EVS4009
Honours Project Thesis

Anti-predatory behaviour of wild vs. captive Freshwater Angelfish, *Pterophyllum scalare*

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Winter/Summer 2009

Abstract

In an attempt to reduce species extinction, captive breeding and reintroduction programs are frequently used. Wild animals raised in captivity often exhibit domesticated behaviour making them unfit for release into the wild. For fish, hatchery raised individuals tend to seek refuge less, making them more prone to predation. We tested this hypothesis with Freshwater Angelfish, *Pterophyllum scalare* by exposing them to artificial and natural predatory threats and measuring their pausing durations. A marginally significant difference in reactions was recorded between captive and wild fish in control conditions and in only one of the artificial stimuli, with captive fish showing shorter pauses than wild fish. A significant difference was obtained for natural stimuli, with wild fish showing shorter freezing durations than captive fish. Our results demonstrate that captive breeding changes the behaviour of fish enhancing their risk taking characteristic and putting them at higher risk of predation. They also demonstrate overreaction of captive fish to natural predatory stimuli, particularly non native ones.

Introduction:

Conservation biologists consider the Earth is in its sixth mass extinction episode (Avise et al, 2008). This phenomenon is currently a foremost worldwide concern (Brito, 2008). Significant efforts are underway to halt the disappearance of species (Avise et al, 2008). For some species, captive breeding programs are deemed necessary for their maintenance when they are in imminent danger of extinction in the wild (de Azevedo & Young, 2006). In captivity, animals are protected from natural threats including disease, competition, and predators. This protection sometimes extends for several generations before reintroduction is attempted. Whether captive bred individuals retain their wild behaviour and fair as well upon release as wild individuals remains largely debated (Alving & Kardong, 1994; Mathews et al, 2005).

Several reintroductions of mammalian species bred in captivity were successful (Wolf et al, 1996). However, the rates of success of reintroductions of captive bred individuals in to the wild are highly debated (Jule et al, 2008; Mathews et al, 2005). Reintroduction projects with individuals coming from a wild source had a higher success rate than those using animals from a captive source (Jule et al, 2008). Captive environments promote domesticated behaviour of wild animals (Kohane et al, 1988, as cited by Kelley et al, 2006) which incorporate change in predator recognition (de Azevedo & Young, 2006), foraging, and reproduction behaviour (Kelley et al, 2006). Such differences have been observed in old field mice (McPhee, 2003), mussels (Hoftyzer et al., 2008), and in steelheads (Lee & Berejikian, 2008). In fish, captive bred individuals seek refuge less than wild fish (Kelley et al, 2006) putting them at a higher risk of predation. As a result, in an attempt to enrich declining populations, larger numbers of reintroduced animals are required (McPhee, 2003). Because captive breeding and reintroduction of endangered species are very expensive (Wolf et al, 1996; Mathews et al, 2005), investigating the influence of captive breeding on animal fitness is crucial for such conservation measures to be successful. Therefore, my general goal in this thesis was to compare the anti predatory behaviour; comprised of the ability to recognize and avoid predator cues; of wild caught and captive bred Freshwater Angelfish, *Pterophyllum scalare*.

Freshwater angelfishs are cichlids native to the Amazon (Yamamoto et al, 1999). These Brazilian fish naturally feed on invertebrates (Degani, 1993) and are preyed upon by larger piscivores (Praetorius, 1932 as cited by Gomez-Laplaza, 2002). The exploratory behaviour of these fish has been observed in previous research. When presented with a stimulus, they exhibit a period of immobilization followed by an investigatory movement towards the stimulus (Gomez-Laplaza & Morgan, 2000).

A jack dempsey cichlid, *Cichlasoma octofasciatum,* was used as a visual stimulus of a natural predator. This is a larger and more aggressive cichlid native to Central America (Goldstein, 1988 as cited by Brown et al, 1999).

