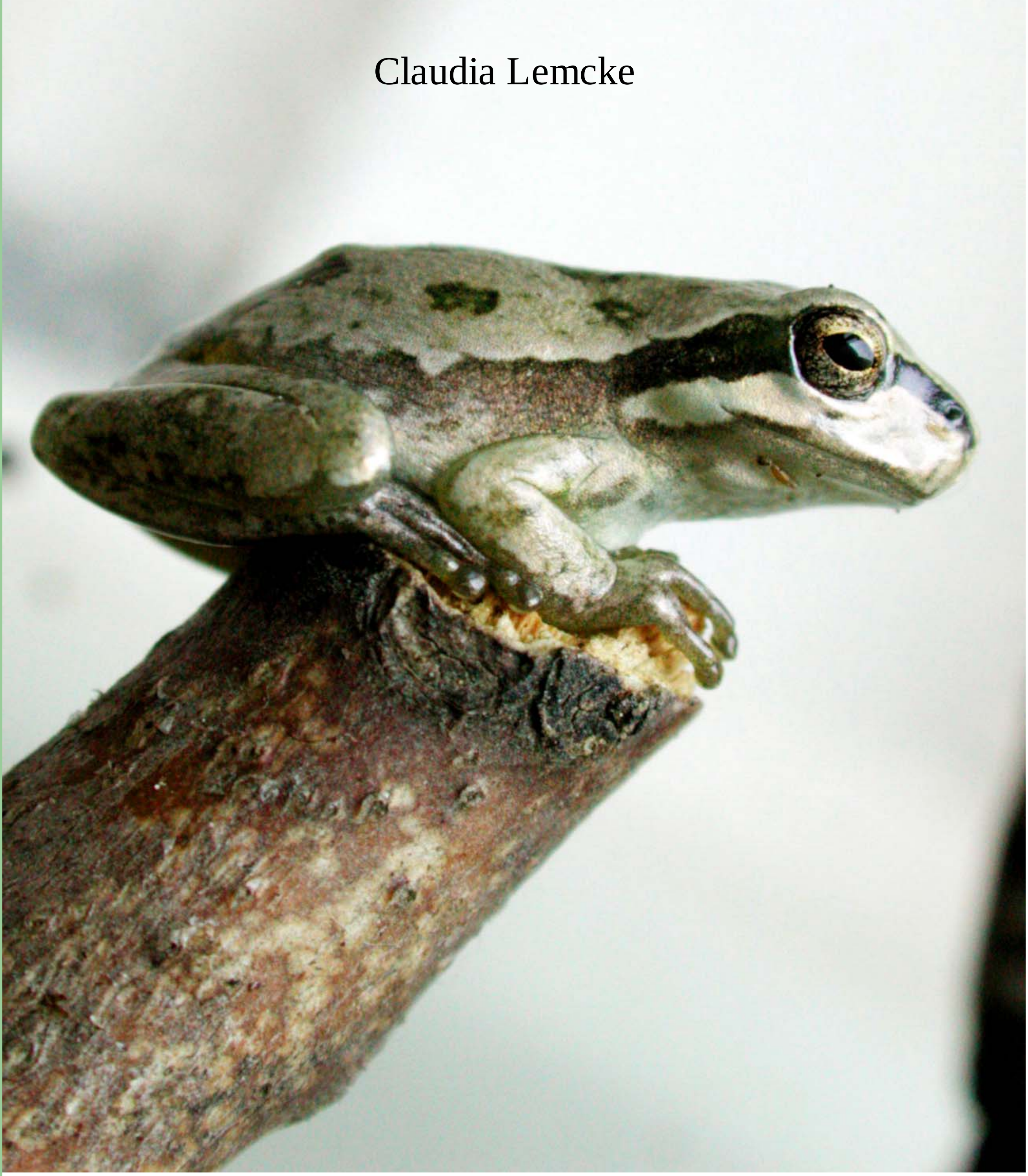
Phänotypische Plastizität bei Kaulquappen des Europäischen Laubfrosches, *Hyla arborea.*

Claudia Lemcke



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Phänotypische Plastizität bei Kaulquappen des Europäischen Laubfrosches, *Hyla arborea*

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Phänotypische Plastizität bei Kaulquappen des Europäischen Laubfrosches, *Hyla arborea*

1. Zusammenfassung

Viele Theorien besagen, dass phänotypische Plastizität, definiert als die Fähigkeit eines Genotyps, verschiedene Phänotypen in unterschiedlicher Umgebung zu produzieren, einen Vorteil bei Räuber-Beute-Konfrontationen darstellt. Amphibien sind als Nachweisführung eines solchen Phänomens besonders geeignete Versuchsobjekte, da sich bei ihnen morphologische Veränderungen deutlich ausprägen und gut sichtbar werden. Ziel meiner Arbeit war es, phänotypische Plastizität bei europäischen Laubfroschkaulquappen (*Hyla arborea*) und ihre möglichen Vorteile bei direkten Räuberkonfrontationen nachzuweisen. Dabei wurden sowohl verschiedene mögliche Auslöser einer morphologischen Veränderung getestet, als auch deren Mechanismus und Auswirkung auf das Verhalten der Kaulquappen bei veränderten Umweltbedingungen.

In meiner Arbeit konnte ich nachweisen, dass *Hyla arborea* Kaulquappen deutlich sichtbare morphologische Veränderungen in Form eines höheren Schwanzsaumes in Gegenwart von Libellenlarven der Art Blaugrüne Mosaikjungfer (*Aeshna cyanea*) ausbildeten. *H. arborea* Kaulquappen wiesen zudem einen deutlich verringerten Aktivitätsgrad bei Räuberpräsenz auf als Kontrolltiere ohne Räuberanwesenheit. Dieses Verhalten kehrte sich jedoch in der Nacht um. Des Weiteren konnte ich klären, dass Laubfroschkaulquappen nicht die Fähigkeit besitzen, auch heterospezifische chemische Stoffe wahrzunehmen.

Erstmalig konnte ich in meiner Arbeit zeigen, dass Libellenlarven beim Jagen sich nicht nur an der Bewegung der Beute orientieren, sondern auch chemische Substanzen als Informationsquelle benutzen. Folglich stellen Libellenlarven der Art *Aeshna cyanea* nicht nur eine Gefahr für Kaulquappen dar, sondern auch bereits für den frischen Laubfroschlaich. Dies könnte einer der vielen Gründe für die deutliche Dezimierung der Laubfroschpopulation in Deutschland in den letzten Jahren darstellen. Um festzustellen, ob zwischenartliche Konkurrenten ebenfalls eine Bedrohung für Laubfroschlaich sind, wurden die mit Laubfröschen häufig koexistierenden Erdkrötenkaulquappen (*Bufo bufo*) als Versuchstiere zu

Forschungszwecken eingesetzt. Dabei erwies sich der Verlust von Laubfroschlaich in Anwesenheit von Erdkrötenkaulquappen als sehr hoch.

Folgende Zusammenfassung zeigt einen kurzen Überblick aller erstellten Hypothesen, durchgeführten Experimente und deren Ergebnisse:

1. **Ausprägung morphologischer Plastizität**

   Hypothese: *Hyla arborea* Larven entwickeln morphologische Plastizität in Anwesenheit eines Räubers.

   Experiment: *H. arborea* Kaulquappen wurden in Anwesenheit von nicht letalen Räubern (*Aeshna cyanea*) gezüchtet. Anschließend wurden morphologische Veränderungen der *H. arborea* Larven mit Hilfe von Körper- sowie Schwanzvermessungen quantifiziert.

   Ergebnis: *H. arborea* entwickelte signifikant höhere Schwanzflossen in Räuberpräsenz als in Räuberabwesenheit. Zusätzlich konnte ich schwarze Pigmentflecke an den Schwanzflossen induzierter Tiere feststellen.

2. **Vorteil induzierbarer Verteidigung**

   Hypothese: Räuber-induzierte morphologische Veränderungen bei Kaulquappen erhöhen die Überlebenschance bei Räuberangriffen.

   Experiment: Induzierte und nicht induzierte *H. arborea* Kaulquappen wurden direkt mit letalen Räubern (*Aeshna cyanea*) konfrontiert. Die Angriffe auf die Beute und die überlebenden Kaulquappen wurden beobachtet und notiert.

   Ergebnis: Meine Ergebnisse zeigten, dass induzierte Tiere eine signifikant höhere Überlebenschance haben als nicht induzierte Kaulquappen. Die ausgebildeten schwarzen Pigmentflecke dienten nicht wie bisher angenommen als Tarnung, sondern als eine Art Ablenkung weg vom verletzlicheren Kopf hin zum Schwanz.

3. **Artspezifität der phänotypischen Plastizität**

   Hypothese: *H. arborea* Larven reagieren morphologisch auf artfremde Alarmpheromone, um ihre Überlebenschancen dadurch zu erhöhen.

   Experiment: Nicht letalen Räubern wurden verschiedene Futterquellen angeboten (*H. arborea* und *Bufo bufo* Larven sowie adulte Wasserflöhe (*Daphnia magna*). Kurz vor ihrer Metamorphose wurden ihre morphologischen Veränderungen erfasst.

Ergebnis: *H. arborea* Kaulquappen bildeten entgegen der Hypothese die höchsten Schwanzflossen bei Freigabe arteigener Alarmpheromone.

4. **Verhaltensänderungen bei Feindpräsenz auf den Tag und Nacht Rhythmus in Abhängigkeit der Populationsdichte und der Futtermenge**

Hypothese: In Anwesenheit von Räubern verändern *H. arborea* Kaulquappen in Abhängigkeit ihrer Populationsdichte und der Futterkonzentration ihre Tag-Nacht Rhythmus Aktivität.

Experiment: *H. arborea* Kaulquappen, aufgezogen in unterschiedlicher Populationsdichte und gefüttert mit verschiedenen Futterkonzentrationen wurden mit nicht letalen Räubern konfrontiert. Ihr Verhalten (Schwimmen, Fressen und Ruhen) wurde während einer festgelegten Zeitspanne Tag und Nacht digitalisiert.

Ergebnis: Die Aktivität induzierter Kaulquappen war abhängig sowohl von der Populationsdichte als auch vom Tag und Nacht Rhythmus. Die Futtermenge hatte nur auf das Fraßverhalten einen Einfluss. Induzierte Kaulquappen verstärkten ihre Aktivität bei Nacht.

In den letzten Jahren konnte ein drastischer Rückgang der Laubfroschpopulation in Deutschland beobachtet werden. Da ich dieses Phänomen ebenfalls an den *H. arborea* Laichsammelplätzen in den Jahren meiner Dissertation feststellen konnte, beschäftigte ich mich zusätzlich mit der Fragestellung nach dem Grund eines solchen Rückgangs. Ich testete daher Tiere, die mit Laubfroschkaulquappen koexistieren auf ihren Gefahrengrad gegenüber Laubfroschkaulquappen:

5. ***Hyla arborea*** **Laich Prädation**

Hypothese: *H. arborea* Laich wird weder von Libellenlarven (*Aeshna cyanea)* noch von artfremden Konkurrenten gefressen.

Experiment: Die Überlebenswahrscheinlichkeit von frischem *H. arborea* Laich wurde in Anwesenheit von gefütterten Erdkrötenkaulquappen (*Bufo bufo*) und gefütterten Libellenlarven der Art *Aeshna cyanea* getestet.

Ergebnis: Im Gegensatz zu der bisher angenommenen Eigenschaft, dass Libellenlarven ausschließlich bewegungsorientiert jagen, wurden *H. arborea* Laichballen von den Libellenlarven gefressen. *B. bufo* Kaulquappen fügten dem Laubfroschlaich ebenfalls letalen Schaden zu.

2. Abstract

Phenotypic plasticity is defined as the ability of a genotype to produce different phenotypes in different environments. Studies have shown that induced morphological defences often occur in aquatic vertebrates and it is said to positively influence the morphological defences of anuran larvae. Amphibians serve as a very popular studying object, for they show very obvious morphological changes in shape and colour. The aim of this study was to prove the existence of phenotypic plasticity in European tree frog, *Hyla arborea*, tadpoles, its mechanisms and its possible advantages in predator-prey confrontations. And to find out more about the triggers that lead to phenotypic plasticity in *Hyla arborea* tadpoles.

Adequate experiments showed definite morphological reactions as *H. arborea* tadpoles increased tail depth and produced dark spots on their tails in the presence predators (*Aeshna cyanea*). When tadpoles that were raised in the presence predators (induced) and tadpoles raised in the absence of predators (control) were directly confronted with a predator, induced tadpoles showed a significantly higher survival rate than control animals. Detailed analysis even pointed altering tail color as a predatory reaction that deflects predator strikes from the vulnerable body to the tail rather than serving as a kind of camouflage.

However, little is known about the actual triggers that lead to phenotypic plasticity. Few studies have suggested that inducing factors in anurans appear to be chemical cues released by either some metabolite of dragonfly digestion of prey or alarm pheromones released by tadpoles when injured by predators. Considering this, it is still to resolve whether alarm cues are species specific or if tadpoles of one species are able to detect heterospecific cues to increase the possibility of sensing predators which would lead to an increase in survival rates. This would amplify the importance of a predators diet. Consequently this study used experiments varying the predators diet. When dragonflies were fed with conspecifics *H. arborea* larvae showed great morphological reactions, while this was not the case, when fed with heterospecifics. This means *H. arborea* react strongest to their own cues.

Are morphological changes the only reactions to the presence of predators, or do tadpoles also vary their behaviour in the presence of predators in order to avoid predatory confrontations and therefore increase survival rate? The results of the experiment dealing with this problem confirmed the assumption of decreasing activity, as it showed a significant decrease in time spend swimming in induced tadpoles.

This study also showed for the first time that dragonfly larvae (*Aeshna cyanea*) not only hunt by sensing movement, they also tend to use chemical sensors. This was hypothesised, when an experiment showed fed dragonfly larvae feasted on freshly laid *Hyla arborea* eggs, where no movement took place. We also found that not only dragonfly larvae like to feast on freshly laid *H. arborea* eggs, but also the often coexisting common toad, *Bufo bufo*, tadpoles tended to eliminate them.

3. Einleitung

3.1. Induzierbare Verteidigung

Der Begriff phänotypische Plastizität ist definiert als die Fähigkeit von Organismen, als Reaktion auf variable Umweltbedingungen unterschiedliche Phänotypen zu produzieren. Induzierbare Verteidigungen sind phänotypische Veränderungen, welche durch verschiedenartige Reize ausgelöst werden können. Oft stehen diese Reize in Zusammenhang mit einer drohenden Gefahr für den Organismus. Die plastischen Veränderungen bedeuten für den Organismus einen Vorteil bei direkten Räuber-Beute-Konfrontationen.

Es existieren unter anderem zwei durch Forschung bekannte Arten von induzierbarer Verteidigung. Die erste wird durch hohe Konkurrenz verursacht und wird daher als konkurrenz-induzierte Verteidigung bezeichnet. Tiere reagieren auf Konkurrenz oftmals mit verstärkter Nahrungssuche oder variieren sogar ihr Futter (Akre und Johnson, 1979; Crowley, 1979). Zum Beispiel gibt es Amphibien, die von Omnivorie auf Karnivorie umstellen können und bei hoher Konkurrenz sogar zu Kannibalen werden (Bragg, 1956).

Die zweite induzierbare Verteidigung wird durch Prädatoren verursacht und wird daher räuber-induzierte Verteidigung genannt. Dieses Phänomen ist im Pflanzenreich ebenso präsent wie im Tierreich. Viele Pflanzen zum Beispiel reagieren auf Herbivorie indem sie kurzzeitig anti-herbivore chemische Stoffe produzieren (Karban und Baldwin, 1997). Tiere reagieren in Anwesenheit von Prädatoren, indem sie ihr Verhalten ändern (zum Beispiel durch Reduktion der Aktivität oder durch räumliche Trennung von dem Prädator; Lima und Dill, 1990), oder sie verändern ihre Morphologie (zum Beispiel Helmbildung bei *Daphnia cucullata*; Tollrian, 1990). Diese räuber-induzierten Reaktionen führen in der Regel zu einer erhöhten Überlebenschance.

Die Ausprägung der phänotypischen Plastizität ist stark an Umweltbedingungen gebunden. Folgende variable Rahmenbedingungen werden als Voraussetzungen für induzierte Verteidigung angenommen:

1. Hinweis auf die Existenz eines Prädators zur Aktivierung der Verteidigung nötig.
2. Variabilität des Selektionsdruckes des Räubers, da bei permanentem Raubdruck eine konstitutive Verteidigung zu erwarten wäre.
3. Existenz eines Vorteils der Verteidigung.

4. Zugleich Existenz eines Nachteils der Verteidigung, der bei Situationen, in denen der Vorteil nicht zutrifft, überwiegt, da ansonsten eine permanente Verteidigung zu erwarten wäre.

