dubois, A., and M. Breuil. 1983. Découverte de *Triturus alpestris* (Laurenti, 1768) en Calabre (sud de l'Italie). Alytes 2:9-18.

Fasola, M., and L. Canova. 1992. Residence in water by the newts *Triturus vulgaris*, *T. cristatus* and *T. alpestris* in a pond in northern Italy. Amphibia-Reptilia 13:227-233.

Ferracin, A., M. Lunadei, and N. Falcone. 1980a. An ecological note on *Triturus alpestris apuanus* (Bonaparte) and *Triturus cristatus carnifex* (Laurenti) in the Garfagnana (Lucca, Central Italy). Bolletino Zool. 47:143-147.

Ferracin, A., M. Lunadei, and N. Falcone. 1980b. Atti del osservazioni sull'ecologia del genere *Triturus* (Rafinesque) in Garfagnana (Lucca). Pages 477-481 in A. Moroni, O. Ravera, and A. Anelli, editors. Primo congresso nazionale della Societa Italiana di Ecologia. Societa Italiana di Ecologia, Salsaggiore Terme, Italy.

Fraser, D. J., and L. Bernatchez. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Conservation Biology 10:2741-2752.

Gabrion, J., P. Sentein, and C. Gabrion. 1977. Les populations néoténiques de *Triturus helveticus* Raz. des Causses et du Bas-Languedoc. I. Répartition et caractéristiques. La Terre et la Vie 31:489-506.

Gamradt, S. C., and L. B. Kats. 1996. Effect of introduced crayfish and mosquitofish on California newts. Conservation Biology 10:1155-1162.

Gibbs, J. P., and A. R. Breisch. 2001. Climate warming and calling phenology of frogs near Ithaca, New York, 1900-1999. Conservation Biology 15:1175-1178.

Goodsell, J. A., and L. B. Kats. 1999. Effect of introduced mosquitofish on pacific treefrogs and the role of alternative prey. Conservation Biology 13:921-924.

Hecnar, S. J., and R. T. M'Closkey. 1997. The effects of predatory fish on amphibian species richness and distribution. Biological Conservation 79:123-131.

Herbeck, L. A., and D. R. Larsen. 1998. Plethodontid salamander response to silvicultural practices in Missouri Ozark Forests. Conservation Biology 13:623-632.

Herrero, P., B. Arano, and C. G. Delavega. 1989. Chromosome differentiation in the *Triturus alpestris* complex (Amphibia, Caudata). Genetica 79:27-35.

Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. Nature 404:752-755.

Jackson, M. E., and R. D. Semlitsch. 1993. Paedomorphosis in the salamander *Ambystoma talpoideum*: effects of a fish predator. Ecology 74:342-350.

Joly, P., C. Miaud, A. Lehmann, and O. Grolet. 2001. Habitat matrix effects on pond occupancy in newts. Conservation Biology 15:239-248.

Kalezic, M. L., and G. Dzukic. 1985. Ecological aspects of the smooth newt (*Triturus vulgaris*) paedomorphosis from Montenegro. Arhiv Bioloskih Nauka 37:43-50.

Kalezic, M. L., G. Dzukic, and A. Popadic. 1989. Paedomorphosis in Yugoslav Alpine newt (*Triturus alpestris*) populations: morphometric variability and sex ratio. Arhiv Bioloskih Nauka 41:67-79.

Kats, L.B., and R.P Ferrer. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9:99-110.

Kiesecker, J. M., A. R. Blaustein, and C. L. Miller. 2001. Transfer of a pathogen from fish to amphibians. Conservation Biology 15:1064-1070.

Knapp, R. A., and K. R. Matthews. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology 14:428-438.

Knapp, R. A., K. R. Matthews, and O. Sarnelle. 2001. Resistance and resilience of alpine lake fauna to fish introductions. Ecological Monographs 71:401-421.

Kosoric, D., and Z. Pocrnic. 1966. Der Einfluss der Ansiedlung von Regenbogenfirellen auf die Populationen des Neotenischen Bergmolches. Ribarstvo Jugoslavije 21:137-139.

Lawler, S. P., D. Dritz, T. Strange, and M. Holyoak. 1999. Effects of introduced mosquitofish and bullfrogs on the threatened California red-legged frog. Conservation Biology 13:613-622.

Meyer, A.H., B.R. Schmidt, and K. Grossenbacher. 1998. Analysis of three amphibian populations with quarter-century long time-series. Proceedings of the Royal Society of London B 265:523-528.

Middleton, E. M., J. R. Herman, E. A. Celarier, J. W. Wilkinson, C. Carey, and R. J. Rusin. 2001. Evaluating ultraviolet radiation exposure with satellite data at sites of amphibian declines in Central and South America. Conservation Biology 15:914-929.

Monello, R.J., and R.G. Wright. 2001. Predation by goldfish (*Carassius auratus*) on eggs and larvae of the eastern long-toed salamander (*Ambystoma macrodactylum columbianium*). Journal of Herpetology 35:350-353.

