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dizertační práce

Vliv invazní želvy nádherné na vývoj pulců skokana hnědého v různých etapách jeho vývoje

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V Českých Budějovicích dne 15. 8. 2022 Ing. Magda Vodrážková

Podpis…………………..

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Souhrn

Interakce predátor-kořist řídí vývoj mnoha behaviorálních a morfologických změn u živočichů. Ve vodním prostředí chemické podněty spolehlivě umožňují kořisti posoudit a vyhnout se riziku predace. Přítomnost predátora ovlivňuje populace kořisti buď přímou predací snížením její početnosti a změnou jejího chování, nebo modifikací různých částí jejich životní historie. Efekt vyvolaný rizikem predace může mít na populace silnější dopad, než přímý efekt mortality

V posledních desetiletích se četnost a význam invazních druhů zvyšuje především v důsledku neúměrného růstu světového obchodu se zvířaty a rostlinami. Nepůvodní želva nádherná (*Trachemys scripta elegans*) má potenciál narušit vodní ekosystémy ve střední Evropě kvůli svým silným konkurenčním schopnostem a oportunní stravě. Tato práce přináší výsledky experimentů, ověřujících vliv přítomnosti nepůvodní a potenciálně invazní želvy nádherné na parametry životní historie embryí a larev skokana hnědého (*Rana temporaria*). Konkrétně se jedná o to, zda chemické podněty přenášené predátory způsobují změny v době líhnutí, vývojové fázi a velikosti těla při líhnutí pulců skokana a při jejich metamorfóze. současně jsem experimentálně ověřila dynamiku ontogeneze pulců v různých časových vzorcích přítomnosti invazního predátora.

Přítomnost predátora ovlivnila všechny zkoumané faktory. Zjistila jsem, že při absenci želvy se embrya vylíhla za 12 dní, zatímco za přítomnosti predátora bylo líhnutí urychleno o dva dny. Embrya se se navíc vylíhla menší a v nižším stupni vývoje než bez přítomnosti predátora.

Přítomnost želv zkrátila dobu metamorfózy pulců ze 110 ± 11,7 dnů na 93 ± 13,0 dnů (průměr ± S.D.). První metamorfovaní jedinci byli zaznamenáni 65. den a 80. den od vylíhnutí v přítomnosti predátora a v kontrolní skupině, resp. Metamorfovaní jedinci byli statisticky významně menší (12,8 ± 0,99 mm) v přítomnosti predátora než v kontrolní skupině (15,2 ± 1,27 mm). Trajektorie rychlosti růstu byly podobné u obou variant. Obrana pulců vyvolaná predátory byla tedy evidentní ve vyšších rychlostech vývoje a menších velikostech při metamorfóze bez významných změn v růstu.

Pulci měli delší larvální období a byli menší velikosti při metamorfóze a nižší tělesné hmotnosti, když byl predátor přítomen v raném vývoji, než když se pulci vyvíjeli bez predátora. Časná přítomnost predátora nápadně snižovala růstové přírůstky pulců v raném vývoji. Po odstranění predátora se růst zrychlil nad úroveň naměřenou v podmínkách, kdy byl predátor přítomen v pozdní fázi vývoje, i bez přítomnosti predátora. Tyto rychlosti růstu však nepřesáhly rychlosti růstu stejně velkých pulců v ostatních treatmentech, a proto nebyly dostatečné pro kompenzaci zpomalení růstu v první části vývoje. Přítomnost predátora v pozdním vývoji pulce neovlivnila ani dobu do metamorfózy, ani velikost/hmotnost těla při metamorfóze.

Výsledky ukazují spíše vzácně dokumentované typy reakcí kořisti obojživelníků na predátory. Tato studie rozšiřuje okruh studovaných predátorů, včetně vlivu na různé fáze vývoje potenciální kořisti.

klíčová slova

antipredátorská reakce, *Rana temporaria*, *Trachemys scripta*, adaptivní plasticita, doba vývoje, kairomony

Summary

In the last few decades, the frequency and importance of invasive species has been increasing mainly due to the disproportionate growth of world trade in animals and plants. Predator-prey interactions drive the evolution of many behavioral and morphological traits in aquatic animals. In aquatic environments, chemical cues reliably enable prey to assess and avoid predation risk. The presence of a predator affects prey populations either by direct predation by reducing its abundance and changing its behavior, or by modifying various parts of its life history. The effect induced by the risk of predation can have a stronger impact on populations than the direct effect of mortality.

Non-native red-eared sliders (*Trachemys scripta elegans*) have the potential to disrupt aquatic ecosystems in Central Europe because of their superior competitive abilities and omnivorous diets. I investigated whether continuous predator-borne cues are tied to changes in the hatching time, developmental stage, and body size at hatching of common frog tadpoles (*Rana temporaria*). Whether the developmental rates, growth rates and sizes at metamorphosis would alter in the presence of a predator and examined the dynamics of the ontogeny of tadpoles under different time patterns of an alien predator presence.

The presence of a predator affected all factors examined. I found that in the absence of the slider, the embryos hatched in 12 days, while hatching was accelerated by two days in slider treatment. At the same time, the embryos hatched smaller and at a lower stage of development with the slider than without it.

The presence of turtles shortened the time at metamorphosis of tadpoles from 110 ± 11.7 days to 93 ± 13.0 days (mean ± S.D.). The froglets were significantly smaller (12.8 ± 0.99 mm) in the presence of the predator than in the control treatment (15.2 ± 1.27 mm). The growth rate trajectories were similar between the predator treatment and the control. Thus, predator-induced tadpole defences were evident in higher developmental rates and smaller sizes at metamorphosis without significant changes in growth.

I also found out that the tadpoles had a longer larval period and were smaller in size at metamorphosis and lower in body mass when the predator was present in early development than when the tadpoles developed without a predator. The early presence of a predator conspicuously reduced the growth increments of the tadpoles at early development. After the removal of the predator, growth accelerated above the level measured under the conditions of both the late predator and no predator. However, these growth rates did not exceed the growth rates of equally sized tadpoles in the other treatments and therefore were not sufficient to compensate for the growth slowdown in the first part of development. The presence of a predator in late tadpole development influenced neither the time to metamorphosis nor size/body mass at metamorphosis.

Results show rather rarely documented types of amphibian prey responses to caged predators. This study extends the range of predators studied, including the effect on different phases of development of potential amphibian prey.

keywords

antipredator response, *Rana temporaria, Trachemys scripta*, adaptive plasticity, development time, kairomones

Obsah

1. Úvod

Predace je všudypřítomná. Všechny heterotrofní organismy jsou v určité fázi svého životního cyklu kořistí pro ostatní a mnoho druhů se po celý život setkává s jejím rizikem (MacCallum *et al.* 1995). Účinky predace jsou různé a zahrnují snížení populace nebo dokonce eliminaci druhů z regionu, omezení využívání stanovišť a času stráveného hledáním potravy. Tyto faktory ovlivňují rychlost růstu a snižují zdatnost a adaptaci prostřednictvím přirozené selekce na trvalé riziko predace (Polo-Cavia *et al.* 2010).

Predátoři mohou způsobit potenciální kaskádu interakcí prostřednictvím trofické sítě, přímo ovlivnit kořist snížením její početnosti a změnou jejího chování při hledání potravy a nepřímo ovlivnit další druhy, s nimiž je kořist spojena jako potrava nebo jako konkurent. Změny v energetických tocích a druhovém složení mohou mít důsledky pro využití živin a regeneraci. Predace může rovněž spustit trofické kaskády, které mají důsledky pro celý ekosystém (Allan *et al.* 2021). Predátor tak může silně ovlivňovat celý ekosystém, i když nezpůsobí přímou úmrtnost kořisti (Lima 1998). Provedené experimenty a teoretické práce dokonce naznačují, že efekt vyvolaný rizikem predace může mít na populace silnější dopad, než přímý efekt mortality (Anholt *et* Werner 1995). Riziko spojené s přítomností predátora způsobuje změny v chování, morfologii a vývoji jedince (Sih 1992, Ferrari *et al.* 2010). Tyto změny se projevují změnami různých parametrů v životní historii jedince. Speciálním případem, jehož význam narůstá v poslední době, jsou nepůvodní predátoři, z nichž se řada v novém prostředí dokáže úspěšně šířit a stát se invazními druhy. Nerozpoznání kořisti přispívá k úspěchu invazivních predátorů a usnadňuje jejich šíření do nových prostředí. Pokud nedojde u populace kořisti k vyhynutí krátce po příchodu invazního predátora, rozpoznání predátorů a účinná obrana proti nim se mohou vyvinout během několika generací a tím přispět ke koexistenci kdysi invazivních predátorů a jejich původní kořisti (Allan *et al.* 2021).

V posledních několika desetiletích vzrůstá četnost a významnost invazních druhů kvůli nepřiměřenému růstu světového obchodu (Daisie 2009). Celosvětový obchod a cestování umožnilo mnoha druhům rozšířit se do vzdálených biogeografických oblastí, které by jinak nebyly schopné obsadit (Pimentel *et al.* 2005, Nentwig 2010). Spolu s dalšími účinky na degradaci ekosystémů jako je změna klimatu, znečištění a přeměna biotopů, jsou biologické invaze považovány za jednu z hlavních příčin poklesu biologické rozmanitosti na celém světě

(Millennium Ecosystem Assessment 2005). Obvykle platí, že čím je větší populace invazivních druhů a čím rychleji se šíří, tím vyšší je její potenciální dopad a tím složitější a dražší bude kontrola nebo vymýcení těchto druhů. Z těchto důvodů se doporučuje co nejdříve reagovat na invazivní druhy (Sakai *et al*. 2001, Wittenberg *et* Cock 2001, Leung *et al*. 2002).

Nepůvodní druhy živočichů i rostlin jsou lidmi zavlékány mimo svůj přirozený areál rozšíření již po tisíciletí. Nicméně zlepšení dopravy, mezinárodního obchodu a globalizační procesy toto zavlékání velmi usnadnilo a do budoucna se předpokládá, že šíření nepůvodních druhů bude pokračovat (Sundseth 2014). Na rozdíl od přirozeného šíření druhů je u zavlékání nepůvodních druhů nezbytným faktorem člověk. Právě jeho činnost napomáhá nepůvodním druhům překonávat biogeografické bariéry neboli hranice jejich přirozeného prostoru. Ve většině případů se nepůvodní druhy nedokáží novému okolí přizpůsobit a zavlečení je tedy neúspěšné (Plesník 2011). Jindy však druhy přežijí, zdomácní a začnou se rozmnožovat. V horším případě začnou být invazní, mají tudíž negativní vliv na přirozenou biodiverzitu. Kromě ohrožení biologické rozmanitosti představují invazní nepůvodní druhy také ekonomickou hrozbu. Škody, které způsobují a náklady nezbytné na jejich kontrolu dosahují jen v Evropské unii každoročně několika miliard eur. Zároveň nesmíme zapomenout ani na jejich dopad na lidské zdraví, kvůli schopnosti přenášet parazity a patogeny (Cambray 2003). Z výše uvedeného vyplývá, že nepůvodní druhy jsou opravdu velkým problémem, který je potřeba řešit.

Původní společenstva jsou nucena čelit novým neznámým predátorům, a obojživelníci nejsou v tomto směru výjimkou (Falaschi *et al.* 2020). Predátoři uvolňují charakteristické pachy, které kořist používá k detekci rizika predace. Schopnost detekovat, rozpoznat a reagovat na tyto látky vylučované potenciálními predátory je důležitou součástí antipredačního chování obojživelníků ve všech jejich vývojových stádiích (Johnson *et al*. 2003, Bennett *et al*. 2013). Introdukce nepůvodních druhů přinesla značné problémy i na evropském kontinentu včetně České republiky. Je známo, že obojživelníci i jejich larvy jsou schopni rozpoznat a reagovat na místní predátory bez předchozí zkušenosti, ale nemusí rozpoznat nové druhy, protože s nimi nesdíleli společnou evoluční historii. V Evropě je z tohoto hlediska jedním z nejvýznamnějších invazních druhů želva nádherná (*Trachemys scripta elegans*). Její negativní dopad na autochtonní faunu již byl zdokumentován v řadě zemí jižní Evropy. V České republice je její dopad prozatím omezený, ale s rostoucím oteplováním se dá očekávat jeho nárůst. Cílem této práce bylo získat nové poznatky v oblasti autekologie vybraných vodních organismů na základě sledování vlivu přítomnosti želvy nádherné na parametry ontogenetického vývoje embryonálního a larválního stádia skokana hnědého (*Rana temporaria*).

2. Obecná problematika invazních druhů

2.1. Invaze

Zavlečení druhů do oblastí mimo jejich původní areály bylo výsledkem lidské činnosti po tisíciletí. Během této doby byly takové aktivity z velké části považovány za přínosné nebo nedůležité. Dnes však už víme, že zavlečení cizí bioty způsobené člověkem je ekologickým narušením, jehož důsledky se vyrovnají těm nejzávažnějším, jako je chemické znečištění, ztráta přirozeného prostředí a změna klimatu (Kraus 2009). Neschopnost likvidace většiny zavlečených invazních druhů je skutečně činí problematičtějšími než mnoho jiných ekologických problémů (Sundseth 2014).

Invazní predátoři při zavlečení často způsobují značné ekologické a ekonomické škody (Blackburn *et al.* 2004). Vymírání druhů, narušení potravinové sítě, změny komunity, přeměna ekosystémů, změny v koloběhu živin, kolaps rybolovu, degradace vodních toků, ztráty v zemědělství, poškození budov a epidemie nemocí patří mezi destruktivní a často nepředvídatelné ekologické a ekonomické dopady, které mohou cizí invazní druhy způsobit (Kraus 2009).

Působení invazních predátorů můžeme rozdělit na tři úrovně. Invaze 1. úrovně nastává, když kořist nerozpoznává riziko predace a nevykazuje antipredační chování. Například nelétaví ptáci na Novém Zélandu, kteří nemají původní savčí predátory, nerozpoznávají invazní lasice nebo krysy, které tak mají katastrofický dopad na místní avifaunu (Blackburn *et al.* 2004). Invaze 2. úrovně nastává, když kořist rozpozná predátora, ale zvolí špatnou antipredační odpověď. Australské kolihy a menší klokani v reakci na lišky zaujímají pozice při kterých splývají s pozadím. Tato antipredační strategie je účinná proti vizuálně orientovaným predátorům (ptáci), ale ne proti liškám lovícím čichem. Invaze 3. úrovně nastává, když kořist rozpozná predátora jako nebezpečného, má i vhodnou obranu, ale „překoná“ ji vynikající taktika lovu invazních druhů. Klokani obrovští (*Macropus giganteus*) se v přítomnosti lišek pasou ve větších skupinách a blíže úkrytů (Moyle 1986), přesto je populace stále ovlivněna liščí predací (Vermeij 1991). Tyto úrovně invaze se vztahují na suchozemské i vodní ekosystémy a upřesňují předpoklad jaký typ predátora může ovlivnit místní faunu.

2.2. Evoluční historie

Rozlišování a rozpoznávání predátorů kořistí je rozděleno podle toho, zda jou schopnosti kořisti úměrné době trvání koevoluce (Blumstein 2002, Banks *et* Dickman 2007) nebo jsou výsledkem ontogenetické zkušenosti (Relyea 2003, Gazzola *et al*. 2017) s konkrétními predátory, a nebo, zda kořist jednoduše zobecňuje svou reakci na všechny predátory na základě charakteristik sdílených a obecných mezi predátory (Cox *et* Lima 2006, Apfelbach *et al*. 2015). Druhy, které měly dlouhou koevoluci s predátorem, mohou mít „pevně nastavené" antipredační reakce. Kořist tedy může vykazovat vrozené schopnosti rozpoznávat a reagovat na predátora, protože se vyvíjela společně s predátorem (Blumstein *et al*. 2002, Apfelbach *et al*. 2005, Monclús *et al*. 2005). Naproti tomu druhy kořisti, které nebyly evolučně vystaveny predátorům, se mohou prostřednictvím ontogenetických zkušeností naučit rozpoznávat a reagovat na čichové podněty predátorů (Berger *et al*. 2001, Anson *et* Dickman 2013) nebo na jejich vizuální podněty (Atkins *et al*. 2016). Podle některých autorů schopnost kořisti rozpoznat introdukované predátory závisí na podobnostech mezi cizími a původními predátory a na obecnosti "informace" používané původní potenciální kořistí k posouzení rizika predace (Sih *et al*. 1987, Warkentin 1995).

I když druh vykazuje schopnost reagovat na oba typy predátorů, intenzita reakce může být různá. Studie, které zkoumaly pozorování schopností rozpoznávat původní a nepůvodní predátory podobných skupin například dokládají, že pulci *Rana iberica* reagovali na chemické podněty původních i nepůvodních druhů pstruhovitých ryb snížením své aktivity, ačkoli reakce na původního pstruha obecného (*Salmo trutta*) byla silnější než na invazního sivena amerického (*Salvelinus fontinalis*) (Bosch *et al*. 2006).

Druhy sousedících kontinentů by měly být vůči nepůvodním predátorům méně zranitelné, i když jsou to konkrétně druhy, s nimiž se v evolučním čase nesetkaly (Frankel *et* Soulé 1981). Dopad invazních suchozemských predátorů na sousední kontinenty je vyšší než u ostrovů (Atkinson 1989, Short *et* Smith 1994). Kontinentální (neendemické) druhy na ostrovech se zdají být méně náchylné k vyhubení zavlečenými predátory, než ostrovní endemické druhy (Atkinson 1985, Case *et al*. 1992, Blackburn *et al*. 2004). Introdukovaná křepelka čínská (*Coturnix chinensis*) na ostrově Guam na rozdíl od původních druhů odolává predaci bojgy hnědé (*Boiga irregularis*), ačkoli mnoho z těchto introdukovaných ptáků nemá s tímto hadem žádné evoluční zkušenosti (Fritts 1988). Kontinentální suchozemští živočichové i obyvatelé mořských ekosystémů jsou zřídka zranitelní vůči novým predátorům kvůli častým historickým a biotickým změnám. Naproti tomu sladkovodní ekosystémy vykazují v predačních režimech stálost, čímž vytváří zranitelnost vůči nepůvodním predátorům (Diamond 1989, Case *et al*. 1992, Blackburn *et al*. 2004).

Existují určité výjimky ze vzoru invazních obratlovců s nízkým dopadem na sousedící kontinenty. Například v Evropě norek americký (*Neovison vison*) snižuje populace původního hryzce vodního (*Arvicola terrestris*). I když hryzec rozpoznává norka jako predátora, jeho obvyklé antipredační chování (potápění se do vody, vyhrabávání substrátu a schovávání se v noře) není proti norkovi účinné (MaCdonald *et* Harrington 2003). Kvůli migračním schopnostem ptáků vykazují ptačí predátoři kosmopolitní historické rozšíření oproti savcům. V tomto srovnání by se tedy dalo očekávat menší ovlivnění vysazením ptačích predátorů. Introdukce savců má více než 12krát větší pravděpodobnost, že povede k velkým negativním dopadům než u ptáků (Ebenhard 1988, Blackburn *et al*. 2004). Existují však některé příklady ničivých invazivních ptačích predátorů jako chřástal weka (*Gallirallus australis*) na ostrovech kolem Nového Zélandu (Ebenhard 1988).

Bezobratlým predátorům se obecně dostává menší pozornosti než zavlečeným obratlovcům. Vzhledem k omezenému historickému rozšíření budou mít pravděpodobně velký vliv, zejména na ostrovech. Suchozemský plž *Euglandina rosea* například zdecimoval původní populace několika druhů měkkýšů na Havaji a jinde v Pacifiku (Loope *et al*. 1998). Na Havaji také přispěli k úbytku mnoha původních členovců nepůvodní mravenci a vosy. Mravenec *Solenopsis invicta* v USA mimo jiné výrazně potlačuje přirozenou kořist bezobratlých (Allen *et al*. 2004).

2.3. Vlastnosti invazních druhů

Rané studie o invazních druzích se skládaly především z popisů úspěšných predátorů. V posledních letech ekologové analyzovali nejen šíření nepůvodních druhů a jejich škodlivé účinky na místní komunity (Elton 2020), ale začali se zabývat společnými rysy, které oddělují úspěšné predátory od neúspěšných (Ehrlich 1986, Warkentin 1995, Simberloff 2013). Úspěch invaze závisí na dvou skupinách všeobecných vlastností, které umožňují lepší predikci druhů. A to vlastnosti, které umožňují nepůvodním druhům osídlit nové stanoviště (disperze) a schopnosti v něm uspět (Ehrlich 1986). Studie z povodí řeky Colorado zjistila, že invazivní rybí druhy byly charakterizovány brzkým rozmnožováním a produkcí menších vajíček ve větších počtech (Olden *et al*. 2006). Přesto mnoho druhů patří mezi velké ryby s velkými vajíčky a/nebo druhy s péčí o potomstvo (*Lates niloticus*, *Salmo trutta*).

Geografický rozsah a tolerance daného prostředí jsou také užitečnými prediktory úspěchu invaze. Mezi invazivními druhy sladkovodních a mořských bezobratlých a ryb jsou zastoupeny druhy s větším zeměpisným rozsahem (Kolar *et* Lodge 2002, Bates *et al*. 2013). Navíc druhy s vyšší tolerancí vůči teplu mají tendenci být ve vyšší početnosti, což má závažné důsledky pro interakce mezi invazivními druhy a změnou klimatu (Bates *et al*. 2013). U některých úspěšně invazních druhů byla také zaznamenána zvýšená tolerance k znečištění ve srovnání s původními druhy (Karatayev *et al*. 2009, Früh *et al*. 2012). Invazní měkkýši používají rychlý růst a reprodukci k rychlému zotavení populace po jejím kolapsu nebo po velkém snížení jejího počtu (McMahon 2002). To zvyšuje jejich kolonizační schopnosti.

Úspěšné invazní druhy budou mít pravděpodobně vlastnosti, které zahrnují širokou toleranci prostředí, kombinaci nepohlavního rozmnožování a vysoký reprodukční počet, nebo budou zavlečeni do sladkovodního prostředí (Havel *et al*. 2015). Předvídání invazí nicméně závisí také na charakteristikách ohrožených původních druhů.

Obrázek 1: Rozšíření invazních druhů na Zemi. (Čím tmavší barva, tím vyšší podíl invazních druhů) (http://atraktivnibiologie.upol.cz/docs/pdf/Biologicke%20invaze.pdf).

Obrázek 2: Celosvětové rozšíření *Trachemys scripta* a záznamy o rozmnožování na daných lokalitách (Heidy *et al*. 2010).

2.4. Náklady a dopady invazních druhů

Vyčíslení nákladů na dopady biologické invaze poukazuje na skutečnost, že tyto invaze mohou mít obrovské ekonomické důsledky. Odhad provedený pro vodní ekosystémy USA ukázal přibližné roční náklady 7,7 miliard USD spojené se škodami způsobenými invazními druhy. Tyto náklady jsou spojeny hlavně s rybami (cca 5,4 miliardy), měkkýši (2,2 miliardy) a vodními rostlinami (0,1 miliardy) (Pimentel *et al*. 2005). Tyto odhady nákladů jsou založené jak na přímých ztrátách a škodách, tak na nákladech na eradikaci.

Ryby způsobují vyhynutí původních druhů, u kapra navíc například snížení kvality vody. Invazivní slávičky (*Dreissena* sp.) snižují množství potravu a koncentraci kyslíku pro původní faunu a ucpávají potrubí a filtry (důležité pro člověka a průmysl). Invazivní vodní rostliny blokují světlo původním rostlinám, dusí vodní toky, mění koloběh živin a omezují rekreační využití řek a jezer.

Dalším problémem mohou být kombinované dopady více invazních druhů. V jezerech jsou běžné mnohonásobné invaze (Mills *et al*. 1993). Jsou jejich účinky doplňkové, synergické nebo antagonistické? Lze tyto interakční efekty prozkoumat pomocí faktoriálových návrhových experimentů prováděných v dostatečně velkém měřítku, aby byly zachyceny klíčové odpovědi?

Analýzy nákladů a přínosů pro řízení a hodnocení rizik vyžadují lepší metodiku pro kvantifikaci dopadů (Simberloff 2013). Je potřeba přesunout pozornost od dopadů výlučně souvisejících s biodiverzitou na hodnocení funkčních změn, protože ztráta biodiverzity a nekonzistentní metodika odráží dramatický dopad a reorganizaci, kterou zažívá mnoho biologických komunit (Dornelas *et al.* 2014, Pandolfi *et* Lovelock 2014). Samotná biologická rozmanitost často neodráží základní funkční změny (Säterberg *et al.* 2013). Je zapotřebí dalších prací ke kvantifikaci a interpretaci těchto funkčních změn ve sladkovodních ekosystémech. Jedním z odrazů lidských hodnot je pohled na invazní versus původní druhy. Proběhlo několik debat o tom, zda by se s invazivními druhy mělo zacházet stejně jako s původními a bylo dokonce navrženo ukončení biologie invaze (Valéry *et al.* 2013). Proti této myšlence Simberloff a Vitule (2014) poskytují přesvědčivý argument, že se invazivní druhy zásadně liší od většiny původních a mohou vytvářet dopady až po časových prodlevách (Simberloff 2013). Dopady způsobené invazivními druhy se ukázaly být závažnější než dopady způsobené přemnožením původních druhů (Simberloff *et* Rejmánek 2011, Hassan *et* Ricciardi 2014).

V neposlední řadě je otázka s cílem omezit zavlečení například prostřednictvím lepší legislativy týkající se přepravy druhů napříč zeměmi a mezi povodími. Opatření v oblasti řízení a eradikace jsou špatně koordinovány. Kromě toho existuje potřeba adekvátních distribučních databází pro sledování invazivních druhů a pro stanovení priorit při řízení akcí (Bobeldyk *et al.* 2015). Pokud jsou předpovědi o změně klimatu a invazivních druzích správné, ekosystémy pocítí rostoucí tlaky od invazivních druhů. Země a regiony budou muset zdvojnásobit úsilí v oblasti prevence invazivních druhů a v oblasti eradikace nově zavlečených druhů. Historické „vyčkejte" přístupům k invazivním druhům nebude životaschopné a k zachování současných ekosystémů bude nutný agresivnější přístup k prevenci a eradikaci.

2.5. Eradikace invazních druhů

Je možné vyhubení invazivních druhů? Nepůvodní druhy napadly mnoho lokalit, a proto se s nimi musíme vypořádat nyní i v budoucnu. Studie navrhly, že je potřeba zaměřit úsilí na včasnou detekci a rychlou reakci na eradikaci k invazivním druhům (Westbrooks *et* Eplee 2011). Tento přístup se osvědčil u některých suchozemských a vodních rostlin (Westbrooks 2004). Vidíme rostoucí důraz kladený na eradikaci a obnovu stanovišť do podmínek, v nichž se nacházely před zavlečením nepůvodních druhů. Například kombinace zaplavení a odstranění dvou invazních rostlin umožnila rychlé obnovení původních rostlin a zvýšené bohatství původních ryb v tropické australské nivě (Perna *et al.* 2012). Tam, kde se obnova zdá jako obtížná nebo nemožná, se ekologové zaměřili na strategie, které minimalizují potenciální dopady. Eradikace nepůvodních druhů byla úspěšná jak v suchozemských, tak ve vodních komunitách (Simberloff 2013). Invazivní druhy byly izolovány a stanoviště bylo chemicky ošetřeno nebo vysušeno. V potocích nebo na více otevřených vodních plochách takové přístupy pravděpodobně praktikovat nelze. Fyzické odstranění odchytem např. raků (Hansen *et al.* 2013) nebo vytrháváním rostlin může výrazně snížit hustotu, ale nedokáže zcela vymýtit populaci. Několik studií uvádí, že po odstranění nepůvodních druhů došlo k úspěšnému vrácení dominance druhům původním (Perna *et* Burrows 2005, Perna *et al.* 2012).

Ryby a vyšší obratlovci se vyznačují větší odolností jako invazní druhy. Jakmile se redukce populací zastaví, dochází k vyšší populační hustotě (Gaeta *et al.* 2015). Knapp a kol. (2007) zjistili, že malá populace vzácných původních žab se začala rychle zotavovat po odlovení pstruhů (*Oncorhynchus* spp. a *Salvelinus fontinalis*) z vysokohorských jezer. Pokud byl pstruh přítomen déle než 50 let, obnova zooplanktonu byla pomalejší a některé původní druhy nedokázaly populaci obnovit (Knapp *et* Sarnelle 2008). Stejně jako často existuje časová prodleva mezi kolonizací zavlečeného druhu a následnými dopady na vodní společenstvo, je evidentní také časová prodleva v obnově původních druhů po úspěšném odstranění invazivního druhu.

Komplexní přehled úspěšných invazních eradikací v Evropě ukázal, že většina projektů zahrnovala likvidaci konkrétních suchozemských druhů z ostrovů nebo nepůvodních savců (Genovesi 2005). Například za účelem ochrany původních populací malých savců v severovýchodním Skotsku byly týmy dobrovolníků proškoleny na odchyt norků amerických (*Neovison vison*). Odchyt probíhal od vysokohorských jezer a dál pokračoval po řekách. I když existovala dobře koordinovaná a systematická strategie pro odstranění invazivního savce, nebyla úplně úspěšná. Vědci doufají, že norkové mohou být zlikvidováni v severním Skotsku, ale bude to víceleté dobrovolnické úsilí s velkými ekonomickými náklady (Bryce *et al.* 2011). Tento příklad nám připomíná, že co se týká invazí, měli by se ekologové zaměřit na prevenci, protože obnova může být obtížná, ne-li nemožná.

2.6. ČR a Evropa

Existuje řada případů, kdy neuvážené vysazování (introdukce) druhů přineslo značné problémy i na evropském kontinentu (Daisie 2009, Kraus 2009, GISD 2022). Připomeňme jen historii expanze ondatry pižmové (*Ondatra zibethica*), která je dodnes vážným problémem pro umělé hráze v Nizozemsku, psíka mývalovitého (*Nyctereutes procyonoides*), který likviduje unikátní ptačí kolonie na mnoha místech baltského pobřeží, či norka amerického (*Neovison vison*) jehož rostoucí populace vznikla ze zvířat ze zaniklých farem, urychlila vymizení norka evropského (*M. lutreola*) a začíná ohrožovat potravní konkurencí a predačním tlakem existenci dalších původních druhů (Hackländer *et* Zachos 2020, Hutterer *et* Kryštufek 2020). Problémy spojené s nepůvodními druhy nejsou pochopitelně omezeny pouze na savce. Existuje dlouhý seznam nepůvodních druhů z celé živočišné říše, kteří stejným způsobem zasáhly do vývoje přírodních společenstev nebo se projevily jako škůdci na polních či lesních kulturách (Lowe *et al*. 2000, Kraus 2009, GISD 2022). Stačí připomenout mandelinku bramborovou (*Leptinotarsa decemlineata*), či problém posledních let – klíněnku jírovcovou (*Cameraria ohridella*), drobného motýla decimujícího stromořadí jírovců v celé střední Evropě. Dalším příkladem je střevlička východní (*Pseudorasbora parva*), která díky rychlému reprodukčnímu cyklu dosahuje v rybnících v krátké době takové biomasy, že představuje vážného potravního konkurenta komerčním druhům ryb (Van der Veer *et* Nentwig 2015).

3. Obojživelníci a invazní druhy

3.1. Predace obojživelníků a její detekce

Aby kořist mohla aktivovat obranné strategie vůči riziku predace, musí správně rozpoznat a vyhodnotit zdroj rizika a jeho intenzitu (Ferrari *et al.* 2010). Rozpoznání predátora je klíčové pro přežití jedinců, a proto se u různých druhů vyvinulo široké spektrum způsobů, jak predátora rozpoznat. Mechanismy použité při získávání informací z prostředí se dále mohou lišit v závislosti na typu zdroje, na morfologii jedince a denní aktivitě (Sazima *et* Eterovick 2000). Ve vodním prostředí je nejčastější detekce vizuální, mechanická a chemická (Relyea 2002, Relyea 2007).

Ve sladkovodních biotopech mohou být predátoři přítomni regionálně, lokálně nebo mohou zcela chybět. Jedná se o sladkovodní „ostrovy“, kde se nevyskytuje většina predátorů. To vytváří vhodné podmínky pro rozvoj invaze, výslednou ekologickou zranitelnost a může způsobit i podstatné změny v populaci jejich kořisti. Například vysazování pstruhů do izolovaných jezer v pohoří Sierra Nevada mělo za následek téměř úplnou eliminaci většiny obojživelníků, bentických živočichů a zooplanktonu (Knapp *et al.* 2001). Když se invazivní druhy chovají jako ekosystémoví inženýři, mohou rychle transformovat celé ekosystémy (Strayer 2012).

Mnoho obojživelníků, kteří obývají proměnlivé prostředí, musí projevit určitou fenotypovou plasticitu, aby dokázali čelit a přizpůsobit se náhlým změnám stanovišť, které obývají. Predace je pro larvy a vajíčka obojživelníků jedním z limitujících faktorů v tomto prostředí. Predátoři mohou v určitých případech vyhubit veškeré společenstvo pulců, které se vyskytuje na dané lokalitě (MacCallum *et al.* 1995). U vajec obojživelníků by se dalo očekávat, že budou obzvláště zranitelná zejména kvůli tomu, že jsou malá, relativně nechráněná, a hlavně se nemohou přemístit, aby unikla před predátory. Avšak predátoři mohou být odrazeni ochrannými adaptacemi vajec, včetně chemické nebo mechanické obrany obalů vajíčka. Přežití embryí může být ovlivněno správným načasováním reprodukce. U vajec obojživelníků jde nejen o správné načasování kladení snůšek, ale také o načasování líhnutí. U *Rana temporaria* dochází k synchronizované reprodukci. Jedná se o strategickou odpověď na kanibalismus pulců. Pulci, kteří se vylíhli dříve, často konzumují ostatní vajíčka. Aby tomu bylo zamezeno, dochází k synchronizovanému líhnutí (Crump 1986).

U pulců se ke snížení predačního tlaku vyvinula řada obranných mechanismů, které se projevují ve změnách morfologických, fyziologických i behaviorálních (Harvell 1990, DeWitt *et al.* 1998, Tollrian *et* Harvell 1999). Pro detekci predátora pulec využívá několik senzorických podnětů, včetně dotykových, vizuálních a chemických. Mezi obranné mechanismy patří nepoživatelnost (Brodie *et* Formankowicz 1987), změna velikosti těla, načasování doby metamorfózy (Richards *et* Bull 1990), shlukování (Nicieza 1999) a snížení pohybové aktivity (Skelly 1994, Relyea 2001, Polo-Cavia *et al.* 2010).

Přítomnost predátora může také snížit dostupnost a příjem potravy, a tím ovlivnit rychlost larválního růstu a dobu metamorfózy (Laurila *et* Kujasalo 1999, Gomez-Mestre *et al.* 2010). Snížená růstová rychlost, která je způsobená přítomností predátora, nebo menším přísunem potravy, aktivuje produkci žláz s vnitřní sekrecí, které vyvolávají metamorfózu (Wilbur *et* Collins 1973, Laurila *et* Kujasalo 1999). Pulci, kteří žijí v prostředí s predátorem nebo mají omezený příjem potravy, metamorfují dříve než jedinci, kteří žijí ve vhodnějších podmínkách bez přítomnosti predátora (Richards *et* Bull 1990).

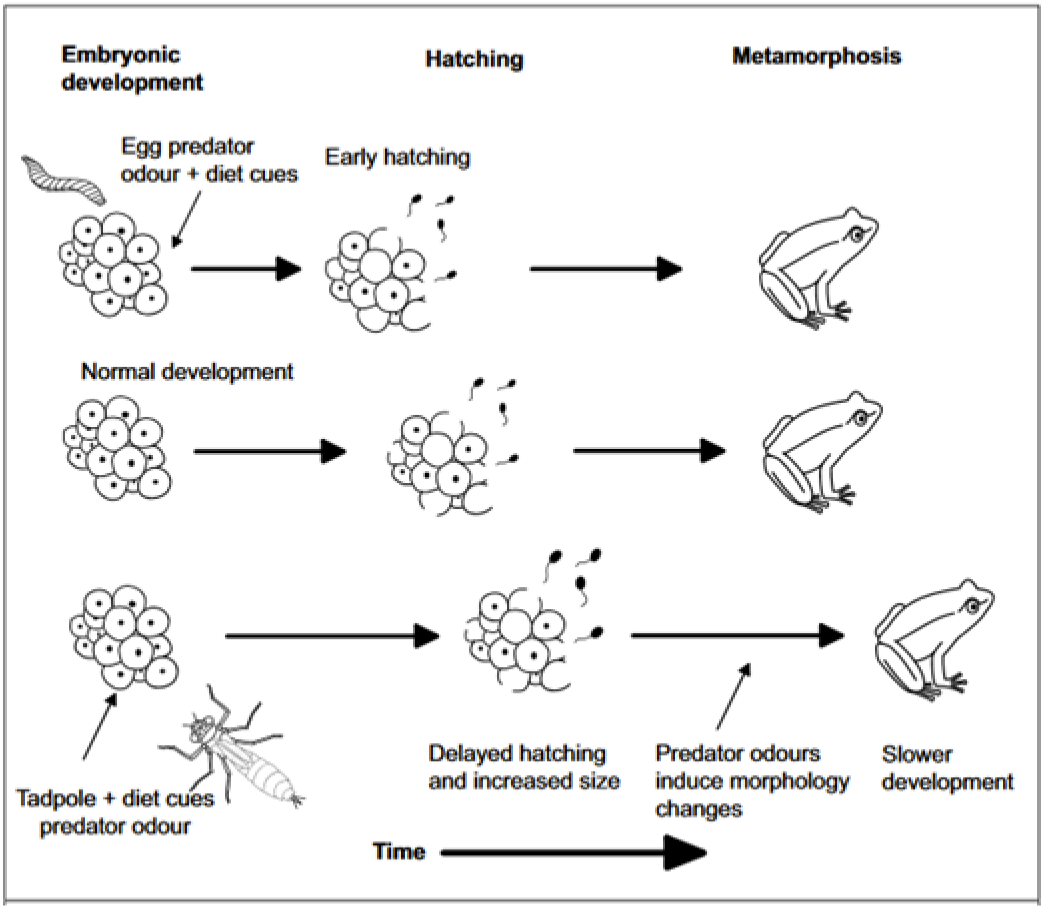
3.1.1. Chemické podněty

Larvy obojživelníků ke zjištění možné predace nevyužívají jen zrakové a mechanické podněty, ale i podněty chemické. Ve vodě rozpuštěné chemické látky jsou vodními organismy využívány k rozpoznání rizika predace zejména proto, že poskytují specifickou informaci o identitě kořisti a predátora a o míře ohrožení (Ferrari *et al.* 2010). Kořist může detekovat jak predátora, který je v klidu, tak samotný akt predace. Chemické stimuly mohou být produkovány predátorem i samotnou kořistí. Bylo zjištěno, že pulci reagují na predátora pomocí registrace určitých chemických látek obsažených v sekretech predátora, ale i v sekretech vlastních (Maag *et al.* 2012). Některé larvy obojživelníků nemají vrozenou reakci na chemické látky produkované predátorem, a proto se musí naučit rozpoznat tyto látky, aby na ně mohly reagovat (Gonzalo *et al.* 2009, Chivers *et al.* 2015). V důsledku toho jsou behaviorální a fyziologické reakce na predátora často závislé na předchozí zkušenosti s daným predátorem (Orr *et al.* 2009, DeSantis *et al.* 2013).