3.2. Induzierbare Verteidigung bei *Amphibien*

Das zunehmende Interesse an Amphibienpopulationen und ihrem weltweiten Rückgang begann 1989 beim ersten Weltkongress der Herpetologie. Seit der Erfassung von Amphibienbeständen wurden bis 1993 mehr als 500 Frosch- und Salamanderpopulationen auf fünf Kontinenten entweder ausgerottet, oder galten als vom Aussterben bedroht. Dies wurde als ein alarmierendes Zeichen gedeutet, wobei die Gründe des Rückgangs im Zusammenspiel vieler Faktoren liegen mögen. Zum Beispiel spielen UV-Strahlung, Prädation sowie Habitatmodifikation, erhöhte Konzentrationen von Toxinen im Habitat, Klimaveränderungen und auch Krankheiten hierbei eine große Rolle. Da die Amphibien zu verschiedenen Zeiten ihres Lebenszyklus sowohl terrestrischen als auch aquatischen Habitaten ausgesetzt sind, und da sie eine sehr permeable Haut besitzen, könnten dies Erklärungen für ihre sensible Reaktion auf Toxine oder Temperaturschwankungen darstellen (Alford und Richards, 1999).

Interaktionen zwischen Amphibien und anderen Organismen spielen eine sehr große Rolle bei der Wahl des Habitats und der Populationsdynamik der Frösche. Larvale Amphibien sind vertebraten und invertebraten Prädatoren ausgesetzt. Daher ist ihre Diversität in Habitaten, die räuberische Fische enthalten, um einiges herabgesetzt (Alford, 1999). Normalerweise bilden Kaulquappen, welche mit ihren natürlichen Feinden koexistieren, Verteidigungsmechanismen aus, um den Räubern zu entkommen (Chovanec, 1992). Da es aber immer mehr künstlich eingesetzte Fischarten oder andere zugewanderte Feinde gibt, mit denen die Kaulquappen zuvor noch nie in Kontakt standen, haben sie gegen diese ‚neuen’ Feinde keine Abwehrmöglichkeiten. Solch ein Fall ist beispielsweise aus der Sierra Nevada beschrieben. Dort sind inzwischen in sechzig Prozent der Seen, die früher mit Fröschen besiedelt waren, nur noch eingesetzte Fische vorzufinden, während die Frösche ausgerottet wurden. Besonders betroffen hiervon ist *Rana muscosa* (Gebirgs-Gelbschenkelfrosch). Diese Art laicht in besonders tiefen Gewässern (Bradford, 1989), und wird somit zu leichter Fischbeute. Selbst eine der in Nordamerika eingeführten Froscharten, *Rana catesbeiana*, bedroht die dort heimischen Gelbschenkelfrösche, da sie sich von genau diesen ernährt (Hayes und Jennings, 1986).

Um sich vor möglichen Bedrohungen zu schützen, besitzen Amphibien die Fähigkeit, ihre äußere Gestalt zu verändern. In Experimenten, in denen *Hyla versicolor* Kaulquappen mit Räubern konfrontiert worden waren, trat phänotypische Plastizität in Form von längeren und breiteren Schwanzflossen auf (Van Buskirk und McCollum, 2000). Weitere Versuche zeigten, dass die Tiere, die den Feinden ausgesetzt waren, vor der Metamorphose langsamer wuchsen als ihre Artgenossen (Van Buskirk und Schmidt, 2000). Diese Entdeckung deutet auf einen ‚Trade-off' hin. Das heißt, die Ausprägung morphologische Plastizität wird zum Beispiel mit einem langsameren Wachstum erkauft. Jedoch lohnt es sich für die Kaulquappen diesen Nachteil zu tragen, da trotz höherer Wachstumskosten eine körperliche Veränderung einen erheblichen Überlebensvorteil bedeutet (Van Buskirk, 2000).

Das Auftreten phänotypischer Plastizität in Amphibien wurde oftmals demonstriert. Dennoch ist man sich nicht einig über die Auslöser und Mechanismen dieses Phänomens. Studien von Kaulquappen der Art *Bufo* (Erdkröte) zeigten, dass bei feindlichen Attacken auf Artgenossen Alarmpheromone von verletzten Tieren abgegeben werden (Waldman und Adler, 1979). Diese Pheromone, welche in epidermischen Zellen gelagert werden können nur freigegeben werden, wenn die Haut direkt verletzt wird, sie können jedoch nicht aktiv ins Wasser abgegeben werden. Normalerweise reagieren die Tiere auf diesen Stoff, indem sie fliehen, oder sich in Gruppen horten. Darauf aufbauende Studien fanden zusätzlich heraus, dass nicht nur die bei einer Verletzung einer Kaulquappe freiwerdenden chemischen Stoffe eine Rolle bei der induzierten Verteidigung spielen, sondern dass auch die Stoffwechselprodukte, die von Prädatoren nach der Verdauung einer Beute ins Wasser abgegeben werden, von Kaulquappen wahrgenommen werden können (Laurila et al., 1998; Petranka und Hayes, 1998).

4. Studienobjekte

4.1. Laubfrosch, *Hyla arborea (Anura, Hylidae)*

Abb. 1: Europäischer Laubfrosch der Art *Hyla arborea*

„... im Allgemeinen zeichnet sich die ganze Oberseite durch ein freudiges lebhaftes Blattgrün aus, das von dem Gelblichweiß der Unterseite durch einen schwärzlichen Streifen geschieden wird.“ (Dürigen, 1897)

Wie die Mehrzahl der einheimischen Amphibien lebt auch der europäische Laubfrosch (Abb. 1) das Jahr über an Land, d.h. er sucht lediglich zum Ablaichen Gewässer auf.
Ursprünglich waren Weiher, Teiche und Altwässer mit 61% Häufigkeit die mit Abstand bevorzugten Paarungs-Ablaichbiotope; nur mit 7% Häufigkeit wurden temporäre Kleinstgewässer gewählt (Grosse, 1994). Da jedoch nur wenige permanente Gewässer erhalten sind, verschiebt sich dieses Verhältnis heutzutage deutlich in Richtung temporärer Gewässer (Abb. 2 und 3).

Abb. 2 (oben) + 3 (unten): Typische *Hyla arborea* Laichgewässer in der Fröttmanninger Heide bei München

Laubfroschweibchen Laichen üblicherweise von Mai bis Ende Juni ab. Die Kaulquappenentwicklung dauert in Abhängigkeit von den Umweltbedingungen ca. 50 Tage. Eine ihrer größten Feinde stellen sicherlich alle möglichen Arten von Libellenlarven dar.

4.2. Prädator: Libellenlarve Blaugrüne Mosaikjungfer, *Aeshna cyanea (Odonata, Aeshnidae)*

Abb. 4: Adulte Libelle (links) und Libellenlarve (rechts) der Art *Aeshna cyanea*

Die Blaugrüne Mosaikjungfer (Abb. 4) hat eine Körpergröße von ca. 70 bis 80 mm. Sie lebt vor allem an kleineren stehenden Gewässern, wie Waldtümpel, Kiesgrubengewässern und oder selbst Gartenteichen. Aber auch an großen Fischteichen, Baggerseen und Weihern ist sie präsent. Libellenlarven leben entsprechend ebenfalls sowohl in temporären als auch permanenten Gewässern. Die Entwicklungszeit beträgt im Allgemeinen zwei Jahre, manchmal auch nur ein Jahr. Das Wachstum der Libellenlarven erfolgt durch Häutungen (Bellmann, 1993).

5. Zusammenfassung der einzelnen Artikel

Zu Beginn meiner Studie konnte ich die Ausprägung von phänotypischer Plastizität in *H. arborea* Kaulquappen in Anwesenheit eines Räubers nachweisen. Jedoch führte mich diese Erkenntnis zu der Frage, welche Mechanismen und Auslöser hinter einer solchen morphologischen Veränderung stecken? Daher behandelte ich diese Fragestellung ebenfalls in meiner ersten Studie. Die darauf folgende Studie setzte sich ebenfalls mit der Frage nach dem Auslöser einer solchen plastischen Reaktion auseinander: Sind diese Auslöser artspezifisch, oder artübergreifend? Nachdem ich phänotypische Reaktionen in *H. arborea* Kaulquappen nachweisen konnte, wollte ich in meiner dritten Studie herausfinden, ob es Aktivitätsänderungen der Kaulquappen in Räuberanwesenheit gab, und welche Umweltbedingungen darauf Einfluss haben. Die letzte Studie baute schließlich auf meinen Beobachtungen im Freiland auf, bei denen ich feststellte, dass die Populationsdichte in meinen Laichsammelgewässern mit den Jahren stetig abnahm. Aufgrund dieser Erkenntnis beobachtete ich frisch abgelegten *H. arborea* Laich im Freiland, und fand dabei heraus, dass Laichfraß sowohl durch Libellenlarven, als auch durch zwischenartliche Konkurrenz in den Laichhabitaten stattfand.

5.1. Predator induced phenotypic plasticity in the European tree frog, *Hyla arborea*: Morphological changes, defensive effect and mechanisms of the defense

Vorangegangene Studien haben gezeigt, dass phänotypische Plastizität in einer Vielzahl von Organismen in ihren natürlichen Umgebungen tatsächlich auftritt. Viele Pflanzen und Tiere reagieren zum Beispiel auf Attacken durch Feinde mit einer morphologischen Veränderung (Schlichting, 1986; Dodson, 1989). Induzierte Verteidigung tritt auf bei einzelligen Organismen bis hin zu höher entwickelten Vertebraten (Tollrian und Harvell, 1999). Untersuchungen an Vertebraten, wie zum Beispiel an verschiedenen Amphibienarten, führten zu der Entdeckung unterschiedlicher Ausprägungen morphologischer Plastizität. So änderten beispielsweise Amphibienlarven in Anwesenheit von Räubern sowohl ihre Schwanzform und Größe, als auch ihre Schwanzfarbe (Smith und Van Buskirk, 1995; McCollum und Van Buskirk, 1996; Relyea, 2001; Van Buskirk et al., 2004). Einige durchgeführte Experimente mit Kaulquappen der Art *Hyla chrysoscelis* (McCollum und Leimberger, 1997) und *Rana* *dalmatina* (Teplitsky et al. 2003) zeigten bei Räuberpräsenz ebenfalls deutliche morphologische Veränderungen.

Während viele Studien jedoch lediglich die Ausprägung morphologischer Plastizität demonstrieren konnten, haben sich nur wenige davon mit den Vorteilen einer solchen befasst. Noch weniger ist darüber hinaus über die Auslöser oder Mechanismen, die dahinter stecken, bekannt. Die von mir durchgeführte Studie konzentrierte sich aus diesem Grund auf die Mechanismen und Auslöser, die einer morphologischen Veränderung zu Grunde liegen. Europäische Laubfroschkaulquappen der Art *Hyla arborea* wurden in naturnahen Umgebungen ohne Prädatoren und mit nicht letalen räuberischen Libellenlarven der Art *Aeshna cyanea* aufgezogen. Dabei konnte eine ausgeprägte phänotypische Plastizität bei induzierten Tieren nachgewiesen werden. Die induzierten Kaulquappen zeigten deutliche morphologische Veränderungen in Schwanzform und Farbe. Schwanzflossen wurden höher und es entstanden dunkle Pigmentflecken am Schwanzsaum (Abb. 5).

Abb. 5: Induzierte und nicht induzierte *Hyla arborea* Kaulquappe. Links: Induziert, mit einer höheren Schwanzflosse und dunklen Pigmentflecken auf dem Schwanz; rechts: Kontrolltier.

Bei direktem Kontakt der induzierten und der Kontrolltiere mit einer hungrigen Libellenlarve wurden signifikant mehr Kontrolltiere gefressen, obwohl die Anzahl der Attacken sich nicht signifikant bei beiden unterschied. Meine Studie zeigt zum ersten Mal die Mechanismen, die zur Ausprägung von phänotypischer Plastizität in *Hyla arborea* Kaulquappen führt. So dient die Farbänderung am Schwanz nicht als Tarnung, sondern als eine Art Ablenkungsmanöver weg vom sehr verletzlichen Kopf oder Rumpf hin zum Schwanz.

Ein anderer Mechanismus, der einer morphologischen Veränderung (Ausbildung einer höheren Schwanzflosse) bei *H. arborea* Larven zu Grunde liegt, ist der Vorteil einer erhöhten Entkommenswahrscheinlichkeit nach dem Fang. Induzierte Tiere konnten sich dank der höheren Schwanzflossen deutlich öfter aus den Fängen der Libellenlarve befreien als Kontrolltiere. Diese Mechanismen stellen relevante Überlebensvorteile von induzierten Kaulquappen gegenüber Kontrolltieren dar, und verdeutlichen somit die Wichtigkeit, phänotypische Plastizität zu initiieren.

5.2. The effect of predator diet on phenotypic plasticity of the European tree frog, *Hyla arborea*, tadpoles

Viele Studien über Räuber-Beute Interaktionen haben die Wichtigkeit chemischer Stoffe im aquatischen Bereich hervorgehoben. In einer Umgebung, in der oftmals visuelle Signale nicht ausreichend vorhanden sind, dienen chemische Stoffe als verlässliche Kommunikationsmöglichkeit. So können zum Beispiel auch Ausscheidungsprodukte eines Räubers (Kairomone) oder durch Verletzung einer Beute hervorgerufene freigegebene Alarmpheromone (Laurila et al., 1997; La Fiandra und Babbitt, 2004) im Wasser wahrgenommen werden. Dies verleiht aquatischen Organismen die Fähigkeit, sensitiv gegenüber einer Vielzahl an chemischen Signalen zu reagieren. Insbesondere aquatische Amphibienlarven reagieren äußerst sensibel auf chemische Reize, die durch Räuberanwesenheit ausgelöst werden. Sie können dadurch sowohl ihre Aktivität als auch ihre morphologische Struktur verändern. Leider ist bisher nur wenig darüber bekannt, wie chemische Stoffe, die durch Stoffwechselprodukte, welche von Prädatoren nach der Verdauung sowohl zwischenartlicher als auch innerartlicher Beute ins Wasser abgegeben werden die morphologischen Veränderungen in innerartlichen Anurenlarven beeinflussen. Ziel meiner Studie war es daher, aufzuklären, ob diese chemischen Stoffe artspezifisch sind, oder ob Kaulquappen einer Art auch artfremde Stoffe wahrnehmen können. Bei eigener niedriger Populationsdichte würde das für die Kaulquappen die Fähigkeit Räuber wahrzunehmen, erhöhen. Eine frühere Räubererkennung würde wiederum zu einer höheren Überlebensrate führen und daher einen großen Überlebensvorteil für die Kaulquappen bedeuten, vorausgesetzt, die Fähigkeit zur Erkennung von artfremden Stoffen ist gegeben. Um dies zu testen, dienten europäische Laubfroschkaulquappen (*Hyla arborea*) als Versuchsobjekte. Sie wurden in naturnahen Habitaten in Anwesenheit und Abwesenheit von nicht letalen Libellenlarven der Art *Aeshna cyanea* aufgezogen. Bei artspezifischer Fütterung der Prädatoren zeigten induzierte Tiere deutliche morphologische Veränderungen der Schwanzform (höhere Schwanzflossen, Abb. 6) und der Farbe.

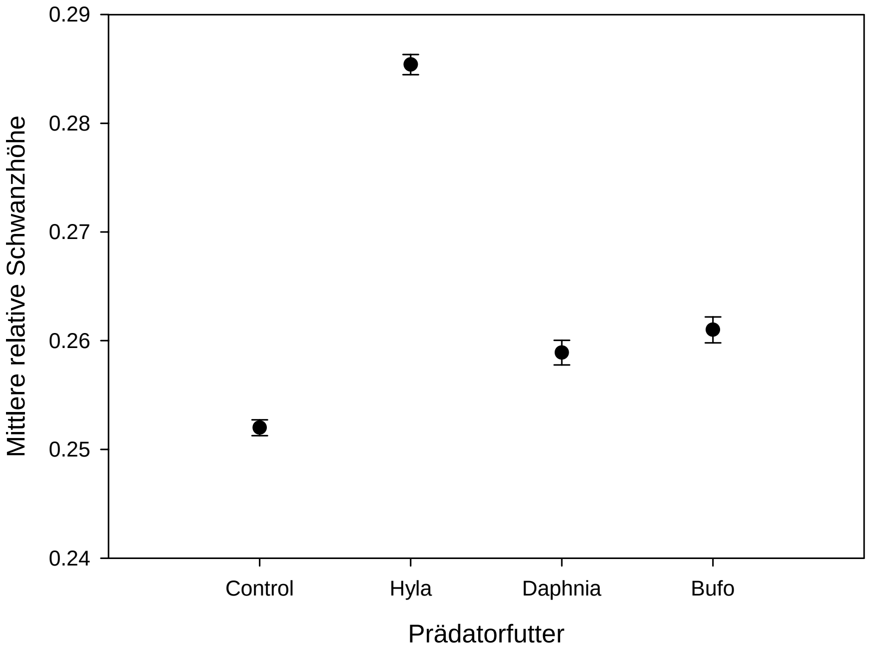

Abb. 6: Mittlere relative Schwanzhöhe (± SE) in Abhängigkeit vom Prädatorfutter.

