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Amphibian Response to the Non-Native Fish, *Lepomis gibbosus*: The Case of the Pinail Nature Reserve, France

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***Abstract.*— Amphibians are a highly endangered taxonomic group, and invasion of alien species in wetland systems has been involved in this decline. The Pinail Nature Reserve, Vienne, France, contains more than 5,000 ponds, many of which are occupied by introduced Pumpkinseed Sunfish (*Lepomis gibbosus*). We sampled ponds with and without sunfish for amphibian species presence multiple times over 2 y. The number of amphibian species was significantly greater in ponds without sunfish (3.3 ± 0.2 [SD] species in 2004; 3.5 ± 0.2 in 2005) than in ponds with sunfish (1.6 ± 0.4 in 2004; 0.9 ± 0.2 in 2005). Sunfish presence was negatively related to the frequency of occurrence in ponds for *Triturus marmoratus* (Marbled Newt), *Hyla arborea* (European Tree Frog), *Lissotriton helveticus* (Palmate Newt), and *Pelophylax* spp. (Green Frog complex). The effect was especially pronounced for *Hyla arborea* and *Triturus marmoratus*, with occupancy nearly 50% and 100%, respectively, in ponds without sunfish, but 0% and 20%, respectively, in ponds with sunfish. Our study did not reveal a significant effect of sunfish presence on three other infrequently encountered amphibian species: *Bufo spinosus* (Spiny Toad), *Rana dalmatina* (Agile Frog), and *Triturus cristatus* (Great Crested Newt). Stomach content analyses confirmed predation on larval amphibians by sunfish. This study showed that Pumpkinseek Sunfish can negatively affect amphibians with species-specific impacts.**

Key Words.—aquatic ecology; freshwater; ponds; Pumpkinseed Sunfish

***Résumé.*—Les amphibiens sont un groupe taxinomique menacé et cela est en partie dû aux invasions biologiques dans les milieux humides. Nous avons décrit la communauté d'amphibiens de la Réserve Naturelle Nationale du Pinail (Vienne, France) et évalué les corrélations entre leur présence et celle d'une espèce exotique de poissons dans certaines mares. Nous avons inventorié la présence des amphibiens pendant deux ans dans les mares avec et sans Perches Soleil (*Lepomis gibbosus*). Le nombre moyen d'espèces était de 3,3 ± (SD) 0,2 dans les mares sans perches, contre 1,6 ± (SD) 0,4 avec perches en 2004 et 3,5 ± (SD) 0,2 dans les mares sans perches contre 0,9 ± (SD) 0,2 avec perches en 2005. Nous avons constaté que la présence des perches avait un effet négatif sur la fréquence du *Triturus marmoratus* (Triton Marbré), de *Hyla arborea* (Rainette Arboricole), du *Lissotriton helveticus* (Triton Palmé), et du *Pelophylax* spp. (complexe de Grenouilles Vertes). L'effet était particulièrement prononcé pour *Hyla arborea* et *Triturus marmoratus*, où l'occupation était de près de 50% et 100% respectivement dans les amres sans perches, mais de 0% et 20% dans les mares avec perches. Notre étude n'a pas permis de mettre en évidence un effet significatif pour les espèces les moins abondantes: *Bufo spinosus* (Crapaud Epineux), *Rana dalmatina* (Grenouille Agile), et *Triturus cristatus* (Triton Crêté). L'étude des contenus stomacaux a confirmé la prédation des larves d'amphibiens. Cette étude a montré que la Perche Soleil peut affecter négativement les amphibiens avec un impact plus ou moins important selon les espèces.**

Mots-clés.—écologie aquatique ; eau douce; espèce exotique; mares; perche soleil

Introduction

Many amphibian species are undergoing population decline (Denoël 2012; Hof et al. 2011; Sillero et al. 2014). These declines are attributed to various threats such as habitat destruction and fragmentation, climate change, invasive species, emergent diseases, over-exploitation, pollution, and UV-B radiation (Collins and

