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Predator-Induced Changes in Metabolism Cannot Explain the Growth/Predation Risk Tradeoff

Ulrich K. Steiner¹ 2, Josh Van Buskirk¹

Abstract
Defence against predators is usually accompanied by declining rates of growth or development. The classical growth/predation risk tradeoff assumes reduced activity as the cause of these declines. However, in many cases these costs cannot be explained by reduced foraging effort or enhanced allocation to defensive structures under predation risk. Here, we tested for a physiological origin of defence costs by measuring oxygen consumption in tadpoles (Rana temporaria) exposed to predation risk over short and long periods of time. The short term reaction was an increase in oxygen consumption, consistent with the "fight-or-flight" response observed in many organisms. The long term reaction showed the opposite pattern: tadpoles reduced oxygen consumption after three weeks exposure to predators, which would act to reduce the growth cost of predator defence. The results point to an instantaneous and reversible stress response to predation risk. This suggests that the tradeoff between avoiding predators and growing rapidly is not caused by changes in metabolic rate, and must be sought in other behavioural or physiological processes.

Introduction
Organisms protect themselves against predators using a range of defence mechanisms, many of which are plastic and expressed only under predation risk [1]. In animals, most attention has been given to predator-induced changes in external morphology, behaviour, and life history, while underlying physiological responses remain little explored [2,3]. The traditional view of induced behavioural defences is that predation risk leads to reduced activity of prey individuals, in turn reducing their encounter rate with, and detection by, predators [4]. However, reduced activity carries a cost, because less active animals spend less time searching for food and feeding. This leads to the so-called growth/predation risk tradeoff, which arises because the survival benefits of defence can only be obtained at the cost of reduced growth or development [3-5]. A similar argument applies to morphological defences, because resources invested in defensive morphologies are unavailable for growth [5,6].

Recent work suggests that this traditional view is too simplistic; a more complex interplay between multiple interacting responses determines the effects of predators on traits such as growth, age, and size at metamorphosis. Although many studies confirm that predation risk causes reduced activity or increased refuge use [7,8], and such reduced activity lowers predation rates [9,10], these behavioural changes are often not directly associated with reduced growth or development [3,11-15]. Consistent evidence of growth costs is also lacking for some well-studied morphological defences [1,16,17]. Two resolutions of this problem have been proposed. One is that decreased activity need not cause decreased food consumption, and therefore a growth or development cost is not an inevitable consequence of the behavioural response to predators [3,13]. The second possibility is that, even if consumption is reduced in the presence of predators, compensatory physiological mechanisms can decouple growth rate from food consumption [3,11,15]. Physiological plasticity could occur in digestion and energy storage or in metabolism and respiration [15,18,19]. Data available so far suggest that digestive explanations cannot always explain the decoupling of behaviour and growth. For example, Steiner [13] discovered that amphibian larvae exposed to predators ingested the same amount of food with less feeding effort, and digested food more efficiently, compared to non-exposed individuals. Steiner therefore expected predator exposed tadpoles to grow or develop faster, but they did not. There is somewhat better support for the metabolic explanation, because brief exposure to predator cues causes increased ventilation, high heart beat rates, or high respiration rates in Daphnia [20], mussels [21], and fish [22,23]. Thus, the growth/predation risk tradeoff may arise not only because prey reduce activity in dangerous situations, but also because predator-induced defences are associated with a costly increase in metabolic rate [24-26].

Our study focused on the metabolic explanation for the tradeoff between predator avoidance and growth or development. We tested whether the increase in oxygen consumption observed under short-term exposure to predators in other organisms occurs also in an amphibian larva, and whether that same metabolic response is maintained under more realistic conditions of chronic exposure over several weeks. Increased oxygen consumption – indicative of an increased metabolic rate – could explain growth
and development costs of responding to predators despite no reduction in food consumption or digestion efficiency. Identifying physiological mechanisms that help shape the growth/predation risk tradeoff is important for understanding the costs and benefits of phenotypic plasticity and how they influence species distributions with respect to predators [2,3,27,28].

Results
Conditioned tadpoles (reared with predators) were smaller than naïve tadpoles on average (mass±SE: 466±21 mg versus 579±25 mg; F_{1,19}=21.54, \( p<0.0002 \); based on 6 individuals per pool sampled at age 28 days). Mass after conditioning is a direct measure of growth rate, because sizes of randomly-assigned tadpoles did not differ at the onset of the experiment. This confirms many previous studies showing reduced growth of predator-exposed tadpoles [29].

