En predators påverkan på hjärtfrekvens, stress och personligheter i oval dammsnäcka Radix balthica.

Predator-induced cardiac response, stress and personalities in the freshwater snail Radix balthica.

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**ABSTRACT**

Anti-predator behavior has been extensively studied, but the physical impact a predator has on its prey has been somewhat neglected. The study was conducted using the freshwater snail, *Radix balthica*, and a common molluscivorous fish predator, *Carassius carassius*. Snails have previously been shown to react to the mere smell of this predator, and the present study investigates stress caused by predator cues in terms of changes in cardiac activity of individual snails in relation to their behavior. The degree of shy/boldness expressed by *R. balthica* individuals, used as a measure of personality, was measured and found to be repeatable over time. However, cardiac activity was not related to personality, nor to the presence or absence of predator chemical cues. It seems that predator chemical cues cause only a moderate stress reaction in the snails, and future research might evaluate responses by snails upon actual predator encounters.

**Key words:** Ecology, *Radix balthica*, *Carassius carassius*, personality, behavioral syndrome, stress, heart rate, anti-predator behavior.

**INTRODUCTION**

In the big question of eat or be eaten, prey have to adopt certain precautions to avoid ending up in the gape of a predator. Prey organisms have evolved a variety of defenses to reduce the risk of predation, such as spines or shells (Blumstein 2006). In systems with a variable presence of predators, such permanent protection might be unnecessary and costly in the absence of danger. The presence of a predator can induce a plastic morphological change in the prey species that makes it more difficult to consume. Such morphological changes can provide a benefit in the presence of predators, but a cost in predator-free environments, in terms of e.g. reduced mobility (Tollrian & Harvell 2003). A disadvantage of phenotypic plasticity, however, is the time lag between predator detection and obtained morphological defense, as well as the change back when predators disappear (Brönmark et al 2011; Dall et al 2004).

Prey may react behaviorally to predator threats to avoid risk, such as increased use of shelter (Turner et al 2000; Dewitt et al 1999). Such behavioral change can provide direct and efficient refuge from
predation, but may also include costs to other important aspects of life, such as mating and foraging, and thus individuals may have to trade off different aspects of their life-history (Křivan 1998; Blumstein 2006).

Upon predator encounter, prey may react with a fright response. Fear is a condition where the body is in an aroused, stressed state, ready to flee or fight (Blumstein 2006). A variety of studies have focused on the influence of a predator, or cues from a predator, on stress-related hormones in prey (Oswald et al 2012), as well as the relationship between heart rate and stress (Koolhas et al 1999). In spite of this, only a few have investigated the influence a predator may have on prey stress measured as cardiac activity, which could give a direct link to energy-allocation trade-offs. Examples include the mussel *Mytilus edulis*, which shows a significant change in heartbeat in the presence of waterborne chemical cues from dogwhelks *Nucella lapillus* (Rovero et al 1999), as well as the black turban snail *Chlorostoma funebralis*, which shows a similar response to the ocher sea star *Pisaster ochraceus* (Pruitt et al 2013). On the other hand, a study of the freshwater snail *Lymnaea stagnalis* revealed heart beat as a non-reliable indicator of stress level (Orr et al 2007).

Animals, including humans, can vary in their response to stressed situations, both between species and within populations. Consistent individual differences in behavior across time and/or context are often referred to as animal personalities or behavioral syndromes (Bell 2007, Sih et al. 2004). Some individuals tend to constantly be bolder across context situations, whereas shy individuals show more reserved behavioral strategies (Sih et al 2004). Bolder individuals respond less to threats, and tend to forage more actively also in dangerous situations (Pruitt et al 2012; Coleman & Wilson 1998). In zebrafish *Danio rerio* and dark-eyed junco *Junco hyemalis*, there is indication that bolder individuals have lower stress responses than shyer individuals (Oswald et al 2012; Atwell et al 2012). Also, more aggressive female spiders tend to stay in shelter a shorter time after a simulated attack (Reichert & Hedrick 1993).

The evolutionary and ecological importance of animal personalities can be explained by trade-offs between seeking shelter and foraging. Fluctuating environmental factors, such as predation pressure, can make it more profitable to use one strategy, being bold or shy, rather than having complete behavioral plasticity (Sih et al 2004; Dewitt et al 1999). Frequency-dependent selection where predators with different personality types tend to forage dissimilarly is crucial to maintain different behavioral syndromes within prey and vice versa (Pruitt et al 2012; Dall et al 2004).

