Size, Sex, and Stress: Factors Affecting Lateralized Behaviour and Boldness in the Convict Cichlid (*Amatitlania nigrofasciata*)

by

Michele Kathryn Moscicki

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Psychology
University of Alberta

Abstract

Cerebral lateralization, the partitioning of cognitive functions preferentially into one hemisphere of the brain, is a trait ubiquitous among vertebrates. Although this trait is pervasive, not all individuals show the same degree or direction of lateralized behaviour. Individual differences in the expression of lateralized behaviour have been linked to factors such as growth rate, neuroanatomical asymmetry, and individual personality traits. The research presented in this thesis was conducted to examine the influence of these variables, as well as additional factors, on the expression of lateralized and other behaviours in a species of Central American fish, the convict cichlid (*Amatitlania nigrofasciata*). Study 1 revealed that differences in growth rate during the juvenile stage affected lateralized behaviour when viewing social stimuli. Study 2 showed that the type of stimuli, social or non-social, as well as the sex of the subject, influenced lateralized behaviour. In Study 3, a stressor altered lateralized behaviour such that the right hemisphere became more heavily involved in processing stimuli. This effect was predominantly found in females. This study also revealed a relationship between lateralized behaviour and neuroanatomical asymmetry that was not apparent when the stressor was absent. Study 4 showed that neuroanatomical asymmetry was unrelated to boldness in convict cichlids. Boldness did, however, influence the response of fish to stressors; this response was modulated by previous experience with stressors in females. These studies reveal that size, sex, stimulus type, stress, and experience interact to affect lateralized and boldness behaviour in the convict cichlid.

Preface

This thesis is an original work by Michele Kathryn Moscicki. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Biological Sciences Animal Policy and Welfare Committee, Project Name "Social Determination of Sex and Social Behaviour in a Cichlid Fish", protocol number AUP00000055, expires 05/31/2015. This research adheres to the guidelines set forth by the Canadian Council for Animal Care.

A portion of Chapter 3 of this thesis has been published as Moscicki, M.K., Reddon, A.R., & Hurd P.L. (2011). Lateralized behaviour of a non-social cichlid fish (*Amatitlania nigrofasciata*) in a social and a non-social environment. Behavioural Processes, 88, 27-32. I was responsible for the data collection and analysis as well as the writing of the manuscript. A.R. Reddon assisted with conceptual ideas and discussion. P.L. Hurd was the supervisory author and was involved with concept formation and manuscript composition.

Acknowledgements

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) discovery grant to PLH. MKM was supported by an NSERC CGS-D, an Alberta Ingenuity Studentship, a Queen Elizabeth II Doctoral Scholarship, and a Dissertation Fellowship from the University of Alberta. ARR was supported by an NSERC CGS-D.

I wish to warmly and heartily thank my supervisor, Dr. Pete Hurd. Pete gave me the tools I needed to succeed and then stepped aside to allow me to ‘rock on’. With his confidence in my abilities I was able to develop the skills needed to train and lead my own army of undergraduate researchers. His faith in me allowed me to have faith in myself and for that I will be forever grateful. Thank you, Pete!

I would also like to extend a heartfelt thank you to the members of the extraordinary Psychology Shop Team. Harold and Lou were always there to help me find silicone after I had busted an experimental apparatus. Philip saved my life, and my sanity, multiple times when my laptop was afflicted with variously coloured screens of death. Without Isaac’s deft hands and keen eye for design I would have no testing tanks and no data. I am greatly indebted to him and the Shop team for the hours of research assistance and delightful conversation.

I also want to thank all the members of the Sex and Violence Lab, past, present, and honorary. This group of individuals has made coming to the lab a pleasure. I don’t feel like I have worked a single day during my PhD – I have instead played intellectually with the best playmates a girl could hope for. We have been a supportive family to one another through the years, celebrating each other’s successes and lending man hours,

comforting shoulders, and intellectual sounding boards when times got tough. To our undergraduates, too many to name here, know that I have appreciated every hour you contributed to the running of fish, the measuring of brains, the analyzing of data, and most of all, the sharing of good times through laser tag, beer drinking, and lab bowling parties.

To my fellow graduate student, Cheryl Seaver, thank you for taking over the running of the wet lab when I went gallivanting away on random trips to catch fish in the wild and wrangle Canada's next generation of science superstars. To Ian Helgesen, thank you for welcoming me into the lab with kind words and a promise that this was a safe and supportive place to thrive as a scientist. You are missed.

Lastly, to my loved ones, thank you to my parents, Linda and Gene, for always having faith in me and supporting my dreams no matter how outrageous or unemployable they may have seemed at the time. Thank you for the unwavering confidence and choruses of 'this too shall pass' when things seemed never-ending. Thank you to my big brother, Mark, for being a sounding board and a shining example that following your passion can actually work out. Finally, to Erik, my partner through everything, I am eternally grateful for your love, wise words, colourful support, comfy spot, and unshakable belief in me even when I stop believing in myself. I would not have made it here or become the person I am without you. Thank you does not even begin to cover it.

Table of Contents

List of Tables

Table 5-3b. Results of type II sums of squares analyses of Total Squares Travelled in the open field task. Habenular ALI (strength of asymmetry in the habenula), Sex (male or female), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as between subjects factors.

Table 5-3c. Results of type II sums of squares analyses of Corner Time in the open field task. Habenular LI (directional asymmetry in the habenula), Sex (male or female), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as between subjects factors.

Table 5-3d. Results of type II sums of squares analyses of Corner Time in the open field task. Habenular ALI (strength of asymmetry in the habenula), Sex (male or female), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as between subjects factors.

List of Figures

preferences for one eye or the other when viewing an empty environment (both ps ≤ 0.001) and when viewing their own mirror images (both ps ≤ 0.001).

Figure 3-3. There was no significant relationship between laterality index (LI) in the detour task and LI in the mirror task for either females (filled circles, $p = 0.695$) or males (empty triangles, $p = 0.653$), indicating that eye preference in one task is unrelated to eye preference in the other task.

Figure 3-4. There was no significant relationship between absolute laterality index (Absolute LI) in the detour task and absolute LI in the mirror task for either females (filled circles, $p = 0.937$) or males (empty triangles, $p = 0.854$), indicating that strength of eye preference in one task was unrelated to strength of eye preference in the other task.

Figure 3-5. Direction (Hb LI) and strength (Hb ALI) of asymmetry in the habenula (Mean ± SEM). Females (dark grey bars) had significantly left-biased habenulae (negative values) at the population level ($p = 0.04$) while males (light grey bars) showed a trend in this direction ($p = 0.082$). Both female and male fish had significantly asymmetrical habenulae at the individual level, regardless of the direction of that asymmetry ($p < 0.001$ for both).

Figure 4-1. Directional eye preference (LI) scores (Mean ± SEM) for females and males in the detour task with either alarm cues absent (Control – shaded bars) or present (Alarm Cues – open bars). N = 30 for all groups. Positive scores indicate a right eye preference to view the empty environment behind the barrier while negative scores indicate a left eye preference. Females switched from right eye to left eye preference when alarm cues were present ($p = 0.002$) while males showed an increased preference to use the left eye when alarm cues were present ($p = 0.077$).

Figure 5-3. I found a significant positive relationship between time to emerge into a novel environment and time spent in corner squares in the open field task in male fish ($p = 0.008$).

Figure 5-4. The relationship between time to emerge into a novel environment and time spent in corner squares of an open field tank in female fish in 4 different stress conditions. (1) Not pre-exposed to net chasing and alarm cues not present in the open field tank (Ctl, Ctl; filled circles, solid line); (2) Not pre-exposed to net chasing and alarm cues present in the open field tank (Ctl, AC; open inverted triangles, long dashed line); (3) Chased with a net and alarm cues not present in the open field tank (Net, Ctl; filled squares, medium dashed line); (4) Chased with a net and alarm cues present in the open field tank (Net, AC; open diamonds, short dashed line). The relationship between emerge time and time spent in corner squares was modulated by the presence of alarm cues in the open field tank in female fish that were not pre-exposed to the net chasing stressor (solid line vs. long dashed line, $p = 0.015$).

Figure 5-5. Both female and male fish had significantly left-biased habenulae at the population level (HbLI: Females = dark grey bars, $p < 0.001$; Males = open bars, $p < 0.001$). There was no difference between the sexes in either direction or strength of lateralization of the habenula. Both females and males showed significant asymmetry of the habenula at the individual level (HbALI: $p < 0.001$ for both). Bars show Mean ± SEM. $N = 64$ for each sex.

1. GENERAL INTRODUCTION

1.1 Cerebral Lateralization

Cerebral lateralization, the partitioning of cognitive functions preferentially into one hemisphere of the brain, has been found in every vertebrate species studied to date (see Rogers & Andrew, 2002 and reviews by Bisazza et al., 1998; Vallortigara et al., 1999). This ubiquity suggests that there must be some benefit to brain lateralization. Indeed, evidence indicates that possessing lateralized cerebral capabilities allows individuals to process information more quickly and attend to multiple stimuli simultaneously (reviewed in Vallortigara, 2006). Similarities have been found between hemispheric functions in species as diverse as humans and birds. In these species, the right hemisphere is more specialized for global attention (Zaidel, 1987), attending to novel stimuli and unexpected events (MacNeilage et al., 2009), and the recognition of complex but unfamiliar stimuli (Andrew, 1991). In contrast, the left hemisphere is more involved in focused attention while ignoring distractors and performing behaviours according to learned routines (MacNeilage et al., 2009). The left hemisphere is also more specialized for speech and language in humans, and producing and perceiving vocalizations in animals, which reflects a more general categorizing function of this hemisphere (Geshwind, 1971; Vallortigara et al., 1999). These differing functions of the cerebral hemispheres suggest that brain lateralization may have evolved to overcome the issue of functional incompatibility (Sherry & Schachter, 1987; Vallortigara et al., 1999). For example, upon encountering a stimulus an organism must first determine the degree of novelty of that stimulus and then categorize that stimulus for future encounters. To determine novelty, an organism must attend to unique features of a stimulus; however, to categorize it, an organism must attend to features of the stimulus that are similar to other stimuli it has previously encountered (Vallortigara et al., 1999). Vallortigara and colleagues (1999) suggest that these types of stimulus assessments are mutually exclusive problems and might best be solved by functionally separate systems, giving rise to the prevalence of lateralized brains. Additionally, due to the similarity between lateralized functions in the distantly related human and avian brains, several authors have suggested that lateralization of brain function is evolutionarily ancient (Rogers & Andrew, 2002; see reviews by Bisazza et al., 1998; Vallortigara et al., 1999; MacNeilage et al., 2009). This idea has led to the burgeoning research field of cerebral lateralization studies in more evolutionarily ancient vertebrate species such as reptiles, amphibians, and fish (see reviews by Bisazza et al., 1998 and Roussigne et al., 2012).

1.2 Population and Individual Level Lateralization

In most studies examining lateralization of either cerebral structure or behaviour, a distinction is made between lateralization at the population level or at the individual level. Population-level lateralization refers to all individuals within a group displaying asymmetries in the same direction. Individual-level lateralization, on the other hand, indicates that individuals do exhibit asymmetries, but not all necessarily in the same direction (Vallortigara, 2006). Sociality, or group living, is a potential explanatory factor for the existence of population-level lateralization (e.g., Bisazza et al., 2000 and reviewed in Vallortigara, 2006). Individuals with similar asymmetries are better able to coordinate behaviours and form more cohesive shoals (Bisazza & Dadda, 2005). However, groups that act in similar ways, such as performing stereotyped escape behaviours from predators, may be exploited by adversaries that learn and respond to typical group behaviours. In this way, individuals that dissent from the group may have a fitness advantage (reviewed in Vallortigara, 2006). Indeed, frequency-dependent selection may operate on the direction of lateralization within a group to maintain variation in direction and strength of asymmetry (e.g., Hori, 1993; reviewed in Vallortigara & Rogers, 2005).

1.3 Life History Strategy and Lateralization

Frequency-dependent selection is not the only proposed mechanism to explain variation in lateralization between individuals within a population. Reddon and colleagues (2009), using a species of South American cichlid fish (*Geophagus brasiliensis*), hypothesized that growth depensation effects contribute to variation in both behavioural and cerebral lateralization. Variation in growth rate among individuals from a single cohort is common in fish species. The work with *G. brasiliensis* showed that larger individuals of a sibling cohort were male and that larger males displayed stronger behavioural lateralization than their smaller male siblings (Reddon et al., 2009). Reddon and Hurd (2009a) showed that more strongly lateralized fish are also bolder than their more symmetrically-behaving conspecifics. These two studies led Reddon and colleagues (2009) to propose that life history strategy was a potential factor affecting lateralized behaviour such that individuals with a more ‘live fast, die young’ approach to fitness were larger, bolder, and more strongly lateralized. Support for this theory comes from studies examining the relationship between lateralization and risk-taking behaviours. For example, zebrafish (*Danio rerio*) larvae that were raised in darkness showed reduced behavioural asymmetry as well as increased shyness in response to a predator model (Budaev & Andrew, 2009). Male topminnows (*Girardinus falcatus*) that are more strongly lateralized resume mating behaviours when placed in a novel environment sooner than less lateralized fish (Dadda et al., 2007). Strongly lateralized chicks produce fewer distress calls when viewing a model predator than weakly lateralized chicks (Dharmaretnam & Rogers, 2005). Fish inhabiting high predation areas show stronger lateralization and increased boldness (Brown et al., 2007a,b). These studies suggest that a more risk-prone life history strategy may be associated with stronger lateralization.

1.4 Stimulus Type and Lateralization

While frequency-dependent selection and life history strategy may both affect the direction and strength of lateralized behaviour, a more proximal variable also affects behavioural lateralization: the type of stimulus eliciting the behaviour. Many studies examine only one aspect of lateralized behaviour, for example, detouring around a barrier to view an empty environment, and then make general statements about asymmetry of behaviour within individuals and groups. However, experiments examining different species have shown that there is not always great consistency in how groups perform in laterality tasks when viewing different stimuli (e.g., Bisazza et al., 1997, 1998, 2001; Facchin et al., 1999; Dadda et al., 2010). Bisazza and colleagues (1998) conducted one of the first laterality experiments on fish in the detour task and showed that two species of poeciliids (*Gambusia holbrooki* and *G. falcatus*) show a right eye bias for viewing predators but a left eye bias when viewing an empty environment. Same sex conspecifics elicited a left eye bias in females but not males and opposite sex conspecifics elicited a right eye bias in females deprived of male contact but no bias in non-deprived females (Bisazza et al., 1998). This study is a classic example of the importance of stimulus type when examining lateralized behaviour. This study did not, however, mention whether there was consistency in either strength or direction of eye preference in individual fish across the different tasks. Indeed, most studies examining behavioural asymmetries with multiple stimuli do not report information on individual consistency.

1.5 Internal State and Lateralization

The study by Bisazza and colleagues (1998) mentioned previously introduces the issue of internal state affecting lateralized behaviour. Females in that study that were deprived of male contact showed a different pattern of eye use towards a sexual stimulus than non-deprived females. Davidson (1995) first proposed that cognitive state, whether positive or negative, can influence which hemisphere is used to process stimuli. He drew on evidence from stroke patients and those with neurological lesions to suggest that negative emotions and withdrawal responses are controlled by the right hemisphere, while positive emotions and approach are controlled by the left hemisphere (Davidson, 1995). This idea is known as the laterality-valence hypothesis. There is disagreement, however, on the validity of the laterality-valence hypothesis in humans as some evidence indicates a dominant role of the right hemisphere in all types of emotional processing, whether positive or negative (Rogers, 2010).

Rogers (2010) advanced the laterality-valence hypothesis and suggested that the present state of an animal may influence which hemisphere that animal uses to process ambiguous stimuli. An animal presently in a negative state, perhaps due to currently experiencing a stressor, may process ambiguous stimuli predominantly with the right hemisphere. In contrast, an animal in a positive state may process the same ambiguous stimulus preferentially with the left hemisphere. Rogers (2010) also suggests that cognitive bias, a persistent positive or negative state, may be related to hemispheric processing. Cognitive bias has been shown in animals trained to associate one stimulus (e.g., a black lid) with a food reward and another stimulus (e.g., a white lid) with punishment. Animals are subsequently presented with an ambiguous grey lid. If they respond as though expecting reward they are said to have a positive cognitive bias; if they respond as though expecting punishment they are deemed to have a negative cognitive bias (Rogers, 2010; Bateson & Matheson, 2007). Rogers (2010) proposes that animals with a negative cognitive bias have increased right hemisphere control of behaviour while those with a positive cognitive bias have increased left hemisphere control. She suggests that stressful events during development may lead to increased control by the right hemisphere. Rogers' (2010) hypothesis brings to the forefront the idea that lateralized behaviour may be altered not only by frequency-dependent selection, life history strategy, and stimulus type, but also by the internal affective or cognitive state of the organism.

1.6 The Habenula

Neural asymmetries in primate brains have been studied for quite some time (reviewed in Walker, 1980); however, only within the last two decades have asymmetries in the structure of non-primate animal brains, and the relation of those asymmetries to lateralized behaviour, garnered substantial research interest (reviewed in Bisazza et al., 1998 and Frasnelli et al., 2012). One of the main targets of this research interest is a structure that presents noticeable asymmetry in many organisms, the habenula (Concha & Wilson, 2001; Vallortigara & Bisazza, 2002). The habenula is part of an evolutionarily conserved pathway in the limbic system and connects the forebrain with the ventral midbrain (Sutherland, 1982). Proposed functions involving the habenula include learning and memory, feeding and mating, behavioural responses to sleep, pain, anxiety, and stress, and inhibition of motor behaviour when a reward is not obtained or when an aversive outcome is anticipated (reviewed in Hikosaka, 2010).

In most species, the habenula is larger in one hemisphere, unlike most other cerebral structures (Concha & Wilson, 2001). It is for this reason that the habenula became a main neural target to study in relation to lateralized behaviour. Fish studies have been the main source of information linking habenular asymmetries with behavioural asymmetries (e.g., Barth et al., 2005; Dadda et al., 2010). Most fish species have laterally placed eyes with little binocular overlap. Due to total decussation at the optic chiasma, information from each eye is processed predominantly by the contralateral hemisphere with little interhemispheric cross-talk. This fact makes fish a useful animal model to study the relationship between behavioural and cerebral asymmetry.

1.7 Habenular and Behavioural Asymmetry

In zebrafish, the parapineal organ forms on the left side of the brain in 95% of wild-type fish (Concha et al., 2000; Gamse et al., 2003) and is in close proximity to the left habenular nucleus, which is typically larger than the right habenula in these fish (Concha et al., 2003). When the parapineal organ is destroyed, the left and right habenulae of the zebrafish develop more similarly (Conch et al., 2003; Gamse et al., 2003). Barth and colleagues (2005) showed that in a *frequent-situs-inversus* (*fsi*) line of zebrafish, a mutant line in which up to 25% of fish show a reversal in parapineal position (i.e., the parapineal organ is close to the right habenula), a subset of lateralized responses, mirror-image viewing and approaching a target to bite, were reversed from those of wild-type fish. Dadda and colleagues (2010) identified *fsi* fish with either left or right parapineal position and found that they differed in behaviour in four different laterality tasks. Facchin and colleagues (2009) selected lines of zebrafish that had a strong tendency to view mirror images with either the right or left eye and maintained these lines for five generations. They found that the intensity of *lov* staining in each hemisphere of the habenula was reversed in a substantial number of fish selected for right eye mirror image viewing compared to a control line not selected for eye preference. The frequency of reverse hemispheric *lov* staining was reduced in fish selected for left eye mirror image viewing. These results together suggest that asymmetry of the habenula may relate to asymmetry in behaviour at the group level; however, these studies did not relate individual asymmetry in the habenula to individual lateralized behaviour.

Only two studies to date have examined the relationship between quantitative asymmetry in the habenula and individual behavioural lateralization (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011). Reddon and colleagues (2009) showed that, in a sibling cohort of South American cichlid fish, *G. brasiliensis*, fish with stronger habenular asymmetry also showed stronger behavioural asymmetry in a detour task. Gutiérrez-Ibáñez and colleagues (2011) showed that, in a non-sibling cohort of Central American cichlids, the convict cichlid (*Amatitlania nigrofasciata*), males with larger left habenulae tended to turn left in a detour task, view the empty environment with their right eye, and process the information with their left hemisphere. These studies suggest that quantitative asymmetry in the habenula is linked to lateralized behaviour in individual cichlid fish.

1.8 Behavioural Syndromes in Fishes

Similar to the movement to study patterns of lateralization within individuals as well as groups, there has also been a shift in the last two decades to study the patterns of behaviour within individuals as well as groups. The existence of behavioural syndromes, consistent behaviour within an individual across contexts (e.g., mating and foraging), or across situations within the same context (e.g., foraging while in the presence or absence of predators), has been shown in all vertebrate species studied to date (see reviews by Dall et al., 2004; Sih et al., 2004a,b; Sih & Bell, 2008; Sih et al., 2012). Behavioural syndromes have also been referred to as animal personality, temperament, coping styles, strategies, axes, and constructs (see Sih et al., 2004a and Réale et al., 2007 for reviews), though coping styles relate particularly to how an individual deals with a stressful situation (e.g., Koolhaas et al. 2010). Several model species of fish have been used to study behavioural syndromes (e.g., sticklebacks (*Gasterosteus aculeatus*), guppies (*Poecilia reticulata*), zebrafish, rainbow trout (*Oncorhynchus mykiss*), and cichlids; see Toms et al., 2010).

One of the most well-studied behavioural axes in fish is the boldness-shyness continuum (Toms et al., 2010). Boldness is typically described as the propensity to take risks (Wilson et al., 1994) and is generally studied by exposing fish to something novel (e.g., food, an object, or an environment) and measuring latency to approach the novel stimulus (Toms et al., 2010; Conrad et al., 2011). While boldness has been shown to be consistent within an individual (Irving & Brown, 2013), it can also be influenced by size (Brown & Braithwaite, 2004), predation pressure (Brown et al., 2005), and experience (Brown et al., 2007).

Boldness has also been linked to stress responsiveness in fish. *Brachyraphis episcopi* from high predation areas, which are bolder than their low-predation counterparts, have lower release rates of cortisol in response to a mild stressor (Archard et al., 2012). Through artificial selection studies it has been possible to breed individuals that differ in stress responsiveness, which tends to also lead to differences in behaviour (e.g., rainbow trout; Øverli et al., 2005, 2007). Generally, 'proactive' individuals, those that show low responsiveness to stressors (e.g., low hypothalamic-pituitary-interrenal (HPI)-axis activity, low release of cortisol), are bolder, remain active in risky situations, and show low behavioural flexibility. In contrast, 'reactive' individuals, characterized by high stress responsiveness, are less bold, respond to risk with immobility, and are flexible in their behaviour (Koolhaas et al., 1999; Øverli et al., 2007).

1.9 The Habenula and Behavioural Syndromes

As previously mentioned, the habenula is involved in functions concerning emotional behaviours, such as responses to aversive stimuli, anxiety, and stress (reviewed in Sutherland, 1982; Klemm, 2004; and Hikosaka, 2010). Rogers (2010) hypothesized that hemispheric dominance may influence cognitive bias (see section 1.5 above), which may ultimately affect an individual's behaviour when faced with certain stimuli. Following this logic, it is reasonable to hypothesize that asymmetry in the habenula may relate to personality traits. Recent studies on zebrafish have shown a link between parapineal position, affecting habenular asymmetry in this species, and behaviour. Zebrafish with the parapineal organ on the right side, the minority position for this species, took longer to begin swimming and covered a shorter distance in a mirror image viewing task (Facchin et al., 2009), were bolder when faced with a novel object (Barth et al., 2005), swam closer to a predator, and travelled a shorter distance in the dark (Dadda et al., 2010) than fish with the parapineal organ on the left side. Aside from these zebrafish studies, there is little work investigating the relationship between asymmetry in the habenula and behaviour. Studies quantifying asymmetry in the habenula (beyond a gross measure of parapineal position) and linking that asymmetry to individual differences in behaviour are totally lacking.

1.10 Research Objectives

In this thesis, I sought to examine the influence of size, stimulus type, sex, and stressors on lateralized behaviour in the convict cichlid (*A. nigrofasciata*). I also wanted to examine how quantitative asymmetry in the habenula was related to lateralized behaviour under stressful and non-stressful conditions. Finally, I explored the effect of stressors on boldness and how that behaviour was related to asymmetry in the habenula.

The convict cichlid is a small, freshwater cichlid native to Central America. These fish are a model species for studying aggressive behaviour (e.g., Reddon & Hurd, 2008; Arnott & Elwood, 2009a,b; Copeland et al., 2011) and recently have been used to investigate cerebral lateralization and behaviour (Reddon & Hurd, 2008, 2009a,b; Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011; Moscicki et al., 2011).

This dissertation will examine the relationship between growth rate and behavioural lateralization in a sibling cohort of juvenile convict cichlid fish (chapter 2); the relationship between lateralized behaviour and habenular asymmetry in individuals when viewing different stimuli (chapter 3); the effect of a stressor on the relationships established in chapter 3 (chapter 4); and the effects of prior exposure to a stressor and habenular asymmetry on boldness when in different situations (i.e., stressful or non-stressful; chapter 5). These studies will expand the currently limited knowledge regarding the relationship between quantitative asymmetry in the habenula and behaviour. As well, the roll that stressors play in altering both behavioural lateralization and boldness will be clarified.

1.11 References

Andrew, R.J. (1991). The nature of behavioural lateralization in the chick. in: Andrew, R.J. (Ed.), *Neural and Behavioural Plasticity. The Use of the Chick as a Model*. Oxford University Press, Oxford, pp. 536–554.

Archard, G.A., Earley, R.L., Hanninen, A.F., & Braithwaite, V.A. (2012). Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. *Functional Ecology, 26,* 637-645.

Arnott, G., & Elwood, R. (2009a). Gender differences in aggressive behaviour in convict cichlids. *Animal Behaviour, 78,* 1221-1227.

Arnott, G., & Elwood, R. (2009b). Probing aggressive motivation in a cichlid fish. *Biology Letters, 5,* 762-764.

Barth, K.A., Miklósi, A., Watkins, J., Bianco, I.H., Wilson, S.W., & Andrew, R.J. (2005). *fsi* zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. *Current Biology, 15,* 844–850.

Bateson, M., & Matheson, S.M. (2007). Performance on a categorisation task suggests that removal of environmental enrichment induces ‘pessimism’ in captive European starlings (*Sturnus vulgaris*). *Animal Welfare, 16,* 33–36.

Bisazza, A., Cantalupo, C., Capocchiano, M., & Vallortigara, G. (2000). Population lateralisation and social behavior: A study with 16 species of fish. *Laterality, 5,* 269-284.

Bisazza, A., & Dadda, M. (2005). Enhanced schooling performance in lateralized fishes. *Proceedings of the Royal Society B – Biological Sciences, 272,* 1677-1681.

Bisazza, A., Pignatti, R., & Vallortigara, G. (1997). Detour tests reveal task- and stimulus-specific behavioural lateralization in mosquitofish (*Gambusia holbrooki*). *Behavioural Brain Research, 89,* 237-242.

Bisazza, A., Rogers, L.J., & Vallortigara, G. (1998). The origins of cerebral asymmetry: A review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neuroscience & Biobehavioral Reviews, 22,* 411-426.

Bisazza, A., Sovrano, V.A., & Vallortigara, G. (2001). Consistency among different tasks of left-right asymmetries in lines of fish originally selected for opposite direction of lateralization in a detour task. *Neuropsychologia, 39,* 1077-1085.