Některé studie prokázaly, že nejen chemické látky produkované predátorem, ale i složení jeho potravy může vyvolat antipredační chování (Schoeppner *et* Relyea 2005, 2009). Pulci navíc dokáží rozeznat chemickou stopu od predátora, kterou vylučuje současně při žvýkání a trávení kořisti (Schoeppner *et* Relyea 2009). V různých ekologických systémech mohou být preferovány různé druhy podnětů. Ve vodním prostředí - a to zejména v zakalených vodách - je pro detekci predátorů považováno za nejdůležitější smyslové vnímání pomocí chemických signálů (Kats *et* Dill 1998, Tollrian *et* Harvell 1999, Brönmark *et* Hansson 2000). Schoeppner a Relyea (2009) pozorovali signifikantní snížení pohybové aktivity pulců *Rana pipiens* v případech, kdy predátor *Anax junis* žvýkal kořist a kdy ji trávil. Dále pozorovali, že pulci se více skrývali v přítomnosti látek z rozmačkaných těl pulců. Pulci *Rana temporalis* posuzují hrozbu predace založenou výhradně na chemických podnětech pocházejících z potravy predátora. Jsou-li v potravě predátora přítomny látky pocházející z pulců stejného druhu, pulci vnímají hrozbu a reagují na ni antipredačním chováním. Vystavení pulců vizuálním podnětům možné predace a vystavení pulců chemickým látkám z hladovějícího predátora nevyvolalo antipredační chování (Mogali *et al*. 2012).

Ve studii účinků chemických podnětů vylučovaných predátorem na vliv pohybové aktivity u dvou larválních stádií žab *Rana temporaria* a *Bufo bufo* vykazovaly oba druhy mírné snížení aktivity plavání při konfrontaci s chemickými látkami od sympatrických predátorů (Marquis *et al*. 2004). Také Takahara (2008) se ve své studii zabýval změnou aktivity plavání pulců *Hyla japonica* v přítomnosti predátora nymf vážky *Anax parthenope Julius.* Rychlost plavání byla vyšší a uplavaná vzdálenost delší u pulců, kteří byli vystaveni chemickým látkám od nymf.

Častá je změna trajektorie pohybu a snaha úniku před predátory, což zpravidla vede ke snížení růstu kořisti (Sih 1987, Relyea *et* Werner 1999, Berec *et al*. 2016). Určitá míra vnímavosti látek produkovaných predátorem je možná již u vajec při embryonálním vývoji. Například Orizaola a Braña (2004) zkoumali charakteristiky líhnutí a morfologii vylíhlých larev čtyř druhů čolků (rod *Triturus*), jež byly vystaveny chemickým látkám signalizujících přítomnost predátora, kterým byl pstruh obecný (*Salmo trutta*). Jedinci vystaveni nebezpečí se líhli v ranějším stádiu vývoje a byli také poměrně menší. V porovnání s kontrolní skupinou zde byly přítomné také změny v morfologii hlavy a ocasu, avšak podle autorů takováto změna v morfologii nezvyšuje pravděpodobnost přežití pod predačním tlakem. Warkentin (2000, 2007) naopak zjistila, že určité změny v načasování líhnutí a jisté změny v morfologii mohou u pulců napomáhat přežívání vůči predaci.



Obrázek 3: Vliv přítomnosti predátora (detekce pomocí chemických látek) na načasování líhnutí, metamorfózy a morfologie (Mitchell *et al.* 2017).

3.2. Antipredační chování

Obranné neboli antipredační chování obojživelníků může mít několik forem. Nejobvyklejší obrannou strategií mobilních zvířat je utéct co nejrychleji od svého potenciálního predátora, ale mnoho druhů také využívá pasivní obrany (Toledo *et al.* 2011). Existuje však široká škála obranných strategií, které se alternativně používají ke zvládnutí rizika představovaného predátorem.

Různé obranné strategie působí v různých fázích predace: lokalizace, identifikace, odhalení, požití a trávení. Primární (nezávisí na přítomnosti predátora) a sekundární (vyvolané přítomností predátora) obranné strategie se vyvinuly, aby zabraňovaly nebo zamezily útoku predátorů (Van Buskirk 2004). Krypticky zbarvení obojživelníci se vyhnou detekci a jedovatí pozření. Následkem toho je vývoj obranných strategií přímo spojen se smysly, které predátoři používají k nalezení své kořisti (Greenbaum 2004).

Pulci se tak svou zranitelnost snaží snižovat pozměňováním svého fenotypu, například vznikem kryptického zbarvení (Caldwell 1982), růstem do větší velikosti těla (Persson 1988, Wilbur 1988, Moore *et* Townsend 1998), vlastní nechutností (Formanowicz *et* Brodie 1982, Brodie *et* Formanowicz, 1987), vývinem kožních jedových žláz - zpravidla mají svou jedovatost spojenou s aposematickým zbarvením (Crossland 1998) nebo nejrůznějšími modifikacemi ocasní ploutve, jako její zvýšená pigmentace upoutávající predátorovu pozornost od důležitějších částí těla (Van Buskirk *et al.* 2003, McIntyre *et al.* 2004), nebo efektivnějším uzpůsobením svých proporcí pro lepší únik před predátorem (McIntyre *et al.* 2004, Teplitsky *et al.* 2005).

Zvláště u tropických žab je poměrně často využívaný pasivní způsob obrany, kdy příslušný druh spoléhá na svou nenápadnost danou především kryptickým zbarvením, popř. i tvarem těla a kdy při setkání s predátorem setrvává v nehybné pozici (např. Bufonidae, Hylidae, Odontophrynidae, Megophryidae) (Jablonski 2014).

3.2.1. Vývojová a fenotypová plasticita

Dalším způsobem reakce na přítomnost predátora je fenotypová plasticita. Fenotypová plasticita je schopnost organismu reagovat na vnitřní nebo vnější podněty změnou fenotypu (West-Eberhard 2003), přesněji řečeno schopnost jednoho genotypu produkovat více alternativních fenotypů v závislosti na životním prostředí. V takové situaci může docházet ke změnám morfologie (McCollum *et* Van Buskirk 1996, Van Buskirk *et* Schmidt 2000), rychlosti vývoje, pohybu, chování (Stearns 1989, Skelly 1997, Nylin *et* Gotthard 1998, West-Eberhard 2003) nebo načasování bodů životní historie (Wilbur *et* Fauth 1990, Sih *et* Moore 1993, Warkentin 1995). Fenotypová plasticita může jedinci přinášet řadu výhod. Například plasticita v chování a vývoji může vybalancovat požadavky na příjem potravy a vyhýbání se predátorovi. V důsledku toho vede plasticita k vyššímu fitness u organismů, jejichž velikost v dospělosti ovlivňuje schopnost rozmnožování (Laurila *et al.* 2004).

Organismy obývající prostředí s proměnlivým, ale předvídatelným složením predátorů, si často vyvíjejí alternativní fenotypy, které zvyšují jejich přežití (Tollrian *et* Harvell 1999).

U obojživelníků je jedním z nejdůležitějších faktorů životního cyklu trvání larválního období - načasování líhnutí (Sih *et* Moore 1993, Warkentin 1995) a metamorfózy (Wilbur *et* Fauth 1990, Hensley 1993). Toto načasování je ovlivněno nejen geneticky, ale také environmentálními faktory. V konečném důsledku je vyjádřeno prostřednictvím změn v činnosti několika žláz s vnitřní sekrecí (Wilbur *et* Collins 1973, Laurila *et* Kujasalo 1999). Druhy žijící v lokalitách, které jsou stálé a předvídatelné, mají obecně delší larvální období, zatímco druhy, které obývají prostředí s nepředvídatelnými a měnícími se podmínkami, mají toto období kratší a mají tedy rychlejší růst a vývoj. Plasticita například umožňuje druhům chovaným v pouštních podmínkách individuální prodloužení larválního období a tím dosažení maximální velikosti potřebné pro metamorfózu (Stephens *et al.* 2017).

Limitující změna plasticity může vzniknout, pokud fenotypový vývoj je časově zpožděný nebo došlo k nepředvídatelné změně prostředí. To může snížit míru fenotypové odpovědi. Čas potřebný k vytvoření fixace fenotypových znaků je omezen nebo může dojít k nesouladu mezi fenotypem a prostředím (Van Buskirk 2002). Několik modelů ukazuje, že časové rozmezí reakce na změnu prostředí může ovlivnit rozsah plasticity (Clark *et* Harvell 1992, Moran 1992, Padilla *et* Adolph 1996, Gabriel 1999). Plasticitu způsobují vlastnosti, které lze rychle upravit během změn okolního prostředí. U takových vlastností je fenotypová odezva v úzkém vztahu se signály stavu prostředí a také zvyšuje pravděpodobnost, že fenotyp bude vhodný pro podmínky, ve kterých živočichové žijí (West-Eberhard 1989).

Denver (1997) se zabýval fenotypovou plasticitou pulců obojživelníků. Zaměřoval se zejména na biochemické a fyziologické mechanismy, které zprostředkovávají reakci organismu na podmínky prostředí. Různé kombinace biotických a abiotických faktorů ovlivňují nejen vývoj obojživelníků, ale také jejich růst (Wilbur 1980). Mezi biotické faktory patří dostupnost živin, vnitrodruhové vztahy, konkurence, kvalita potravy, ale také predace. Abiotické faktory působící na obojživelníky jsou zejména teplota, fotoperioda a množství vody (Wilbur *et* Collins 1973, Smith-Gill *et* Berven 1979, Pandian *et* Marian 1985, Alford *et* Harris 1988, Ruthsatz *et al.* 2019).

Účinky faktorů životního prostředí se mohou lišit v závislosti na konkrétní fázi růstu a vývoje organismu. Stejný faktor může působit inhibičně na růst organismu, pokud se nachází v larválním období, nebo může stimulovat rozvoj, pokud je přítomen v průběhu prometamorfózy - fáze růstu, kdy se začíná tvořit štítná žláza (Denver 1997, Ruthsatz *et al.* 2019).

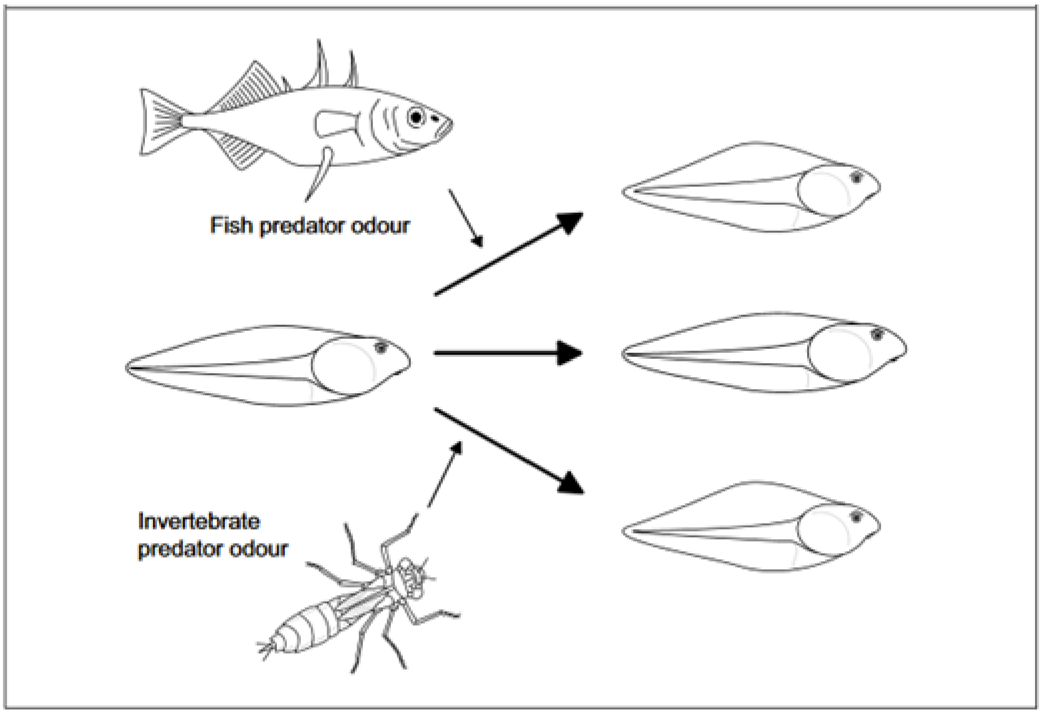
Výzkumy dokázaly, že obojživelníci žijící v nepředvídatelných podmínkách dokáží urychlit průběh metamorfózy v důsledku vysychání vody v rybníce, zatímco druhy žijící ve stálých podmínkách tuto schopnost neovládají. *Bufo americanus* chovaný v dočasných rybnících byl schopen urychlit metamorfózu v reakci na vysoušení rybníka. Oproti tomu *Rana utricularia*, který se vyskytuje v trvalých vodních tůních, nebyl schopen metamorfózu při vysychání vody urychlit. Pravděpodobně se tato vlastnost vyvinula během ontogeneze v závislosti na přírodním výběru (Newman 1992). Další podmínka, která může vyvolat fenotypovou plasticitu u obojživelníků, je teplota vody. Teplota prostředí pozitivně koreluje s rychlostí metamorfózy a růstu. Newman (1989) zjistil, že teplota mělkých rybníků byla při vysychání zvýšena o několik stupňů oproti hlubšímu rybníku. Pokud je tedy rychlejší metamorfóza vyvolána vysycháním rybníka, je tedy poháněna i zvýšenou teplotou rybníka. Další studie provedená v Izraeli (Blaustein *et al*. 1999) uváděla, že sluneční záření, které ovlivňuje teplotu u periodických vod, silně ovlivňovalo tempo růstu pulců *Hyla savignyi*.

3.2.1.1. Morfologické změny

Jedním z příkladů antipredační strategie v přítomnosti predátora je vznik morfologické obrany (Mikolajewski *et* Johansson 2004). Predace může zvýhodnit jedince kořisti s odlišnou morfologií nebo chováním a tím zvýšit variabilitu znaků v její populaci. Vystavení predátorovi na pouhé 3-18 dní zahájí u pulců morfologické změny, které se liší v závislosti na druhu predátora. Vystavení bezobratlým predátorům má za následek pozdější vylíhnutí z vajíček (Ireland *et al*. 2007), snížení růstu a zkrácení velikosti těla pro zvýšení únikové rychlosti (Wilson *et* Lefort 1993, Relyea 2001, Maher *et al*. 2013). Ryby mají podobné účinky na morfologické změny, ale pulci navíc více rozvíjejí svaly ocasu (Relyea 2001, Teplitsky *et al*. 2005).

Van Buskirk a McCollum (2000) také zkoumali vliv tvaru ocasu pulců na jejich schopnost pohybu. Za tímto účelem chirurgickým zákrokem zredukovali délku ocasu o 21 %, 34 % a 55 %. Výšku ocasu zredukovali o 11 %, 34 % a 59 %. Pokles maximální rychlosti a zvýšení doby potřebné k uplavání vzdálenosti 2,5 cm došlo u nejvíce redukovaných ocasů, ať už u jeho délky nebo výšky. V případech redukce ocasu v menší míře (11-34 %) nebyl prokázán významný vliv na pohybové vlastnosti. Trajektorie a úhel útěku nevykazoval závislost k žádné míře redukce ocasu. Morfologické změny vyvolané vlivem predačního tlaku vedoucí ke zvětšení výšky ocasu (Van Buskirk a Relyea 1998) nezvyšují pohybové vlastnosti pulců, proto se předpokládá, že mají význam v jiném obranném mechanismu (Laurila *et al*. 2001, Relyea 2001).

Výsledky studie Van Buskirka (2002) ukazují, že pulci reagovali na zvyšující se riziko predace tím, že snižovali svou aktivitu a viditelnost, snižovali délku a šířku těla, délku ocasu, ale šířka ocasu se zvyšovala. Tato změna v morfologii zvyšuje šanci na přežití pulce, možnost rychleji se vyhnout útoku predátora a opustit místa jeho pravděpodobného výskytu. Větší ocasní ploutev pak může být užitečná i v predátorově nepřítomnosti, protože zvyšuje pohybové schopnosti larev, a tak i například vyšší možnost příjmu potravy (Van Buskirk 2000, 2002).



Obrázek 4: Změny v morfologii pulců vlivem působení predátorů. Bezobratlý predátoři (*Anax* spp.) indukují menší tělo s relativně větším a vyšším ocasem, které zvyšují rychlost úniku (Maher *et al*. 2013), ryby indukují větší ocasní svaly, menší velikost těla a širší ocasy (Teplitsky *et al*. 2005, Mitchell *et al*. 2017).

3.2.1.2. Růstové strategie

Obecně existují dvě možné strategie, jak uniknout riziku predátora. Minimalizovat kontakt s predátorem zkrácením doby vývoje nebo rychlým růstem, aby měla kořist větší než ideální velikost pro přítomného predátora, avšak přítomnost neznámého predátora zvyšuje riziko pro larvy a tím neúčinnost velikostní strategie. Většina predátorů bere velikost kořisti v omezeném rozsahu, takže některé larvy obojživelníků jsou nakonec příliš velké na to, aby je bylo možné pozřít (Heyer *et al.* 1975, Caldwell *et al.* 1980, Brodie *et* Formanowicz 1983, Crump 1984, Alford 1986, Cronin *et* Travis 1986, Formanowicz 1986, Semlitsch *et* Gibbons 1988). Přestože se vliv velikosti pulců liší podle typu predátora a velikosti predátora, pulci se s růstem obecně stávají méně zranitelnými (Alford *et* Richards 1999). Zdá se, že to vyplývá z větší schopnosti velkých pulců uniknout predátorům.

Obecně platí, že rychlost plavání pulce roste s rostoucí velikostí (Huey 1980, Richards *et* Bull 1990), i když lokomoční výkonnost může klesat, když pulec dosáhne metamorfózy (Brown *et* Taylor 1995). Někteří predátoři jsou v zacházení s velkými pulci zkušenější než jiní a většina druhů pravděpodobně nikdy nedosáhne velikosti, která je činí zcela nezranitelnými vůči útoku. Někteří vodní a suchozemští predátoři, jako jsou ptáci, mohou být ve skutečnosti úspěšnější při chytání velkých pulců než malých (Crump *et* Vaira 1991, Tejedo 1993). Nicméně, predace je pravděpodobně důležitou selektivní silou působící na rychlost růstu larválních obojživelníků (Travis 1984, Semlitsch *et* Gibbons 1988). Travis (1983) prokázal genetické variace v rychlosti růstu larev, přičemž vysoké rychlosti korelovaly s vyšším přežitím. Wilbur a Collins (1973) předpokládali, že každý druh má charakteristickou minimální velikost, které musí být dosaženo, než může dojít k metamorfóze, a maximální velikost, kterou nelze v larválním stádiu překročit.

Pulci jsou při predaci nuceni urychlit svůj růst v důsledku velikostně selektivní predace, aby se rychleji dostali do méně zranitelných stádií (Travise 1984). Větší velikost pulce umožnila uniknout predačnímu tlaku nejvíce studovaných velikostně selektivních bezobratlých predátorů (*Notonecta, Anax* a larvy brouků). Na druhou stranu, *Trachemys scripta* může představovat druhý, velikostně neselektivní typ predátora, u kterého strategie většího těla není adaptivní.

4. Cíle disertační práce

Cílem této práce bylo experimentálně ověřit, zda přítomnost invazní želvy *Trachemys scripta elegans* ovlivňuje:

1) embryonální vývoj *Rana temporaria*, dobu líhnutí a vývojové stádium, ve kterém se embrya líhnou;

2) parametry ontogenetického vývoje (velikost při metamorfóze, délku larválního vývoje) larválního stadia *Rana temporaria* při kontinuální přítomnosti želvy;

3) dynamiku ontogeneze (velikost a hmotnost při metamorfóze, délku larválního vývoje) pulců *Rana temporaria* v případě přítomnosti želvy v různých etapách jeho vývoje.

5. Vliv invazní želvy nádherné na vývoj pulců skokana hnědého v různých etapách jeho vývoje

Přítomnost predátorů představuje jednu z nejvýznamnějších výzev pro úspěšnou reprodukci obojživelníků. Pro velkou většinu žab vyžaduje stanoviště pro vajíčka nebo pulce určitou formu stojaté vody (Wells *et* Schwartz 2007) a výběr vhodného místa k rozmnožování bude pravděpodobně zvláště důležitý, protože většina těchto druhů neposkytuje žádnou rodičovskou péči, embrya a larvy jsou tedy ohrožené predací až do metamorfózy (Binckley *et* Resetarits 2003). Vodní prostředí představuje ideální médium pro rozptýlení rozpustných chemických sloučenin (Wisenden 2000), které jsou charakteristické pro predátory a mohou kořisti poskytnout důležité informace k bezprostřednímu riziku predace (Bronmark *et* Hansson 2000).

Predátoři mohou způsobit nejen přímou úmrtnost mláďat, ale mohou také vyvolat morfologické, behaviorální a imunologické změny larev, které mohou ovlivnit přežití v postmetamorfních stádiích (Wilbur 1997, Relyea 2007, Groner *et al.* 2013). Konkurenti mohou nepřímo ovlivnit přežití larev a dobu larválního vývoje a velikost při metamorfóze (Wilbur 1987) a mohou způsobit škodlivé přenosové efekty v pozdějších fázích života (Wilbur 1997, Van Allen *et al.* 2010, Groner *et al.* 2013).

Aby došlo k rychlé evoluci, musí být selekční tlak vyvolaný nepůvodním predátorem silný a konzistentní a původní populace musí být geneticky variabilní, pokud jde o náchylnost k predátorovi (Strauss *et al.* 2006). Některá kořist je však schopna vrozeně detekovat a vyvolat adaptivní reakce na podněty nových predátorů i přes absenci společné evoluční historie (Epp *et* Gabor 2008, Peluc *et al.* 2008, Rehage *et al.* 2009, Nunes *et al.* 2014). Tato schopnost může být důsledkem fylogenetické nebo fenotypové podobnosti s původními predátory, kteří se vyskytují společně s kořistí (Epp *et* Gabor 2008, Rehage *et al.* 2009).

Předmětem této práce je želva *Trachemys scripta elegans*, která představuje v Evropě nový druh predátora s negativním vlivem na místní faunu. Ve svém původním areálu je *Trachemys scripta* oportunní všežravec. Všechny velikostní třídy želv konzumují významný podíl živočišné hmoty, což je činí potenciálně nebezpečnými pro původní druhy (TTWG 2017, GISD 2022). Tento druh želvy je v současné době celosvětově nejrozšířenější sladkovodní želvou, protože byl široce introdukován ať už záměrně nebo jako vedlejší efekt obchodu s exotickými zvířaty, a to po celém světě s výjimkou Antarktidy (Kraus 2009, Ficetola *et al*. 2012). Invazivní potenciál a oportunní strava želvy nádherné mají významný dopad na původní obojživelníky (Ernst *et* Lovich 2009).

Pulci dvou testovaných druhů žab *Rana nigromaculata* a *R. limnocharis* byli schopni reagovat na původní želvu Reevesovu (*Chinemys reevesii*), ale ne na invazní želvu *Platysternon megacephalum* (Zhang *et al*. 2015). Podobně v jedné z pouhých dvou předešlých studií (Berec *at el*. 2016, Polo-Cavia *et al*. 2010), ve kterých byla *Trachemys scripta* konkrétně použita jako potenciální invazní predátor, v jedné zjistili neschopnost rozpoznat nepůvodní želvy jako predátora u čtyř druhů žab *Bufo calamita*, *Hyla arborea*, *Pelobates cultripes* a *Pelophylax perezi* (Polo-Cavia *et al*. 2010). Naproti tomu druhá studie potvrdila stejné výsledky jako v mé práci, že pulci *Rana temporaria* reagují na invazní želvu *Trachemys scripta*, bez předchozí expozice nebo kontaktu a jsou schopni vrozeně rozpoznat a reagovat na chemické podněty želvy přenášené vodou, konkrétně změnou jejich plaveckého chování (téměř o 50 %) (Berec *et al*. 2016). Očekává se, že tento typ antipredačního chování bude nést náklady ve smyslu kratších časových příležitostí k hledání potravy. Zkoumala jsem, zda původní pulci *Rana temporaria* reagují adaptivně na přítomnost želvích kairomonů.

5.1. Přítomnost nepůvodní želvy urychluje líhnutí pulců skokanů hnědých (*Rana temporaria*)

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Q1 (Animal Science and Zoology; Aquatic Science; Ecology; Ecology, Evolution, Behavior and Systematics)

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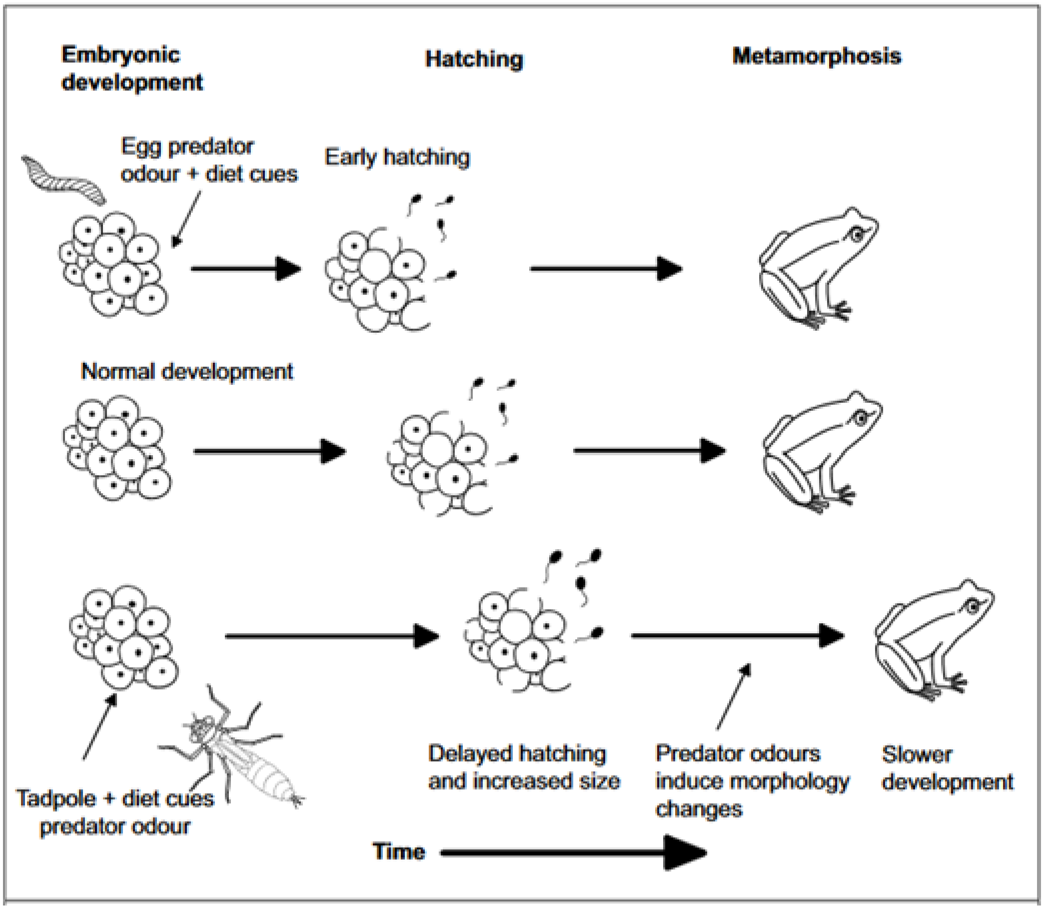
Žáby jsou schopny rozpoznat přítomnost predátorů nebo konkurentů v místě rozmnožování a podle toho se rozhodnout, zda vejce nebo pulce nakladou nebo nekladou (Buxton *et al*. 2016). Toto rozhodnutí předpokládá minimálně současný výskyt predátora a reprodukčního chování žab nebo předchozí výskyt predátora na lokalitě. Takové rozhodování však není možné, pokud uložení vajíček nebo pulců předchází přítomnost predátora na lokalitě. Jako první obojživelníci se na jaře začínají rozmnožovat hnědě zbarvení skokani (rod *Rana*) (Speybroeck *et al.* 2016). V té době mnoho jejich potenciálních predátorů stále hibernuje, a tak dochází ke kontaktu s potenciálními predátory až po nakladení vajíček nebo během vývoje larev.

Změny v načasování líhnutí v důsledku přítomnosti predátorů mohou vést k morfologickým rozdílům mezi vylíhnutými mláďaty (Buckley *et al.* 2005, Touchon *et* Warkentin 2008). Kratší načasování líhnutí může naznačovat menší velikost při líhnutí a v nižších vývojových fázích (Sih *et* Moore 1993, Moore *et al.* 1996, Warkentin 1999, Benard 2004). Odložení líhnutí může larvám umožnit dosáhnout větší tělesné velikosti a pokročilejšího vývojového stadia a zvýšit tak pravděpodobnost jejich přežití pod rizikem predace (Sih *et* Moore 1993, Moore *et al.* 1996). V přítomnosti predátorů specifických pro stádium mohou obojživelníci modifikovat trvání příslušné vývojové fáze (Chivers *et al.* 2001, Ireland *et al.* 2007, Mitchell *et al.* 2017). Konkrétně u žabích embryí bylo většinou prokázáno, že přítomnost predátorů vajíček vyvolává časné líhnutí embryí (Warkentin 1995, 2000, Chivers *et al.* 2001, Laurila *et al.* 2001, Johnson *et al.* 2003, Segev *et al.* 2015), zatímco predátoři pulců vyvolávají opožděné líhnutí (Sih *et* Moore 1993, Laurila *et al.* 2002, Schalk *et al.* 2002, Mitchell *et al.* 2017), čímž zvyšují svou šanci na přežití tím, že unikají možným útokům (Obrázek 5). Laurila a kol. (2002) zjistili, že se později vylíhla embrya *Rana temporaria* vystavená predaci koljušky tříostné (*Gasterosteus aculeatus*), která je predátorem larev.

Studie zkoumající účinky predátorů na rychlost vývoje vajíček i larev jsou vzácné, protože jen málo predátorů konzumuje vajíčka i larvy současně. Zajímavé je, že současná expozice predátorů vajec a predátorů pulců (pijavice a nymfy vážek) nezpůsobila žádnou změnu v načasování líhnutí (Ireland *et al.* 2007). *Trachemys scripta* však není predátorem specifickým pro stádium, protože je schopna konzumovat vajíčka i larvy obojživelníků (Storm *et al.* 1995, Chen 2006, Ernst *et* Lovich 2009). V mé studii přítomnost nepůvodního predátora představuje stimul, při kterém embrya zkracují dobu líhnutí, snižují velikost při líhnutí a líhnou se v nižší vývojové fázi. To je v souladu s Mandrillon a Saglio (2007) a Marquis a kol. (2004), kteří uvádí, že expozice vyhladovělých predátorů ovlivnila behaviorální reakci u ropuchy obecné (*Bufo bufo*) a snížila plavecké chování u dvou druhů žab (*Rana temporaria*, *Bufo bufo*). Capellán a Nicieza (2007) uvedli stejný výsledek pro *Rana temporaria* s predátorem vajec - pijavicí *Haemopsis sanguisuga*.



Obrázek 5: Vliv přítomnosti predátora (detekce pomocí chemických látek) na načasování líhnutí, metamorfózy a morfologie (Mitchell *et al*. 2017).

Pouhá dvoudenní (30%) změna v načasování líhnutí listovnice červenooké (*Agalychnis callidryas*) ovlivnila životaschopnost larev a metamorfní fenotyp (Touchon *et al*. 2013). Vonesh a Bolker (2005) zjistili, že 30% snížení velikosti vylíhnutých žab *Hyperolius spinigularis* výrazně změnilo míru úmrtnosti larev (o 90 %) a po vylíhnutí došlo k malému (tj. <10%) přírůstku velikosti vylíhnutých larev, což odpovídá výrazným změnám v přežití. Snížená velikost vylíhnutých mláďat může tedy mít za následek vyšší mortalitu larev v prostředích s intenzivní predací, protože malé larvy jsou vůči predátorům zranitelnější (Nicieza *et al*. 2006). Navíc jediný predátor může zlikvidovat celou snůšku vajec, což má za následek každoroční selhání reprodukce jedince (Grözinger *et al*. 2012).

V této studii bylo cílem zjistit, zda se doba líhnutí, vývojové stádium a velikost těla při vylíhnutí embryí *Rana temporaria* změní v přítomnosti invazních želv *Trachemys scripta elegans* jako predátora. Přítomnost predátora ovlivnila všechny zkoumané faktory. Zjistila jsem, že při absenci želvy se embrya vylíhla za 12 dní, zatímco za přítomnosti predátora bylo líhnutí urychleno o dva dny. Embrya se se navíc vylíhla menší a v nižším stupni vývoje než bez přítomnosti predátora.

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Presence of an alien turtle accelerates hatching of common frog (*Rana temporaria*) tadpoles

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Abstract

The presence of a predator affects prey populations either by direct predation or by modifying various parts of their life history. We investigated whether the hatching time, developmental stage, and body size at hatching of common frog (*Rana temporaria*) embryos would alter in the presence of a red-eared slider (*Trachemys scripta elegans*) as a predator. The presence of a predator affected all factors examined. We found that in the absence of the slider, the embryos hatched in 12 days, while hatching was accelerated by two days in slider treatment. At the same time, the embryos hatched smaller and at a lower stage of development with the slider than without it. Our study extends the range of predators studied, including the effect on different phases of development of potential amphibian prey.

Keywords

Antipredator defence, embryonic development, invasive predator, predator-cued hatching, predation risk, *Rana temporaria*, *Trachemys scripta elegans*

Introduction

The impacts of invasive species on native communities are still difficult to generalise due to the limited number of species and environments researched (Rolim et al. 2015; Tricarico et al. 2016; Griesemer et al. 2018; Ramírez-Albores et al. 2019). However, inappropriate responses of individuals to invasive predators can strongly affect native populations (Mooney and Cleland 2001). In amphibians, predation can account for a significant proportion of the total mortality of all their developmental stages (Nyström et al. 1997; Chivers et al. 2001; Laurila et al. 2002; Gunzburger and Travis 2005). The ability to detect, recognise, and respond to potential predators is, therefore, an important part of antipredatory behaviour (Bennett et al. 2013; Polo-Cavia and Gomez-Mestre 2014), and native populations can have especially serious problems facing the presence of new alien predators (Polo-Cavia et al. 2010; Gomez-Mestre and Díaz-Paniagua 2011; Nunes et al. 2019). In general, embryonic and early larval stages are the most vulnerable to predation (Laurila et al. 2002; Wells 2007), and the ability to respond to the presence of a predator can therefore significantly increase the fitness of an individual and thus the viability of the entire population (Warkentin 1995; Vonesh and Bolker 2005).

Whether intentionally or unintentionally introduced, the recent wide occurrence of the red-eared slider (*Trachemys scripta elegans*) in Europe (GISD 2021) presents a new opportunity to investigate the responses of naive native amphibian populations to a new predator. Although the red-eared slider (hereafter referred to as slider) is not reproductively successful throughout Europe (Cadi et al. 2004; Ficetola et al. 2009; Mikátová and Šandera 2015; Standfuss et al. 2016), even the mere presence of adults may pose a certain risk to native species. In previous studies, we found that the presence of the sliders affects several life history parameters of common frog (*Rana temporaria*) tadpoles, such as movement activity, trajectory of movement (Berec et al. 2016), time to metamorphosis, or size at metamorphosis (Vodrážková et al. 2020). Although sliders are usually still hibernating at the time of common frog breeding (Gibbons et al. 1990; Speybroeck et al. 2016), which eliminates the risk of direct predation, chemical cues (kairomones) released by sliders into the aquatic environment provide amphibians with information about their presence. Since the slider is an opportunistic predator and can consume frog eggs (Ernst and Lovich 2009), some response of common frog embryos is to be expected.