Oxygen consumption, corrected for body mass, was reduced 10.0% in conditioned tadpoles (0.287±0.02 \( \mu \)g/min per 100 mg mass) compared to naïve tadpoles (0.319±0.01 \( \mu \)g/min per 100 mg mass; Fig. 1, Table 1). Oxygen consumption increased 16.8% when tadpoles were measured in kairomone water (water containing predator cues; 0.312±0.01 \( \mu \)g/min per 100 mg mass) compared to blank water (water lacking predator cues; 0.267±0.01 \( \mu \)g/min per 100 mg mass). Kairomones are chemical cues emitted by predators that have fed upon prey [30]. There was no interaction between rearing and measuring environments. Time of day did not influence oxygen consumption, but temperature had a significant positive effect (Table 1; increase 0.0211 \( \mu \)g/min per \( ^\circ C \) for 100 mg mass).

Discussion
We found that physiological responses to predation risk were highly plastic, and depended on the time scale of exposure to risk.

![Figure 1. Relationship between oxygen consumption and tadpole mass for conditioned and naïve tadpoles measured in environments with and without kairomones. Each point is the average of three tadpoles measured during three 4-minute intervals. doi:10.1371/journal.pone.0006160.g001](image)

Table 1. Mixed-effects model testing for the influence of body mass, temperature, time of day, rearing environment, and measuring environment on oxygen consumption of tadpoles.

| Source                  | Estimate±1 SE | Test statistic | \( p \)-value |
|-------------------------|---------------|----------------|--------------|
| Fixed effects           |               |                |              |
| Body mass               | 7.343±0.822   | 8.932          | 0.0001       |
| Temperature             | 1.210±0.313   | 3.862          | 0.0005       |
| Time of measurement     | −5.974±3.278  | −1.623         | 0.0778       |
| Rearing environment     | −3.030±0.697  | −4.348         | 0.0001       |
| Measuring environment   | −2.838±0.745  | −3.806         | 0.0006       |
| Rearing* Measuring      | 1.082±0.623   | 1.739          | 0.0917       |
| Random effect           |               |                |              |
| Rearing                 | 1.354         | 5.134          | 0.0189       |

Tadpole oxygen consumption increased during short term exposure to predation risk but declined after long term exposure. Our interpretation of these results assumes that oxygen consumption is correlated with metabolic rate [31,32]; metabolism reflects energetic demand, which in turn links to our interest in the growth/predation risk tradeoff. Although our study does not reveal the origin of this tradeoff, it adds to our understanding of its underlying physiological mechanisms.

Increased oxygen consumption by naïve tadpoles under short-term exposure to predators parallels similar findings in other organisms [20–23]. This reaction is interpreted as a component of the “fight-or-flight” response, in which release of stress hormones triggers (among other things) increased respiration and heart rate, redirection of energy to locomotory structures, and an enhanced ability to escape predators [19,33]. Naïve tadpoles might be expected to show a particularly strong response to short-term predator exposure, because kairomones represented a novel threat to them. This was not observed. The change in oxygen consumption caused by short-term exposure was roughly the same for both kinds of tadpoles; that is, naïve tadpoles increased their oxygen consumption when faced with kairomones by about the same amount as conditioned tadpoles reduced oxygen consumption when suddenly released from predation risk. The physiological response to predation risk is therefore nearly instantaneous, which implies a rapid and accurate assessment of the chemical environment. This result also shows that oxygen consumption is not closely linked to behaviour, because tadpoles released from predation risk do not show an immediate change in feeding or swimming activity to match the novel predator-free environment [29,34,35].