Brown, C., & Braithwaite, V.A. (2004). Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour, 68,* 1325–1329.

Brown, C., Jones, F., & Braithwaite, V.A. (2005). In situ examination of boldness-shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Animal Behaviour, 70,* 1003–1009.

Brown, C., Burgess, F., & Braithwaite, V.A. (2007a). Heritable and experiential effects on boldness in a tropical poeciliid. *Behavioural Ecology and Sociobiology, 62,* 237–243.

Brown, C., Western, J., & Braithwaite, V.A. (2007b). The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour, 74,* 231-238.

Budaev, S.V., & Andrew, R.J. (2009). Shyness and behavioural asymmetries in larval zebrafish (*Brachydanio rerio*) developed in light and dark. *Behaviour, 146,* 1037-1052.

Concha, M.L., Burdine, R.D., Russell, C., Schier, A.F., & Wilson, S.W. (2000). A nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain. *Neuron, 28,* 399–409.

Concha, M.L., & Wilson, S.W. (2001). Asymmetry in the epithalamus of vertebrates. *Journal of Anatomy, 199,* 63–84.

Concha, M.L. *et al.*, (2003). Local tissue interactions across the dorsal midline of the forebrain establish CNS laterality. *Neuron, 39,* 423–438.

Conrad, J.L., Weinersmith, K.L., Brodin, T., Saltz, J.B., & Sih, A. (2011). Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. *Journal of Fish Biology, 78,* 395-435.

Copeland, D.L., Levay, B., Sivaraman, B., Beebe-Fugloni, C., & Earley, R.L. (2011). Metabolic costs of fighting are driven by contest performance in male convict cichlid fish. *Animal Behaviour, 82,* 271-280.

Dadda, M., Domenichini, A., Piffer, L., Argenton, F., & Bisazza, A. (2010). Early differences in epithalamic left-right asymmetry influence lateralization and personality of adult zebrafish. *Behavioural Brain Research, 206,* 208-215.

Dadda, M., Zandona, E., & Bisazza, A. (2007). Emotional responsiveness in fish from lines artificially selected for a high or low degree of laterality. *Physiology & Behavior, 92,* 764-772.

Dall, S.R.X., Houston, A.I., & McNamara, J.M. (2004). The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters, 7,* 734-739.

Davidson, R.J. (1995). Cerebral asymmetry, emotion, and affective style. in: Davidson, R.J., & Hugdahl, K. (Eds.), *Brain Asymmetry*. MIT Press, Cambridge, MA, pp. 361–387.

Dharmaretnam, M., & Rogers, L.J. (2005). Hemispheric specialization and dual processing in strongly versus weakly lateralized chicks. *Behavioural Brain Research, 162,* 62–70.

Facchin, L., Bisazza, A., & Vallortigara, G. (1999). What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behavioural Brain Research, 103,* 229-234.

Facchin, L., Burgess, H.A., Siddiqi, M., Granato, M., & Halpern, M.E. (2009). Determining the function of zebrafish epithalamic asymmetry. *Philosophical Transactions of the Royal Society of London. Series B - Biological Sciences, 364,* 1021-1032.

Frasnelli, E., Vallortigara, G., & Rogers, L.J. (2012). Left-right asymmetries of behaviour and nervous system in invertebrates. *Neuroscience and Biobehavioural Reviews, 36,* 1273-1291.

Gamse, J.T., Thisse, C., Thisse, B., & Halpern, M.E. (2003). The parapineal mediates left–right asymmetry in the zebrafish diencephalon. *Development, 130,* 1059–1068.

Geschwind, N. (1971). Current Concepts - Aphasia. *The New England Journal of Medicine, 284,* 654-656.

Gutiérrez-Ibáñez, C., Reddon, A.R., Kreuzer, M.B., Wylie, D.R., & Hurd, P.L. (2011). Variation in the asymmetry of the habenular nucleus correlates with behavioural asymmetry in a cichlid fish. *Behavioural Brain Research, 221,* 189-196.

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nature Reviews Neuroscience, 11,* 503-513.

Hori, M. (1993). Frequency-dependent natural selection in handedness of scale-eating cichlid fish. *Science, 260,* 216–219.

Irving, E., & Brown, C. (2013). Examining the link between personality and laterality in a feral guppy *Poecilia reticulata* population. *Journal of Fish Biology, 83,* 311-325.

Klemm, W.R. (2004). Habenular and interpeduncularis nuclei: shared components in multiple-function networks. *Medical Science Monitor, 10,* 261-273.

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinologi, 31,* 307-321.

Koolhaas, J.M., Korte, S.M., de Boer, S.F., van der Vegt, B.J., van Reenen, C.G., Hopster, H., de Jong, I.C., Ruis, M.A.W., & Blokhuis, H.J. (1999). Coping styles in animals: current status in behaviour and stress-physiology. *Neuroscience and Biobehavioral Reviews, 23,* 925-935.

MacNeilage, P.F., Rogers, L.J., & Vallortigara, G. (2009). Origins of the left and right brain. *Scientific American, July,* 60–67.

Moscicki, M.K., Reddon, A.R., & Hurd, P.L. (2011). Lateralized behaviour of a non-social cichlid fish (*Amatitlania nigrofasciata*) in a social and a non-social environment. *Behavioural Processes, 88,* 27-32.

Øverli, Ø., Sorensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W.J., Summers, C.H., & Nilsson, G.E. (2007). Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neuroscience and Biobehavioral Reviews, 31,* 396-412.

Øverli, Ø., Winberg, S., & Pottinger, T.G. (2005). Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout - A review. *Integrative and Comparative Biology, 45,* 463-474.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., & Dingemanse, N.J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82,* 291-318.

Reddon, A.R., Gutiérrez-Ibáñez, C., Wylie, D.R., & Hurd, P.L. (2009). The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. *Behavioural Brain Research, 201,* 223-228.

Reddon, A.R., & Hurd, P.L. (2008). Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology Letters, 4,* 338-340.

Reddon, A.R., & Hurd, P.L. (2009a). Individual differences in cerebral lateralization are associated with shy-bold variation in the convict cichlid. *Animal Behaviour, 77,* 189-193.

Reddon, A.R., & Hurd, P.L. (2009b). Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. *Behavioural Processes, 82,* 25-29.

Rogers, L.J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Applied Animal Behaviour Science, 127,* 1-11.

Rogers, L.J., & Andrew, R.J. (2002). *Comparative Vertebrate Lateralization*. University Press, Cambridge.

Roussigne, M., Blader, P., & Wilson, S.W. (2012). Breaking symmetry: The zebrafish as a model for understanding left-right asymmetry in the developing brain. *Developmental Neurobiology, 72,* 269-281.

Sherry, D.F., & Schachter, D.L. (1987). The evolution of multiple memory systems. *Psychological Review, 94,* 439–454.

Sih, A., & Bell, A.M. (2008). Insights for behavioural ecology from behavioural syndromes. *Advances in the Study of Behavior, 38,* 227-281.

Sih, A., Bell, A.M., & Johnson, J.C. (2004a). Behavioural syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution, 19,* 372-378.

Sih, A., Bell, A.M., Johnson, J.C., & Ziemba, R.E. (2004b). Behavioral syndromes: An integrative overview. *Quarterly Review of Biology, 79,* 241-277.

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecology Letters, 15,* 278-289.

Sutherland, R.J. (1982). The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. *Neuroscience and Biobehavioral Reviews, 6,* 1-13.

Toms, C.N., Echevarria, D.J., & Jouandot, D.J. (2010). A methodological review of personality-related studies in fish: Focus on the shy-bold axis of behavior. *International Journal of Comparative Psychology, 23,* 1-25.

Vallortigara, G. (2006). The evolutionary psychology of left and right: Costs and benefits of lateralization. *Developmental Psychobiology, 48,* 418-427.

Vallortigara, G., & Bisazza, A. (2002). How ancient is brain lateralization? in: Rogers, L.J., & Andrew, R.J., (Eds.), *Comparative Vertebrate Lateralization*. University Press, Cambridge, UK, pp. 9–69.

Vallortigara, G., & Rogers, L.J. (2005). Survival with an asymmetrical brain: Advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences, 28,* 575-633.

Vallortigara, G., Rogers, L.J., & Bisazza, A. (1999). Possible evolutionary origins of cognitive brain lateralization. *Brain Research Reviews, 30,* 164-175.

Walker, S.F. (1980). Lateralization of functions in the vertebrate brain. *British Journal of Psychology, 71,* 329–367.

Wilson, D. S., Clark, A. B., Coleman, K., & Dearstyne, T. (1994). Shyness and boldness in humans and other animals. *Trends in Ecology and Evolution, 9,* 442–446.

Zaidel, D.W. (1987). Hemispheric asymmetry in memory for pictorial semantics in normal subjects. *Neuropsychologia, 25,* 487– 495.

2. GROWTH RATE IS RELATED TO EYE PREFERENCE IN A PERCEIVED SOCIAL TASK IN JUVENILE CONVICT CICHLIDS (*AMATITLANIA NIGROFASCIATA*)

2.1 Introduction

Empirical studies of cerebral lateralization in non-human animals have increased dramatically within the last two decades (see Bisazza et al., 1998a; Vallortigara, 2006; Vallortigara and Rogers, 2005; Rogers & Andrew, 2002 for reviews). Cerebral lateralization is thought to be a ubiquitous trait among vertebrates. Two selective advantages proposed to explain the existence of this trait are: increased processing speed and enhanced ability to simultaneously attend to two conflicting stimuli (e.g., foraging while remaining vigilant for predators; Rogers, 2002; Vallortigara & Rogers, 2005; Vallortigara, 2006). This latter advantage may be particularly important for organisms with laterally placed eyes and little interhemispheric connectivity, such as birds and fishes (Bisazza et al., 1998). Empirical studies provide evidence for these proposed advantages of cerebral lateralization (e.g., Sovrano et al., 2005; Dadda & Bisazza, 2006a,b).

Despite these advantages, and the taxonomic ubiquity of cerebral lateralization, the degree to which individuals display lateralized traits is variable within a species (Vallortigara & Bisazza, 2002). If lateralization is advantageous, it is puzzling why all individuals would not possess this trait either maximally or at least to the same degree. This variation may be explained by counterbalancing disadvantages of having a lateralized brain. Animals with strong visual or perceptual asymmetries may be ill-equipped to respond to predators or prey on their non-preferred side (Vallortigara & Rogers, 2005). For example, toads react more quickly to predators appearing on their left side (Lippolis et al., 2002) and to prey appearing on their right side (Vallortigara et al., 1998); however, predators and prey are equally likely to appear on either side. It is likely that frequency-dependent selection acts on the trade-offs between a symmetrical and asymmetrical brain to maintain variation in lateralization within a population (Vallortigara & Rogers, 2005).

Other factors that have been empirically linked to lateralization may also act to maintain variation in this trait within a population. For example, personality traits such as boldness have been linked to cerebral lateralization (Reddon & Hurd, 2009a; Irving & Brown, 2013; Brown & Bibost, 2014). Strongly lateralized individuals may be bolder because, theoretically, each hemisphere would be able to make quicker unilateral decisions (Reddon & Hurd, 2009b; Vallortigara & Rogers, 2005). In addition, aggressive male convict cichlids (*Amatitlania nigrofasciata*) are more strongly lateralized than non-aggressive males, indicating another personality variable linked to cerebral lateralization (Reddon & Hurd, 2008). Finally, growth rate has been linked to lateralized behaviour in cichlid fish (Reddon et al., 2009). Reddon and colleagues (2009) showed that, in a sibling cohort of the South American cichlid fish *Geophagus brasiliensis,* larger males were more strongly lateralized in the detour task than smaller males of the same age. It is possible that boldness, aggression, fast growth, and lateralization may be components of a more risky life history strategy (Reddon et al., 2009).

Growth rate, the rate of increase in body size per unit time, can vary substantially within a cohort of fish and may be linked to fitness (Hunt & Hodgson, 2010). Rapid growth during the juvenile stage is generally critically important for fish to quickly move out of the size class most vulnerable to predators (Houde, 1987). All fish in the study by

Reddon and colleagues (2009) were adults of a single cohort reared together since birth. Final adult size may obscure differences in growth rate that are apparent when fish are developing in the juvenile stage (i.e., some fish may grow to a certain size faster than others but ultimately their siblings may grow to a comparable size). It is currently unclear how growth rate during the juvenile stage, before full adult size is reached, may affect lateralized behaviour.

Here I examined the relationship between growth rate and behavioural lateralization in a sibling cohort of juvenile convict cichlids using a perceived social stimulus task. I assessed behavioural lateralization by examining eye preference for mirror image viewing (a standard method of testing lateralization for social stimuli in fish; Bisazza et al., 1997; Sovrano et al., 2001; Reddon & Balshine, 2010; Moscicki et al., 2011). Convict cichlids, while solitary and aggressive fish as adults, school for a brief period as juveniles (Wisenden, 1994); thus, I hypothesized that social stimuli would be particularly salient to juvenile fish of this species. Based on previous research using adult cichlids (Reddon et al., 2009), I hypothesized that faster growing (i.e., larger) fish would show stronger behavioural lateralization. In addition, I hypothesized that faster growing fish would prefer to view mirror images with the left eye because these fish may be closer to their adult size compared to slower growing fish and there is evidence that adult fish tend to view mirror images preferentially with the left eye (Sovrano et al., 1999, 2001; De Santi et al., 2001; Sovrano, 2004; Sovrano & Andrew, 2006; Andrew et al., 2009; Moscicki et al., 2011).

2.2 Materials and Methods

2.2.1 Subjects and Housing

Subjects consisted of two cohorts of juvenile convict cichlid fish (sex undetermined); cohort 1 contained 17 fish and cohort 2 contained 46 fish (see details of fish size in each cohort in section 2.3.1 below). Each cohort originated from different parents, was raised in the lab, and consisted of all sibling fish that were six months of age at the time of testing. All fish from a cohort were housed in the same 95 L communal aquarium (76 cm X 31 cm X 47 cm). Fish were fed a mixture of dried prepared fish foods *ad libitum* once a day. Water temperature was maintained at 26 ± 1 °C and the light cycle was maintained at 12L:12D. All fish were naïve to the testing apparatus. All protocols were approved by the University of Alberta Biological Sciences Animal Policy and Welfare Committee (protocol number 5441006) and adhered to the guidelines of the Canadian Council for Animal Care.

2.2.2 Apparatus

Behavioural lateralization was assessed in fish using an octagonal mirror apparatus. This apparatus has been described previously for adult fish (Sovrano et al., 2001; Reddon & Balshine, 2010; Moscicki et al., 2011); I used a smaller version to test juvenile fish. My apparatus consisted of a square outer tank (33 cm X 33 cm) constructed from white plexiglass. Eight identical mirror panels (each 20 cm X 13 cm) were arranged in an octagon shape inside the square tank. An octagon-shaped start box, constructed from eight identical white plexiglass panels (each 20 cm X 6 cm), was placed inside the mirrored octagon equidistant from all mirrored panels. One panel of the start box contained a sliding door (20 cm X 6 cm) to allow the fish to leave the box. The sliding door was attached to a string and could be opened remotely by an experimenter hidden behind a white curtain. The entire apparatus was filled with water to a depth of 6 cm and was lit from above by four 23 w compact fluorescent lamps. A webcam (Logitech LT-QCNBDX, Logitech, Apples, Switzerland) was mounted above the apparatus and was connected to a laptop running the media program Cheese (2.30.1, Siegel, 2007).

2.2.3 Procedure

A trial consisted of gently placing a fish into the start box for a two minute acclimation period. After this period, the sliding door was raised and the fish was free to leave the start box. The experimenter could observe the fish on the laptop screen from behind the white curtain. Once the fish left the start box, the trial began. Trials lasted 10 minutes – during this time an image was captured every 2 seconds for a total of 300 images. Each fish was tested only once. After completing the trial, fish were removed from the apparatus and standard length (the distance from the anterior tip of the body to the posterior end of the vertebral column; cm) and mass (g) were measured.

Because fish have laterally placed eyes, and stimuli from each eye project mainly to the contralateral hemisphere, eye use has been widely used as a measure of cerebral lateralization in fish (e.g., Bisazza et al., 1997; Facchin et al., 1999; Sovrano et al., 2001). Each of the 300 images for a trial was scored to determine which eye of the fish was facing the closest mirror. Eye use was determined based on the angle of the fish's body with respect to the closest mirror (see Sovrano et al., 1999). If the fish was facing the mirror at a perpendicular angle, or the fish's body was greater than 90° from the closest mirror, no eye use preference was recorded and that image was removed from analysis. A laterality index (LI; Bisazza et al., 1997) was calculated using the following formula to determine the degree of lateralized eye preference for mirror image viewing for each fish:

Laterality Index (LI) = (Right eye use – Left eye use) / (Right eye use + Left eye use)

An absolute laterality index (ALI) was calculated to assess the strength of lateralization in my sample regardless of direction. Previous research on cichlid species has shown that the strength, and not necessarily the direction, of behavioural lateralization correlates with other behaviours (Reddon et al., 2009; Reddon & Hurd, 2008, 2009b).

2.2.4 Data Analysis

All statistical analyses were carried out using RKWard 0.5.2 (R Core Development Team, Vienna, Austria). For both cohorts 1 and 2, the distribution of fish standard lengths did not differ significantly from normality (Shapiro-Wilk: Cohort 1: $W = 0.934$, $p = 0.257$; Cohort 2: $W = 0.959$, $p = 0.604$), but their masses did (Shapiro-Wilk: Cohort 1: $W = 0.850$, $p = 0.011$; Cohort 2: $W = 0.926$, $p = 0.007$). All non-normally distributed data were log transformed to conform to normality (post-transformation Shapiro-Wilk: Cohort 1: $W = 0.964$, $p = 0.708$; Cohort 2: $W = 0.969$, $p = 0.269$). Population-level and individual-level lateralization for eye use preference were assessed using one-sample two-tailed t-tests. Relationships between size measures (Length and LogMass) and behavioural lateralization (LI and ALI) were assessed with Pearson correlations. Analyses were performed on each cohort separately. Size comparisons between cohorts were assessed using two-sample two-tailed t-tests. A significance criterion of $p < 0.05$ was used for all tests.

2.3 Results

2.3.1 Fry Size

The fish in cohort 1 ranged in mass from 0.14g to 1.84g (Mass Mean ± SEM: 0.69g ± 0.12g) while the fish in cohort 2 ranged from 0.02g to 0.26g (Mass Mean ± SEM: 0.11g ± 0.01g). Cohort 1 ranged in length from 1.6cm to 2.8cm (Length Mean ± SEM: 2.0 cm ± 0.8 cm). Fish in cohort 2 ranged in length from 1.0cm to 2.0cm (Length Mean ± SEM: 1.4cm ± 0.04cm). Fish in cohort 1 were both longer (two-sample *t*-test: $t(61) = 7.00$, $p < 0.001$) and heavier (two-sample *t*-test: $t(61) = 5.08$, $p < 0.001$) than those in cohort 2, likely due to the increased density of fish in the home tank of cohort 2.

2.3.2 Behavioural Lateralization

Neither cohort of fry in my study showed a significant population-level preference to view mirror images with a particular eye (Cohort 1: LI Mean ± SEM: -0.02 ± 0.04; one-sample *t*-test: $t(16) = 0.629$, $p = 0.538$; Cohort 2: LI Mean ± SEM: 0.09 ± 0.07; one-sample *t*-test: $t(45) = 1.306$, $p = 0.198$). Both cohorts in my sample did, however, show individual preferences to view mirror images with a particular eye (Cohort 1: ALI Mean ± SEM: 0.11 ± 0.02; one-sample *t*-test: $t(16) = 4.81$, $p < 0.001$; Cohort 2: ALI Mean ±SEM: 0.41 ± 0.04; one-sample *t*-test: $t(45) = 10.97$, $p < 0.001$).

2.3.3 Behavioural Lateralization and Fry Size

Fry mass was significantly related to the direction of behavioural lateralization (LI) such that heavier fry viewed their mirror image more often with their left eye while lighter fry showed the opposite relationship. This relationship was consistent for both cohorts (Pearson correlation: Cohort 1: $r(15) = -0.566$, $p = 0.018$; Fig. 2-1A; Cohort 2:

r(44) = -0.344, *p* = 0.021; Fig. 2-1B). Fry length was also significantly negatively related to behavioural LI in both cohorts (Pearson correlation: Cohort 1: *r*(15) = -0.675, *p* = 0.003; Fig. 2-1C; Cohort 2: *r*(44) = -0.332, *p* = 0.026; Fig. 2-1D). The strength of behavioural lateralization (ALI) was not significantly related to either mass or length in either cohort (Pearson correlation: Cohort 1: *r*(15) = 0.343, 0.118, *p* = 0.178, 0.652, respectively; Cohort 2: *r*(44) = 0.114, 0.146, *p* = 0.451, 0.338, respectively; Fig. 2-2A-D).

2.4 Discussion

Here I have shown that larger/faster growing juvenile convict cichlids viewed their mirror image more with their left eye than their smaller/slower growing siblings. I found no relationship between either mass or length of fish and the strength of behavioural lateralization. I also found no population-level preference for direction of eye bias in our samples of fish.

A lack of population-level eye preference has been reported for convict cichlids in a standard detour task when turning to view an open, yet empty, environment (Reddon & Hurd, 2008, 2009b but see Gutiérrez-Ibáñez et al., 2011 and Moscicki et al., 2011 for contrasting results). Moscicki and colleagues (2011) also reported a lack of population-level eye preference in adult male convict cichlids when viewing mirror images in a task identical to the one I used here. Motivation, or in the cases above, lack of motivation, to view the target stimulus may contribute to a lack of population-level eye bias. For example, Bisazza and colleagues (1998b) showed that when females of two species of poeciliid fish (*Gambusia holbrooki* and *Girardinus falcatus*) were deprived of males for 2 months they showed significantly stronger lateralization when detouring to view males than females that had not been male-deprived. Presumably, the lack of male contact for 2 months made the males a more salient stimulus for the deprived females. Like many fishes, convict cichlids, though solitary as adults, depend on schooling behaviour for protection from predators when juveniles. Therefore, social stimuli (like the mirror octagon task used here) may be more salient than solitary stimuli (like the detour task) when assessing behavioural lateralization in juvenile fish. There is currently no data available regarding how juvenile convict cichlids perform in a standard detour task.

While I saw no population-level eye bias in the juvenile fish, this result can be explained by the relationship I found between fish size and direction of eye bias. Individual fish did have significant side biases, and these biases were correlated with the size of the fish, with larger fish viewing their mirror image more with their left eye. Left eye bias for mirror image viewing has been reported in a variety of adult fish species using a variety of mirror viewing tasks (e.g., Sovrano et al., 1999, 2001; De Santi et al., 2001; Sovrano, 2004; Sovrano & Andrew, 2006; Andrew et al., 2009; Moscicki et al., 2011). In addition, viewing of conspecifics with the left eye, and thus processing this information with the right hemisphere, has been observed in several vertebrate taxa (reviewed in Bisazza & Brown, 2011). This evidence may indicate that left eye preference for this type of social stimulus develops as fish increase in size. However, Bisazza and colleagues (2002) tested five species of juvenile anurans and found that all species tested showed a left eye bias for mirror image viewing. It is currently unclear whether a left eye bias for mirror image viewing is established at birth or whether this preference develops over time in relation to growth rate.

Behavioural lateralization has been linked to growth rate in another species of cichlid fish, *G. brasiliensis* (Reddon et al., 2009). In this cichlid, Reddon and colleagues

(2009) found a positive relationship between the strength of behavioural lateralization and size in male fish, but not in females. This experiment used a standard detour apparatus in which the fish turns to view an empty environment. Reddon and colleagues (2009) suggest that the relationship between growth rate and strength of behavioural lateralization may be mediated by different life history strategies; larger fish devote more energy to immediate growth and reproduction and behave in a more risk-prone way. However, in their sample of *G. brasiliensis*, the larger fish tended to be male while the smaller fish were female; thus, gender may have played a role in determining strength of behavioural lateralization. These fish had yet to develop secondary sexual characteristics and remained unsexed.

No relationship was found between fish size and direction of behavioural lateralization in adult *G. brasiliensis* (Reddon et al., 2009). My data contradict this study by showing a significant relationship between growth rate and direction of lateralization in juvenile convict cichlids. A possible explanation for the different results between these two studies is that slower growing fish may eventually catch up to faster growing fish in absolute size but still retain their juvenile pattern of side bias. In this way, direction of behavioural lateralization may be more related to juvenile growth rate than to ultimate adult size. Studies examining the relationship between growth rate and lateralization over the lifespan within the same cohort are needed to address this possibility.

In addition to absolute growth rate, relative growth rate, or position within the size hierarchy of a cohort, may influence direction of eye bias for social stimuli. When convict cichlids engage in conflict, the larger fish is generally victorious (Koops & Grant, 1993). Hence, the larger fish in this cohort may be more likely to win in an aggressive encounter with a smaller fish, and may engage more frequently in aggressive behaviour. Since all the fish were housed in the same tank for 6 months, the smaller fish may have learned to inhibit attacks against the larger fish because they would be unlikely to win. It has been suggested that the left hemisphere is involved in inhibition of attack (Andrew & Rogers, 2002; Miklosi & Andrew, 1999; Bisazza & De Santi, 2003). Therefore, smaller fish may have come to rely more on processing of social stimuli with the left hemisphere and thus view social stimuli more with the right eye. It would be interesting to determine whether the pattern of lateralized viewing of social stimuli would change if the larger fish were removed from the cohort and smaller fish were added, thus propelling the initially smaller fish to the head of the size hierarchy. Looking at the plasticity of lateralized behaviour in this manner is an intriguing area for future research.

In conclusion, I have shown that, within the same cohort of juveniles, convict cichlids show a relationship between body size/growth rate and behavioural lateralization when viewing social stimuli. Larger/faster growing fish view their mirror image more with their left eye and smaller/slower growing fish view their mirror image more with their right eye. I suggest that growth rate may affect the development of lateralized stimulus processing and that where a fish falls in the size hierarchy, and not necessarily absolute growth rate, may be a substantial contributing factor to the development of cerebral lateralization.

2.5 Figures

Figure 2-1. Panels A and B show the significant negative relationship between fish mass and eye preference (LI) in a perceived social mirror image viewing task. Heavier fish viewed their mirror image more with their left eye while lighter fish preferentially used their right eye for this task. Panels C and D show this same significant negative relationship between fish length and eye preference (LI) in the mirror image viewing task. Longer fish used their left eye more for mirror image viewing while shorter fish preferentially used their right eye.