The objective of this experiment was to compare the anti predatory behaviour of wild caught and captive bred freshwater angelfish, *Pterophyllum scalare*. This was done by observing the effect of captivity on the ability to recognize and avoid predator cues. The fish were exposed to three treatments for detection of visual predatory cues. Different responses to the presented stimuli were recorded. With pausing (immobilization/freezing) being acknowledged as a trustworthy antipredator response to visual cues (Malavasi et al, 2008; Mesquita & Young, 2007; Petersson & Järvi, 2006), we decided to use this response as well as its duration as an indication of antipredator reaction.

Methods

Animal maintenance

Twelve captive-bred marble (henceforth referred to as captive) and fourteen wild-caught zebra (henceforth referred to as wild) angelfish bought from the local pet store (Big Al's Aquarium Services Warehouse Outlets Inc.) were kept in two separate circular opaque plastic containers (diameter: 66 cm, water height: 31 cm). The opaqueness of the containers minimized external disturbance. The wild fish were 5-7 cm and the captive fish were 5 cm. Fish were given at least 20 days after their arrival to acclimate to their new environment. They were under a 12L: 12D photoperiod. Water temperature was sustained at 25±1˚C using regular aquarium heaters. Water quality was maintained (0 ppm Nitrate, 0 ppm ammonia) using submersible carbon filters. When nitrate and ammonia exceeded the above mentioned concentrations, a 50-75% water change was performed. Such water changes were not performed the day fish were going to have a treatment. Fish were fed tropical flake feed once a day after completion of the treatments, therefore at a different time everyday.

Each fish underwent one treatment per day. Fish undergoing the treatments were kept individually in rectangular perforated cages (length: 7 cm× width: 4 cm× water depth: 24 cm) that allowed visual and olfactory contact with other fish (no isolation of fish). These cages helped us identify the fish without using stressful tagging methods. The external sides of the cage were covered with brown paper to reduce external interference. Fish that underwent all three treatments were placed together in a separate rectangular tank (60 cm × 31 cm × 24 cm) under the same conditions mentioned above.

The jack dempsey (henceforth referred to as JD) was kept in a separate rectangular tank (60 cm × 31 cm × 34 cm) under similar conditions, however it was provided with pellet food instead of the tropical flakes.

Treatments

The three experimental treatments were run on each of the fish in two rectangular experimental tanks (60 cm × 31 cm × 24 cm; 50 cm × 28 cm × 26 cm). The tanks were filled with dechlorinated water pre-heated to the required temperature. A video camera was placed in front of the short side of the experimental tank during the procedures and was connected to a large television. The tank and the camera were then covered with fabric sheets. This isolated the fish from any external disturbance, but still allowed observation of the response through the camera. Durations were recorded using a stop watch. After each treatment, experimental tanks were drained and cleaned.

The sequence of treatments was randomly selected using a draw to eliminate potential carry over effects. In all treatments, fish were moved into the experimental tank and left for an hour to acclimate and re-establish normal behaviour. For the sake of this experiment, we considered pausing to be motion inhibition for more than five seconds. Motion includes in place rotation, flinching, or translational forward or backward motion.

Control

Fish were observed for 10 minutes under normal stimulus free conditions. Maximum pausing duration (maxpause) in seconds was recorded. This variable gave an estimate of how long fish would pause in such conditions.

A description of the fish's regular activities was also recorded for 10 minutes of observation. This description was used to identify the onset of normal behaviour in the other two treatments, as some studies have shown such responses to diminish as an antipredator reaction (Brown et al, 2009). Some of the activities included pecking on the bottom of the tank or on the surface of the water (feeding); and continuous swimming along the whole extent of the experimental tank (swimming).

Netting

Dip netting has been used as a predatory stimulus in several experiments (Giles, 1987; Yue et al, 2004; Seppälä et al, 2005). In this treatment, the fish were chased with a net under the water surface for 10 seconds in the experimental tank with the net coming out from between the sheets keeping the experimenter hidden. The duration of pause after presenting the netting stimulus was recorded (netpause) in seconds.

Duration until the fish resumed normal behaviour was also recorded (normal net) in minutes.