For frog embryos, there are two basic strategies for avoiding predation or significantly reducing its effects: the development of egg unpalatability and hatching plasticity (Wells 2007). The unpalatability of eggs is a passive strategy in which the embryo relies on the predator's inability or unwillingness to consume eggs, which imposes costs on its host even if the host never comes in contact with the predator; environmentally cued hatching is characterised by an embryo's active capability to alter the time of hatching according to the conditions it encounters during embryonic development. Hatching plasticity has been documented many times in amphibian embryos, and predator presence has been shown to trigger early hatching from eggs incubated in both air and water (Chivers et al. 2001; Warkentin 2011). In terrestrially laid eggs, hatching can be stimulated by vibrational cues during the direct physical attacks of predators, such as snakes (Warkentin 1995; Jung et al. 2019), frogs (Vonesh and Bolker 2005), katydids (Poo and Bickford 2014), wasps (Warkentin 2000), or egg-eating fly larvae (Vonesh and Bolker 2005). In aquatic environments, these responses are induced mainly by chemical cues from predators (kairomones) or by chemical cues that are released from injured prey during predation events (Petranka et al. 1987; Dodson 1988; Tollrian 1994; Nicieza 1999, 2000; Laurila et al. 2002; Smith and Fortune 2009).

This study aimed to shift our previous focus (Berec et al. 2016; Vodrážková et al. 2020, 2022) to a different developmental stage, namely, embryos in eggs. We investigated whether the presence of a slider can alter the hatching time of common frog embryos. We hypothesised that the presence of a slider would accelerate the hatching time, so the ontogenetic stage and body size at hatching were also measured. The uniqueness of this study lies in the use of a stage-nonspecific predator, which is virtually absent in the literature. At the same time, it is an alien predator from a taxonomic group to which the prey has no common history.

Materials and methods

Five freshly laid clutches of common frogs were collected in a pool between Holubov and Vrábče, South Bohemia, the Czech Republic (48.9078633°N, 14.3485608°E), on 2 April 2021. Collection locality was monitored daily to collect egg clutches laid during the night before. Neither the slider nor any other species of turtle occurs at the collection locality, so the eggs and their parents are naive prey relative to the turtles. The experiment was performed in six glass tanks – three replications with the sliders and three replications of control without the sliders. Glass tanks (size: 100 × 55 × 50 cm) filled with 20 cm of aged tap water were equipped with a Claro 300 filter pump (300 $L.h^{-1}$) and rinsed three times a week. The room temperature was set at 15 °C and the datalogger (Dostman LOG200 PDF) recorded a mean air temperature of 14.8 ± 0.4 °C (± S.D.; measured at hourly intervals) during the experiment. Fluorescent tubes (2 × 36 W) with a light regime of 12 h/12 h were used. During the dark phase of the day, the glass tanks were illuminated with red light to allow permanent monitoring of egg hatching.

Three adult sliders (carapace length: 18 cm, 20 cm, and 21 cm) were used as predators. A slider was placed in each of three glass tanks over the course of three days to release kairomones into the water before the experiment was initiated and fed three times a week with ReptoMin Tetra turtle gammarus. To prevent physical but not chemical contact between the slider and frog eggs, a glass barrier was placed inside each glass tank with a 6 cm gap at both ends so that water could flow freely throughout the tank. On the other side of this barrier, five perforated opaque boxes (20 × 14 cm) with holes 1 mm in diameter were glued to the bottom of the glass tanks to contain the eggs (Fig. 1).

Six fragments of approximately 150 eggs each were taken from the collected clutch and randomly placed in five boxes, one in each glass tank. This procedure was repeated for all five clutches, so that there were five boxes in each tank with a fragment from each clutch. Each glass tank was continuously monitored using a camera (Niceboy Stream Pro) to distinguish hatched tadpoles occasionally returned to the inside of the egg capsules from tadpoles just before hatching. Hatched tadpoles were counted every 24 h. Hatching was defined as the moment at which the whole hatchling had left the protective jelly of the eggs. To maintain a good processing of the camera recordings

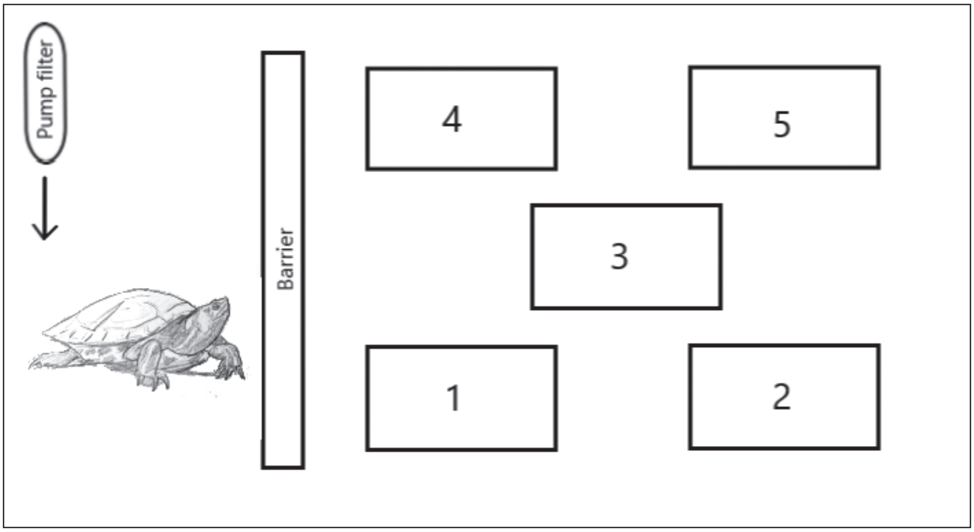

Figure 1. Diagram of the glass tank showing the position of the slider (if present) and the boxes for clutch fragments. These were placed randomly in the boxes in each glass tank (see Materials and methods). Three replications with the sliders and three replications without them (control) were used. Slider drawing by Jakub Berec.

(the large number of hatched tadpoles in a small box makes it difficult to count them), hatched tadpoles were transferred every six hours to a depot tank. At the time when half of the eggs in each box had hatched, two tadpoles were taken from the group of tadpoles hatched in the last six hours. These tadpoles were photographed under a stereomicroscope (Olympus SZX 7) and measured (to the nearest 0.01 mm) using QuickPHOTO MICRO 3.2 software. Their developmental phase was determined according to Gosner (1960).

Three different dependent variables connected with different aspects of the hatching of common frog tadpoles with/without the presence of a slider were measured as described above: hatching time, developmental stage, and size at hatching. The potential impact of four independent variables – (1) slider: presence/absence, (2) glass tank: three tanks with slider and three controls without slider, (3) box: five boxes in each glass tank at the same position within the glass tank, and (4) clutch: six fragments of each clutch – on each of the dependent variables was then analysed. Thus, three separate analyses were performed to fulfil the aim of the present study. According to the experimental design, a linear main effect ANOVA model from the general linear model family (GLM) was used for analysis of experimental data (Quinn and Keough 2002). The factor 'slider' was set in all three analyses as a fixed factor, as both levels of this factor (presence/absence) were of direct interest to our study and are not interdependent. The other three factors (glass tank, clutch, and box) were set as random factors (Allen 2017). The overall fit of all parameters in the GLM was tested using a test of the sum of squares of whole model versus sum of squares of residuals. An overparameterized model based on the indicator variable approach (Midway 2019) was used in type III sums of squares test (TIBCO 2017) to represent the effects of all four independent variables (factors) on the dependent variable. Adjusted R-squared was used in the overall fit of all parameters as a measure of the variability explained by the GLM because more than one independent variable was used in the model. The effect sizes of all four partial factors were evaluated using partial eta-squared (Richardson 2011). Given the number of eggs, the statistical significance was assessed at the 99.9% level (Steel et al. 2013). All calculations were performed in Tibco Statistica (TIBCO 2017).

Results

The GLM for hatching time with the effects of all factors analysed (overall fit of all parameters) was statistically significant (all effect: $F_{11,2988} = 809.2$, $p<<0.001$; adjusted $R^2 = 0.75$). In the partial effects analysis, we found a significant difference in hatching time between the presence and absence of the slider ($F_{1,2988} = 8672.4$; $p<<0.001$). The effect of this factor on hatching time (partial eta-squared 0.74) prevailed over the effect of the other factors. In the absence of the slider, the embryos hatched in 12 ± 0.6 days (mean ± S.D.). The presence of the slider accelerated hatching by two days (10 ± 0.6 days) (Fig. 2A). The partial effects of the random factors were also significant: glass tank ($F_{2,2988} = 11.7$; $p < 0.001$), box ($F_{4,2988} = 7.6$; $p < 0.001$), and clutch ($F_{4,2988} = 44.1$; $p<<0.001$). However, compared to the effect of slider presence, the effect sizes of these factors were negligible (partial eta-squared for clutch: 0.06, glass tank: 0.01, and box: 0.01) (Suppl. material 3: Table S1).

The GLM for developmental stage of all the factors analysed (overall fit of all parameters) was statistically significant (all effect: $F_{11,48} = 33.2$, $p<<0.001$; adjusted $R^2 = 0.86$). In the analyses of partial effects, the presence of the slider was the only significant factor in the model ($F_{1,48} = 358.0$; $p<<0.01$). In the presence of a slider, embryos hatched at developmental stage 20 ± 1.5 (mean ± S.D.), while in control, freshly hatched embryos had developed to stage 23 ± 1.0 (Fig. 2B). The developmental stage was not significantly influenced by glass tank, clutch, and box (Suppl. material 3: Table S2).

Similarly to the previous life history parameters, the GLM for size at hatching of all factors analysed (overall fit of all parameters) was statistically significant (all effect: $F_{11,48} = 23.8$, $p<<0.001$; adjusted $R^2 = 0.81$). In the analyses of partial effects, the significant difference was found between the size of freshly hatched embryos in the presence of the slider and without it ($F_{1,48} = 245.3$; $p<<0.001$). In the presence of a slider, the embryos hatched with an average size of 5.92 ± 1.460 mm (mean ± S.D.), while in the control, the average size of the freshly hatched embryos was 10.77 ± 1.042 mm (Fig. 2C). As for developmental stage, the presence of the slider was the only significant factor in the model (Suppl. material 3: Table S3).

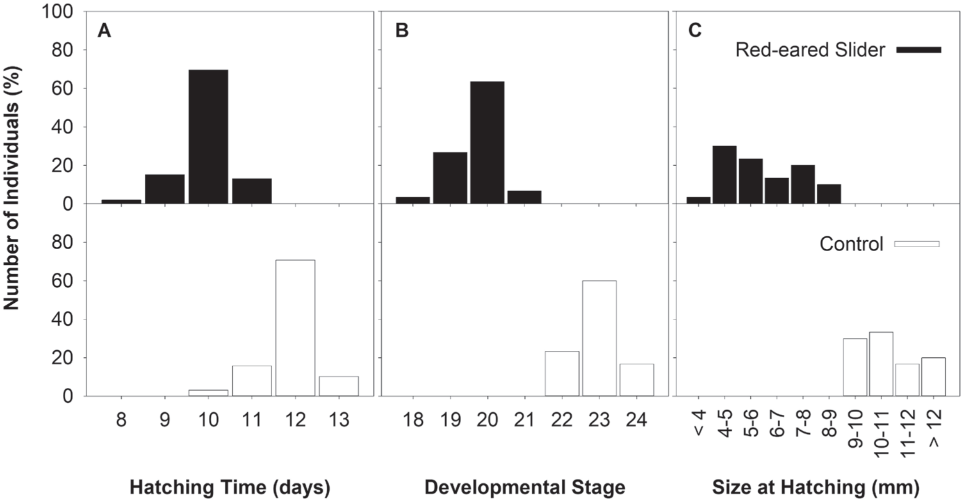

Figure 2. Histogram of **A** hatching time **B** developmental (Gosner) stage, and **C** size at hatching of the embryos of common frogs in the presence of red-eared slider and control.

Discussion

Developmental plasticity is an adaptive response of anuran embryos and larvae to the risk of predation (Altig and McDiarmid 1999; Benard 2004; Warkentin 2011). Here, we present evidence for the developmental plasticity of common frog embryos in the presence of a red-eared slider and, in addition to a previous study (Vodrážková et al. 2020), provide a comprehensive insight into the influence of this alien predator on the early phases of the common frog life cycle. We have previously shown (Vodrážková et al. 2020) that, in the slider presence, tadpoles of common frogs are capable to modify the duration of larval development. In the present study, we confirmed a similar response in common frog embryos, which hatched earlier in the presence of a slider. At the same time, the embryos were smaller and less developed when exposed to the chemical signals of a predator.

In the presence of stage-specific predators, amphibians can modify the duration of the relevant developmental stage (Chivers et al. 2001; Ireland et al. 2007; Mitchell et al. 2017). In anuran embryos, specifically, the presence of egg predators has mostly been shown to induce early hatching of embryos (Warkentin 1995, 2000; Chivers et al. 2001; Laurila et al. 2001; Johnson et al. 2003; Segev et al. 2015), while tadpole predators induce delayed hatching (Sih and Moore 1993; Laurila et al. 2002; Schalk et al. 2002; Mitchell et al. 2017), thus increasing their chance of survival by escaping possible attacks. However, the slider is not a stage-specific predator, as it is capable of consuming both amphibian eggs and larvae (Brown et al. 1995; Chen 2006; Ernst and Lovich 2009); thus, the allocation of risk between developmental stages of the frog may be more complex in this case (Warkentin 2011). Studies that examine predator effects on the developmental rates of both eggs and larvae are rare because few predators consume both eggs and larvae simultaneously. Muraro et al. (2021) used a stage-nonspecific predator (*Procambarus clarkii*) and found, in concordance with our results, a reduction in hatching time in *Rana latastei* embryos. However, they did not study larval development. Ireland et al. (2007) solved the problem of predator stage specificity by simultaneously exposing frog eggs to stage-specific predators of eggs (leech: *Nephelopsis obscura*) and larvae (dragonfly: *Aeshna canadensis* nymphs), which resulted in no change in hatching time, while tests with separately acting predators produced the expected response of a reduction in hatching time in the egg predator treatment and an increase in hatching time in the larval predator treatment. This study on embryos and a previous study on tadpoles (Vodrážková et al. 2020) jointly clarify that the embryos/tadpoles of the common frog responded to the presence of a predator by shortening the stage of development during which the embryo/tadpole would be exposed to the predator. It would be interesting to analyse how common frog tadpoles react to the presence of a slider if the entire development from eggs to metamorphosis was taking place with this predator present.

However, some studies have shown that frog embryos, including the common frog, do not always respond specifically to stage-specific predators by shortening hatching time (Laurila et al. 2001, 2002; Schalk et al. 2002; Saglio and Mandrillon 2006; Touchon et al. 2006; Capellán and Nicieza 2010; Touchon and Wojdak 2014). The published differences in embryo responses may correspond to different signal intensities of the presence of a specific predator, and thus the responses to indirect waterborne cues might be weaker than those to the direct, mechanical cues of a predator attack (Warkentin 2011). An evident response to water-borne cues of sliders may be related to a markedly stronger signal of a much larger-sized predator in our experiment compared to commonly tested invertebrate predators. The ability to scale predator danger and adjust hatching time accordingly has been found, for example, in embryos of southern leopard frogs (*Lithobates sphenocephalus*) (Johnson et al. 2003). Moreover, a possible absence of a change in hatching time does not necessarily imply a complete lack of response to the presence of a predator. It may be manifested by other types of response, such as changes in the body shape of tadpoles (Laurila et al. 2001; Saglio and Mandrillon 2006; Mandrillon and Saglio 2007; Touchon and Wojdak 2014) or their behaviour (Saglio and Mandrillon 2006; Touchon and Wojdak 2014).

Native and naive prey can fail to detect the novel predator adequately as a dangerous threat, resulting in no (Cox and Lima 2006; Sih et al. 2010) or inefficient antipredator responses to counter the predator's attack strategies (Strauss et al. 2006; Sih et al. 2010). However, when responses in hatching time in naive prey are detected, they are often explained by the presence of syntopic, taxonomically related predators (Sih et al. 2010; Melotto et al. 2021; Muraro et al. 2021), although the time since invasion may also play an important role (Gomez-Mestre and Díaz-Paniagua 2011; Nunes et al. 2013). Our results suggested that a common evolutionary history is not necessary for a detectable response. Such a result has already been published for tadpole development time (Stav et al. 2007; Vodrážková et al. 2020), but as far as we know, it has not yet been published for the hatching time in frog embryos. An explanation for embryo response to an alien slider may be in the ability of embryos to detect a kind of general "smell of fear" that is elicited by most predators, regardless of taxonomic classification (Sih et al. 2010).

Finding a general tendency in the phenotypic plasticity responses of prey across a broad range of animal predators (different taxa and feeding spectra), environmental and experimental conditions is a challenge even in anurans themselves (Relyea et al. 2018). However, in frogs, the earlier hatching time was generally associated with smaller size at hatching (Chivers et al. 2001; Laurila et al. 2002; Capellán and Nicieza 2007; Ireland et al. 2007) and lower developmental stage (Chivers et al. 2001; Laurila et al. 2002; Capellán and Nicieza 2007; Ireland et al. 2007; Muraro et al. 2021), and our results confirm this relationship. In some cases, earlier hatched tadpoles performed higher growth rate and reached the size of later hatched tadpoles at metamorphosis (Capellán and Nicieza 2007). However, if tadpoles are unable to compensate for their smaller size at hatching, this can impose significant costs in later developmental phases. These costs have been demonstrated through increased mortality during the larval stage (Smith 1987; Warkentin 1995; but see Vonesh and Bolker (2005) where early hatchlings survived better), reduced size at metamorphosis (Vonesh and Bolker 2005; Vodrážková et al. 2020), lower post-metamorphic survival (Berven 1990; Altwegg and Reyer 2003), change of behaviour (Buckley et al. 2005; Capellán and Nicieza 2007), delayed maturity (Smith 1987) and lower reproductive success (Smith 1987).

The hatching time was also influenced by the clutch, glass tank, and box. The clutch effect can be explained by a possible difference in the age of the collected clutches. Although freshly laid clutches were always collected in the morning after the actual reproductive event, differences of several hours in the age of the clutches cannot be excluded. The box effect could be attributed to the different distances of each box from the pump filter and/or the slider compartment. We can rule out a temperature gradient in the experimental room as the most likely cause of the glass tank effect, as regular temperature measurements during the experiment did not detect one. Nevertheless, all partial eta-squared of clutch, glass tank, and box are an order of magnitude lower than partial eta-squared for slider presence/absence. This confirms the importance of the slider presence/absence on the hatching time. Moreover, the statistical significance of the above-mentioned random factors need not be functionally relevant.

Although the results are fairly straightforward, we are aware of certain limitations of our experiment. First, the five clutches used originated from a single pool. For this reason, the general validity of our results cannot be confirmed, as some studies also point to a genetic component of variability in some features of ontogenetic development (Lind et al. 2008; to our knowledge, the genetic component of hatching time variability in frogs has not yet been investigated). An additional potential statistical complication could be the placement of multiple boxes in a glass tank and multiple eggs (a fragment of clutch) in a box. Having individual eggs in individual tanks with their own maintenance system and with a separate water supply from the glass tank with or without a slider would prevent this issue. At the same time, it solves the problem of different box distances from the pump filter and/or the slider compartment. However, although this solution is technically feasible, division of the clutches into individual eggs remains problematic in terms of embryo survival.

Our work added a slider as an additional predator inducing changes in the embryonic developmental rate in Ranidae. Since the impact of earlier embryo hatching (lower body size and lower stage of development) on fitness has been confirmed in several frog species (Warkentin 1995; Laurila et al. 2002; Vonesh and Bolker 2005; Touchon et al. 2013), the same impact can be expected for the common frog. The existence of defensive responses in slider-exposed embryos may reduce the threat that the spreading of this invasive species poses in Europe. On the other hand, the reduced size at hatching and developmental stage of common frog hatchlings represents additional risks of negative fitness impacts, and at the very least, the presence of sliders in non-native areas should receive increased attention.

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All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Czech Ministry of Agriculture, Department of Animal Welfare according to article No. 15, section 2 of the act registered under number 9103/2009-17210.

References

Allen M (2017) The sage encyclopedia of communication research methods (Vols. 1–4). SAGE Publications, Inc., (Thousand Oaks, CA): 1–2064. https://doi.org/10.4135/9781483381411

Altig R, McDiarmid RW (1999) Tadpoles: The biology of anuran larvae. University of Chicago Press, (Chicago): 1–454.

Altwegg R, Reyer HU (2003) Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57(4): 872–882. https://doi.org/10.1111/j.0014-3820.2003.tb00298.x

Benard MF (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. Annual Review of Ecology, Evolution, and Systematics 35(1): 651–673. https://doi.org/10.1146/annurev.ecolsys.35.021004.112426

Bennett AM, Pereira D, Murray DL (2013) Investment into defensive traits by anuran prey (*Lithobates pipiens*) is mediated by the starvation-predation risk trade-off. PLoS ONE 8(12): e82344. https://doi.org/10.1371/journal.pone.0082344

Berec M, Klapka V, Zemek R (2016) Effect of an alien turtle predator on movement activity of European brown frog tadpoles. The Italian Journal of Zoology 83(1): 68–76. https://doi.org/10.1080/11250003.2016.1139195

Berven KA (1990) Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71(4): 1599–1608. https://doi.org/10.2307/1938295

Brown HA, Bury RB, Darda DM, Diller L, Peterson C, Storm R (1995) Reptiles of Washington and Oregon. Seattle Audubon Society, (Seattle, WA): 1–176.

Buckley CR, Michael SF, Irschick DJ (2005) Early hatching decreases jumping performance in a direct-developing frog, *Eleutherodactylus coqui*. Functional Ecology 19(1): 67–72. https://doi.org/10.1111/j.0269-8463.2005.00931.x

Cadi AVD, Prévot-Julliard AC, Joly P, Pieau C, Girondot M (2004) Successful reproduction of the introduced slider turtle (*Trachemys scripta elegans*) in the South of France. Aquatic Conservation: Marine and Freshwater Ecosvstems 14(3): 237–246. https://doi.org/10.1002/aqc.607

Capellán E, Nicieza AG (2007) Trade-offs across life stages: Does predator-induced hatching plasticity reduce anuran post-metamorphic performance? Evolutionary Ecology 21(4): 445–458. https://doi.org/10.1007/s10682-006-9133-9

Capellán E, Nicieza AG (2010) Constrained plasticity in switching across life stages: Pre-and post-switch predators elicit early hatching. Evolutionary Ecology 24(1): 49–57. https://doi.org/10.1007/s10682-008-9289-6

Chen T-H (2006) Distribution and status of the introduced red-eared slider (*Trachemys scripta elegans*) in Taiwan. In: Koike F, Cloud M, Kawamichi M, De Poorter M, Iwatsuki K (Eds) Assessment and control of biological invasion risks. Shoukadoh Book Sellers (Kyoto, Japan and IUCN, Gland, Switzerland): 187–195.

Chivers DP, Kiesecker JM, Marco A, Devito J, Anderson MT, Blaustein AR (2001) Predator-induced life history changes in amphibians: Egg predation induces hatching. Oikos 92(1): 135–142. https://doi.org/10.1034/j.1600-0706.2001.920116.x

Cox JG, Lima SL (2006) Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. Trends in Ecology & Evolution 21(12): 674–680. https://doi.org/10.1016/j.tree.2006.07.011

Dodson S (1988) The ecological role of chemical stimuli for the zooplankton: Predator-avoidance behavior in *Daphnia*. Limnology and Oceanography 33(6_part_2): 1431–1439. https://doi.org/10.4319/lo.1988.33.6_part_2.1431

Ernst CH, Lovich JE (2009) Turtles of the United States and Canada. JHU Press, (Baltimore): 1–840.

Ficetola GF, Thuiller W, Padoa-Schioppa E (2009) From introduction to the establishment of alien species: Bioclimatic differences between presence and reproduction localities in the slider turtle. Diversity & Distributions 15(1): 108–116. https://doi.org/10.1111/j.1472-4642.2008.00516.x

Gibbons J, Greene J, Congdon J (1990) Temporal and spatial movement patterns of sliders and other turtles. In: Gibbons J (Ed) Life history and ecology of the slider turtle. Smithsonian Institution Press (Washington, D.C.): 201–215.

GISD (2021). Global Invasive Species Database. http://www.issg.org/database [accessed 14/10/2021]

Gomez-Mestre I, Díaz-Paniagua C (2011) Invasive predatory crayfish do not trigger inducible defences in tadpoles. Proceedings of the Royal Society B: Biological Sciences 278(1723): 3364–3370. https://doi.org/10.1098/rspb.2010.2762

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183–190.

Griesemer J, Jeschke J, Heger T (2018) Mapping theoretical and evidential landscapes in ecological science: Levin's virtue trade-off and the hierarchy-of-hypotheses approach. In: Jeschke J, Heger T (Eds) Invasion biology: Hypotheses and evidence. CABI (Boston, MA): 23–29.

Gunzburger MS, Travis J (2005) Critical literature review of the evidence for unpalatability of amphibian eggs and larvae. Journal of Herpetology 39(4): 547–571. https://doi.org/10.1670/1-05A.1

Ireland D, Wirsing A, Murray D (2007) Phenotypically plastic responses of green frog embryos to conflicting predation risk. Oecologia 152(1): 162–168. https://doi.org/10.1007/s00442-006-0637-3

Johnson JB, Saenz D, Adams CK, Conner RN (2003) The influence of predator threat on the timing of a life-history switch point: Predator-induced hatching in the southern leopard frog (*Rana sphenocephala*). Canadian Journal of Zoology 81(9): 1608–1613. https://doi.org/10.1139/z03-148

Jung J, Kim SJ, Pérez Arias SM, McDaniel JG, Warkentin KM (2019) How do red-eyed treefrog embryos sense motion in predator attacks? Assessing the role of vestibular mechanoreception. The Journal of Experimental Biology 222: jeb206052. https://doi.org/10.1242/jeb.206052

Laurila A, Crochet P-A, Merilä J (2001) Predation-induced effects on hatchling morphology in the common frog (*Rana temporaria*). Canadian Journal of Zoology 79(5): 926–930. https://doi.org/10.1139/z01-045

Laurila A, Pakkasmaa S, Crochet P-A, Merilä J (2002) Predator-induced plasticity in early life history and morphology in two anuran amphibians. Oecologia 132(4): 524–530. https://doi.org/10.1007/s00442-002-0984-7

Lind MI, Persbo F, Johansson F (2008) Pool desiccation and developmental thresholds in the common frog, *Rana temporaria*. Proceedings of the Royal Society B: Biological Sciences 275(1638): 1073–1080. https://doi.org/10.1098/rspb.2007.1737

Mandrillon A-L, Saglio P (2007) Herbicide exposure affects the chemical recognition of a non native predator in common toad tadpoles (*Bufo bufo*). Chemoecology 17(1): 31–36. https://doi.org/10.1007/s00049-006-0354-8

Melotto A, Ficetola GF, Alari E, Romagnoli S, Manenti R (2021) Visual recognition and coevolutionary history drive responses of amphibians to an invasive predator. Behavioral Ecology 32(6): 1352–1362. https://doi.org/10.1093/beheco/arab101

Midway S (2019) Bayesian Hierarchical Models in Ecology. Bookdown. https://bookdown.org/steve_midway/BHME/ [accessed 27/04/2022]

Mikátová B, Šandera M (2015) První rozmnožování volně žijící želvy nádherné (*Trachemys scripta*) na území České republiky. Herpeta 1: 5–6.

Mitchell MD, Bairos-Novak KR, Ferrari MC (2017) Mechanisms underlying the control of responses to predator odours in aquatic prey. The Journal of Experimental Biology 220(11): 1937–1946. https://doi.org/10.1242/jeb.135137

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences of the United States of America 98(10): 5446–5451. https://doi.org/10.1073/pnas.091093398

Moore RD, Newton B, Sih A (1996) Delayed hatching as a response of streamside salamander eggs to chemical cues from predatory sunfish. Oikos 331–335(2): e331. https://doi.org/10.2307/3546073

Muraro M, Romagnoli S, Barzaghi B, Falaschi M, Manenti R, Ficetola GF (2021) Invasive predators induce plastic and adaptive responses during embryo development in a threatened frog. NeoBiota 70: 69–86. https://doi.org/10.3897/neobiota.70.65454

Nicieza AG (1999) Context-dependent aggregation in Common Frog *Rana temporaria* tadpoles: Influence of developmental stage, predation risk and social environment. Functional Ecology 13(6): 852–858. https://doi.org/10.1046/j.1365-2435.1999.00375.x

Nicieza AG (2000) Interacting effects of predation risk and food availability on larval anuran behaviour and development. Oecologia 123(4): 497–505. https://doi.org/10.1007/s004420000343

Nunes AL, Richter-Boix A, Laurila A, Rebelo R (2013) Do anuran larvae respond behaviourally to chemical cues from an invasive crayfish predator? A community-wide study. Oecologia 171(1): 115–127. https://doi.org/10.1007/s00442-012-2389-6

Nunes AL, Fill JM, Davies SJ, Louw M, Rebelo AD, Thorp CJ, Vimercati G, Measey J (2019) A global meta-analysis of the ecological impacts of alien species on native amphibians. Proceedings of the Royal Society B 286(1897): e20182528. https://doi.org/10.1098/rspb.2018.2528

Nyström P, Axelsson E, Sidenmark J, Brönmark C (1997) Crayfish predation on amphibian eggs and larvae. Amphibia-Reptilia 18(3): 217–228. https://doi.org/10.1163/156853897X00107

Petranka JW, Kats LB, Sih A (1987) Predator-prey interactions among fish and larval amphibians: Use of chemical cues to detect predatory fish. Animal Behaviour 35(2): 420–425. https://doi.org/10.1016/S0003-3472(87)80266-X

Polo-Cavia N, Gomez-Mestre I (2014) Learned recognition of introduced predators determines survival of tadpole prey. Functional Ecology 28(2): 432–439. https://doi.org/10.1111/1365-2435.12175

Polo-Cavia N, Gonzalo A, López P, Martín J (2010) Predator recognition of native but not invasive turtle predators by naïve anuran tadpoles. Animal Behaviour 80(3): 461–466. https://doi.org/10.1016/j.anbehav.2010.06.004

Poo S, Bickford DP (2014) Hatching plasticity in a Southeast Asian tree frog. Behavioral Ecology and Sociobiology 68(11): 1733–1740. https://doi.org/10.1007/s00265-014-1781-0

Quinn GP, Keough MJ (2002) Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge, 537 pp. https://doi.org/10.1017/CBO9780511806384

Ramírez-Albores JE, Badano EI, Flores J, Flores-Flores JL, Yáñez-Espinosa L (2019) Scientific literature on invasive alien species in a megadiverse country: advances and challenges in Mexico. NeoBiota 48: 113–127. https://doi.org/10.3897/neobiota.48.36201

Relyea RA, Stephens PR, Barrow LN, Blaustein AR, Bradley PW, Buck JC, Chang A, Collins JP, Crother B, Earl J, Gervasi SS, Hoverman JT, Hyman O, Lemmon EM, Luhring TM, Michelson M, Murray C, Price S, Semlitsch RD, Sih A, Stoler AB, VandenBroek N, Warwick A, Wengert G, Hammond JI (2018) Phylogenetic patterns of trait and trait plasticity evolution: Insights from amphibian embryos. Evolution 72(3): 663–678. https://doi.org/10.1111/evo.13428

Richardson JTE (2011) Eta squared and partial eta squared as measures of effect size in educational research. Educational Research Review 6(2): 135–147. https://doi.org/10.1016/j.edurev.2010.12.001

Rolim RG, de Ferreira PMA, Schneider AA, Overbeck GE (2015) How much do we know about distribution and ecology of naturalized and invasive alien plant species? A case study from subtropical southern Brazil. Biological Invasions 17(5): 1497–1518. https://doi.org/10.1007/s10530-014-0811-1

Saglio P, Mandrillon A-L (2006) Embryonic experience to predation risk affects tadpoles of the common frog (*Rana temporaria*). Archiv für Hydrobiologie 166(4): 505–523. https://doi.org/10.1127/0003-9136/2006/0166-0505

Schalk G, Forbes MR, Weatherhead PJ (2002) Developmental plasticity and growth rates of green frog (*Rana clamitans*) embryos and tadpoles in relation to a leech (*Macrobdella decora*) predator. Copeia 2002(2): 445–449. https://doi.org/10.1643/0045-8511(2002)002[0445:DPAGRO]2.0.CO;2

Segev O, Rodríguez A, Hauswaldt S, Hugemann K, Vences M (2015) Flatworms (*Schmidtea nova*) prey upon embryos of the common frog (*Rana temporaria*) and induce minor developmental acceleration. Amphibia-Reptilia 36(2): 155–163. https://doi.org/10.1163/15685381-00002992

Sih A, Moore RD (1993) Delayed hatching of salamander eggs in response to enhanced larval predation risk. American Naturalist 142(6): 947–960. https://doi.org/10.1086/285583

Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD, Pintor LM, Preisser E, Rehage JS, Vonesh JR (2010) Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. Oikos 119(4): 610–621. https://doi.org/10.1111/j.1600-0706.2009.18039.x

Smith DC (1987) Adult recruitment in chorus frogs: Effects of size and date at metamorphosis. Ecology 68(2): 344–350. https://doi.org/10.2307/1939265

Smith GR, Fortune DT (2009) Hatching plasticity of wood frog (*Rana sylvatica*) eggs in response to mosquitofish (*Gambusia affinis*) cues. Herpetological Conservation and Biology 4: 43–47.

Speybroeck J, Beukema W, Bok B, Van Der Voort J (2016) Field guide to the amphibians and reptiles of Britain and Europe. Bloomsbury Publishing, London, UK, 434 pp.

Standfuss B, Lipovšek G, Fritz U, Vamberger M (2016) Threat or fiction: Is the pond slider (*Trachemys scripta*) really invasive in Central Europe? A case study from Slovenia. Conservation Genetics 17(3): 557–563. https://doi.org/10.1007/s10592-015-0805-2

Stav G, Kotler BP, Blaustein L (2007) Direct and indirect effects of dragonfly (*Anax imperator*) nymphs on green toad (*Bufo viridis*) tadpoles. Hydrobiologia 579(1): 85–93. https://doi.org/10.1007/s10750-006-0388-5

Steel EA, Kennedy MC, Cunningham PG, Stanovick JS (2013) Applied statistics in ecology: Common pitfalls and simple solutions. Ecosphere 4(9): e115. https://doi.org/10.1890/ES13-00160.1

Strauss SY, Lau JA, Carroll SP (2006) Evolutionary responses of natives to introduced species: What do introductions tell us about natural communities? Ecology Letters 9(3): 357–374. https://doi.org/10.1111/j.1461-0248.2005.00874.x

TIBCO (2017) Tibco Statistica Quick Reference. https://docs.tibco.com/pub/stat/13.3.0/doc/pdf/TIB_stat_13.3_quick_ref.pdf [accessed 6/2/2022]

Tollrian R (1994) Fish-kairomone induced morphological changes in *Daphnia lumholtzi* (Sars). Archiv für Hydrobiologie 130(1): 69–75. https://doi.org/10.1127/archiv-hydrobiol/130/1994/69

Touchon JC, Wojdak JM (2014) Plastic hatching timing by red-eyed treefrog embryos interacts with larval predator identity and sublethal predation to affect prey morphology but not performance. PLoS ONE 9(6): e100623. https://doi.org/10.1371/journal.pone.0100623

Touchon J, Gomez-Mestre I, Warkentin K (2006) Hatching plasticity in two temperate anurans: Responses to a pathogen and predation cues. Canadian Journal of Zoology 84(4): 556–563. https://doi.org/10.1139/z06-058

Touchon JC, McCoy MW, Vonesh JR, Warkentin KM (2013) Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. Ecology 94(4): 850–860. https://doi.org/10.1890/12-0194.1

Tricarico E, Junqueira AO, Dudgeon D (2016) Alien species in aquatic environments: A selective comparison of coastal and inland waters in tropical and temperate latitudes. Aquatic Conservation 26(5): 872–891. https://doi.org/10.1002/aqc.2711

Vodrážková M, Šetlíková I, Berec M (2020) Chemical cues of an invasive turtle reduce development time and size at metamorphosis in the common frog. Scientific Reports 10(7978): 1–6. https://doi.org/10.1038/s41598-020-64899-0

Vodrážková M, Šetlíková I, Navrátil J, Berec M (2022) Different time patterns of the presence of red-eared slider influence the ontogeny dynamics of common frog tadpoles. Scientific Reports 12(7876): 1–9. https://doi.org/10.1038/s41598-022-11561-6

Vonesh JR, Bolker BM (2005) Compensatory larval responses shift trade-offs associated with predator-induced hatching plasticity. Ecology 86(6): 1580–1591. https://doi.org/10.1890/04-0535

Warkentin KM (1995) Adaptive plasticity in hatching age: A response to predation risk trade-offs. Proceedings of the National Academy of Sciences of the United States of America 92(8): 3507–3510. https://doi.org/10.1073/pnas.92.8.3507

Warkentin KM (2000) Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. Animal Behaviour 60(4): 503–510. https://doi.org/10.1006/anbe.2000.1508

Warkentin KM (2011) Plasticity of hatching in amphibians: Evolution, trade-offs, cues and mechanisms. Integrative and Comparative Biology 51(1): 111–127. https://doi.org/10.1093/icb/icr046

Wells KD (2007) The Ecology and Behavior of Amphibians. The University of Chicago Press, Chicago, 114 pp. https://doi.org/10.7208/chicago/9780226893334.001.0001

Supplementary material 1

Hatching time

Authors: Magda Vodrážková, Irena Šetlíková, Josef Navrátil, Michal Berec

Data type: excel file.

Explanation note: Data of hatching time (days) with four independent variables – slider presence (0/1), glass tank (1–6), box (1–5), and clutch (1–5) (n = 3000).