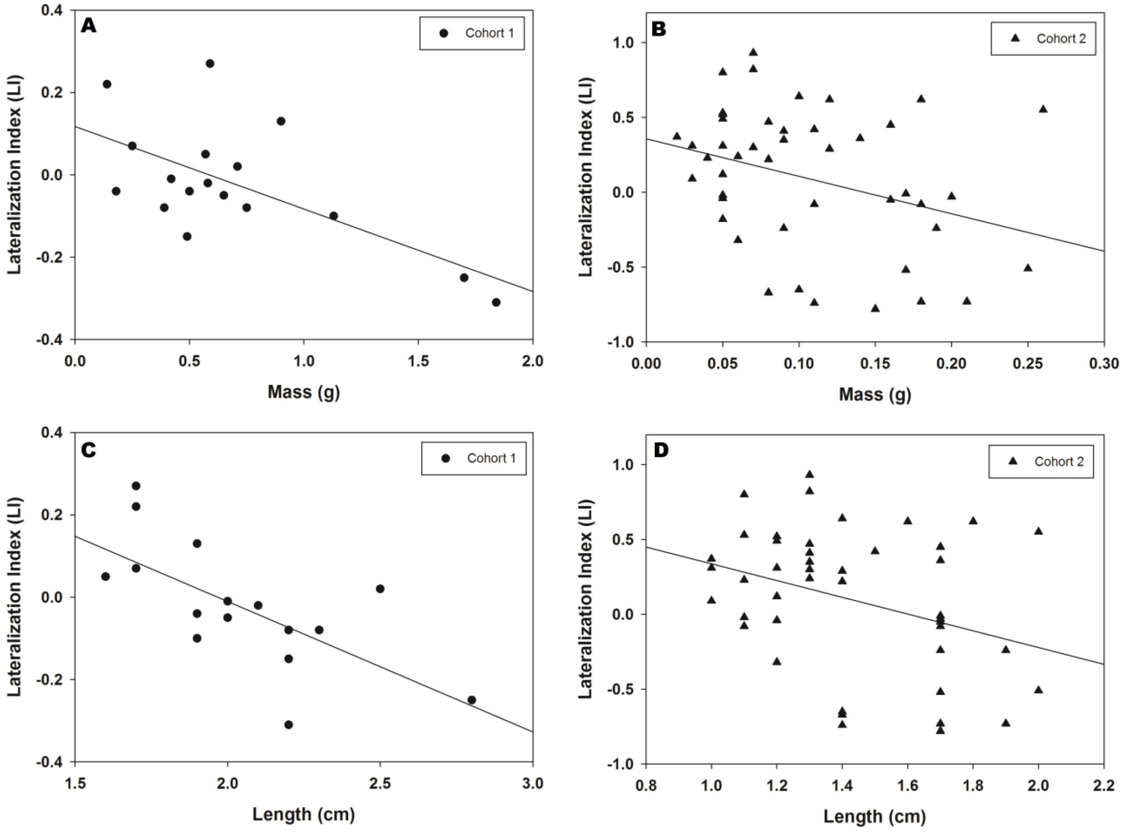
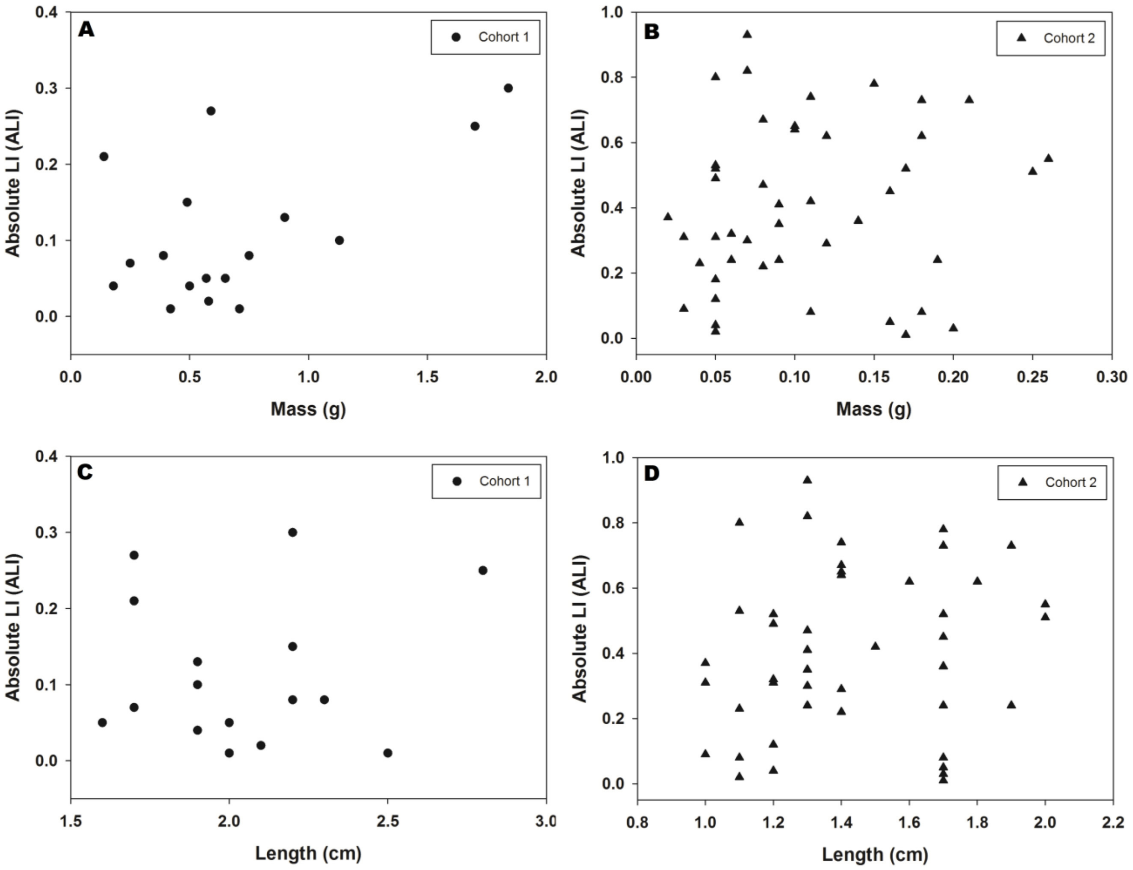


Figure 2-2. Panels A and B illustrate the relationship between fish mass and strength of eye preference (ALI) for mirror image viewing in two sibling cohorts of juvenile convict cichlid fish. Panels C and D show the relationship between fish length and strength of eye preference (ALI) for mirror image viewing.



2.6 References

Andrew, R.J., & Rogers, L.J. (2002). The nature of lateralization in tetrapods, in: Rogers, L.J., Andrew, R.J. (Eds.), *Comparative Vertebrate Lateralization*. University Press, Cambridge, pp. 94-125.

Andrew, R.J., Dharmaretnam, M., Gyori, B., Miklosi, A., Watkins, J.A.S., & Sovrano, V.A. (2009). Precise endogenous control of involvement of right and left visual structures in assessment by zebrafish. *Behavioural Brain Research, 196,* 99–105.

Bisazza, A., De Santi, A., Bonso, S., & Sovrano, V.A. (2002). Frogs and toads in front of a mirror: Lateralisation of response to social stimuli in tadpoles of five anuran species. *Behavioural Brain Research, 134,* 417–424.

Bisazza, A., Facchin, L., Pignatti, R., & Vallortigara, G. (1998). Lateralization of detour behaviour in poeciliid fish: The effect of species, gender and sexual motivation. *Behavioural Brain Research, 91,* 157-164.

Bisazza, A., & De Santi, A. (2003). Lateralization of aggression in fish. *Behavioural Brain Research, 141,* 131–136.

Bisazza, A., Rogers, L.J., & Vallortigara, G. (1998). The origins of cerebral asymmetry: A review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neuroscience & Biobehavioral Reviews, 22,* 411-426.

Bisazza, A., Pignatti, R., & Vallortigara, G. (1997). Detour tests reveal task- and stimulus-specific behavioural lateralization in mosquitofish (*Gambusia holbrooki*). *Behavioural Brain Research, 89,* 237-242.

Blaxter, J.H.S. (1988). Pattern and variety in development. in: Hoar, W.S., & Randall, D.J. (Eds.), *Fish Physiology. Vol. 11,* Academic Press, San Diego, pp. 1–58.

Brown, C., & Bibost, A-L. (2014). Laterality is linked to personality in the black-lined rainbowfish, *Melanotaenia nigrans*. *Behavioural Ecology and Sociobiology, 68,* 999-1005.

Dadda, M., & Bisazza, A. (2006a). Does brain asymmetry allow efficient performance of simultaneous tasks? *Animal Behaviour, 72,* 523-529.

Dadda, M., & Bisazza, A. (2006b). Lateralized female topminnows can forage and attend to a harassing male simultaneously. *Behavioral Ecology, 17,* 358-363.

Dall, S.R.X., Houston, A.I., & McNamara, J.M. (2004). The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters, 7,* 734-739.

De Santi, A., Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Animal Behaviour, 61,* 305-310.

Facchin, L., Bisazza, A., & Vallortigara, G. (1999). What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behavioural Brain Research, 103,* 229-234.

Gutiérrez-Ibáñez, C., Reddon, A.R., Kreuzer, M.B., Wylie, D.R., & Hurd, P.L. (2011). Variation in the asymmetry of the habenular neucleus correlates with behavioural asymmetry in a cichlid fish. *Behavioural Brain Research, 221,* 189-196.

Houde, E.D. (1987). Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium, 2,* 17-29.

Hunt, J., & Hodgson, D. (2010). What is fitness, and how do we measure it?, in: Westneat, D.E., Fox, C.W. (Eds.), *Evolutionary Behavioral Ecology*. University Press, Oxford, pp. 46-70.

Irving, E., & Brown, C. (2013). Examining the link between personality and laterality in a feral guppy *Poecilia reticulata* population. *Journal of Fish Biology, 83,* 311-325.

Koops, M.A., & Grant, J.W.A. (1993). Weight asymmetry and sequential assessment in convict cichlid contests. *Canadian Journal of Zoology, 71,* 475-479.

Lippolis, G., Bisazza, A., Rogers, L.J., & Vallortigara, G. (2002). Lateralization of predator avoidance responses in three species of toads. *Laterality, 7,* 163–183.

Magnuson, J.J. (1962). An analysis of aggressive behavior, growth, and competition for food and space in medaka (*Oryzias latipes* (Pisces, Cyprinodontidae)). *Canadian Journal of Zoology, 40,* 313–363.

Miklósi, Á., & Andrew, R.J. (1999). Right eye use associated with decision to bite in zebrafish. *Behavioural Brain Research, 105,* 199–205.

Moscicki, M.K., Reddon, A.R., & Hurd P.L. (2011). Lateralized behaviour of a non-social cichlid fish (*Amatitlania nigrofasciata*) in a social and a non-social environment. *Behavioural Processes, 88,* 27-32.

R Core Development Team. (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reddon, A.R., & Balshine, S. (2010). Lateralization in response to social stimuli in a cooperatively breeding cichlid fish. *Behavioural Processes, 85,* 68-71.

Reddon, A.R., & Hurd, P.L. (2008). Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology Letters, 4,* 338-340.

Reddon, A.R., & Hurd, P.L. (2009a). Individual differences in cerebral lateralization are associated with shy-bold variation in the convict cichlid. *Animal Behaviour, 77,* 189-193.

Reddon, A.R., & Hurd, P.L. (2009b). Acting unilaterally: Why do animals with strongly lateralized brains behave differently than those with weakly lateralized brains? *Bioscience Hypotheses, 2,* 383-387.

Reddon, A.R., Gutiérrez-Ibáñez, C., Wylie, D.R., & Hurd, P.L. (2009). The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. *Behavioural Brain Research, 201,* 223-228.

Rogers, L.J., & Andrew, R.J. (2002). *Comparative Vertebrate Lateralization*. University Press, Cambridge.

Rogers, L.J. (2002). Advantages and disadvantages of lateralization. in: Rogers, L.J., Andrew, R.J. (Eds.), *Comparative Vertebrate Lateralization*. University Press, Cambridge, pp. 126–153.

Siegel, D.G. (2007). Cheese Webcam Booth v.2.30.1 [computer program]. www.projects.gnome.org/cheese. Accessed 21 March 2011.

Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Lateralization of response to social stimuli in fishes: A comparison between different methods and species. *Physiology & Behavior, 74,* 237-244.

Sovrano, V.A., Rainoldi, C., Bisazza, A., & Vallortigara, G. (1999). Roots of brain specializations: Preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behavioural Brain Research, 106,* 175-180.

Sovrano, V.A. (2004). Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behavioural Brain Research, 152,* 385–391.

Sovrano, V.A., & Andrew, R.J. (2006). Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae. *Behavioural Brain Research, 167,* 226–231.

Sovrano, V.A., Dadda, M., & Bisazza, A. (2005). Lateralized fish perform better than non-lateralized fish in spatial reorientation tasks. *Behavioural Brain Research, 163,* 122–127.

Vallortigara, G. (2006). The evolutionary psychology of left and right: Costs and benefits of lateralization. *Developmental Psychobiology, 48,* 418-427.

Vallortigara, G., & Bisazza, A. (2002). How ancient is brain lateralization? in: Rogers, L.J., & Andrew, R.J., (Eds.), *Comparative Vertebrate Lateralization*. University Press, Cambridge, UK, pp. 9–69.

Vallortigara, G., & Rogers, L.J. (2005). Survival with an asymmetrical brain: Advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences, 28,* 575-633.

Vallortigara, G., Rogers, L.J., Bisazza, A., Lippolis, G., & Robins, A. (1998). Complementary right and left hemifield use for predatory and agonistic behaviour in toads. *Neuroreport, 9,* 3341-3344.

Wisenden, B.D. (1994). Factors affecting mate desertion by males in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). *Behavioral Ecology, 5,* 439-447.

Wilson, D. (1998). Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society B – Biological Sciences, 353,* 199-205.

3. LATERALIZED BEHAVIOUR OF A NON-SOCIAL CICHLID FISH (*AMATITLANIA NIGROFASCIATA*) IN SOCIAL AND NON-SOCIAL ENVIRONMENTS

3.1 Introduction

Cerebral lateralization, the partitioning of cognitive functioning into one hemisphere of the brain or the other, has been demonstrated in a variety of vertebrate species, including humans, other primates, birds, reptiles, amphibians, and fish (reviewed in Bisazza et al., 1998; Rogers & Andrew, 2002; MacNeilage et al., 2009; Vallortigara et al., 2010; Rogers, 2010). Several benefits of lateralized brains have been identified, including increased manual dexterity (Fabre-Thorpe et al., 1993; McGrew & Marchant, 1999), increased ability to attend to multiple stimuli simultaneously (Rogers, 2000; Rogers et al., 2004; Dadda & Bisazza, 2006a, 2006b), enhanced spatial abilities (Bisazza & Dadda, 2005; Sovrano et al., 2005), avoiding the simultaneous activation of incompatible behavioural responses (Vallortigara et al., 1998; De Santi et al., 2001), and greater problem solving ability (Magat & Brown, 2009). Many species show behavioural lateralization at the population level with most members of the species behaving in the same side-biased way in standardized testing situations (see Bisazza et al., 1998; Vallortigara et al., 1999; Andrew, 2002; Rogers, 2002; Vallortigara & Rogers, 2005 for reviews).

The aforementioned benefits of lateralization would in theory accrue to an individual regardless of the direction of side bias within that individual (Vallortigara, 2000; Bisazza & Dadda, 2005; Dadda & Bisazza, 2006a, 200b; Corbalis, 2009) and alignment of side bias in individuals at the population level may in fact be costly. For example, if most members of a species preferentially identify predators using one eye, resulting in side biases in escape behaviour, predators may learn these biases and anticipate the escape route. Similarly, if most members of a predator species prefer to attack prey on a particular side, the prey species may use this predictable pattern of behaviour to their advantage (Vallortigara, 2006). The existence of population-level lateralization is puzzling and leads to the speculation that there must be some additional selective force acting upon the direction of lateralization at the individual level that results in population-level biases (Vallortigara & Rogers, 2005; MacNeilage et al., 2009; Vallortigara et al., 2010). Population-level lateralization may be beneficial for species that live in social groups because it may allow these animals to more effectively coordinate their behaviour (Rogers, 1989; Bisazza et al., 2002; Ghirlanda & Vallortigara, 2004; Vallortigara & Rogers, 2005; Ghirlanda et al., 2009; Anfora et al., 2010; Reddon & Balshine, 2010) and maintain cohesive groups during complex group actions (e.g., shoaling behaviour in fish; Bisazza et al., 2000; Sovrano et al., 2005). Individual-level lateralization in the absence of population-level lateralization may be more prevalent in species that do not live in complex social environments. Bisazza et al. (2000) studied 16 species of fish with varying levels of shoaling behaviour. They found that all 6 species that formed shoals showed population-level lateralization while only 4 of the 10 species that did not form shoals displayed this trait. Thus, social structure may be a factor contributing to the development and maintenance of population-level lateralized behaviour. It is important to note that ontogenetic shifts in social behaviour may have lasting effects on the organization of the lateralized brain. For example, if an animal is social as a juvenile, but solitary as an adult, the need to coordinate behaviour with conspecifics early in life may have persistent effects on lateralized behaviour in the non-social adults (Rogers, 2002; Andrew & Rogers, 2002).

While all the shoaling species in Bisazza and colleagues' (2000) study showed population-level lateralization, the direction of this bias varied among species. Many studies examining eye preference when detouring to view a stimulus have shown that the precise stimulus used (e.g., an empty environment, a predator, a group of conspecifics) affects the eye with which the focal organism prefers to view the stimulus (Bisazza et al., 1997a; Facchin et al., 1999; De Santi et al., 2002). Several authors have suggested that stimuli are processed differently depending on the hemisphere doing the processing and that the type of processing a stimulus receives determines what kind of behavioural response will be elicited by that stimulus (Vallortigara & Andrew, 1991; Rogers et al., 2004; Wiltschko et al., 2007). A species that shows population-level lateralized responding to one stimulus does not necessarily show this response to other stimuli (e.g., Bisazza et al., 1997b, 1998). Following this logic, fish species that do not shoal may show behavioural lateralization at the population level to some stimuli but not others.

Recently, a few studies have investigated the relationship between a highly asymmetrical brain structure, the habenula, and behavioural lateralization in cichlid fish. The habenula is a highly conserved brain region present in all vertebrates; it is part of the limbic system and connects the forebrain to the ventral midbrain (Sutherland, 1982). Hypothesized functions of the habenula include inhibition, reward, learning and memory, feeding, and mating behaviours (Klemm, 2004). Reddon and colleagues (2009) investigated the relationship between asymmetry in the habenula and behaviour in a non-social detour task in the South American cichlid, *Geophagus brasiliensis*. They found that fish in a sibling cohort showed a relationship between fish size and habenular asymmetry; larger fish had a larger right habenula while smaller fish had a larger left habenula. They also found a relationship between habenular asymmetry and behavioural asymmetry such that fish with more asymmetrical habenulae, regardless of the direction of that asymmetry, showed stronger behavioural asymmetry in the detour task. In a similar study conducted with the Central American convict cichlid (*Amatitlania nigrofasciata*), Gutiérrez-Ibáñez and colleagues (2011) found that both males and females had larger left habenulae. They also found a positive relationship between habenular asymmetry and behavioural asymmetry in males; males with larger left habenulae tended to turn left in the detour task and males with more symmetrical or larger right habenulae tended to turn right. They did not find this same relationship for females. As mentioned, stimulus type likely affects hemispheric preference for processing. These previous studies have looked at only one type of lateralized responding (i.e., detouring to view an empty environment behind a barrier). As the habenula also functions in motivation (see Hikosaka 2010 for a review), and motivation to view certain stimuli will likely differ, particularly for biologically relevant stimuli (e.g., conspecifics or predators), it will be informative to determine if habenular asymmetry relates to behavioural asymmetry when viewing different types of stimuli, particularly those that may be more biologically motivating than an empty environment.

Here I investigated whether a fish that shoals during parental dependence, but is territorial, aggressive and non-shoaling during the adult stage (Wisenden, 1994) shows population-level lateralization to social stimuli. This idea is consistent with the hypothesis that social behaviour is an important selective pressure driving the evolution of population-level lateralization (Vallortigara & Rogers, 2005). I also investigated whether the direction and strength of lateralization within an individual was consistent across two different lateralization tasks. Consistency would be expected if social behaviour during ontogeny serves to align lateralized functioning in a population. Consistency would not be expected if sociality only acts on lateralization regarding social, or biologically relevant, stimuli. Finally, I investigated whether individual asymmetry in the habenula was related to individual lateralized behaviour for social stimuli. As the habenula is involved in motivation, I hypothesize I will find a relationship between habenular asymmetry and asymmetry in eye use for biologically-relevant social stimuli in the form of mirror images. Most studies of cerebral and behavioural asymmetry report only group-level data. This study will enhance knowledge about cerebral lateralization by elucidating whether lateralized responding to different stimuli is consistent within an individual. This work will also help to clarify whether an asymmetrical habenula is related to lateralized behaviour within an individual on a general scale or whether this asymmetry is particularly related to lateralized viewing of certain types of stimuli.

3.2 Materials and Methods

3.2.1 Subjects

I used 66 adult convict cichlids, 33 males and 33 females, obtained from commercial fish suppliers. The fish had no previous experience with either testing apparatus used in this study. Fish were housed in the laboratory in 95 L (75 cm X 31 cm X 41 cm) mixed-sex communal aquaria at densities of approximately 10 fish per aquarium for at least one week before behavioural testing. Water in communal aquaria was maintained at 25 °C ± 2 °C with a light cycle of 12L:12D. Fish were fed *ab libitum* five days a week with various prepared dried fish foods. Protocols were approved by the University of Alberta Biological Sciences Animal Policy and Welfare Committee (protocol number 5441006) and adhered to the guidelines of the Canadian Council for Animal Care.

3.2.2 Procedure

3.2.2.1 Non-Social Detour Task

Fish were tested for eye use preference in a non-social context using a detour apparatus described in detail elsewhere (Bisazza et al., 1997a; Reddon & Hurd, 2008). Briefly, the apparatus consisted of a large opaque plexiglass aquarium (195 cm X 30 cm X 29 cm), filled with water to a depth of 11 cm, divided into two equal-sized holding areas at each end (57 cm X 30 cm X 29 cm) connected by a narrow channel in the middle (10 cm X 75 cm). Holding areas could be separated from the channel with a black plexiglass blockade (28 cm X 28 cm) to keep the fish in the holding area during the acclimation period and between testing trials. A barrier 16 cm wide, consisting of alternating opaque and clear bars (each 0.75 cm wide), was placed at the end of the channel so the fish had to detour around the barrier to enter the holding area.

A trial consisted of gently removing a fish from the communal aquarium using a dip net and placing the fish in one randomly chosen holding area of the test tank with the blockade in place. The fish was given 2 minutes to acclimatize before the blockade was raised. The experimenter then maneuvered the fish into the middle of the mouth of the channel. The fish was allowed to swim down the channel and around the barrier on the other end and into the other holding area. The blockade was then inserted between the channel and the holding area containing the fish and the striped barrier was moved to the opposite side of the channel. Trials were separated by 2 minutes. In total, 10 trials were completed for each fish, 5 swimming in one direction down the channel and 5 swimming in the opposite direction. Upon completion of the 10th trial, fish were removed from the detour apparatus and placed in a solitary aquarium to preserve individual identity before testing in the second apparatus.

For each trial, I visually scored an eye preference of left or right to view the area behind the striped barrier; I considered an eye preference to be established when the main axis of the fish's body was parallel to the striped barrier and the fish could only gaze through the barrier with one eye. Convict cichlids have laterally placed eyes with limited overlap in their optic fields and each eye projects almost entirely to the contralateral hemisphere. Measuring eye use is a well-established method for measuring hemisphere use in fish (Bisazza et al., 1997a; Facchin et al., 1999; Sovrano et al., 2001). I used the eye preference scores on the 10 trials to calculate a laterality index (LI; Bisazza et al., 1997a) for each fish using the following formula:

Laterality Index (LI) = (Right eye use - Left eye use) / (Right eye use + Left eye use)

In addition to the directional laterality index, I also calculated the absolute value of the LI for each fish. Strong signed laterality indices will cancel one another when averaging, thus obscuring the strength of lateralization irrespective of direction (Brown et al., 2007). Examining absolute laterality values allowed us to ask whether individual fish have eye preferences even if, as a population, they do not have a consistent direction of eye preference (Brown et al., 2007; Clotfelter & Kuperberg, 2007).

3.2.2.2 Social Mirror Octagon Task

Fish were tested for eye use preference in a simulated social context using an octagonal mirror apparatus described in detail elsewhere (Sovrano et al., 2001; Reddon & Balshine, 2010). Briefly, the apparatus consisted of a large square aquarium (74 cm X 74 cm X 38 cm) inside which 8 identical rectangular mirrors (30 cm X 30 cm) were arranged in an octagon shape. An octagonal start box (41 cm X 41 cm X 20 cm), made of 8 identical opaque plexiglass rectangles (17 cm X 20 cm), was located in the middle of the aquarium equidistant from all mirrors. One of the start box walls contained a sliding door (5 cm X 20 cm) attached to a string that led behind a white curtain so the door could be opened remotely. The aquarium was filled with water to a depth of 11 cm. Trials were recorded by a webcam (Creative Live! Cam Optia Pro VF0380, Creative Labs, Inc., Milpitas, California, USA) mounted above the apparatus and connected to a laptop running the media program Cheese Webcam Booth v.2.30.1 (Siegel, 2007).

All octagonal mirror trials were conducted one week after detour apparatus testing. A trial consisted of gently removing a fish from its individual aquarium using a dip net and placing the fish in the octagonal start box. The fish was given 2 minutes to acclimatize to the new aquarium and then the sliding door was raised from behind the curtain. The trial was visible via the laptop behind the curtain. Once the fish emerged from the start box the trial began. Trials lasted 10 minutes – during this time the webcam above the aquarium captured one image every 2 seconds for a total of 300 images for each trial. After 10 minutes, the trial was stopped and the fish was returned to its individual holding aquarium.

A fish was considered to be using a certain eye to view its mirror image based on the angle of the fish's body with respect to the closest mirror (see Sovrano et al., 1999). If the fish was facing the mirror at a perpendicular angle, or was greater than 90° from any mirror, no eye use preference was recorded for that image and it was excluded from subsequent analyses. Based on this scoring, I calculated a laterality index (LI) for eye use preference using the same formula as that used in the non-social detour apparatus (see section 3.2.2.1 above).

As with the detour task described above, I also calculated absolute LI for each fish to examine the strength of lateralized eye preference in a perceived social environment.

3.2.3 Neuroanatomical Measurements

Measurements were taken of both the habenula as well as the cortical pretectal nucleus (COPn) which was used as a control nucleus. The COPn was chosen as a control nucleus because it has very clear margins and is similar in size to the habenula. Analysis of this nucleus allowed differentiation between asymmetry specific to the habenula and asymmetry of the entire brain.

Upon completion of behavioural experiments fish were sacrificed by decapitation. Fish heads were stored in jars of 4% paraformaldehyde until later processing. Processing consisted of extracting the brain and storing it in a 30% sucrose solution in 0.1M phosphate buffered saline (PBS) for 24 hours for cryoprotection. Subsequently, the brain was gelatin embedded and stored in 30% sucrose for another 24 hours. The brain was then frozen via dry ice and sectioned at 30 µm using a freezing stage microtome. Brain sections were stored in 24-well plates filled with 0.1M PBS and then mounted onto gelatinized slides. Once mounted, sections were stained with thionin, dehydrated using a degraded ethanol series, cleared with Hemo-D, and coverslipped with Permount (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011).