Vision

The short side of the experimental tank was placed facing the short side of the JD tank. We visually separated the two tanks with a partition before positioning them together to avoid visual contact between the fish prior to the beginning of the treatment. After the acclimation period, the partition was removed from between the sheets and the two fish had visual contact through the glass. The variables recorded were: duration of pause after partition removal (partitionshock) in minutes; duration of pause when eye contact between the two fish occurred (JDpause) in seconds; duration for the angelfish to register the first investigatory trip towards JD (firstinvest) in minutes; and duration to resume normal behaviour (normal vision) in minutes. Moreover, the type of movement the fish exhibited after removal of the partition altered between a pause and a sideway drift. This was also recorded as a discrete variable labelled (firstrxn).

Statistical Analysis

Statistical analysis was conducted using S-plus 8.0. Data transformations were performed to fulfill the normality and homoscadasticity assumptions of a one tailed two sampled t-test, which was used to asses the difference between the means of the responses of wild and captive. When the assumptions could not be met, a non parametric Wilcoxon rank-sum test was run. 'firstrxn' was compared using Pearson's chi-square test.

Results

Control

The difference between the means of 'maxpause' for captive and wild fish was not significant ($p = 0.14$, Fig. 1). In addition, almost all fish displayed 'swimming' and 'feeding' behaviour.

Netting

The difference in 'netpause' between wild and captive fish was not significant (p= 0.68). A non significant difference (p= 0.33) was also observed in 'normal net'.

Vision

In terms of the fish's first reaction, only 2 wild and 4 captive fish exhibited a drift as their first reaction after partition removal, with the rest exhibiting a pause (Fig. 2). Considering the non significant p-value obtained (0.46), we decided to ignore drifting and only consider pause durations even if they were not the first response after removal of partition between the experimental and JD tank.

Discussion:

In all the treatments, the presentation of a predatory stimulus caused both types of fish to exhibit a freezing response. All but two of the measured variables gave non significant results in the expected trend, with wild fish taking longer durations than captive. However, in the vision treatment, 'JDpause' and 'normal vision' gave significant p-values in the opposite trend, with captive fish taking longer than wild to resume activity and normal behaviour after presentation of a stimulus, respectively.

A marginally significant p-value registered for 'maxpause' in the control treatment illustrates the difference originally present between the two types of fish. It also supports the suggestion made by Kelly and colleagues (2006) that captivity promotes aggressive behaviour in fish, making them take more risks and therefore being more susceptible to predation.

In the netting treatment, no difference was recorded between wild and captive fish. That might be due to the fact that dip-netting is known to produce an anti-predator response when it involves a sudden overhead stimulus and a change in the testing environment (Giles, 1987; Giles & Huntingford, 1984). This includes chasing, capture, anoxic transfer, and placement in a new setting, all of which engender physiological stress (Ferguson & Tufts, 1992; Rotllant & Tort, 1997; Barton et al, 1980; Brydges et al, 2009). Comparing both methods, ours would not be as stressful as a regular dip netting procedure and is therefore closer to an artificial visual stimulus than to dip netting. Moreover, this net was also used for all transportation carried on the fish. This might have caused habituation of the angelfish to the net as has been seen in Panamanian bishops (Brydges et al, 2009) and in sticklebacks (Milinski, 1985) which could be the reason for the lack of significance of our results.

Furthermore, it seems logical to consider ‘partitionshock’ as another visual artificial stimulus. This variable caused both fish to pause with captive fish resuming activity almost significantly faster than wild. These results are similar to those of Solanen and Peuhkuri (2006) who found that hatchery reared European graylings stop aggressive behaviour after the overhead movement of a black rubber glove for a slightly shorter duration than wild. They are also compatible with experiments that attacked the fish with an artificial overhead predatory stimulus suddenly disturbing the water surface (Malavasi et al, 2008; Petersson & Järvi, 2006). These observations also indicate that captivity promotes boldness and risk taking in fish (Solanen & Peuhkuri, 2006; Sundström et al, 2004; Kelly et al, 2006). Unlike our results, the latter two experiments obtained significant differences between captive and wild fish. The lack of significance of our results might be due to the large and unequal variance around the means. This is most likely attributed to inter-individual variation, inexperienced data collection, and un-standardized hunger levels that are known to affect the duration of antipredator responses (Gotceitas & Godin, 1991). A significant p-value would have been obtained had we had a slightly larger sample size. A post hoc power analysis run for 'partitionshock' gave a 59.8% power to detect a statistically significant result if present. It also indicated the need of a ($N_{captive}$= N_{wild} = 22) 44 fish sample size to obtain a significant result of p = 0.05.