Link: https://doi.org/10.3897/neobiota.74.82250.suppl1

Supplementary material 2

Developmental stage, size

Authors: Magda Vodrážková, Irena Šetlíková, Josef Navrátil, Michal Berec

Data type: excel file.

Explanation note: Data of developmental (Gosner) stage and size at hatching (mm) with four independent variables – slider presence (0/1), glass tank (1–6), box (1–5), and clutch (1–5) (n = 30).

Link: https://doi.org/10.3897/neobiota.74.82250.suppl2

Supplementary material 3

Tables 1–3

Authors: Magda Vodrážková, Irena Šetlíková, Josef Navrátil, Michal Berec

Data type: Docx file.

Explanation note: Results of linear main effect ANOVA models for hatching time (Table S1), developmental stage (Table S2), and size at hatching (Table S3). All three tables include the overall fit of all parameters including adjusted R2 and then univariate results for all factors tested including partial eta-squared. Abbreviations: SS = Sum of Squares, d.f. = Degrees of Freedom, and MS = Mean Square.

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5.2. Chemické podněty invazivní želvy snižují dobu vývoje a velikost při metamorfóze u skokanů hnědých

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Nový predátor ve vodním prostředí může zásadně narušit biotop pro larvy obojživelníků. Vystavení predátorům může také změnit rychlost vývoje směrem k metamorfóze. Většina studií překvapivě prokázala, že vystavení predátorům mělo za následek delší nebo stejnou dobu trvání larválního období a větší nebo stejnou velikost při metamorfóze. Flexibilita načasování a velikosti při metamorfóze může být adaptivní, což umožňuje larvám obojživelníků reagovat na změny v kvalitě jejich vodního prostředí (Stearns 1989, Newman 1992, Gomez-Mestre *et al.* 2010) a zvýšit úspěšnost vývoje (Semlitsch *et* Caldwell 1982, Denver 1997, Kishida *et al.* 2014).

Vývojová plasticita u žab, včetně skokanů hnědých, byla již mnohokrát prokázána (Laurila 2000, Wells *et* Schwartz 2007 a odkazy v nich uvedené). Pulci různých druhů žab však na nového predátora nereagovali konzistentně. Kiesecker a Blaustein (1997) prokázali, že syntopičtí pulci skokana rudonohého (*Rana aurora*) výrazně snížili svou aktivitu a zvýšili využití úkrytů, když byli vystaveni chemickým podnětům jak pulců, tak dospělých jedinců skokana volského (*Rana catesbeiana*), kteří se jimi živí. Naopak alotopičtí pulci své chování nezměnili.

Pulci se snaží uniknout před predátorem tím, že rychleji vyrostou, a tudíž by byli velcí na pozření, nebo se snaží opustit vodní prostředí co nejdříve, ale bohužel dosáhnou minimální velikost, při které mohou metamorfovat. Zkracují tím larvální období, což nakonec snižuje expozici predátorům (Bulen *et* Distel 2011, Benard 2004). Například pulci *Bufo boreas*, kteří byli vystaveni dravým znakoplavkám (*Notonecta*) nebo podnětům od zraněných pulců, dosáhli metamorfózy rychleji než pulci bez přítomnosti predátora (Chivers *et al.* 1999). Tento výsledek byl interpretován jako adaptivní změna v historii života, která umožnila pulcům uniknout z nebezpečných podmínek ve vodním prostředí. Větší velikost při metamorfóze má za následek lepší fyziologické a pohybové schopnosti v suchozemském prostředí, větší šance na přežití juvenilních jedinců, dřívější reprodukci a větší velikost jedinců při prvním rozmnožování (Wilbur *et* Collins 1973, Smith 1983, Benard 2004). Vysoká růstová rychlost umožňuje pulcům rychle metamorfovat, a tím co nejdříve uniknout vodním predátorům. Jedná se tedy o velmi častý trade-off: jedinci, kteří zůstávají déle ve vodním prostředí dorůstají větší velikosti při metamorfóze, a tak dosahují i větších velikostí v dospělosti. Větší velikost během metamorfózy je spojena se zvýšeným fitness dospělých jedinců (Wilbur *et* Collins 1973, Vonesh *et* Bolker 2005, Bennett *et al.* 2013), avšak ve vodách, kde jsou přítomni predátoři, zvyšuje rychlý larvální vývoj a brzká metamorfóza míru přežití. Tento trade-off jsem pozorovala i ve svém pokusu. Potvrdilo se, že u pulců, kde probíhal larvální vývoj v přítomnosti predátora, dorůstali jedinci menší velikosti a dříve metamorfovali. Neprojevilo se však zvýšení rychlosti růstu oproti jedincům, kteří se vyvíjeli bez přítomnosti predátora a mohli tak dorůst do větší velikosti bez obavy z predace a tím zvýšit svou budoucí životaschopnost. Pulcům se podařilo zkrátit trvání larválního období urychlením jejich vývoje, ale nikoli rychlosti růstu, když byli vystaveni neustálé přítomnosti želvích chemických podnětů (Van Buskirk *et* Schmidt 2000, Capellán *et* Nicieza 2007, Bennett *et al.* 2013).

U obojživelníků je větší velikost při metamorfóze spojena se zvýšeným růstem v dospělosti a přežitím, větší velikostí těla v dospělosti a zvýšeným reprodukčním úspěchem (Relyea 2002, Vonesh *et* Bolker 2005, Takatsu *et* Kishida 2013). Naopak později metamorfovaní dospělí mohou mít nižší míru přežití a nižší růst (Altwegg *et* Reyer 2003).

Rozdíly ve velikostech při metamorfóze mohou vyplývat ze změn buď ve vývoji a/nebo rychlosti růstu. Relyea (2007) identifikovala pomalejší tempo jak ve vývoji larev, tak v růstu, jako nejběžnější vzorce reakce pulců na tlak predátora. V mém experimentu však zůstala rychlost růstu (na základě celkové délky) po celou dobu vývoje stabilní mezi pulci chovanými s želvou i bez ní s jednou výjimkou ve třetím vývojovém období (29. - 42. den). Dřívější metamorfóza v menší velikosti byla většinou výsledkem zrychlení rychlosti vývoje. To je v souladu s nálezy výsledky Laurily a Kujasala (1999), kteří prokázali zrychlení vývojové rychlosti pulců, které se neprojevilo ve změnách velikosti těla v přítomnosti larev vážek během raného období vývoje.

V této studii jsem tedy zkoumala, zda chemické podněty produkované predátory mají vliv na změny ve vývojové rychlosti, rychlosti růstu a velikosti při metamorfóze pulců *Rana temporaria*. Výsledky ukazují spíše vzácně dokumentované typy reakcí kořisti obojživelníků na predátory. Přítomnost želv zkrátila dobu metamorfózy pulců ze 110 ± 11,7 dnů na 93 ± 13,0 dnů (průměr ± S.D.). První metamorfovaní jedinci byli zaznamenáni 65. den a 80. den od vylíhnutí v přítomnosti predátora a v kontrolní skupině, resp. metamorfovaní jedinci byli statisticky významně menší (12,8 ± 0,99 mm) v přítomnosti predátora než v kontrolní skupině (15,2 ± 1,27 mm). Trajektorie rychlosti růstu byly podobné u obou variant. Obrana pulců vyvolaná predátory byla tedy evidentní ve vyšších rychlostech vývoje a menších velikostech při metamorfóze bez významných změn v růstu.

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Chemical cues of an invasive turtle reduce development time and size at metamorphosis in the common frog

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In aquatic systems, chemical cues are one of the major sources of information through which animals can assess local predation risk. Non-native red-eared sliders (*Trachemys scripta elegans*) have the potential to disrupt aquatic ecosystems in Central Europe because of their superior competitive abilities and omnivorous diets. In this study, we examined whether continuous predator-borne cues are tied to changes in the developmental rates, growth rates and sizes at metamorphosis of common frog tadpoles (*Rana temporaria*). Our results show rather rarely documented types of amphibian prey responses to caged predators. The presence of turtles shortened the time at metamorphosis of tadpoles from 110 ± 11.7 days to 93 ± 13.0 days (mean ± S.D.). The first metamorphosed individuals were recorded on the 65th day and on the 80th day from hatching in the predator treatment and in the control group, respectively. The froglets were significantly smaller (12.8 ± 0.99 mm) in the presence of the predator than in the control treatment (15.2 ± 1.27 mm). The growth rate trajectories were similar between the predator treatment and the control. Thus, predator-induced tadpole defences were evident in higher developmental rates and smaller sizes at metamorphosis without significant changes in growth.

Alien species are recognized as important drivers of global environmental change[1,2]. Although only a fraction of alien species become invasive[3], the massive increase in the rate of biological invasions due to rapid globalization of trade is reflected in the corresponding increase in research into the impacts of invasive alien species[4]. In addition to damage to human health and economies[5], alien species can have a significant impact on the functioning of parts of communities, entire communities or local ecosystems[6,7]. Alien species can interfere with the life histories of autochthonous fauna in many ways, including through predation, competition, transmission of new diseases, immune system compromisation, or hybridization[8,9].

Many amphibian species are unable to respond to non-native predators[10] however, there is a plethora of studies that documented measurable behavioural, physiological or morphological reactions. The ability to detect and recognize potential predators is an important component of the anti-predatory behaviour of amphibians in all their developmental stages[11]. Prey responses to the threat of predation are multi-faceted and include increased refuge use[12,13], increased distance from the threat[14], reduction of activity[15–18], shortening of feeding time[19,20], change in development time[21–23], change in growth rate[24], reduced size at metamorphosis[25], changes in body shape[26–28], changes in colour[29] and inedibility[13,30].

Werner[31] predicted that the exact point at which metamorphosis is favoured depends on the equilibrium of size-specific growth and mortality rates in aquatic and terrestrial habitats. A new predator in the aquatic environment can fundamentally impairs the habitat for amphibian larvae. A rapid growth theoretically provides the best protection against the costs of predation risk. Exposure to predators can also alter rates of development and progress toward metamorphosis. Surprisingly, the majority of studies demonstrated that exposure to predators resulted in longer or the same time of aquatic phase duration and larger or the same size at metamorphosis[32].

In their native range, red-eared sliders (*Trachemys scripta elegans*) are opportunistic omnivores. All turtle size classes consume significant proportion of animal matter, which makes them potentially dangerous to native species[33,34]. This turtle species is currently the world's most widespread freshwater turtle, because it has been widely introduced, both purposely or as the side effect of the pet trade, all over the world with the exception of

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Antarctica[35,36]. Although turtles were not mentioned among the non-native predators of amphibians[8], our previous research[18] revealed that tadpoles of common frogs (*Rana temporaria*) recognize red-eared sliders as potential predators. In that study[18], we documented that in the presence of turtle kairomones, tadpoles significantly reduce their movement activity (by almost 50%) and modify their swimming paths into more zigzagged trajectories. Because this type of antipredator behaviour is expected to convey costs in terms of lost foraging opportunities, we examined whether naïve common frog tadpoles respond adaptively to the presence of turtle kairomones[37]. Specifically, we asked if the presence of turtle cues accelerate the developmental or growth rate in tadpoles of common frog in laboratory conditions as predicted by theoretical models[31] or decelerate as was often experimentally documented[32]. Secondly, we addressed the question of whether tadpoles respond to the presence of turtle cues by shifting body size at metamorphosis.

Materials and methods

In the experiment, two adult red-eared sliders (carapax length: 18 cm and 21 cm) and the tadpoles of common frogs were used. Six clutches of common frog eggs were collected in pools around Holubov, South Bohemia, Czech Republic (48.9078633N, 14.3485608E) on 15-Apr-2017. Neither the eggs nor their parents came into contact with red-eared sliders at this locality. Clutches were placed in a glass tank of 220 L volume with tap water and a pump filter in a temperature-controlled laboratory room (20 ± 1 °C) for fourteen days. The pump filter was rinsed twice a week. The turtles were fed three times a week with chicken meat and ReptoMin Tetra turtle gammarus. The tadpoles were fed daily with TetraMin aquarium flakes for ornamental fish. The light source was a fluorescent tube (2 × 36 W) with a light regime of 12 h/12 h.

Four glass tanks of 220 L volume (size: 100 × 40 × 55 cm) and 6 cm water depth were used for the experiment. A Claro 300 filter pump ($300\,\mathrm{L.h}^{-1}$) was installed in each glass tank. To prevent physical, but not chemical, contact between turtle and tadpoles, a glass barrier was placed inside each glass tank with a 6 cm gap at both ends so that water could flow freely throughout the tank. On the other side of the barriers, 25 individual perforated opaque boxes (8 × 8 cm) with holes 2 mm in diameter were glued to the bottoms of the glass tanks (5 rows with 5 boxes in each row). Tadpoles of the same size (16.6 ± 0.16 mm) at stages 26 and 27 according to Gosner[38] were stocked individually in each box, and turtles were placed in two of the glass tanks prior to the experiment. During the experiment, the same feeding regime as that in the preparation phase was used. The tadpoles were photographed under a stereomicroscope (Olympus SZX 7) and measured using the QuickPHOTO MICRO 3.1 program every 14 days for 112 days. Tadpoles who died prior to metamorphosis (2%; one individual in each control glass tank) were eliminated from the experiment.

Linear regression between size and time was used to obtain slopes of individual growth trajectories (for all cases, $R^2 > 0.820$, $P < 0.05$). A hierarchical ANOVA with predator presence/absence as a fixed factor and glass tank identity (1–4) as a random factor nested in predator was used to test the differences in development time, final size of froglets at metamorphosis and slopes of individual growth. The normality and the homogeneity of the variances were checked using the Shapiro-Wilk test and the Bartlett test, respectively. All data analyses were performed in Statistica 13.

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Czech Ministry of Agriculture, Department of Animal Welfare according to article No. 15, section 2 of the act registered under number 9103/2009-17210.

Results

The time to metamorphosis differed significantly between the treatment with predator presence and control treatment ($F_{1,2} = 56.99$, $P = 0.017$). The average time to metamorphosis were 93 ± 13.0 days and 110 ± 11.7 days (mean ± S.D.) in the group where tadpoles were exposed to the predator and in the control group, respectively (Fig. 1). In the predator treatment group, the first metamorphosed individuals were recorded on the 65th day after hatching. The tadpoles in the control group achieved metamorphosis for the first time on the 80th day. The impact of the individual glass tanks was not proven for time to metamorphosis ($F_{2,94} = 0.74$, $P = 0.481$).

Similarly, we found a significant difference in the size at metamorphosis of froglets raised with the predator and without it ($F_{1,2} = 130.76$, $P = 0.008$). The froglets were significantly smaller (12.8 ± 0.99 mm) in the presence of the predator than those in the control group (15.2 ± 1.27 mm) (mean ± S.D.) (Fig. 2). The impact of the individual glass tanks was not proven for size at metamorphosis ($F_{2,94} = 0.81$, $P = 0.447$). Slopes of individual growth trajectories did not differ between the treatments ($F_{1,2} = 0.51$, $P = 0.549$), although individual glass tanks differed significantly ($F_{2,94} = 8.18$, $P = 0.001$), specifically one control tank differed from the others (Fig. 3).

Discussion

This study clearly shows that common frog tadpoles recognize the presence of alien red-eared sliders through water-borne chemical cues and react to the potential danger by shaping their developmental trajectories through a physiological life-history shift, in which final size was traded off against development rate. Our previous study[18] of common frog tadpoles from the same population showed that naïve tadpoles without any previous exposure or contact to red-eared sliders are able to innately face and respond to the water-borne chemical cues of the turtles by modifying their swimming behaviour. This study further corroborates these findings by finding that the presence of alien turtles also induces developmental plasticity of tadpoles. Invasive predators may often not be recognized as enemies by native prey ("prey naiveté hypothesis")[39–41]. The red-eared slider was specifically used as a potential alien predator of tadpoles in only two studies what we are aware of, in which an ability to recognize non-native turtles as a predator was either demonstrated[18] or not[42]. If alien predator is innately recognized as an enemy, this ability is attributed to the cue similarities between alien and native predators or to the generality of the cues used by naïve prey to assess risk predation[42,43]. However, this might not be the case in our study, because the tadpoles in our experiment originated from a population that lives in a geographic area with a complete absence

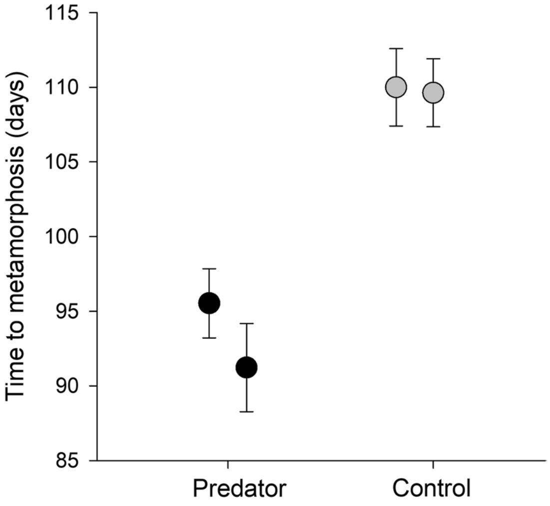

Figure 1. Time to metamorphosis of *Rana temporaria* tadpoles in the presence of predator (*Trachemys scripta elegans*) and those in the control group (mean ± S.E.). The average time to metamorphosis were longer when tadpoles were exposed to the predator. Each point represents one glass tank.

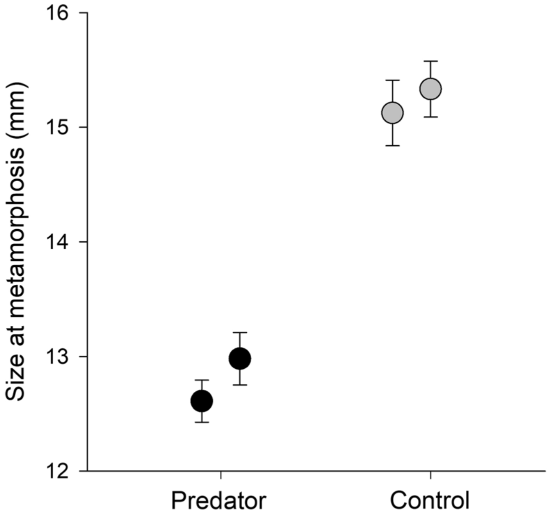

Figure 2. Size at metamorphosis of *Rana temporaria* froglets in the presence of predator (*Trachemys scripta elegans*) and those in the control group (mean ± S.E.). The average size at metamorphosis were larger when tadpoles were exposed to the predator. Each point represents one glass tank.

of turtles. An alternative explanation, although much less probable[44–46], would assume the similarity of the cues between the red-eared slider and some of the non-turtle native predators.

The flexibility of the timing of and size at metamorphosis can be adaptive, allowing amphibian larvae to respond to changes in the quality of their aquatic environment[22,47,48] and to increase developmental success[49,50]. In this experiment, tadpoles managed to shorten the duration of the larval period by accelerating their development rate, but not growth rate, when exposed to the constant presence of turtle chemical cues. Specifically, tadpoles metamorphosed on average 17 days earlier and at smaller size (by 16% on average) in the continual presence of the turtles than in the control treatment. This is consistent with the model of amphibian metamorphosis[31,51]. However, this type of response was only rarely demonstrated in amphibians - smaller size at metamorphosis was confirmed in only 14% of the studies, and a shorter time to metamorphose in only 5% of the studies reviewed by Relyea[32], i.e., in the northern red-legged frog, *Rana aurora*[52], and in the common toad, *Bufo bufo*, for shorter time to metamorphose only[53]. Comparison of growth trajectories is missing in the literature, the metamorphic changes are expressed only as body size (body mass) at the time of metamorphosis and/or time to metamorphosis[32]. However, it is also evident from these data that although acceleration of growth is one of the predicted options to avoid predation pressure, this method is very rare in amphibians[52,54]. Like most other amphibians tested, tadpoles of common frog did not accelerate growth.

The question arises, why our results are consistent with the model predictions[31,51] unlike most other studies demonstrating mostly longer time to metamorphosis at the same size in the presence of a predator[32]. Reduction of

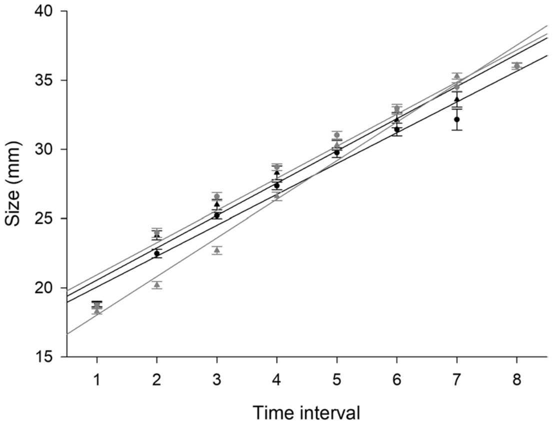

Figure 3. Growth trajectories of tadpoles of *Rana temporaria* in the presence of the predator (*Trachemys scripta elegans*) (black) and in the control group (grey). Slopes of growth trajectories were similar between the treatments but differed among the glass tanks. Each time interval corresponds to 14 days from the start of the experiment (mean ± S.E.). Each line represents one glass tank in the presence of the predator and in the control group.

tadpoles' activity due to predator presence itself is sufficient to induce earlier metamorphosis at the smaller size[55] via the increase level of corticotropin-releasing hormone[56,57]. We previously documented that common frog tadpoles decrease their activity in the presence of turtle cues[18]. A red-eared slider used as a predator model expanded the spectrum of less used vertebrate predator types and is exceptional in some features among experimental predators used so far. Moreover, this turtle is by far the largest predator ever used in this type of experiment which could lead to a significantly greater amount of released kairomones. Big size difference between predator and prey clearly limited the efficiency of tadpole to avoid predator pressure by achieving larger size[58]. Turtles therefore can act as a super stimulus and the tadpoles thus clearly responded to their presence by a rapid metamorphosis. Tadpoles try to avoid predator pressure by growing faster to be too large to become prey or to try to leave the aquatic environment as soon as possible, i.e., when tadpoles reach the minimum size at which they can metamorphose[25]. In addition, Relyea[32] suggested that developmental plasticity can be phylogenetically constrained. Thus, the ratio of published outcome possibilities may be also biased because some of the results are due to the absence of the ability to plastically change the developmental trajectory under any conditions[32].

In conclusion, our results suggested that common frog tadpoles are, despite the absence of a common evolutionary history with red-eared sliders, innately able to discriminate predator-specific scents of the invasive turtles and to subsequently respond to the predation risk by shortening their larval period in exchange for a smaller body size, which may affect the survival and fitness of a metamorphosed individual[51,59]. Given the probability of creating stable populations within Europe only at its southern edge, the red-eared slider is considered to have a limited impact on indigenous fauna[60]. However, the ability of escaped or released individuals to survive for a long time in the suboptimal conditions in the more northern parts of Europe[35] makes them a potential threat to frog populations in this region. It would be very appropriate to pay future attention to the study of demecology of the post-metamorphic phase of development.

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References

1. Levine, J. M. & D'Antonio, C. M. Forecasting biological invasions with increasing international trade. *Conserv. Biol.* **17**, 322–326 (2003).
2. Simberloff, D. *et al*. Impacts of biological invasions: what's what and the way forward. *Trends Ecol. Evol.* **28**, 58–66 (2013).
3. Pimentel, D. *Biological invasions: economic and environmental costs of alien plant, animal, and microbe species*. (CRC Press, 2014).
4. Lockwood, J. L., Hoopes, M. F. & Marchetti, M. P. *Invasion ecology*. (John Wiley & Sons, 2013).
5. Pimentel, D., Zuniga, R. & Morrison, D. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* **52**, 273–288 (2005).
6. Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. Environmental and economic costs of nonindigenous species in the United States. *Bioscience* **50**, 53–66 (2000).
7. Vilà, M. & Hulme, P. E. *Impact of biological invasions on ecosystem services*. Vol. 12 (Springer, 2017).
8. Bucciarelli, G. M., Blaustein, A. R., Garcia, T. S. & Kats, L. B. Invasion complexities: the diverse impacts of nonnative species on amphibians. *Copeia* **2014**, 611–632 (2014).
9. Nunes, A. L. *et al*. A global meta-analysis of the ecological impacts of alien species on native amphibians. *Proc. R. Soc. Lond. [Biol.]* **286**, 20182528 (2019).
10. Gall, B. G. & Mathis, A. Innate predator recognition and the problem of introduced trout. *Ethology* **116**, 47–58 (2010).
11. Bennett, A. M., Pereira, D. & Murray, D. L. Investment into defensive traits by anuran prey (*Lithobates pipiens*) is mediated by the starvation-predation risk trade-off. *PLoS One* **8**, e82344 (2013).
12. Cadi, A. & Joly, P. Impact of the introduction of the red-eared slider (*Trachemys scripta elegans*) on survival rates of the European pond turtle (*Emys orbicularis*). *Biodivers. Conserv.* **13**, 2511–2518 (2004).
13. Pearl, C. A., Adams, M. J., Schuytema, G. S. & Nebeker, A. V. Behavioral responses of anuran larvae to chemical cues of native and introduced predators in the Pacific Northwestern United States. *J. Herpetol.* **37**, 572–577 (2003).

14. Lima, S. L. & Dill, L. M. Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619–640 (1990).
15. Petranka, J. W., Kats, L. B. & Sih, A. Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. *Anim. Behav.* **35**, 420–425 (1987).
16. Lefcort, H. & Eiger, S. M. Antipredatory behaviour of feverish tadpoles: implications for pathogen transmission. *Behaviour* **126**, 13–27 (1993).
17. Kats, L. B. & Dill, L. M. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**, 361–394 (1998).
18. Berec, M., Klapka, V. & Zemek, R. Effect of an alien turtle predator on movement activity of European brown frog tadpoles. *Ital. J. Zool.* **83**, 68–76 (2016).
19. Lenda, M., Witek, M., Skórka, P., Moroń, D. & Woyciechowski, M. Invasive alien plants affect grassland ant communities, colony size and foraging behaviour. *Biol. Invasions* **15**, 2403–2414 (2013).
20. Robbins, T. R., Freidenfelds, N. A. & Langkilde, T. Native predator eats invasive toxic prey: evidence for increased incidence of consumption rather than aversion-learning. *Biol. Invasions* **15**, 407–415 (2013).
21. Márquez-García, A. Z., Campos-Verduzco, R. & Castro-Soriano, B. S. Sedimentología y morfología de la playa de anidación para tortugas marinas, El Carrizal, Coyuca de Benítez, Guerrero. *Hidrobiológica* **20**, 101–112 (2010).
22. Gomez-Mestre, I. *et al*. The shape of things to come: linking developmental plasticity to post-metamorphic morphology in anurans. *J. Evol. Biol.* **23**, 1364–1373 (2010).
23. Goldberg, T., Nevo, E. & Degani, G. Phenotypic plasticity in larval development of six amphibian species in stressful natural environments. *Zool. Stud.* **51**, 345–361 (2012).
24. Van Buskirk, J. The costs of an inducible defense in anuran larvae. *Ecology* **81**, 2813–2821 (2000).
25. Bulen, B. J. & Distel, C. A. Carbaryl concentration gradients in realistic environments and their influence on our understanding of the tadpole food web. *Arch. Env. Contam. Toxicol.* **60**, 343–350 (2011).
26. Nunes, A. *et al*. Antipredator responses of two anurans towards native and exotic predators. *Amphibia-Reptilia* **32**, 341–350 (2011).
27. Phillips, B. L. & Shine, R. An invasive species induces rapid adaptive change in a native predator: cane toads and black snakes in Australia. *Proc. R. Soc. Lond. [Biol.]* **273**, 1545–1550 (2006).
28. Berthon, K. How do native species respond to invaders? Mechanistic and trait-based perspectives. *Biol. Invasions* **17**, 2199–2211 (2015).
29. Berchtold, A. *Behavioural and physiological responses of prey fish to an invasive predator* MSc thesis, Simon Fraser University, (2017).
30. Zhang, F., Zhao, J., Zhang, Y., Messenger, K. & Wang, Y. Antipredator behavioral responses of native and exotic tadpoles to novel predator. *Asian Herpetol. Res.* **6**, 51–58 (2015).
31. Werner, E. E. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am. Nat.* **128**, 319–341 (1986).
32. Relyea, R. A. Getting out alive: how predators affect the decision to metamorphose. *Oecologia* **152**, 389–400 (2007).
33. Ernst, C. H. & Lovich, J. E. *Turtles of the United States and Canada*. (JHU Press, 2009).
34. Works, A. J. & Olson, D. H. Diets of two nonnative freshwater turtle species (*Trachemys scripta* and *Pelodiscus sinensis*) in Kawai Nui Marsh, Hawaii. *J. Herpetol.* **52**, 444–452 (2018).
35. Ficetola, G. F., Rödder, D. & Padoa-Schioppa, E. In *Handbook of global freshwater invasive species* (ed. Francis, R.) 331–339 (Earthscan, Taylor and Francis Group, 2012).
36. Kraus, F. *Alien reptiles and amphibians: a scientific compendium and analysis*. Vol. 4 (Springer, 2009).
37. West-Eberhard, M. J. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* **20**, 249–278 (1989).
38. Gosner, K. L. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190 (1960).
39. Cox, J. G. & Lima, S. L. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. *Trends Ecol. Evol.* **21**, 674–680 (2006).
40. Banks, P. B. & Dickman, C. R. Alien predation and the effects of multiple levels of prey naiveté. *Trends Ecol. Evol.* **5**, 229–230 (2007).
41. Hettyey, A. *et al*. Naive tadpoles do not recognize recent invasive predatory fishes as dangerous. *Ecology* **97**, 2975–2985 (2016).
42. Sih, A. *et al*. Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos* **119**, 610–621 (2010).
43. Ferrari, M. C., Gonzalo, A., Messier, F. & Chivers, D. P. Generalization of learned predator recognition: an experimental test and framework for future studies. *Proc. R. Soc. Lond. [Biol.]* **274**, 1853–1859 (2007).
44. Chalcraft, D. R. & Resetarits, W. J. Jr. Predator identity and ecological impacts: functional redundancy or functional diversity? *Ecology* **84**, 2407–2418 (2003).
45. Sih, A., Englund, G. & Wooster, D. Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.* **13**, 350–355 (1998).
46. Soluk, D. A. & Collins, N. C. Synergistic interactions between fish and stoneflies: facilitation and interference among stream predators. *Oikos* **52**, 94–100 (1988).
47. Stearns, S. C. The evolutionary significance of phenotypic plasticity. *Bioscience* **39**, 436–445 (1989).
48. Newman, R. A. Adaptive plasticity in amphibian metamorphosis. *Bioscience* **42**, 671–678 (1992).
49. Semlitsch, R. D. & Caldwell, J. P. Effects of density of growth, metamorphosis, and survivorship in tadpoles of *Scaphiopus holbrooki*. *Ecology* **63**, 905–911 (1982).
50. Denver, R. J. Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. *Horm. Behav.* **31**, 169–179 (1997).
51. Wilbur, H. M. & Collins, J. P. Ecological aspects of amphibian metamorphosis: nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. *Science* **182**, 1305–1314 (1973).
52. Kiesecker, J. M., Chivers, D. P., Anderson, M. & Blaustein, A. R. Effect of predator diet on life history shifts of red-legged frogs, *Rana aurora*. *J. Chem. Ecol.* **28**, 1007–1015 (2002).
53. Laurila, A., Kujasalo, J. & Ranta, E. Predator-induced changes in life history in two anuran tadpoles: effects of predator diet. *Oikos* **83**, 307–317 (1998).
54. Kurzava, L. M. & Morin, P. J. Tests of functional equivalence: complementary roles of salamanders and fish in community organization. *Ecology* **79**, 477–489 (1998).
55. Laurila, A. & Kujasalo, J. Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *J. Anim. Ecol.* **68**, 1123–1132 (1999).
56. Boorse, G. C. & Denver, R. J. Acceleration of *Ambystoma tigrinum* metamorphosis by corticotropin-releasing hormone. *J. Exp. Zool.* **293**, 94–98 (2002).
57. Ruthsatz, K. *et al*. Endocrine disruption alters developmental energy allocation and performance in *Rana temporaria*. *Integr. Comp. Biol.* **59**, 70–88 (2019).
58. Formanowicz, D. R. Jr. Anuran tadpole/aquatic insect predator-prey interactions: tadpole size and predator capture success. *Herpetologica* **42**, 367–373 (1986).
59. Smith, D. C. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. *Ecology* **64**, 501–510 (1983).
60. Bringsøe, H. In *Handbuch der Reptilien und Amphibien Europas* Vol. 3/IIIA Schildkröten (Testudines) I. (ed. Fritz, U.) 525–583 (Aula, 2001).

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Author contributions

M.V. and M.B. conceived and designed the experiment. M.V. collected the data. All authors developed the statistical analyses and contributed to the writing of the manuscript.

Competing interests

The authors declare no competing interests.

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5.3. Různé časové vzorce přítomnosti želvy nádherné ovlivňují dynamiku ontogeneze pulců skokanů hnědých

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Koexistence a interakce mezi druhy v daném společenstvu nezávisí pouze na samotném druhovém složení, ale také na vzorcích časové aktivity příslušných druhů (Gerber *et al*. 2012, Zapata-Ríos *et* Branch 2016, Azevedo *et al*. 2018). Specifické vzorce aktivity jsou důležitým atributem chování druhu a jsou utvářeny různými faktory, jako jsou fyziologické adaptace (Willmer *et* Stone 2004), abiotické podmínky (Scheibe *et al*. 1999) a životní strategie druhů (např. sociální organizace, vyhýbání se konkurenci) (Ferrari *et al*. 2008, Kloskowski *et al*. 2020). Jakákoliv změna sezónní aktivity u jednoho druhu může mít důsledky na interagující druhy v rámci trofických úrovní i mezi nimi (Damien *et* Tougeron 2019).

Obojživelníci pravděpodobněji reagují na predátory, když jsou jejich potomci vůči predátorovi vysoce zranitelní (tj. predátor způsobuje vysokou úmrtnost), když se predátor vyskytuje nerovnoměrně v celé krajině a je relativně běžný a předvídatelný (např. predátor nevstupuje a neopouští lokality náhodně), a když dospělí mají na výběr řadu dostupných míst k rozmnožování. Tyto podmínky jsou často splněny u žab, které umisťují své snůšky do dočasných nebo periodických tůní (Blaustein *et al*. 1999, Chivers *et* Ferrari 2013). Vzhledem k tomu, že jedinci jsou schopni vnímat rozdíly v míře rizika, měli by se rozhodnout, zda nakladou snůšky do tůní bez těchto biotických rizikových faktorů.

Predace během raných fází života, kdy jsou organismy vnímavé k biotickým i abiotickým faktorům, bude mít pravděpodobně všudypřítomné účinky na vývoj populace (Vieira *et al*. 2012). Jak predace v raných životních etapách ovlivňuje složení společenstev je daleko méně známé. Některé studie naznačují, že predace během raného období po vylíhnutí, kdy jsou jedinci obecně malí a stále se nemohou bránit, může určovat početnost a distribuci populací nezávisle na predaci dospělých (Young *et* Chia 1984, Epelbaum *et al.* 2009, Willink *et al.* 2014). Obojživelníci jsou zvláště dobrým modelem pro studium fyziologických a behaviorálních reakcí na plasticitu růstu, protože velikost při metamorfóze pozitivně koreluje s přežitím v následných stadiích terestrické životní fáze.

Stejně jako v mé studii i Laurila a Kujasalo (1999) uvádí, že na začátku experimentu se pulci v přítomnosti predátorů vyvíjeli pomaleji. Při metamorfóze neměla přítomnost larvy vážky žádný vliv.

V tomto článku jsem sledovala dynamiku ontogeneze pulců *Rana temporaria* v různých časových vzorcích přítomnosti predátora *Trachemys scripta elegans*. Zjistila jsem, že pulci měli delší larvální období a byli menší velikosti při metamorfóze a nižší tělesné hmotnosti, když byl predátor přítomen v raném vývoji, než když se pulci vyvíjeli bez predátora. Časná přítomnost predátora nápadně snižovala růstové přírůstky pulců v raném vývoji. Po odstranění predátora se růst zrychlil nad úroveň naměřenou v podmínkách, kdy byl predátor přítomen v pozdní fázi vývoje, i bez přítomnosti predátora. Tyto rychlosti růstu však nepřesáhly rychlosti růstu stejně velkých pulců v ostatních treatmentech, a proto nebyly dostatečné pro kompenzaci zpomalení růstu v první části vývoje. Přítomnost predátora v pozdním vývoji pulce neovlivnila ani dobu do metamorfózy, ani velikost/hmotnost těla při metamorfóze. Závěrem lze říci, že predátor měl vliv na znaky metamorfózy pouze tehdy, pokud byl přítomen v raném vývoji pulců.

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Different time patterns of the presence of red-eared slider influence the ontogeny dynamics of common frog tadpoles

M. Vodrážková, I. Šetlíková, J. Navrátil & M. Berec

The coexistence of species in a given community depends on the set of species involved and the timing of their interactions. Many native communities are increasingly forced to face both direct and indirect pressures from new alien predators, which, in extreme cases, can lead to the extinction of prey populations. In this study, we examine the dynamics of the ontogeny of common frog (*Rana temporaria*) tadpoles under different time patterns of an alien predator—the red-eared slider (*Trachemys scripta elegans*) presence. We found that the tadpoles had a longer larval period and were smaller in size at metamorphosis and lower in body mass when the predator was present in early development than when the tadpoles developed without a predator. The early presence of a predator conspicuously reduced the growth increments of the tadpoles at early development. After the removal of the predator, growth accelerated above the level measured under the conditions of both the late predator and no predator. However, these growth rates did not exceed the growth rates of equally sized tadpoles in the other treatments and therefore were not sufficient to compensate for the growth slowdown in the first part of development. The presence of a predator in late tadpole development influenced neither the time to metamorphosis nor size/body mass at metamorphosis. In conclusion, the predator had the effect on metamorphosis traits only if it was present in the early development of tadpoles.