Eine sehr geringe phänotypische Plastizität konnte dagegen in den Fällen nachgewiesen werden, in denen die Räuber mit artfremden Tieren, wie zum Beispiel Wasserflöhen (*Daphnia magna*) oder Erdkrötenlarven (*Bufo bufo*) gefüttert wurden. Die Ergebnisse meiner Studie deuten darauf hin, dass die Auslöser einer phänotypischen Plastizität vor allem durch artspezifische chemische Stoffe, freigesetzt durch Verletzung eines Artgenossen, oder durch Ausscheidungsprodukte der Prädatoren hervorgerufen werden. Dies zeigt sich auch daran, dass *H. arborea* auf fremde Alarmsubstanzen offensichtlich kaum reagieren, da die Fütterung mit *D. magna* und *B. bufo* zu keinem signifikanten morphologischen Unterschied in der Schwanzhöhe der Kaulquappen führte (Abb. 6).

5.3. Effects of predator presence, food level, density and day-night rhythm on the behavior of European tree frog, *Hyla arborea*, tadpoles

Seit langem gilt Prädation als Ursache vieler sowohl morphologischer Veränderungen als auch Änderungen im Verhaltensmuster bei Tieren. Studien bezeugten Aktivitätsänderungen bei Tieren in Räuberpräsenz. So, zum Beispiel reduzierten Guppies ihre Fraßaktivität in Anwesenheit räuberischer Fische (Fraser und Gilliam 1987). Ebenso reagierten

Odonatenlarven, wie *Coenagrion puella* und *Ischnura verticalis,* wenn sie lebensbedrohliche Fische entdeckten (Convey, 1988; Dixon und Baker, 1988). Im Allgemeinen bedingt eine Nahrungsaufnahme gewisse Aktivität. Jedoch ist mit einer höheren Aktivitätsrate stets ein größeres Prädationsrisiko verbunden, da mehr Aktivität gleichzeitig eine größere Gefahr, dem Räuber zu begegnen, mit sich bringt. Daher ist es oft der Fall, dass Beutetiere ihre Fraßaktivitäten verringern um, in einer Art „Handel" (trade-off) zwischen Wachstum und Überleben, Räuberbegegnungen zu vermeiden. Eine beliebte Tierart bei der der Zusammenhang von Aktivitätsveränderungen bei Fraßverhalten und Prädationsrisiko oftmals studiert wurde, sind Anurenlarven. Zum Beispiel konnte verringerte Aktivität bei *Hyla versicolor* (grauer Laubfrosch) Larven in Gegenwart von *Ambystoma tigrinum* (Tiger-Salamander) festgestellt werden (Skelly, 1992). Jedoch spielt Habitatvariabilität bei Prädator-Kaulquappen-Interaktionen eine besonders große Rolle, da Prädation eine erhebliche Mortalitätsquelle für Kaulquappen darstellt. Zahlreiche Umweltfaktoren wie zum Beispiel Futterresource oder Populationsdichte der Beute haben ebenfalls einen starken Einfluss auf biotische Interaktionen, wie beispielsweise die Tag und Nacht Aktivität.

Ziel meiner Studie war es, an europäischen Laubfroschkaulquappen (*Hyla arborea),* Verhaltensänderungen am Tag und in der Nacht in Anwesenheit und Abwesenheit von nicht letalen Libellenlarven der Art *Aeshna cyanea* zu beobachten. Faktoren wie Futtermenge und Versuchstierdichte wurden dabei variiert. Die Resultate ergaben, dass die Aktivität (Fressen, Schwimmen und Ruhen) der Kaulquappen sich sowohl mit der Versuchstierdichte, als auch während der Tag-bzw. Nacht-Zeit verändert. Die Futtermenge spielt dagegen hauptsächlich bei dem Fraßverhalten eine tragende Rolle. Des Weiteren konnte eine Aktivitätssteigerung von induzierten Tieren bei Nacht festgestellt werden, während sich die Kontrolltiere eher tagsüber aktiver verhielten.

5.4. Inter- and intraguild predation on eggs of the European tree frog, *Hyla arborea,* by predators and competitors

In den letzten Jahren konnte ein erheblicher Rückgang bei europäischen Laubfroschpopulationen der Art *Hyla arborea* beobachtet werden. Da adulte Amphibien eine wichtige Nahrungsquelle zum Beispiel für Vögel darstellen und Anurenlarven wichtige Algenkonsumenten in stillen Gewässern sind, bedeutet ein Amphibienrückgang, einen erheblichen Einschnitt in das Ökosystem. Dieser Rückgang ist hauptsächlich auf den Verlust von Habitaten durch antropogene Einflüsse zurückzuführen. Dennoch spielen andere Faktoren, wie zum Beispiel Prädatoren und zwischenartliche Konkurrenz, ebenfalls eine wichtige Rolle. Beide Faktoren beeinflussen das Brutverhalten von Amphibien, welches ein wichtiges Element in ihrer „life-history“ Strategie darstellt. Artspezifische Brutstrategien sind adaptiv und korrelieren oft mit dem Bruthabitat, den Jahreszeiten, sowie der Anwesenheit von Prädatoren und Konkurrenten (Petranka et al., 1995). Ein frühes Ablaichen erlaubt zum Beispiel den geschlüpften Kaulquappen mehr Zeit bis zur Metamorphose, bevor die temporären Gewässer austrocknen. Zusätzlich ermöglicht es einen Größenvorteil bei zwischenartlicher Konkurrenz, was zu Laichprädation bei später ablaichenden artfremden Konkurrenten führen kann. Dagegen bringt späteres Ablaichen den Vorteil mit sich, Räuber und Konkurrenten möglicherweise während der Larvalphase zu vermeiden. Eine Studie zeigte zum Beispiel, dass *Rana sylvatica* (Waldfrosch) Larven Laich der später ablaichenden Amerikanischen Kröte (*Bufo americanus*) fraßen (Petranka et al., 1994). Ähnlich wie *B. americanus* laicht der europäische Laubfrosch, *Hyla arborea*, ebenfalls später im Jahr in mehreren Laichschüben ab. Die koexistierende Erdkröte (*Bufo bufo*) laicht dagegen früher ab. Obwohl es sich nur um eine sehr geringe Zeitspanne zwischen dem Ablaichen der Erdkröte und dem Ablaichen des Laubfrosches handelt (ca. 10-14 Tage), reicht die Zeit für *B. bufo* aus, eine beträchtliche Größe bis zum Anfang der *H. arborea* Laichabgabe zu entwickeln. Es könnte daher zu einem zwischenartlichen Laichfraß von *B. bufo* an *H. arborea* Laich kommen.

Prädatoren sind aufgrund ihrer bisher angenommenen bewegungsorientierten Beutejagd (Pritchard, 1965) dagegen vermutlich eher Kaulquappenräuber als Laichräuber. Dies würde zu der Annahme führen, dass die von mir in meinen Experimenten verwendete und in natürlichen *H. arborea* Habitaten vorkommende Libellenlarve (*Aeshna cyanea*) sich nicht von Anureneiern ernähren würde. Ich konnte jedoch in vorangegangenen Experimenten beobachten, dass die Mortalität von *H. arborea* Laich in Anwesenheit von Libellenlarven stark anstieg (Abb. 7).

Abb. 7: Libellenlarve an *Hyla arborea* Laich

Eigene Beobachtungen im Freiland lieferten ebenfalls Hinweise auf Rückgänge von Laichgelegen in Anwesenheit sowohl von Libellenlarven als auch von *B. bufo* Kaulquappen. Aufgrund dieser Erkenntnisse führte ich Versuche zu dem Thema Laichfraß an *Hyla arborea* im Labor durch. Das Fraßverhalten von regelmäßig gefütterten Libellenlarven (*Aeshna cyanea*) und Kaulquappen der Erdkröte (*B. bufo*) wurde an frisch gelegtem Laubfroschlaich observiert. Zusätzlich wurden Libellenlarven auf Gelbbauchunken (*Bombina variegate)* Laichballen angesetzt. Alle Eier beider Anurenspezies wurden komplett von der Libellenlarve konsumiert.

Zudem fand ich in meiner Studie heraus, dass Erdkrötenlarven ebenfalls durch Fraßversuche die Entwicklung von frisch gelegten Laubfroscheiern weitgehend verhindern. Diese Resultate deuten darauf hin, dass im Fall einer fortwährenden Habitatzerstörung die Folge davon wäre, dass Generalisten wie Erdkröten mehr und mehr in Laubfrosch Habitate gedrängt werden. Auf Grund der dadurch entstehenden zunehmenden Laichzerstörung und dem steigenden Konkurrenzdruck hätte dies einen negativen Einfluss auf die Abundanz von *Hyla arborea*. Meine Ergebnisse führen daher zu der Annahme, dass der Rückgang von Anurenarten neben Habitatzerstörung zudem auf Laichfraß sowohl von Prädatoren als auch durch Konkurrenz zurückzuführen ist.

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7. Artikel

7.1. Predator induced phenotypic plasticity in the European tree frog, *Hyla arborea*: Morphological changes, defensive effect and mechanisms of the defense

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ABSTRACT

Inducible morphological defences in amphibian have only recently been discovered and the functional mechanisms of the defensive traits are widely unknown. We tested for phenotypic plasticity of European tree frog (*Hyla arborea*) tadpoles in environments with and without predatory dragonfly (*Aeshna cyanea*) larvae. Tadpoles displayed significant morphological plasticity and formed deeper tails with dark pigmented spots in the presence of cues from odonate larvae. When directly exposed to these predators significantly more control individuals were killed, although induced and control animals both suffered the same rate of attacks. Our analysis of the defensive mechanisms revealed that altering tail pigmentation deflects predator strikes from the vulnerable body to the tail. It does not provide a camouflage effect. The deeper tail fins prevent the odonate larvae to get a firm grasp on their prey and allow the tadpoles to struggle free once caught. We found no evidence for a difference in escape behavior which might be caused by the longer tails. Our study shows the induced morphology protects during different steps of the predation cycle and this multi-stage function increases the effectivity of this phenotypically plastic defense.

INTRODUCTION

Former studies revealed that phenotypic plasticity, defined as the ability of a genotype to produce different phenotypes in different environments, occurs in a variety of organisms in many natural habitats. Many plants and animals display adaptive plasticity in response to attacks by predators or herbivores (Schlichting, 1986; Dodson, 1989). Induced morphological defenses occur from unicellular organisms to vertebrates (Tollrian and Harvell, 1999). Among invertebrates ciliates (Kuhlmann et al., 1999), rotifers (Gilbert, 1999), bryozoa (Harvell, 1986; 1990) and *Daphnia* (Tollrian and Dodson, 1999) have been shown to form a wide range of inducible defenses. Examining vertebrates, research on fish (Brönmark and Miner, 1992) and amphibia led to the discovery of several inducible defenses. Amphibian larvae often react on predator presence by initializing morphological changes such as tail shape and size and tail color (Smith and Van Buskirk, 1995; Mc Collum and Van Buskirk, 1996; Relyea, 2001; Van Buskirk et al., 2004). Previous experiments on amphibians have indicated that tadpoles of the grey tree frog *Hyla chrysoscelis* (McCollum and Leimberger, 1997) and *Rana* tadpoles

(Teplitsky et al., 2003) show flexible morphological responses to predators. While several studies reported morphological changes, only few studies tested the benefits and even less is known about the functional mechanisms of these phenotypic changes. Van Buskirk and Schmidt (2000) for example showed that newt tadpoles of two species (*T. alpestris* and *T. helveticus*) had darker pigmentation in the tail fin and developed deeper tail fins and longer tails relative to their body size in the presence of *Aeshna* dragonfly larvae. They could demonstrate that induced individuals had twice the survival advantage in predator trials compared to control individuals. Many hypotheses have been developed for the mechanisms behind the defense. Van Buskirk and McCollum (2000) suggested that the mechanism does not involve fast start performance or swimming speed. They hypothesized that a larger tail may allow a tadpole to lose part of the fin without affecting subsequent swimming performance. Loss of up to 30% of the tail depth can be sustained without adverse consequences for swimming performances. This implies that a predator induced tadpole (with about 15% extra tail depth to begin with) may be able to sustain more tail damage without affecting swimming than can control tadpole. Chovanec (1992) hypothesized, that a deeper tail fin allows the tadpoles to escape from the dragonfly’s labium by intensive tail movements.

Similarly, plasticity in tail coloration has been reported from anuran larvae (McCollum and Leimberger, 1997; Teplitsky et al., 2003;Van Buskirk et al., 2004). Mechanisms behind these changes are suggested to be either a kind of camouflage for the prey or a mechanism for deflection of attacks from the more vulnerable body to the tail (Van Buskirk et al., 2004).

The European tree frog *Hyla arborea* breeds in a wide range of habitats, which differ strongly in the associated predators. Thus, the tadpoles dwell in environments with variable predation risk rendering them prime candidates for the evolution of inducible defenses. Our own field observations and data from Van Buskirk and Arioli (2002) indicated phenotypic plasticity in *H. arborea* tadpoles in the presence of predators. Hence we studied the effect of odonate predators on morphological changes in *H. arborea* larvae. The experiments were focused on three major aims: The first aim was to test the hypothesis that *H. arborea* tadpoles exhibit phenotypic plasticity in response to predator cues. To prove this we performed induction experiments with caged predators. Our second hypothesis that morphological changes in *H. arborea* function as anti-predator defense was tested in predation experiments. The third aim was to unravel the functional mechanisms behind the defensive strategy. We performed short term predation experiments to analyze the effects of induced tail pigmentation and induced changes in tail shape. If tail pigmentation would act as camouflage it would lead to a reduced attack rate. If it would act as deflection mechanism it would redirect the attacks from the body to the tail. The reported deeper or longer tail fins could potentially lead to a higher acceleration i.e., `fast start`, or to a higher swimming speed. Both effects would be measurable as a larger flight distance in behavioral experiments. Therefore we measured the flight distance of attacked tadpoles of both morphs in a separate experiment.

MATERIAL AND METHODS

Eggs of the European tree frog, *Hyla arborea* were collected at the end of May 2003 from temporary ponds located in the Fröttmanninger Heide in Munich. The eggs were maintained in the laboratory in 3L glass beakers until the plasticity experiment began, 5-8 days after the tadpoles had hatched. The tadpoles were raised in the caged presence and absence of the naturally co-occurring dragonfly larvae, *Aeshna cyanea*.

Plasticity experiment

Morphological plasticity was measured in *H. arborea* exposed to the presence or absence of dragonfly larvae. The experiment was conducted in a laboratory at the Ludwig-Maximilians-University in Munich in artificial tanks under natural light conditions until the tadpoles metamorphosed. Ponds were established in black plastic tanks filled with 60L of tap water, at an average temperature of 25°C.

The presence or absence of caged *Aeshna cyanea* larvae (Odonata: Aeshnidae) was manipulated in the tanks and each of the two treatments was replicated four times. Each tank contained two floating cages (6.5 cm diameter x 10.0 cm length) constructed of Perspex frames covered with gauze to allow the diffusion of chemical cues. In the predator treatment one *A. cyanea* larvae was placed in each cage, together with some pond plants for habitat structure, while in the control treatments the cages contained no *A. cyanea* larvae. Dragonflies were fed 3-5 *H. arborea* tadpoles every second day, the number depending on the size of the tadpoles. Tadpoles were fed stinging nettle (*Urtica dioica*). The dried stinging nettle was cut and subsequently mixed with water and shortly heated up in the microwave, to soften the leaves. It was then cooled down, sieved and distributed among the tanks. Tadpoles were fed dry per capita rations (15% wet body mass per day) that promote growth and development (LaFiandra and Babitt, 2004), enough to exclude food availability as a limiting factor. Food rations were calculated weekly based on the mean mass of the animals in the control treatments. All uneaten food and wastes were removed prior to each subsequent feeding. Half of the water from each tank was removed weekly to maintain a good water quality but to avoid complete removal of predator cues.

The eight tanks were initiated on the 5th of June, when 80 tadpoles of the larval stages 26 (Gosner, 1960) were added to each tank and ended on the 19th of August 2003, when tadpoles started to metamorphose. Samples of 20 randomly chosen tadpoles per tank were measured every seventh day. Selection of parameters for measurement was based upon those traits that were considered likely to be under selection from predators (e.g., Van Buskirk and Relyea, 1998; Van Buskirk and McCollum, 1999). These were body length, body depth, tail length, and maximum tail fin depth defined as the distance from the upper to the lower side of the tail fin at the widest point, orthogonal to the spine. The changes in body proportion and size of the tadpoles were measured using a camera connected to a computer installed with analysis software (AnalySIS V 3.0, Soft Imaging Software, Münster). Relative tail fin depth was calculated relative to the tadpoles overall body size.

The data received from individuals in larval stages 29-32 (Wilbur, 1973) were then analyzed using a nested ANOVA. We combined larval stages 29 and 30 into larval range 3, and stages 31 and 32 into larval range 4. Earlier larval stages did not show any indication of morphological changes. A similar observation has been reported by Relyea (2003) who showed that *Hyla versicolor* tadpoles had a greater morphological change in later ontogenetic stages.

Benefits of the induced morphology: Predation experiments

Three different predation trials were performed to test possible advantages of predator induced morphological plasticity in tadpoles. We conducted experiments, in which induced and control tadpoles were directly confronted with dragonfly larvae (all dragonfly larvae had passed the fourth instar). The first experiment was a long term trial, aimed to unravel a potential defensive effect of the induced morphology. The second experiment was a short term trial especially designed to provide information about the functional mechanisms underpinning a defensive effect of the induced morphology. In the third predation experiment we studied potential effects of the wider and longer tail fins.