Images were taken using a camera (Retiga EXi FAST Cooled mono 12-bit camera; Qimaging, Burnaby, BC) mounted on a compound light microscope (Leica DMRE, Rich-mond Hill, ON) of each hemisphere of the two areas of interest, the habenula and COPn, throughout the rostral-caudal region in which these two areas appear in the brain. The area of each image was measured using ImageJ (U.S. National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/). The area of each image was multiplied by the thickness of the section (30 µm) to obtain the volume of the nucleus of interest for that section. The volumes were summed for all images of the nucleus of interest within a hemisphere to obtain the total volume of that nucleus within a hemisphere. I then calculated a laterality index (LI) to determine the degree of asymmetry of the nuclei of interest:

$$\text{Laterality Index (LI): } \frac{\text{Volume of Right Nucleus} - \text{Volume of Left Nucleus}}{\text{Volume of Right Nucleus} + \text{Volume of Left Nucleus}}$$

I calculated this value for both the habenula and the COPn. I also calculated the absolute value of the laterality index (ALI) to determine the degree of asymmetry of the habenula and COPn regardless of the direction of that asymmetry (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011).

I measured habenular and COPn asymmetry in 56 fish, 28 males and 28 females. Data was not collected for all 66 behaviour-tested fish as some brains were damaged at various stages of processing.

3.2.4 Data Analysis

All statistical analyses were carried out using RKWard 0.5.2 (R Core Development Team, Vienna, Austria). Population-level and individual-level lateralization for eye use preference and neuroanatomical asymmetry were assessed using one-sample two-tailed *t*-tests. All two-sample comparisons were assessed using robust parametric *t*-tests employing the Welch-Satterthwaite correction (as per Ruxton, 2006). Relationships between performance in the detour task, performance in the octagonal mirror task, neuroanatomical asymmetry, and size were assessed with Pearson correlations. Significant *p*-values were set at 0.05.

3.3 Results

3.3.1 Non-Social Detour Task

The fish in my sample showed a significant preference to turn left, resulting in viewing the empty environment behind the barrier with their right eye (LI Mean ± SEM: 0.18 ± 0.04; one-sample *t*-test: $t(65) = 4.28$, $p \leq 0.001$). Both males and females preferred to use their right eye (LI Mean ± SEM: Males: 0.23 ± 0.06; one-sample *t*-test: $t(32) = 3.63$, $p \leq 0.001$; Females: 0.13 ± 0.06; one-sample *t*-test: $t(32) = 2.37$, $p = 0.024$, Fig. 3-1). There was no difference between males and females in their eye use preference (two-sample *t*-test: $t(63.08) = 1.144$, $p = 0.257$). In addition to population-level lateralized eye preference, males and females in my sample showed individual-level lateralized eye preference in the detour task (Absolute LI Mean ± SEM: Males: 0.35 ± 0.04; one-sample *t*-test: $t(32) = 8.24$, $p \leq 0.001$; Females: 0.28 ± 0.04; one-sample *t*-test: $t(32) = 7.78$, $p \leq$

0.001, Fig. 3-2). There was no difference between the sexes in strength of eye preference (two-sample *t*-test: $t(62.14) = 1.305$, $p = 0.197$).

3.3.2 Social Mirror Octagon Task

In my sample, I found no significant population-level eye preference for viewing mirror images, though there was a trend towards viewing mirror images with the left eye (LI Mean ± SEM: -0.05 ± 0.03; one-sample *t*-test: $t(65) = -1.74$, $p = 0.087$). However, when I looked at males and females separately I found a population-level preference for females to view their mirror image with the left eye (LI Mean ± SEM: -0.07 ± 0.03; one-sample *t*-test: $t(32) = -2.25$, $p = 0.031$, Fig. 3-1). I found no such population-level eye preference in males (LI Mean ± SEM: -0.03 ± 0.05; one-sample *t*-test: $t(32) = -0.562$, $p = 0.578$, Fig. 3-1). There was, however, no significant difference in directional laterality between males and females when viewing mirror images (two-sample *t*-test: $t(56.35) = 0.80$, $p = 0.426$). Males and females both showed individual lateralization in eye preference for mirror image viewing (Absolute LI Mean ± SEM: Males: 0.18 ± 0.03; one-sample *t*-test: $t(32) = 5.75$, $p \leq 0.001$; Females: 0.16 ± 0.02; one-sample *t*-test: $t(32) = 9.61$, $p \leq 0.001$, Fig. 3-2). There was no difference between males and females in the strength of their individual eye preference for viewing mirror images (two-sample *t*-test: $t(48.48) = 0.61$, $p = 0.543$).

3.3.3 Comparison between Non-Social and Social Laterality Tasks

I found no significant relationships between eye preference for viewing an empty environment in the non-social task and eye preference for viewing mirror images in the perceived social task with respect to either direction or strength of lateralized behaviour

(Direction (LI): Pearson correlation: $r(64) = -0.058$, $p = 0.641$, Fig. 3-3; strength (Absolute LI): Pearson correlation: $r(64) = -0.016$, $p = 0.901$, Fig. 3-4). When looking at the sexes individually I found no relationship between eye preference in the non-social and perceived social tasks in either direction or strength of lateralized behaviour for either sex (Direction (LI): Pearson correlation: Males: $r(31) = -0.078$, $p = 0.666$; Females: $r(31) = -0.068$, $p = 0.706$; strength (Absolute LI): Pearson correlation: Males: $r(31) = -0.033$, $p = 0.853$; Females: $r(31) = -0.019$, $p = 0.917$).

3.3.4 Neuroanatomical Asymmetry

Females showed significantly left-biased habenulae at the population level while males showed a trend towards the same (LI: Mean ± SEM: Females: -0.04 ± 0.02, one-sample *t*-test: $t(27) = -2.158$, $p = 0.040$; Males: -0.03 ± 0.02, one-sample *t*-test: $t(27) = -1.806$, $p = 0.082$; Fig. 3-5). Both females and males showed significantly asymmetrical habenulae at the individual level (ALI: Mean ± SEM: Females: 0.08 ± 0.01, one-sample *t*-test: $t(27) = 5.744$, $p < 0.001$; Males: 0.06 ± 0.01, one-sample *t*-test: $t(27) = 4.898$, $p < 0.001$; Fig. 3-5). There were no sex differences in either habenular LI or ALI (two-sample *t*-test: $t(54) = -0.554$, $p = 0.582$; $t(54) = 1.203$, $p = 0.234$, respectively).

I found no significant relationships when I analyzed the COPn. Neither females nor males had significantly asymmetrical COPns at the population level (LI: Mean ± SEM: Females: -0.02 ± 0.03, one-sample *t*-test: $t(27) = -0.639$, $p = 0.557$; Males: -0.01 ± 0.02, one-sample *t*-test: $t(27) = -0.689$, $p = 0.508$) or at the individual level (ALI: Females: 0.04 ± 0.02, one-sample *t*-test: $t(27) = 2.048$, $p = 0.110$; Males: 0.03 ± 0.04, one-sample *t*-test: $t(27) = 1.795$, $p = 0.133$). There were no sex differences in either COPn

LI or ALI (two-sample *t*-test: $t(54) = -0.842$, $p = 0.422$; $t(54) = 0.292$, $p = 0.777$, respectively).

3.3.5 Comparison between Neuroanatomical Asymmetry and Behaviour

There were no significant relationships for either females or males between degree of habenular asymmetry (LI or ALI) and behaviour in either the non-social laterality task (LI or ALI) or the perceived social laterality task (LI or ALI; Pearson correlation: all *r*s < |0.399|, all *p*s > 0.158). There were also no significant relationships for females or males between habenular asymmetry and fish size (Pearson correlation: all *r*s < |0.326|, all *p*s > 0.090). I found no significant relationships between asymmetry in the COPn and lateralized behaviour (Pearson correlation: all *r*s < |0.702|, all *p*s > 0.120) or between size and asymmetry in the COPn (Pearson correlation: all *r*s < |0.510|, all *p*s > 0.261). There was no significant relationship between asymmetry in the habenula and asymmetry in the COPn for either females or males (Pearson correlation: Females: LI: $r(26) = 0.092$, $p = 0.863$; ALI: $r(26) = 0.068$, $p = 0.825$; Males: LI: $r(26) = -0.392$, $p = 0.442$; ALI: $r(26) = 0.337$, $p = 0.514$).

3.4 Discussion

I have shown that convict cichlids prefer to use their right eye to view an empty space but have different eye use preferences to view their mirror image. I have also shown that eye use preference differs between the sexes in a perceived social environment, with females showing a significant population-level preference to use their left eye when viewing mirror images and males showing only individual-level eye preferences. My data replicate previous results showing that both females and males have larger left habenulae in this species. However, these results contrast with previous studies in that I found no significant relationship between habenular asymmetry and eye preference in either behavioural task.

Previous studies looking at preferential eye use in the convict cichlid in a standard detour task did not find a population-level eye preference in this species when detouring to view an empty environment (Reddon & Hurd, 2008, 2009a). In contrast to these studies, I found here that, in their first encounter with the detour apparatus, convict cichlids show a population-level preference to use their right eye in this task. In agreement with the current study, Gutiérrez-Ibáñez and colleagues (2011) found that female convicts prefer to view an unfamiliar empty environment with their right eye; however, in contrast to this study, Gutiérrez-Ibáñez and colleagues (2011) found that males had a slight bias to use their left eye in the detour task. The difference in results between this study and previous experiments using convict cichlids may be due to a subtle difference in methodology. In the present experiment, I took care to release each fish in the middle of the opening to the narrow channel down which they must swim. In previous studies using a variety of fish species in the detour task, fish have been coaxed out of the holding area using dip nets (Bisazza et al., 1997a; Facchin et al., 1999; Reddon & Hurd, 2008, 2009a, 2009b; Gutiérrez-Ibáñez et al., 2011). By releasing the fish in the middle of the entrance to the channel any bias to remain near the wall that the fish is already closest to is removed and the fish is forced to choose which wall to swim along and which direction to detour around the barrier. This slight procedural modification may result in greater power to detect a turning preference in the detour task.

This slight variation in methodology may also explain why I did not find any relationship between asymmetry in the habenula and behaviour in the detour task when a positive relationship was previously found between these two variables by Gutiérrez-Ibáñez and colleagues (2011). I did, however, replicate the results of Gutiérrez-Ibáñez and colleagues (2011) by showing that both male and female convict cichlids have larger left habenulae. I did not find any significant relationship between habenular asymmetry and behavioural asymmetry in either of my two tasks which indicates that asymmetry in the habenula may not be heavily involved in lateralized behaviour when fish view empty environments or mirror images in this species.

In contrast to the population-level eye preference I found in the solitary detour task, I found no overall population-level eye preference in the perceived social environment task, though I did find a population-level preference for females to use their left eye to view their mirror image in this task. My data correspond well with the results of previous studies showing that other fish species prefer to view mirror images with their left eye (e.g., Sovrano et al., 1999, 2001; De Santi et al., 2001; Sovrano 2004; Sovrano & Andrew, 2006; Andrew et al., 2009). Bisazza and colleagues (1998) found that males of two species of poeciliid fishes (*Gambusia holbrooki* and *Girardinus falcatus*) showed no population-level eye preference when viewing male conspecifics while females preferred to use their left eye when viewing female conspecifics. However, Bisazza and colleagues (1998) found no sex difference in eye use when detouring to view a predator, with both sexes preferring to use their right eye for this task. These results suggest that males and females may be differentially motivated to view social stimuli in the form of conspecifics but perhaps are not differentially motivated to view predators.

Interestingly, I did not find a population-level eye use preference for males in the perceived social environment task. This result is in direct contrast to results found with a highly social species of cichlid (*Neolamprologous pulcher*) studied using the same apparatus; males of this species exhibited a population-level preference to view mirror images with their right eye while females exhibited no significant population-level preference for viewing mirror images (although there was a trend in the same direction as males; Reddon & Balshine, 2010). Reddon and Balshine (2010) explain the sex differences in their results by suggesting that it may be more beneficial for males to be lateralized at the population level because males of this species are the sex that typically must disperse and join new breeding groups; accordingly, being aligned at the population level would help unfamiliar fish behave cohesively with a new group, an important consideration for highly social species. Motivational differences between the sexes when viewing conspecific stimuli may stem from the natural history of the species in question and the differing ecological demands faced by each sex of that species.

In addition to determining lateralized behaviour, the motivational state of a fish may determine how it processes certain kinds of information. Reddon and Hurd (2008) showed that lateralized eye use in convict cichlids was related to aggressive behaviour and that this relationship was mediated by sex; aggressive males were more strongly lateralized than aggressive females and non-aggressive females were more strongly lateralized than non-aggressive males. None of the fish in this study showed any instances of attacking the mirror images in the octagonal tank so I may assume they were not behaving aggressively during the task. Thus, I may see a lateralization difference between the sexes based on their current level of aggressive motivation, such that, when females are not behaving aggressively, they view their mirror image in a more lateralized manner than males when males are not behaving aggressively, leading to a population-level lateralization effect in females but not in males in this task. However, the aggressive and non-aggressive labels designated by Reddon and Hurd (2008) were determined in a test separate from that which measured lateralization. A fruitful area of future research would be to determine if fish change their pattern of lateralized responding depending on their current state (i.e., does lateralized behaviour change when a fish is currently behaving aggressively vs. non-aggressively). Previous studies have shown that some fish species, including convict cichlids, view their mirror image preferentially with the right eye when they are interacting aggressively with that image (e.g., Bisazza & De Santi, 2003; Arnott et al., 2011). Thus, current aggressive state may have a significant effect on lateralized viewing of perceived conspecifics. Schaafsma and Groothuis (2011) found that pharmacological treatment with exogenous testosterone, a hormone that may increase aggressive motivation, induced population-level lateralization in males but not females of another new world cichlid *Aequidens rivulatus*, suggesting a possible mechanistic basis for the sex differences in lateralized behaviour frequently reported in cichlids (Reddon & Hurd, 2008; Reddon & Balshine, 2010; the current study).

In conclusion, I have shown that convict cichlids exhibit a population-level preference to view an empty environment behind a barrier with the right eye. I have also shown that females, but not males, show a population-level preference to use their left eye to view their own mirror image. Finally, I report that neither the strength nor direction of lateralization of these two biases are correlated across individuals or with asymmetry in the habenula. I suggest that my reported sex differences may be driven by a tendency for the sexes to view conspecifics in different ways.[1]

---

[1] *A version of this chapter has been published. Moscicki, M.K., Reddon, A.R., & Hurd, P.L. (2011). Lateralized behaviour of a non-social cichlid fish (Amatitlania nigrofasciata) in a social and a non-social environment. Behavioural Processes, 88, 27-32.*

3.5 Figures

Figure 3-1. Mean (± SEM) laterality index (LI) scores for females (dark grey bars) and males (light grey bars) when viewing an empty environment (Detour) or their own mirror image (Mirror). Positive scores indicate a right eye preference to view the stimulus while negative scores show a left eye preference. Females and males showed a population-level right eye preference when viewing an empty environment (*p* = 0.024, 0.001, respectively) while only females showed a population-level left eye preference when viewing their own mirror image (*p* = 0.032). Males showed no population-level eye preference when viewing mirror images (*p* = 0.572).

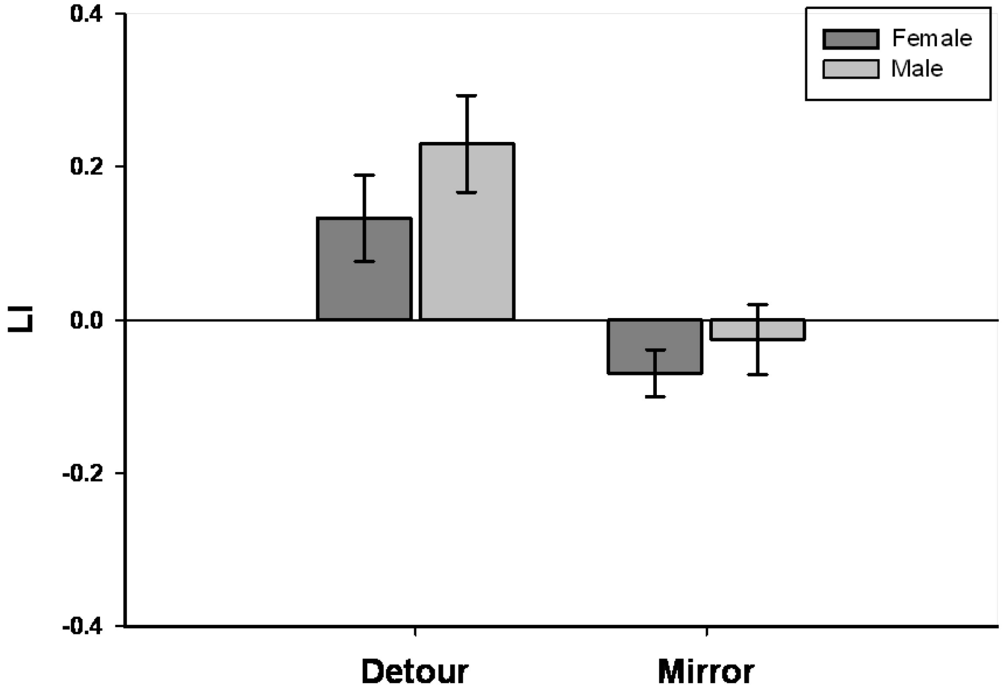


Figure 3-2. Mean (± SEM) absolute laterality index (Absolute LI) scores for females (dark grey bars) and males (light grey bars) when viewing an empty environment (Detour) or their own mirror image (Mirror). Females and males showed individual preferences for one eye or the other when viewing an empty environment (both *p*s $\leq$ 0.001) and when viewing their own mirror images (both *p*s $\leq$ 0.001).

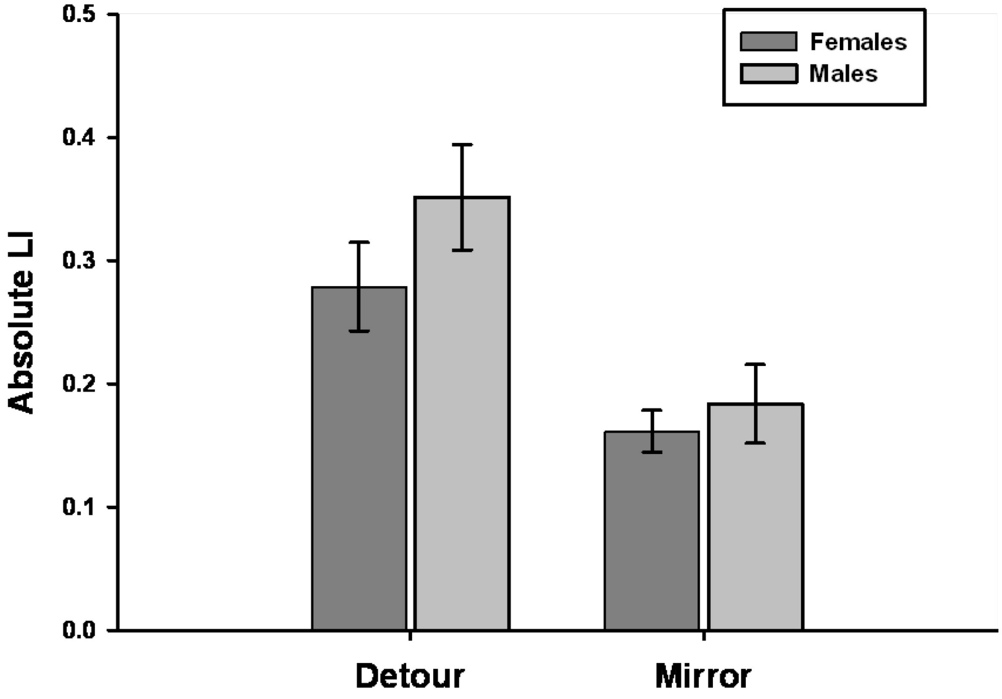


Figure 3-3. There was no significant relationship between laterality index (LI) in the detour task and LI in the mirror task for either females (filled circles, $p = 0.695$) or males (empty triangles, $p = 0.653$), indicating that eye preference in one task is unrelated to eye preference in the other task.

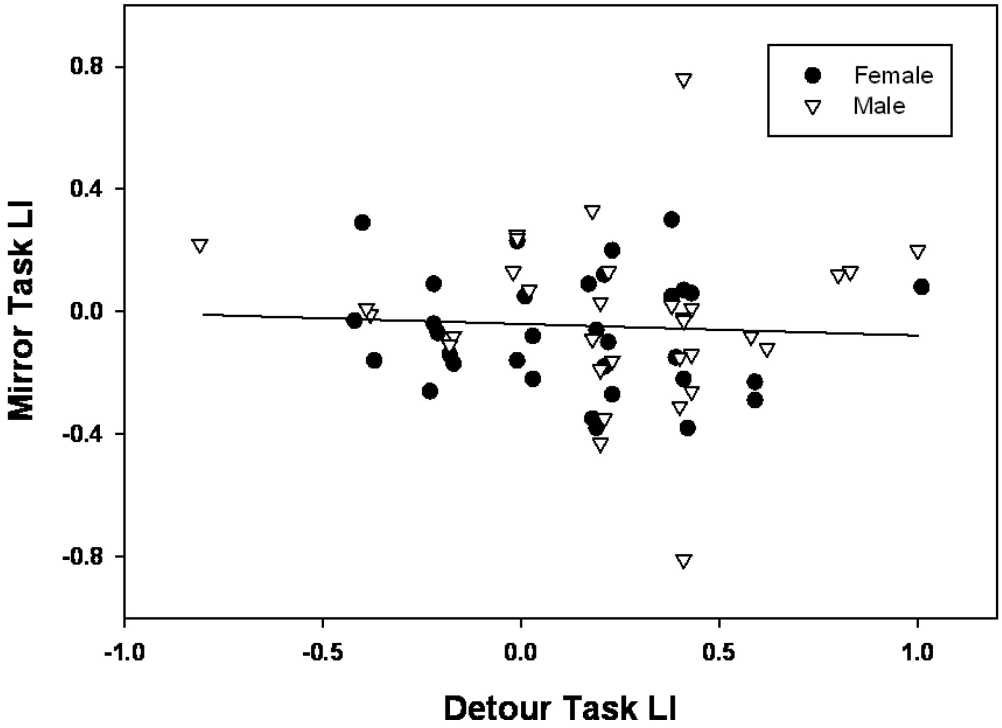


Figure 3-4. There was no significant relationship between absolute laterality index (Absolute LI) in the detour task and absolute LI in the mirror task for either females (filled circles, *p* = 0.937) or males (empty triangles, *p* = 0.854), indicating that strength of eye preference in one task was unrelated to strength of eye preference in the other task.

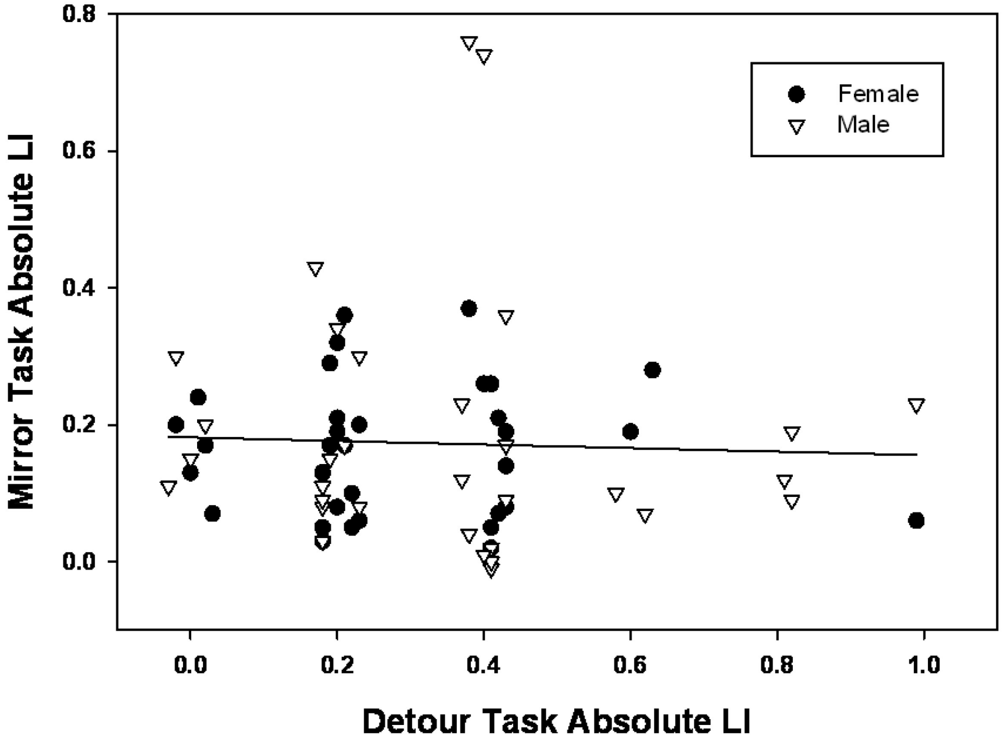
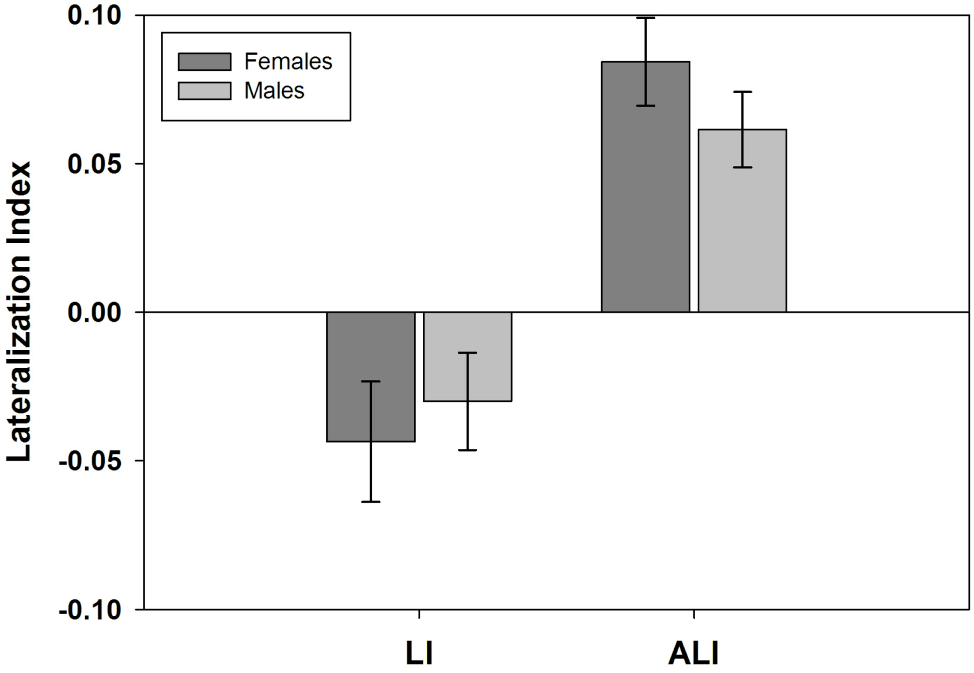


Figure 3-5. Direction (Hb LI) and strength (Hb ALI) of asymmetry in the habenula (Mean ± SEM). Females (dark grey bars) had significantly left-biased habenulae (negative values) at the population level ($p = 0.04$) while males (light grey bars) showed a trend in this direction ($p = 0.082$). Both female and male fish had significantly asymmetrical habenulae at the individual level, regardless of the direction of that asymmetry ($p < 0.001$ for both).



3.6 References

Andrew, R.J. (2002). Behavioral development and lateralization, in: Rogers, L.J., & Andrew, R.J. (Eds.), *Comparative Vertebrate Lateralization.* University Press, Cambridge, pp. 157–205.

Andrew, R.J., & Rogers, L.J. (2002). The nature of lateralization in tetrapods, in: Rogers, L.J., & Andrew, R.J. (Eds.), *Comparative Vertebrate Lateralization.* University Press, Cambridge, pp. 94-125.