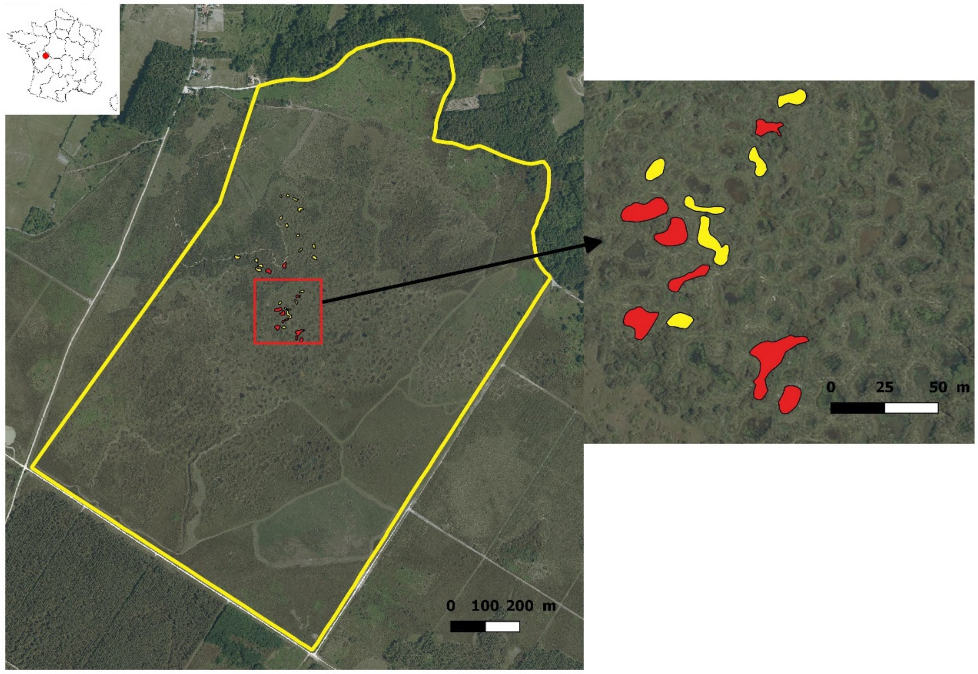

FIGURE 1. Pinail Nature Reserve, Vienne, France, which contains more than 5,000 ponds (boundaries in yellow). Control ponds are in yellow (n = 20) and study ponds with Pumpkinseed Sunfish (*Lepomis gibbosus*) are in red (n = 10). (Taken from BD Ortho 2007, Institut National de l'Information Géographique et Forestière, Saint Mandé, France).

Storfer 2003; Beebee and Griffiths 2005; Hof et al. 2011). Invasive fish species are a major concern for amphibian conservation (Kats and Ferrer 2003). For example, in Europe, fish introduction is a threat to metamorphic and especially to paedomorphic newts, whose disappearance has led to the loss of genetic diversity (Braña et al. 1996; Crochet et al. 2004; Denoël et al. 2005). Fishes impact amphibian communities either directly (by predation) or indirectly (e.g., by reducing growth rate, reducing metamorphic size and rate, and altering habitat use or activity; Kats and Ferrer 2003; Winandy et al. 2017). Introduced fishes can compete for resources (Hartel et al. 2007) and act as vectors for pathogens (Kiesecker et al. 2001; Gray et al. 2009).

The Pinail Nature Reserve (Réserve Naturelle Nationale du Pinail, Vienne, France) protects a relictual ecosystem of heathlands that has had centuries of millstone extraction, and the site shelters a rich biodiversity (Baron 1985) that includes several species of amphibians. The surrounding human population introduced fish for aquaculture in many ponds of the Reserve before 1980 (Copp and Fox 2007). Pumpkinseed Sunfish (*Lepomis gibbosus*), which originates from eastern North America, is now well established in Europe and Asia (Copp and Fox 2004). It is considered a nuisance fish and is spreading in Europe (Copp and Fox 2007). This predatory centrarchid feeds on various invertebrates and strongly decreases macroinvertebrate abundance (van Kleef et al. 2008). Thus, the fish may compete for food with adult amphibians. This sunfish has also been described as aggressive (threat, attack, pursuit) with amphibians for territorial defense, as shown for *Pelophylax perzi* (Perez's Frog; Almeida et al. 2014). More directly, the fish could prey on amphibian larvae because of its flexible diet (Hartel et al. 2007).