The coexistence of species in a given community depends on the set of species involved[1,2]. Any change in species composition can have consequences on interacting species, both within and among trophic levels[3–5], the result of which depends on the timing of presence/activity of individual species[6–9]. Globally, many native communities are currently increasingly forced to face new alien predators[10], which, in extreme cases, can lead to the extinction of whole prey populations[11,12]. In addition to these predators causing direct mortality, current theoretical and empirical studies of trophic cascades suggest that the indirect effect of the "landscape of fear" created by predators may be more important than direct killing[13–16].

Amphibians are a particularly good model for studying morphological, physiological, and behavioural responses to changes in community composition[17]. These responses can, inter alia, consist of changes in body morphology[18,19], behaviour[20], or timing of life-history switch points, such as hatching[21–23] or metamorphosis[24–26]. Specifically, predation during early life-history stages, when organisms are particularly susceptible to both biotic and abiotic factors, is likely to have pervasive effects on community development[27]. The fitness consequences of phenological shifts that appear early in the ontogeny of a species can also be fundamentally different from the effects observed at later ontogenetic stages. For example, the relatively early seasonal appearance (or accelerated developmental rate) of a prey species may decrease its predation risk early in life, but increase the negative effects of competition with its former predator later in life[28].

Tadpoles of various frog species respond differently to novel predators. Some species innately detect and elicit adaptive responses to stimuli of novel predators in the absence of a common evolutionary history[29–31]. In others, no such responses have been documented[32,33]. One of the 100 worst world invaders[34] is a red-eared slider (*Trachemys scripta elegans*), which is also among the most widespread animal species outside its native range[35,36]. Berec, et al. [37] and Vodrážková, et al.[38] recently found a response in the behaviour and growth of common frog (*Rana temporaria*) tadpoles to the presence of red-eared sliders in the form of reduced movement activity associated with more zigzagged movement trajectories, smaller size at metamorphosis, and shorter time to metamorphosis.

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In these studies, the response of the common frog tadpoles was examined under the continuous presence of a red-eared slider throughout larval development. However, such a situation may not correspond to reality, as turtles frequently change their place of occurrence in a habitat during a period of activity[39]; therefore, at least in areas where the red-eared slider does not naturally reproduce and thus its density is relatively low, different populations may encounter red-eared sliders at different parts of development and for different time intervals.

In this study, we examine the dynamics of the ontogeny of common frog tadpoles under different time patterns of red-eared slider presence. Because the presence of red-eared sliders reduces the activities of tadpoles and consequently their growth rates[37,38], we expect that the presence of turtles in any part of larval ontogenesis will lead to a growth slowdown. As the level of this slowdown decreases with increasing size as an effect of better movement abilities in terms of faster escape reaction to predator[40–42], we expect that the presence of red-eared sliders in the early stages of ontogeny will have a more pronounced effect than their presence in the later stages. In the early red-eared slider presence, this could lead to a smaller size and/or weight of tadpoles at metamorphosis with the same time to metamorphosis as in tadpoles with the late red-eared slider presence[43] or to the same size and weight of tadpoles at metamorphosis with a longer time to metamorphosis than the tadpoles with the late red-eared slider presence[44]. Alternatively, if it is more advantageous for tadpoles to leave the hazardous environment with tadpoles as soon as possible, we might expect a reduction in the time to metamorphosis together with a smaller total size.

Materials and methods

Common frog tadpoles originated from six clutches that were collected in pools near Holubov, South Bohemia, Czech Republic (48.9078633°N, 14.3485608°E) on 5 April 2020. Clutches were placed in a 220 L glass tank with tap water in a temperature-controlled laboratory. Each glass tank was equipped with a pump filter that was cleaned twice a week along with removing debris from the bottom of the tanks, and the tanks were replenished with an equal volume of the tap water (up to 5% of glass tank volume). The acclimatization temperature was gradually raised from 16 to 21 °C for fourteen days. The tadpoles were fed ad libitum with TetraMin aquarium flakes for ornamental fish. The light source was fluorescent tubes (2 × 36 W) with a light regime of 12 h/12 h.

Six glass tanks that were each 220 L (size: 100 × 55 × 40 cm) with an 8 cm water depth were used for the experiment. A Claro 300 filter pump (300 L h^{-1}) was installed in each glass tank. Two adult red-eared sliders (carapax length: 21 cm and 18 cm) obtained from Hluboká nad Vltavou zoo were used as the predators. Turtles were placed in two of the glass tanks before the experiment and were fed three times a week with ReptoMin Tetra turtle food consisting of *Gammarus*. To prevent physical, but not chemical, contact between a turtle and tadpoles, a glass barrier was placed inside each glass tank three days before the start of the experiment with a 6 cm gap at both ends so that water could flow freely throughout the tank. On the other side of the barriers, 21 individual perforated opaque boxes (8 × 8 cm) with holes 2 mm in diameter were glued to the bottoms of the glass tanks (7 rows with 3 boxes).

Tadpole development was observed in three treatments, each with two independent repetitions (glass tanks). Based on a pilot experiment, the development time at a given temperature was determined to be approximately six weeks. In the first treatment, tadpoles were present in the tanks with red-eared sliders for the first three weeks of development (predator early—PE). In the second treatment, tadpoles developed with turtles from the start of the 4th week until the completion of metamorphosis (predator late—PL). At the time of the switch of predator presence, the tadpoles of the PE treatment were exchanged with those of the PL treatment and vice versa. This transfer of tadpoles took place during the regular 7-day measurement period (see below), so handling was the same in all treatments, although the tadpoles were returned to the same glass tank in the control. Water maintenance in the glass tanks was carried out at regular intervals, that is, three days before the replacement of tadpoles. In the control, the tadpoles developed without a slider all the time. Neither the eggs nor their parents came into contact with a red-eared slider at the collection locality.

At the beginning of the experiment, similarly sized tadpoles (18.9 ± 1.27 mm) at stages 26 and 27 according to Gosner[45] were stocked individually in each box. During the experiment, the same feeding and light regime as that in the acclimation phase was used. The water temperature was 21 ± 1.7 °C (mean ± S.D.). During the experiment, the tadpoles were photographed under a stereomicroscope (Olympus SZX 7) and measured (to the nearest 0.5 mm) using QuickPHOTO MICRO 3.2 software every seven days. After reaching metamorphosis (Gosner phase 46), the time to metamorphosis was recorded in days, the size of every froglet was measured to the nearest 0.5 mm, and after gently blotting them dry on absorbent paper, each froglet was weighed to the nearest 0.01 g.

To compare the growth trajectories of tadpoles among treatments, the growth rate at a particular measurement (time interval) and the growth rate related to a particular total size (body + tail length) were used. The growth was expressed as a percentage increase in size between the two subsequent measurements. Negative growth increment values, indicating the onset of metamorphosis, were not used. Individual linear growth curves (i.e., the relationship between growth increments and total size) were calculated. To determine the ability to compensate for the reduced growth rate in the presence of a predator in the PE treatment, the slopes and intercepts of individual growth curves (percentage of growth increment versus size) were compared among the PE treatment, the PL treatment and the control. To eliminate the effect of absolute total size (due to the upper size limit, i.e., larger tadpoles have less potential growth capacity), we compared growth curves only in the tadpole size range detected in the turtle-free period (i.e., from the 3rd time interval) in the PE treatment with the tadpole growth rate of the same size interval in the PL treatment and the control.

The normality of the residuals and homogeneity of the variances were checked using the Shapiro–Wilk test and the Bartlett test, respectively. Differences in time to metamorphosis among the treatments were tested by survival analysis for comparison of multiple groups. To do this, a score was assigned to each survival time using Mantel's procedure and next a Chi-square value was computed based on the sums (for each group) of this score.

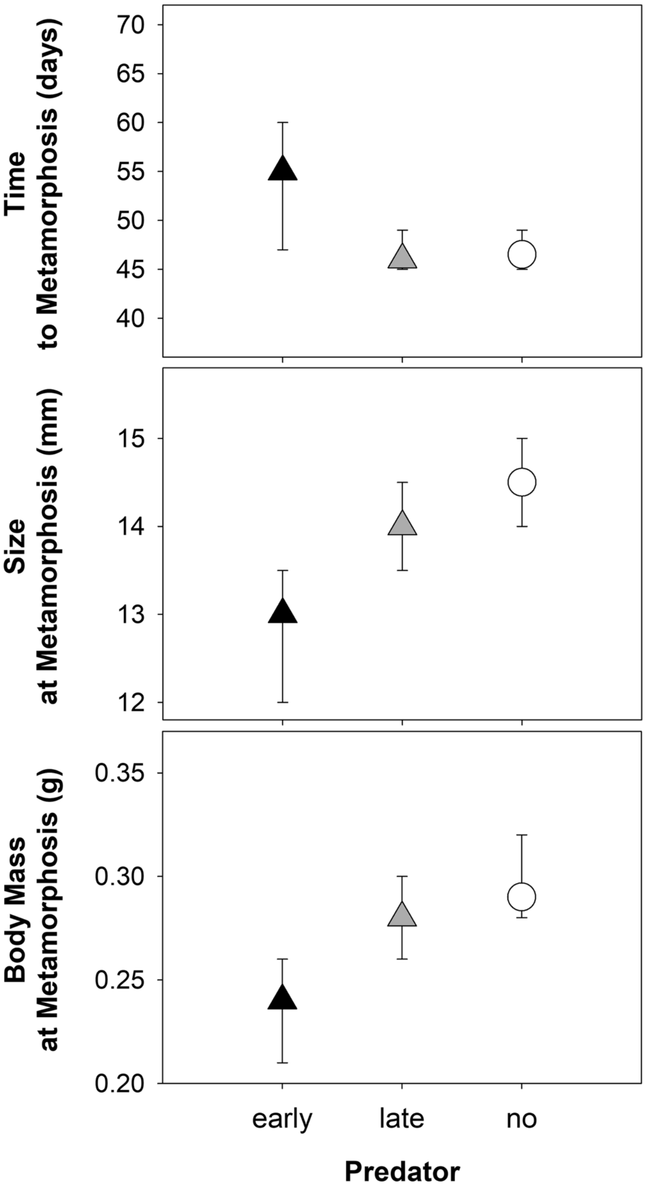

Figure 1. Time to metamorphosis and size and body mass at metamorphosis (median and interquartile range) in the treatments.

Differences in final size and body mass at metamorphosis, the growth increments at particular time intervals of development, and the slopes and intercepts of the individual growth lines among the treatments were tested using repeated measures ANOVA (as tadpoles in one glass tank could not be handled as completely independent one from the another) with predator presence/absence as three-level categorical factor followed by post-hoc comparisons using Tukey HSD method. All data analyses were performed using Survival Analysis and General Linear Models in Tibco Statistica 13.

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Czech Ministry of Agriculture, Department of Animal Welfare according to article No. 15, Section 2 of the act registered under number MZP/2019/630/437. Appropriate permission (No. 2/2020) was taken from the zoo authorities for obtaining the turtles. We adhered the recommendations in the ARRIVE guidelines.

Results

Metamorphosis time and size. Time to metamorphosis (Chi-square = 23.5, df = 2, $p < 0.001$), size (F(2,3) = 25.7, $p = 0.013$) and body mass (F(2,3) = 10.1, $p = 0.047$) at metamorphosis did significantly differ between treatments. Compared to the other treatments, in the PE treatment, tadpoles achieved metamorphosis later and were smaller with respect to their size and body mass (Fig. 1). In contrast, the presence of a predator in the later phase of development had no effect on all factors measured (Fig. 1). Specifically, the tadpoles without

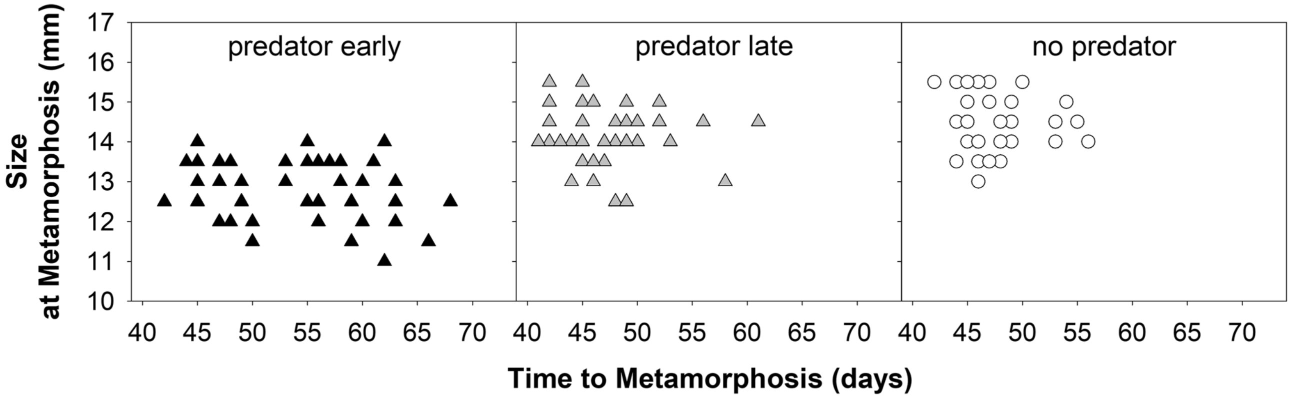

Figure 2. Size at metamorphosis and time to metamorphosis of individual tadpoles in the treatments. Note that some dots overlap.

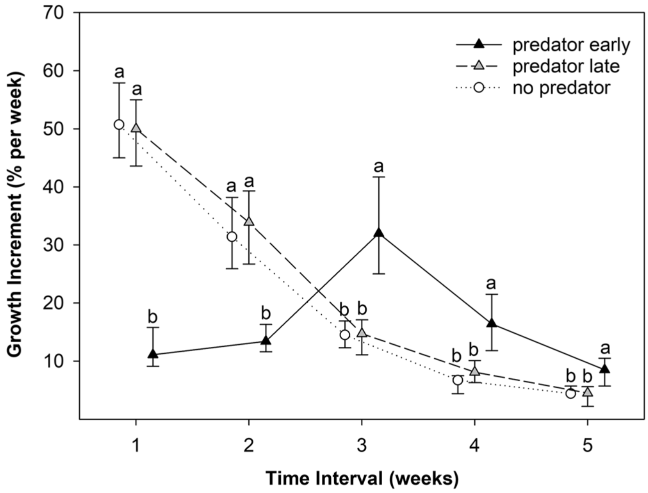

Figure 3. Median and interquartile range of growth increment in particular time intervals in the treatments. Different letters indicate a significant difference.

red-eared sliders metamorphosed in 46.5 days (median). In comparison to the control, the early presence of a predator delayed metamorphosis by 8.5 days. In the PL treatment, the tadpoles metamorphosed in 46 days. Size and body mass at metamorphosis were the lowest (medians 13.0 mm and 0.24 g) in the PE treatment. Tadpoles metamorphosed at similar size (14.0 mm and 14.5 mm in the PL treatment and the control, respectively) and body mass (0.28 g and 0.29 g in the PL treatment and the control, respectively) in the other two treatments (Fig. 1).

Furthermore, the presence of a predator increased the interquartile range in the time to metamorphosis of the tadpoles in the PE treatment to 13 days in comparison to 4 days in both the PL treatment and the control, while the interquartile range in size at metamorphosis was comparable among all treatments (Figs. 1 and 2). The first individuals metamorphosed on the 41st day in the PE treatment and on the 42nd day in the PL treatment and the control. The last individual metamorphosed on the 68th day, 61st day and 56th day in the PE treatment, the PL treatment and the control, respectively (Fig. 2).

Growth of tadpoles. The growth increments significantly differed in each time interval ($F(2,3)=306.6$, $p<0.001$, $F(2,3)=31.4$, $p=0.010$, $F(2,3)=9.2$, $p=0.049$, $F(2,3)=78.2$, $p=0.003$, $F(2,3)=20.0$, $p=0.047$). In the PE treatment, the growth increments of the tadpoles differed from those in the PL treatment and the control at all time intervals, while they were similar in the PL treatment and the control (Fig. 3). The presence of predators conspicuously reduced the growth increments of the tadpoles at early development. After the removal of the predator, growth accelerated above the level in the PL treatment and the control (Fig. 3). The growth increments decreased with time and size in the PL treatment and the control (Figs. 3 and 4).

However, the growth rate increase after the removal of the predator in the PE treatment was not enough to compensate for the previous growth slowdown (see final size at metamorphosis; Fig. 1). The slopes and intercepts of the individual growth trajectories calculated for the same tadpole size ranges (from 21.5 to 43.0 mm, i.e., the size range available for tadpoles in the PE treatment for intervals 3–5) in all treatments did not differ ($F_{slopes}(2,3)=0.1$, $p=0.899$, $F_{intercept}(2,3)=0.2$, $p=0.862$). Thus, the growth increments of tadpoles that were the same

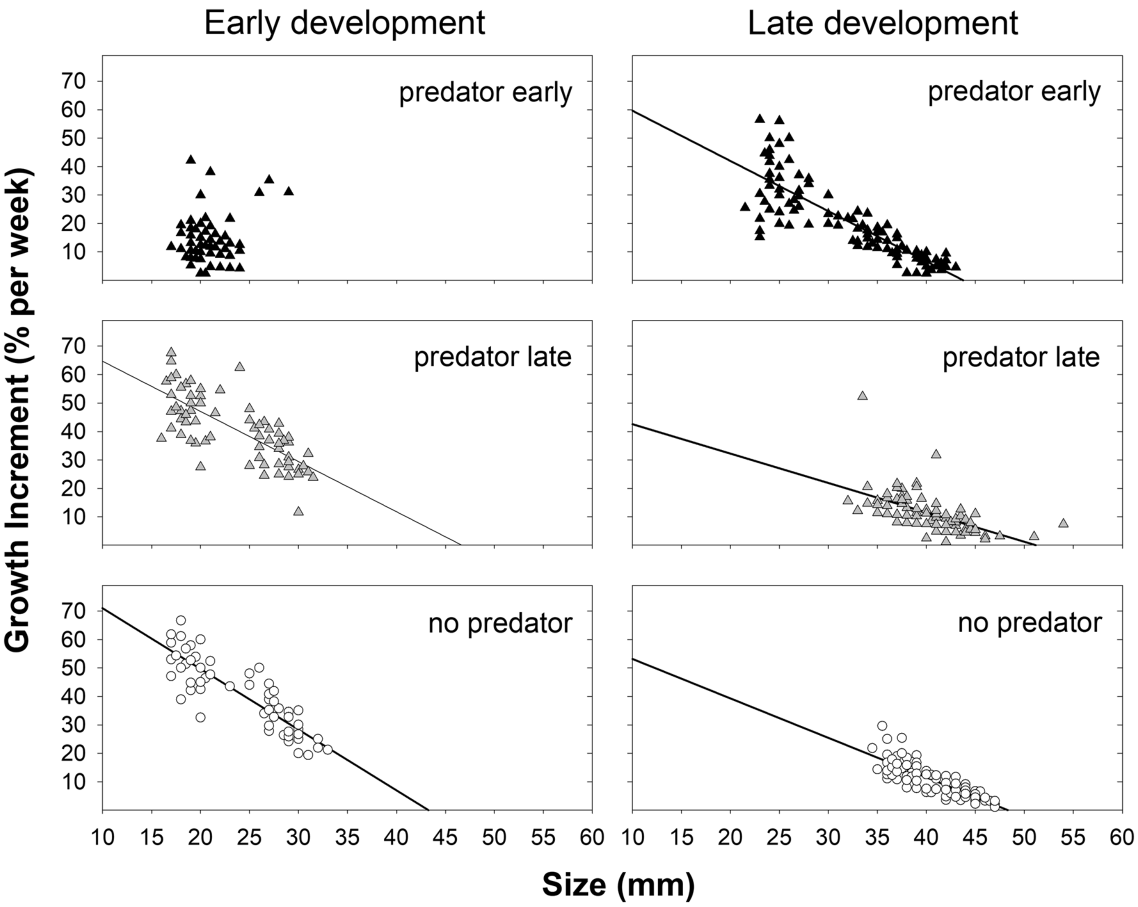

Figure 4. Relationship between growth increment and size throughout two intervals of development. PE treatment—early development: regression NS (p = 0.14), PE treatment—late development: GI (growth increment) = −1.76 size + 77.24, R-square = 0.73; PL treatment—early development: GI = −1.77 size + 82.37, R-square = 0.56, PL treatment—late development: GI = −1.03 size + 52.87, R-square = 0.34; control—early development: GI = −2.13 size + 92.36, R-square = 0.76, control—late development: GI = −1.39 size + 67.00, R-square = 0.71.

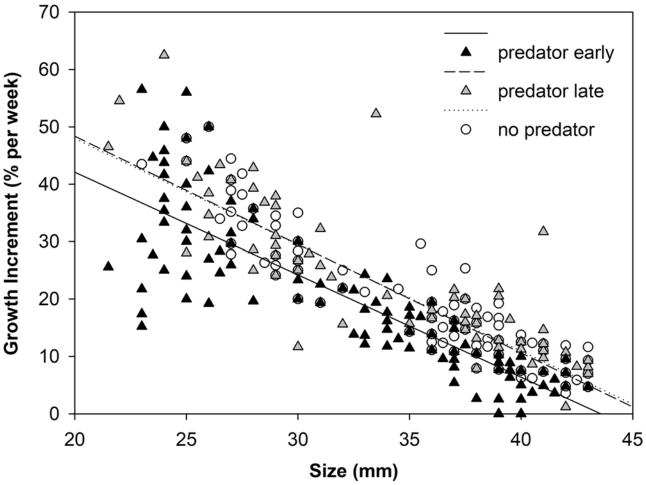

Figure 5. Relationship between growth increment and size for the size categories from 21.5 to 43.0 mm.

size in the PE and PL treatments and the control were comparable (Fig. 5). Growth compensation after predator removal was therefore not indicated in the PE treatment.

Discussion

The interaction between predator and prey was investigated in a less frequently studied vertebrate-vertebrate model without the prey being able to attain a "size refuge" (i.e., prey cannot avoid the risk of predation by reaching a threshold size[46]) and with caged predators present only for a defined part of larval prey development. Most previous studies[47–49] and references there in have addressed the permanent presence of an invertebrate predator and/or fish that occur together throughout the development of tadpoles. In contrast to these studies, we have studied the response of tadpoles to a large gape-unlimited vertebrate predator, which is able to frequently alter its presence in water bodies where tadpoles develop[39]. Moreover, the red-eared slider represents a completely new type of predator in specific places as it is able to colonize even localities in which no other species of turtles live.

Most studies have shown that the continuous presence of a predator has no effect or prolongs the time to metamorphosis and at the same time has no effect or increases the size of the body at metamorphosis[18,47,50–53]. Our results do not match any of these combinations, which could be due to the type of predator used[38]. In accordance with previous results[38], when a red-eared slider was specifically used as a permanently present predator, common frog tadpoles reached a smaller size at metamorphosis and metamorphosed earlier than without predator. In the present study, we divided the presence of the predator into two intervals, which led to different responses in time to metamorphosis and size/body mass at metamorphosis. The early presence of a predator prolonged the developmental time by 8.5 days (18%), and tadpoles metamorphosed when they were 1.5 mm smaller size (10%) and 0.05 g (17%) lighter than those in the control, while the late presence of a predator did not affect any of these parameters.

Since the presence of a predator reduces the movement activity of tadpoles[37], and, therefore, food consumption rate, we can compare our results with experiments in which the ontogeny was studied at different food availability[54,55]. The growth trajectory of tadpoles in the PE treatment exactly matches the situation described by Leips and Travis[54]. At an early age, most of the incoming resources are allocated to development, and some resources are allocated to growth; development takes priority. Decreases in food level (corresponding to reduced movement activity[37] and therefore reduced food intake rate in our PE treatment) at this stage will delay development and prolong the time to metamorphosis. Beyond a certain point of development (late predator presence), the rate of development is fixed, and changes in food level affect only size at metamorphosis[54]. The fact that in our case the PL tadpoles were similar in size and body mass to the control tadpoles may be due to the fact that our switching point lies later than where the developmental rate is fixed, and thus the tadpoles do not have enough time for the difference to become apparent.

Our results for the PL treatment are consistent with the predictions of the Travis model of anuran metamorphosis[43] (see also Fig. 2b, curves 2 or 3 in Alford and Harris[55]). However, for PE treatment, our results fit neither the Wilbur-Collins[44] nor the Travis model (see Figs. 1a and 2a, in Alford and Harris[55]). While the Wilbur-Collins model predicts the same size of tadpoles at different times to metamorphosis, the Travis model predicts different sizes at the same time to metamorphosis. We believe that PE tadpoles in our case could grow in agreement with the Wilbur-Collins model. The difference in body size between PE tadpoles and the control, which is not predicted by the model, could then arise because tadpoles are forced to metamorphose within a certain period of time[56,57], and this time from the release of predator pressure in the PE treatment was not sufficient to allow tadpoles to grow to the same size as tadpoles in the control.

Tadpole vulnerability to predation by both vertebrate and invertebrate predators appears to decrease with increasing tadpole size and age until the onset of metamorphosis[46,58–61]. The lower susceptibility of larger tadpoles to a predator is often explained by the increase in their swimming ability[62,63] and the decrease in the foraging efficiency of predators on larger tadpoles[58]. The nonsignificant 3% reduction of both size (0.5 mm) and body mass (0.01 g) at metamorphosis in the presence of a red-eared slider in later development with larger tadpoles in comparison to that in the control supports these predictions.

Growth increments were similar in the PL treatment and the control throughout development. In contrast, in the PE treatment, the tadpole increments were conspicuously lower in the presence of a predator and higher in the absence of a predator than in the PL treatment and the control. This result is consistent with the results of Laurila and Kujasalo[64], where common frog tadpoles developed more slowly early in the experiment in the presence of predators (dragonfly larvae). Yet, their tadpoles metamorphosed later and at larger sizes and were able to catch up with the delay. Other studies have similarly described that when developing individual is placed in better conditions after a period of nutritional deficiency, compensatory growth can occur[65,66]. However, although tadpoles in our study grew faster in the PE treatment after removal of the predator compared to same-age tadpoles in the PL treatment and the control, they were unable to grow to the same size at metamorphosis. The observed higher growth rate in the tadpoles with the early presence of red-eared sliders was the result of the higher growth capacity of smaller tadpoles in this treatment alone, as the growth rates of tadpoles of the same size in the PL treatment and the control were the same. Thus, in our experiment, tadpoles were unable to initiate faster growth above the usual level at a given size after removal of predation pressure but only delayed the normal growth rate until later development.

Conclusion

The timing of predator–prey interactions has fundamentally different effects at various ontogenetic stages of prey. The predator had the effect on time to metamorphosis, size, and body mass at metamorphosis only if it was present in the early development of tadpoles. In our experiment, the common occurrence of red-eared sliders and common frog tadpoles in early development resulted in a longer time to metamorphosis and a smaller size/

body mass of tadpoles than when they occurred together in the later stages of larval development. In amphibians, later metamorphosed individuals may often have a lower survival rate and growth, and a smaller size at metamorphosis is also associated with a smaller size at maturity and reduced reproduction[30,67–69], but see also[70,71]. Future studies on post-metamorphic stages may provide additional insights into the role of alien predators in common frog antipredator defences.

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References

1. Gerber, B. D., Karpanty, S. M. & Randrianantenaina, J. Activity patterns of carnivores in the rain forests of Madagascar: Implications for species coexistence. *J. Mammal.* **93**, 667–676 (2012).
2. Azevedo, F., Lemos, F., Freitas-Junior, M., Rocha, D. & Azevedo, F. Puma activity patterns and temporal overlap with prey in a human-modified landscape at Southeastern Brazil. *J. Zool.* **305**, 246–255 (2018).
3. Wellborn, G. A., Skelly, D. K. & Werner, E. E. Mechanisms creating community structure across a freshwater habitat gradient. *Annu. Rev. Ecol. Syst.* **27**, 337–363 (1996).
4. Sitvarin, M. I., Rypstra, A. L. & Harwood, J. D. Linking the green and brown worlds through nonconsumptive predator effects. *Oikos* **125**, 1057–1068 (2016).
5. Damien, M. & Tougeron, K. Prey–predator phenological mismatch under climate change. *Curr. Opin. Insect Sci.* **35**, 60–68 (2019).
6. Relyea, R. A. & Werner, E. E. Quantifying the relation between predator-induced behavior and growth performance in larval anurans. *Ecology* **80**, 2117–2124 (1999).
7. Relyea, R. A. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* **82**, 523–540 (2001).
8. Osman, R. W. & Whitlatch, R. B. The control of the development of a marine benthic community by predation on recruits. *J. Exp. Mar. Biol. Ecol.* **311**, 117–145 (2004).
9. Schmidt, B. R., Băncilă, R. I., Hartel, T., Grossenbacher, K. & Schaub, M. Shifts in amphibian population dynamics in response to a change in the predator community. *Ecosphere* **12**, e03528 (2021).
10. Falaschi, M., Melotto, A., Manenti, R. & Ficetola, G. F. Invasive species and amphibian conservation. *Herpetologica* **76**, 216–227 (2020).
11. Gamradt, S. C. & Kats, L. B. Effect of introduced crayfish and mosquito fish on California newts. *Conserv. Biol.* **10**, 1155–1162 (1996).
12. Matthews, K. R., Knapp, R. A. & Pope, K. L. Garter snake distributions in high-elevation aquatic ecosystems: Is there a link with declining amphibian populations and nonnative trout introductions?. *J. Herpetol.* **36**, 16–22 (2002).
13. Dodds, W. K. & Whiles, M. R. *Freshwater Ecology: Concepts and Environmental Applications* 3rd edn. (Elsevier, 2002).
14. Preisser, E. L., Bolnick, D. I. & Benard, M. F. Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology* **86**, 501–509 (2005).
15. Le Roux, E., Kerley, G. I. & Cromsigt, J. P. Megaherbivores modify trophic cascades triggered by fear of predation in an African savanna ecosystem. *Curr. Biol.* **28**, 2493–2499 (2018).
16. Daversa, D. *et al.* Broadening the ecology of fear: Non-lethal effects arise from diverse responses to predation and parasitism. *Proc. R. Soc. B* **288**, 20202966 (2021).
17. Benard, M. F. Predator-induced phenotypic plasticity in organisms with complex life histories. *Annu. Rev. Ecol. Evol. Syst.* **35**, 651–673 (2004).
18. Van Buskirk, J. & Schmidt, B. R. Predator-induced phenotypic plasticity in larval newts: Trade-offs, selection, and variation in nature. *Ecology* **81**, 3009–3028 (2000).
19. McCollum, S. A. & Van Buskirk, J. Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* **50**, 583–593 (1996).
20. Skelly, D. K. Tadpole communities: pond permanence and predation are powerful forces shaping the structure of tadpole communities. *Am. Sci.* **85**, 36–45 (1997).
21. Sih, A. & Moore, R. D. Delayed hatching of salamander eggs in response to enhanced larval predation risk. *Am. Nat.* **142**, 947–960 (1993).
22. Warkentin, K. M. Adaptive plasticity in hatching age: A response to predation risk trade-offs. *Proc. Natl. Acad. Sci.* **92**, 3507–3510 (1995).
23. Johnson, J. B., Saenz, D., Adams, C. K. & Conner, R. N. The influence of predator threat on the timing of a life-history switch point: Predator-induced hatching in the southern leopard frog (*Rana sphenocephala*). *Can. J. Zool.* **81**, 1608–1613 (2003).
24. Wilbur, H. M. & Fauth, J. E. Experimental aquatic food webs: Interactions between two predators and two prey. *Am. Nat.* **135**, 176–204 (1990).
25. Laurila, A. Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. *Oikos* **88**, 159–168 (2000).
26. Gomez-Mestre, I. *et al.* The shape of things to come: Linking developmental plasticity to post-metamorphic morphology in anurans. *J. Evol. Biol.* **23**, 1364–1373 (2010).
27. Vieira, E. A., Duarte, L. F. L. & Dias, G. M. How the timing of predation affects composition and diversity of species in a marine sessile community?. *J. Exp. Mar. Biol. Ecol.* **412**, 126–133 (2012).
28. Andrade, M. R., Albeny-Simões, D., Breaux, J. A., Juliano, S. A. & Lima, E. Are behavioural responses to predation cues linked across life cycle stages?. *Ecol. Entomol.* **42**, 77–85 (2017).
29. Knapp, R. A. Effects of nonnative fish and habitat characteristics on lentic herpetofauna in Yosemite National Park, USA. *Biol. Conserv.* **121**, 265–279 (2005).
30. Kiesecker, J. M. & Blaustein, A. R. Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs. *Ecology* **78**, 1752–1760 (1997).
31. Nunes, A. L., Orizaola, G., Laurila, A. & Rebelo, R. Rapid evolution of constitutive and inducible defenses against an invasive predator. *Ecology* **95**, 1520–1530 (2014).
32. Polo-Cavia, N., Gonzalo, A., López, P. & Martín, J. Predator recognition of native but not invasive turtle predators by naïve anuran tadpoles. *Anim. Behav.* **80**, 461–466 (2010).
33. Zhang, F., Zhao, J., Zhang, Y., Messenger, K. & Wang, Y. Antipredator behavioral responses of native and exotic tadpoles to novel predator. *Asian Herpetol. Res.* **6**, 51–58 (2015).
34. Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. *100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database* (Invasive Species Specialist Group, 2000).
35. TTWG. *Conservation biology of freshwater turtles and tortoises: A compilation project of the IUCN/SSC tortoise and freshwater turtle specialist group*. in *Chelonian Research Monographs 7 Turtle of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy,*

Distribution, and Conversation Status. 8th edn. (eds. Rhodin, A.G.J., Iverson, J.B., van Dijk, P.P., Saumure, R.A., Buhlmann, K.A., Pritchard, P.C.H., Mittermeier, R.A.). 1–292. (Chelonian Research Foundation and Turtle Conservancy, 2017).

36. GISD. *Global Invasive Species Database*. http://www.issg.org/database (2021).
37. Berec, M., Klapka, V. & Zemek, R. Effect of an alien turtle predator on movement activity of European brown frog tadpoles. *Ital. J. Zool.* **83**, 68–76 (2016).
38. Vodrážková, M., Šetlíková, I. & Berec, M. Chemical cues of an invasive turtle reduce development time and size at metamorphosis in the common frog. *Sci. Rep.* **10**, 1–6 (2020).
39. Gibbons, J., Greene, J. & Congdon, J. *Life history and ecology of the slider turtle*. in *Temporal and Spatial Movement Patterns of Sliders and Other Turtles* (ed. Gibbons, J.). 201–215. (Smithsonian Institution Press, 1990).
40. Formanowicz, D. R. Anuran tadpole/aquatic insect predator-prey interactions: tadpole size and predator capture success. *Herpetologica* **42**, 367–373 (1986).
41. Semlitsch, R. D. & Gibbons, J. W. Fish predation in size-structured populations of treefrog tadpoles. *Oecologia* **75**, 321–326 (1988).
42. Teplitsky, C., Piha, H., Laurila, A. & Merilä, J. Common pesticide increases costs of antipredator defenses in *Rana temporaria* tadpoles. *Environ. Sci. Technol.* **39**, 6079–6085 (2005).
43. Travis, J. Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. *Ecology* **65**, 1155–1160 (1984).
44. Wilbur, H. M. & Collins, J. P. Ecological aspects of amphibian metamorphosis: Nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. *Science* **182**, 1305–1314 (1973).
45. Gosner, K. L. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190 (1960).
46. Woodward, G. & Hildrew, A. G. Body-size determinants of niche overlap and intraguild predation within a complex food web. *J. Anim. Ecol.* **71**, 1063–1074 (2002).
47. Relyea, R. A. Getting out alive: How predators affect the decision to metamorphose. *Oecologia* **152**, 389–400 (2007).
48. Pujol-Buxó, E., San Sebastián, O., Garriga, N. & Llorente, G. A. How does the invasive/native nature of species influence tadpoles' plastic responses to predators?. *Oikos* **122**, 19–29 (2013).
49. Phuge, S., Shetye, K. & Pandit, R. Effect of water level on insect-tadpole predator-prey interactions. *Acta Oecol.* **108**, 103649 (2020).
50. Loman, J. Early metamorphosis in common frog *Rana temporaria* tadpoles at risk of drying: An experimental demonstration. *Amphibia-Reptilia* **20**, 421–430 (1999).
51. Stav, G., Kotler, B. P. & Blaustein, L. Direct and indirect effects of dragonfly (*Anax imperator*) nymphs on green toad (*Bufo viridis*) tadpoles. *Hydrobiologia* **579**, 85–93 (2007).
52. Goldberg, T., Nevo, E. & Degani, G. Phenotypic plasticity in larval development of six amphibian species in stressful natural environments. *Zool. Stud.* **51**, 345–361 (2012).
53. Kishida, O., Costa, Z., Tezuka, A. & Michimae, H. Inducible offences affect predator–prey interactions and life-history plasticity in both predators and prey. *J. Anim. Ecol.* **83**, 899–906 (2014).
54. Leips, J. & Travis, J. Metamorphic responses to changing food levels in two species of hylid frogs. *Ecology* **75**, 1345–1356 (1994).
55. Alford, R. A. & Harris, R. N. Effects of larval growth history on anuran metamorphosis. *Am. Nat.* **131**, 91–106 (1988).
56. Loman, J. Temperature, genetic and hydroperiod effects on metamorphosis of brown frogs *Rana arvalis* and *R. temporaria* in the field. *J. Zool.* **258**, 115–129 (2002).
57. Laugen, A. T. *et al.* Quantitative genetics of larval life-history traits in *Rana temporaria* in different environmental conditions. *Genet. Res.* **86**, 161–170 (2005).
58. Brodie, E. D. & Formanowicz, D. R. Prey size preference of predators: Differential vulnerability of larval anurans. *Herpetologica* **39**, 67–75 (1983).
59. Eklöv, P. & Werner, E. E. Multiple predator effects on size-dependent behavior and mortality of two species of anuran larvae. *Oikos* **88**, 250–258 (2000).
60. Urban, M. C. Predator size and phenology shape prey survival in temporary ponds. *Oecologia* **154**, 571–580 (2007).
61. Jara, F. G. & Perotti, M. G. Risk of predation and behavioural response in three anuran species: influence of tadpole size and predator type. *Hydrobiologia* **644**, 313–324 (2010).
62. Wassersug, R. J. & Sperry, D. G. The relationships of locomotion to differential predation on *Pseudacris triseriata* (Anura: Hylidae). *Ecology* **58**, 830–839 (1977).
63. Huey, R. B. Sprint velocity of tadpoles (*Bufo boreas*) through metamorphosis. *Copeia* **1980**, 537–540 (1980).
64. Laurila, A. & Kujasalo, J. Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *J. Anim. Ecol.* **68**, 1123–1132 (1999).
65. Metcalfe, N. B. & Monaghan, P. Compensation for a bad start: grow now, pay later?. *Trends Ecol. Evol.* **16**, 254–260 (2001).
66. Downie, J. & Weir, A. Developmental arrest in *Leptodactylus fuscus* tadpoles (Anura: Leptodactylidae) III effect of length of arrest period on growth potential. *Herpetol. J.* **7**, 85–92 (1997).
67. Smith, D. C. Adult recruitment in chorus frogs: Effects of size and date at metamorphosis. *Ecology* **68**, 344–350 (1987).
68. Altwegg, R. & Reyer, H. U. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* **57**, 872–882 (2003).
69. Brunelli, E. *et al.* Environmentally relevant concentrations of endosulfan impair development, metamorphosis and behaviour in *Bufo bufo* tadpoles. *Aquat. Toxicol.* **91**, 135–142 (2009).
70. Boone, M. D. Juvenile frogs compensate for small metamorph size with terrestrial growth: Overcoming the effects of larval density and insecticide exposure. *J. Herpetol.* **39**, 416–423 (2005).
71. Schmidt, B. R., Hödl, W. & Schaub, M. From metamorphosis to maturity in complex life cycles: Equal performance of different juvenile life history pathways. *Ecology* **93**, 657–667 (2012).