*Radix balthica* is a freshwater snail consumed by fish and different invertebrates. It is known to change shell morphology according to predator presence, to the extent that it was first believed to be
two different species (Hubendick 1951). Snails can detect the presence of predatory fish by chemical cues in the water, which is an important type of cue for many aquatic animals (Kats & Dill 1998; Turner et al 2000; Brönmark et al 2011), and increase refuge accordingly (Rundle & Brönmark 2001; Ahlgren & Brönmark 2011). The aim of this work is to investigate the difference in snail stress response, measured as heart rate, depending on whether or not there are chemical cues from fish, to learn more about predator-induced responses in cardiac activity in light of individual differences in behavior. I pursue this by investigating the differences between baseline pulse and the pulse after a simulated attack in connection to snail personality and fish cue treatments. I hypothesize that bolder snails will have a higher baseline pulse than shyer individuals, and that bolder snails will have a relatively lower difference between resting pulse and stressed pulse.
METHODS

Around 20 snails where collected from three different ponds: Frihult, Bosarp and Krutmöllan in Scania. They were held in a 70 l tank and all eggs produced and found were moved to a new tank. All snails were fed a diet of lettuce and spirulina. The molluscivorus fish used in this study were crucian carp, *Carassius carassius*, collected by netting a pond in Lund. Four individual crucian carp (91.5-92.5 mm total length) were kept in a 15 l tank at 18°C in a 12h:12h light:dark regime, and fed 12 snails every second day.

Experiments started when snail offspring were three months old. Snails used in this study (originally N=349) were completely naïve to predators. The snails were individually assayed for boldness in a Petri dish containing tap water. Each snail was acclimatized for 30 minutes, and was then poked on the shell with tweezers to make it withdraw its entire body into its shell. The time (seconds) taken to emerge from the shell (until both antennas were visible) was then measured as an indicator of individual boldness. This procedure was repeated after one week. Individuals with both emergence times <10sec were considered bold, while emergence times >15sec were used as a shy personality criterion. The two emergence times where added for each individual to give a boldness index (low sum indicating high boldness). The personality assays were later used in two ways; 1) to compare pulses between the bold and shy groups of snails, and 2) as a correlate to snail heart rates using all individual snails (see below).

For the cardiac activity study, a heart rate sensor was attached to individual snails, and two days later individual baseline heart rates were measured during 50 minutes. Approximately half of the snails were then exposed to predator cues from water from the tank holding crucian carp, while the remaining snails were exposed to a tap water control. Pulses of each individual were then measured for another 50 minutes and this period is referred to as the treatment period. The cardiac activity was measured with infrared technology, where a transmitter and a receiver of infrared light were placed on the opposite sides of the shell, and movement in the circulatory system was measured as electric currents that were filtered, amplified and finally analyzed by the program Picoscope 6. For more details, see Burnett et al (2013). Heart frequency outputs were manually interpreted and only individuals with at least 300 seconds of clearly visible heart beats in both periods of measurements were included in analysis, for a total of 77 individuals. Out of these 77 individuals, 29 were classified as bold, 23 as shy and 25 as intermediate, according to the boldness assay. Each individual’s pulse rate during the treatment period was later compared to its pulse during the baseline period, and a possible correlation for pulses measures during these two periods was also evaluated. Heart rates from the baseline and treatment period, as well as the difference between these two periods were compared between bold and shy snail groups. The mean pulse rate during the treatment period was compared
between snails experiencing fish cues and tap water (control). Finally, the pulse rate during the two periods was correlated against the individual boldness index to evaluate personality-dependent heart rates. All statistical analyses of data were performed in IBM SPSS statistics 2.0.

RESULTS

The time taken to emerge from the shell after being poked was measured twice. There was a positive correlation between these two emergence times, indicating there was a repeatable personality difference (Spearman’s rank correlation \( \rho=0.227 \), \( N=77 \), \( p=0.047 \), Fig. 1).

There was a significant difference in heart rate between the baseline and treatment period (paired samples t-test, \( t_{66}=-2.607 \), \( p=0.011 \)). There was also a positive correlation between heart rates measured during these two periods (Spearman’s rank correlation \( \rho=0.831 \), \( N=77 \), \( p<0.001 \), Fig. 2).