Our discovery that tadpoles decreased oxygen consumption after long term exposure to predators is unexpected in light of the short-term response to kairomones. But this result is supported by other work showing that vertebrates can have distinct short-term and long-term physiological responses to stress. While metabolic rate typically increases under sudden exposure to stress [20,22,33], it can decline over long-term stress [36] or long-term implantation of stress hormones such as corticosterone [37]. Thus, the conditioned tadpoles in our study reacted as other vertebrates do when they experience extended exposure to stress hormones.
What are the consequences of these short- and long-term physiological responses for the growth/predation risk tradeoff? Over short time periods, there are potentially costly reactions at the physiological level (oxygen consumption is increased [20–23, this study]) and the behavioural level (feeding is curtailed [8,39]). The physiological reaction diverts energy from growth or storage into metabolism [24–26] and the behavioural reaction affects food intake [7,8]. But the impact of these events on individual growth rate will be small if the fight-or-flight response lasts for a relatively short time. Our study was not designed to detect the duration of the short-term metabolic response, but reversibility of various predator-induced responses suggests that the impact might not be long lasting [34]. Over the long-term, there is acclimation to predation risk at both physiological and behavioural levels, such that oxygen consumption declines (this study) and food intake rebounds to that observed in low-risk situations [3,13]. Thus, allocation theory suggests that long-term changes in metabolism and food consumption cannot explain the growth costs of responding to predators found in this study. In fact, a plausible interpretation of our results is that the metabolic response has evolved to minimize costs of anti-predator defense. However, those costs that remain must originate elsewhere.

This conclusion may at first seem discouraging, but we prefer to emphasize that a physiological approach to inducible defenses holds much promise for understanding the growth/predation risk tradeoff. For instance, the short term response observed here and in previous studies demonstrates a highly accurate and rapid assessment of the environment, enabling instantaneous and reversible plasticity. Recent studies of anurans and other taxa likewise illustrate complex interactions among predation risk, behaviour, metabolism, and enzyme physiology [13,15,19,20, this study]. Many more induced physiological mechanisms surely await discovery.

Materials and Methods

Tadpoles of Rana temporaria Linnaeus, 1758, react to predators by decreasing feeding and swimming activity and increasing the depth of their tail fins, both of which reduce vulnerability to predation [9,39]. Predators also cause reductions in growth and development rates, which are usually construed as costs of defence [12,29,40]. We first reared tadpoles for three weeks with and without non-lethal predators (termed conditioned and naïve tadpoles), and then tested the oxygen consumption of both types of tadpole in the presence and absence of predator kairomones. Our experiment had a two-by-two factorial design, with long-term conditioning environment crossed with testing environment.

Rearing of conditioned and naïve tadpoles

Tadpoles were reared outdoors in 20 plastic pools (0.28 m², 80 litres volume), giving 10 replicates each of two treatments (with and without predators). The pools were filled with aged tap water, covered with shade cloth to prevent colonization by predators, and stocked with zooplankton, 5 g of rabbit food, and 60 g of dried leaf litter. We arranged pools in a field at the University of Zurich, Switzerland, and assigned treatments at random. The predator pools contained a floating cage (~1 litre volume) containing one final instar dragonfly larva (Aeshna cyanea Muller, 1764). Throughout the rearing period the predators were fed 300 mg of R. temporaria tadpoles three times a week and were rotated to equalize any possible differences between individual dragonfly larvae. Pools without predators contained empty cages, which were also rotated to control for effects of disturbance. Tadpoles were derived from three clutches collected on the university campus; each pool received 15 (five from each clutch) randomly assigned, six day old tadpoles on 5 April 2004.

Oxygen consumption

We measured oxygen consumption over three consecutive days (27–29 April 2004) using an intermittently closed respirometer in which a measuring period alternated with a flow-through period [41,42]. The respirometer consisted of an aquarium pump, a sequencing valve system, a stirring chamber with a HQ20 LDO sensor (Hach-Lange GmbH, Hegnau, Switzerland), and two experimental chambers (each 125 ml volume), all immersed in a 120 L aquarium. We conducted 20 trials comprising 40 groups in all. Each trial included two groups of three tadpoles, each of which was randomly assigned to one of the two experimental chambers. In each trial one group originated from a rearing pool with predators (conditioned tadpoles), while the other group originated from a pool without predators (naïve tadpoles). Trials lasted for 30 minutes, during which 5-min intervals of flow-through were alternated with five minutes of measuring. While one chamber was measured the other was flushed. The oxygen sensor made recordings every 30 sec. Immediately after a chamber switched from flow-through to measurement, there was a brief period during which the remaining water in the hoses and stirring chamber mixed with the water from the measurement chamber. We therefore discarded data from the first minute of each measuring period. For logistical reasons we could not randomize the sequence of exposure to water with kairomones and blank water (without kairomones). Thus, we started each day with trials in blank water and thereafter added 200 ml of water containing kairomones (from three A. cyanea larvae each held in 200 ml water and fed 300 mg R. temporaria tadpoles two days earlier). We allowed the kairomones to mix for 15 minutes before initiating trials under kairomone conditions. Each aquarium and respirometer were cleaned and refilled for the following day’s trials.