The goal of this study was to determine whether sunfish have negatively affected the amphibian populations in this ecosystem with many small ponds. We predicted that sunfish have reduced the frequency of amphibian species occurrence in ponds with sunfish. We further predicted that sunfish prey on amphibian larvae.

MATERIALS AND METHODS

***Study site*.**—The Pinail Nature Reserve in Vienne, France (46°42'2.698"N, 0°31'13.378"E), is a unique ecosystem on 135 ha with more than 5,000 ponds, of which 3,000 have permanent water (Figs. 1 and 2; www.reserve-pinail.org [Accessed 08 August 2017]; Beaune et al., in press). It is surrounded by 4,166 ha of forest, classified into several protection statuses, including Birds and Habitats European Directives (Special Area of Conservation, Special Protection Area, Important Bird Area; Dubech and Sellier 2010). The reserve is covered with heath (*Erica* moors) on acidic and poor soil (podzol) resulting from human pasturing and

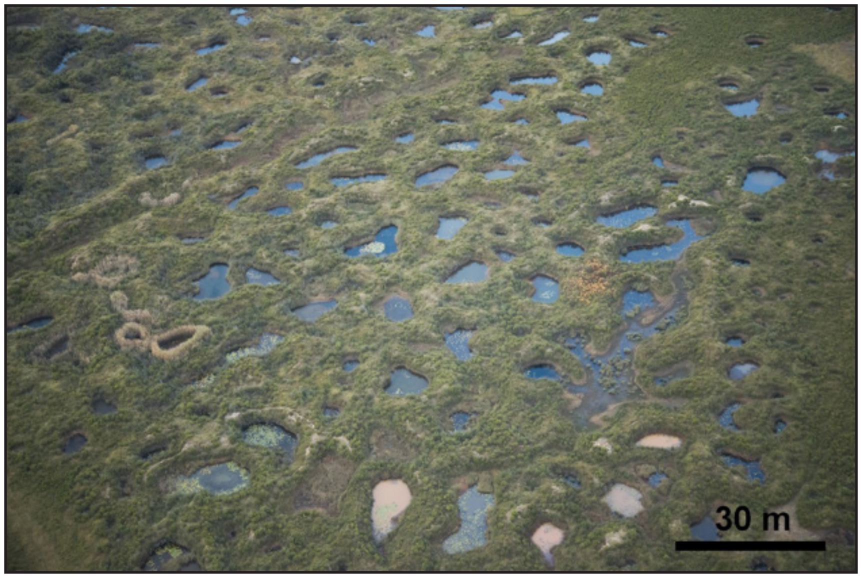

Figure 2. Aerial view of ponds at the Pinail Nature Reserve, Vienne, France. (Photographed by Jean-Guy Couteau).

burning activities. Geology of the site is sedimentary from Oxfordian to Ludian (163 to 42 mya; Dubech and Sellier 2010). Humans have exploited the area since at least the 9th Century for millstone, resulting in thousands of ponds (Baron 1985). The uppermost geological layer used for millstone is gray and silty clay from the Pliocene-Quaternary (0.5 to 3 m thickness); some ponds may be in contact with other layers, leading to subtle physicochemical differences (marl, sandy clay, limestone; Dubech and Sellier 2010). This site forms a very rich ecological complex with more than 2,600 species of fauna, flora, fungi (Dubech and Sellier 2010).