Andrew, R.J., Dharmaretnam, M., Gyori, B., Miklosi, A., Watkins, J.A.S., & Sovrano, V.A. (2009). Precise endogenous control of involvement of right and left visual structures in assessment by zebrafish. *Behavioural Brain Research, 196,* 99-105.

Anfora, G., Frasnelli, E., Maccagnani, B., Rogers, L.J., & Vallortigara, G. (2010). Behavioural and electrophysiological lateralization in a social (*Apis mellifera*) but not in a non-social (*Osmia cornuta*) species of bee. *Behavioural Brain Research, 206,* 236-239.

Arnott, G., Ashton, C., & Elwood, R.W. (2011). Lateralization of lateral displays in convict cichlids. *Biology Letters, 7,* 683-685.

Bizassa, A., Cantalupo, C., Capocchiano, M., & Vallortigara, G. (2000). Population lateralisation and social behavior: A study with 16 species of fish. *Laterality, 5,* 269-284.

Bisazza, A., & Dadda, M. (2005). Enhanced schooling performance in lateralized fishes. *Proceedings of the Royal Society B – Biological Sciences, 272,* 1677-1681.

Bisazza, A., & De Santi, A. (2003). Lateralization of aggression in fish. *Behavioral Brain Research, 141,* 131-136.

Bisazza, A., De Santi, A., Bonso, S., & Sovrano, V.A. (2002). Frogs and toads in front of a mirror: Lateralisation of response to social stimuli in tadpoles of five anuran species. *Behavioural Brain Research, 134,* 417–424.

Bisazza, A., Facchin, L., Pignatti, R., & Vallortigara, G. (1998). Lateralization of detour behaviour in poeciliid fish: The effect of species, gender and sexual motivation. *Behavioural Brain Research, 91,* 157-164.

Bisazza, A., Pignatti, R., & Vallortigara, G. (1997a). Detour tests reveal task- and stimulus-specific behavioural lateralization in mosquitofish (*Gambusia holbrooki*). *Behavioural Brain Research, 89,* 237-242.

Bisazza, A., Pignatti, R., & Vallortigara, G. (1997b). Laterality in detour behaviour: Interspecific variation in poeciliid fish. *Animal Behaviour, 54,* 1273–1281.

Brown, C., Western, J., & Braithwaite, V.A. (2007). The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour, 74,* 231-238.

Clotfelter, E.D., & Kuperberg, E.S. (2007). Cerebral lateralization and its relationship to phylogeny and aggression in anabantoid fishes. *Brain, Behavior and Evolution, 69,* 169-175.

Corballis, M.C. (2009). The evolution and genetics of cerebral asymmetry. *Philosophical Transactions of the Royal Society B – Biological Sciences, 364,* 867-879.

Dadda, M., & Bisazza, A. (2006a). Does brain asymmetry allow efficient performance of simultaneous tasks? *Animal Behaviour, 72,* 523-529.

Dadda, M., & Bisazza, A. (2006b). Lateralized female topminnows can forage and attend to a harassing male simultaneously. *Behavioral Ecology, 17,* 358-363.

De Santi, A., Bisazza, A., & Vallortigara, G. (2002). Complementary left and right eye use during predator inspection and shoal-mate scrutiny in minnows. *Journal of Fish Biology, 60,* 1116-1125.

De Santi, A., Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Animal Behaviour, 61,* 305-310.

Fabre-Thorpe, M., Fagot, J., Lorincz, E., Levesque, F., & Vauclair, J. (1993). Laterality in cats: Paw preference and performance in a visuomotor activity. *Cortex, 29,* 15-24.

Facchin, L., Bisazza, A., & Vallortigara, G. (1999). What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behavioural Brain Research, 103,* 229-234.

Ghirlanda, S., & Vallortigara, G. (2004). The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proceedings of the Royal Society B – Biological Sciences, 271,* 853-857.

Ghirlanda, S. Frasnelli, E., & Vallortigara, G. (2009). Intraspecific competition and coordination in the evolution of lateralization. *Philosophical Transactions of the Royal Society B – Biological Sciences, 364,* 861-866.

Gutiérrez-Ibáñez, C., Reddon, A.R., Kreuzer, M.B., Wylie, D.R., & Hurd, P.L. (2011). Variation in the asymmetry of the habenular neucleus correlates with behavioural asymmetry in a cichlid fish. *Behavioural Brain Research, 221,* 189-196.

Klemm, W.R. (2004). Habenular and interpeduncularis nuclei: shared components in multiple-function networks. *Medical Science Monitor, 10,* 261-273.

MacNeilage, P.F., Rogers, L.J., & Vallortigara, G. (2009). Origins of the left and right brain. *Scientific American, 301,* 60-67.

Magat, M., & Brown, C. (2009). Laterality enhances cognition in Austrailian parrots. *Proceedings of the Royal Society B – Biological Sciences, 276,* 4155-4162.

McGrew, W.C., & Marchant, L.F. (1999). Laterality of hand use pay off in foraging success for wild chimpanzees. *Primates, 40,* 509-513.

R Core Development Team. (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reddon, A.R., & Balshine, S. (2010). Lateralization in response to social stimuli in a cooperatively breeding cichlid fish. *Behavioural Processes, 85,* 68-71.

Reddon, A.R., & Hurd, P.L. (2008). Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology Letters, 4,* 338-340.

Reddon, A.R., Gutiérrez-Ibáñez, C., Wylie, D.R., & Hurd, P.L. (2009). The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. *Behavioural Brain Research, 201,* 223-228.

Reddon, A.R., & Hurd, P.L. (2009a). Individual differences in cerebral lateralization are associated with shy-bold variation in the convict cichlid. *Animal Behaviour, 77,* 189-193.

Reddon, A.R., & Hurd, P.L. (2009b). Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. *Behavioural Processes, 82,* 25-29.

Rogers, L.J. (1989). Laterality in animals. *International Journal of Comparative Psychology, 3,* 5–25.

Rogers, L.J. (2000). Evolution of hemispheric specialization: Advantages and disadvantages. *Brain and Language, 73,* 236-253.

Rogers, L.J. (2002). Advantages and disadvantages of lateralization. in: Rogers, L.J., & Andrew, R.J. (Eds.), *Comparative Vertebrate Lateralization.* University Press, Cambridge, pp. 126–153.

Rogers, L.J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Applied Animal Behaviour Science, 127,* 1-11.

Rogers, L.J., & Andrew, R.J. (2002). *Comparative Vertebrate Lateralization.* University Press, Cambridge.

Rogers, L.J., Zucca, P., & Vallortigara, G. (2004). Advantages of having a lateralized brain. *Proceedings of the Royal Society B – Biological Sciences, 271,* S420-S422.

Ruxton, G.D. (2006). The unequal variance t-test is an underused alternative to Student's t-test and the Mann-Whitney U test. *Behavioral Ecology, 17,* 688-690.

Schaafsma, S.M., & Groothuis, T.G.G. (2011). Sex specific effects of postnatal testosterone on lateralization in cichlid fish. *Animal Behaviour, 81,* 283-288.

Siegel, D.G. (2007). Cheese Webcam Booth v.2.30.1 [computer program]. www.projects.gnome.org/cheese. Accessed 21 March 2011.

Sovrano, V.A. (2004). Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behavioral Brain Research, 152,* 385-391.

Sovrano, V.A., & Andrew, R.J. (2006). Eye use during viewing a reflection: Behavioural lateralisation in zebrafish larvae. *Behavioural Brain Research, 167,* 226-231.

Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Lateralization of response to social stimuli in fishes: A comparison between different methods and species. *Physiology & Behavior, 74,* 237-244.

Sovrano, V.A., Dadda, M., & Bisazza, A. (2005). Lateralized fish perform better than non-lateralized fish in spatial reorientation tasks. *Behavioural Brain Research, 163,* 122-127.

Sovrano, V.A., Rainoldi, C., Bisazza, A., & Vallortigara, G. (1999). Roots of brain specializations: Preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behavioural Brain Research, 106,* 175-180.

Sutherland, R.J. (1982). The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. *Neuroscience & Biobehavioral Reviews, 6,* 1-13.

Vallortigara, G. (2000). Comparative neuropsychology of the dual brain: A stroll through animals' left and right perceptual worlds. *Brain and Language, 73,* 189-219.

Vallortigara, G. (2006). The evolutionary psychology of left and right: Costs and benefits of lateralization. *Developmental Psychobiology, 48,* 418-427.

Vallortigara, G., & Andrew, R.J. (1991). Lateralization of response by chicks to change in a model partner. *Animal Behaviour, 41,* 187–94.

Vallortigara, G., Chiandetti, C., & Sovrano, V.A. (2010). Brain asymmetry (animal). *WIREs Cognitive Science, 2,* 146-157.

Vallortigara, G., Rogers, L.J., & Bisazza, A. (1999). Possible evolutionary origins of cognitive brain lateralization. *Brain Research Reviews, 30,* 164-175.

Vallortigara, G., Rogers, L.J., Bisazza, A., Lippolis, G., & Robins, A. (1998). Complementary right and left hemifield use for predatory and agonistic behaviour in toads. *Neuroreport, 9,* 3341-3344.

Vallortigara, G., & Rogers, L.J. (2005). Survival with an asymmetrical brain: Advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences, 28,* 575-633.

Wiltschko, W., Freire, R., Munro, U., Ritz, T., Rogers, L., Thalau, P., & Wiltschko, R. (2007). The magnetic compass of domestic chickens *Gallus gallus*. *Journal of Experimental Biology, 210,* 2300–2310.

Wisenden, B.D. (1994). Factors affecting mate desertion by males in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). *Behavioral Ecology, 5,* 439-447.

4. EFFECT OF STIMULUS TYPE AND ALARM CUES ON CONVICT CICHLID (*AMATITLANIA NIGROFASCIATA*) EYE PREFERENCE

4.1 Introduction

The partitioning of cognitive functions into one hemisphere of the brain, known as cerebral lateralization, is a trait shared by all vertebrate species studied to date (reviewed in Bisazza et al., 1998; MacNeilage et al., 2009; Rogers, 2010; Rogers & Andrew, 2002; Vallortigara et al., 2010). Many species show an agreement at the population level with respect to lateralized behaviour (i.e., most members are lateralized in the same direction), which is referred to as population-level lateralization. This type of lateralized behaviour may confer benefits on a population, such as more effective coordination of behaviours in large social groups (e.g., Anfora et al., 2010; Bisazza & Dadda, 2005; Ghirlanda et al., 2009; Ghirlanda & Vallortigara, 2004; Reddon & Balshine, 2010; Rogers, 1989; Vallortigara & Rogers, 2005).

In addition to population-level lateralization within a species, some cognitive functions appear to be lateralized in a similar manner across a range of species (see Vallortigara, 2000 and Rogers & Andrew, 2002 for reviews). For example, it has been suggested that the processing of negative emotions is predominantly the domain of the right hemisphere (see Rogers, 2010 and Gainotti, 2012). Dharmaretnam and Rogers (2005) showed that chicks that see a predator with their left eye, and process that stimulus with the right hemisphere, make more distress calls than when they see the same predator with their right eye. Further, a variety of species exhibit aggression or fear when the right hemisphere is active. Dogs normally process the barks of other dogs with the left hemisphere; however, when those barks induce fear, dogs switch to right hemisphere processing (Siniscalchi et al., 2008). Rogers (2010) has suggested that predominant processing by the right hemisphere could result in a negative cognitive bias, or vice-versa. For example, left-handed marmosets, which are right-hemisphere dominant for most tasks (Rogers, 2009), showed a negative cognitive bias compared to right-handed marmosets (Gordon & Rogers, 2010). In addition, Kalin and colleagues (1998) showed that rhesus monkeys with increased activity in the right hemisphere had higher cortisol levels and were more fearful than monkeys with greater left hemisphere activity. Stressful conditions may result in a negative cognitive bias, and subsequent increased right hemisphere processing. Harding and colleagues (2004) showed that rats that experienced the stress of unpredictable housing responded to ambiguous stimuli as though they were negative more often than rats experiencing predictable housing. The link between negative emotionality and right hemisphere processing may be mediated by predominant right hemisphere control of endocrine function, the hypothalamic-pituitary-adrenal (HPA) axis (Wittling & Pfluger, 1990), and heart and blood pressure (Wittling et al., 1998).

Stressors, both natural and conditioned, can affect not only the direction of lateralized behaviour, but also the strength of that lateralization. Brown and colleagues (2007) showed that female *Brachyraphis episcopi* from areas of high predation are more strongly lateralized when detouring to view a novel object than females from low predation areas. Reddon and Hurd (2009a) conditioned convict cichlids to associate a neutral stimulus with positive emotional valence (by pairing it with food) and another stimulus with negative emotional valence (by pairing it with damage-induced alarm cues). Females showed stronger lateralized behaviour when detouring to view the positive-valence stimulus while males were more strongly lateralized when viewing the negative-valence stimulus.

Damage-induced alarm cues, like those used by Reddon and Hurd (2009a) to negatively condition stimuli, are produced by some fish species when the epidermal cells of the body are mechanically damaged. These cues indicate to conspecifics that a predator may be in the area (Smith, 1992). The response to alarm cues has been well-documented in convict cichlids (e.g., Wisenden & Sargent, 1997; Brown et al., 2004; Pollock et al., 2005). When alarm cues are added to tank water these fish show species-typical anti-predator responses such as freezing or fleeing to shelters. Pollock and colleagues (2005) showed that convict cichlids exposed to damage-induced alarm cues for 41 days grew significantly less and bred faster than fish exposed to either extract from an unknown heterospecific (swordtail, *Xiphophorus helleri*) or a distilled water control. Damage-induced alarm cues are a useful method to induce stimulus processing under stressful conditions in fish without the confound of a visual predator stimulus or the actual threat of predation.

Recent studies have shown a link between asymmetry in a specific brain nucleus, the habenula, and lateralized behaviour in cichlid fish. The habenula is one of the most well-known asymmetrical structures in the vertebrate brain. This nucleus is part of the limbic system and connects the forebrain to the ventral midbrain (Sutherland, 1982). The habenula is involved in responses to pain, anxiety, and stress. In addition, the habenula is implicated in inhibition of motor behaviour when an animal anticipates an unpleasant outcome (Hikosaka, 2010). Reddon and colleagues (2009) showed a positive relationship between strength of habenular asymmetry and strength of behavioural asymmetry in a detour task in a sibling cohort of the South American cichlid *Geophagus brasiliensis*. In the convict cichlid, Gutiérrez-Ibáñez and colleagues (2011) found a positive relationship between direction of habenular asymmetry and direction of turn bias in a detour task in male fish but not females. Considering that the habenula is potentially involved in responses to stress, and it has already been linked to lateralized behaviour in cichlid fish, it might be expected that fish performing lateralized behaviours when under stress would show a relationship between asymmetry in the habenula and those lateralized behaviours.

Previously, I showed that adult convict cichlids exhibit a population-level bias to view an empty environment with the right eye. I also showed that females display a population-level bias to view mirror images with the left eye, though this effect was not seen in males (Moscicki et al., 2011; Chapter 3 of this thesis). Here, I assessed the lateralized behaviour of adult convict cichlids in the presence or absence of a stressor (i.e., damage-induced alarm cues). I measured lateralized behaviour in both a social and a non-social context to determine if competition from another salient stimulus (i.e., perceived conspecifics) would alter lateralized behaviour in the presence of a stressor. My hypotheses were fourfold: (1) that fish would switch from viewing the empty environment with their right eye to viewing it with their left eye in the presence of the stressor, in order to preferentially use the right hemisphere for this more negatively-valent stimulus processing; (2) that females and males would both show population-level biases to view social stimuli with the left eye in the presence of a stressor, again to facilitate right hemisphere processing; (3) that females in the presence of a stressor would show stronger lateralized behaviour than control females, as stressors like predation pressure tend to have stronger effects in females (e.g., Brown et al. 2007); and (4) that I would find a relationship between asymmetry in the habenula and lateralized behaviour when a stressor was present; particularly, that fish with larger right habenulae would show stronger lateralized behaviour in the presence of a stressor.

4.2 Materials and Methods

4.2.1 Subjects

In total, 120 convict cichlid fish were used in this experiment. 60 fish, 30 males and 30 females, were used to assess behavioural lateralization in the control condition (i.e., without damage-induced alarm cues added to tank water). An additional 30 males and 30 females were used to assess behavioural lateralization in the stressed condition (i.e., damage-induced alarm cues added to tank water). I used a between-subjects design because it has been previously shown that when convict cichlids were familiar with the detour task they behaved differently than during their first presentation with the apparatus (Reddon & Hurd, 2009b). All fish were obtained from commercial fish suppliers in Edmonton, AB and no fish had previous experience with either testing apparatus. Prior to testing, fish were housed communally in a large 460 L (184 cm X 49 cm X 51 cm) stock tank for at least one week. Fish were fed dry prepared cichlid flakes *ad libitum* once a day five days a week. Water temperature was maintained at 26 ˚C ± 2 ˚C and fluorescent lights above the tank were maintained on a 12L:12D schedule. All protocols were approved by the University of Alberta Biological Sciences Animal Policy and Welfare Committee (protocol number 5441006).

4.2.2 Non-Social Detour Task

4.2.2.1 Apparatus

Convict cichlids have laterally placed eyes with little binocular overlap; information viewed through each eye projects mainly to the contralateral hemisphere. Eye preference has been used as a proxy for cerebral lateralization in fishes (e.g., Bisazza et al., 1997; Facchin et al., 1999). I used a standard detour task to assess behavioural lateralization in a non-social context. This task has been widely used for this purpose and has been described in detail elsewhere (e.g., Bisazza et al., 1997; Brown et al., 2007; Reddon & Hurd, 2008, 2009a,b; Moscicki et al., 2011). The apparatus consisted of an opaque plexiglass enclosure (195 cm X 30 cm X 29 cm) with two holding areas on either side (57 cm X 30 cm X 29 cm). A narrow channel (10 cm X 75 cm) connected the holding areas which could be separated from the channel by a black plexiglass blockade (28 cm X 28 cm). A 16 cm wide black and white striped barrier (each stripe 0.75 cm wide) could be placed at the end of the channel. The tank was filled with water to a height of 11cm.

4.2.2.2 Procedure

The holding area for the first trial was decided by coin flip. A trial consisted of placing the fish in the holding area for a 2 minute acclimation period with the black blockade preventing access to the channel. After acclimation, the blockade was lifted and the fish was free to swim down the channel. At the end of the channel, the fish encountered the striped barrier and had to detour either left or right around the barrier to reach the other holding area. When the fish detoured around the barrier it could view the empty environment beyond with only one eye.

Each fish completed ten trials in the detour apparatus, alternating the start holding area between trials. Trials were separated by 2 minute acclimation periods. After the tenth trial was completed I calculated a laterality index (LI; Bisazza et al., 1997) using the formula below:

Laterality Index (LI) = (Right eye use - Left eye use) / (Right eye use + Left eye use)

Right eye and left eye indicated the eye facing the empty environment as the fish detoured around the striped barrier. I considered a turn decision to be made when the main axis of the fish's body was parallel to the striped barrier (Bisazza et al., 1997). The laterality index indicated the preferential eye with which each fish viewed the empty environment. Positive values indicated a right eye preference while negative values indicated a left eye preference. I also calculated the absolute value of this index to indicate the strength of eye preference for each fish regardless of direction.

After testing in the detour apparatus, each fish was placed in a separate 40 L (51 cm X 26 cm X 31 cm) tank, to preserve individual identity, for at least one week before being tested in the octagonal mirror task (see section 4.2.3 below).

4.2.3 Social Mirror Octagon Task

4.2.3.1 Apparatus

Fish were tested in an octagonal mirror apparatus to assess lateralized behaviour in a perceived social environment. This apparatus has been described in detail elsewhere (Sovrano et al., 2001; Reddon & Balshine, 2010; Moscicki et al., 2011). The apparatus consisted of a large opaque square plexiglass aquarium (74 cm X 74 cm X 38 cm) inside which an octagon was constructed from 8 identical mirrored panels (each 30 cm X 30 cm). In the centre of this mirrored octagon was an octagonal-shaped start box (41 cm X 41 cm X 20 cm) constructed from 8 identical white plexiglass panels; (each 17 cm X 20 cm) one of these panels contained a sliding door (5 cm X 20 cm) from which the fish could exit. The apparatus was filled with water to a depth of 11cm. A webcam (Creative Live! Cam Optia Pro VF0380, Creative Labs, Inc., Milpitas, CA, USA) was mounted above the apparatus and was connected to a laptop running the media program Cheese Webcam Booth v.2.30.1 (Siegel, 2007).

4.2.3.2 Procedure

A trial consisted of placing a fish into the octagonal start box with the sliding door closed. After a 2 minute acclimation period the door was opened remotely by an experimenter behind a white curtain pulling on a string. The trial was visible in real time on the laptop also located behind the curtain. Once the fish emerged from the start box the timed trial began. A photo of the apparatus was taken every 2 seconds for a total of ten minutes (300 photos per trial). After ten minutes, the trial was ended and the fish was returned to the solitary holding aquarium.

4.2.3.3 Data Scoring

I scored each photo for eye preference for viewing mirror images (a proxy for assessing behavioural lateralization in response to social stimuli; Sovrano et al., 2001; Reddon & Balshine, 2010; Moscicki et al., 2011). To do this, I recorded whether the fish faced the closest mirror with either its right or left eye. If a fish was perpendicular to the closest mirror, or greater than 90° from any mirror, that photo was eliminated from further analyses (see Sovrano et al., 1999). I calculated a laterality index (LI) for each fish in the same manner as that calculated for the detour task.

I again calculated both a signed and an absolute value of the laterality index for each fish. The same 30 males and 30 females as tested in the detour apparatus without alarm cues were tested in the mirror octagon without alarm cues. An additional 30 males and 30 females were tested in both the detour and mirror octagon tests with damage-induced alarm cues added to the tank water.

4.2.4 Damage-Induced Alarm Cues

4.2.4.1 Preparation

I collected damage-induced alarm cues following the method of Pollock and colleagues (2005) and Reddon and Hurd (2009a). I used 14 fish (7 males and 7 females: Standard Length: Mean ± SEM = 6.17 cm ± 0.43 cm) to prepare a stock solution of alarm cues. I euthanized fish by decapitation and then removed a skin fillet from each flank with a scalpel. Each fillet was finely chopped and the resulting pieces were placed into a beaker filled with 196 mL of chilled distilled water. After fillets from all fish were added to the beaker the entire mixture was filtered through 125 mm diameter filter paper to remove any particulate matter. After filtering, 392 mL of distilled water was added to bring the final volume to 588 mL. This stock solution was separated into 15 mL aliquots and frozen at -20 ˚C until used.

4.2.4.2 Administration to Apparatus

Fish in the stressed condition were tested in tanks with damage-induced alarm cues added to the water. To administer the alarm cues to the detour tank I placed an air stone attached to a length of tubing in each of the holding areas. I used a 15 mL syringe to inject 7.5 mL of alarm cue mixture into each length of tubing. The tubing was then attached to an aerator and air was pumped through the air stones for 2 minutes. Dye tests showed this procedure was successful in dispersing the substance throughout the detour apparatus. After 2 minutes, the aerator was disconnected and a clean dip net was used to further mix the water throughout the apparatus. When filled to a depth of 11 cm, both the detour tank and the mirror octagon tank contained 60 L of water; therefore, the final concentration of alarm cues in both tanks was 0.25 mL alarm cue / L water.

To administer alarm cues to the mirror octagon tank the same general procedure was used; however, instead of air stones added to each holding area, they were placed on opposite sides of the octagon and the sliding door to the start box remained closed while air was being pumped through the air stones.

4.2.5 Neuroanatomical Measurements

Upon completion of behavioural testing in the mirror octagon task all fish were sacrificed by decapitation. Fish heads were removed and stored in 4% paraformaldehyde until subsequent processing. During processing, brains were extracted, submerged in a 30% sucrose solution in 0.1 M phosphate buffered saline (PBS) for 24 hours for cryoprotection, embedded in gelatin, placed in 30% sucrose for an additional 24 hours, frozen, and sectioned to a thickness of 30 μm using a freezing-stage microtome. Sections were mounted on gelatinized slides, allowed to dry for at least 24 hours, stained with thionin, dehydrated through a graded ethanol series, cleared using Hemo-D, and coverslipped using Permount (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011).

Images of stained sections containing the nucleus of interest, the habenula, and a control nucleus, the cortical pretectal nucleus (COPn), were captured using a camera (Retiga EXi FAST Cooled mono 12-bit camera; Qimaging, Burnaby, BC) mounted on a compound light microscope (Leica DMRE, Rich-mond Hill, ON). The areas of the left and right hemispheres of the two nuclei were measured for each stained section using Image J (U.S. National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/). The area of each section was multiplied by the thickness of the section (30 μm) to obtain a volume for each section; these volumes were summed together for each hemisphere of each nucleus to determine the total volume of each hemisphere of the nucleus of interest (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011).

I measured habenula and COPn asymmetry in 28 males and 27 females tested in the behavioural tasks with damage-induced alarm cues added to the tanks. I did not process brains for fish in the control condition as neuroanatomical asymmetry data has already been reported on fish in these behavioural tasks without alarm cues present in the tank water (see Gutiérrez-Ibáñez et al., 2011; chapter 3 of this thesis). Due to damage during processing, I did not have neuroanatomical measurements for all the fish that were run in the behavioural studies. I did not have neuroanatomical data for 2 males and 3 females.

To examine neuroanatomical asymmetry I calculated a laterality index for each nucleus in each fish using the following formula:

$$\text{Laterality Index (LI)} = \frac{\text{Volume of Right Nucleus} - \text{Volume of Left Nucleus}}{\text{Volume of Right Nucleus} + \text{Volume of Left Nucleus}}$$

In addition, I calculated the absolute value of the laterality index (ALI) to assess the strength of side bias regardless of the direction of that bias (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011).

4.2.6 Data Analysis

All analyses were performed using R v. 2.12.2 (R Core Development Team, Vienna, Austria). I used univariate ANOVAs and simple effects analyses to assess differences between sexes and alarm cue treatment in direction and strength of eye preference in each behavioural lateralization task. Population-level and individual-level neuroanatomical asymmetry were assessed using one-sample t-tests. All two-sample comparisons were assessed using two-sample t-tests employing the Welch-Satterthwaite correction (Ruxton, 2006). I used Pearson correlations to assess the relationship between neuroanatomical asymmetry and behaviour in the lateralization tasks. All tests were two-tailed. I used an α level of 0.05 to determine significance.

4.3 Results

4.3.1 Non-Social Detour Task

I found no sex difference in eye preference in the detour task ($F(1, 116) = 0.610$, $p = 0.436$). However, fish tested without alarm cues preferred to use their right eye to view the empty environment while fish tested with alarm cues preferred to use their left eye (LI Mean ± SEM: Control: 0.16 ± 0.05; Treatment: -0.14 ± 0.07; $F(1, 116) = 12.574$, $p = 0.001$, Fig. 4-1). I found no significant interaction between sex and treatment ($F(1, 116) =$

1.044, *p* = 0.309). I examined the simple effect of treatment and found that females in tanks without alarm cues preferred to use their right eye while females with alarm cues present preferred to use their left eye (LI Mean ± SEM: Control: 0.17 ± 0.07; Treatment: -0.21 ± 0.10; *F*(1,116) = 10.432, *p* = 0.002). I found this same trend for males (LI Mean ± SEM: Control: 0.15 ± 0.08; Treatment: -0.06 ± 0.08; *F*(1,116) = 3.186, *p* = 0.077, Fig. 4-1).