After each trial the wet mass of both groups of three tadpoles was recorded. The temperature in the blank environment (18.17 ± 0.20°C) was lower than that in the kairomone environment (19.39 ± 0.17°C). We analyzed the data using a mixed effect model with average oxygen consumption across the three 4-min measuring periods (µg/min) as the response variable, rearing pool as a random factor, mass, temperature, time of measurement, and measuring environment, as fixed effects measured at the level of the group, and the rearing treatment as a fixed effect at the level of the pool. Time was included to account for changes in metabolic rate during the day [43,44], because trials in blank water were performed prior to the trials in kairomone water. We judged the significance of fixed effects from 10,000 Markov chain Monte Carlo samples drawn from the posterior distribution of the parameters in a Bayesian version of the model [45]. Analyses were done using the R function in R [46]. One group of tadpoles was discarded because their experimental chamber opened prematurely.

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Author Contributions

Conceived and designed the experiments: UKS. Performed the experiments: UKS. Analyzed the data: UKS JVB. Wrote the paper: UKS JVB.
References

1. Tollrian R, Harvell CD (1999) The ecology and evolution of inducible defenses. Princeton: Princeton University Press.
2. Noonburg EG, Nisbet RM (2005) Behavioural and physiological responses to food availability and predator risk. Evolutionary Ecology Research 7: 89–104.
3. McPeek MA (2004) The growth/predation risk trade-off: So what is the mechanism? American Naturalist 163: E38–E111.
4. Werner EE, Anholt BR (1993) Ecological consequences of the trade-off between growth and mortality-rates mediated by foraging activity. American Naturalist 142: 242–272.
5. Steiner UK, Pfeiffer T (2007) Optimizing time and resource allocation trade-offs for investment into morphological and behavioural defense. American Naturalist 169: 118–129.
6. Harvell CD (1990) The ecology and evolution of inducible defenses. Quarterly Review of Biology 65: 323–340.
7. Lima SL (1998) Stress and decision making under the risk of predation: Recent developments from behavioral, reproductive, and ecological perspectives. Advances in the Study of Behavior 27: 215–290.
8. Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation - a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie 68: 619–640.
9. Van Buskirk J, McCollum NA (2000) Functional mechanisms of an inducible defense in tadpoles: morphology and behaviour influence mortality risk from predation. Journal of Evolutionary Biology 13: 336–347.
10. Brodin T, Jehansson F (2004) Conflicting selection pressures on the growth/predation risk trade-off in a damselfly. Ecology 85: 2927–2932.
11. McPeek MA, Grace M, Richardson JML (2001) Physiological and behavioral responses to predators shape the growth/predation risk trade-off in damselflies. Ecology 82: 1535–1545.
12. Steiner UK (2007) Investment in defense and cost of predator-induced defense along a resource gradient. Oecologia 152: 201–210.
13. Steiner UK (2007) Linking antipredator behavior, ingestion, gut evacuation and costs of predator-induced responses in tadpoles. Animal Behaviour 74: 1473–1479.
14. Stoks R (2001) Food stress and predator-induced stress shape developmental performance in a damselfly. Oecologia 127: 222–229.
15. Stoks R, De Block M, Van De Meuter F, Johansson F (2005) Predation cost of rapid growth: behavioural coupling and physiological decoupling. Journal of Animal Ecology 74: 708–713.
16. Tollrian R (1993) Predator-induced morphological defenses - costs, life-history shifts, and maternal effects in Daphnia pulex. Ecology 76: 1691–1705.
17. Spitze K (1992) Predator-mediated plasticity of prey life-history and morphology - Chaoborus ambrosi predation on Daphnia pulex. American Naturalist 139: 229–247.
18. Stoks R, De Block M, McPeek MA (2005) Alternative growth and energy storage responses to mortality threats in damselflies. Ecology Letters 8: 1307–1316.
19. Slos S, Stoks R (2008) Predator stress induces stress proteins and reduces antioxidant defense. Functional Ecology 22: 637–642.
20. Beckerman AP, Wieski K, Baird DJ (2007) Behavioural versus physiological mediation of life history under predator risk. Oecologia 152: 335–345.