Removing the partition provided the angelfish with a through the glass stimulus of a natural visual threat. Such an approach has been shown to be a stressful predatory stimulus in zebrafish (Barcellos et al, 2007), Japanese flounder (Miyazaki et al, 2004), and slimy sculpins (Chivers et al, 2001). Instant responses to stressful stimuli vary; however freezing and its duration have been often recorded as an anti-predator response, with first drift or dash ignored (Solanen &Peuhkuri, 2006; Brown et al, 2009, Malavasi et al, 2008). This agrees with our decision to ignore the type of first reaction and focus on the duration of freezing. A significant difference in pause duration was recorded between captive and wild fish when the predator was spotted, however, wild fish resumed activity faster than captives. Similarly, wild fish resumed normal behaviour significantly faster than captives. These results are in compliance with those of Epp and Gabor (2008) that found that predator naïve aquatic salamanders significantly decreased their activity when presented with the chemical cues of a non-native predator, unlike the predator experienced salamanders (Epp & Babor, 2008). Considering that jack dempseys are Central American cichlids (Goldstein, 1988 as cited by Brown et al, 1999) and therefore not a native predator for South American freshwater angelfish, we would expect captive fish to have longer freezing durations than wild fish. Our results also agree with those of Oliver et al (2008) who found that lobsters raised without predators in the experimental tank tended to reduce their activity significantly more in the presence of a threat than those raised together with predators. They labelled such lengthy stagnations as overreactions to predatory stimuli as has been suggested by Lafrance et al (2003) for cultured sea scallops. The fact that both of these experiments used visual stimulus complemented with chemical cues to obtain significant results puts greater confidence in our results that were significant in the absence of olfactory complementation.

In terms of investigatory trips towards the predator, no significant difference was recorded. This is in contradiction with several studies that found captive fish always launching investigatory trips sooner than wild fish (Petersson & Järvi, 2006; Malavasi et al, 2004; Sundstrom et al, 2004). This well established phenomenon was also attributed to the risk taking characteristic of captive fish.

Mirza and Chivers (2000) found brook trout loosing their wild traits ten days after being captured from the wild. Although we did not quantify the observation, four of our wild fish that were moved into the recovery tank after going through the treatments established similar behaviour. After spending a few days in one tank with captive fish, wild fish learned to come up to feed after being used to feeding on flakes sinking to the bottom.

Acknowledgments

I would like to thank my supervisor Gabriel Blouin Demers for all his guidance and for funding my project. I would also like to thank the Animal Care Personnel for their help caring for the animals, particularly William Fletcher. I extend my acknowledgments as well to the University of Ottawa and the department of Biology.