In addition to direction of eye preference, I also examined strength of eye preference in the detour task. I found no sex difference (*F*(1, 116) = 0.785, *p* = 0.377). I did find that fish tested with alarm cues in the water showed a significantly stronger eye preference in the detour task than fish tested with control water (ALI Mean ± SEM: Control: 0.34 ± 0.03; Treatment: 0.45 ± 0.04; *F*(1, 116) = 4.985, *p* = 0.027; Fig. 4-2). There was no significant interaction between sex and treatment (*F*(1, 116) = 2.543, *p* = 0.113). I examined the simple effect of treatment and found that females tested with alarm cues showed significantly stronger eye preference compared to females tested without alarm cues (ALI Mean ± SEM: Treatment: 0.51 ± 0.06; Control: 0.33 ± 0.04; *F*(1, 116) = 7.325, *p* = 0.008). I did not find this effect in males (ALI Mean ± SEM: Treatment: 0.39 ± 0.04; Control: 0.36 ± 0.05; *F*(1, 116) = 0.203, *p* = 0.653; Fig. 4-2).

4.3.2 Social Mirror Octagon Task

I found no sex difference in eye preference for mirror image viewing (*F*(1, 116) = 0.013, *p* = 0.910), I found a trend for fish to alter eye preference when alarm cues were present (*F*(1, 116) = 2.782, *p* = 0.098), and I found no interaction between sex and treatment (*F*(1, 116) = 0.664, *p* = 0.417, Fig. 4-3).

Females showed a population-level preference to view mirror images with their left eye when alarm cues were not present; males had no population-level eye preference (LI Mean ± SEM: Females: -0.12 ± 0.04; Males: -0.07 ± 0.06; $t(29) = -2.652, -1.239$, $p = 0.013, 0.225$, respectively). When alarm cues were present, neither females nor males showed a population-level eye preference (LI Mean ± SEM: Females: 0.06 ± 0.07; Males: 0.004 ± 0.08; $t(29) = 0.870, 0.052$, $p = 0.391, 0.959$, respectively).

There was no sex difference in strength of eye preference for mirror image viewing ($F(1, 116) = 0.041$, $p = 0.840$). Fish with alarm cues added to the tank had a stronger eye preference than control fish (ALI: Mean ± SEM: Control: 0.21 ± 0.03; Treatment: 0.29 ± 0.04; $F(1, 116) = 3.968$, $p = 0.049$; Fig. 4-4). I found no significant interaction between sex and treatment ($F(1, 116) = 0.354$, $p = 0.553$). I examined the simple effect of treatment and found that females tested with alarm cues showed significantly stronger eye preference than females tested without alarm cues (ALI Mean ± SEM: Treatment: 0.30 ± 0.05; Control: 0.20 ± 0.03; $F(1, 116) = 3.940$, $p = 0.050$). I did not find this effect in males (ALI Mean ± SEM: Treatment: 0.27 ± 0.06; Control: 0.22 ± 0.04; $F(1, 116) = 0.692$, $p = 0.407$; Fig. 4-4).

4.3.3 Comparison between Non-Social and Social Laterality Tasks

I found no significant relationship for either females or males in direction or strength of eye preference between the detour and mirror octagon tasks when control water was in the testing apparatus (Direction (LI): Females: $r(28) = 0.127$, $p = 0.503$; Males: $r(28) = 0.008$, $p = 0.969$, Fig. 4-5a; Strength (ALI): Females: $r(28) = -0.260$, $p = 0.166$; Males: $r(28) = -0.242$, $p = 0.198$, Fig. 4-5b). When alarm cues were added to the testing apparatus I found the same pattern of results, with no significant relationships for either males or females in direction or strength of eye preference between the non-social and perceived social laterality tasks (Direction (LI): Females: $r(28) = -0.060$, $p = 0.754$; Males: $r(28) = -0.272$, $p = 0.145$, Fig. 4-5c; Strength (ALI): Females: $r(28) = -0.096$, $p = 0.615$; Males: $r(28) < 0.001$, $p = 0.998$, Fig. 4-5d).

4.3.4 Neuroanatomical Asymmetry

I measured habenular asymmetry only in fish behaviour tested in the presence of alarm cues. For fish that were tested with alarm cues in the testing apparatus, both male and female fish showed significantly left biased habenulae at the population level (LI: Mean ± SEM: Male: -0.04 ± 0.01; Female: -0.03 ± 0.01; $t(29) = -3.014, -2.285$, $p = 0.007, 0.033$, respectively) and significantly asymmetrical habenulae at the individual level (ALI: Mean ± SEM: Male: 0.05 ± 0.01; Female: 0.05 ± 0.01; $t(29) = 5.631, 7.239$, $p < 0.001$ for both, Fig. 4-6). There were no sex differences in either directional or absolute asymmetry of the habenula (LI: $t(49.57) = 0.617$, $p = 0.540$; ALI: $t(46.34) = -0.099$, $p = 0.922$). I found no evidence of directional asymmetry in the COPn in either males or females (LI: Mean ± SEM: Males: 0.002 ± 0.01; $t(27) = 0.180$, $p = 0.858$; Females: -0.004 ± 0.02; $t(26) = -0.212$, $p = 0.834$).

4.3.5 Comparison between Neuroanatomical Asymmetry and Behaviour

In female fish there were no significant relationships between habenular asymmetry (LI or ALI) and behaviour in either the non-social laterality task (LI or ALI) or the perceived social laterality task (LI or ALI; all rs < $|0.223|$, all ps > 0.319). In male fish there was a significant positive relationship between habenular asymmetry (LI) and eye preference in the non-social laterality task (LI: $r(28) = 0.619$, $p = 0.004$, Fig. 4-7).

There were no other significant relationships between male fish habenular asymmetry (LI or ALI) and behaviour in either the non-social laterality task (LI or ALI) or the perceived social laterality task (LI or ALI; all rs < |0.423|, all ps > 0.063). I found no relationship between the COPn and behavioural lateralization in either males or females (LI or ALI; all rs < |0.480|, all ps > 0.413).

4.4 Discussion

I demonstrated an effect of exposure to a stressor on behavioural lateralization. In general, when a stressor is present fish switch from right eye preference to left eye preference in the detour task when no explicit stimulus was present. Females showed this effect more than males. I did not find an eye preference switch in the presence of a stressor when fish were viewing mirror images. The addition of a stressor also increased the strength of eye preference in both behavioural tasks. This effect was driven by females. Males showed a relationship between habenular asymmetry and eye preference when a stressor was present in the detour task, an effect not found in females. Both females and males had left-biased habenulae, which has been previously reported in this species (Gutiérrez-Ibáñez et al., 2011; chapter three of this thesis). To the best of my knowledge, this is the first test of lateralization in a fish species in the presence of damage-induced alarm cues, a stressful predator cue in the absence of an actual predator stimulus.

The right hemisphere has long been linked to the processing of emotions. In fact, three main hypotheses exist about the role of the right hemisphere in emotional processing. The valence hypothesis suggests that the right hemisphere is dominant for negative emotions while the left hemisphere is dominant for positive emotions (Perria et al., 1961). The right hemisphere hypothesis suggests right hemisphere dominance for all emotional processing regardless of valence (Gainotti, 1972). Finally, the emotional type hypothesis presumes right hemisphere dominance for basic emotions and left hemisphere dominance for more evolved social emotions (Ross et al., 1994). All hypotheses indicate a function of the right hemisphere in negative or stressful emotional processing (reviewed in Gainotti, 2012). Recently, a few authors have reviewed the literature on non-human animal species and have shown support for this hypothesis (Rogers, 2010; Leliveld et al. 2013). In particular, evidence examining eye use in response to, and escape attempts from, predators in a variety of species supports this idea. Australian lungfish *(Neoceratodus forsteri)* initiate left-biased C-start escape responses from a predator indicating use of the right hemisphere (Lippolis et al., 2009). Domestic chicks (*Gallus gallus*) interrupt foraging to attend to a simulated overhead avian predator more quickly when the predator is viewed with the left visual field (Rogers, 2000). This same pattern of stronger responses to predators seen on the left side was shown in three different species of toads (Lippolis et al., 2002). Common wall lizards *(Podarcis muralis)* show left eye preference for inspecting predators (Bonati et al., 2010). My data fit well with these studies in that, when a predator cue was present, female fish switched from a right eye to a left eye preference to view the area behind a barrier in the detour task. These results appear to indicate a preference for right hemisphere processing when in a stressful situation in female convict cichlids.

Research on predator inspection in fishes appears, at least on the surface, to contradict the idea that predator stimuli are primarily processed with the left eye/right hemisphere. Bisazza and colleagues (1999) showed that when mosquitofish (*Gambusia* *holbrooki*) were placed in an apparatus in which they could view their mirror image with one eye and maintain visual fixation on a predator with the other eye, these fish approached the predator more closely when their mirror image could be seen with the left eye and the predator with the right eye. A subsequent experiment confirmed that, in the absence of a mirror image to contend for eye preference, mosquitofish preferentially inspect a predator with the right eye when close to the predator (De Santi et al., 2001). These results could be explained by the need of a fish that is approaching a predator for inspection to inhibit the initial response to escape. Evidence suggests that the left hemisphere is involved in inhibition of response (Andrew & Rogers, 2002; Miklósi & Andrew, 1999); thus, for a fish to approach a predator, rather than perform an escape response, it may be beneficial for the fish to view the predator with the right eye. This idea is supported by evidence that mosquitofish turn right (and use the left eye) to escape a simulated predator in their first exposure to that predator; however, on subsequent exposures the turning preference changes to left (Cantalupo et al., 1995). These results may indicate that the initial fear response to the unknown predator evoked right hemisphere processing (left eye use). Upon subsequent predator presentations, the fish may be inspecting the predator more than initiating a fear response, as the predator did not harm the fish in the initial presentations; thus, preference for left hemisphere control (right eye use) becomes dominant (Cantalupo et al., 1995). In my task, there was no physical predator present and no visual predator stimulus to orient towards. These factors may explain why I see more left eye/right hemisphere responding in the presence of chemical cues that indicate a fearful stimulus while previous studies using physical predators have shown more right eye/left hemisphere use.

I found a preference for females to view social stimuli with the left eye when in the control condition but not when alarm cues were present. Males showed no eye preference in either condition. Studies using fish and other species have shown a trend for animals to view mirror images or same sex conspecifics with their left eye (e.g., Andrew et al., 2009; Bisazza et al., 2002; De Santi et al., 2001, 2002; Sovrano, 2004; Sovrano & Andrew, 2006; Sovrano et al., 1999, 2001; Vallortigara, 1992; Vallortigara & Andrew, 1991, 1994). When alarm cues are present, fish may become more vigilant for unseen predators. If fish prefer to use their left eye/right hemisphere to maintain vigilance for unseen threats and to process information about conspecifics, then they may have to split their left-eye viewing (i.e., occasionally view the social stimuli and also scan for predators with the same eye). This would lead each fish to decrease viewing social stimuli with the left eye and show a concomitant decreased strength of lateralized eye use when alarm cues are present. However, this is not what I found. I found a significant increase in the strength of lateralized eye-viewing of mirror images in the presence of alarm cues, at least in females. Each individual fish was not splitting eye use between the right and left eye; but rather, each fish had a strong preference for one eye. It is possible that individual characteristics, such as boldness or styles of coping with stressors, may influence the perceived risk and subsequent response elicited by individuals in the presence of stressful cues (e.g., Clement et al., 2005; Koolhaas et al., 2010; Øverli et al., 2004). Indeed, Reddon and Hurd (2008, 2009b) showed that the personality traits of both boldness and aggression affect lateralized eye use in the detour task in the convict cichlid. Assessing behavioural traits and style of coping with stressors in conjunction with lateralized responses in the presence of stressors is a fruitful area for further research.

Females showed increase strength of eye preference when alarm cues were present in both behavioural tasks; males did not show this effect. This result is consistent with data showing that female *Brachyraphis episcopi* from high predation rivers, and their first generation offspring in the lab, show stronger lateralized behaviour than females of the same species from low predation rivers (Brown et al., 2007). These data suggest that females respond to threats of predation with increased use of the preferred eye for stimulus viewing.

It is possible that the sex difference in lateralized responding to alarm cues in convict cichlids may reflect differential sensitivity to those cues rather than differential behavioural responses. In zebrafish, females are more sensitive to alarm cues than males (Gandolfi et al., 1968). In addition, female zebrafish and female European minnows have more alarm substance producing cells than males (Smith, 1986; Irving, 1996). A few studies have examined the response of juvenile convict cichlids to different concentrations of alarm cues (Brown et al., 2006; Roh et al., 2004; Vavrek & Brown, 2009); however, these studies do not report sex differences. To the best of my knowledge, differential sensitivity of adult male and female convict cichlids to various concentrations of alarm cues has not been studied.

Both male and female fish tested in behavioural tasks with alarm cues had larger left than right habenulae. Larger left habenulae have been previously reported in this species (Gutiérrez-Ibáñez et al., 2011; chapter three of this thesis). One of the proposed functions of the habenula is inhibition of motor responses when an aversive stimulus is anticipated (Klemm, 2004). If the left hemisphere is also involved in inhibition of responses, as previously suggested (Andrew & Rogers, 2002; Miklósi & Andrew, 1999), then there may be a link between degree of asymmetry favouring the left habenula and degree of behavioural inhibition in individual fish. It would be worthwhile to examine if fish that show greater inhibition in tasks such as exploring an open field also have larger left habenulae than fish that are more willing to explore a novel environment.

In addition to leftward-biased habenulae for both sexes, I found a significant positive relationship between habenular asymmetry and eye preference in the detour task when a stressor was present for male fish but not for females. Males with larger left habenulae tended to view an empty environment more with their left eye. This result is contrary to the pattern found by Gutiérrez-Ibáñez and colleagues (2011) in which male convict cichlids with larger left habenulae tended to use their right eye more in the detour task, though there was no stressor present in that task. Development of asymmetry in the habenula has been extensively studied in the zebrafish. In wildtype fish, the parapineal, an unpaired accessory to the pineal organ, forms on the left side in more than 95% of larvae (Concha et al., 2000). This unpaired structure has dramatic effects on the development of the left habenula, leading to differential gene expression, more dense neuropil, and a larger size than the right habenula (Gamse et al., 2003). In the *frequent situs inversus* (*fsi*) strain of zebrafish, a greater percentage of larvae present L-R reversed asymmetry in both parapineal position and viscera compared to wildtype fish (Barth et al., 2005). Barth and colleagues (2005) showed that fish with reversed parapineal position showed opposite eye preference to wildtype fish in a series of lateralization tasks, though this reversal was not seen in all tasks. In addition, fish with reversed parapineal also exhibited differences from wildtype fish in personality dimensions, such as quicker emergence into a swimway when a model predator face was present. These studies examined gross asymmetry in the habenula (i.e., parapineal position); there are only a handful of studies to my knowledge that measure more fine-grain asymmetry in the area of the habenula (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011; chapter three of this thesis). These fine-grain analyses were all conducted in cichlid fish, for which the link between parapineal position and habenular asymmetry has not been studied as it has been in the zebrafish model. Currently, it is difficult to say how fine-grain asymmetry in the habenula may relate to lateralized behaviour; it is likely that this relationship is complex and involves other aspects of behaviour such as personality characteristics, particularly in response to stressors.

I have shown that a stressor in the form of damage-induced alarm cues affects both direction and strength of eye preference, particularly in female convict cichlids. In addition, I have shown a link between habenular asymmetry and behavioural lateralization in the presence of alarm cues. I suggest that individual differences in personality traits and responsiveness to stressors may interact with individual asymmetry in the habenula to influence individual patterns of lateralized behaviour.

4.5 Figures

Figure 4-1. Directional eye preference (LI) scores (Mean ± SEM) for females and males in the detour task with either alarm cues absent (Control – shaded bars) or present (Alarm Cues – open bars). N = 30 for all groups. Positive scores indicate a right eye preference to view the empty environment behind the barrier while negative scores indicate a left eye preference. Females switched from right eye to left eye preference when alarm cues were present (*p* = 0.002) while males showed an increased preference to use the left eye when alarm cues were present (*p* = 0.077).

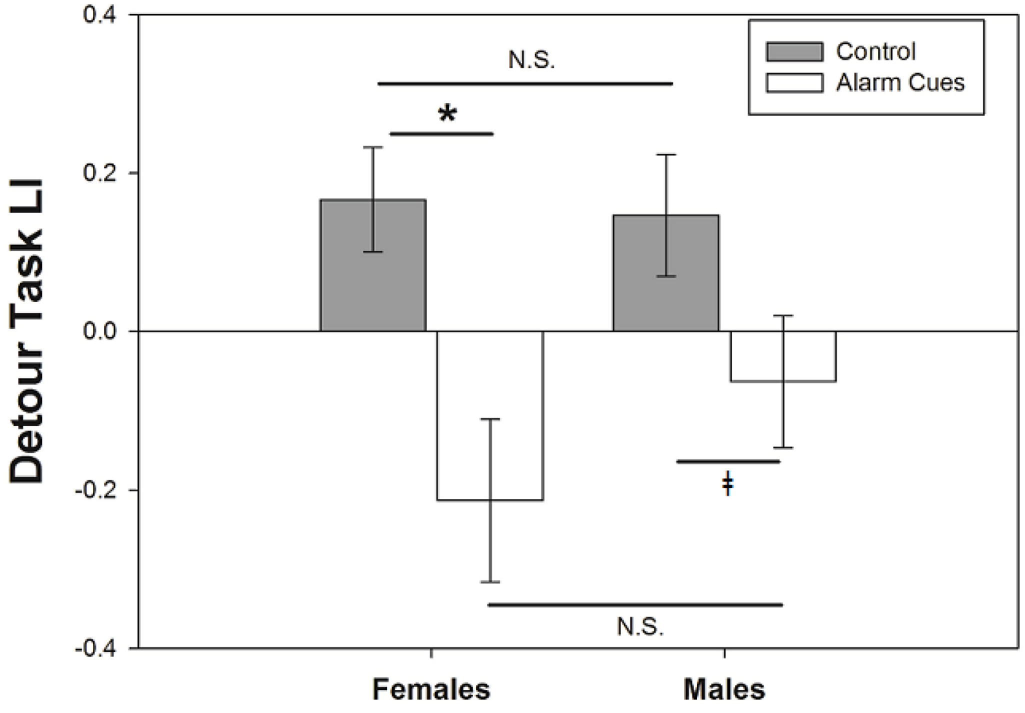


Figure 4-2. Strength of eye preference (Absolute LI) scores (Mean ± SEM) for females and males in the detour task with either alarm cues absent (Control – shaded bars) or present (Alarm Cues – open bars). N = 30 for all groups. Females showed a significantly stronger eye preference when detouring around a barrier when alarm cues were present in the tank vs. when alarm cues were absent (*p* = 0.008). Males did not show this effect (*p* = 0.653).

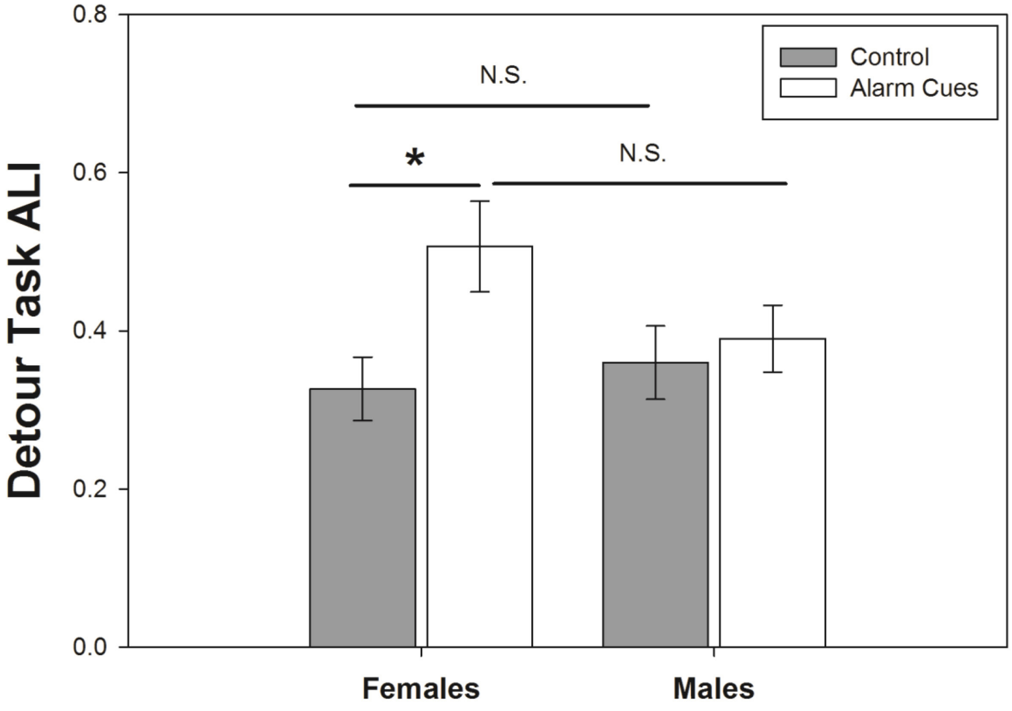


Figure 4-3. Directional eye preference (LI) scores (Mean ± SEM) for females and males in the mirror task with either alarm cues absent (Control – shaded bars) or present (Alarm Cues – open bars). N = 30 for all groups. Positive scores indicate a right eye preference to view mirror images while negative scores indicate a left eye preference. I found no effect of sex (*p* = 0.910) or alarm cues (*p* = 0.098) on the direction of eye preference in this task.

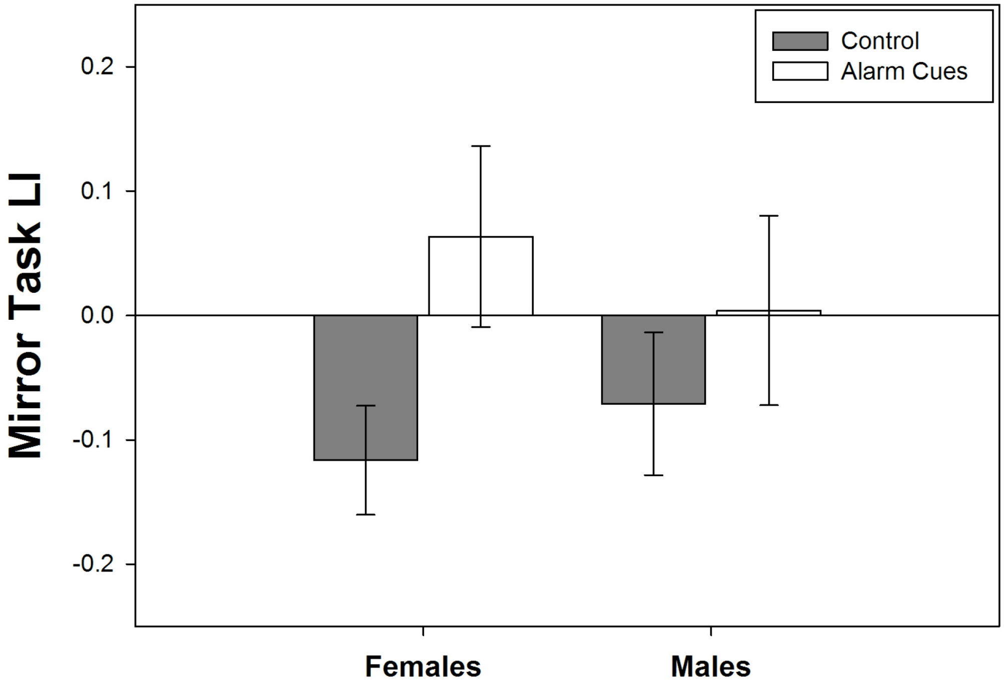


Figure 4-4. Strength of eye preference (Absolute LI) scores (Mean ± SEM) for females and males in the mirror task with either alarm cues absent (Control – shaded bars) or present (Alarm Cues – open bars). N = 30 for all groups. Females, but not males, showed a significantly stronger eye preference when viewing mirror images when alarm cues were present in the tank vs. when alarm cues were absent (Females: $p = 0.050$; Males: $p = 0.407$).

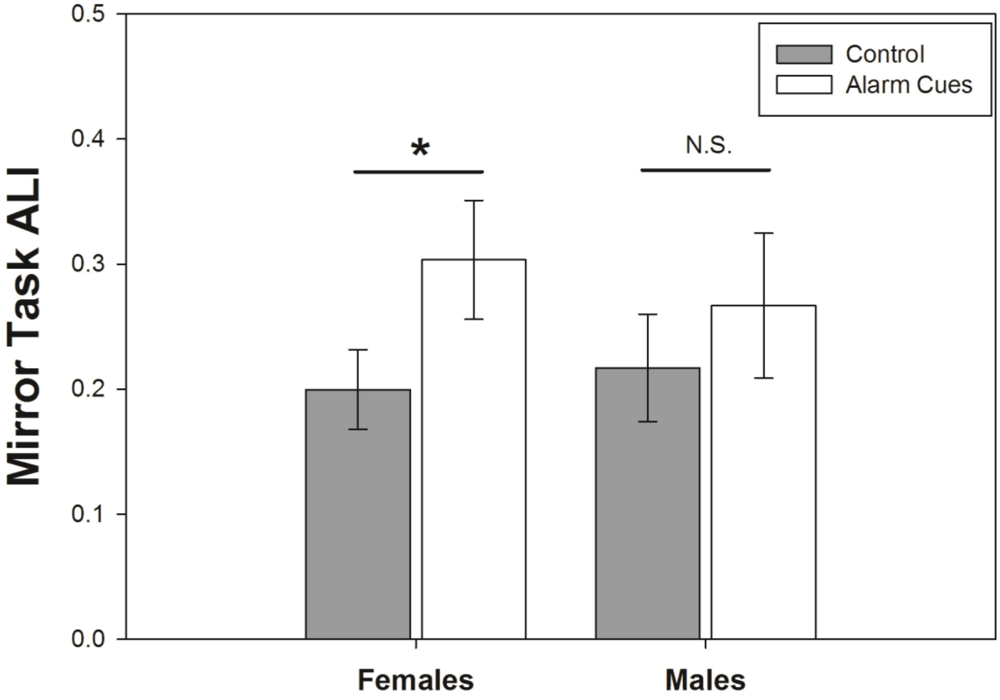


Figure 4-5. There were no significant relationships between direction of eye preference (LI) or strength of eye preference (ALI) in the detour and mirror tasks when alarm cues were absent or present for either females (open circles) or males (filled triangles). Panel A: Direction of eye preference (LI) when alarm cues were absent. Panel B: Strength of eye preference (ALI) when alarm cues were absent. Panel C: Direction of eye preference (LI) when alarm cues were present. Panel D: Strength of eye preference (ALI) when alarm cues were present.