The surrounding human population introduced fish for aquaculture before the creation of the Pinail Nature Reserve (Baron 1985). The following fish species have been recorded at the reserve: *Ameiurus melas* (Black Bullhead), *Anguilla anguilla* (European Eel), *Carassius auratus* (Goldfish), *Carassius carassius* (Crucian Carp), wild *Cyprinus carpio* (Common Carp), *Esox lucius* (Northern Pike), *Lepomis gibbosus*, *Leucaspius delineatus* (Moderlieschen), *Rutilus rutilus* (Roach), *Scardinius erythrophthalmus* (Rudd), and *Tinca tinca* (Tench; Dubech and Sellier 2010). The amphibian community of the Pinail Nature Reserve is composed of 13 taxa: *Epidalea calamita* (Natterjack Toad), *Bufo spinosus* (Spiny Toad), *Hyla arborea* (European Tree Frog), *Lissotriton helveticus* (Palmate Newt), *Pelodytes punctatus* (Parsley Frog), *Pelophylax lessonae* (Pool Frog), *Pelophylax kl. esculentus* (Edible Frog), *Pelophylax ridibundus* (Eurasian Marsh Frog), *Rana dalmatina* (Agile Frog), *Salamandra salamandra* (Common Fire Salamander), *Triturus cristatus* (Great Crested Newt), *Triturus marmoratus* (Marbled Newt), and hybrid *Triturus cristatus* × *T. marmoratus* (Yann Sellier, unpubl. data). We selected a study area within the reserve because it was in a sector that was easily accessed and the pond density of this sector was the greatest (Fig. 1).

Impact on amphibian diversity.—In 2004, we randomly selected 20 control ponds and 10 ponds in the study area for which we had knowledge of sunfish presence/absence. Ponds shared the same typology, i.e., acidic, oligotrophic ponds, with permanent water and 50 to 100% of the bottom covered by vegetation. The main vegetation cover in the ponds was composed of *Myriophyllum alterniflorum* (Alternate Water-milfoil), *Nymphaea alba* (European White Waterlily), *Potamogeton polygonifolius* (Bog Pondweed), *Carex elata* (Tufted Sedge), *Eleocharis multicaulis* (Many-stalked Spike-rush), *Hydrocotyle vulgaris* (Marsh Pennywort), *Hypericum elodes* (St. John's-wort), *Juncus* sp. (rush), *Lythrum salicaria* (Purple Loosestrife), and *Scirpus fluitans* (Floating Club-rush). The ponds were between 1 and 1.5 m deep, and pond surface area was between 40.07 and 248.35 m². We used minnow traps with meat baits to confirm presence or absence of fish in the 30 selected ponds in April 2004. We found no fish species other than *Lepomis gibbosus*. In 2004 and 2005, we searched for amphibian larvae in each of the 30 ponds, once per month from May to August, which corresponds to the period of presence of larvae of the studied species in ponds (Miaud and Muratet 2004). In 2004, we dip-netted in every pond, three times a month, with 10 sweeps at the periphery of the pond. We did not sample the central part of ponds to avoid being too intrusive, which may have limited our ability to detect larvae. We detected eggs at the surface of ponds by

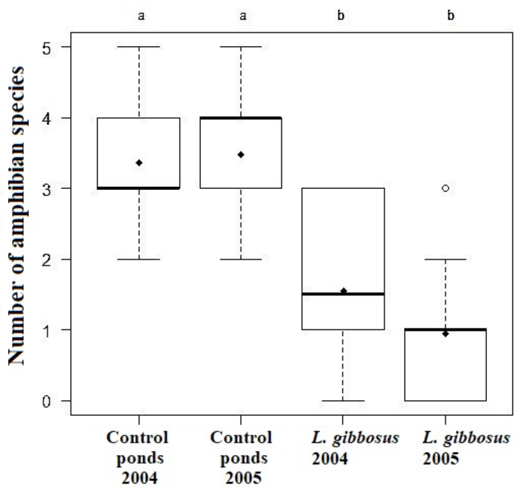

Figure 3. Number of amphibian species in control ponds (n = 20) and ponds with Pumpkinseed Sunfish (*Lepomis gibbosus*; n = 10) at the Pinail Nature Reserve, Vienne, France. Dark horizontal lines represent median, dark points represent mean, box outlines represent 25th percentile and 75th percentile, vertical lines represent minimum and maximum observations, and isolated point is an extreme value. Different letters (a or b) indicate significant differences among groups.

sight. The dip net was a standard sampler with an open base of 1/20 m² and mesh-size of 250 µm. Because the number of species found in ponds reached a maximum after five dip-nettings per ponds, we adjusted the number of dip-nettings to five per pond in 2005. Thus, we searched each pond to determine presence or absence of amphibian species 12 times per year, with 120 sweeps in 2004 and 60 sweeps in 2005.