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Author contributions

M.V. and M.B. conceived and designed the experiment. M.V. collected the data. I.Š. designed the figures. All authors developed the statistical analyses, discussed the results and contributed to the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Competing interests

The authors declare no competing interests.

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5.4. Vyvolává i krátkodobá přítomnost nepůvodního predátora u pulců žáby chronický stres?

V další sérii experimentů nad rámec cílů této práce jsem studovala reakce na invazního predátora stanovením míry stresu u pulců měřením kortikosteronu (CORT) - hlavního stresového hormonu u obojživelníků. Měření glukokortikoidních hormonů jako primárního mediátoru stresu spojeného se stresovou reakcí u obratlovců poskytuje způsob, jak kvantifikovat fyziologické reakce na stresory. Pokud je expozice stresorům chronická, jedinci pociťují náklady na opětovné zahájení glukokortikoidové reakce a dochází ke změně hormonů osy hypotalamus–hypofýza–interrenální, jako jsou glukokortikoidy. U obojživelníků je tato osa primárním endokrinním systémem řídícím fyziologickou a behaviorální reakci na vnější stresory prostřednictvím regulace kortikosteronu. Testovala jsem, zda nový invazivní typ predátora *Trachemys scripta elegans*, vyvolává chronickou hormonální odpověď u pulců. Hladina CORT v celém těle u pulců byla měřena u jedinců chovaných za trvalé a dočasné přítomnosti predátora (pulci ve stádiu 46 dle Gosnera 1960). V obou treatmentech měli pulci nižší hladinu CORT než pulci bez vlivu predátora (12,6 ± 7,56 ng.g^{-1}). Srovnatelné hodnoty hladiny CORT v trvalé a krátkodobé přítomnosti želvy (6,8 ± 3,81 ng.g^{-1} oproti 5,4 ± 2,52 ng.g^{-1}) naznačovaly, že i krátkodobý kontakt s predátorem vede k hormonální odezvě na úroveň chronického stresu. Chronický stres je obvykle spojen s trvale sníženými hladinami CORT, což může být škodlivé a může nepříznivě ovlivnit růst a vývoj postmetamorfních jedinců.

Přiložená verze rukopisu je nedokončenou pracovní verzí, která dosud neprošla recenzí.

Short-term presence of a non-native predator induce chronic stress in common frog tadpoles

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Abstract

Amphibian tadpoles show extensive phenotypic plasticity against predators. Among others, they reduce locomotor activity and develop smaller and earlier when exposed to predators. Measuring glucocorticoid hormones as the primary stress mediator associated with the stress response in vertebrates, provides a way to quantify physiological responses to stressors. When exposure to stressors is chronic, individuals experience the cost of reinitiating a glucocorticoids response and there is a change in the hormones of the hypothalamic–pituitary–interrenal axis such as glucocorticoids. In amphibians, this axis is the primary endocrine system controlling the physiological and behavioral response to external stressors via the regulation of corticosterone (CORT). In our experiment, we tested whether a new invasive type of predator of frog tadpoles, the slider (*Trachemys scripta elegans*), induces a chronic hormonal response in common frog tadpoles (*Rana temporaria*). The whole body CORT-level of froglets of common frog was measured for individuals reared under permanent and temporal presence (i.e. tadpoles from Gosner stage 46) of caged slider. In both slider treatments, the tadpoles had a lower CORT-level than the tadpoles reared without it (12.6 ± 7.56 ng g^{-1}). Comparable CORT-level values in the permanent and short-term presence (6.8 ± 3.81 ng g^{-1} versus 5.4 ± 2.52 ng g^{-1}) of the slider indicated that even short-term contact with the slider leads to a hormonal response at the level of chronic stress. Chronic stress is usually associated with persistently reduced CORT-levels, which can be detrimental and can adversely affect the growth and development of post-metamorphic individuals.

Keywords

Antipredator defense, predation risk, corticosterone, glucocorticoids, stress physiology

Introduction

Nowadays, it is considered indisputable that the combination of multiple sources of environmental disturbance threatens the biodiversity of fauna. Amphibians are one of the most affected groups of animals globally (Stuart *et al*. 2004, Cox *et al*. 2022). Stressors that contribute to amphibian population decline include global climate change, invasive species, over exploitation, emerging infectious diseases, pesticides/pollution, and habitat loss/alteration (Collins *et* Storfer 2003, Wake *et* Vredenburg 2008, Rollins-Smith 2009, Blaustein *et al.* 2010, Hof *et al*. 2011). Understanding stress responses to sublethal anthropogenic changes has become an important issue because stress-related physiological variables can predict population declines and, consequently, can be effective tools in conservation management.

Responses to stress are often divided into two categories: acute and chronic. Acute responses are those that take place in response to short-term stressors and have a definitive onset and last for only a few hours. Chronic stress is defined as either multiple, frequent exposure to stressors and/or long term constant exposure to stressors, and is usually measured by changes in hormones of the hypothalamic–pituitary–interrenal (HPI) axis such as glucocorticoids (GCs) and corticotropin releasing factor, the catecholamines epinephrine and norepinephrine, and changes in immune factors such as cytokines and lymphocytes. These hormones and cytokines are often referred to as primary stress mediators (reviewed in McEwen 1998, Cyr *et* Romero 2009).

A way to quantify physiological responses to stressors is provided by measuring glucocorticoid (GC) hormones associated with the stress response in vertebrates. In amphibians, the hypothalamo–pituitary–interrenal (HPI) axis is the primary endocrine system controlling the physiological and behavioral response to external stressors via the regulation of corticosterone (CORT), the main glucocorticoid hormone in amphibians (Idler 2012). When individuals are exposed to acute or short-term stressors, circulating CORT levels frequently become elevated (Romero 2004). When a perturbation is perceived as a stressor by the brain, the hypothalamus secretes corticotropin releasing hormone (CRH) which induces the pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH is transported by blood to the adrenal cortex, which then releases glucocorticoids (GCs) (Denver 2009). This adaptive temporary increase in CORT mobilizes available energy by down-regulating functions not essential to immediate survival, such as reproduction, immune function and growth (Sapolsky *et al*. 2000, Bridges 2002, Jermacz *et al*. 2020). In the short term, a GC response is advantageous as it increases the chances of surviving a life-threatening situation; however, it can have detrimental effects on the future fitness of an individual, particularly when stress situations occur frequently or become chronic (Sapolsky 2002). When exposure to stressors is chronic, individuals experience costs of repeatedly mounting a CORT response, and may eventually lose the ability to respond physiologically (Cyr *et* Romero 2009). Hence, the GC response, initially evolved to survive stressors, can also contribute negatively to many fitness components, such as reproductive output, growth and developmental rate (Moore *et* Jessop 2003, Ellis *et al*. 2006). Reduction in metamorphic size and lipid levels can lead to slower growth and lower survival rates during the terrestrial stage (Altwegg *et* Reyer 2003, Scott *et al*. 2007). Additionally, frequent exposure to early life stressors can alter the CORT response to stressors at later life stages. Tadpoles exposed to frequent stressors grow more slowly and have downregulated CORT responses to handling stress, as well as reduced fat storage and growth rates as adults, although some individuals have exhibited catch-up growth depending on the stressor they experienced (Hu *et al*. 2008, Warne *et* Crespi 2015).

While the deleterious effects of chronic stress are theoretically well described, few empirical studies showing the loss of fitness or pathology and subsequent demographic consequences of allostatic overload in wild populations have been published so far (Boonstra 2013, Jessop *et al*. 2013, see also Sapolsky *et al*. 2000). Exceptions include several studies in wild populations that have shown that predation pressure causes indirect and negative glucocorticoid-mediated effects on individual fitness, leading to reduced prey population growth (Boonstra *et al*. 1998, Creel *et al*. 2009, Zanette *et al*. 2011). Additionally, a number of studies attribute population declines to the effects of chronic stress in wild mammals (Boonstra *et* Singleton 1993, Pride 2004, Charbonnel *et al*. 2008, Middlemis *et al*. 2013).

Whether intentionally or unintentionally introduced, the recent wide occurrence of the red-eared slider (*Trachemys scripta elegans*) in Europe (GISD 2022) presents a new opportunity to investigate the responses of naive native amphibian populations to a new predator. In their native range, red-eared sliders are opportunistic omnivores (Ernst *et* Lovich 2009). Although turtles were not mentioned among the non-native predators of amphibians (Bucciarelli *et al*. 2014), our previous research (Berec *et al*. 2016, Vodrážková *et al*. 2020, 2022a, 2022b) revealed that tadpoles of common frogs (*Rana temporaria*) recognize red-eared sliders as potential predators. Specifically, we found that the presence of the sliders affect several life history parameters of common frog (*Rana temporaria*) tadpoles, such as movement activity, trajectory of movement (Berec *et al*. 2016), time to metamorphosis, or size at metamorphosis in the permanent presence of a predator (Vodrážková *et al*. 2020), hatching time and Gosner stage during hatching (Vodražková *et al*. 2022b) and if the red-eared slider is present early in tadpole development, individuals grow more slowly and metamorphose to smaller sizes and lower body mass (Vodrážková *et al*. 2022a).

Here, we studied the stress response of common frog tadpoles under permanent and short-term presence of a slider. Specifically, we quantified stress hormone (CORT) of tadpoles under permanent presence of predator and short-term presence of predator shortly before metamorphosis. If predator presence is perceived as an acute stressor, then we predicted higher CORT level than CORT level in tadpoles kept without predator. Contrary, if predator presence is perceived as a chronic stressor, then we predicted comparable or lower CORT level than CORT level in tadpoles kept without predator. These results can be of great importance in slider management in non-native areas.

Methods (in preparation)

Experimental design: Each of three treatments (permanent presence, short-term presence (3 days prior to metamorphosis), and control) contained two glass tanks, for a total of 6 tanks in the experiment, with each glass tank containing 24 tadpoles. In the control treatment, no slider was added to the glass tanks. In the permanent and short-term slider presence treatments, one slider was added to each of the two replicate glass tanks in each treatment.

Statistical analysis

The data of CORT-level was normalized using log transformation. We tested the effect of slider presence on CORT-level using an analysis of covariance (ANCOVA) with measurement time as an independent continuous variable. Slider presence/absence and glass tank were used as independent discrete variables. We confirmed the homogeneity of regression slopes of day time on CORT-level in all treatments. The normality and the homogeneity of the variances were checked using the Shapiro-Wilk test and the Bartlett test, respectively. All data analyses were performed in Statistica 13.

Results

The presence of the slider significantly influenced the CORT-level of tadpoles (F(2,67)=11.44, $p<0.001$). In both slider treatments, the CORT-levels were approximately half of that in the control (Fig. 1). The values of CORT-levels in the permanent and short-term slider presence were 6.8 ± 3.81 ng g^{-1} and 5.4 ± 2.52 ng g^{-1}, respectively, while in the control the mean CORT-level was 12.6 ± 7.56 ng g^{-1} (mean ± S.D.). The effects of both the measurement time and the glass tank were insignificant (F(1,67)=1.28, $p=0.261$ and F(1,67)=0.88, $p = 0.351$, respectively).

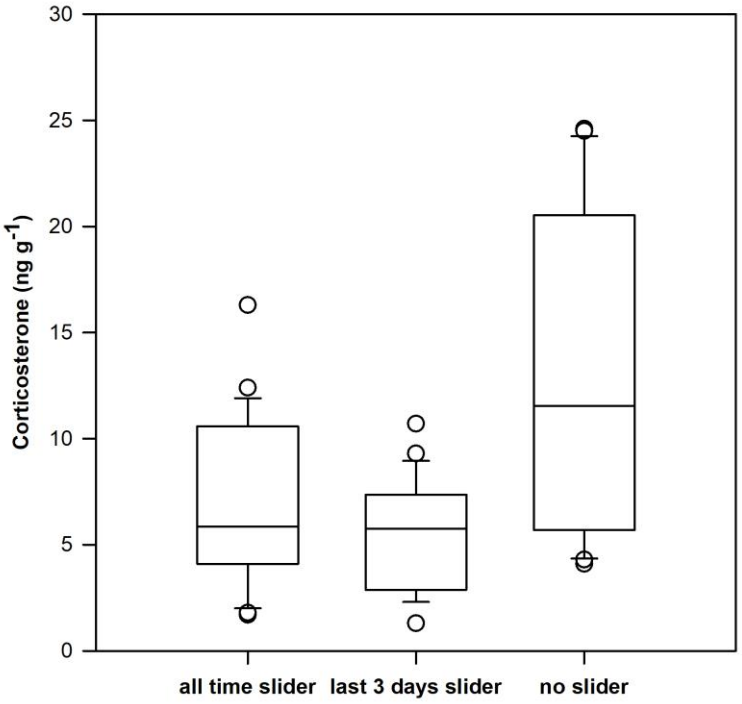

Fig. 1. Concentration of corticosterone (ng g^{-1}) in the treatments. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles.

Discussion (in preparation)

Whole-body corticosterone determination is useful as a general indicator of stress. The stress response is being used increasingly asa biomonitor for potentially threatened populations (e.g. Creel *et al*. 1997, Wasser *et al*. 1997), and elevated glucocorticoid concentrations may be useful in predicting survival of individuals in stressed populations (Romero *et* Wikelski 2001). Glucocorticoid hormones are released in response to stressful environmental stimuli (such as predators) and can have beneficial short-term effects, such as temporary suppression of reproduction, increased foraging, immune system regulation and increased gluconeogenesis (e.g.Wingfield et al. 1997, Romero *et* Wikelski 2001). If the stressor is long lasting (i.e. chronic), however,glucocorticoids can have negative consequences for an animal, such as shutdown of reproduction, chronic suppression of both immune function and growth, and neuronal cell death (Sapolsky 1992, Romero *et* Wikelski 2001). Evidence from many species indicates that robust corticosterone increases in response to an acute stressor(e.g. capture) are indicative of a healthy individual free of long-term chronic stress (Romero *et* Wikelski 2001).

Our results are consistent with the study Bryant *et al*. (2022), amphibian larvae responded to the presence of invasive predators by lowering corticosterone levels than individuals without predators. These studies indicate that predator presence alters glucocorticoid regulation, which may allow species to better cope with native and introduced predators.

Our studies (Vodrážková *et al*. 2020, 2022a, 2022b) suggest that the corticosteroid pathway may mediate tadpole morphological response to perceived predation risk. Our results suggest that corticosteroid adjustment may impact prey survival through phenotypic change upon exposure to predation risk and thereby suggest a possible functional role of this hormonal pathway in amphibian physiological ecology.

6. Závěr

Tato studie přináší nové poznatky v oblasti autekologie vodních organismů na základě sledování rychlosti ontogenetického vývoje skokana *Rana temporaria* ovlivněného kairomony želvy *Trachemys scripta elegans*.

Přítomnost invazní želvy, jako predátora, ovlivnila všechny zkoumané faktory uvedené v této práci. V přítomnosti predátora se embrya líhla dříve, menší a v nižším vývojovém stádiu vývoje než bez něj. Kontinuální přítomnost želv v larválním stádiu zkrátila dobu metamorfózy a výsledná velikost při metamorfóze byla menší než v kontrolní skupině. Pokud byl predátor přítomen v raném vývoji larválního období, v době metamorfózy měli jedinci při metamorfóze menší velikost a nižší tělesnou hmotnost než pulci, kteří se vyvíjeli bez predátora. Časná přítomnost predátora navíc snižovala růstové přírůstky pulců. Výsledky ukazují spíše vzácně dokumentované typy reakcí kořisti obojživelníků na predátory. Tato studie rozšiřuje okruh studovaných predátorů, včetně vlivu na různé fáze vývoje potenciální kořisti.

Závěrem lze konstatovat, že výsledky mého výzkumu naznačují, že pulci *Rana temporaria* jsou, navzdory absenci společné evoluční historie s želvou *Trachemys scripta*, schopni rozlišovat chemické stimuly tohoto druhu specifické pro predátory a následně reagovat na riziko predace zkrácením larválního i embryonálního období svého vývoje. Přestože úspěšné rozmnožování *Trachemys scripta* nebylo zaznamenáno ve všech oblastech jejího nepůvodního areálu, včetně České republiky (Heidy *et al*. 2010, Ficetola *et al*. 2012), její schopnost dlouhodobého přežití v suboptimálních podmínkách z něj činí hrozbu pro populace našich obojživelníků.

7. Seznam použité literatury

Alford, R. A. (1986). Effects of parentage on competitive ability and vulnerability to predation in Hyla chrysoscelis tadpoles. Oecologia, 68(2), 199-204.

Alford, R. A., & Harris, R. N. (1988). Effects of larval growth history on anuran metamorphosis. The American Naturalist, 131(1), 91-106.

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

Allan, J. D., Castillo, M. M., & Capps, K. A. (2021). Stream ecology: structure and function of running waters. Springer Nature.

Allen, C. R., Epperson, D. M., & Garmestani, A. S. (2004). Red imported fire ant impacts on wildlife: a decade of research. The American Midland Naturalist, 152(1), 88-103.

Altwegg, R., & Reyer, H. U. (2003). Patterns of natural selection on size at metamorphosis in water frogs. Evolution, 57(4), 872-882.

Anholt, B. R., & Werner, E. E. (1995). Interaction between food availability and predation mortality mediated by adaptive behavior. Ecology, 76(7), 2230-2234.

Anson, J. R., & Dickman, C. R. (2013). Behavioral responses of native prey to disparate predators: naiveté and predator recognition. Oecologia, 171(2), 367-377.

Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A., & McGregor, I. S. (2005). The effects of predator odors in mammalian prey species: a review of field and laboratory studies. Neuroscience & Biobehavioral Reviews, 29(8), 1123-1144.

Apfelbach, R., Parsons, M. H., Soini, H. A., & Novotny, M. V. (2015). Are single odorous components of a predator sufficient to elicit defensive behaviors in prey species?. Frontiers in Neuroscience, 9, 263.

Atkinson, I. A. (1985). The spread of commensal species of *Rattus* to oceanic islands and their effects on island avifaunas. ICPB Tech Publ, 3, 35-81.

Atkinson, I. (1989). Introduced animals and extinctions. Conservation for the twenty-first century., 59-75.

Atkins, R., Blumstein, D. T., Moseby, K. E., West, R., Hyatt, M., & Letnic, M. (2016). Deep evolutionary experience explains mammalian responses to predators. Behavioral Ecology and Sociobiology, 70(10), 1755-1763.

Azevedo, F. C., Lemos, F. G., Freitas-Junior, M. C., Rocha, D. G., & Azevedo, F. C. C. (2018). Puma activity patterns and temporal overlap with prey in a human-modified landscape at Southeastern Brazil. Journal of Zoology, 305(4), 246-255.

Balian, E. V., Segers, H., Martens, K., & Lévéque, C. (2007). The freshwater animal diversity assessment: an overview of the results. Freshwater animal diversity assessment, 627-637.

Banks, P. B., & Dickman, C. R. (2007). Alien predation and the effects of multiple levels of prey naiveté. Trends in ecology & evolution, 22(5), 229-30.

Bates, A. E. McKelvie, C. M. Sorte, C. J. Morley, S. A. Jones, N. A. Mondon, J. A. ... & Quinn, G. (2013). Geographical range, heat tolerance and invasion success in aquatic species. Proceedings of the Royal Society B: Biological Sciences, 280(1772), 20131958.

Benard, M. F. (2004). Predator-induced phenotypic plasticity in organisms with complex life histories. Annual Review of Ecology, Evolution, and Systematics, 651-673.

Bennett, A. M., Pereira, D., & Murray, D. L. (2013). Investment into defensive traits by anuran prey (*Lithobates pipiens*) is mediated by the starvation-predation risk trade-off. PLoS One, 8(12), e82344.

Berec, M., Klapka, V., & Zemek, R. (2016). Effect of an alien turtle predator on movement activity of European brown frog tadpoles. Italian Journal of Zoology, 83(1), 68-76.

Berger, J., Stacey, P. B., Bellis, L., & Johnson, M. P. (2001). A mammalian predator–prey imbalance: grizzly bear and wolf extinction affect avian neotropical migrants. Ecological Applications, 11(4), 947-960.

Binckley, C. A., & Resetarits Jr, W. J. (2003). Functional equivalence of non-lethal effects: generalized fish avoidance determines distribution of gray treefrog, *Hyla chrysoscelis*, larvae. Oikos, 102(3), 623-629.

Blackburn, T. M., Cassey, P., Duncan, R. P., Evans, K. L., & Gaston, K. J. (2004). Avian extinction and mammalian introductions on oceanic islands. Science, 305(5692), 1955-1958.

Blaustein, L., Garb, J. E., Shebitz, D., & Nevo, E. (1999). Microclimate, developmental plasticity and community structure in artificial temporary pools. Hydrobiologia, 392(2), 187-196.

Blaustein, A. R., Walls, S. C., Bancroft, B. A., Lawler, J. J., Searle, C. L., & Gervasi, S. S. (2010). Direct and indirect effects of climate change on amphibian populations. Diversity, 2(2), 281-313.

Blumstein, D. T. (2002). Moving to suburbia: ontogenetic and evolutionary consequences of life on predator-free islands. Journal of Biogeography, 29(5-6), 685-692.

Blumstein, D. T., Mari, M., Daniel, J. C., Ardron, J. G., Griffin, A. S., & Evans, C. S. (2002). Olfactory predator recognition: wallabies may have to learn to be wary. In Animal Conservation Forum (Vol. 5, No. 2, pp. 87-93). Cambridge University Press.

Bobeldyk, A. M., Rüegg, J., & Lamberti, G. A. (2015). Freshwater hotspots of biological invasion are a function of species–pathway interactions. Hydrobiologia, 746(1), 363-373.

Boonstra, R. (2013). Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. Functional Ecology, 27(1), 11-23.

Boonstra, R., Hik, D., Singleton, G. R., & Tinnikov, A. (1998). The impact of predator-induced stress on the snowshoe hare cycle. Ecological monographs, 68(3), 371-394.

Boonstra, R., & Singleton, G. R. (1993). Population declines in the snowshoe hare and the role of stress. General and comparative endocrinology, 91(2), 126-143.

Bosch, J., Rincón, P. A., Boyero, L. U. Z., & Martínez-Solano, I. Ñ. I. G. O. (2006). Effects of introduced salmonids on a montane population of Iberian frogs. Conservation Biology, 20(1), 180-189.

Bridges, C. M. (2002). Tadpoles balance foraging and predator avoidance: effects of predation, pond drying, and hunger. Journal of Herpetology, 627-634.

Brodie Jr, E. D., & Formanowicz Jr, D. R. (1983). Prey size preference of predators: differential vulnerability of larval anurans. Herpetologica, 67-75.

Brodie Jr, E. D., & Formanowicz Jr, D. R. (1987). Antipredator mechanisms of larval anurans: protection of palatable individuals. Herpetologica, 369-373.

Brown, J. H., & Lomolino, M. V. (1998). Biogeography, 2nd Sinauer Associates: Sunderland. MA, USA.

Brönmark, C., & Hansson, L. A. (2000). Chemical communication in aquatic systems: an introduction. Oikos, 88(1), 103-109.

Bryant, A. R., Gabor, C. R., Swartz, L. K., Wagner, R., Cochrane, M. M., & Lowe, W. H. (2022). Differences in Corticosterone Release Rates of Larval Spring Salamanders (*Gyrinophilus porphyriticus*) in Response to Native Fish Presence. Biology, 11(4), 484.

Bryce, R., Oliver, M. K., Davies, L., Gray, H., Urquhart, J., & Lambin, X. (2011). Turning back the tide of American mink invasion at an unprecedented scale through community participation and adaptive management. Biological conservation, 144(1), 575-583.

Bucciarelli, G. M., Blaustein, A. R., Garcia, T. S., & Kats, L. B. (2014). Invasion complexities: the diverse impacts of nonnative species on amphibians. Copeia, 2014(4), 611-632.

Buchan, L. A., & Padilla, D. K. (1999). Estimating the probability of long-distance overland dispersal of invading aquatic species. Ecological applications, 9(1), 254-265.

Buckley, C. R., Michael, S. F., & Irschick, D. J. (2005). Early hatching decreases jumping performance in a direct-developing frog, *Eleutherodactylus coqui*. Functional ecology, 67-72.

Bulen, B. J., & Distel, C. A. (2011). Carbaryl concentration gradients in realistic environments and their influence on our understanding of the tadpole food web. Archives of environmental contamination and toxicology, 60(2), 343-350.

Buxton, V., Ward, M. P., & Sperry, C. (2016). Conspecific Attraction in Anuran Amphibians. In Great Waters, Great Lands, Great Responsibilities: 76th Midwest Fish & Wildlife Conference, January 24-27, 2016, Grand Rapids Michigan.

Caldwell, J. P. (1982). Disruptive selection: a tail color polymorphism in *Acris* tadpoles in response to differential predation. Canadian Journal of Zoology, 60(11), 2818-2827.

Caldwell, J. P., Thorp, J. H., & Jervey, T. O. (1980). Predator-prey relationships among larval dragonflies, salamanders, and frogs. Oecologia, 46(3), 285-289.

Cambray, J. A. (2003). Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. Hydrobiologia, 500(1), 217-230.

Capellán, E., & Nicieza, A. G. (2007). Trade-offs across life stages: does predator–induced hatching plasticity reduce anuran post-metamorphic performance?. Evolutionary Ecology, 21(4), 445-458.

Case, T. J., Bolger, D. T., & Richman, A. D. (1992). Reptilian extinctions: the last ten thousand years. In Conservation biology. Springer, Boston, MA, 91-125.

Charbonnel, N., Chaval, Y., Berthier, K., Deter, J., Morand, S., Palme, R., & Cosson, J. F. (2008). Stress and demographic decline: a potential effect mediated by impairment of reproduction and immune function in cyclic vole populations. Physiological and Biochemical Zoology, 81(1), 63-73.

Chen, T. H. (2006). Distribution and status of the introduced red-eared slider (*Trachemys scripta elegans*) in Taiwan. Assessment and Control of Biological Invasion Risks. Shoukadoh Book Sellers, Kyoto, Japan and IUCN, Gland, Switzerland, 187-195.

Chivers, D. P., & Ferrari, M. C. (2013). Tadpole antipredator responses change over time: what is the role of learning and generalization?. Behavioral Ecology, 24(5), 1114-1121.

Chivers, D. P., Kiesecker, J. M., Marco, A., Wildy, E. L., & Blaustein, A. R. (1999). Shifts in life history as a response to predation in western toads (*Bufo boreas*). Journal of Chemical Ecology, 25(11), 2455-2463.

Chivers, D. P., Kiesecker, J. M., Marco, A., Devito, J., Anderson, M. T., & Blaustein, A. R. (2001). Predator-induced life history changes in amphibians: egg predation induces hatching. Oikos, 92(1), 135-142.

Chivers, D. P., Mathiron, A., Sloychuk, J. R., & Ferrari, M. C. (2015). Responses of tadpoles to hybrid predator odours: strong maternal signatures and the potential risk/response mismatch. Proceedings of the Royal Society B: Biological Sciences, 282(1809), 20150365.

Clark, C. W., & Harvell, C. D. (1992). Inducible defenses and the allocation of resources: a minimal model. The American Naturalist, 139(3), 521-539.

Collins, J. P., & Storfer, A. (2003). Global amphibian declines: sorting the hypotheses. Diversity and distributions, 9(2), 89-98.

Cox, J. G., & Lima, S. L. (2006). Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. Trends in Ecology & Evolution, 21(12), 674-680.

Cox, N., Young, B. E., Bowles, P., Fernandez, M., Marin, J., Rapacciuolo, G., ... & Xie, Y. (2022). A global reptile assessment highlights shared conservation needs of tetrapods. Nature, 605(7909), 285-290.

Creel, S., Creel, N. M., & Monfort, S. L. (1997). Radiocollaring and stress hormones in African wild dogs. Conservation Biology, 11(2), 54.

Creel, S., Winnie Jr, J. A., & Christianson, D. (2009). Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. Proceedings of the National Academy of Sciences, 106(30), 12388-12393.

Cronin, J. T., & Travis, J. (1986). Size-limited predation on larval *Rana areolata* (Anura: Ranidae) by two species of backswimmer (Insecta: Hemiptera: Notonectidae). Herpetologica, 171-174.

Crossland, M. R. (1998). A comparison of cane toad and native tadpoles as predators of native anuran eggs, hatchlings and larvae. Wildlife Research, 25(4), 373-381.

Crump, M. L. (1984). Ontogenetic changes in vulnerability to predation in tadpoles of *Hyla pseudopuma*. Herpetologica, 265-271.

Crump, M. L. (1986). Cannibalism by younger tadpoles: another hazard of metamorphosis. Copeia, 1986(4), 1007-1009.

Crump, M. L., & Vaira, M. (1991). Vulnerability of *Pleurodema borelli* tadpoles to an avian predator: effect of body size and density. Herpetologica, 316-321.

Cyr, N. E., & Romero, L. M. (2009). Identifying hormonal habituation in field studies of stress. General and comparative endocrinology, 161(3), 295-303.

Daisie (2009). Handbook of alien species in Europe. Springer, Dordrecht, 428.

Damien, M., & Tougeron, K. (2019). Prey–predator phenological mismatch under climate change. Current opinion in insect science, 35, 60-68.

Denver, R. J. (1997). Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. Hormones and behavior, 31(2), 169-179.

Denver, R. J. (2009). Stress hormones mediate environment-genotype interactions during amphibian development. General and comparative endocrinology, 164(1), 20-31.

DeSantis, D. L., Davis, D. R., & Gabor, C. R. (2013). Chemically mediated predator avoidance in the Barton Springs salamander (*Eurycea sosorum*). Herpetologica, 69(3), 291-297.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. Trends in ecology & evolution, 13(2), 77-81.

Diamond, J. M. (1989). Overview of recent extinctions. Conservation for the twenty-first century, 37–41.

Dodson, S. & Frey, D. (2001). In: J. Thorp, and A. Covich (Eds) Ecology and Classification of North American Freshwater Invertebrates, 849-913.

Dornelas, M., Gotelli, N. J., McGill, B., Shimadzu, H., Moyes, F., Sievers, C., & Magurran, A. E. (2014). Assemblage time series reveal biodiversity change but not systematic loss. Science, 344(6181), 296-299.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., ... & Sullivan, C. A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews, 81(2), 163-182.

Ebenhard, T. (1988). Introduced birds and mammals and their ecological effects. Swedish Wildlife Research (Sweden).

Ellis, B. J., Jackson, J. J., & Boyce, W. T. (2006). The stress response systems: Universality and adaptive individual differences. Developmental Review, 26(2), 175-212.

Ehrlich, P. R. (1986). Ecology of biological invasions of North America and Hawaii Mooney HA, Drake JA, editors, 79-95.

Elton, C. S. (2020). The ecology of invasions by animals and plants. Springer Nature.

Epelbaum, A., Pearce, C. M., Barker, D. J., Paulson, A., & Therriault, T. W. (2009). Susceptibility of non-indigenous ascidian species in British Columbia (Canada) to invertebrate predation. Marine Biology, 156(6), 1311-1320.

Epp, K. J., & Gabor, C. R. (2008). Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. Ethology, 114(6), 607-615.

Ernst, C. H., & Lovich, J. E. (2009). Turtles of the united states and Canada. JHU Press.

Falaschi, M., Melotto, A., Manenti, R., & Ficetola, G. F. (2020). Invasive species and amphibian conservation. Herpetologica, 76(2), 216-227.

Ferrari, M. C., Messier, F., & Chivers, D. P. (2008). Can prey exhibit threat-sensitive generalization of predator recognition? Extending the predator recognition continuum hypothesis. Proceedings of the Royal Society B: Biological Sciences, 275(1644), 1811-1816.

Ferrari, M. C., Wisenden, B. D., & Chivers, D. P. (2010). Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. Canadian Journal of Zoology, 88(7), 698-724.

Ficetola, G. F., Rödder, D. & Padoa-Schioppa, E. (2012). In Handbook of global freshwater invasive species (ed R Francis) 331-339 (Earthscan, Taylor and Francis Group).

Formanowicz Jr, D. R., & Brodie Jr, E. D. (1982). Relative palatabilities of members of a larval amphibian community. Copeia, 91-97.

Formanowicz Jr, D. R. (1986). Anuran tadpole/aquatic insect predator-prey interactions: tadpole size and predator capture success. Herpetologica, 367-373.

Frankel, O., & Soulé, M. E. (1981). Conservation and evolution. CUP Archive.

Fritts, T. H. (1988). Demise of an insular avifauna: the brown tree snake on Guam. Transactions of the Western Section of the Wildlife Society, 24, 31-37.

Früh, D. Stoll, S. & Haase, P. (2012). Physico-chemical variables determining the invasion risk of freshwater habitats by alien mollusks and crustaceans. Ecology and Evolution, 2(11), 2843-2853.

Gabriel, W. (1999). Evolution of reversible plastic responses: inducible defenses and environmental tolerance. The Ecology and Evolution of inducible defenses, 286-305.

Gaeta, J. W., Hrabik, T. R., Sass, G. G., Roth, B. M., Gilbert, S. J., & Vander Zanden, M. J. (2015). A whole-lake experiment to control invasive rainbow smelt (Actinoperygii, Osmeridae) via overharvest and a food web manipulation. Hydrobiologia, 746(1), 433-444.

Gazzola, A., Balestrieri, A., Ghitti, M., Paganelli, D., & Galeotti, P. (2017). Behavioural and life history responses to predation risk by common frog tadpoles exposed to two predators during ontogeny. acta ethologica, 20(3), 235-241.

Genovesi, P. (2005). Eradications of invasive alien species in Europe: a review. Biological invasions, 7(1), 127-133.

Gerber, B. D., Karpanty, S. M., & Randrianantenaina, J. (2012). Activity patterns of carnivores in the rain forests of Madagascar: implications for species coexistence. Journal of Mammalogy, 93(3), 667-676.

GISD. Global Invasive Species Database. http://www.issg.org/database (2022).

Gomez-Mestre, I., Saccoccio, V. L., Iijima, T., Collins, E. M., Rosenthal, G. G., & Warkentin, K. M. (2010). The shape of things to come: linking developmental plasticity to post-metamorphic morphology in anurans. Journal of Evolutionary Biology, 23(7), 1364-1373.

Gonzalo, A., López, P., & Martín, J. (2009). Learning, memorizing and apparent forgetting of chemical cues from new predators by Iberian green frog tadpoles. Animal cognition, 12(5), 745-750.

Gordon, D. R. Onderdonk, D. A. Fox, A. M. & Stocker, R. K. (2008). Consistent accuracy of the Australian weed risk assessment system across varied geographies. Diversity and Distributions, 14(2), 234-242.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica, 16(3), 183-190.

Greenbaum, E. (2004). The influence of prey-scent stimuli on predatory behavior of the North American copperhead *Agkistrodon contortrix* (Serpentes: Viperidae). Behavioral Ecology, 15(2), 345-350.

Groner, M. L., Buck, J. C., Gervasi, S., Blaustein, A. R., Reinert, L. K., Rollins-Smith, L. A., ... & Relyea, R. A. (2013). Larval exposure to predator cues alters immune function and response to a fungal pathogen in post-metamorphic wood frogs. Ecological Applications, 23(6), 1443-1454.

Grözinger, F., Wertz, A., Thein, J., Feldhaar, H., & Rödel, M. O. (2012). Environmental factors fail to explain oviposition site use in the European common frog. Journal of Zoology, 288(2), 103-111.