Long term predation trial

For the long term experiment the bottom of a 3L beaker was filled with gravel. Pond plants were added and a brown background was created with cardboard surrounding the beaker, to simulate a natural habitat structure for predator and prey. The beakers were filled with 2L of tap water. Subsequently eight randomly chosen tadpoles of the same larval stages consisting of four control and four induced individuals with pigmented tails were placed into the habitat. Some food was provided for the tadpoles and one starved (for two days) dragonfly larvae was added. The experiment lasted 24 hours at natural light conditions (16:8h; day:night). Killed and injured individuals were then scored. This experiment was replicated 40 times, using 40 different dragonfly larvae. The data were analyzed using a Wilcoxon Signed Ranks Test for two related samples.

Short term predation trial

Except for the amount of water, which was reduced to 1L, the same artificial environment was constructed as in the first trial. Two tadpoles of the larval stages 30-32 (Gosner, 1960) randomly chosen one each from the induced (only individuals with black pigmented spots were used) and predator-free tanks were placed into the beakers. A dragonfly larvae which had been starved for two days, was added. The following predator-prey interaction was then observed until one of the two tadpoles was captured and killed. We recorded the number of attacks on both tadpoles as well as the part of the body, where the attacks aimed at. During the observations the escapes after capture were scored and which tadpole was killed by the predator was noted. This experiment was replicated 40 times, using 40 different dragonfly larvae.

For analysis, a Chi-square test was performed to determine if there were overall significant differences among the killed tadpoles of both induced and control treatments. All values concerning the attacks on tadpoles were compared using a nonparametric Wilcoxon U-test for two related samples.

Prey escape-behavior trial

Induced and control *H. arborea* tadpoles from the plasticity experiment were used to study escape behavior. During the late larval stages (30-32; Gosner, 1960), where phenotypic plasticity was visible as in deeper tail fins of the induced animals, one induced and one control tadpole were randomly chosen and added to a 30 L glass tank (80 x 40 x 40cm) filled with 10 L of tap water and plants for natural conditions. A paper with centimeter marks was placed underneath the glass bottom of the tank. A starved (for three days) *A. cyanea* larvae was placed into the tank. Attacks on both tadpoles and their flight distances were recorded with a video camera until one of the two tadpoles was captured and eaten. The latter also being noted. The flight distance of the prey was recorded after each unsuccessful attack. This experiment was replicated 50 times. Subsequently an univariate ANOVA was performed to compare the flight distances between control and induced tadpoles.

RESULTS

Phenotypic Plasticity

We found significant differences in tail shape between induced and control treatments. The results for the relative tail fin depth were significant in all cases (Fig. 1). Induced tadpoles in both, larval range 3 and 4 showed significant deeper tail fins compared to the control tadpoles (relative tail fin depth larval range 3: $F_{1/235} = 46.202$, $p < 0.002$, larval range 4: $F_{1/157} = 46.826$, $p < 0.002$) . Tadpoles in larval range 3 also showed a significantly longer relative tail length of the predator-induced tadpoles (relative tail length: $F_{1/235} = 17.568$, $p < 0.013$). In larval range 4 there was no significant difference (relative tail length: $F_{1/157} = 3.801$, $p < 0.117$). Within all treatments the replicates were not significantly different. In most cases (95.7%) the induced individuals additionally to deeper tail fins developed a color polymorphism with black spotted tails (Fig.4).

Benefits of the induced morphology: Predation experiments

Long term predation trial

There was a significantly higher survival for induced individuals ($p < 0.001$; 37df; $Z = -4.568$) in the presence of predators. There was a survival rate of 62.8% for induced tadpoles and 38.2% for control tadpoles. A significantly higher relative number of sliced tail fins occurred in the induced surviving tadpoles ($p < 0.049$; $N=35$; $Z = -1.971$) (Fig.2). There was no significant difference in the relative number of bitten-off tails in the surviving tadpoles ($p < 1.00$)

Short term predation trial

Significantly more control individuals were killed ($p < 0.046$; $N = 36$). Predators attacked both induced and control tadpoles several times until one of them was caught and eaten (mean attacks on induced tadpoles 4.08, and control tadpoles 4.25). The amount of attacks performed by *A. cyanea* larvae on induced and control individuals did not significantly differ from each other ($p < 0.910$; N = 36). The predators attack efficiency (captures / attacks) for induced tadpoles was 0.08, for control tadpoles: 0.16. The orientation of the attacks differed between both treatments (Fig. 3). More attacks occurred on the black spotted tails of the induced tadpoles ($p < 0.011$; df = 35; N = 36; Z = -2.549), while more attacks were aimed at the bodies of the control individuals ($p < 0.029$; N = 36; df = 35; Z = -2.186). Comparing the attacks on tail and body within the induced individuals, there was no significant difference evident ($p < 0.421$; N = 36; Z = -0.804). Whereas predation in control treatments showed more attacks on the body ($p < 0.003$; N = 36; Z = -2.957). All of these results indicate that the pigmented tail functions as a deflection mechanism to divert the attack of the *A. cyanea* to the tail of the tadpole and away from the more vulnerable body.

Prey escape-behavior trial

The result showed no significant difference in flight distances ($p < 0.803$; F = 0.062) between control and induced tadpoles. Mean flight distance of control tadpoles was 4.82 cm, while induced individuals managed 4.7 cm.

DISCUSSION

Our study shows that tadpoles of the European tree frog *H. arborea* form phenotypic plastic changes in tail morphology, induced by chemical cues which are related to the presence of their predators. We demonstrated a protective effect of these changes and we elucidated their functional mechanisms.

Several studies have proven the existence of predator induced phenotypic plasticity in anurans in natural as well as in laboratory conditions (e.g. Van Buskirk and McCollum, 1999; Van Buskirk and Relyea, 1998; Van Buskirk, 2002; Teplitsky et al., 2003). In all studies, all anuran larvae increased tail area and in several studies anurans even changed tail color in the presence of predators. These changes have been shown to be induced by chemical cues, such as alarm pheromones released from prey upon attack or metabolites of digestion released by the predator following consumption of prey. Many compounds emitted by either predators themselves, digested metabolites produced by predators or cues allocated by injured prey (Laurila et al., 1997; LaFiandra and Babbitt, 2004) can dissolve in water. The relevance of chemical cues could also be confirmed in our study, where the presence of caged dragonfly led to the induction of deeper tail fins and dark pigment spots on the tails.

Former studies have reported defensive benefits for phenotypic plasticity in morphological traits in anurans. Similarily, we found that *H. arborea* larvae initiating morphological changes in the presence of a predator have a higher survival rate than control animals. The exact functional mechanisms of the morphological traits have only briefly been studied. Van Buskirk and McCollum 2000 hypothesized that a possible function of the large and conspicuous tail is that it may draw the predators attention away from the more vulnerable body to the darker pigmented tail. Caldwell (1981) underlined this hypothesis considering black spotted and deeper tail fins. Van Buskirk et al. (2004) tested this hypothesis by presenting model tadpoles that differed only in coloration to *Aeshna* dragonfly larvae. The models had a dark body and a pale tail, a dark spot in the middle of the tail, or a dark spot near the tip of the tail. They observed that models with plain tails were struck on the body, whereas those with dark spots in the tail were struck more often on the tail. The results of our short term predation trial verify this hypothesis, as we directly observed more attacks to the body of control tadpoles and vice versa more attacks to the tail of induced animals. Also our long term predation results support this assumption because we found a significantly higher relative number of sliced tail fins in the surviving tadpoles. Relatively more sliced tail fins indicate that due to the pigment spots on the tail, a significantly higher probability of attacks occurred to this part of the tail. Thus, our results confirm the hypothesis that pigment spots on the tail fin deflect the attention away from the vulnerable body to the tail.

However, pigment spots possibly could also act as camouflage or in contrast could render induced tadpoles more conspicuous. Caldwell (1981) found in his experiments with cricket frog tadpoles of the genus *Acris* in the presence of aeshnid dragonfly *Anax junius* that tadpoles with black tails suffered significantly more attacks. This result is not supported by our study. Our results show similar attack rates on both induced (pigmented) and control tadpoles. The difference to Caldwell’s study could result from different conditions in these studies. We used a naturally dark background where the pigmented spots on the tails are not as easily visible for the predator. The predator is only able to see the pigmented tail across a short distance against a dark background, while against a bright background it might attract predators over a wider distance.

In the presence of a predator, *H. arborea* not only produced pigmented tails but also varied tail shape by forming deeper tail fins. Tail shape might influence swimming properties. Van Buskirk and McCollum (2000) proved in their study that the defensive mechanism does not involve escape responses like fast start performance or swimming speed due to longer tails or deeper tail fins. Zottoli et al. (2001) proved in their experiment, that naïve tadpoles do not initiate a flight response before being struck by the dragonfly labium and he therefore hypothesized that an escape response may help only after labial contact. Our observations similarly indicate that predator attacks are much faster than escaping speed. Hence, fast starts are not likely to be effective. Tadpoles only speed up, after they are attacked, but only a few seconds later start to slow down again into their normal swimming pace. In our study, the prey-flight-distance trial showed no difference between control and induced tadpoles. Thus, there is neither an indication of a higher acceleration nor of a faster swimming speed caused by the different tail shape, because both factors should lead to a longer flight distance. But why do tadpoles form deeper tail fins? Most likely a deeper tail fin could be an anti-predator tool preventing the predators from grasping the prey. Results from Van Buskirk et al. (2003) indicate that attacks to the tail are less successful. They found that living tadpoles only escaped in 10% of the attacks to the body but in 29.4% of attacks on the tail. This suggests that tail attacks are less dangerous than body attacks. Thus, it is possible that the higher tail fins prevent the labium of the predator to catch the tail muscle and instead push the tail away. This “anti-grasp mechanism” would be a pre-capture defense. A second hypothesis would be that induced *H. arborea* larvae are less susceptible to predation due to their greater possibility of escape, once caught, from the dragonfly’s labium by intensive tail movements (Chovanec, 1992). This “struggle-free mechanism” would be a post-capture defense. Similarily, Doherty et al. (1998) suggested that the tail fin could be acting as a protective but expendable wrap around the core muscle tissue.

The numbers of sliced tail fins can be used to differentiate between both mechanisms. Sliced tail fins reflect a post-capture escape. In our study the relative rate of sliced tails was significantly higher for the induced morph, indicating that the chance to escape after capture is higher for animals with deeper tail fins. Furthermore the relative number of ripped tails was not significantly different, indicating that the post-capture mechanism is responsible for the higher survival rate of induced *H. arborea* tadpoles. The observation that morphological changes are initiated late in ontogeny in *Hyla versicolor* tadpoles (Relyea, 2003), together with our observation that pigmented tails are initialized in late larval development in *H. arborea* tadpoles, might suggest that tadpoles form their deflection mechanisms when they are larger and have a better ability of escaping out of the dragonfly’s labium by intensive movements.

However the late formation of defenses could also be a strategy to reduce costs of defenses by forming them only when they are needed most. Activity level rises with the age of the tadpole and therefore increases the predation risk in later ontogeny (Lawler, 1989), especially since many odonate larvae are able to feed on large tadpoles (Caldwell et al., 1980).

Our results directly support the assumption of a post-capture mechanism, but do not rule out that the pre-capture mechanism might act synergistically.

In this paper we reported phenotypic plasticity in defensive traits of the European tree frog *H. arborea* and identified the functional mechanisms of the defense. We found that tail pigmentation, as a pre-capture defense, deflects predator strikes from the vulnerable body to the tail, while deeper tail fins additionally serve as a post-capture defense preventing a firm grasp and allowing tadpoles to struggle free after capture. Thus, the induced tail morphology in *H. arborea* reduces mortality during different steps of the predation cycle, similar to other phenotypically plastic morphological traits, e.g. helmets in *Daphnia cucullata* (Laforsch and Tollrian, 2004). This multi-stage mechanism likely increases the defensive effect. Our study once more emphasizes the functional role of morphological plasticity for reducing the effect of predation.

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FIGURE LEGEND

Fig. 1: Induction of relative tail fin depth in larval range 3 (equals Gosner, 1960 stages 29, 30) and larval range 4 (equals stages 31, 32). The difference is significant in both larval ranges.

Fig 2: Mean number of survivals and the mean number of tail injuries (sliced tail fins) in control and induced tadpoles.

Fig. 3: Mean attacks of predators on the body and tail of induced and control tadpoles.

Fig. 4: Induced and control *Hyla arborea* tadpole. Left: induced tadpole, with deeper tail fin and pigment spots on the tail; right: control tadpole.

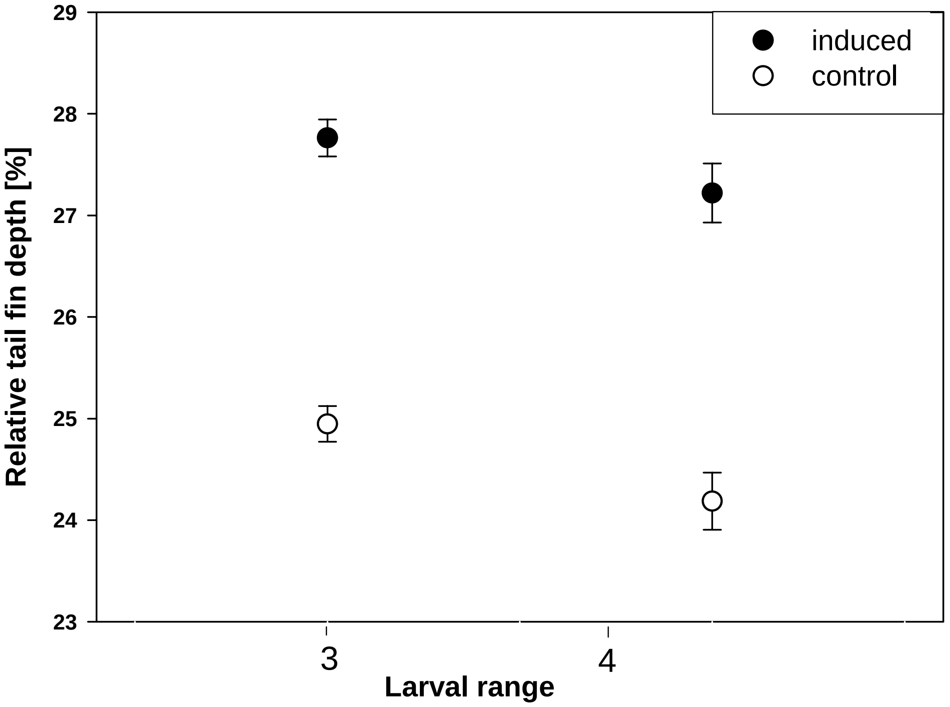
Relative tail fin depth [%]
29
28
27
26
25
24
23
3
4
Larval range
● induced
○ control

Fig. 1

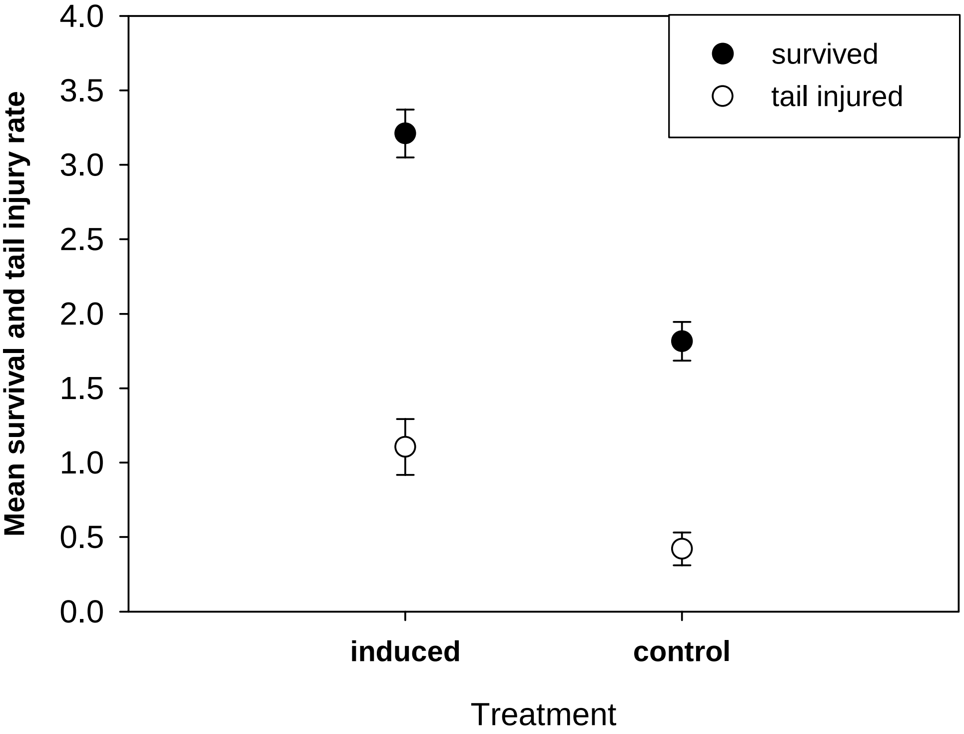
Mean survival and tail injury rate
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
induced
control
Treatment
● survived
○ tail injured