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Figures

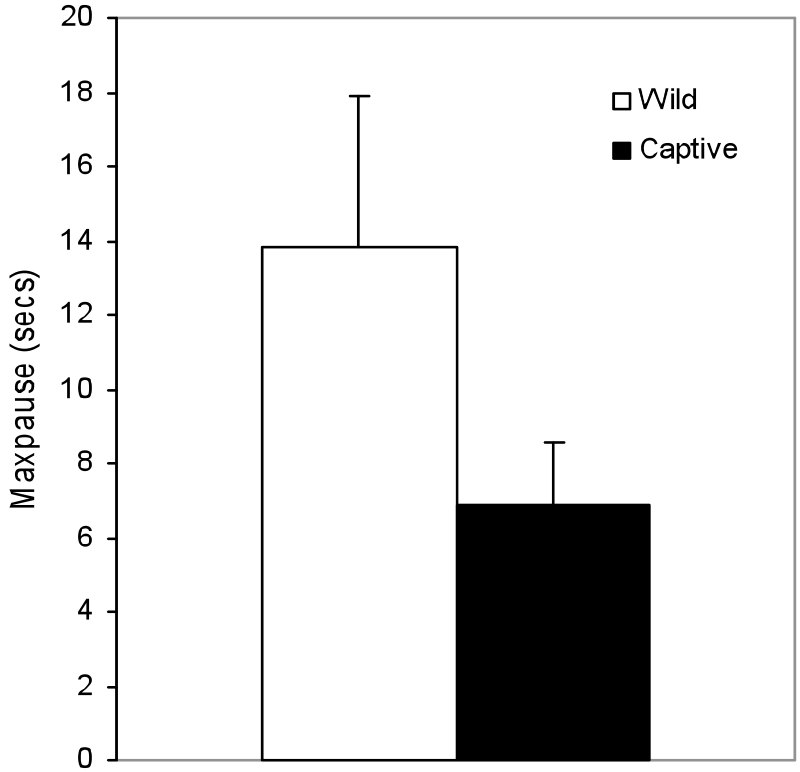

Figure 1. Means (+/- 1SE) of ‘maxpause’ in seconds for wild and captive freshwater angelfish in the control treatment.

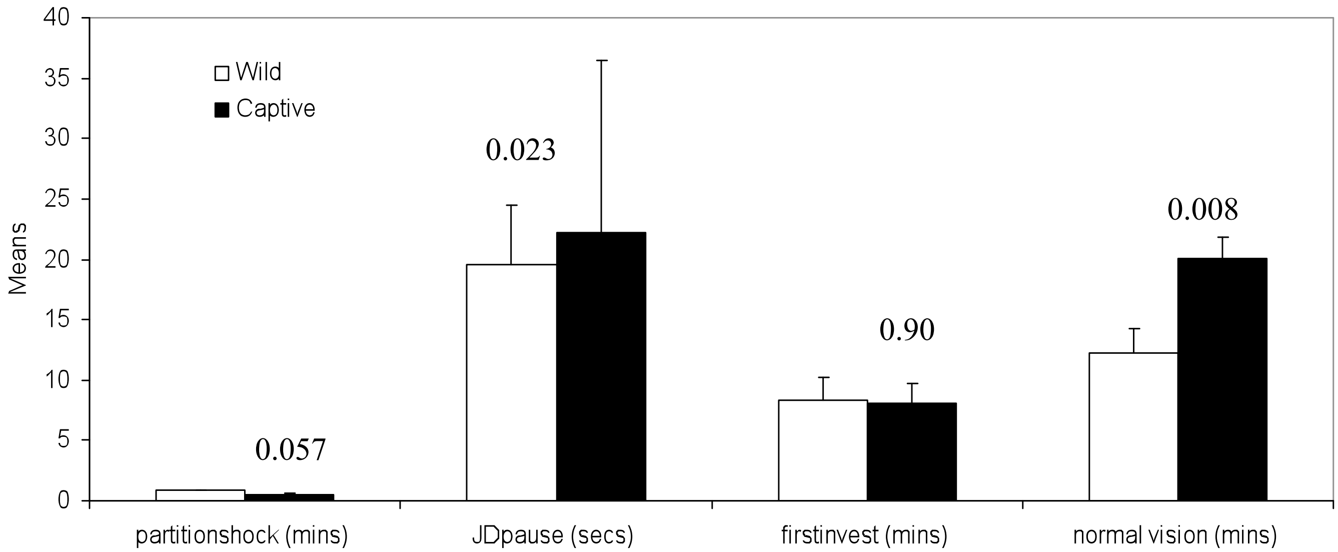

Figure 2: Means (+/- 1SE) of wild and captive freshwater angelfish for the five variables measured after they were able to see a jack dempsey cichlid, a potential predator. The p-values are presented above each bar.