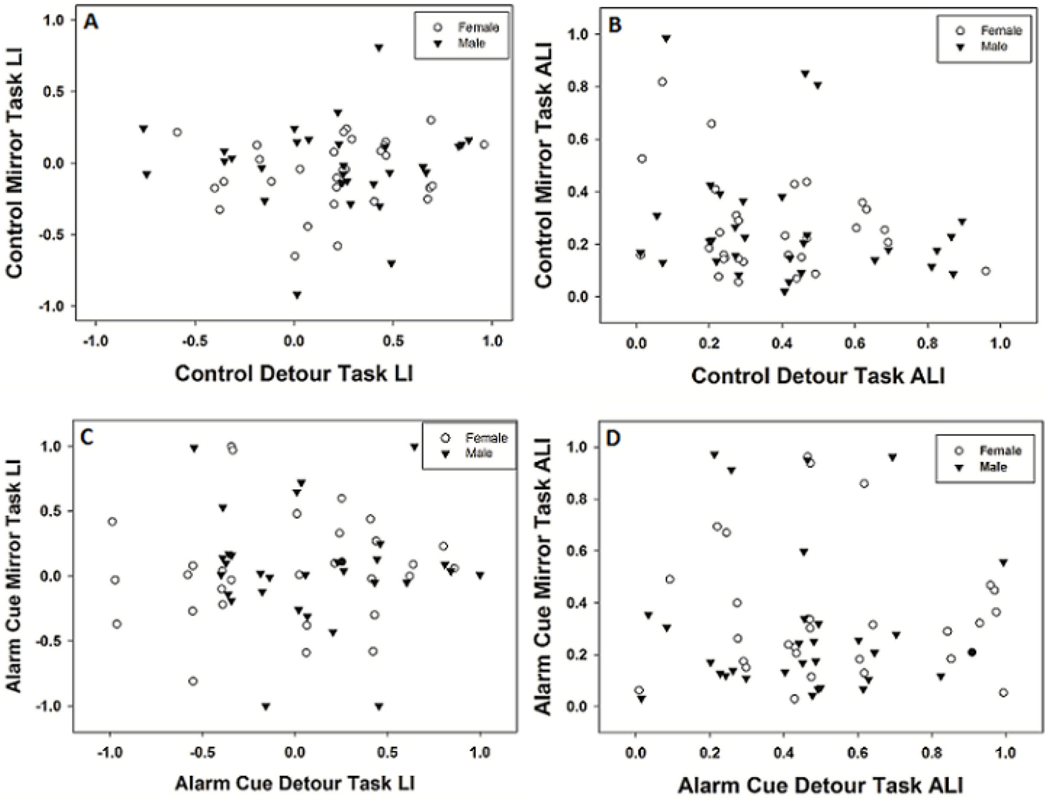


Figure 4-6. Direction (Hb LI) and strength (Hb ALI) of asymmetry in the habenula (Mean ± SEM) for fish that were tested in behavioural tasks with alarm cues present. N = 28 males. N = 27 females. Both females (shaded bars) and males (open bars) had left-biased habenulae (negative values) at the population level (Females: $p = 0.033$; Males: $p = 0.007$). There were no sex differences in either the direction or strength of habenular asymmetry (LI: $p = 0.540$; ALI: $p = 0.922$).

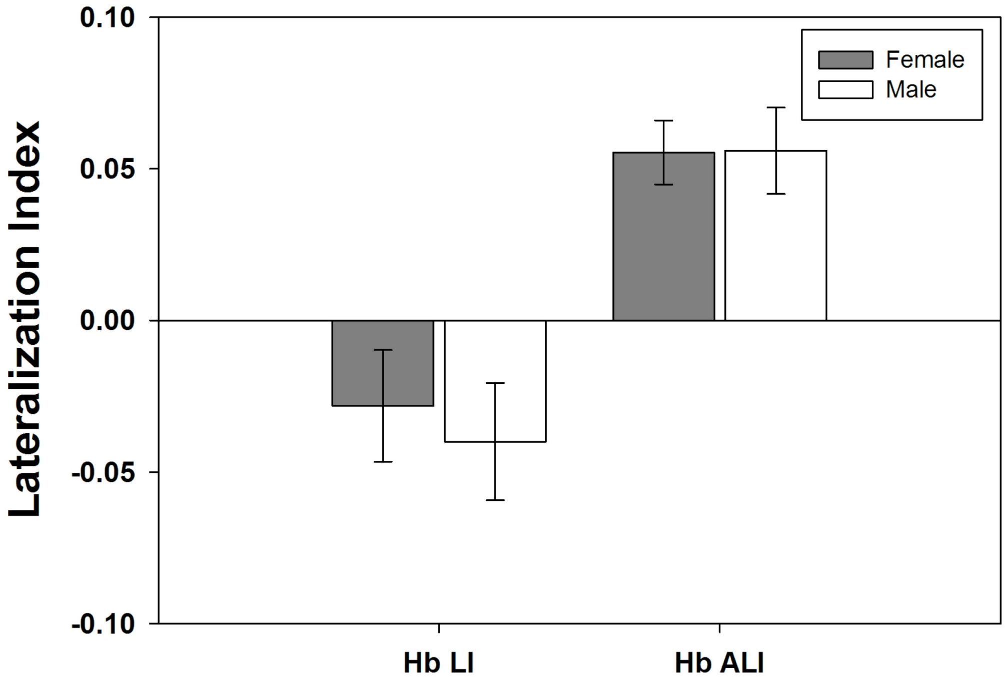
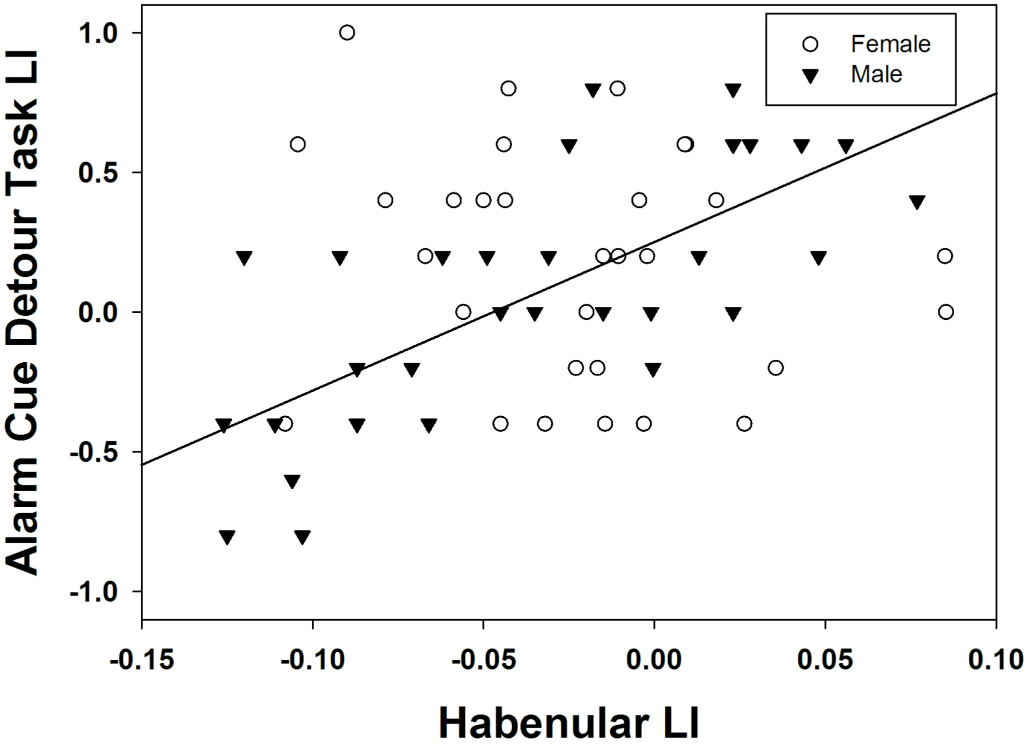


Figure 4-7. There was a significant positive relationship between the direction of habenular asymmetry and direction of eye preference in the detour task in male fish (filled triangles) tested with alarm cues present (*p* = 0.004; solid line). I did not find this same significant relationship for females (open circles).



4.6 References

Andrew, R.J., Osorio, D., & Budaev, S. (2009). Light during embryonic development modulates patterns of lateralization strongly and similarly in both zebrafish and chick. *Philosophical Transactions of the Royal Society B - Biological Sciences, 364,* 983-989.

Andrew, R.J., & Rogers, L.J. (2002). The nature of lateralization in tetrapods. In L. J. Rogers, & R.J. Andrew (Eds.), *Comparative Vertebrate Lateralization* (pp. 94-125). Cambridge: University Press.

Anfora, G., Frasnelli, E., Maccagnani, B., Rogers, L.J., & Vallortigara, G. (2010). Behavioural and electrophysiological lateralization in a social (*Apis mellifera*) but not in a non-social (*Osmia cornuta*) species of bee. *Behavioural Brain Research, 206,* 236-239.

Barth, K.A., Miklósi, A., Watkins, J., Bianco, I.H., Wilson, S.W., & Andrew, R.J. (2005). *fsi* zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. *Current Biology, 15,* 844-850.

Bisazza, A., & Dadda, M. (2005). Enhanced schooling performance in lateralized fishes. *Proceedings of the Royal Society B - Biological Sciences, 272,* 1677-1681.

Bisazza, A., De Santi, A., Bonso, S., & Sovrano, V.A. (2002). Frogs and toads in front of a mirror: Lateralisation of response to social stimuli in tadpoles of five anuran species. *Behavioural Brain Research, 134,* 417–424.

Bisazza, A., De Santi, A., & Vallortigara, G. (1999). Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side. *Animal Behaviour, 57,* 1145-1149.

Bisazza, A., Facchin, L., Pignatti, R., & Vallortigara, G. (1998). Lateralization of detour behaviour in poeciliid fish: The effect of species, gender and sexual motivation. *Behavioural Brain Research, 91,* 157-164.

Bisazza, A., Pignatti, R., & Vallortigara, G. (1997). Detour tests reveal task- and stimulus-specific behavioural lateralization in mosquitofish (*Gambusia holbrooki*). *Behavioural Brain Research, 89,* 237-242.

Bonati, B., Csermely, D., López, P., & Martin, J. (2010). Lateralization in the escape behaviour of the common wall lizard (*Podarcis muralis*). *Behavioural Brain Research, 207,* 1-6.

Brown, C., Western, J., & Braithwaite, V.A. (2007). The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour, 74,* 231-238.

Brown, G.E., Foam, P.E., Cowell, H.E., Guevara Fiore, P., & Chivers, D.P. (2004). Production of chemical alarm cues in convict cichlids: the effects of diet, body condition and ontogeny. *Annales Zoologici Fennici, 41,* 487-499.

Brown, G.E., Rive, A.C., Ferrari, M.C.O., & Chivers, D.P. (2006). The dynamic nature of antipredator behavior: prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. *Behavioral Ecology and Sociobiology, 61,* 9-16.

Cantalupo, C., Bisazza, A., & Vallortigara, G. (1995). Lateralization of predator-evasion response in a teleost fish (Girardinus falcatus). *Neuropsychologia, 33(12),* 1637-1646.

Clement, T.S., Parikh, V., Schrumpf, M., & Fernald, R.D. (2005). Behavioral coping strategies in a cichlid fish: the role of social status and acute stress response in direct and displaced aggression. *Hormones and Behavior, 47,* 336-342.

Concha, M.L., Burdine, R.D., Russell, C., Schier, A.F., & Wilson, S.W. (2000). A nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain. *Neuron, 28,* 399-409.

De Santi, A., Bisazza, A., & Vallortigara, G. (2002). Complementary left and right eye use during predator inspection and shoal-mate scrutiny in minnows. *Journal of Fish Biology, 60,* 1116-1125.

De Santi, A., Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Animal Behaviour, 61,* 305-310.

Dharmaretnam, M., & Rogers, L.J. (2005). Hemispheric specialization and dual processing in strongly versus weakly lateralized chicks. *Behavioural Brain Research, 162,* 62–70.

Facchin, L., Bisazza, A., & Vallortigara, G. (1999). What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behavioural Brain Research, 103,* 229-234.

Gainotti, G. (1972). Emotional behavior and hemispheric side of lesion. *Cortex, 8,* 41–55.

Gainotti, G. (2012). Unconscious processing of emotions and the right hemisphere. *Neuropsychologia, 50,* 205-218.

Gamse, J.T., Thisse, C., Thisse, B., & Halpern, M.E. (2003). The parapineal mediates left-right asymmetry in the zebrafish diencephalon. *Development, 130,* 1059-1068.

Gandolfi, G., Mainardi, D., & Rossi, A.C. (1968). The fright reaction of zebra fish. *Atti della Societa Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 107,* 74–88.

Ghirlanda, S., Fransnelli, E., & Vallortigara, G. (2009). Intraspecific competition and coordination in the evolution of lateralization. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364,* 861–866.

Ghirlanda, S., & Vallortigara, G. (2004). The evolution of brain lateralization: A game-theoretical analysis of population structure. *Proceedings of the Royal Society B: Biological Sciences, 271,* 853-857.

Gordon, D.J., & Rogers, L.J. (2010). Differences in social and vocal behavior between left- and right-handed common marmosets. *Journal of Comparative Psychology, 124,* 402-411.

Gutiérrez-Ibáñez, C., Reddon, A.R., Kreuzer, M.B., Wylie, D.R., & Hurd, P.L. (2011). Variation in the asymmetry of the habenular nucleus correlates with behavioural asymmetry in a cichlid fish. *Behavioural Brain Research, 221,* 189-196.

Harding, E.J., Paul, E.S., & Mendl, M. (2004). Cognitive bias and affective state. *Nature, 427,* 312.

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nature Reviews Neuroscience, 11,* 503-513.

Irving, P.W. (1996). Sexual dimorphism in club cell distribution in the European minnow and immunocompetence signalling. *Journal of Fish Biology, 48,* 80-88.

Kalin, N.H., Larson, C., Shelton, S.E., & Davidson, R.J. (1998). Asymmetric frontal brain activity, cortisol, and behavior associated with fearful temperament in rhesus monkeys. *Behavioral Neuroscience, 112,* 286-292.

Klemm, W.R. (2004). Habenular and interpeduncularis nuclei: shared components in multiple-function networks. *Medical Science Monitor, 10,* 261-273.

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinologi, 31,* 307-321.

Leliveld, L.M.C., Langbein, J., & Puppe, B. (2013). The emergence of emotional lateralization: Evidence in non-human vertebrates and implications for farm animals. *Applied Animal Behaviour Science, 145,* 1-14.

Lippolis, G., Bisazza, A., Rogers, L.J., & Vallortigara, G. (2002). Lateralization of predator avoidance responses in three species of toads. *Laterality, 7,* 163–183.

Lippolis, G., Joss, J.M.P., & Rogers, L.J. (2009). Australian lungfish (*Neoceratodus forsteri*): a missing link in the evolution of complimentary side biases for predator avoidance and prey capture. *Brain, Behavior and Evolution, 73,* 295-303.

MacNeilage, P.F., Rogers, L.J., & Vallortigara, G. (2009). Origins of the left and right brain. *Scientific American, 301,* 60-67.

Miklosi, A., & Andrew, R.J. (1999). Right eye use associated with decision to bite in zebrafish. *Behavioural Brain Research, 105,* 199–205.

Moscicki, M.K., Reddon, A.R., & Hurd, P.L. (2011). Lateralized behaviour of a non-social cichlid fish (*Amatitlania nigrofasciata*) in a social and a non-social environment. *Behavioural Processes, 88,* 27-32.

Øverli, O., Korzan, W.J., Hoglund, E., Winberg, S., Bollig, H., Watt, M., Forster, G.L., Øverli, E., Renner, K.J., & Summers, C.H. (2004). Stress coping style predicts aggression and social dominance in rainbow trout. *Hormones and Behavior, 45,* 235-241.

Perria, L., Rosadini, G., & Rossi, G.F. (1961). Determination of side of cerebral dominance with Amobarbital. *Archives of Neurology, 4,* 173–181.

Pollock, M.S., Zhao, X., Brown, G.E., Kusch, R.C., Pollock, R., & Chivers, D.P. (2005). The response of convict cichlids to chemical alarm cues: an integrated study of behaviour, growth and reproduction. *Annales Zoologici Fennici., 42,* 485-495.

R Core Development Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. url:http://www.R-project.org/.

Reddon, A.R., & Balshine, S. (2010). Lateralization in response to social stimuli in a cooperatively breeding cichlid fish. *Behavioural Processes, 85(1),* 68-71.

Reddon, A.R., & Hurd, P.L. (2008). Aggression, sex and individual differences in cerebrallateralization in a cichlid fish. *Biology Letters, 4(4),* 338-340.

Reddon, A.R., Gutiérrez-Ibáñez, C., Wylie, D.R., Hurd, P.L. (2009). The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. *Behavioural Brain Research, 201,* 223-228.

Reddon, A.R., & Hurd, P.L. (2009a). Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. *Behavioural Processes, 82,* 25-29.

Reddon, A.R., & Hurd, P.L. (2009b). Individual differences in cerebral lateralization are associated with shy-bold variation in the convict cichlid. *Animal Behaviour, 77,* 189-193.

Rogers, L.J. (1989). Laterality in animals. *International Journal of Comparative Psychology, 3,* 5–25.

Rogers, L.J. (2000). Evolution of hemispheric specialization: Advantages and disadvantages. *Brain and Language, 73,* 236-253.

Rogers, L.J. (2009). Hand and paw preferences in relation to the lateralized brain. *Philosophical Transactions of the Royal Society of London B – Biological Sciences, 364,* 943–954.

Rogers, L.J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Applied Animal Behaviour Science, 127,* 1-11.

Rogers, L.J., & Andrew, R.J. (2002). *Comparative Vertebrate Lateralization*. Cambridge: University Press.

Roh, E., Mirza, R.S., & Brown, G.E. (2004). Quality or quantity? The role of donor condition in the production of chemical alarm cues in juvenile convict cichlids. *Behaviour, 141,* 1235-1248.

Ross, E.D., Homan, R.W., & Buck, R. (1994). Differential hemispheric lateralization of primary and social emotions. *Neuropsychiatry, Neuropsychology, and Behavioral Neurology, 7,* 1–19.

Ruxton, G.D. (2006). The unequal variance t-test is an underused alternative to Student's t-test and the Mann-Whitney U test. *Behavioral Ecology, 17,* 688-690.

Siegel, D.G. (2007). Cheese Webcam Booth (version 2.30.1) [Computer software]. www.projects.gnome.org/cheese. Accessed 18 April 2012.

Siniscalchi, M., Quaranta, A., & Rogers, L.J. (2008). Hemispheric specialization in dogs for processing different acoustic stimuli. *PloS ONE, 3,* e3349.

Smith, R.J.F. (1986). Reduced alarm substance cell counts in male and androgen treated zebra danios (*Brachydanio rerio*). *Canadian Journal of Zoology, 64,* 551-553.

Smith, R.J.F. (1992). Alarm signals in fishes. *Reviews in Fish Biology and Fisheries, 2,* 33-63.

Sovrano, V.A. (2004). Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behavioural Brain Research, 152,* 385-391.

Sovrano, V.A., & Andrew, R.J. (2006). Eye use during viewing a reflection: Behavioural lateralisation in zebrafish larvae. *Behavioural Brain Research, 167,* 226-231.

Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Lateralization of response to social stimuli in fishes: A comparison between different methods and species. *Physiology & Behavior, 74,* 237-244.

Sovrano, V.A., Rainoldi, C., Bisazza, A., & Vallortigara, G. (1999). Roots of brain specializations: Preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behavioural Brain Research, 106,* 175-180.

Sutherland, R.J. (1982). The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. *Neuroscience and Biobehavioral Reviews, 6,* 1-13.

Vallortigara, G. (1992). Right hemisphere advantage for social recognition in the chick. *Neuropsychologia, 30,* 761–8.

Vallortigara, G. (2000). Comparative neuropsychology of the dual brain: A stroll through animals' left and right perceptual worlds. *Brain and Language, 73,* 189-219.

Vallortigara, G., & Andrew, R.J. (1991). Lateralization of response by chicks to change in a model partner. *Animal Behaviour, 41,* 187–94.

Vallortigara, G., & Andrew, R.J. (1994). Differential involvement of right and left hemisphere in individual recognition in the domestic chick. *Behavioural Processes, 33,* 41–58.

Vallortigara, G., Chiandetti, C., & Sovrano, V.A. (2010). Brain asymmetry (animal). *WIREs Cognitive Science, 2,* 146-157.

Vallortigara, G., & Rogers, L.J. (2005). Survival with an asymmetrical brain: Advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences, 28,* 575-633.

Vavrek, M.A., & Brown, G.E. (2009). Threat-sensitive responses to disturbance cues in juvenile convict cichlids and rainbow trout. *Annales Zoologici Fennici, 46,* 171-180.

Wisenden, B.D., & Sargent, R.C. (1997). Antipredator behavior and suppressed aggression by convict cichlids in response to injury-released chemical cues of conspecifics but not to those of an allopatric heterospecific. *Ethology, 103,* 283–291.

Wittling, W., Block, A., Schweiger, E., & Genzel, S. (1998). Hemisphere asymmetry in sympathetic control of the human myocardium. *Brain and Cognition, 38,* 17–35.

Wittling, W., & Pfluger, M. (1990). Neuroendocrine hemisphere asymmetries: salivary cortisol secretion during lateralized viewing of emotion-related and neutral films. *Brain and Cognition, 14,* 243–265.

5. SEX DIFFERENCES IN BEHAVIOURAL RESPONSE AFTER STRESS EXPERIENCE IN CONVICT CICHLIDS (*AMATITLANIA NIGROFASCIATA*)

5.1 Introduction

Interest in the study of personality traits in animals has increased substantially in the last two decades (e.g., Dall et al., 2004; Sih et al., 2004a,b; Sih & Bell, 2008; Sih et al., 2012). The animal personality literature has adopted several different terms to describe stable behaviour within an individual across time and/or situations (e.g., behavioural syndromes, temperament, coping styles, behavioural tendencies, strategies, axes, and constructs; see Sih et al., 2004a for a review). A behavioural trait may be stable across different contexts (e.g., an animal may be aggressive both while fighting and while mating), or within a context across different situations (e.g., an animal may be bold while foraging in the presence and absence of predators; Gosling, 2001; Sih et al., 2004a,b). In addition to the existence of stable individual behavioural traits, such as aggression or boldness, suites of traits may be correlated in a stable manner (e.g., an animal may be both consistently aggressive and consistently bold).

Although consistency may imply that behaviours are rigid and always predictable based on an animal's personality that is not necessarily the case. Personality may describe typical ways of acting over a long time span but behaviour may still be quite flexible (Budaev & Brown, 2011). Dingemanse and colleagues (2009) proposed the concept of behavioural reaction norms to show that individual behaviour may vary across different environmental conditions. For example, shy fish may remain shy regardless of whether or not predators are present. In contrast, bold fish may show more plasticity in behaviour and behave less boldly when predators are present while still being bolder than shy conspecifics in this scenario. These reaction norms can highlight differences in plasticity between individuals based on initial assessment of a personality trait (Dingemanse et al., 2009).

Fish have become a useful model for studying personality traits. Fish are relatively easy to breed and house in the laboratory and can be collected from different habitats, allowing for more evolutionary questions about stable behavioural traits to be posed (Budaev and Brown, 2011). The most well-studied personality dimension in fish is the boldness-shyness continuum (Toms et al., 2010; Conrad et al., 2011). Boldness has been defined as an individual's reaction to a situation perceived as dangerous (Réale et al., 2007). Common assays to measure boldness in fish include latency to approach a novel object, eat novel food, explore a novel environment, or emerge from a shelter. Freezing behaviours are common in an open field task, a novel environment to the subject, and are thought to be related to lack of boldness (Burns, 2008); however, some authors suggest this task may also measure tendency to explore. Additionally, latency to leave a 'safe' enclosed refuge and enter a novel, open, 'less-safe' area, has been used as a measure of boldness. It is presently unclear whether these commonly used behavioural tasks all relate to the underlying concept of boldness (Conrad et al., 2011). Toms and colleagues (2010) ultimately suggest that novelty is the key to investigating boldness.

A variety of factors can affect boldness in fish. Brown and colleagues (2005a) showed that bishops (*Brachyraphis episcopi*) from high predation areas were bolder than their low-predation counterparts. In a subsequent experiment, first generation lab-reared fish from high- and low-predation parents revealed a heritable component to boldness in that lab-reared fish from high-predation parents were bolder than those from low-predation parents (Brown et al., 2007). Males were also bolder than females in this species. In this same study, Brown and colleagues (2007) showed that experience can influence boldness. Fish chased with a net for two minutes a day for two weeks were bolder than undisturbed fish, regardless of parental predation pressure. Guppies showed similar boldness behaviour to bishops in that fish from high-predation areas were bolder than those from low-predation habitats and males were bolder than females (Harris et al., 2010).

A relationship between boldness and stress responsiveness has been shown in many fish species. *B. episcopi* from high predation areas, which were bolder than their low predation counterparts (Brown et al. 2005a), had lower ventilation rates (Brown et al., 2005b) and lower release rates of cortisol in response to a stressor than fish from low predation areas (Archard et al., 2012). Bold juvenile mulloway (*Argyosomus japonicus*) had significantly lower plasma cortisol concentrations after handling stress than shy fish (Raoult et al., 2012). Thomson and colleagues (2012) showed that bolder rainbow trout were able to modulate their behaviour in response to stressors (i.e., decreased food availability and increased predation threat) more than shy individuals, which remained more rigid in their behaviour. Contrary to Thomson and colleagues' (2012) study, it has been suggested that bold animals have a more proactive stress coping style, characterized by inflexible routines and quick, superficial exploration of novel stimuli. Shy animals, in contrast, tend to display a reactive stress coping style, attending more to environmental stimuli and showing flexibility in adapting to changing environments (Koolhaas et al., 1999; Øverli et al., 2007).

Damage-induced alarm cues, chemical cues released when the epidermis of a prey species is damaged, invoke anti-predator defenses in conspecifics (Wisenden and Sargent, 1997). These cues are often used to study the assessment of threat in fishes (Brown et al., 2011). Responses to alarm cues have been extensively studied in the convict cichlid (*Amatitlania nigrofasciata*), a species of Central American fish. Convict cichlids exposed to alarm cues increased antipredator behaviours such as freezing and also decreased the frequency of aggressive interactions (Wisenden and Sargent, 1997). Convict cichlid fry, whether or not independent from parental care, engaged in more grouping behaviours and avoided areas in which alarm cues were present. Pollock and colleagues (2005) showed that convict cichlids exposed to alarm cues ingested less food and engaged in less movement than when exposed to a distilled water control. In addition, after 41 days of exposure to alarm cues, fish grew significantly less and reproduced faster than controls. Reddon and Hurd (2009a) showed a sex difference in lateralized response to alarm cues in convict cichlids. Males had a stronger eye bias than females when viewing a stimulus that had been previously paired with alarm cues. When alarm cues were actually present in lateralization tasks, females, but not males, showed stronger eye preference both when viewing an empty environment and when viewing their own mirror image (Chapter 4 of this thesis). It is clear that alarm cues are perceived as a substantial stressor in this species and have dramatic effects on behaviour and physiology. However, no studies to my knowledge have looked at the effect of prior exposure to stressors on subsequent behavioural response to alarm cues in this species.