Fig. 2

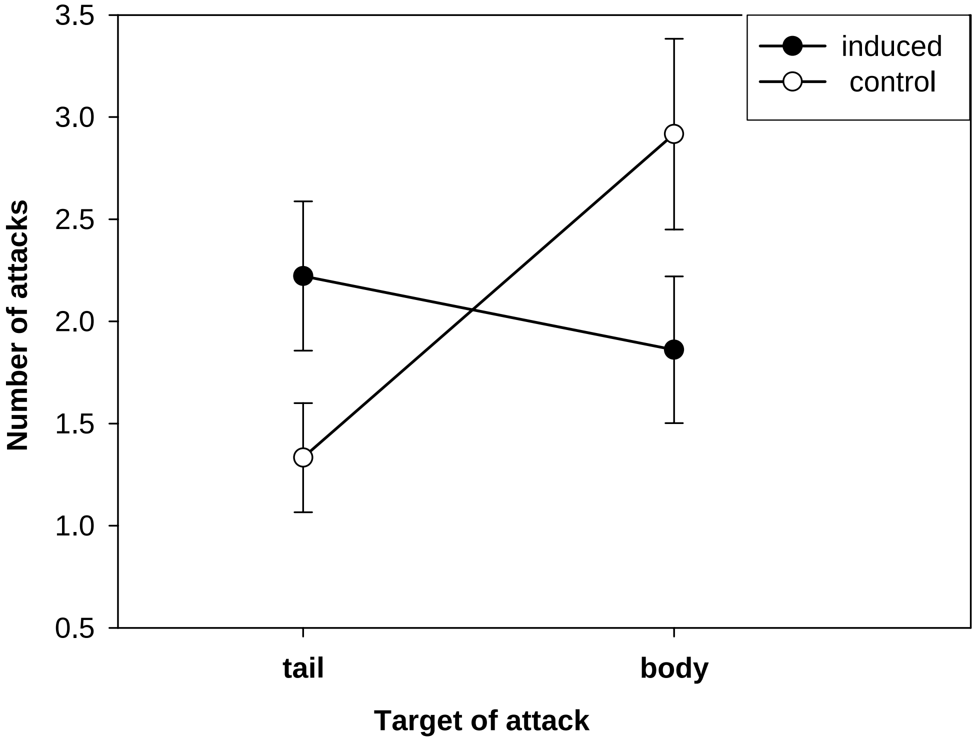
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Number of attacks
● induced
○ control
tail
body
Target of attack

Fig. 3

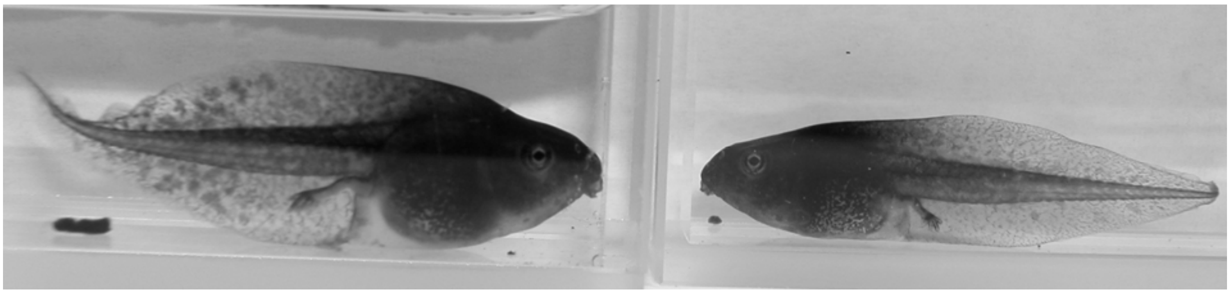

Fig. 4

7.2. The effect of predator diet on phenotypic plasticity of the European tree frog, *Hyla arborea*, tadpoles

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ABSTRACT

While many anuran species have been shown to produce inducible defenses in the presence of their predators, previous studies have suggested that chemical cues trigger these reactions in anurans. However, not much is known about the origin of the chemical cues and their specificity. Cues can either be released by the predator independent of its diet or they can be alarm cues released by tadpoles when injured or digested by predators. If alarm cues provide the chemical signals it should be an advantage for tadpoles to develop sensitivity to alarm cues of coexisting species especially in situations when their own population density is low. Considering this, we tested the effect of different predator diets on morphological reactions of the European tree frog (*Hyla arborea*) tadpoles in experiments with predatory dragonfly (*Aeshna cyanea)* larvae. Tadpoles displayed significant morphological plasticity in tail shape (increased tail fin depth) when reared in the presence of caged dragonflies fed with conspecifics. When dragonflies were fed with invertebrates and heterospecifics *Bufo bufo* tadpoles, *H. arborea* larvae showed weaker morphological reaction. Thus, our results suggest that a species-specific alarm cue or species-specific digestive metabolite released by the predator after feeding on conspecifics should be involved in triggering the defense reactions. Additionally, a predator kairomone evoked a weak response. *H. arborea* obviously did not develop sensitivity to heterospecific alarm cues, as the induced morphological difference between treatments with odonates fed with *B. bufo* or with *D. magna* was not significant. Both, species-specific cues and kairomones might allow a good estimate of the predation risk.

INTRODUCTION

Many predator-prey interactions have shown the importance of chemical cues in aquatic environments. Chemical cues provide reliable signals in these often turbid environments where visual cues are sometimes not available. Many compounds emitted by either predators themselves, digested metabolites produced by predators or cues released by injured prey (Laurila et al., 1997; LaFiandra and Babitt, 2004) can dissolve in water. This enables aquatic organisms to evolve sensitivity to a large number of chemical signals. Experiments have shown that the ability of organisms to produce defensive phenotypes under different conditions depends on the presence and detection of reliable cues (Harvell, 1986; Van Buskirk et al., 1997; Tollrian and Harvell, 1999).

Many studies proved that amphibians show a remarkable high ability of morphological adaptations to predation risk. For example, Van Buskirk and Schmidt (2000) found that newts of two species (*T. alpestris* and *T. helveticus*) had darker pigmented tail fins and developed larger tails relative to their body size in the presence of caged *Aeshna* dragonfly larvae. Previous experiments have indicated that the grey tree frog *Hyla chrysoscelis* (McCollum and Leimberger, 1997) and *Rana* tadpoles (Teplitsky et al., 2003) show flexible morphological responses to predators. The trigger of these morphological changes is said to lie in released chemical cues. Petranka and Hayes (1998) demonstrated that wood frog and American toad tadpoles (*Rana sylvatica* and *Bufo americanus*) exposed to direct cues from starved predators reduce activity, while McCollum and Leimberger (1997) found that grey tree frog tadpoles (*Hyla chrysoscelis*) do not demonstrate morphological responses to cues from starved predators alone. The cues for morphological reactions could, but must not necessarily be, identical with behavior inducing cues. Recent studies have begun to examine behavioral, morphological and life historical changes of prey animals to predator diet cues. Few previous experiments have indicated, that different antipredator reactions (larval life history as well as behavioral response) in anuran tadpoles depending on the predators diet and the predator exist (Laurila et al., 1998). They found that local adaptations to predator presence are important in the evolution of antipredator reactions. For example Chivers et al. (2001) proved that tree frogs from a population co-occurring with bullfrogs showed a strong avoidance of chemical cues of bullfrogs. In contrast, tree frogs from a population not co-occurring with bullfrogs did not respond to the bullfrog cues. Tree frogs from both populations avoided chemical alarm cues from injured conspecifics. Former experiments affirmed the conspecific effect of chemical cues (Mathis and Smith, 1993; McCollum and Leimberger, 1997; Belden et al., 2000). Chivers and Mirza (2001) for example demonstrated a stronger response of *R. sylvatica* tadpoles to *Anax* fed conspecific tadpoles than fed invertebrates.

The ability of recognizing heterospecific alarm cues would increase the possibility of sensing predators in situations when conspecifics density is low and the cues might be below a detection threshold. To increase survival rates it should be therefore relevant for tadpoles to be able to detect not only conspecific cues but also heterospecific alarm signals.

In this study, we examined the effects of predator diet on morphological antipredator reactions in *Hyla arborea* tadpoles. The predator diet consisted of conspecifics, an invertebrate (*Daphnia magna*) and a heterospecific species (*Bufo bufo*). By comparing the morphological reactions of *H. arborea* tadpoles to predators being fed with *Daphnia magna* and *H. arborea,* we can test the existence of diet-specific cues (e.g. alarm cues) versus generally feeding related predator released cues (mainly kairomones). Cues released by injured *Bufo* tadpoles could demonstrate the relevance of heterospecific chemical alarm cues and species specificity. *B. bufo* was chosen, because former studies proved *B. bufo* to possess alarm cues (Pfeiffer, 1966; Laurila, 1997).

H. arborea and *B. bufo* often lay eggs in the same temporary ponds and therefore often co-occur in the same habitat. Thus, the evolution of sensitivity against the cues of these co-occurring species could provide additional information for *H. arborea* and would help to lower the predation risk.

Most former studies have focused on behavioral reactions to predator presence. In our study, we tested cues for morphological reactions in anuran tadpoles.

MATERIAL AND METHODS

To test the existence of phenotypic plasticity responses of different predator diets in *Hyla arborea* tadpoles, an experiment was conducted altering *H. arborea*, *B.bufo* tadpoles and *D. magna* as predator diet.

Hyla arborea eggs were collected on the 23rd of May 2002 from temporary ponds located in the Fröttmanninger Heide near Munich. The eggs were maintained in the laboratory in 3L glass beakers until 5-8 days after tadpoles hatched.

Morphological plasticity was measured in *H. arborea* exposed to the presence or absence of caged dragonfly predators. The experiment was conducted in a laboratory at the Ludwig-Maximilians-University in Munich in artificial ponds under artificial light conditions (16:8 h; day:night). The experiment was terminated when the tadpoles metamorphosed. For the experiment we used 14 tanks (9 induced and 5 control tanks) filled with 60L of tap water. The presence or absence of a predator of caged *Aeshna cyanea* larvae (*Odonata: Aeshnidae*) was manipulated in the tanks. We established three different predator-diet treatments. Each predator-treatment was replicated three times adding up to the 9 induced treatments. In the first treatment, the predators were fed with *H. arborea* tadpoles (every second day one tadpole) while in the second and third group they received *B. bufo* tadpoles (every second day one tadpole) and *D. magna* (30 adults) respectively. There were five control replicates without predators.

Each of the 9 predator-induced tanks contained one floating cage with one odonate larvae (which equals a predator concentration of 0.017/L) constructed of perspex tubes (6.5 cm diameter x 10.0 cm length) capped with gauze screen. In the predator treatment one *Aeshna* larvae, including part of some pond plants for habitat structure was placed in each cage. The cages allowed the chemical cues emitted by predators to diffuse through the water while preventing the predators from killing the target animals. Dragonflies were fed 3-5 *H. arborea* tadpoles every second day, the number depending on the size of the tadpoles. The tadpoles were fed every second day 50g of dry stinging nettle per tank, enough to exclude food availability as a limiting factor. The stinging nettle was mixed with water and shortly heated up in the microwave, to soften the leaves. It was then cooled down, sieved and distributed among the tanks.

The experiment was initiated on the 5th of June, when 40 tadpoles were added to each of the 14 tanks and ended on the 19th of August 2003. This equals a tadpole concentration of 0.7/L. The water temperature was kept at a constant 20°C during the experiment. Every seven days 20 randomly chosen tadpoles from each tank were measured. Selection of parameters for measurement was based upon those traits that were considered likely to be under selection from predators (e.g. Van Buskirk and Relyea, 1998; Van Buskirk and McCollum, 1999). These were body length, body depth, tail length, and maximum tail fin depth defined as the distance from the upper to the lower side of the tail fin at the widest point, orthogonal to the spine. The changes in body proportion and size of the tadpoles were measured using a digital image analysis system (AnalySIS V 2.11, Soft Imaging Software, Münster, Germany). Relative tail fin depth was calculated relative to the tadpoles body size. A repeated measures analysis was conducted on relative tail depth of the morphological measurements using measurement weeks as within subject factors and the different predators diet as between subject factors. Subsequently a Tamhane post-hoc test was performed to identify differences between treatments.

RESULTS

Using the relative tail depth of the morphological measurements, the repeated measures ANOVA showed a significant difference between treatments ($p < 0.001$; $F_{3/350} = 306.336$). Clearly tadpoles reacted morphologically different depending on the predators diet (Fig. 1). The Tamhane test showed that tadpoles in treatments where predators were fed conspecifics developed significantly deeper tail fins compared to control tadpoles and tadpoles fed with *B. bufo* and *D. magna* (all $p < 0.001$). Tail depth of tadpoles in the *B. bufo* treatment and the *D. magna* treatment were homogenous ($p < 0.734$) but were significantly larger compared to control animals (both $p < 0.001$). Mean relative tail depth of *H. arborea* tadpoles for the different treatments were control: 0.252; *H. arborea*: 0.285; *D. magna*: 0.251; *B. bufo*: 0.261. During this experiment 89.9% of the induced tadpoles in treatments where predators were fed with *Hyla arborea* tadpoles showed pigmented spots on their tails.

DISCUSSION

Chemical cues are known to occur in many different species. In anuran larvae, for example, McCollum and Leimberger (1997) found that grey tree frog tadpoles reared with predatory dragonfly larvae differ in shape and color from tadpoles reared in the absence of predators. Similarly, our study proved the effect of chemical cues on *H. arborea* tadpoles. In the presence of predators they showed significantly different tail morphology (tail depth) compared to control animals. McCollum and Leimberger (1997) suggested that these phenotypic differences were induced by either some metabolite of dragonfly digestion of tadpoles or an alarm pheromone released by tadpoles when attacked.

Former studies have demonstrated that species-specific cues exist in many vertebrate species, such as fish as well as in anuran larvae. For example Mathis and Smith (1993) found that naïve fathead minnows (*Pimephales promelas*) show no behavioral response (fright) to chemical stimuli from a fish (pike) predator fed on heterospecific prey (swordtails). But they do respond strongly when fed on conspecific minnows. In a similar study, Chivers and Mirza (2001) showed that wood frog (*Rana sylvatica*) tadpoles responded more to larval dragonflies (*Anax spp*.) fed conspecific tadpoles than to predators fed invertebrates. Wilson and Lefcort (1993) showed that tadpoles of the red-legged frog (*Rana aurora*) avoid chemical cues from predatory newts (*Taricha granulosa*) that had eaten tadpoles but did not avoid insect-fed newts. Similarily, our study shows that *Hyla arborea* tadpoles react morphologically strong to species-specific cues, but show a weaker reaction to cues from predators fed with *B. bufo* tadpoles and *Daphnia*. Pfeiffer (1966) found that fright reactions occur in *Bufo bufo* and in the family Bufonidae. He furthermore demonstrated that a response to heterospecific cues from *Bufo bufo* could be observed only in closely related species within Bufonidae, but not in Ranidae and Discoglossidae. This would explain the lack of reaction of *H. arborea* tadpoles to *B. bufo* cues in our experiment, where the response to *B. bufo* cues was not significantly stronger compared to predators fed with invertebrates which do not release alarm cues.

Schoeppner and Relyea (2005) documented that when *Hyla versicolor* was confronted either with ten different crushed prey or prey consumed by predators, crushed prey only induced a minor reaction. They confirmed the phylogenetic-relatedness hypothesis, which predicts that an organism´s defensive response will be strong when closely related prey are killed but weak when distantly related prey are killed. Our study similarly found minor reactions from *H. arborea* tadpoles to distantly related *B. bufo* tadpoles and the invertebrates, *D. magna*.

La Fiandra and Babitt (2004) showed that a combination of chemicals released by injured tadpoles as well as metabolites released by predator digestion (*Anax*) are responsible for the degree of antipredator response in *Hyla femoralis*. In their study the degree of responses to a combination of ´alarm pheromones´ and metabolites were greater than the degree of response to metabolites alone. This means either the metabolites are sufficient for defensive response or it means chemicals released upon consumption are the same as during digestion. Combining both metabolites and alarm pheromones would therefore result in a higher concentration of chemical cues (La Fiandra and Babitt, 2004). Our results show that a predator compound evoked a weak response, which shows that *H. arborea* tadpoles developed sensitivity to predator kairomones. *H. arborea* obviously did not develop sensitivity to heterospecific alarm cues, as the induced morphological difference between treatments with odonates fed with *B. bufo* or with *D. magna* was not significant.

Our study does not allow to differentiate between the release of species-specific alarm pheromones emitted by wounded conspecifics and species-specific cues which label the predator and which are released during digestion. However, our study has clearly shown that species-specific cues are relevant for triggering the morphological reaction of *Hyla arborea*. It has also proven that *H. arborea* tadpoles possess the ability to differentiate between species-specific cues and predator kairomones. Indeed, the evolution of species-specific “alarm” cues should be favored in many anurans as in *H. arborea* because spawning produces a high degree of relatedness of tadpoles hatching from clutches within a puddle. Therefore both, emission of and sensitivity to alarm cues would be favored and kinselection could act. In contrast, release of predator kairomones would only benefit the sender and would be disadvantageous for the emitter. Thus, release of these cues should be selected against which might restrict the availability of reliable cues from predators.