Sunfish capture and stomach content analyses.—We visited 39 ponds with sunfish, and where amphibian reproduction had been confirmed in previous studies (Yann Sellier, unpubl. data), in April 2016 between 0900 and 1700. The main method to catch sunfish was angling with maggots as bait. If we did not catch fish by angling, we used fish traps. We killed captured fish by manual percussion, which results in rapid death (Robb and Kestin 2002). We collected contents from 24 stomachs and preserved these in a sample container with 90% ethanol. We analyzed the contents with a binocular microscope to identify prey items at this time of year.

Statistical analysis.—We performed analyses using R 2.11R (R Development Core Team 2011). We evaluated mean number of species in ponds with and without sunfish in 2004 and 2005 with the Wilcoxon test. We evaluated differences in species percentage occupancy between ponds with and without sunfish in 2004 and 2005 separately by parametric Chi-square proportion tests. For both tests, $\alpha = 0.05$.

Results

We found a significant difference in number of amphibian species between ponds with and without sunfish (W = 175.5, P < 0.001 in 2004 and W = 191.5, P < 0.001 in 2005; Fig. 3). The average number of species was higher in ponds without sunfish than in ponds with sunfish in 2004 and 2005. We found significant differences in the percentage of ponds occupied by *Hyla arborea* ($\chi^2 = 4.46$, df = 1, $P < 0.05$ in 2004 and $\chi^2 = 7.66$, df = 1, $P < 0.01$ in 2005), *Triturus marmoratus* ($\chi^2 = 17.91$, df = 1, $P < 0.01$ in 2004 and $\chi^2 = 11.27$, df = 1, $P < 0.01$ in 2005), *Lissotriton helveticus* ($\chi^2 = 6.09$, df = 1, $P < 0.05$ in 2005), and *Pelophylax* spp. ($\chi^2 = 735$, df = 1, $P < 0.01$ in 2005), between control ponds and sunfish ponds (Fig. 4). The impact of sunfish was negative for the four species, with percentage occupancy lower in ponds with fish than in ponds without fish. The effect was especially pronounced for *Hyla arborea* and *Triturus marmoratus*, with occupancy nearly 50% and 100%, respectively, in ponds without sunfish but roughly 0% and 20%, respectively, in ponds with sunfish.

We identified prey items from 24 sunfish stomachs. Most prey items were macroinvertebrates: Diptera (n = 428), Crustacea (n = 185), Odonata (n = 7), Megaloptera (n = 6), Bivalva (n = 5), Trichoptera (n = 4), Ephemeroptera (n = 3), Hemiptera (n = 1), and Trombidiformes (n = 1). The only vertebrate found was a larval caudate (*Triturus* sp.).

Discussion

Our study shows that introduction of the non-native Pumpkinseed Sunfish has had a negative impact on the amphibian community of the Pinail Nature Reserve. Sunfish presence severely impacted *Triturus marmoratus* and *Hyla arborea* in both 2004 and 2005. Furthermore, the presence of the sunfish also negatively affected the frequency of occurrence of *Lissotriton helveticus* and *Pelophylax* spp. Consequently, in ponds with sunfish, amphibian species richness has been reduced by more than half (from 3.3 to 1.6 species in 2004, and from 3.5 to 0.9 species in 2005). We did not observe a significant effect of the sunfish on *Bufo spinosus*, *Rana dalmatina* or *Triturus cristatus*, but these species occurred in much lower frequencies in all ponds than the other four species. The differences in effects among species suggests species-specific sensitivity to the sunfish. Crochet et al. (2004) found that *Lissotriton helveticus* and *Triturus marmoratus* were also negatively affected by introduced fishes, whereas they found no effect on *Bufo bufo*.