Hackländer, K., & Zachos, F. E. (Eds.). (2020). Mammals of Europe-Past, Present, and Future. London: Springer.

Hansen, G. J., Hein, C. L., Roth, B. M., Vander Zanden, M. J., Gaeta, J. W., Latzka, A. W., & Carpenter, S. R. (2013). Food web consequences of long-term invasive crayfish control. Canadian Journal of Fisheries and Aquatic Sciences, 70(7), 1109-1122.

Harvell, C. D. (1990). The ecology and evolution of inducible defenses. The Quarterly review of biology, 65(3), 323-340.

Hassan, A., & Ricciardi, A. (2014). Are non-native species more likely to become pests? Influence of biogeographic origin on the impacts of freshwater organisms 3. Frontiers in Ecology and the Environment, 12(4), 218-223.

Havel, J. E., Shurin, J. B., & Jones, J. R. (2002). Estimating dispersal from patterns of spread: spatial and local control of lake invasions. Ecology, 83(12), 3306-3318.

Havel, J. E. Kovalenko, K. E. Thomaz, S. M. Amalfitano, S. & Kats, L. B. (2015). Aquatic invasive species: challenges for the future. Hydrobiologia, 750(1), 147-170.

Heidy Kikillus, K., Hare, K. M., & Hartley, S. (2010). Minimizing false-negatives when predicting the potential distribution of an invasive species: A bioclimatic envelope for the red-eared slider at global and regional scales. Animal Conservation, 13, 5-15.

Hensley, F. R. (1993). Ontogenetic loss of phenotypic plasticity of age at metamorphosis in tadpoles. Ecology, 74(8), 2405-2412.

Hettyey, A., Roelli, F., THUeRLIMANN, N. I. N. A., ZUeRCHER, A. C., & Van Buskirk, J. (2012). Visual cues contribute to predator detection in anuran larvae. Biological Journal of the Linnean Society, 106(4), 820-827.

Heyer, W. R., McDiarmid, R. W., & Weigmann, D. L. (1975). Tadpoles, predation and pond habitats in the tropics. Biotropica, 100-111.

Hof, C., Araújo, M. B., Jetz, W., & Rahbek, C. (2011). Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature, 480*(7378), 516-519.

Hu, F., Crespi, E. J., & Denver, R. J. (2008). Programming neuroendocrine stress axis activity by exposure to glucocorticoids during postembryonic development of the frog, *Xenopus laevis*. Endocrinology, 149(11), 5470-5481.

Huey, R. B. (1980). Sprint velocity of tadpoles (*Bufo boreas*) through metamorphosis. Copeia, 1980(3), 537-540.

Hulme, P. E., Pyšek, P., Jarošík, V., Pergl, J., Schaffner, U., & Vila, M. (2013). Bias and error in understanding plant invasion impacts. Trends in ecology & evolution, 28(4), 212-218.

Hutterer, R., & Kryštufek, B. (2020). A history of mammal research in Europe. Mammals of Europe-Past, Present, and Future, 9-30.

Idler, D. R. (Ed.). (2012). Steroids in nonmammalian vertebrates. Elsevier.

Ireland, D. H., Wirsing, A. J., & Murray, D. L. (2007). Phenotypically plastic responses of green frog embryos to conflicting predation risk. Oecologia, 152(1), 162-168.

Jablonski, D., & Balej, P. (2014). Case of defensive behavior in *Pelobates syriacus* (Amphibia: Pelobatidae). Herpetol Notes, 7, 141-143.

Jeschke, J., Aparicio, L. G., Haider, S., Heger, T., Lortie, C., Pyšek, P., & Strayer, D. (2012). Support for major hypotheses in invasion biology is uneven and declining. NeoBiota, 14, 1-20.

Jessop, T. S., Woodford, R., & Symonds, M. R. (2013). Macrostress: do large-scale ecological patterns exist in the glucocorticoid stress response of vertebrates?. Functional Ecology, 27(1), 120-130.

Johnson, L. E., & Padilla, D. K. (1996). Geographic spread of exotic species: ecological lessons and opportunities from the invasion of the zebra mussel *Dreissena polymorpha*. Biological conservation, 78(1-2), 23-33.

Johnson, J. B., Saenz, D., Adams, C. K., Conner, R. N. (2003) The influence of predator threat on the timing of a life-history switch point: predator-induced hatching in the southern leopard frog (*Rana sphenocephala*). Canadian Journal of Zoology 81: 1608-1613.

Kats, L. B., & Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience, 5(3), 361-394.

Karatayev, A. Y. Burlakova, L. E. Padilla, D. K. Mastitsky, S. E. & Olenin, S. (2009). Invaders are not a random selection of species. Biological Invasions, 11(9).

Kiesecker, J. M., & Blaustein, A. R. (1997). Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs. Ecology, 78(6), 1752-1760.

Kishida, O., Costa, Z., Tezuka, A., & Michimae, H. (2014). Inducible offences affect predator–prey interactions and life-history plasticity in both predators and prey. Journal of Animal Ecology, 83(4), 899-906.

Kloskowski, J., Nieoczym, M., & Stryjecki, R. (2020). Between-habitat distributions of pond tadpoles and their insect predators in response to fish presence. Hydrobiologia, 847(5), 1343-1356.

Knapp, R. A., Matthews, K. R., & Sarnelle, O. (2001). Resistance and resilience of alpine lake fauna to fish introductions. Ecological monographs, 71(3), 401-421.

Knapp, R. A., Boiano, D. M., & Vredenburg, V. T. (2007). Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog, *Rana muscosa*). Biological Conservation, 135(1), 11-20.

Knapp, R. A., & Sarnelle, O. (2008). Recovery after local extinction: factors affecting re-establishment of alpine lake zooplankton. Ecological Applications, 18(8), 1850-1859.

Kolar, C. S. & Lodge, D. M. (2002). Ecological predictions and risk assessment for alien fishes in North America. Science, 298(5596), 1233-1236.

Kraus, F. (Eds.). (2009). Alien reptiles and amphibians: a scientific compendium and analysis. Dordrecht: Springer Netherlands.

Laurila, A. (2000). Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. Oikos, 88(1), 159-168.

Laurila, A., Järvi-Laturi, M., Pakkasmaa, S., & Merilä, J. (2004). Temporal variation in predation risk: stage-dependency, graded responses and fitness costs in tadpole antipredator defences. Oikos, 107(1), 90-99.

Laurila, A., & Kujasalo, J. (1999). Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. Journal of Animal Ecology, 68(6), 1123-1132.

Laurila, A., Pakkasmaa, S., Crochet, P. A., & Merilä, J. (2002). Predator-induced plasticity in early life history and morphology in two anuran amphibians. Oecologia, 132(4), 524-530.

Laurila, A., Pakkasmaa, S., & Merilä, J. (2001). Influence of seasonal time constraints on growth and development of common frog tadpoles: a photoperiod experiment. Oikos, 95(3), 451-460.

Leung, B. Lodge, D. M. Finnoff, D. Shogren, J. F. Lewis, M. A. & Lamberti, G. (2002). An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society of London B: Biological Sciences, 269(1508), 2407-2413.

Lima, S. L. (1998). Nonlethal effects in the ecology of predator-prey interactions. Bioscience, 48(1), 25-34.

Lodge, D. M., Taylor, C. A., Holdich, D. M., & Skurdal, J. (2000). Nonindigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. Fisheries, 25(8), 7-20.

Loope, L. L., Hamann, O., & Stone, C. P. (1988). Comparative conservation biology of oceanic archipelagoes: Hawaii and the Galapagos. BioScience, 272-282.

Lowe, S. Browne, M. Boudjelas, S. & De Poorter, M. (2000). 100 of the world's worst invasive alien species: a selection from the global invasive species database (Vol. 12). Auckland: Invasive Species Specialist Group.

MacCallum, C. J., Nürnberger, B., & Barton, N. H. (1995). Experimental evidence for habitat dependent selection in a *Bombina* hybrid zone. Proceedings of the Royal Society of London. Series B: Biological Sciences, 260(1359), 257-264.

MaCdonald, D. W., & Harrington, L. A. (2003). The American mink: the triumph and tragedy of adaptation out of context. New Zealand Journal of Zoology, 30(4), 421-441.

Maag, N., Gehrer, L., & Woodhams, D. C. (2012). Sink or swim: a test of tadpole behavioral responses to predator cues and potential alarm pheromones from skin secretions. Journal of Comparative Physiology A, 198(11), 841-846.

Middlemis Maher, J., Werner, E. E., & Denver, R. J. (2013). Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. Proceedings of the Royal Society B: Biological Sciences, 280(1758), 20123075.

Mandrillon, A. L., & Saglio, P. (2007). Herbicide exposure affects the chemical recognition of a non native predator in common toad tadpoles (*Bufo bufo*). Chemoecology, 17(1), 31-36.

Marquis, O., Saglio, P., & Neveu, A. (2004). Effects of predators and conspecific chemical cues on the swimming activity of *Rana temporaria* and *Bufo bufo* tadpoles. Archiv für Hydrobiologie, 153-170.

McCollum, S. A., & Van Buskirk, J. (1996). Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. Evolution, 50(2), 583-593.

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. New England journal of medicine, 338(3), 171-179.

McMahon, R. F. (2002). Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Canadian Journal of Fisheries and Aquatic Sciences, 59(7), 1235-1244.

McPeek, M. A. (1990). Behavioral differences between *Enallagma* species (Odonata) influencing differential vulnerability to predators. Ecology, 71(5), 1714-1726.

Mikolajewski, D. J., & Johansson, F. (2004). Morphological and behavioral defenses in dragonfly larvae: trait compensation and cospecialization. Behavioral Ecology, 15(4), 614-620.

Millennium Ecosystem Assessment, M. E. (2005). Ecosystems and human well-being: wetlands and water synthesis. World resources Resources instituteInstitute, Washington, DC, 5.

Mills, E. L., Leach, J. H., Carlton, J. T., & Secor, C. L. (1993). Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. Journal of Great Lakes Research, 19(1), 1-54.

Mitchell, M. D., Bairos-Novak, K. R., & Ferrari, M. C. (2017). Mechanisms underlying the control of responses to predator odours in aquatic prey. Journal of Experimental Biology, 220(11), 1937-1946.

Mogali, S. M., Saidapur, S. K., & Shanbhag, B. A. (2012). Tadpoles of the bronze frog (*Rana temporalis*) assess predation risk before evoking antipredator defense behavior. Journal of ethology, 30(3), 379-386.

Monclús, R., Rödel, H. G., Von Holst, D., & De Miguel, J. (2005). Behavioural and physiological responses of naive European rabbits to predator odour. Animal Behaviour, 70(4), 753-761.

Moore, R. D., Newton, B., & Sih, A. (1996). Delayed hatching as a response of streamside salamander eggs to chemical cues from predatory sunfish. Oikos, 331-335.

Moore, I. T., & Jessop, T. S. (2003). Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and Behavior, 43(1), 39-47.

Moore, M. K., & Townsend Jr, V. R. (1998). The interaction of temperature, dissolved oxygen and predation pressure in an aquatic predator-prey system. Oikos, 329-336.

Moran, N. A. (1992). The evolutionary maintenance of alternative phenotypes. The American Naturalist, 139(5), 971-989.

Moyle, P. B. (1986). Fish introductions into North America: patterns and ecological impact. In Ecology of biological invasions of North America and Hawaii (pp. 27-43). Springer, New York, NY.

Moyle, P. B. & Light, T. (1996). Biological invasions of fresh water: empirical rules and assembly theory. Biological conservation, 78(1-2), 149-161.

Nentwig, W. Kühnel, E. & Bacher, S. (2010). A Generic Impact-Scoring System Applied to Alien Mammals in Europe. Conservation Biology, 24(1), 302-311.

Newman, R. A. (1989). Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. Ecology, 70(6), 1775-1787.Newman, R. A. (1992). Adaptive plasticity in amphibian metamorphosis. BioScience, 42(9), 671-678.

Nicieza, A. G. (1999). Context-dependent aggregation in Common Frog *Rana temporaria* tadpoles: influence of developmental stage, predation risk and social environment. Functional Ecology, 13(6), 852-858.

Nicieza, A. G., Alvarez, D., & Atienza, E. M. S. (2006). Delayed effects of larval predation risk and food quality on anuran juvenile performance. Journal of Evolutionary Biology, 19(4), 1092-1103.

Nunes, A. L., Orizaola, G., Laurila, A., & Rebelo, R. (2014). Rapid evolution of constitutive and inducible defenses against an invasive predator. Ecology, 95(6), 1520-1530.

Nylin, S., & Gotthard, K. (1998). Plasticity in life-history traits. Annual review of entomology, 43(1), 63-83.

Olden, J. D. Poff, N. L. & Bestgen, K. R. (2006). Life-history strategies predict fish invasions and extirpations in the Colorado River Basin. Ecological Monographs, 76(1), 25-40.

Orizaola, G., & Braña, F. (2004). Hatching responses of four newt species to predatory fish chemical cues. In Annales Zoologici Fennici. Finnish Zoological and Botanical Publishing Boar, 635-645.

Orr, M. V., Hittel, K., & Lukowiak, K. (2009). Different strokes for different folks': geographically isolated strains of Lymnaea stagnalis only respond to sympatric predators and have different memory forming capabilities. Journal of Experimental Biology, 212(14), 2237-2247.

Osman, R. W., & Whitlatch, R. B. (2004). The control of the development of a marine benthic community by predation on recruits. Journal of Experimental Marine Biology and Ecology, 311(1), 117-145.

Padilla, D. K., & Adolph, S. C. (1996). Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. Evolutionary Ecology, 10(1), 105-117.

Pandian, T. J., & Marian, M. P. (1985). Predicting anuran metamorphosis and energetics. Physiological Zoology, 58(5), 538-552.

Pandolfi, J. M., & Lovelock, C. E. (2014). Novelty trumps loss in global biodiversity. Science, 344(6181), 266-267.

Peluc, S. I., Sillett, T. S., Rotenberry, J. T., & Ghalambor, C. K. (2008). Adaptive phenotypic plasticity in an island songbird exposed to a novel predation risk. Behavioral Ecology, 19(4), 830-835.

Perna, C., & Burrows, D. (2005). Improved dissolved oxygen status following removal of exotic weed mats in important fish habitat lagoons of the tropical Burdekin River floodplain, Australia. Marine Pollution Bulletin, 51(1-4), 138-148.

Perna, C. N., Cappo, M., Pusey, B. J., Burrows, D. W., & Pearson, R. G. (2012). Removal of aquatic weeds greatly enhances fish community richness and diversity: an example from the Burdekin River floodplain, tropical Australia. River Research and Applications, 28(8), 1093-1104.

Persson, L. (1988). Asymmetries in competitive and predatory interactions in fish populations. In Size-structured populations. Springer, Berlin, Heidelberg, 203-218.

Pimentel, D. Zuniga, R. & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological economics, 52(3), 273-288.

Plesník, J. (2011). Někdo to rád horké. Invazní nepůvodní druhy. Ochrana přírody, 5, 37-29.

Polo-Cavia, N., Gonzalo, A., López, P., & Martín, J. (2010). Predator recognition of native but not invasive turtle predators by naïve anuran tadpoles. Animal Behaviour, 80(3), 461-466.

Pride, E. R. (2005). High faecal glucocorticoid levels predict mortality in ring-tailed lemurs (*Lemur catta*). Biology letters, 1(1), 60-63.

Rehage, J. S., Dunlop, K. L., & Loftus, W. F. (2009). Antipredator responses by native mosquitofish to non-native cichlids: an examination of the role of prey naiveté. Ethology, 115(11), 1046-1056.

Relyea, R. A. (2001). Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology, 82(2), 523-540.

Relyea, R. A. (2002). Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. Ecological Monographs, 72(4), 523-540.

Relyea, R. A. (2003). Predators come and predators go: the reversibility of predator-induced traits. Ecology, 84(7), 1840-1848.

Relyea, R. A. (2007). Getting out alive: how predators affect the decision to metamorphose. Oecologia, 152(3), 389-400.

Relyea, R. A., & Werner, E. E. (1999). Quantifying the relation between predator-induced behavior and growth performance in larval anurans. Ecology, 80(6), 2117-2124.

Richards, S. J., & Bull, C. M. (1990). Size-limited predation on tadpoles of three Australian frogs. Copeia, 1041-1046.

Rollins-Smith, L. A. (2009). The role of amphibian antimicrobial peptides in protection of amphibians from pathogens linked to global amphibian declines. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1788(8), 1593-1599.

Romanuk, T. N. Zhou, Y. Brose, U. Berlow, E. L. Williams, R. J. & Martinez, N. D. (2009). Predicting invasion success in complex ecological networks. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1524), 1743-1754.

Romero, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. Trends in ecology & evolution, 19(5), 249-255.

Romero, L. M., & Wingfield, J. C. (2001). Regulation of the hypothalamic-pituitary-adrenal axis in free-living pigeons. Journal of Comparative Physiology B, 171(3), 231-235.

Ruthsatz, K., Dausmann, K. H., Reinhardt, S., Robinson, T., Sabatino, N. M., Peck, M. A., & Glos, J. (2019). Endocrine disruption alters developmental energy allocation and performance in *Rana temporaria*. Integrative and Comparative Biology, 59(1), 70-88.

Sakai, A. K. Allendorf, F. W. Holt, J. S. Lodge, D. M. Molofsky, J. With, K. A. McCauley, D. E. (2001). The population biology of invasive species. Annual review of ecology and systematics, 32(1), 305-332.

Sapolsky, R. M. (1992). Stress, the aging brain, and the mechanisms of neuron death. the MIT Press.

Sapolsky, R. M. (2002). Endocrinology of the stress-response.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine reviews, 21(1), 55-89.

Sazima, I., & Eterovick, P. C. (2000). Structure of an anuran community in a montane meadow in southeastern Brazil: effects of seasonality, habitat, and predation. Amphibia-Reptilia, 21(4), 439-461.

Säterberg, T., Sellman, S., & Ebenman, B. (2013). High frequency of functional extinctions in ecological networks. Nature, 499(7459), 468-470.

Schalk, G., Forbes, M. R., & Weatherhead, P. J. (2002). Developmental plasticity and growth rates of green frog (*Rana clamitans*) embryos and tadpoles in relation to a leech (*Macrobdella decora*) predator. Copeia, 2002(2), 445-449.

Scheibe, K. M., Berger, A., Langbein, J., Streich, W. J., & Eichhorn, K. (1999). Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. Biological rhythm research, 30(2), 216-233.

Scheffer, M. (2020). Critical transitions in nature and society (Vol. 16). Princeton University Press.

Schoeppner, N. M., & Relyea, R. A. (2005). Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecology letters, 8(5), 505-512.

Schoeppner, N. M., & Relyea, R. A. (2009). Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. Functional Ecology, 23(6), 1114-1121.

Scott, D. E., Casey, E. D., Donovan, M. F., & Lynch, T. K. (2007). Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. Oecologia, 153(3), 521-532.

Segev, O., Rodríguez, A., Hauswaldt, S., Hugemann, K., & Vences, M. (2015). Flatworms (*Schmidtea nova*) prey upon embryos of the common frog (*Rana temporaria*) and induce minor developmental acceleration. Amphibia-Reptilia, 36(2), 155-163.

Semlitsch, R. D., & Caldwell, J. P. (1982). Effects of density of growth, metamorphosis, and survivorship in tadpoles of *Scaphiopus holbrooki*. Ecology, 63(4), 905-911.

Semlitsch, R. D., & Gibbons, J. W. (1988). Fish predation in size-structured populations of treefrog tadpoles. Oecologia, 75(3), 321-326.

Short, J., & Smith, A. (1994). Mammal decline and recovery in Australia. Journal of Mammalogy, 75(2), 288-297.

Sih, A. (1987). Predator and prey lifestyles: an evolutionary and ecological overview. Predation: direct and indirect impacts on aquatic communities, 203-224.

Sih, A. (1992). Prey uncertainty and the balancing of antipredator and feeding needs. The American Naturalist, 139(5), 1052-1069.

Sih, A., & Moore, R. D. (1993). Delayed hatching of salamander eggs in response to enhanced larval predation risk. The American Naturalist, 142(6), 947-960.

Simberloff, D., & Rejmánek, M. (Eds.). (2011). Encyclopedia of biological invasions (No. 3). Univ of California Press.

Simberloff, D. (2013). Invasive species: what everyone needs to know. Oxford University Press.

Simberloff, D., & Vitule, J. R. (2014). A call for an end to calls for the end of invasion biology. Oikos, 123(4), 408-413.

Skelly, D. K. (1994). Activity level and the susceptibility of anuran larvae to predation.

Skelly, D. K. (1997). Tadpole communities: pond permanence and predation are powerful forces shaping the structure of tadpole communities. American Scientist, 85(1), 36-45.

Smith, D. C. (1983). Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. Ecology, 64(3), 501-510.

Smith-Gill, S. J., & Berven, K. A. (1979). Predicting amphibian metamorphosis. The American Naturalist, 113(4), 563-585.

Speybroeck, J., Beukema, W., Bok, B., & Van Der Voort, J. (2016). Field guide to the amphibians and reptiles of Britain and Europe. Bloomsbury publishing.

Stearns, S. C. (1989). The evolutionary significance of phenotypic plasticity. Bioscience, 39(7), 436-445.

Stephens, J. P., Stoler, A. B., Sckrabulis, J. P., Fetzer, A. J., Berven, K. A., Tiegs, S. D., & Raffel, T. R. (2017). Ontogenetic changes in sensitivity to nutrient limitation of tadpole growth. Oecologia, 183(1), 263-273

Stoks, R., & McPeek, M. A. (2003). Antipredator behavior and physiology determine Lestes species turnover along the pond-permanence gradient. Ecology, 84(12), 3327-3338.

Storm, R. M., Leonard, W. P., Brown, H. A., Bury, R. B., Darda, D. M., Diller, L. V., & Peterson, C. R. (1995). Reptiles of Washington and Oregon. Seattle Audubon Society, Seattle, WA.

Strauss, S. Y., Lau, J. A., & Carroll, S. P. (2006). Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities?. Ecology letters, 9(3), 357-374.

Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater biology, 55, 152-174.

Strayer, D. L. (2012). Eight questions about invasions and ecosystem functioning. Ecology letters, 15(10), 1199-1210.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. Science, 306(5702), 1783-1786.

Sundseth, K. (2014). Invasive alien species: a European response. European Commission DG Environment Units B, 2.

Takahara, T., Kohmatsu, Y., Maruyama, A., & Yamaoka, R. (2008). Benefit of suites of defensive behavior induced by predator chemical cues on anuran tadpoles, *Hyla japonica*. Behavioral ecology and sociobiology, 63(2), 235-240.

Takatsu, K., & Kishida, O. (2013). An offensive predator phenotype selects for an amplified defensive phenotype in its prey. Evolutionary Ecology, 27(1), 1-11.

Tejedo, M. (1993). Size-dependent vulnerability and behavioral responses of tadpoles of two anuran species to beetle larvae predators. Herpetologica, 287-294.

Teplitsky, C., Plénet, S., Léna, J. P., Mermet, N., Malet, E., & Joly, P. (2005). Escape behaviour and ultimate causes of specific induced defences in an anuran tadpole. Journal of evolutionary biology, 18(1), 180-190.

Toledo, L. F., Rodrigues, M. T., Jared, C., Verdade, V. K., & Antoniazzi, M. M. (2011). The Amazonian toad *Rhaebo guttatus* is able to voluntarily squirt poison from the paratoid macroglands. Amphibia-Reptilia, 32(4), 546-549.

Tollrian, R., & Harvell, C. D. (Eds.). (1999). The ecology and evolution of inducible defenses. Princeton university press.

Touchon, J. C., McCoy, M. W., Vonesh, J. R., & Warkentin, K. M. (2013). Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. Ecology, 94(4), 850-860.

Touchon, J. C., & Warkentin, K. M. (2008). Fish and dragonfly nymph predators induce opposite shifts in color and morphology of tadpoles. Oikos, 117(4), 634-640.

Travis, J. (1984). Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. Ecology, 65(4), 1155-1160.

Turtle Taxonomy Working Group (2017): Turtles of the World: Annotated checklist and atlas of taxonomy, synonymy, distribution, and conservation status (8th edition). IN: Rhodin, A.G.J., et al. (Editors), Conservation Biology of Freshwater Turtles and Tortoises. Chelonian Research Monographs 7: 1–292.

Van Allen, B. G., Briggs, V. S., McCoy, M. W., & Vonesh, J. R. (2010). Carry-over effects of the larval environment on post-metamorphic performance in two hylid frogs. Oecologia, 164(4), 891-898.

Van Buskirk, J., Anderwald, P., Lüpold, S., Reinhardt, L., & Schuler, H. (2003). The lure effect, tadpole tail shape, and the target of dragonfly strikes. Journal of Herpetology, 420-424.

Van Buskirk, J., & Arioli, M. (2002). Dosage response of an induced defense: how sensitive are tadpoles to predation risk?. Ecology, 83(6), 1580-1585.

Van Buskirk, J., Aschwanden, J., Buckelmüller, I., Reolon, S., & Rüttiman, S. (2004). Bold tail coloration protects tadpoles from dragonfly strikes. Copeia, 2004(3), 599-602.

Van Buskirk, J. O. S. H., & McCollum, S. A. (2000). Influence of tail shape on tadpole swimming performance. Journal of Experimental Biology, 203(14), 2149-2158.

Van Buskirk, J., & Schmidt, B. R. (2000). Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. Ecology, 81(11), 3009-3028.

Van der Veer, G., & Nentwig, W. (2015). Environmental and economic impact assessment of alien and invasive fish species in Europe using the generic impact scoring system. Ecology of Freshwater fish, 24(4), 646-656.

Van Kleunen, M. Weber, E. & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology letters, 13(2), 235-245.

Vermeij, G. J. (1991). When biotas meet: understanding biotic interchange. Science, 253(5024), 1099-1104.

Vieira, E. A., Duarte, L. F. L., & Dias, G. M. (2012). How the timing of predation affects composition and diversity of species in a marine sessile community?. Journal of Experimental Marine Biology and Ecology, 412, 126-133.

Vodrážková, M., Šetlíková, I., & Berec, M. (2020). Chemical cues of an invasive turtle reduce development time and size at metamorphosis in the common frog. Scientific Reports, 10(1), 1-6.

Vodrážková, M., Šetlíková, I., Navrátil, J., & Berec, M. (2022a). Different time patterns of the presence of red-eared slider influence the ontogeny dynamics of common frog tadpoles. Scientific Reports, 12(1), 1-9.

Vodrážková M., Šetlíková I., Navrátil J., Berec M. (2022b). Presence of an alien turtle accelerates hatching of common frog (*Rana temporaria*) tadpoles. NeoBiota 74: 155-169.

Vonesh, J. R., & Bolker, B. M. (2005). Compensatory larval responses shift trade-offs associated with predator-induced hatching plasticity. Ecology, 86(6), 1580-1591.

Wake, D. B., & Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences, 105(supplement_1), 11466-11473.

Warkentin, K. M. (1995). Adaptive plasticity in hatching age: a response to predation risk trade-offs. Proceedings of the National Academy of Sciences, 92(8), 3507-3510.

Warkentin, K. M. (2000). Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. Animal Behaviour, 60(4), 503-510.

Warkentin, K. M. (2007). Oxygen, gills, and embryo behavior: mechanisms of adaptive plasticity in hatching. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 148(4), 720-731.

Warne, R. W., & Crespi, E. J. (2015). Larval growth rate and sex determine resource allocation and stress responsiveness across life stages in juvenile frogs. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 323(3), 191-201.

Wasser, S. K., Bevis, K., King, G., & Hanson, E. (1997). Noninvasive physiological measures of disturbance in the northern spotted owl. Conservation Biology, 11(4), 1019-1022.

Wellborn, G. A., Skelly, D. K., & Werner, E. E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. Annual review of ecology and systematics, 27(1), 337-363.

Wells, K. D., & Schwartz, J. J. (2007). The behavioral ecology of anuran communication. In Hearing and sound communication in amphibians. Springer, New York, NY, 44-86.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. Annual review of Ecology and Systematics, 249-278.

West-Eberhard, M. J. (2003). Developmental plasticity and evolution. Oxford University Press.

Westbrooks, R. G. (2004). New Approaches for Early Detection and Rapid Response to Invasive Plants in the United States1. Weed Technology, 18(sp1), 1468-1471.

Westbrooks, R. G., & Eplee, R. E. (2011). Early detection and rapid response, 169-177.

Wetzel, R. G. (2001). Limnology: lake and river ecosystems. gulf professional publishing.

Wilbur, H. M. (1980). Complex life cycles. Annual review of Ecology and Systematics, 11, 67-93.

Wilbur, H. M. (1987). Regulation of structure in complex systems: experimental temporary pond communities. Ecology, 68(5), 1437-1452.

Wilbur, H. M. (1988). Interactions between growing predators and growing prey. In Size-structured populations. Springer, Berlin, Heidelberg, 157-172.

Wilbur, H. M., & Collins, J. P. (1973). Ecological Aspects of Amphibian Metamorphosis: Nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. Science, 182(4119), 1305-1314.

Wilbur, H. M., & Fauth, J. E. (1990). Experimental aquatic food webs: interactions between two predators and two prey. The American Naturalist, 135(2), 176-204.

Willink, B., Palmer, M. S., Landberg, T., Vonesh, J. R., & Warkentin, K. M. (2014). Environmental context shapes immediate and cumulative costs of risk-induced early hatching. Evolutionary Ecology, 28(1), 103-116.

Willmer, P. G., & Stone, G. N. (2004). Behavioral, ecological, and physiological determinants of the activity patterns of bees. Advances in the Study of Behavior, 34(34), 347-466.

Wingfield, J. C., Hunt, K., Breuner, C., Dunlap, K., Fowler, G. S., Freed, L., & Lepson, J. (1997). Environmental stress, field endocrinology, and conservation biology. Behavioral approaches to conservation in the wild, 95-131.

Wilson, D. J., & Lefcort, H. (1993). The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. Animal Behaviour.

Wisenden, B. D. (2000). Olfactory assessment of predation risk in the aquatic environment. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 355(1401), 1205-1208.

Wissinger, S. A., Whiteman, H. H., Sparks, G. B., Rouse, G. L., & Brown, W. S. (1999). Foraging trade-offs along a predator–permanence gradient in subalpine wetlands. Ecology, 80(6), 2102-2116.

Wittenberg, R. & Cock, M. J. (2001). Invasive alien species: a toolkit of best prevention and management practices. CABI.

Young, C. M., & Chia, F. S. (1984). Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the first 21 days after settlement. Marine Biology, 81(1), 61-68.

Zanette, L. Y., White, A. F., Allen, M. C., & Clinchy, M. (2011). Perceived predation risk reduces the number of offspring songbirds produce per year. Science, 334(6061), 1398-1401.

Zapata-Ríos, G., & Branch, L. C. (2016). Altered activity patterns and reduced abundance of native mammals in sites with feral dogs in the high Andes. Biological Conservation, 193, 9-16.

Zhang, F., Zhao, J., Zhang, Y., Messenger, K., & Wang, Y. (2015). Antipredator behavioral responses of native and exotic tadpoles to novel predator. Asian Herpetol. Res, 6, 51-58.

8. Další publikační činnost

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Short communication

The number of shipments in the CITES Trade Database does not accurately reflect the volume of traded wildlife

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ABSTRACT

Volume estimation of the wildlife trade is crucial for effective biodiversity protection. Recently, Mair et al. (Biol. Conserv. 2019, 239, 108260) used the number of shipments reported in the CITES Trade Database as an estimate of the total traded volume of species to rapidly assess the appropriateness of species listings in CITES Appendices. The aims of the present study were to (1) test the presumed relationship between the number of shipments and the volume of various traded terms converted to whole organism equivalents (WOEs) and (2) compare the distribution of species in defined trade volume categories based on different calculation methods. The number of shipments did not reflect traded volume in 15% of species. Different calculation methods led to different categorisations for more than one-third of the species. In general, the number of shipments underestimates the volume of species traded in small-sized terms that allow trade in larger and less-frequent shipments and vice versa. In contrast, WOEs quantification does not consider the species that are traded in non-convertible terms (e.g., meat) or units (e.g., kilograms). Due to the structure of the data in the CITES Trade Database, it is not possible to find the only objective method of trade quantification for the whole data set. However, we recommend to use shipments approach only to exclude species with zero trade, and evaluate the real volume using WOEs where possible.

1. Introduction

Limited resources for nature conservation (economic, spatial and human activity) force conservationists and scientists to select species that need immediate protection (Davies et al., 2018; Waldron et al., 2013; Wilson et al., 2006). This requires objective criteria according to which species can be classified for nature conservation purposes (Faith, 1992, 2018). Several different approaches and frameworks, such as the IUCN Red List (Mair et al., 2019; Scheffers et al., 2019) and evolutionary distance (Gumbs et al., 2020; Stein et al., 2018), have been used to categorise species and are currently in use.

CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) seeks to protect species actually or potentially endangered by their trade. The publicly accessible CITES Trade Database (UNEP-WCMC, 2020) collects and publishes data on volumes, trading countries, terms, purposes and sources of species listed in the CITES Appendices. Mair et al. (2019) recently used the CITES Trade Database to assess the degree of trade threat to vertebrates, on the basis of which they propose a highly desirable revision of the presence of species in CITES Appendices. Using the simplification of the number of reported trade shipments as an estimate of its total traded volume, Mair et al. (2019) divided CITES-listed vertebrates into discrete groups: species that were highly traded and also in high threat categories according to the IUCN were identified as species of high concern, while the inclusion in CITES Appendices of species that were not highly traded and also in a low threat category according to the IUCN was questioned. Although the shipment-based approach is understandable and unavoidable because the individual units of traded quantities used are not comparable, evaluating all relevant data in the Database is not necessarily a robust approach either. Moreover, Mair et al. (2019) used shipments reported by both exporters and importers; however, for Appendix II species, the importer is not always required to report trade (Robinson and Sinovas, 2018). Counting both importer- and exporter-reported shipments overestimates the real frequency of trade, whereas the magnitude of this overestimation is unknown. This method of data processing can thus affect relevant interpretations and outcomes.

To avoid these two methodological ambiguities, we decided to analyse only shipments reported by exporters by testing their correlation

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to trade volume using the same data source as Mair et al. (2019). The aims of this study were (1) to test the presumed relationship between the number of shipments and the volume of various traded terms converted to whole organism equivalents (WOEs) and (2) to compare the distribution of species in defined trade volume categories based on different calculation methods.

2. Methods

We downloaded the full CITES Trade Database in the shipment-per-shipment format on 14 March 2020 (UNEP-WCMC, 2019). We selected the years 2006–2015 in order to match the time period of Mair et al. (2019). From this dataset, we extracted the Appendix II vertebrate species (n = 2779) assessed by Mair et al. (2019). This resulted in 4,830,737 individual shipments reports (Table A1). To avoid overestimation of the traded volume, we used only exporter data (Reporter. type: E) without re-exports (i.e., all shipments with blank Origin.permit. Random ID). The shipments with an Origin.permit.Random ID was used only if Origin.permit.Random ID was identical with Export.permit. Random ID, and simultaneously both Origin and Export country were the same (if the country of origin is blank, the country of export is also the country of origin (CITES, 2013)). We used only shipments in numbers of individuals (Unit: blank). This procedure further reduced the number of shipments to 1,288,630 (27% of the original dataset) (Table A1).

Instead of using shipments as a proxy of traded volume, we transformed traded volume to whole organism equivalents (WOEs) (Harfoot et al., 2018). WOEs enable the heterogeneous types of products reported in the CITES Trade Database to be summarised. We used the methods and conversion factors of Harfoot et al. (2018) (i.e., bodies, carapaces, fingerlings, fins, genitalia, heads, live, skeletons, skins, skulls, swim bladders, tails, trophies, and tusks in *Monodon monoceros* equal to 1; ears, horns, sides, and tusks (except that of *Monodon monoceros* and *Loxodonta africana*) equal to 0.5; feet equal to 0.25; tusks in *Loxodonta africana* equal to 0.532; teeth in *Hippopotamus amphibius* equal to 0.083). The resulting numbers were rounded up to whole individuals. We assumed that different terms were sourced from independent animals because there is no information indicating whether different terms came from different or identical specimens. The terms that could not be converted to WOEs were excluded from further analyses. The final list contained 1100 species and 235,761 individual shipments (Table A1).

Next, we tested the correlation between the number of WOEs and the number of shipments for each species that was traded in a sufficient number of years for correlation analysis (i.e., at least three years during the study period). To obtain results comparable to those of Mair et al. (2019), the species were categorised into eight categories according to the trade volume with the same range (but note the different units - mean number of traded WOEs per year) as the following WOE categories: no trade; > 0–10; > 10–50; > 50–100; > 100–500; > 500–1000; > 1000–5000; and > 5000. For species that had a non-zero traded volume in WOEs, we compared the categorisation between this study and that of Mair et al. (2019). Thus, only species present in both lists were used for comparison. All analyses were performed using STATISTICA StatSoft® 13.0 software.

3. Results and discussion

Mair et al. (2019) assumed that more frequent trade records indicate a larger number of individuals being traded and suggested that the number of shipments should be used as a proxy for trade quantity when designing a rapid risk assessment framework to improve the efficiency of CITES. We quantified trading volume using WOEs as a more accurate measure of traded volume. This approach led to a reduction in the number of vertebrate species monitored to 1100, as 1679 species were not traded in WOE-converted forms during the study period.