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FIGURE LEGEND

Fig. 1: Mean relative tail depth (±1 SE) of *H. arborea* tadpoles raised in different treatments. Homogeneous groups are indicated by letters.

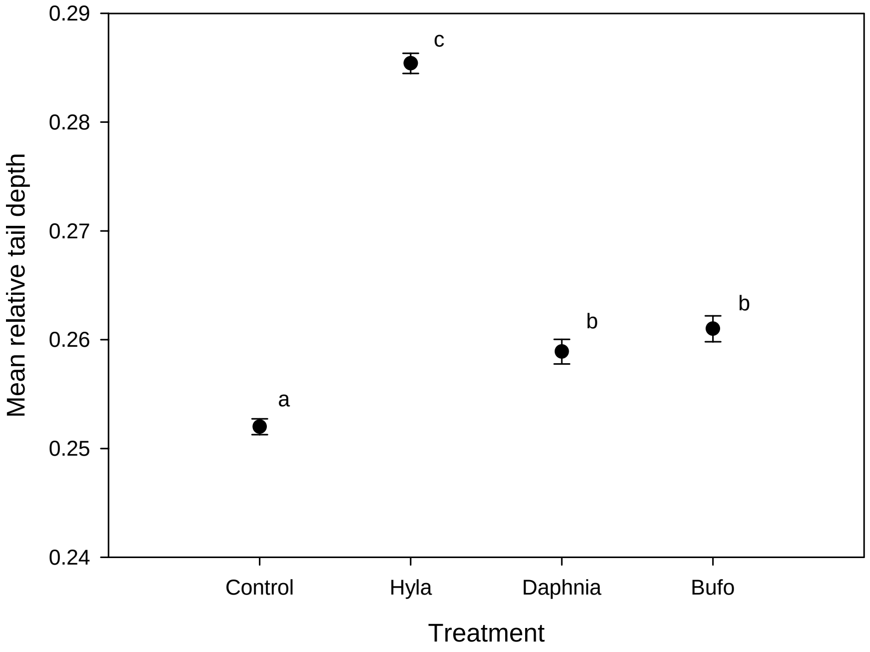

Fig. 1

7.3. Effects of predator presence, food level, density and day-night rhythm on the behavior of European tree frog, *Hyla arborea*, tadpoles

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ABSTRACT

Activity level is a key behavioral trait in many animals which mediates a trade-off between finding food and avoiding predation. Tadpoles for example, tend to reduce activity in the presence of predators, avoiding predator encounters but at the same time reduce food foraging activity, leading to less food uptake. In this study, behavioral responses of the anuran *Hyla arborea* tadpoles in presence and absence of caged dragonfly larvae are observed. We studied interacting environmental conditions such as food level, conspecific density and additionally a day and night effect on the behavioral antipredator response simultaneously. Because dragonfly larvae are visually oriented predators, prey could strive to reduce predator encounters by increasing food foraging at night. Therefore, at night activity should increase for maximum food foraging. A high food level was expected to lead to a decrease in activity, because it allows consumption of a threshold of food level rapidly. A low conspecific density indicates a higher individual predation risk as well as reduced competition and therefore should lead to a decrease in activity. Our results proved induced tadpoles activity behavior (feeding, resting and swimming) to be dependent on density level as well as the night and day rhythm. Food level only plays a major role in feeding behavior. We also found that induced tadpoles increase activity at night time, while control tadpoles increased activity at day time.

INTRODUCTION

Predation has long been implicated as a major selective force in the evolution of several morphological and behavioral changes in animals. Several studies have reported of animals altering their activity levels in the presence (or chemical cues) of predators. The shrimp *Tozeuma* for example altering activity in the presence of pinfish (Main, 1987). Guppies decrease their feeding rate in the presence of predatory cichlids (Fraser and Gilliam, 1987). Larval odonates, *Coenagrion puella* and *Ischnura verticalis* decreased activity level in the presence of fish predators (Convey, 1988; Dixon and Baker, 1988) and *Ischnura elegans* do the same when they detect the presence of predatory notonectids (Heads, 1986).

To search for food most animals have to be active. However, higher activity increases the risk of encounters with predators. Frequently, as a trade-off, prey decreases foraging for food when threatened by predators to avoid predator encounters. The optimal activity level will therefore depend on environmental factors.

A group in which activity level and its relationship to foraging and predation risk has been well studied are larval anurans. Eklöv and Werner (2000) for example, found that bullfrog (*Rana catesbeiana*) and green frog (*R. elamitans*) larvae both decreased activity when exposed to predators. Lefcort (1998) tested chemical cues of the predatory warmouth sunfish on southern toad (*Bufo terrestris*) tadpoles and also could observe a decrease in activity. Similarly, Skelly (1992) found that grey tree frog (*Hyla versicolor*) larvae reduced activity when they sensed chemical cues from tiger salamander (*Ambystoma tigrinum*). However, activity level depends on numerous environmental conditions, such as food level, tadpole density and perhaps day and night rhythm. Some studies show the influence of these factors, for example Lefcort (1998) tested tadpole density on induced southern toad larvae. He found that the size of tadpole groups influence their response to predators. Anholt and Werner (1995) varied food level on predator (dragonfly larvae) induced bullfrog (*Rana catesbeiana*) tadpoles. They found that at low food level tadpoles moved more often and more quickly. Therefore we studied potentially influencing environmental conditions such as food level, conspecific density and additionally a day and night effect on the behavioral response of *H. arborea* tadpoles. Because dragonfly larvae are visually oriented predators, prey could avoid predator encounters by foraging for food at night. We hypothesized that at night, activity of induced animals would increase. Reduced activity incurs the cost of reduced food uptake. Therefore activity should be adjusted according to predation risk. Individual predation risk is inversely correlated to prey density. Therefore we expected a decrease in activity at low density in the induction treatment. Finally, a high food level would allow tadpoles in the vicinity of predators to decrease activity because less time is required to gather a minimum threshold food concentration.

To test our hypotheses, egg clutches of the European (or Common) tree frog *Hyla arborea* were collected and raised in the presence and absence of natural predatory dragonfly larvae (*Aeshna cyanea*). In the experiments food level and density were altered and feeding, resting and swimming behavior was observed at day as well as at night time.

MATERIAL AND METHODS

Hyla arborea eggs were collected on the 19th of May 2004 from temporary ponds located in the Fröttmanninger Heide in Munich. The experiment was conducted in the laboratory at the Ludwig-Maximilians-University in Munich in artificial small ponds under natural light conditions until the tadpoles metamorphosed. Ponds were established in 40 white plastic tanks (30L), divided into 20 induced and 20 control tanks, each filled with 10L of tap water. Two different tadpole densities and food levels were created each consisting of 5 replicates (see treatments 1-4). Each tank contained one floating cage constructed of a perspex frame (6.5 cm diameter x 10.0 cm length) covered with gauze. In the predator treatments (20 tanks) one *Aeshna cyanea* larvae (*Odonata: Aeshnidae*), together with some pond plants for habitat structure, was placed in each cage. The cages allowed the chemical cues emitted by predators to diffuse through the water while preventing the predators from killing the target animals. The twenty control tanks contained no predators.

Dragonflies were fed 3-5 *H. arborea* tadpoles every second day, the number depending on the size of the tadpoles. Also food availability and tadpole concentration were manipulated. Four different treatments were established for control and for induced tadpoles:

1. Low food treatment/low tadpole density: food availability for tadpoles was a limiting factor in five induced and five control tanks with a low tadpole density of 0.3/L
2. Low food treatment/high tadpole density: food availability for tadpoles was a limiting factor in five induced and five control tanks with a high tadpole density of 0.9/L
3. High food treatment/low tadpole density: food availability for tadpoles was not a limiting factor in five induced and five control tanks with a low tadpole density of 0.3/L
4. High food treatment/high tadpole density: food availability for tadpoles was not a limiting factor in five induced and five control tanks with a high tadpole density of 0.9/L

Tadpoles were fed stinging nettle (*Urtica dioica*) by dry weight. The dried stinging nettle was cut and subsequently mixed with water and shortly heated up in the microwave, to soften the leaves. It was then cooled down, sieved and distributed among the tanks. Tadpoles in the low food treatment were given per capita rations (8.3% wet body mass per day) that were intended to be growth limiting without inducing starvation or weight loss. Tadpoles in the high food treatment were fed per capita rations (15% wet body mass per day) that promote growth and development (LaFiandra and Babbitt, 2004). Food rations were recalculated weekly based on the mean mass of the control treatments. All uneaten food and wastes were removed prior to each feeding. Half of the water from each tank was removed weekly to maintain water quality and predator cues.

The experiment was initiated on the 9th of June, one week after the tadpoles had been measured and similar sized animals (larval stages 30-32; Gosner, 1960) had been set into the tanks for brief adaptation. It ended after 10 days. Subsequently the 40 tanks were recorded daily by a camera each for 10 min at day time and again at night time (always at 10am and 10pm). These videos were then observed to analyze the behavior of the tadpoles in each tank at three different time points (after 3 min, 6 min and 9 min).

For analysis, relative values were arcsin-transformed and Kolmogorov-Smirnoff-Tests for each activity level (feeding, swimming, resting) were performed to test for normal distribution. Subsequently repeated measures ANOVAS were performed using control and induced, food level, and density as between subject factors. Univariate ANOVAS were performed in order to test differences between night and day behavior.

RESULTS

Each of the three different environmental conditions: food level, tadpole density and day and night time effect were analyzed comparing control and induced tadpoles as well as interactions between control and induced tadpoles and environmental conditions.

Food level

Control and induced tadpoles showed significantly different feeding behavior ($p < 0.016$; $F = 5.916$) during different food levels. Induced tadpoles increased their resting behavior at high food level. Control tadpoles however decreased the percentage of time spent swimming at low food level (Fig. 1).

Conspecific density

There was a highly significant difference between control and induced tadpoles in their behavioral response to conspecific density concerning feeding ($p < 0.001$; $F = 53.550$, $df = 1$); swimming ($p < 0.001$; $F = 195.968$) and resting ($p < 0.001$; $F = 809.899$) during day time. At night there is a similar effect: feeding ($p < 0.001$; F = 22.789); swimming ($p < 0.001$; F = 193.023) and resting ($p < 0.001$; F = 839.615). Overall, induced tadpoles spend less time swimming at low density, whereas control tadpoles spend less time swimming at high density (Fig. 2). Consequently, the interaction between density and treatment concerning swimming behavior was highly significant concerning their swimming and resting behavior during day and night (day/swimming: $p < 0.001$, F = 13.431; day/resting: $p < 0.001$; F = 36.582; night/swimming: $p < 0.001$; F = 17.012; night/resting: $p < 0.001$; F = 82.158).

Day and night effect

There is also a strong day and night effect ($p < 0.001$; F = 49.279, df = 1). Induced tadpoles spend more time resting, while control animals were more active during night and day time (Fig. 3). Their resting behavior at day or night time also differed significantly ($p < 0.024$; F = 5.234, df = 1). Induced tadpoles spend more time feeding at night time then during the day (Fig. 3).

DISCUSSION

Former studies have shown, that anuran larvae respond to predatory risk by reducing activity in order to avoid predatory encounters (Selmish and Reyer, 1992; Nicieza, 2000). Eklöv and Werner (2000) for example found that bullfrog and green frog (*R. elamitans*) tadpoles both decreased activity when exposed to predatory dragonfly larvae (*Anax junius*). Eklöv (2000) also proved that activity level of small bullfrog tadpoles was lowest in the presence of *Anax* larvae and bluegill sunfish.

We studied interacting environmental conditions such as food level, conspecific density and additionally a day and night effect on the behavioral response. We proved induced tadpoles to respond to different density levels by changing their activity level during day and night time. We also found that induced tadpoles increase activity at night time, while control tadpoles increased activity at day time.

Similar to vertical migration pattern in zooplankton, showing an ascent at dusk and a descent at dawn to reduce predation risk by visually hunting predators, anuran prey could strive to reduce predator encounters by increasing activity for maximum food foraging at night. In zooplankton this behavioral trait offers an escape from predation in space and time since zooplankton avoids water layers of high predation risk during day. In tadpoles a spatial segregation is not possible, but a temporal shift of the activity pattern could reduce mortality by visually hunting odonate larvae. The hypothesis of induced tadpoles increasing their time spent feeding at night was confirmed by our study when pressure from environmental conditions was high. Many herbivores are not pressed for time in their food searching behavior (Jeschke and Tollrian, 2005).Time spend searching for food can be switched to the night if vision is not important in food detection. Although this diurnal behavioral switch has not been reported from anurans before, it should be expected in other anuran systems as well.

Foraging behavior in our study also had been influenced by food level and conspecific density. Former studies have shown that anuran larvae modify their behavioral adaptations to predation risk in response to food levels. Anholt and Werner (1995), for example, predicted that foraging activity should be reduced at higher resource levels, to decrease the likelihood of predator encounters. They tested this hypothesis on bullfrog (*Rana catesbeiana*) tadpoles in the presence of caged and lethal dragonfly larvae. At low food level, they found that induced tadpoles moved more often and more quickly and suffered higher predation mortality because they had to invest more time into foraging for food to achieve a minimum threshold food level.

Their hypothesis could also be confirmed in our study concerning the activity level. We observed that induced tadpoles increased swimming behavior, which is related to searching for food, at low food level. This supports the assumption proposed by Anholt and Werner (1995) that density dependent depletion of resources by prey lead to increased activity levels, which will result in higher per capita predation rates.

Experimenting on *Hyla versicolor* and *Anax* larvae, Van Buskirk and McCollum (2000) confirmed that mortality risk of anuran tadpoles increased with time spend swimming. They assumed that predatory responses are based on an interaction between morphology and behavior, because increasing activity caused higher risk for induced tadpoles with deep tail fins compared to control animals.

But does this decrease in activity still occur with different tadpole densities? Van Buskirk and Yurewicz (1998) found in *Rana sylvatica* tadpoles and predatory *Anax* that at low density, the time spent swimming decreased in induced tadpoles. Similarly, Lefcort (1998) found that the size of tadpole groups of the southern toad (*Bufo terrestris*) influenced their response to predators (warmouth sunfish and eastern lesser sirens). He observed that tadpoles in groups of eight and 20 decreased movement to a similar degree, but lone and paired tadpoles moved even less often freezing during an entire trial. He presumed this behavior to be antipredatory. These observations are very similar to our results, where low density (three tadpoles; 0.3/L) led to a decrease in swimming behavior, while a larger group of induced tadpoles (0.9/L) was more active. Relyea (2001) came to a different conclusion after a similar observation. Using 30 predator-prey combinations, he concluded that larval anurans exhibit predator- and prey-specific behavioral and morphological changes in response to predator- and prey-specific risk. Relyea and Hoverman (2003) found that high larval competition led to an 18% decrease in survival as juvenile *Hyla versicolor* frogs. Tadpoles grew slower and took four days longer to metamorphosis. Relyea and Auld (2004) could prove that *Rana sylvatica* tadpoles raised at high density developed longer guts compared to tadpoles raised in low densities. The last two mentioned studies show different reactions to intra specific competition. In our study, we excluded food limitation as an indirect modifying factor when we tested effects of conspecific density. We tested the response to competitors in control experiments without predators and found a reduced activity at higher densities. Thus, an adaptation to competitors can not explain the density dependent modification of the antipredator behavior. Instead, our observation might indicate, that tadpoles can estimate their individual predation risk, based on predator and prey density, and adjust their behavior accordingly. Theory predicts an adjustment of these inducible defenses to the predation risk, because these defenses evolve in a cost benefit framework (Peacor, 2003).

Other environmental factors modify behavioral reactions to predators. Babbitt and Tanner (1997), for example observed that naturally active squirrel tree frog (*Hyla squirella*) tadpoles maintain high activity levels in the presence of predators (*Anax* and giant water bugs) in higher cover levels (e.g. plants). It is therefore important to consider many environmental factors to be able to predict behavioral reactions of anuran larvae.

Our study shows that a complex association of environmental factors modifies anti-predator reactions. Day and night activity patterns should be studied also in other anurans. It is likely that other species which do not rely on vision in foraging for food also shift their activity to the night when they are safer from predators which detect their prey visually. Clearly a lack of food forces tadpoles to search more and this in turn increases predation risk. However, further studies should show whether the effect of conspecific density, we observed, is due to mere food depletion at higher densities or whether it is an adaptation to a higher predation risk or possibly both.

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FIGURE LEGEND

Fig. 1: Percent of induced and control tadpoles spend swimming during high and low food level.

Fig. 2: Percent of induced and control tadpoles spend swimming during high and low tadpole density.

Fig. 3: Sum number of tadpoles feeding, resting and swimming induced and control animals comparing night and day time during low and high food level and density.