The mechanism(s) for the negative effect of the sunfish on amphibians in the present study is not

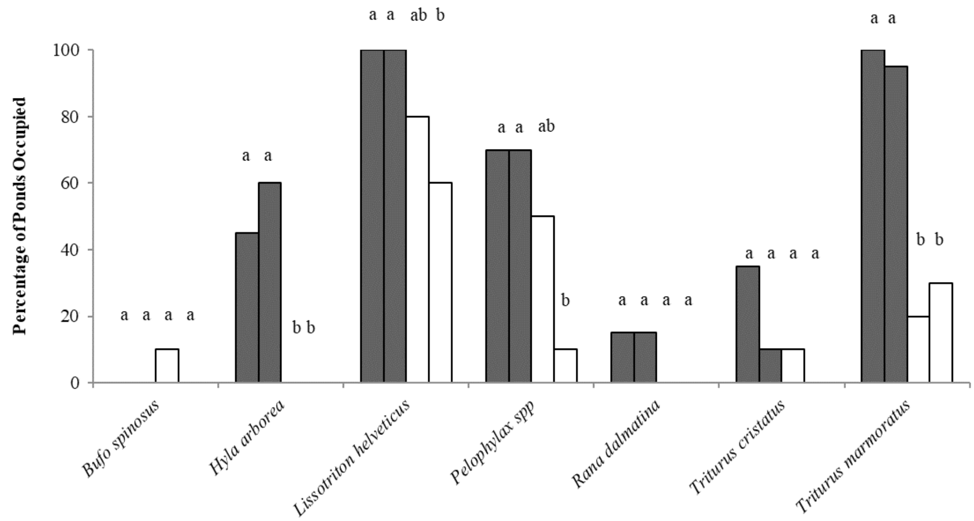

Figure 4. Percentage of ponds occupied by each amphibian species in 2004 (left) and 2005 (right) at the Pinail Nature Reserve, Vienne, France, without (dark bars, n = 20) and with (light bars, n = 10) Pumpkinseed Sunfish (*Lepomis gibbosus*). Different letters (a or b) indicate significant differences among year/fish occupancy groupings.

clear, but several hypotheses can be considered. One hypothesis is the predation of the sunfish on amphibians. The sunfish is too small to prey on adult amphibians and it does not seem to usually feed on amphibian larvae (Gkenas et al. 2016; Locke et al. 2013). Nevertheless, it has been reported to occasionally eat amphibian eggs and larvae (Hartel et al. 2007), and we found a newt larva in one of 24 sunfish stomachs in the present study. Thus, we consider the predation hypothesis to have some support.

A second hypothesis is that sunfish compete with amphibians for food. Based on the stomach content analyses in the present study, the sunfish diet in the Pinail Nature Reserve is composed mostly of invertebrates. A predominantly invertebrate diet was also observed for *L. gibbosus* in other studies (e.g., Wolfram-Wais et al. 1999; van Kleef et al. 2008). This diet is consistent with the opportunistic feeding behavior of sunfish (Almeida et al. 2009), and consequently indicates the potentiality for sunfish to compete for food with amphibians that also feed on invertebrates such as newts. Conversely, however, sunfish prey on direct predators of amphibian larvae, and this could be beneficial to amphibians.

A third hypothesis is the effect of the agonistic interactions initiated by the sunfish in ponds. For example, Winandy and Denoël (2015) showed that aggressive behavior of fish could reduce foraging activity in newts. The presence of sunfish in ponds could also induce variation in antipredator defenses such as egg and larval unpalatability, shown for *Bufo bufo* and *Triturus cristatus* (Gunzburger and Travis 2005). The last hypothesis, is habitat selection (Egan and Paton 2004; Winandy et al. 2015; Winandy et al. 2017). Specifically, some amphibians may avoid entering ponds with sunfish.