21. Rovero F, Hughes RN, Chelazzi C (1999) Cardiac and behavioural responses of muskies to risk of predation by dogfishes. Animal Behaviour 58: 707–714.
22. Hawkins LA, Armstrong JD, Maguran AE (2004) Predator-induced hyper ventilation in wild and hatchery Atlantic salmon fry. Journal of Fish Biology 65: 101–110.
23. Barretto RE, Luchari AC, Marcondes AL (2003) Ventilation frequency indicates visual recognition of an allopatric predator in naive Nile tilapia. Behavioural Processes 60: 235–239.
24. Angilletta MJ, Wilson RS, Navas CA, James RS (2003) Tradeoffs and the evolution of thermal reaction norms. Trends in Ecology & Evolution 18: 234–240.
25. Pernet F, Tremblay R, Redjahl J, Sevigny JM, Gionet C (2008) Physiological and biochemical traits correlate with differences in growth rate and temperature adaptation among groups of the eastern oyster Crassostrea virginica. Journal of Experimental Biology 211: 969–977.
26. Inselnd AK, Foss A, Naerdel G, Coss T, Bonga SW, et al. (2000) Countergradient variation in gut morphology and food conversion efficiency of juvenile turbot. Journal of Fish Biology 57: 1213–1226.
27. Wellborn GA, Skelly DK, Werner EE (1996) Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27: 337–363.
28. Trussell GC, Evanschek PJ, Matsama CM (2006) The fear of being eaten reduces energy transfer in a simple food chain. Ecology 87: 2979–2984.
29. Van Buskirk J (2002) Phenotypic lability and the evolution of predator-induced plasticity in tadpoles. Evolution 56: 361–370.
30. Schoepner NM, Relyea RA (2005) Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecology Letters 8: 565–572.
31. Turner VA (1975) Energetic cost of moving about. American Scientist 63: 413–419.
32. Videler JJ (1993) Fish Swimming. London: Chapman & Hall.
33. Sapolisky RM (2002) Endocrinology of the stress-response. In: Becker JB, Bredlove SM, Gross D, McCarthy MM, eds. Behavioral Endocrinology. Cambridge, MA: MIT Press. pp 409–450.
34. Relyea RA (2003) Predators come and predators go: The reversibility of predator-induced traits. Ecology 84: 1840–1848.
35. Nolte D, Reyer HU (1992) Modification of antipredator behavior in tadpoles by environmental conditioning. Journal of Animal Ecology 61: 353–360.
36. Holopainen JJ, Ahl J, Vormann M, Huuskonen H (1997) Phenotypic plasticity and predator effects on morphology and physiology of ciscoe carp in nature and in the laboratory. Journal of Fish Biology 50: 781–798.
37. Miles DB, Calibbeek R, Sinervo B (2007) Corticosterone, locomotor performance, and metabolism in side-blotched lizards (Uta stansburiana). Hormones and Behavior 51: 540–554.
38. Lima SL, Redenkolz PA (1999) Temporal variation in danger drives antipredator behavior: The predation risk allocation hypothesis. American Naturalist 153: 649–659.
39. Van Buskirk J, Anderwald P, Lupold S, Reinhardt L, Schuler H (2005) The bare effect, tadpole tail shape, and the target of dragonfly strikes. Journal of Herpetology 37: 420–424.
40. Laurila A, Lindgren B, Langen AT (2008) Antipredator defenses along a latitudinal gradient in Rana temporaria. Ecology 89: 1399–1413.
41. Grantner A, Taborsky M (1998) The metabolic rates associated with resting, and aerobic exercise, tadpole tail shape, and the target of dragonfly strikes. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 168: 413–433.
42. Forstner H (1983) An automated multiple-chamber intermittent-flow respirometer. In: Gaugler E, Forstner H, eds. Polarographic oxygen sensors. Berlin Heidelberg New York: Springer: pp 111–126.
43. Abé D, Scale DB, Boraas ME (1992) Periodicities and transient shifts in anuran ( Xenopus laevis, Rana clamitans) oxygen-consumption revealed with flow-through respirometry. Comparative Biochemistry and Physiology a-Physiology 101: 425–432.
44. Moeller E (1981) Effect of body size, trophic state, time of day, and experimental stress on oxygen-consumption of anuran larvae - an experimental assessment and evaluation of the literature. Comparative Biochemistry and Physiology a-Physiology 70: 497–508.
45. Byungh RH, Davidson DJ, Bates DM (2008) Mixed-effects modeling with crossed random effects for subjects and items. Journal of Memory and Language 59: 390–412.
46. R Development Core Team (2009) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.