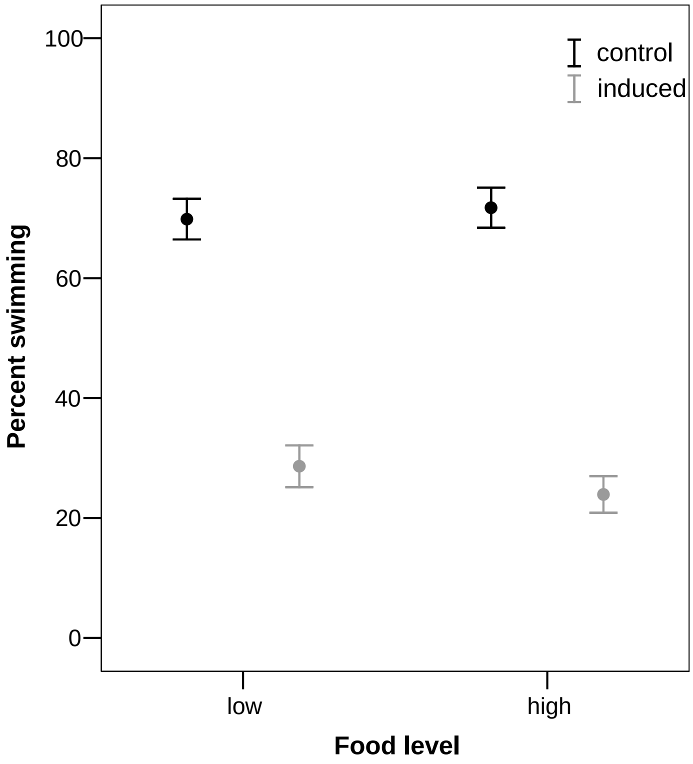
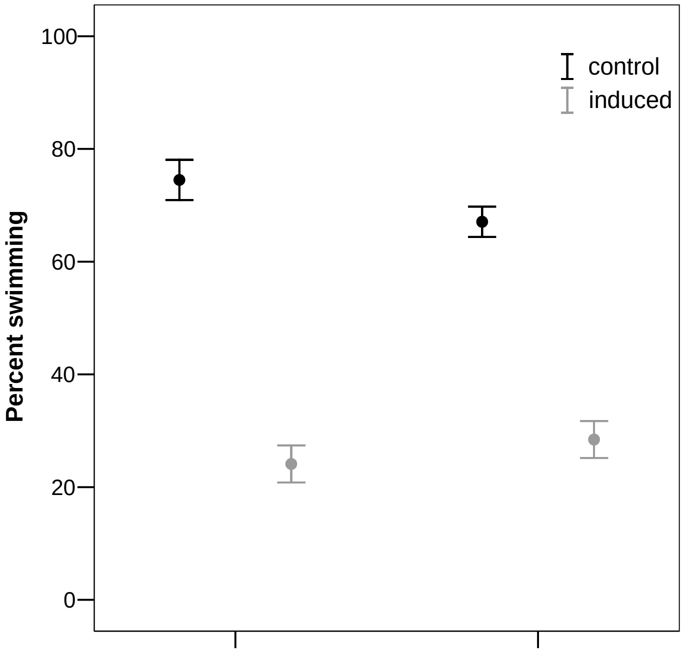

100
80
60
40
20
0
Percent swimming
control
induced
low
high
Food level

Fig. 1


100
80
60
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20
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Percent swimming
control
induced
low
high
Density level

Fig. 2

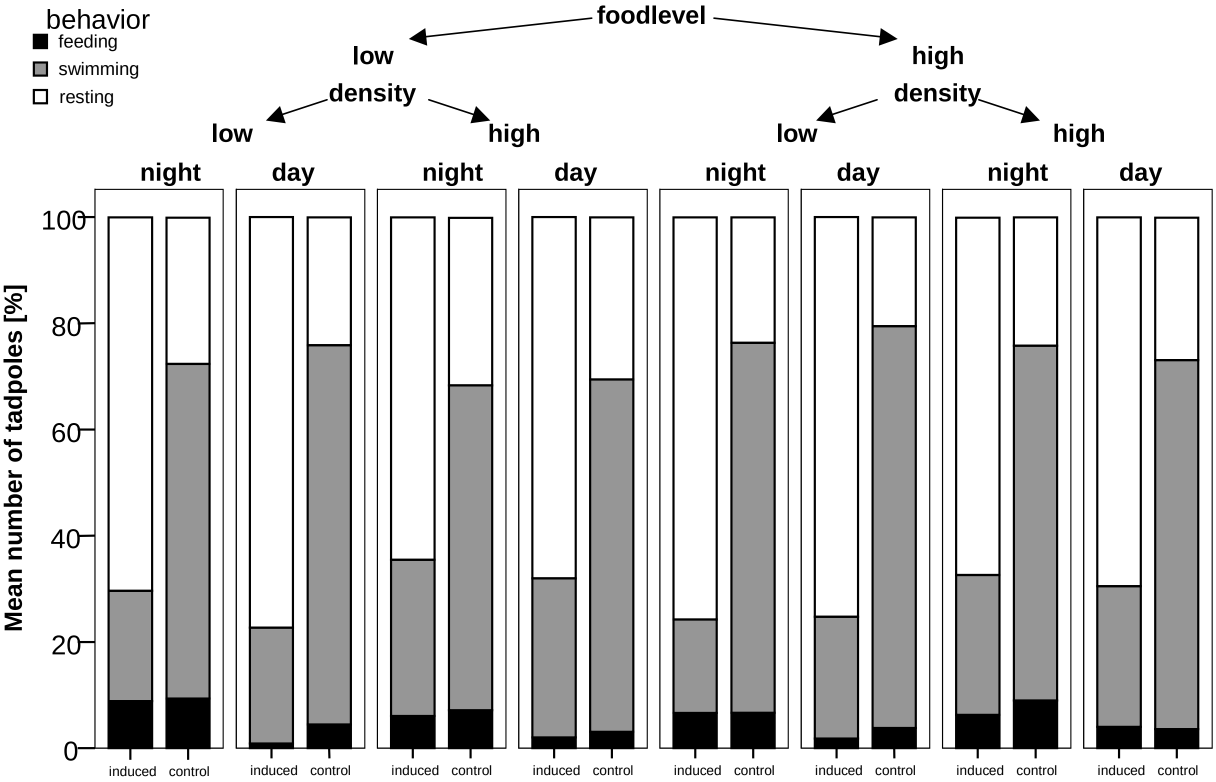
behavior
■ feeding
▨ swimming
☐ resting
foodlevel
low
high
density
density
low
high
low
high
night
day
night
day
night
day
night
day
Mean number of tadpoles [%]
100
80
60
40
20
0
induced
control
induced
control
induced
control
induced
control
induced
control
induced
control
induced
control
induced
control

Fig. 3

7.4. Inter- and intraguild predation on eggs of the European tree frog, *Hyla arborea*, by predators and competitors

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ABSTRACT

In the last years the decline of many anuran species has been well documented. The major reason for the decline is suggested to be the loss of habitats by anthropogenic habitat destruction. However, other factors may contribute, such as predators and competitors. In this study the influence of both of these factors on the development of eggs was tested. We studied the effects of a naturally co-occurring predator, the dragonfly larvae *Aeshna cyanea* and tadpoles of a competitor, the common toad, *Bufo bufo* on freshly laid egg clutches of the common European tree frog *Hyla arborea*. Additionally survival of eggs of the yellow-bellied toad, *Bombina variegata* was tested in the presence of dragonfly larvae.

The dragonfly larvae, said to recognize only moving prey, showed an unexpected behavior when they fed on the egg clutches of both anuran species. Similarly larvae of the earlier hatching competitor *B. bufo* fed on *H. arborea* eggs. When *B. bufo* tadpoles were kept with *H. arborea* eggs survival rate of the *H. arborea* eggs decreased, even when alternative food was abundant, from 84% in the control to 29.5% at low food and 10.9% at high food conditions.

Our study suggests that egg predation by predators and by competitors might also contribute to the decline of anuran species. If habitat destruction continues, more generalistic species like *B. bufo* might be forced into the few remaining *H. arborea* habitats leading to increased competition and egg predation and a further decline of the abundance of *H. arborea*.

INTRODUCTION

A major decline of amphibians has been observed within the last years with a major impact on other species and the entire ecosystem. Adult amphibians are for example important predators as well as prey and larval amphibians may be important herbivores (Blaustein et al., 1994a). Declines and losses of amphibian populations are a global problem with both, global and local causes. The reasons for the decline are supposed to be a combination of many factors such as habitat destruction as well as naturally co-occurring predation and climate changes and consequently the increase of the UV radiation (Alford et al., 1999). Kiesecker et al. (2001), for example, found that climate-induced reduction in water depth at amphibian oviposition sites caused high mortality of embryos by increasing their exposure to UV-B radiation and consequently, their vulnerability to infection by a fungus. The strong UV-radiation could have a lethal impact not only on embryos but also on tadpoles living on pond surfaces (Blaustein et al., 1994b).

Competition and predation may also be relevant. Both factors influence amphibian-breeding behavior, which is an important element of amphibian life-history strategy and can affect community composition. Species-specific breeding strategies are adaptive and often correlate with breeding habitat, seasonal changes, and the presence of predators and competitors (Petranka, 1995). Early breeding may give hatched tadpoles more time to complete larval development before temporary habitats desiccate. Later breeding, in turn, may allow avoiding predators and competitors by maternal selection of the breeding site. Breeding early may additionally improve interspecific competitive ability and allows for intraguild predation (Polis et al., 1989) on later breeding heterospecific competitors. Petranka et al. (1994), for example, reported that *Rana sylvatica* tadpoles are fierce predators on eggs and hatchlings of the later breeding American toads, *Bufo americanus*.

Similar to *B. americanus*, the european tree frog *Hyla arborea* breeds late during several bouts over a prolonged breeding season from Mai until July, depending on the seasonal weather conditions. The co-occurring common toad, *Bufo bufo* tends to breed earlier. Although it is only a minor period of time (about 10-14 days), the Bufo tadpoles already reach a considerable size (Gosner, 1960; stage 29) when *H. arborea* eggs are laid. The habitat overlap between *H. arborea* larvae and *B. bufo* larvae increases competition between both anuran larvae. The ability of *B. bufo* adults to oviposit earlier than *H. arborea* leads to advantages such as allowing the larvae to grow while experiencing no interspecific competition for the first very vulnerable two weeks of larval development.

Predators are assumed to be more relevant for tadpole mortality than for mortality at the egg stage. Pritchard (1965) reported that for *Odonata* movement is important for prey recognition while shape, color and odor are not. This result would lead to the assumption that *Aeshna cyanea* larvae (*Odonata; Anisoptera*) do not feed on immobile anuran eggs. However, in experiments (unpublished Data) with odonate larvae and *H. arborea* we observed a high initial mortality of *H. arborea*, possibly already at the egg stage. Furthermore, several observations in natural *H. arborea* habitats in the Fröttmanninger Heide north of Munich (Germany) indicated egg predation on *H. arborea* egg clutches by *A. cyanea* larvae as well as *B. bufo* larvae. Hence we performed lab experiments to test whether egg predation occurs. In the case of dragonfly larvae we tested whether predation occurs already shortly before the tadpole larvae start to move. We furthermore analyzed whether egg predation occurs also on

Bombina variegata clutches. In the case of intraguild predation by competing *Bufo* larvae we tested whether egg predation is intensified under limited food availability.

MATERIAL AND METHODS

We conducted two experiments to determine the lethal impact of *A. cyanea larvae* and *B. bufo* larvae on freshly laid, immobile *H. arborea* egg clutches. In the second experiment *B. variegata* egg clutches were additionally tested. Because both amphibian species are protected, we used as few animals as possible in our experiments. Surviving amphibians and odonates were returned to their original habitat.

Interguild predation by dragonfly larvae

Thirteen *Hyla arborea* egg-clutches, each containing around 30 eggs, were collected on the 23rd of May 2002 from temporary ponds located in the Fröttmanninger Heide north of Munich (Germany). Additionally thirteen *B. variegata* egg-clutches were collected from Ampfing (southern Germany) in Mai 2002. The experiments were conducted in a laboratory in 6L-beakers, filled with 5.5L of tap water, under natural light conditions. The larval stage of each egg-clutch was determined according to Gosner (1960) under a microscope. The eggs used in this experiment varied between stages 10 and 12 (mid Gastrula) but no clutch exceeded stage 12. Each tank was established to simulate natural conditions by adding pond plants and gravel stones. Each tank was divided by gauze on a wire into two halves. Five liters of tap water were added. All dead or destroyed eggs were removed. The eggs in each clutch were then counted and divided in two parts. One part was placed immediately in the top half of the glass, which equals the control. The other part was placed in the bottom half of the tank, containing one *A. cyanea* larvae (*Odonata: Aeshnidae*) (past fourth instar). Thirteen replicates were constructed. We excluded hunger as a major factor by allowing the predators to consume one *B. bufo* tadpole each day.

The experiment ran for four days, well before the embryos had developed into moving tadpoles (larval stage 20). Every day the eggs of each clutch were counted and digitized. One-Sample Kolmogorov Smirnov Tests were performed in order to test the normal distribution and subsequently an univariate ANOVA and a Post-Hoc-Tamhane-Test were performed.

Intraguild predation by *Bufo bufo* tadpoles

Thirty *H. arborea* egg-clutches, each containing around thirty eggs, were collected during May 2004 from one temporary pond located in the Fröttmanninger Heide in Munich. The experiment was also conducted in a laboratory at the Ludwig-Maximilians-University in Munich in artificial glasses under natural light conditions. The larval stages of all eggs were determined using Gosner (1960) developmental stages under a microscope. The eggs used in this experiment varied between stages 10 and 12 (mid Gastrula) but as in experiment 1 no clutch exceeded stage 12. The eggs were immediately placed in 6L-tanks filled with 5.5L of tap water. Pond plants and gravel stones were then added for habitat structure. Twenty *B. bufo* tadpoles of the larval stages 25 (Gosner, 1960) that had been collected from the same pond isochronal to *H. arborea* egg-clutches were then placed in each glass. Three different treatments were constructed. In the first treatment food availability represented a limiting factor for *B. bufo* tadpoles, whereas the second treatment food concentration was kept high. The third treatment represented the control containing no *B. bufo* tadpoles. Each of these treatments was replicated ten times. Tadpoles were fed stinging nettle (*Urtica dioica*) by dry weight. The dried stinging nettle was cut and subsequently mixed with water and shortly heated up in the microwave, to soften the leaves. It was then cooled down, sieved and distributed among the glasses. Tadpoles in the low food treatment were given per capita rations (8.3% wet body mass per day) that were intended to be growth limiting without inducing starvation or weight loss. Tadpoles in the high food treatment were fed per capita rations (15% wet body mass per day) that do not limit growth and development (LaFiandra and Babbitt 2004). We calculated food rations weekly based on the mean wet mass of tadpoles from control treatments for the high and low food treatments independently. The experiment ran until all *H. arborea* tadpoles had hatched. One-Sample Kolmogorov Smirnov Tests were performed in order to test the normal distribution. Subsequently, an ANOVA as well as a Tamhane post-hoc test were performed to identify differences between treatments.

RESULTS

Interguild predation by dragonfly larvae

After four experimental days, dragonfly larvae had consumed 100% of the *B. variegata* eggs and 96% of the *H. arborea* clutches (Fig. 2), except clutches number 10 and 11, where the beakers had contained slightly smaller predators. In control treatments 91% of the eggs hatched in the absence of a predator. 9% were destroyed by fungal decay. The difference was highly significant ($p < 0.001$, $F_{2,26} = 49.278$).

Intraguild predation by *Bufo bufo* tadpoles

The survival rate of freshly laid *H. arborea* eggs was highest in the control treatment, where the mean relative survival rate (hatched *H. arborea* tadpoles) added up to 84%. In the presence of *B. bufo* tadpoles survival of *H. arborea* eggs decreased down to 29.5% when alternative food was limiting and 10.9% when alternative food was abundant (Fig. 1). All treatments differed significantly from each other (low food vs control, $p < 0.001$; high food vs control $p < 0.001$; low food vs high food $p < 0.014$).

DISCUSSION

Our study shows that egg predation by predators and competitors can be a significant source of mortality. *Aeshna* larvae are believed to attack only moving prey. They detect their prey visually in daylight with their large eyes (Pritchard, 1965). They usually remain immobile, but if hungry, they rush after the prey. Rowe (1994) also observed that prey is not only detected visually, but also vibrations can play an important role. Richard (1960) has suggested that the young stages of *A. cyanea* detect prey by mechanical stimuli, but visual stimuli soon became more important and are dominant after the fourth instar. Kanou et al. (1983) found that immobilized prey were unable to elicit a strike from *Aeshna* larvae. All of these results emphasize the importance of movement of prey for *Aeshna*-larvae hunting behavior. Hence our results come as a surprise. Although all *Aeshna* larvae had past the fourth instar, and were not starved, they vigorously fed on immobile egg clutches of both *H. arborea* and *B. variegata*. Our results indicate that either *Aeshna* larvae have the ability to find food also based on chemical cues or their vision allows them to detect even immobile targets. While we demonstrate for the first time that *A. cyanea* larvae feast on freshly laid, immobile anuran eggs, egg predation by ephydrid and phorid flies has been reported for hyperoliid frogs which deposit their eggs on tree leaves (Vonesh, 2000).