Bold and shy individuals did not differ in their heart rates during the baseline period (independent t-test, \( t_{50}=0.591 \), \( p=0.557 \)), nor did they do so during the treatment period (\( t_{50}=0.242 \), \( p=0.810 \)). In addition, bold and shy individuals did not differ in their heart rate when conducting the test on the difference between the two periods (\( t_{50}=1.415 \), \( p=0.163 \), Fig. 3).

The heart rates of snails treated during the treatment period did not differ depending on whether the snails were exposed to predator
cues or tap water (independent t-test, $t_{77}=-1.082, p=0.285$). There was no correlation between boldness score and pulse rate during both the baseline period (Spearman’s rank correlation $\rho=0.096, N=77, p=0.408$) and the treatment period ($\rho=0.071, N=77, p=0.540$).
DISCUSSION

The main purpose of this study was to investigate the difference in stress responses to predator threat between bold and shy individuals of freshwater snail *Radix balthica*. I hypothesized that bolder individuals would have a higher baseline pulse because of their more active lifestyle, and a lower increase to stressed pulse because of their potentially damper response to threat compared to shyer individuals. The snails were tested for personality, and showed repeatability between the first and second measurement of boldness, indicating that the snail’s had personalities that were consistent across time. The baseline pulse and the pulse after received treatment differed, and showed a positive correlation, which agrees with what would be assumed if response in cardiac activity is linked to personality. There was no difference in baseline pulse, treated pulse or relative increase in pulse between bold and shy individuals, which rejects the hypothesis that pulse should be linked to personality. Furthermore, there was no difference between the control and fish cue treatment during the treatment period. Pulse rate during the baseline and the treatment period were not correlated with the bold-shy score, rejecting the hypothesized link between cardiac activity and personality. Thus, both of my hypotheses were rejected. In contrast to other studies of aquatic prey species (Oswald et al 2012; Rovero et al 1999; Pruitt et al 2013), *R. balthica* in this study does not seem to respond to predator cues with increased cardiac activity. Results from the present study are consistent with the reported results for the freshwater snail *Lymnaea stagnalis*, in which there were individual differences in behavior, but no changes in cardiac activity linked to either personality or predator threat (Orr et al 2007).

One alternative explanation to my results is that the response to predators may be based on previous encounters. If so, one would not expect to see any effect of treatment, since my test subjects were naïve to predators. Personalities are often genetically linked (Sih et al 2004), and the fact that my snails were naïve would not have made a difference, there would still be individual variation in response. It has however been shown that waterborne chemical cues from predators is enough to induce morphological and behavioral defenses in *R. balthica*, and *L. stagnalis* maintained this ability over 250 generations (Orr et al 2007), arguing against this explanation.

My choice of experimental design was intended to imitate natural situations as much as possible, with the scent cues from predators as well as the poke on the shell to simulate an attack. The shell is an important protection for the snail, and it is logical to assume that a foraging predator might come in contact with the shell, causing the snail to withdraw its body. There is, to my knowledge, only one previous study that considers both cardiac activity and personality in freshwater snails, which makes it difficult to draw general conclusions, but it seems that determining stress responses based on heart
rates is not reliable. An alternative could be to measure oxygen consumption, which has been used for mussels (Rovero et al. 1999), but shown to be suboptimal for *L. stagnalis* (Orr et al. 2007). Further studies on the ecology of freshwater snails are needed to find suitable stress indicators. An argument against the use of hormonal indicators is that they do not tell us anything about altered energy use and are therefore not optimal. Moreover, we should maybe not expect a proper difference in cardiac activity until an actual encounter between predator and prey, since an increase in heart rate is associated with a cost. This suggests that the requirements for energy intake will increase, which contradicts the behavioral response of the snails, as they hide to avoid predators rather than become active and forage.

**CONCLUSIONS**

The reaction of a species induced by its predator tells us about a species’ physical reaction to stress and energy allocation. Freshwater snails *R. balthica* vary in response to simulated attack, but the variation is not correlated to pulse responses, nor does the scent of predators affect snail pulse. This may suggest that snails do not show individual variation in stress level when faced with predators. More research is however needed to corroborate these findings, including using alternative measures of stress.
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