In the Pinail Nature Reserve, the connectivity between some ponds, especially in winter, allows the sunfish to spread, which increases the threat to amphibians. At present, 20% of ponds with permanent water have been invaded by sunfish, and it is urgent to restrain further invasion. As more than 600 ponds contain sunfish, sunfish removal would be a laborious and costly task. At a minimum, however, it would be useful to remove fish in a few ponds and test the impact on amphibian populations. Multiple studies have shown that fish removal can allow amphibian recovery (e.g., Vredenburg 2004; Knapp et al. 2007). Denoël and Winandy (2015) showed the ability of the metamorph and paedomorph phenotypes of *Lissotriton helveticus* to recover from decreasing populations after fish removal. Denoël and Winandy (2015) emphasize the importance of preserving common phenotypes to restore intraspecific diversity.

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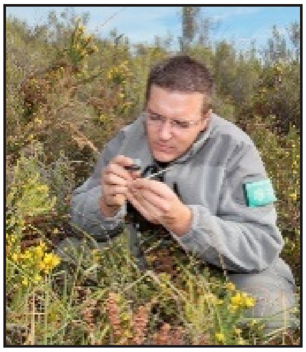
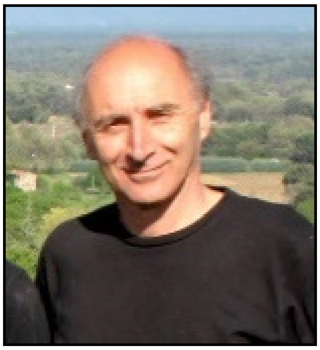
Clémentine Préau is Ph.D. student in Ecology, working on identification and modeling of French amphibian habitats in the context of climate change. She collects data for her dissertation at the Pinail Nature Reserve (Vienne, France), co-directed by the University of Poitiers (Vienne, France) and the University of Tours (Indre-et-Loire, France). At the Pinail Nature Reserve, she works with the managers, studying and managing local biodiversity, particularly amphibians and reptiles. (Photographed by Yann Sellier).

Pascal Dubech has been Manager of the Pinail Nature Reserve since 2005. Previously, he was in charge of studies on the fauna of the reserve, and he mapped the 5,000 ponds there. He studies mostly arachnids, odonatans, lepidopterans, amphibians, and passerines. He has begun work towards a diploma at the École Pratique des Hautes Etudes (EPHE) of Montpellier (France), studying the impacts of perturbations on amphibian reproduction. (Photographed by Yann Sellier).



Yann Sellier has been responsible for scientific missions (fauna, flora, fungus, and naturals habitats) at the Pinail Nature Reserve since 2008. He holds a Master's degree in Ecology and Environment. He is founder of the scientific group Cryptoflore of the French Natural Reserves (RNF), and has been co-leader of this group since 2012. He is a member of experts' groups of regional IUCN Red List (Odonata, Lepidoptera, Amphibia, Reptilia). (Photographed by Thierry Degene DREAL PC).



Marc Cheylan is a Professor at the École Pratique des Hautes Etudes (EPHE), an institute within the PSL Research University (Paris Sciences et Lettres). He currently works at the Centre for Functional and Evolutionary Ecology (CEFE) at the French National Centre for Scientific Research (CNRS) where he teaches conservation biology. Marc is the author of some 200 publications and four books on the ecology, biogeography, and the conservation of amphibians and reptiles around the Mediterranean region. (Photographed by Marc Cheylan).

Franck Castelnau is a Master's degree student at the University of Caen, Basse-Normandie, France. His specialty is ecological management of natural spaces and agro-systems. At the Pinail Nature Reserve, he worked as an intern on the impact of *Lepomis gibbosus* in pond ecosystem and biocontrol methods for that alien species. He wants to focus his research on wetlands and river management, especially on threatened species conservation such as amphibians, odonates, or fishes. (Photographed by Yann Sellier).

David Beaune is a Wildlife Biologist working on various organisms and ecosystems including amphibians and reptiles. He holds a Ph.D. in Ecology (2012) from the Max Planck Institute (Munich, Germany) and the University of Bourgogne (Dijon, France). He was the Manager of the Pinail Nature Reserve. He is currently the Director of the Ornithological Society of Polynesia where reptiles are severely threatened by invasive mammals such as rats and cats. (Photographed by Yann Sellier).