Our study also showed that earlier hatching *B. bufo* tadpoles are able to prey on freshly laid *H. arborea* eggs. Both anuran species have different breeding habits. Offspring of early pond breeders such as *B. bufo* have a size advantage compared to the younger offspring of late pond breeders. This size advantage, besides giving a competitive benefit, allows them to prey on later deposited heterspecifics eggs. Wilbur (1985), for example, demonstrated a negative effect of early spring breeding toad tadpoles (*B. americanus)* on summer-breeding frog tadpoles (*Hyla chrysoscelis*) due to inter specific competition. However egg predation was not considered. Heusser (1970) observed that *Rana temporaria* tadpoles prey upon *H. arborea* eggs. *R. temporaria* is similar to *B. bufo* an early breeder. These results demonstrate the importance of female oviposition behavior. Alford (1985) suggested that optimal oviposition behavior is a complex problem for female frogs for the best timing also depends on the presence of other species. Nyman (1993) argued that although the eggs of early ovipositing species may be likely to gain selective advantages (size specific competition), later breeding may be maintained because early breeders face greater risk due to unpredictable pond conditions (e.g., evaporation, desiccation) and confrontations with early-season predators. Because *H. arborea* larvae have a longer larval period than *B. bufo* tadpoles (Pavignano, 1989), abiotic factors such as pond duration and desiccation are thought to play a more important role for female *H. arborea* frogs in selecting oviposition sites for larval survival than biotic factors such as predators and competitors (Barandun, 1997). However, different studies suggested that biotic factors play a major role in oviposition site selection behavior of anuran females. Petranka (1995) demonstrated that large tadpoles prey heavily on eggs and hatchlings of conspecifics (wood frog). Petranka et al. (1994) studied American toad egg and hatchling mortality in artificial ponds containing natural densities of wood frog tadpoles. They found that toad eggs and hatchlings suffered complete mortality within two days after being placed into the ponds. Resulting from this, Petranka et al. (1994) observed that adult toads completely avoid ovipositing in ponds with wood frog tadpoles. They therefore suggested that the oviosition-site-selection behavior in toads depends on biotic and abiotic factors and that predators, competitors and conspecifics also have an influence.

From an evolutionary point of view, intraguild egg-predation behavior may be selected because of two benefits (Polis et al., 1989): (1) Competitors are eliminated and subsequently more resources are available, and (2) eggs may serve as a valuable and nutritious source of food. Guenther (1990) found that removing egg capsules from hatched *Rana ridibunda* led to an increase in mortality rate. He therefore assumed that either egg capsules or the micro flora that develops around them plays an important role in the early tadpole’s diet. *H. arborea* eggs themselves could serve as a nutritious food source for developing *B. bufo* larvae. In our experiment egg predation by *Bufo* tadpoles was not intensified under conditions of food limitation. Therefore, either competitor exclusion might be more relevant than the direct nutritive effect, or anuran eggs only serve as an add-on to balance the diet. Berninghausen

(1998) had suggested that the jelly-like egg capsules successfully prevent the eggs from being eaten by competing anuran larvae. This hypothesis could not be confirmed in our study. Most eggs of *H. arborea* were totally consumed or damaged to the degree of not being able to develop into tadpoles. Similarily, Heusser (1970) observed in a laboratory experiment that *R. ridibunda* larvae fed on the eggs of *Bufo calamita* (natterjack toad), *H. arborea* and *B. variegata* (yellow- bellied toad).

Although tadpoles of most anurans are considered to be microphageous suspension feeders that occupy lower trophic levels of food chains, they can shift their feeding modes to become macrophageous predators and suppress later hatching competitors and even predators. Petranka et al. (1998) found that wood frog larvae feed on eggs of an upper-level predator (spotted salamander).

Summarizing, this study shows two factors, which may be relevant for the decline of amphibian populations. We found that competing *B. bufo* larvae feed on *H. arborea* eggs, and even dragonfly larvae are able to feed on immobile *H. arborea* egg clutches.

These results show that predation occurs even during early developmental stages and may contribute to the dramatic decrease of *H. arborea* population and other anurans. However, this should not be regarded as a natural process per se. If anthropogenic habitat destruction continues, more generalistic species like *B. bufo* might be forced into the last existing *H. arborea* habitats and competition and egg predation might increase, reducing the abundance of the European tree frog and other anuran species. Egg predation by predators and by competitors might contribute to the decline of anuran species.

ACKNOWLEDGEMENT

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FIGURE LEGEND

Fig. 1: Hatching success of *Hyla arborea* spawn after three days in contact with *Bufo bufo* tadpoles at low and high concentration of alternative food and in the control treatment (Mean ± 1 SE).

Fig. 2: *Aeshna cyanea* larvae feeding on *Hyla arborea* egg clutch.

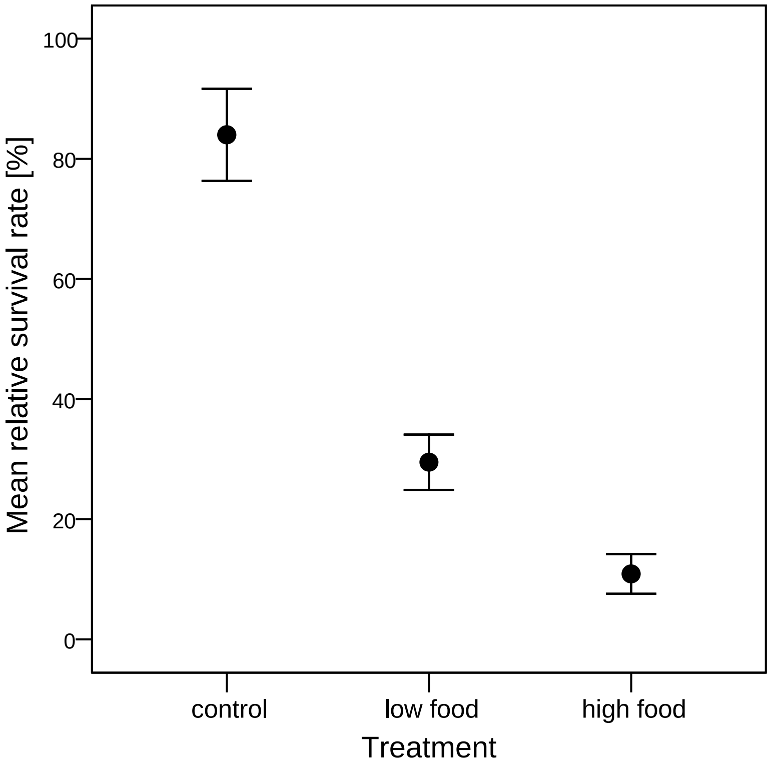
Mean relative survival rate [%]
100
80
60
40
20
0
control
low food
high food
Treatment

Fig. 1

Fig. 2

8. Synopsis

Phänotypische Plastizität wird definiert als die Fähigkeit eines Genotyps, sich in variablen Umweltbedingungen auf verschiedene Weise zu exprimieren. Dieses Phänomen konnte in dieser Arbeit an *Hyla arborea* Kaulquappen demonstriert werden. Aufgrund der erzielten Ergebnisse eignet sich *Hyla arborea* hervorragend als Studienobjekt sowohl zur Erforschung der evolutiven Mechanismen phänotypischer Plastizität als auch zum Zwecke von Verhaltensforschungen.

In meiner Arbeit konnte ich nachweisen, dass *Hyla arborea* Kaulquappen deutlich sichtbare morphologische Veränderungen in Gegenwart von Libellenlarven der Art *Aeshna cyanea* (Blaugrüne Mosaikjungfer) aufwiesen. Die induzierten, bei indirekter Räuberanwesenheit aufgewachsenen, Kaulquappen zeigten einen deutlich höheren Schwanzsaum als Kontrolltiere ohne Räuber. Zusätzlich bildeten sich dunkle Pigmentflecke am Schwanz der induzierten Tiere.

Besonderen Fokus habe ich in meiner Studie auf die Mechanismen morphologischer Veränderungen gesetzt. Bei direkter Konfrontation mit dem Räuber zeigten die induzierten Kaulquappen einen deutlichen Überlebensvorteil gegenüber den Kontrolltieren. Dies bedeutet, dass höhere Schwanzflossen und pigmentierte Schwänze einen Überlebensvorteil mit sich bringen. Mit Hilfe der Auswertungen konnte ich zeigen, dass die dunklen Pigmentflecken am Schwanz Attacken von Prädatoren anziehen und damit als Ablenkung vom eher verletzlichen Körper der Kaulquappe dienen. Dies führt zu einer höheren Überlebenswahrscheinlichkeit, da Kaulquappen trotz eines verletzten Schwanzes zur Metamorphose gelangen können, nicht jedoch mit einer Rumpfverletzung. Zusätzlich dazu hilft ein größerer und kräftigerer Schwanz, wie ihn induzierte Tiere entwickeln (Abb. 8) sich vom Räuber frei zu schlagen. Eben diese Mechanismen konnten durch meine Experimente gezeigt und bestätigt werden.

Abb. 8: Induzierte Kaulquappe der Art *Hyla arborea*

Meine Experimente zur Untersuchung der morphologischen Plastizität verdeutlichten, wie wichtig morphologische Veränderungen zum Überleben der Anurenlarven sind. Doch ist sehr wenig über den Auslöser einer solchen Reaktion bekannt. Man vermutet, dass bestimmte chemische Stoffe zu diesen Reaktionen führen. Dabei unterscheidet man zwei Theorien, die sich mit der Entstehung dieser chemischen Stoffe beschäftigen. Die eine deutet darauf hin, dass bei einer Verletzung durch eine Kaulquappe Stoffe freigesetzt werden, die eine Rolle bei der induzierten Verteidigung spielen. Die andere geht von Stoffwechselprodukten aus, die von Prädatoren nach der Verdauung einer Beute ins Wasser abgegeben werden. Wahrscheinlich ist jedoch das Zusammenspiel beider, da sie unmittelbar zusammen hängen. In meinen Studien konnte ich deutliche artspezifische phänotypische Reaktionen von *H. arborea* auf Räuberpräsenz nachweisen. Von Bedeutung ist des Weiteren die Frage, ob *H. arborea* Kaulquappen die Fähigkeit besitzen, auch auf heterospezifische chemische Stoffe morphologisch zu reagieren, um somit die Wahrscheinlichkeit einer frühen Räubererkennung und damit ihre Überlebenschancen zu erhöhen. Um dies zu erforschen, konzipierte ich Experimente, in denen *Hyla arborea* Larven in Anwesenheit und Abwesenheit von nicht letalen Libellenlarven aufgezogen wurden. Dabei wurde das Futter der Libellenlarven von *Hyla arborea* über *Bufo bufo* (Erdkröte) bis hin zu *Daphnien* (Wasserfloh) variiert. Die Ergebnisse zeigen, dass die größte phänotypische Reaktion von *H. arborea* Kaulquappen durch artspezifische Fütterung hervorgerufen wurde. Dies deutet somit auf eine eingeschränkte Wahrnehmung artfremder chemischer Substanzen hin.

In Freilandbeobachtungen konnte ich *H. arborea* Laichfraß in Anwesenheit von Libellenlarven und zwischenartlichen Konkurrenten entdecken. Daraufhin habe ich entsprechende Versuche konzipiert, die mit interessanten Ergebnissen aufwarten konnten. Libellenlarven fraßen alle *H. arborea* Eier binnen weniger Tage komplett auf. Dies führte zu einer neuen Erkenntnis, denn wie zuvor erwähnt, gelten Libellenlarven allgemein als bewegungsorientierte Jäger. Da sich jedoch die Embryonen der Eier in einem völlig bewegungslosen Zustand befanden, ist in diesem Falle eine bewegungsorientierte Jagd auszuschließen. Dies bedeutet, dass Libellenlarven beim Jagen sich nicht nur an der Bewegung der Beute orientieren, sondern auch chemische Substanzen als Informationsquelle nutzen. Folglich stellen Libellenlarven der Art *Aeshna cyanea* nicht nur eine Gefahr für Kaulquappen dar, sondern auch schon für den frischen Laubfroschlaich. Dies könnte somit auch einer der vielen Gründe für die deutliche Dezimierung der Laubfroschpopulation in Deutschland in den letzten Jahren sein.

Des Weiteren konnte ich in den von mir durchgeführten Freilandbeobachtungen feststellen, dass die mit Laubfröschen häufig koexistierenden Erdkrötenkaulquappen (*Bufo bufo*) ebenfalls eine Gefahr für den *H. arborea* Laich darstellen. So konnte ich Schwärme von *Bufo bufo* Larven in den Laichgewässern von *H. arborea* entdecken (Abb. 9), welche an den *H. arborea* Laichballen zu fressen schienen.

Abb. 9: *Bufo bufo* Kaulquappen in *H. arborea* Laichgewässer

Auf Grund dessen konzipierte ich weitere Laborversuche ähnlich den Libellenlarven Laichfraßexperimenten. Der Verlust von Laubfroschlaich erwies sich hier als sehr hoch in Anwesenheit von Erdkrötenkaulquappen. Die hohe Zerstörungsrate der Eier, die durch *Bufo bufo* Kaulquappen hervorgerufen wurde, lässt sich entweder dadurch erklären, dass *B. bufo* Larven sich von der Flora ernähren, die sich auf den Eikapseln gebildet hat, oder dass sie versuchen, sich mit fremden, sehr nahrhaften Eiweiß zu stärken. Sicher ist jedoch, dass *B. bufo* Larven für *Hyla arborea* Kaulquappen zu gefährlichen Konkurrenten werden. Möglicherweise hat sich dieses Phänomen erst in den letzten Jahren entwickelt, als immer mehr Lebensraum, auch der von Erdkröten, zerstört wurde, und sie daher auf andere Gebiete, in diesem Fall zu *H. arborea* Laichplätzen, ausweichen mussten.

Aus den Ergebnissen dieser beider Versuche kann man schließen, dass nicht allein der Rückgang an Lebensraum auf Laubfrösche einen negativen Einfluss hat, sondern auch verstärkte Anwesenheit von Räubern, sowie erhöhter Konkurrenzdruck durch artfremde Kaulquappen. Diese werden möglicherweise durch den Rückgang an eigenen Lebensräumen verstärkt in die traditionellen Lebensräume der Laubfrösche gedrängt, und tragen somit vor Ort zu deren Dezimierung bei.

9. Ausblick

In den letzten Jahren konnte in Deutschland ein erheblicher Rückgang bei europäischen Laubfroschpopulationen der Art *Hyla arborea* beobachtet werden. Dieser Rückgang ist, wie zuvor bereits erwähnt, hauptsächlich auf den Verlust von Habitaten durch antropogene Einflüsse zurückzuführen. Dennoch spielen andere Faktoren wie zum Beispiel Prädatoren und zwischenartliche Konkurrenz ebenfalls eine wichtige Rolle. Meine Studien zeigten deutlich die Bedeutung der zuletzt genannten Faktoren. In der Regel liegen die Gründe des Rückgangs oftmals im Zusammenspiel vieler Umwelteinflüsse. Zum Beispiel spielen UV-Strahlung, erhöhte Konzentration toxischer Stoffe im Habitat, Klimaveränderungen und auch Krankheiten hierbei eine große Rolle. Im Rahmen der Ergebnisse meiner Arbeit konnte ich deutliche Hinweise finden, dass Habitatveränderungen zu neuen Konkurrenzdynamiken in *H. arborea* Lebensräumen führen können (zum Beispiel *Bufo bufo* dringt in *H. arborea* traditionelle Lebensräume ein). Da sich aber auch abiotische Faktoren wie zum Beispiel die UV-Strahlung durch Verringerung der Ozonschicht, in den letzten Jahren drastisch verändert hat und da Kaulquappen bekannt sind für eine besonders sensible und leicht verletzliche Haut, wäre es interessant, zu erforschen, ob heutige UV-Strahlung bei flachen Gewässern einen negativen Einfluss auf das Wachstum oder sogar das Überleben von *H. arborea* Kaulquappen besitzt.

Meine Studien trugen weiterhin dazu bei, die Ausprägung phänotypischer Plastizität bei einheimischen Laubfröschen zu erforschen. Die phänotypische Reaktion auf Räubersubstanzen lässt darauf schließen, dass natürliche Feinde in der Evolution von *Hyla arborea* eine wichtige Rolle spielen. Es besteht die Möglichkeit, dass durch Umweltveränderungen (z.B. Habitatzerstörung) neue Feinde in die angestammten Laichgewässer von *H. arborea* Populationen eindringen. *H. arborea* Populationen konnten gegen diese neuen Feinde noch keine wirksamen Verteidigungsstrategien evolviert haben. Auf der anderen Seite könnte die hohe phänotypische Plastizität von *H. arborea* jedoch aber auch ein Hinweis darauf sein, dass *H. arborea* Kaulquappen durchaus in der Lage sind, rasch Verteidigungsstrategien gegenüber neuen Feinden zu entwickeln.

Untersuchungen im Rahmen des Forschungsgebietes morphologische Plastizität am Modellorganismus *Hyla arborea* ist demnach weiterhin ein spannendes Thema, das verschiedenste Bereiche der Biologie vereint.

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Lebenslauf

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und nur mit den darin angegebenen Hilfsmitteln und Quellen durchgeführt und verfasst habe.

Ort, Datum: ……………………………..

………………………………………
Unterschrift