Overall, 413 out of 1100 species were traded in only one or two years during the study period; therefore, a correlation with the number of shipments could not be established. Of the 687 species that were traded in at least three years in a given period, we found that there was no correlation between the number of shipments and WOEs in 102 (or 15%) of species (Table A2). Conversely, this correlation was significant in 585 species (mean $R^2 = 0.83 \pm 0.16$ (± S.D.), 0.41–1.00 (min-max), $p < 0.05$). However, there are two important limitations of shipment data, even if they correlate with WOEs. Firstly, the probability of a significant correlation is a function of the number of years in which species has been traded (in our case, the proportion of non-correlating species increased from 5% to 33% in species that were traded in three and ten years, respectively; Fig. A1), and if a longer study period were used, the proportion of non-correlated species would likely increase. Therefore, the assumption of Mair et al. (2019) that the number of shipments equals traded volume was not entirely confirmed, especially in frequently traded species. Secondly, the number of shipments can be used as a proxy of WOEs only in species with a correlation slope value close to one (meaning that the number of shipments equals the volume traded, which occurred in 14% of analysed species; Table A2). For example, in Channel-billed toucan (*Ramphastos vitellinus*) the number of shipments was 250 and the volume expressed in WOEs was 1184 individuals (slope = 6.3). On the contrary, for species with a high slope, the increase in WOEs per shipment number is much steeper. For example, Yellow-spotted river turtle (*Podocnemis unifilis*) was traded in just five more shipments (i.e., 255) than Channel-billed toucan, but the volume expressed in WOEs was 652,834 individuals. This means that the number of individuals is on average four orders of magnitude higher than the number of shipments (slope = 4668.7). Therefore, the value of slope markedly influences species inclusion in the trade category. The shipment approach resulted in the same trade category 4 for both species in Mair et al. (2019), while Channel-billed toucan and Yellow-spotted river turtle were evaluated in trade category 5 and 8, respectively, using our method.

Using WOEs as a measure of traded volume, we obtained different distributions of species in defined categories than those expressed in terms of the number of shipments (compare our Fig. 1 to Fig. 1 in Mair et al. (2019)) which was reflected by an increase in the share of species in higher trade categories in all of the IUCN Red List categories (Table A3). Subsequent comparison between these two categorisations resulted in a change in the category of 409 out of 1100 species (i.e., 37%), while 691 species (i.e., 63%) remained in the same category (Figs. 2 and 3). In addition, WOE quantification ameliorates the shortcomings of the shipment-based approach. Given the nature of the wildlife trade, the shipment-based approach leads to an underestimation of the volume of trade in species traded in small-sized terms that allow trade in larger and less-frequent shipments (e.g., fish fingerlings, seahorses, frogs, and tortoise juveniles). The predominance of species traded in small terms was reflected by a 2.5-times greater number of species for which the category has been increased compared to species for which the category decreased (Fig. 2), which is reflected in the higher proportion of species in WOE categories with a higher volume of trade (Fig. 3). Conversely, the volume of trade in species traded in large terms may be overestimated using shipment numbers. For example, the African elephant (*Loxodonta africana*), Brown bear (*Ursus arctos*), Polar bear (*Ursus maritimus*), Bald eagle (*Haliaeetus leucocephalus*), and Sandhill crane (*Grus canadensis*) decreased by three categories of trade. However, for species that are traded either in terms or in units not convertible to WOEs (which applies to bodies, meat, eggs and ivory carvings expressed in kg), the actual volume of trade using our approach may be underestimated; thus the approach using the number of shipments is therefore superior. The two most significant cases we identified were the meat of the European eel (*Anguilla anguilla*) and eggs of the American paddlefish (*Polyodon spathula*), which formed dominant terms of traded volume.

This is fair to note that there is a possibility to convert more data to WOEs, especially the ones expressed in kilograms using average mass of

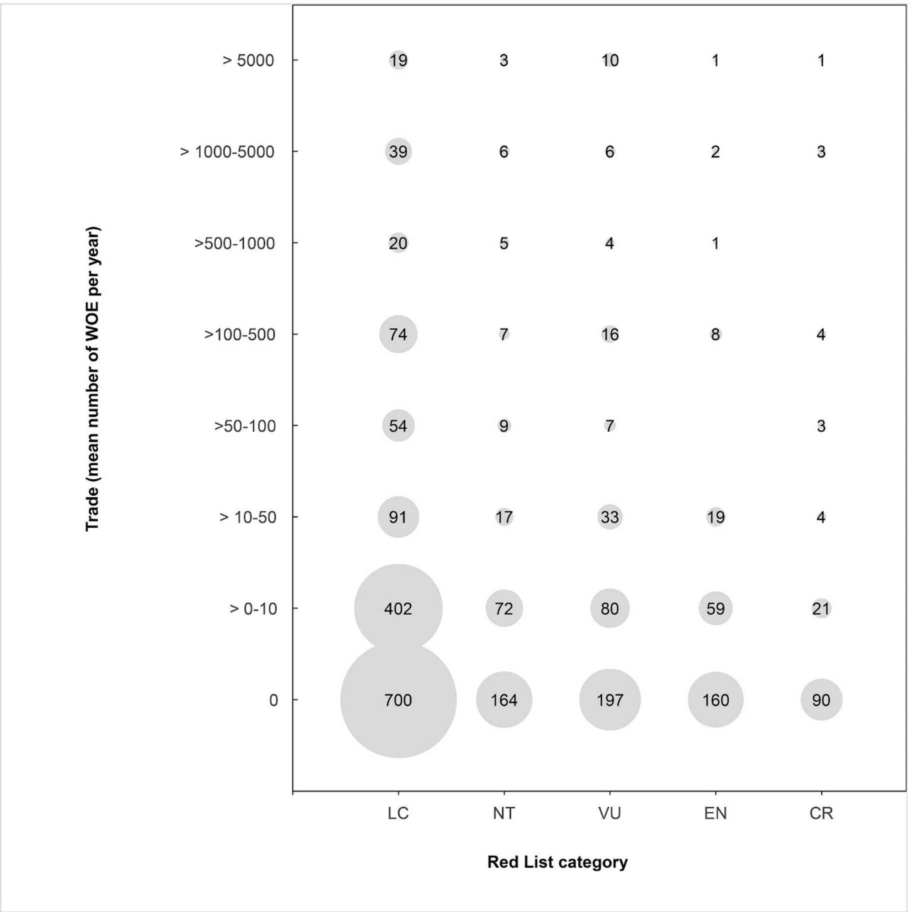

Fig. 1. The number of vertebrate species within each category of trade volume (measured as average WOE per year) by species IUCN Red List conservation status ($n = 2411$; the number of vertebrate species on Appendix II categorised on the IUCN Red List as LC, NT, VU, EN or CR). The size of the circles is indicative of the number of species in each group. Trade category “0” depicts the species not convertible to WOEs under our methods or not traded at all.

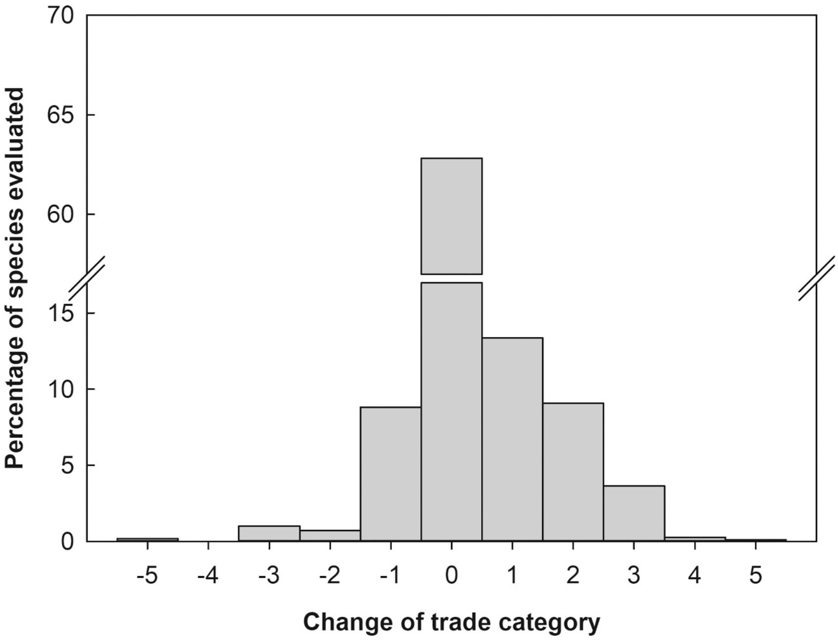

Fig. 2. Histogram of the relative frequencies of species ($n = 1100$; species without trade convertible to WOEs were excluded) according to trade category change (i.e., the difference between traded volume category according to WOEs and the number of shipments).

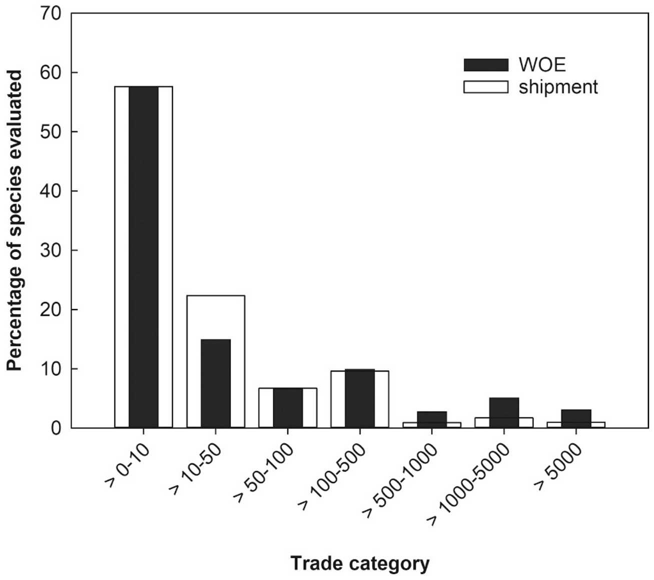

Fig. 3. The relative number of species (n = 1100; species without trade convertible to WOEs were excluded) according to the trade category (WOEs and the number of shipments).

an organism or its part traded, although it concerns a few specific cases (i.e., frog legs, gall bladders, horns, or pangolin scales (Schloegel et al., 2009; Zhou et al., 2012)). However, even in these cases, the conversion to WOEs is ambiguous, as the conversion values for the same term are given differently, if at all. For example, estimates of average individual weights of young yellow eels were reported at 3.5 g for Denmark, 20 g for Germany, 33 g for the Netherlands and 90 g for Sweden (CITES, 2007). Similarly, Basson et al. (1991) state that the conversion factor, which is related to the number of elephants killed and the weight of the ivory, is not constant. Despite these inconsistencies, to further enlarge the dataset of biologically sensible data, a standardized list of WOE conversion indexes would be highly appreciated, but this is beyond the aims of this study.

The need to quantify wildlife trade is crucial for the effective protection of species. Mair et al. (2019) published the first results of the assessment of CITES-listed species based on data quantification. Here, we provide a different method of an assessment using the same data source. Both approaches have their advantages and disadvantages. Despite some controversial approaches to the data set (use of importer, exporter and also re-export shipments), the advantages of the Mair et al. (2019) are the use of all shipments and rapid data processing, but it does not reflect the actual volume of trade. In contrast, our method evaluates data based on volumes expressed in WOEs that are closer to the actual traded quantity, but at the cost of usability of only about a quarter of the shipment data and the use of a complex counting algorithm. Here, we have shown that the categorisation of species based on the CITES Trade Database is highly sensitive to the selection of data and methods of their processing. Unfortunately, because of the structure of the data in the CITES Trade Database, in particular the use of several incomparable volume units, it is not possible to find the only objective method of quantification for the whole data set. If we were to recommend any procedure, it would be as follows: (1) use shipments approach to exclude species with zero trade, (2) evaluate the real volume using our method for the species with non-zero shipment numbers and terms and units convertible to WOEs, (3) no unambiguous method is applicable for the species with non-zero shipment numbers and no terms and units convertible to WOEs.

CRediT authorship contribution statement

Markéta Slábová: Conceptualization; Data curation; Formal analysis; Methodology; Validation; Writing - original draft.
Irena Šetlíková: Conceptualization; Data curation; Formal analysis; Methodology; Visualization; Validation; Writing - original draft.
Magda Vodrážková: Data curation; Writing - review.
Zuzana Richtrová: Data curation; Writing - review.
Michal Berec: Conceptualization; Data curation; Formal analysis; Methodology; Supervision; Validation; Writing - original draft.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2020.108917.

References

Basson, M., Beddington, J., May, R., 1991. An assessment of the maximum sustainable yield of ivory from African elephant populations. Math. Biosci. 104, 73–95. https://doi.org/10.1016/0025-5564(91)90031-D.

CITES, 2007. Consideration of proposals for amendment of Appendices I and II. https://speciesplus.net/api/v1/documents/627).

CITES, 2013. A Guide to Using the CITES Trade Database. United Kingdom, Cambridge.

Davies, T., Cowley, A., Bennie, J., Leyshon, C., Inger, R., Carter, H., Robinson, B., Duffy, J., Casalegno, S., Lambert, G., Kevin, G., 2018. Popular interest in vertebrates does not reflect extinction risk and is associated with bias in conservation investment. PLoS One 13, e0203694. https://doi.org/10.1371/journal.pone.0203694.

Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. Biol. Conserv. 61, 1–10. https://doi.org/10.1016/0006-3207(92)91201-3.

Faith, D.P., 2018. Phylogenetic diversity and conservation evaluation: Perspectives on multiple values, indices, and scales of application. In: Scherson, R.A., Faith, D.P. (Eds.), Phylogenetic Diversity. Springer, Cham, pp. 1–26.

Gumbs, R., Gray, C.L., Böhm, M., Hoffmann, M., Grenyer, R., Jetz, W., Meiri, S., Roll, U., Owen, N.R., Rosindell, J., 2020. Global priorities for conservation of reptilian phylogenetic diversity in the face of human impacts. Nat. Commun. 11, 1–13. https://doi.org/10.1038/s41467-020-16410-6.

Harfoot, M., Glaser, S.A., Tittensor, D.P., Britten, G.L., McLardy, C., Malsch, K., Burgess, N.D., 2018. Unveiling the patterns and trends in 40 years of global trade in CITES-listed wildlife. Biol. Conserv. 223, 47–57. https://doi.org/10.1016/j.biocon.2018.04.017.

Mair, L., Ridley, F.A., Fleming, L.V., McGowan, P.J., 2019. A risk assessment framework to improve the efficiency of CITES. Biol. Conserv. 239, 108260. https://doi.org/10.1016/j.biocon.2019.108260.

Robinson, J.E., Sinovas, P., 2018. Challenges of analyzing the global trade in CITES-listed wildlife. Conserv. Biol. 32, 1203–1206. https://doi.org/10.1111/cobi.13095.

Scheffers, B.R., Oliveira, B.F., Lamb, I., Edwards, D.P., 2019. Global wildlife trade across the tree of life. Science 366, 71–76. https://doi.org/10.1126/science.aav5327.

Schloegel, L.M., Picco, A.M., Kilpatrick, A.M., Davies, A.J., Hyatt, A.D., Daszak, P., 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). Biol. Conserv. 142, 1420–1426. https://doi.org/10.1016/j.biocon.2009.02.007.

Stein, R.W., Mull, C.G., Kuhn, T.S., Aschliman, N.C., Davidson, L.N., Joy, J.B., Smith, G. J., Dulvy, N.K., Mooers, A.O., 2018. Global priorities for conserving the evolutionary history of sharks, rays and chimaeras. Nat. Ecol. Evol. 2, 288–298. https://doi.org/10.1038/s41559-017-0448-4.

UNEP-WCMC, 2019. Full CITES Trade Database Download. Version 2019.2. http://www.trade.cites.org (accessed 1 April 2020).

UNEP-WCMC, 2020. The CITES Trade Database. http://trade.cites.org (accessed 14 March 2020).

Waldron, A., Mooers, A.O., Miller, D.C., Nibbelink, N., Redding, D., Kuhn, T.S., Roberts, J.T., Gittleman, J.L., 2013. Targeting global conservation funding to limit immediate biodiversity declines. Proc. Natl. Acad. Sci. 110, 12144–12148. https://doi.org/10.1073/pnas.1221370110.

Wilson, K.A., McBride, M.F., Bode, M., Possingham, H.P., 2006. Prioritizing global conservation efforts. Nature 440, 337–340. https://doi.org/10.1038/nature04366.

Zhou, Z.-M., Zhao, H., Zhang, Z.-X., Wang, Z.-H., Wang, H., 2012. Allometry of scales in Chinese pangolins (*Manis pentadactyla*) and Malayan pangolins (*Manis javanica*) and application in judicial expertise. Zool. Res. Jun. 33, 271–275. https://doi.org/10.3724/SP.J.1141.2012.03271.

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Short communication

Tracking the wildlife trade: Usability of shipment identifiers in the CITES Trade Database

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ABSTRACT

To improve the usability of the CITES Trade Database, UNEP-WCMC recently released data on trade in CITES species in a new shipment-by-shipment format. The aims of this study were to determine the extent to which the shipments of CITES Appendix I species reported by exporters and importers can be matched with respect to individual identifiers and the reported volume of individual combinations of terms and units. The CITES Trade Database contains 618,750 rows (with 373,031 individual identifiers (IDs)) on the trade in animals listed in CITES Appendix I for the period 1996–2018. Only 18.1% of IDs were usable for tracing the movement of CITES samples and appeared in 216,041 (34.9%) rows. Almost two-thirds of IDs appeared only once in the dataset and are therefore not usable in terms of trade tracing. Although another 63,603 IDs appeared more than once in the dataset, they always appeared in only one of the identifier fields and at the same time only as either a reporter type E (exporters) or type I (importers) and therefore also have zero value for trade tracing. Furthermore, no identifier was assigned to shipments in 35,395 rows (5.7%). For the highest number of combinations of terms and units, the matchable volume represented a maximum of 10% of the total volume of trades. Importantly, for the most traded combination of term and unit, i.e., live animals reported in the number of individuals, more than half of the traded volume (56.3%) was in matchable shipments.

1. Introduction

The CITES Trade Database (https://www.trade.cites.org) has been widely used recently to assess the volume of trade in many of the animal and plant taxa listed (Auliya et al., 2016a, 2016b; D'Cruze and Macdonald, 2016; Harfoot et al., 2018; Harris et al., 2015; Hierink et al., 2020; Luiselli et al., 2016). However, the challenges and limitations of working with the CITES Trade Database are well known and have been widely described (Berec et al., 2018; Foster et al., 2016; Robinson and Sinovas, 2018). Despite repeated criticisms, there is still no unambiguous or recommended algorithm for accurately calculating trade volumes. The different calculation methods used in various studies have resulted in highly disparate observations of total trade volumes for the given taxa without the ability to determine which of the results corresponds best to reality (Berec et al., 2018; Janssen and Indenbaum, 2019). Alternatively, the authors (Andersson and Gibson, 2018; Bruckner, 2001; Carpenter et al., 2004; Nijman and Shepherd, 2010; Pernetta, 2009; Russo, 2015; Vall-llosera and Cassey, 2017) report only the results of one of the possible

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methods of quantification, knowing this is only one of the possible approaches. To improve the usability of the CITES Trade Database, UNEP-WCMC (UN Environment Programme - World Conservation Monitoring Centre) recently released data on trade in CITES species in a new shipment-by-shipment format, consisting of an impressive 20,217,368 rows (v2019.2; downloaded 1 April 2020). The availability of this data structure soon led to new analyses of trade volume (Harfoot et al., 2018; Hierink et al., 2020; Mair et al., 2019).

Reportedly, unlike the aggregated format, the only format previously available, individual shipments with permit numbers (in the form of unique ten-character alphanumeric identifiers) are now downloadable to “facilitate the tracking of shipments over time and can enable matching import and export shipments, which helps to identify discrepancies for further investigation” (CITES, 2018a). Previously, the aggregated format offered data only in the form of records (rows) in comparative tables, which contain the traded volume reported by the exporting and importing country aggregated for each year, individual taxa, term, purpose and source (this data format is still available). A maximum of 19 variables can be provided for each shipment report (row) in the non-aggregated shipment format, of which the first 14 are identical to those in the aggregated format: Year, Appendix, Taxon, Class, Order, Family, Genus, Term, Unit, Importer, Exporter, Origin, Purpose and Source. The data on the traded volume and type of reporting country (exporter or importer) are addressed differently between the two formats. In the shipment format, the Reporter.type column indicates whether the shipment data originate from an exporter (E) or importer (I), and the traded volume is reported separately in the Quantity column (Table S1). The traded volume is indicated in the Exporter Reported Quantity and/or Importer Reported Quantity columns in the aggregated format. Three completely new entries are devoted to unique identifiers (IDs): Import.permit.RandomID (I ID), Export.permit.RandomID (E ID), and Origin.permit.RandomID (O ID) (Table S1), which can be useful in terms of identifying the flow of goods.

Here, we analyse the potential of a new shipment-level format for evaluating the volume of trade. For our analysis, we chose the CITES Appendix I species because, unlike for the CITES Appendix II species, the reports of both exporters and importers on trade in these species are obligatory (CITES Convention text, Article III). Therefore, to comply with the above mentioned requirement to match shipments for CITES Appendix I species (Robinson and Sinovas, 2018), each unique identifier for the exporter should have at least one identifier identical to the importer and vice versa, and the total volume traded should be the same between the exporter and the importer for identical identifiers. To estimate the usability of IDs, we tested whether and to what extent shipments of CITES Appendix I species reported by exporters and importers match with respect to (1) the individual identifiers and (2) the reported volume for individual combinations of terms and units.

2. Methods

We analysed trade in CITES Appendix I animal species from 1996 to 2018 using the CITES Trade Database maintained by the UNEP-WCMC on behalf of the CITES Secretariat, specifically the recently released shipment-level dataset (v2019.2; downloaded 1 April 2020) to test the usefulness of this new data format. Shortly after the manuscript of this article was completed, a new version of the CITES Trade Database in shipment-by-shipment format was released; we assume that if the new data affect our results, the impact will be insignificant, as the new data expand the original dataset by 7% (1418,294 rows). If the listing of a taxon in the Appendices changed during study period, we used only shipments during the time when the taxon was traded as an Appendix I species. We have also limited the analysis to data after 1996 because the data is more credible and better controlled beginning in that year (CITES, 2013). Shipments for the year 2018 were included in the analyses, despite probably being incomplete at the time of download.

First, we analysed how much of the CITES Trade Database in terms of shipments and trade volumes could be connected through identifiers (IDs). We assumed that the full trade record would include matchable (i.e., reciprocal) reporting from both the exporter and importer, i.e., the shipment would appear in at least two rows of the shipment-level data. It may include different combinations of shipments from the exporter and importer (e.g., one record from the exporter and several records from the importer).

An ID can be listed as useful in terms of identifying the flow of goods (or “enabling the matching of export and import shipments”) if it appears in at least two combinations of Reporter.type and ID at the same time (Fig. 1). Thus, the movement of goods can be identified either (1) within a single column (e.g., if a shipment was reported under the same identifier in E ID at least once by the importer and at least once by the exporter) (1a+1b in Fig. 1) or (2) between columns (e.g., if a shipment was reported under the same identifier at least once in the E ID entry and at least once by the importer in the I ID entry) (2a+2b in Fig. 1). If an ID appears multiple times in one column and always only as the importer or exporter, it only indicates that the shipment was exported or imported in multiple parts, but nothing can be determined about the shipment’s movement. Origin IDs, indicating re-exports, were not used for shipments matching because all shipments with an O ID should be simultaneously connectable via the E ID and I ID (CITES, 2018a). For the matchable shipments, the share of the number of shipments and volume of trade was calculated for individual combinations of terms and units.

3. Results and discussion

The CITES Trade Database contained 618,750 shipments for trade in animals listed in Appendix I for the period 1996–2018 (Table S2). For this selected dataset on CITES Appendix I animal species, it turns out that the usability of IDs for tracking shipments appears to be relatively limited for three main reasons.

1a

Reporter.type	E ID	I ID
E	x	
I	x	

1b

Reporter.type	E ID	I ID
E		x
I		x

2a

Reporter.type	E ID	I ID
E	x	
I		x

2b

Reporter.type	E ID	I ID
E		x
I	x	

Fig. 1. Types of matchable shipments. Within-column selection for (1a) E ID: same E ID for both the E and I Reporter type, each at least once as the E Reporter type and at least once as the I Reporter type and (1b) I ID: same I ID for the E and I Reporter type, each at least once as the E Reporter type and at least once as the I Reporter type. Between-column selection for the same E ID and I ID, each reported at least once as the E Reporter type and at least once as the I Reporter type: (2a) E ID as the E Reporter type and I ID as the I Reporter type and (2b) E ID as the I Reporter type and I ID as the E Reporter type. Note: Reporter.type E (exporters) and I (importers), E ID: Export.permit.RandomID, I ID: Import.permit.RandomID x: 10-digit alphanumeric code, e.g., 5e99cfb92b.

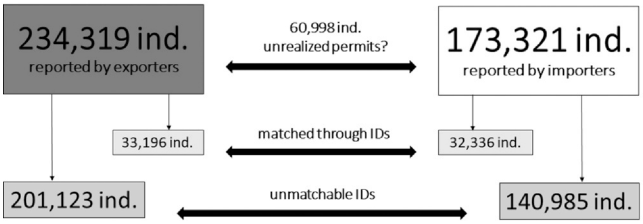

Fig. 2. Volume (number of individuals) in shipments matchable and unmatchable through IDs for live *Scleropages formosus* reported by exporters and importers in 2015 (n = 3927).

In the first place, IDs were not always uniquely used. In 618,750 shipments, a total of 373,031 IDs appeared either as E ID, I ID, or O ID. Unfortunately, some of them were apparently misallocated because they combine unrelated shipments. Indeed, for 24,934 of the 290,651 E IDs, more than one exporting country is listed (maximum of 76 countries are listed for E ID e85fb541b7), although the rules for export permitting allow only one country to be listed (CITES, 2018b). Similarly, 30,174 out of 290,651 E IDs reported trade in more than one year (18 years out of the 23 we covered for E ID 5d567958a6), whereas a permit is valid for a maximum of half a year (CITES, 2018b). If we admit the possibility of exporting specimens at the end of one year and importing at the beginning of the following year (i.e. one E IDs for two subsequent years), the number of E IDs listed in more years would be reduced to 20,950.

Secondly, IDs were missing or did not have a counterpart. In 35,395 shipments (5.7% out of 618,750), no ID was assigned and this occurred four times more often for shipment reports from importing countries (Table S2). However, it is definitely good that these cases are declining over time, as the number of shipments with no ID has been below the overall annual mean (1539 ± 860 shipments) since 2010. In fact, almost two-thirds of individual IDs (242,005 out of 373,031) appeared only once in the dataset and are therefore not usable in terms of trade tracing (of course, if the ID is not given later). Furthermore, although the other 63,603 individual IDs appeared more than once in the dataset, they always appeared in only one of the identifier fields (E ID, I ID, or O ID) and at the same time only as either a Reporter.type E or I, and therefore also have zero value for trade tracing. This means that the remaining 67,423 (18.1%) individual IDs were usable for tracing the movement of CITES samples. These usable IDs appeared in 216,041 (34.9%) shipments. For the most traded combination of term and unit—live animals reported in the number of individuals— matchable shipments accounted for less than half the number of unmatchable shipments (13.9% vs. 28.4% of the total number of shipments, respectively).

Finally, shipments matchable through IDs accounted for only a minor portion of the total traded volume. For the highest number of combinations of terms and units, the matchable volume represented a maximum of 10% of the total volume of trades (Fig. 3). Importantly, for the most traded combination of term and unit, i.e., live animals reported in the number of individuals, more than half of the traded volume (56.3%) was in matchable shipments (65th in order from the highest volume share in matchable shipments of the 263 total combinations).

To illustrate the problem, we are trying to point out, we choose the example of *Scleropages formosus* traded in 2015. According to exporters, a total of 234,319 live fish were traded that year. In contrast, importers reported only 173,321 live fish (Fig. 2). The difference of 60,998 individuals is usually explained as a result of unrealized permits (Robinson and Sinovas, 2018). In fact, this could be true if import and export data were connectable for all 173,321 fish reported by importers by using the same IDs by both trading parties (which, reportedly, is the goal of introducing the use of IDs; CITES (2018a)). However, due to the disconnectivity of the majority of the data in this example, nothing like this can be stated with certainty and the actual

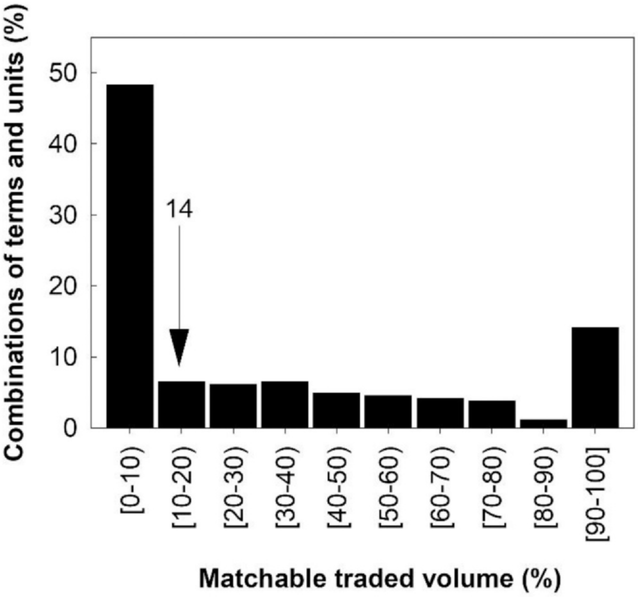

Fig. 3. Relative number of combinations of terms and units (n = 263) by shares of traded volume in matchable shipments relative to the total volume (all shipments). The arrow shows the median.

number of traded individuals may be higher, as the absence of reports on both sides of the trade cannot be ruled out. In our case, a total of 2440 IDs were listed for both types of reports. Of them, 301 IDs connected export and import data in 825 out of 3927 shipments. In volume, it represents 33,196 fish reported by exporters or 32,336 fish reported by importers. All other fish can be the same individuals, reported under different IDs, but also different individuals, reported only by one trading Party. To sum up, out of the total number of 234,319 exported fish, only 32,336 individuals (13.8%) can be matched by connecting unique E IDs or IDs listed by both the exporter and the importer. Other individuals could or could not be the same.

4. Conclusions

We commend UNEP-WCMC for managing of trade data and making them available to the public to explore one of the major threats to biodiversity. Unfortunately, it is clear that the use of new descriptors in the form of unique IDs is not currently helping progress be made in deciphering the volume of trade in CITES Appendix I species. The main problems with the CITES Trade Database are highly varied levels of compliance with CITES reporting requirements across Parties in terms of report submission and report quality and substantial amounts of missing data (Foster et al., 2016), whether unintentional or intentional due to the absence of this requirement from CITES trade rules (e.g., import reports for CITES Appendix II species are not required unless specified by national legislation).

As further use of the CITES Trade Database can be envisaged to assess the impact of trade on threats to plants and animals, unnecessarily complex methods of calculating the traded volume could be replaced by reporting only the actual traded quantity. If it is not possible and Parties have to report permitted quantity, they should indicate this in the permit (CITES, 2018b, 2019). Unfortunately, this information is currently not available in the Database. Tightening the rules at a time of increasing the absolute global trade volume is highly desirable; monitoring through the quantities reported by importing countries appears to be a good way of control.

Overall, it is essential that CITES Secretariat should increase pressure on CITES Parties to provide the most complete trade reports possible. If the situation does not improve, the calculation of trade volume will remain a quixotic effort.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01635.

References

Andersson, A., Gibson, L., 2018. Missing teeth: discordances in the trade of hippo ivory between Africa and Hong Kong. Afr. J. Ecol. 56, 235–243. https://doi.org/10.1111/aje.12441

Auliya, M., Altherr, S., Ariano-Sanchez, D., Baard, E.H., Brown, C., Brown, R.M., Cantu, J.-C., Gentile, G., Gildenhuys, P., Henningheim, E., 2016a. Trade in live reptiles, its impact on wild populations, and the role of the European market. Biol. Conserv. 204, 103–119. https://doi.org/10.1016/j.biocon.2016.05.017

Auliya, M., García-Moreno, J., Schmidt, B.R., Schmeller, D.S., Hoogmoed, M.S., Fisher, M.C., Pasmans, F., Henle, K., Bickford, D., Martel, A., 2016b. The global amphibian trade flows through Europe: the need for enforcing and improving legislation. Biodivers. Conserv. 25, 2581–2595. https://doi.org/10.1007/s10531-016-1193-8

Berec, M., Vršecká, L., Šetlíková, I., 2018. What is the reality of wildlife trade volume? CITES Trade Database limitations. Biol. Conserv. 224, 111–116. https://doi.org/10.1016/j.biocon.2018.05.025

Bruckner, A.W., 2001. Tracking the trade in ornamental coral reef organisms: the importance of CITES and its limitations. Aquar. Sci. Conserv. 3, 79–94. https://doi.org/10.1023/A:1011369015080

Carpenter, A.I., Rowcliffe, J.M., Watkinson, A.R., 2004. The dynamics of the global trade in chameleons. Biol. Conserv. 120, 291–301. https://doi.org/10.1016/j.biocon.2004.03.002

CITES, 2013. A Guide to Using the CITES Trade Database. Cambridge, United Kingdom.

CITES, 2018a. CITES Trade Database: improving access to annual report data. https://cites.org/sites/default/files/eng/com/sc/70/Inf/E-SC70-Inf-01.pdf (accessed 12 July 2020).

CITES, 2018b. Resolution Conf.12.3 Permits and certificates. https://www.cites.org/sites/default/files/document/E-Res-12-03-R18.pdf (accessed 12 July 2020).

CITES, 2019. Guidelines for the preparation and submission of annual reports and of annual illegal trade reports. https://cites.org/eng/node/55474 (accessed 10 April 2021).

D'Cruze, N., Macdonald, D.W., 2016. A review of global trends in CITES live wildlife confiscations. Nat. Conserv. 15, 47–63. https://doi.org/10.3897/natureconservation.15.10005

Foster, S., Wiswedel, S., Vincent, A., 2016. Opportunities and challenges for analysis of wildlife trade using CITES data–seahorses as a case study. Aquat. Conserv. 26, 154–172. https://doi.org/10.1002/aqc.2493

Harfoot, M., Glaser, S.A., Tittensor, D.P., Britten, G.L., McLardy, C., Malsch, K., Burgess, N.D., 2018. Unveiling the patterns and trends in 40 years of global trade in CITES-listed wildlife. Biol. Conserv. 223, 47–57. https://doi.org/10.1016/j.biocon.2018.04.017

Harris, J.B.C., Green, J.M., Prawiradilaga, D.M., Giam, X., Hikmatullah, D., Putra, C.A., Wilcove, D.S., 2015. Using market data and expert opinion to identify overexploited species in the wild bird trade. Biol. Conserv. 187, 51–60. https://doi.org/10.1016/j.biocon.2015.04.009

Hierink, F., Bolon, I., Durso, A.M., de Castañeda, R.R., Zambrana-Torrelio, C., Eskew, E.A., Ray, N., 2020. Forty-four years of global trade in CITES-listed snakes: trends and implications for conservation and public health. Biol. Conserv. 248, 108601. https://doi.org/10.1016/j.biocon.2020.108601

Janssen, J., Indenbaum, R.A., 2019. Endemic Vietnamese reptiles in commercial trade. J. Asia-Pac. Biodivers. 12, 45–48. https://doi.org/10.1016/j.japb.2018.11.010

Luiselli, L., Starita, A., Carpaneto, G.M., Segniagbeto, G.H., Amori, G., 2016. A short review of the international trade of wild tortoises and freshwater turtles across the world and throughout two decades. Chelonian Conserv. Biol. 15, 167–172. https://doi.org/10.2744/CCB-1216.1

Mair, L., Ridley, F.A., Fleming, L.V., McGowan, P.J., 2019. A risk assessment framework to improve the efficiency of CITES. Biol. Conserv. 239, 108260. https://doi.org/10.1016/j.biocon.2019.108260

Nijman, V., Shepherd, C.R., 2010. The role of Asia in the global trade in CITES II-listed poison arrow frogs: hopping from Kazakhstan to Lebanon to Thailand and beyond. Biodivers. Conserv. 19, 1963–1970. https://doi.org/10.1007/s10531-010-9814-0

Pernetta, A.P., 2009. Monitoring the trade: using the CITES database to examine the global trade in live monitor lizards (*Varanus* spp.). Biawak 3, 37–45.

Robinson, J.E., Sinovas, P., 2018. Challenges of analyzing the global trade in CITES-listed wildlife. Conserv. Biol. 32, 1203–1206. https://doi.org/10.1111/cobi.13095

Russo, A., 2015. The Prevalence of Documentation Discrepancies in CITES (Convention on the International Trade in Endangered Species of Wild Fauna and Flora) Trade Data for Appendix I and II Species Exported out of Africa between the Years 2003 and 2012. Ph.D. Thesis, University of Cape Town.

Vall-llosera, M., Cassey, P., 2017. 'Do you come from a land down under?' Characteristics of the international trade in Australian endemic parrots. Biol. Conserv. 207, 38–46. https://doi.org/10.1016/j.biocon.2017.01.015

Články ve sbornících

Vodrážková M., Berec M. (2019). Is the growth of brown frog´s tadpoles influenced by the present od red-eared sliders? In: Conference: XX European Congress of Herpetology. Milano, S.E.H. ISBN: 979-12-200-5284-9;

Vodrážková M., Šetlíková I., Berec M. (2020). Smell of fear: presence of an invasive turtle reduces development time and size at metamorphosis in the common frog. In: Zoologické dny Olomouc, Ústav biologie obratlovců AV ČR, v.v.i., ISBN: 978-80-87189-32-0

Vodrážková M., Šetlíková I., Berec M. (2021). Smell of fear: the presence of an invasive turtle influences embryonic and larval development in the common frog. In: XIII. ročník konference Kostelecké inspirování, Kostelec nad Černými lesy, ISBN: 978-80-213-3145-7