Nyström, P., O. Svensson, L. B., C. Brönmark, and W. Granéli. 2001. The influence of multiple introduced predators on a littoral pond community. Ecology 82:1023-1039.

Pahkala, M., K. Räsänen, A. Laurila, U. Johanson, L. O. Björn, and J. Merilä. 2002. Lethal and sublethal effects of UV-B-pH synergism on common frog embryos. Conservation Biology 16:1063-1073.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington.

Pocrnjic, Z., and D. Kosoric. 1966. New finding-sites of neotenic alpine tritons (*Triturus alpestris*). Bulletin Scientifique, Beograd, A 11:251.

Pounds, J. A., M. P. L. Fogden, and J. H. Campbell. 1999. Biological response to climate change on a tropical mountain. Nature 398:611-615.

Radovanovic, M. 1951. A new race of the Alpine newt from Yugoslavia. British Journal of Herpetology 1:93-97.

Radovanovic, M. 1961. Neue Fundorte Neotenischer Bergmolche in Jugoslawien.206-218.

Seliskar, A., and H. Pehani. 1935. Limnologische Beiträge zum Problem der Amphibienneotenie (Beobachtungen an Tritonen der Triglavseen). Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie 7:263-294.

Semlitsch, R. D. 1987. Paedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. Ecology 68:994-1002.

Semlitsch, R. D., and H. M. Wilbur. 1989. Artificial selection for paedomorphosis in the salamander *Ambystoma talpoideum*. Evolution 43:105-112.

Semlitsch, R.D., D.E. Scott, J.H.K. Pechmann, and J.W. Gibbons 1996. Structure and dynamics of an amphibian community: evidence from a 16-year study of a natural pond. Pages 217-248 in M.L. Cody and J.A. Smallwood, editors. Long-term studies of vertebrate communities. Academic Press, San Diego, California.

Smith, G. R., J. E. Rettig, G. G. Mittzelbach, J. L. Valiulis, and S. R. Schaack. 1999. The effects of fish on assemblages of amphibians in ponds: a field experiment. Freshwater Biology 41:829-837.

Sotiropoulos, K., L. Tomovic, G. Dzukic, and M. L. Kalezic. 2001. Morphological differentiation of the alpine newt (*Triturus alpestris*) in the Balkans: taxonomic implications. Herpetological Journal 11:1-8.

Tyler, T. J., W. J. Liss, R. L. Hoffman, and L. M. Ganio. 1998. Experimental analysis of trout effects on survival, growth, and habitat use of two species of ambystomatid salamanders. Journal of Herpetology 32:345-349.

Voss, S. R. 1995. Genetic basis of paedomorphosis in the axolotl, *Ambystoma mexicanum*: A test of the single-gene hypothesis. Journal of Heredity 86:441-447.

Werner, F. 1902. Eine neue Varietät des Alpenmolches aus Bosnien. Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien 52:7-9.

Whiteman, H. H. 1994. Evolution of facultative paedomorphosis in salamanders. Quarterly Review of Biology 69:205-221.

Whiteman, H. H., and R. D. Howard. 1998. Conserving alternative amphibian phenotypes: Is there anybody out there? Pages 317-324 in M. J. Lannoo, editor. The status and conservation of Midwestern amphibians. Iowa University Press, Iowa city, Iowa.

Whiteman, H. H., R. D. Howard, and K. A. Whitten. 1995. Effects of pH on embryo tolerance and adult behavior in the tiger salamander, *Ambystoma tigrinum tigrinum*. Canadian Journal of Zoology 73:1529-1537.

Whiteman, H. H., S. A. Wissinger, and W. S. Brown. 1996. Growth and foraging consequences of facultative paedomorphosis in the tiger salamander, *Ambystoma tigrinum nebulosum*. Evolutionary Ecology 10:433-446.

Wolterstorff, W. 1938. *Triturus alpestris reiseri* Wern. und *Triturus alpestris alpestris* (=typica) Laur. Vergellschaftet im Prokosko-See. Zoologischer Anzeiger 122:23-30.

Young, B. E. et al. 2001. Population declines and priorities for amphibian conservation in Latin America. Conservation Biology 15:1213-1223.

Zuiderwijk, A. 1997a. *Triturus alpestris* (Laurenti, 1768). Pages 72-73 in Gasc, J.-P. et al., editors. Atlas of amphibians and reptiles in Europe. Societas Europaea Herpetologica, Muséum National d'Histoire Naturelle, Paris, France.

Zuiderwijk, A. 1997b. *Triturus helveticus* (Razoumowski, 1789). Pages 78-79 in Gasc, J.-P. et al., editors. Atlas of amphibians and reptiles in Europe. Societas Europaea Herpetologica, Muséum National d'Histoire Naturelle, Paris, France.

Paper submitted January 2, 2004; revised manuscript accepted May 28, 2004. Article first published online: 19 JAN 2005

DOI: 10.1111/j.1523-1739.2005.00001.x