The habenula, a highly conserved neural pathway in vertebrates, connects limbic regions of the forebrain with motor pathways in the hindbrain (see Bianco & Wilson, 2009 and Hikosaka, 2010 for reviews). This nucleus is involved in inhibition of behaviour when an aversive outcome is anticipated as well as in behavioural responses to pain, stress, and anxiety (reviewed in Hikosaka, 2010). Work on rodents has shown that animals pre-exposed to strong stressors fail to display avoidance behaviour even when this failure results in continued exposure to painful stimuli (Overmier & Seligman, 1967; Weiss & Glazer, 1975). Lesioning of the habenula in rodents has led to a decrease in this maladaptive avoidance deficit (Amat et al., 2001). Recent work on zebrafish, an increasingly used model species for the study of anxiety, has shown that disrupting neural circuitry in the habenula leads to deficits in avoidance behaviour when pre-exposed to stressors in the form of inescapable shocks (Lee et al., 2010). Zebrafish studies have also shown that asymmetry in the habenula, measured via left or right position of the unpaired parapineal organ, is associated with boldness (Barth et al., 2005). Convict cichlids are one of only two species in which fine-grain asymmetry in the habenula has been studied. This asymmetry was related to behavioural asymmetries in eye preference (Gutiérrez-Ibáñez et al., 2011; Chapter 4 of this thesis). In addition, a link between boldness and lateralized eye preference has been found in convict cichlids (Reddon & Hurd, 2009b). However, to date no studies have examined the relationship between fine-grain individual differences in habenular asymmetry and boldness. Moreover, no work has examined how asymmetry in the habenula may relate to behaviour after prior exposure to stressors.

In the current study, I assessed the effect of prior exposure to a stressor, net chasing, on the behaviour of convict cichlid fish in an open field task. I examined whether prior and current stress exposure affected plasticity of behaviour differently based on the initial position of fish along the boldness-shyness continuum. Lastly, I investigated the relationship between directional or absolute asymmetry in the habenula and boldness and open field behaviour when stressors were previously or currently experienced. My hypotheses were fourfold. I hypothesized that, (1) based on the work of Brown and colleagues (2007), net chasing would lead to increased boldness in the open field task; (2) drawing on stress-coping literature in fish, fish that were initially assessed as bold would show less plasticity in behaviour in the open field when a stressor was present compared to shy fish; (3) based on previous research in our lab (Reddon & Hurd, 2009a; Chapter 4 of this thesis), there would be a sex difference in response to alarm cues in the open field; and (4), convict cichlids would have left-biased habenula (Gutiérrez-Ibáñez et al., 2011; Chapters 3 & 4 of this thesis) and that there would be a relationship between asymmetry in the habenula and boldness.

5.2 Materials and Methods

5.2.1 Subjects

I used 128 convict cichlid fish in this experiment, 64 males and 64 females. No fish had prior experience in either testing apparatus. Fish were obtained from commercial fish suppliers in Edmonton, AB and were fed dry prepared cichlid flakes *ad libitum* daily five days a week. Prior to testing, fish were housed communally in a large 460 L (184 cm X 49 cm X 51 cm) stock tank for at least one month. Water temperature was maintained at 26 ˚C ± 2 ˚C and fluorescent lights above the tank were maintained on a 12L:12D schedule. All protocols were approved by the University of Alberta Biological Sciences Animal Policy and Welfare Committee (protocol number 5441006).

5.2.2 Treatment Conditions

I had one stress condition (Net Stress) and a Control condition. Control fish received no treatment between testing in the boldness task (see section 5.2.3) and the open field task (see section 5.2.4). Net Stress fish were chased by a dip net for two minutes a day for 10 days after testing in the boldness task and before testing in the open field task.

I had a Treatment and Control condition for the open field task (see section 5.2.4). In the Treatment condition, damage-induced alarm cues were added to the water in the open field tank; in the Control condition there were no alarm cues present in the tank water. I used these treatments to determine the effects of prior exposure to stress on behaviour in the presence or absence of a stressor. In total I tested 68 Control fish, 34 males and 34 females, and 60 Net Stress fish, 30 males and 30 females. Half the subjects in each group were assigned to the open field Control condition and half to the open field Treatment condition (see sections 5.2.3, 5.2.4.2, and 5.2.5).

5.2.3 Boldness Task

5.2.3.1 Rankings

Fish were initially tested in a boldness task; a standard behavioural assay used to assess placement of fish on the shyness-boldness continuum (Toms et al., 2010). The latency to emerge from a covered area into an open area was scored for each fish. Fish were ranked based on latency scores; a shorter latency indicated a bolder fish (i.e., the boldest fish was given a rank of one). The sexes and treatment groups were ranked separately (i.e., female Control fish had a separate set of rankings from male Net Stress fish). Fish were ranked in order to pair similarly ranked fish and randomly assign one of the pair to each open field condition (Control or Treatment) via a coin flip. I used this method to ensure I would have an equal distribution of boldness scores in each open field condition (see section 5.2.3). Fish were then placed in housing tanks to receive the stress treatment. Fish were distributed into tanks, 5 fish per tank, with 2-3 Control fish and 2-3 Treatment fish per housing tank. All fish were given unique combinations of coloured lines using elastomer dye (Visible Implant Elastomer, Northwest Marine Technology Inc., Shaw Island, WA, USA) injected under the scales for individual identification.

5.2.3.2 Apparatus

The boldness apparatus consisted of a 40 L tank (26.5 cm X 51 cm X 31 cm) filled with 1 cm of aquarium sand and 11 cm of water. An opaque black plexiglass enclosure (20 cm X 20.5 cm X 26 cm) with a steel base (25 cm X 25.5 cm) was placed inside the tank against one wall. The front face of the enclosure was a sliding door (19.5 cm X 25 cm) that could be opened by an experimenter pulling a string behind a curtain so as not to disturb the fish. The apparatus was lit from above by a 30 w fluorescent light. All trials were recorded by a video camera (JVC Everio GZ-MG335, Americas Corp, Wayne, NJ, USA) recording through a hole in a white screen.

5.2.3.3 Procedure

A trial consisted of gently capturing a fish from the stock tank in a dip net and placing the fish in the plexiglass enclosure with the sliding door shut. The fish was given a two minute acclimation period to recover from being captured and moved. After two minutes, the sliding door was raised and the latency for the fish to emerge from the enclosure was recorded. Trials were conducted for a maximum of 5 minutes – if fish did not emerge within this time they were given a ceiling value of 300 seconds. 13 Control fish, 7 males and 6 females, and 7 Net Stress fish, 2 males and 5 females, for a total of 20 out of 128 fish did not emerge from the enclosure within 5 minutes and received this ceiling value.

After testing in the boldness task, fish were moved to smaller 100 L (32 cm X 75.5 cm X 41 cm) housing tanks (see section 5.2.3.1). Tanks were equipped with 5 clay pots, an air stone, a carbon filter, and sand medium. Males were housed separately from females and different treatment groups were housed separately. All tanks were fed 0.30 g of dry prepared cichlid flakes daily. Fish remained in these housing conditions, and received their respective stress treatments (see section 5.2.2), for 10 days until they were tested in the open field task.

5.2.4 Open Field Task

5.2.4.1 Apparatus

The open field apparatus consisted of a 40 L tank (26.5 cm X 51 cm X 31 cm) filled with 11 cm of water. Underneath the tank was a plastic sheet on which a 5 x 10 grid of 5 cm x 5 cm squares was drawn. This grid covered the entire bottom area of the tank. In the middle of the grid was a circle on which an acclimation enclosure was placed. The acclimation enclosure was a piece of 9 cm diameter tubing cut to 6.5 cm tall siliconed to an 11 cm x 11 cm white ceramic tile. The acclimation enclosure was attached to a string so it could be lifted from behind a curtain. The apparatus was lit from above by a 30 w fluorescent bulb. Trials were recorded from above by a webcam (Logitech QuickCam V-

UBV49, Logitech, Apples, Switzerland) attached to a laptop running the program guvcview (1.2.0, Assis, 2008).

5.2.4.2 Procedure

A trial consisted of gently catching a fish from its housing tank in a dip net and placing it in the acclimation enclosure in the open field tank. The fish was given a two minute acclimation period to recover from being captured and moved. After two minutes, the string was pulled to remove the acclimation apparatus. The fish was allowed to swim freely around the tank for five minutes.

After the trial was completed the recording was scored using JWatcher (Blumstein et al. 2010). As a measure of exploration, I counted the total number of squares travelled by the fish (Toms et al., 2010). A square counted as being travelled if the fish passed through the square up to the opercula. As a measure of boldness, I calculated the time spent in the corners of the tank (Toms et al., 2010).

5.2.5 Damage-Induced Alarm Cues

5.2.5.1 Preparation

I followed the methods of Pollock and colleagues (2005) and Reddon and Hurd (2009a) to collect damage-induced alarm cues. 30 fish were used as donors (15 males and 15 females; standard length (Mean ± SD) = 5.08 cm ± 0.90 cm) to prepare a stock solution of alarm cues. Fish were decapitated with scissors and a fillet of skin was taken from each side of the body using a scalpel. Fillets were finely chopped with scissors and placed into a beaker filled with 420 mL of chilled distilled water. The solution was filtered through 125 mm diameter filter paper to remove any particulate matter and was then diluted with an additional 840 mL of distilled water to bring the final volume to 1,260 mL. The stock solution was divided into 15 mL aliquots and frozen at -20˚C until used.

5.2.5.2 Administration to Apparatus

I administered alarm cues to the open field tank for Treatment fish by placing two lengths of tubing on opposite sides of the tank and injecting 7.5 mL of alarm cue mixture into each length of tubing. An air stone was attached to each length of tubing, the tubing was attached to an aerator, and air was pumped through the tubing for two minutes. Dye tests indicated this procedure dispersed the alarm cue substance throughout the tank. After two minutes, a clean dip net was used to further mix the tank water to ensure an even distribution of alarm cues. When filled to a depth of 11 cm, the open field tank contained 15 L of water; the final concentration of alarm cues was therefore 1 mL alarm cue mixture / 1 L water. For the Control condition, the same procedure was followed except 7.5 mL of water was injected into each length of tubing instead of alarm cues.

5.2.6 Neuroanatomical Measurements

Following open field testing all fish were sacrificed by decapitation. Fish heads were stored in 4% paraformaldehyde solution until the brains were extracted. Extracted brains were placed in a 30% sucrose solution in 0.1 M phosphate buffered saline (PBS) for 24 hours for cryoprotection, embedded in gelatin, placed in a 30% sucrose solution for an additional 24 hours, frozen via dried ice, and sectioned to a thickness of 30 µm on a freezing-stage microtome. Sections were stored in 0.1 M PBS-filled well plates before being mounted on gelatinized slides. After drying, slides were stained with thionin, dehydrated through a graded ethanol series, cleared using Hemo-D, and coverslipped using Permount (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011).

Brain sections were examined under a compound light microscope (Leica DMRE, Rich-mond Hill, ON). Images were captured of every section of the two nuclei of interest (the habenula and a control nucleus, the cortical pretectal nucleus (COPn)) throughout the rostro-caudal extent of each nucleus using a camera (Retiga EXi FAST Cooled mono 12-bit camera; Qimaging, Burnaby, BC) mounted on the microscope.

The areas of the left and right hemispheres of the habenula and the COPn were measured for each brain section using Image J (U.S. National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/). These areas were then multiplied by the thickness of the sections (30 µm) and summed together to determine the volume of the nuclei (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011). Habenula and COPn measurements were taken for all 128 fish included in this experiment.

I calculated a laterality index for each nucleus in each fish using the following formula:

$$\text{Laterality Index (LI)} = \frac{\text{Volume of Right Nucleus} - \text{Volume of Left Nucleus}}{\text{Volume of Right Nucleus} + \text{Volume of Left Nucleus}}$$

I also calculated the absolute value of the laterality index (ALI) as a measure of the strength of side bias regardless of the direction of that bias (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011).

5.2.7 Data Analysis

Analyses were performed using R v. 2.12.2 (R Core Development Team, Vienna, Austria). All two sample comparisons were assessed using two-tailed *t*-tests which employed the Welch-Satterthwaite correction (Ruxton, 2006). Pearson correlations were used to assess the relationship between size and emerge time and between habenular asymmetry and emerge time. I used the Anova() function (car package; Fox & Weisberg, 2011) in R to analyze the open field and emerge time data. I also used this function to analyze how behaviour in the open field was influenced by habenular asymmetry. I used type II sums of squares when testing effects that had no *a priori* rationale for hypothesized interactions and type III sums of squares analyses when there was a biological rationale for expecting an interaction or when an interaction would have been meaningful.

To assess neuroanatomical asymmetry I used one-sample two-tailed *t*-tests on LI and ALI values to investigate the population level direction and strength of asymmetry in both the habenula and the COPn. I used an α level of 0.05 to determine significance.

5.3 Results

5.3.1 Fish Size

There was no significant difference in the weight of fish after the boldness task between the control and stress pre-exposure treatment (Mean ± SD: Control = 4.36 ± 2.12; Net Stress = 4.61 ± 1.95; $t(126.99) = -0.708$, $p = 0.480$). Fish also did not differ in weight between the control and stress pre-exposure treatment after open field testing (Mean ± SD: Control = 4.48 ± 2.11; Net Stress = 4.91 ± 2.27; $t(125.67) = -1.146$, $p = 0.254$), which indicated that net chasing for two weeks did not adversely affect growth during the experiment.

5.3.2 Boldness Task

The time taken for both females and males to emerge into a novel environment ranged from one to the maximum 300 seconds (Mean ± SD: Females = 88.71 ± 107.14s; Males = 86.37 ± 101.41s). There was no significant difference between the sexes in emerge time ($t(127.99) = 0.129$, $p = 0.897$), indicating equivalent boldness levels between the sexes. I found no significant relationship between size and emerge time in males (Weight: $r(62) = -0.226$, $p = 0.075$; Length: $r(62) = -0.192$, $p = 0.143$) or females (Weight: $r(62) = -0.147$, $p = 0.231$; Length: $r(62) = -0.234$, $p = 0.061$), although there was a non-significant trend for larger fish to emerge faster. I found no significant difference in emerge time between the control and stress pre-exposure treatment fish ($t(125.99) = 1.065$, $p = 0.289$).

5.3.3 Open Field Task

I had *a priori* reasons to believe sex would affect response to alarm cues (see Chapter 4 of this thesis), so I performed separate analyses for males and females. I also conducted separate analyses for the dependent variables of Total Squares Travelled and Corner Time. In these models, I included Emerge Time, Stress Treatment, and Open Field Treatment as independent variables.

I found a significant main effect of Stress Treatment on Total Squares Travelled for both males and females (Males: $F(1,56) = 3.909$, $p = 0.050$; Females: $F(1,56) = 4.641$, $p = 0.035$), such that fish previously chased with a net visited more squares in the open field task than control fish (Males: Mean ± SEM: Net Stress: 258.40 ± 27.53 squares; Control: 194.59 ± 21.17 squares; Females: Mean ± SEM: Net Stress: 259.29 ± 22.01 squares; Control: 182.35 ± 25.16 squares; Table 5-1a,b; Fig. 5-1).

In males, I found a significant main effect of Stress Treatment on Corner Time ($F(1,56) = 4.958$, $p = 0.030$; Table 5-2a). Males that had been previously chased with a net for two weeks spent significantly less time in corner squares than control males (Mean ± SEM: Net Stress: 126.58s ± 12.20s; Control: 155.60s ± 8.74s; $p = 0.047$). I did not find this same effect for females ($F(1,56) = 1.075$, $p = 0.304$; Fig. 5-2).

When I examined Emerge Time in males, I found a main effect of Emerge Time on Corner Time ($F(1,56) = 7.584$, $p = 0.008$) such that males that emerged quicker in the boldness task spent less time in corner squares in the open field task (Table 5-2a; Fig. 5-3).

For female fish, I did not find significant main effects for any of the independent variables on Corner Time. However, my analysis revealed an interaction between Emerge Time and Open Field Treatment ($F(1,56) = 7.826$, $p = 0.007$; Table 5-2b). In the control condition, females that emerged quicker spent less time in corner squares when there were no alarm cues in the open field task. In contrast, when alarm cues were present in the open field task, females in the control condition that emerged faster spent more time in corner squares (Fig. 5-4).

5.3.4 Neuroanatomical Asymmetry

The lateralization index (LI) of the habenula ranged from -0.36 to 0.12 (Mean ± SD: Females: -0.06 ± 0.06; Males: -0.06 ± 0.07). Only 4 of 64 females and 7 of 64 males presented right-biased habenulae. The absolute laterality index (ALI) for the habenula ranged from 0.001 to 0.36 (Mean ± SD: Females: 0.07 ± 0.05; Males: 0.08 ± 0.05). Both female and male habenular LI and ALI were significantly different from 0 (Female: LI: $t(63) = -8.561$, $p < 0.001$; ALI: $t(63) = 11.473$, $p < 0.001$; Male: LI: $t(63) = -6.872$, $p <$

0.001; ALI: $t(63) = 11.372$, $p < 0.001$; Fig. 5-5), which indicated that both females and males had significantly larger left habenulae. There was no difference between the sexes in either habenular LI or ALI (LI: $t(118.22) = 0.098$, $p = 0.922$; ALI: $t(123.03) = -0.998$, $p = 0.320$). There was no significant relationship between fish size and habenular asymmetry (LI or ALI) for either females or males (all rs < |0.113|, all ps > 0.276).

I measured the cortical pretectal nucleus (COPn) as a control nucleus to ensure any lateralized results I found regarding the habenula were not simply the result of gross hemispheric asymmetries. The lateralization index (LI) of the COPn ranged from -0.17 to 0.15 (Mean ± SD: Females: -0.004 ± 0.06; Males: 0.005 ± 0.07). The absolute laterality index (ALI) for the COPn ranged from 0.001 to 0.17 (Mean ±SD: Females: 0.04 ± 0.04; Males: 0.05 ± 0.05). The LI for the COPn for both females and males did not differ significantly from 0, indicating that habenular asymmetry was not simply the result of a larger left hemisphere in general (Female: LI: $t(63) = -0.245$, $p = 0.810$; Male: LI: $t(63) = 0.398$, $p = 0.694$). There was no difference between the sexes in either COPn LI or ALI (LI: $t(58.99) = -0.451$, $p = 0.655$; ALI: $t(56.65) = -1.008$, $p = 0.320$). There was no significant relationship between fish size and COPn asymmetry (LI or ALI) for either females or males (all rs < |0.254|, all ps > 0.183).

5.3.5 Comparison between Neuroanatomical Asymmetry and Behaviour

I found no significant relationship between habenular asymmetry and boldness in either females or males (all rs < |0.207|, all ps > 0.176).

I again used the Anova() function (car package; Fox & Weisberg, 2011) in R to analyze the open field and habenular asymmetry data. I included Sex, Stress Treatment, Open Field Treatment, and Habenular Asymmetry as independent variables in the model and Corner Time and Total Squares as dependent variables. I found no significant main effects or interactions when examining the relationship between habenular asymmetry and behaviour in the open field task (see Tables 5-3a-d).

5.4 Discussion

I have shown that prior exposure to the stress of net chasing can influence later behaviour in an open field task and that this behaviour can also be influenced by sex and alarm cues present in the open field task. Recent experience with net stress resulted in both male and female fish travelling through more squares in the open field task regardless of whether a stressor was present in that task. Males that had been net chased spent less time in corner squares than un-chased males. Females and males both showed consistency in boldness behaviour across tasks; fish that emerged more quickly into a novel environment also spent less time in corner squares in the open field task. In females that were chased with a net prior to open field testing, the addition of alarm cues to the open field tank did not affect the relationship between emerge time and time spent hiding in corners; however, when females were not pre-exposed to the stressor of net chasing the addition of alarm cues had a dramatic effect on this relationship. This effect was not seen in males. Finally, I showed that both male and female convict cichlids have left-biased habenula and that habenular asymmetry was unrelated to open field behaviour regardless of sex or experience with stressors.

The fish in this study, particularly males, showed an increase in boldness-related behaviours after experience with the stressor of net chasing, as evidenced by greater exploration of the open field and less time spent immobile in corners. This result may seem counterintuitive, as previous experience with stress or predation would likely indicate the environment is frightening and anti-predator responses such as freezing should be employed; however, this is not generally the case. Many studies have shown that animals exposed to high levels of predation, or previously exposed to stressors, subsequently behave more boldly when again faced with stressors. For example, in the bishop *B. episcopi*, Brown and colleagues (2007) found that fish from areas of high predation pressure behaved more boldly than their low-predation counterparts. In addition, both high and low predation fish that had been chased by a net, using the same procedure I employed in this study, showed increased boldness over fish that had not been net chased. Research has shown that fish living in areas of high predation pressure are bolder than those living with fewer predators (e.g., *G. aculeatus*, Brydges et al., 2008; *Poecilia reticulata*, Harris et al., 2010; adult *Perca fluviatilis*, Magnhagen & Borcherding, 2008). More recently, Niemela and colleagues (2012) found that, after exposure to a frog predator, previously shy crickets emerged more quickly into a novel environment. Similar results were found with lizards (*Lacerta monticola)* that spent less time in a refuge after exposure to a predator (Lopez et al., 2005). Potential explanations for an increase in boldness in animals that have substantial experience with predators stem from the idea that in a high predation area organisms have no choice but to carry out the functions of mating and foraging under constant threat; therefore, they must be bold in the face of threats in order to maintain fitness. As such, high predation fish must be bold while experiencing threats in order to balance the trade-offs between hiding from predators and the costs to fitness that result from predator avoidance (Lima & Dill, 1990).

Both male and female convict cichlids showed a consistent boldness trait across tasks in this experiment. Fish that emerged more quickly into a novel environment also spent less time hiding in corner squares in the open field test. Hiding in corners in a novel environment with no other refuges available, or freezing in general, is a species-typical fear or anti-predator response in most fish. The boldness-shyness continuum is a well-studied and prevalent behavioural trait in fish (for reviews see Toms et al., 2010; Conrad et al., 2011). A consistency in boldness across tasks has been shown in juvenile convict cichlids that were attacked by a robotic predator while foraging. Fish that were slow to respond to the predator during foraging were quicker to explore a novel environment when no predation threat was present (Jones & Godin, 2010).

I found that, in female fish but not in males, the relationship between time to emerge into a novel environment and time spent in corner squares in the open field was disrupted by the presence of alarm cues in the open field tank. This result may suggest that a consistency in boldness is adaptive in females but perhaps constrained in males (i.e., it may not be adaptive for fish to be bold in the presence of threat cues). Alternatively, the presence of alarm cues may be interpreted differently by males and females. I have previously shown that female convict cichlids show greater changes in behaviour in response to alarm cues than males (Chapter 4 of this thesis). It is currently unclear whether this difference in response may be due to a difference in the assessment of the threat posed by alarm cues or to a difference in the ability to detect alarm cues between the sexes. Thus far, two studies examining the response of convict cichlids to alarm cues have not reported sex differences in detection ability (although these studies were conducted with juvenile fish; Brown et al., 2004; Roh et al., 2004). Determining the origin of the different responses of males and females to alarm cue threat will be a first valuable step to elucidating the mechanisms underlying consistency in boldness and the potential sex differences therein.

Females that were pre-exposed to net chasing did not show a disruption in the relationship between emerge time and corner hiding time when alarm cues were present in the open field tank. This result suggests that pre-exposure to a stressor allowed females to maintain consistent behaviour later when either in the presence or absence of a stressor. Animal models of stress, particularly using mice and rats, have identified two types of reactivity to stressors: resilience and vulnerability (Ricon et al., 2012; Scharf & Schmidt, 2012). Exposure to early moderate stressors tends to result in resilience while exposure to early chronic or unpredictable stressors tends to result in vulnerability to future stressors (Plotsky & Meaney, 1993; Liu et al., 2000). In most mammalian (i.e., rat and non-human primate) studies of early life stress, the young are separated from the mother for extended periods of time to induce stress. This type of prolonged and intense juvenile stress is generally found to result in maladaptive endocrine and behavioural changes such as elevated stress hormones, anxiety, and learning deficits (see Lupien et al., 2009 and Pryce & Feldon, 2003 for reviews). In contrast, recent studies have shown that stressful challenges during early development can also promote resilience and an enhanced ability to regulate arousal. This phenomenon has been deemed ‘stress inoculation-induced resilience’ (Lyons & Parker, 2007) and primarily results when the stressors are challenging but not overwhelming (Gunnar et al., 2009). This resilience can result in improved stress-coping abilities in later life. Boyce and Ellis (2005) suggest that environments with moderate levels of stress facilitate lower stress reactivity while environments either high or low in stressors lead to heightened stress reactivity. Primarily early environments have been studied in stress resilience research but it is possible that the brief exposure to a moderate stressor in these fish was adequate to provide subsequent resilience to another moderate stressor, negating the effect of alarm cues on open field behaviour in females.

Another potential explanation for the difference in behaviour between net chased and control females is that the females habituated to the moderate stressor of net chasing over the ten day treatment period. This habituation may have transferred to the alarm cue stressor in the subsequent open field task; but then, habituation may simply be another word for resilience in this context. Males showed very little effect of pre-exposure to net chasing or to the presence of alarm cues in the open field tank. It may be that male convict cichlids are already more resilient to stressors than females. This resilience may come from the more prominent role of males in defending territories and broods against predators in the wild in this species. Males spend more time engaged in stressful predator-attack encounters, while females more often split time between helping the male defend the nest and herding the brood into the nest and remaining there with them during a predatory attack (Wisenden, 1995; pers. obs.) Thus, males may have more experience with direct stress such as net chasing and a greater ability to prevent mild stressors from affecting behaviour than females.

I found leftward-biased habenula in both male and female convict cichlids, which has been previously reported elsewhere (Chapters 3 and 4 of this thesis; Gutiérrez-Ibáñez et al., 2011). A proposed function of the habenula is inhibition of motor behaviour when an organism anticipates a negative outcome (Hikosaka, 2010). A proposed function of the left hemisphere is inhibition of responses (Andrew & Rogers, 2002; Miklósi & Andrew, 1999). Following this logic, it has previously been proposed (Chapter 4 of this thesis) that fish with larger left habenulae may show greater inhibition while exploring an open field arena. I did not find this to be true whether or not fish were exposed to prior or current stressors. Dadda and colleagues (2010) showed that zebrafish in which the parapineal organ was located on the right side, which results in a larger and differentially innervated right habenula, were bolder and spent less time in the periphery of an open field than fish with the typical leftward positioned parapineal organ. It is possible that fine-grain asymmetry in the area of the habenula, as I measured here, does not influence behaviour to the same degree as gross habenular asymmetry resulting from reversed parapineal position. In light of the involvement of the right hemisphere in processing negative emotions such as stress (Gainotti, 2012; Leliveld et al., 2013; Rogers, 2010), it is intriguing to suggest that convict cichlids with right-biased habenulae may show different boldness behaviour and response to stressors than their typical left-habenular biased conspecifics. However, due to the fact that I only found 4 females and 7 males with right-biased habenula in my sample of 128 fish I am not able to speak to this possibility. All fish examined in this study were supplied from pet stores and raised in captivity with no previous experience with predators. This lack of stressful experience could be a factor in producing the predominantly leftward-biased habenulae I have found in this species. To my knowledge, no data are available that examine asymmetry in the habenula of wild convict cichlids that have predator experience. Further study is needed to determine if ontogenetic experience with stressors affects asymmetry in the habenula, perhaps creating a rightward-bias, and whether this asymmetry may be related to responses to early and later environmental stressors.

5.5 Tables

Table 5-1a. Results of type II sums of squares analyses of Total Squares Travelled by male fish in the open field task. Emerge Time (time to emerge from the boldness task), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as independent variables.

Source of Variation	df	*F*	*p*
Emerge Time	1,56	0.171	0.681
Stress Treatment	1,56	3.909	0.050*
Open Field Treatment	1,56	0.890	0.350
Emerge Time x Stress Treatment	1,56	0.332	0.567
Emerge Time x Open Field Treatment	1,56	0.923	0.341
Stress Treatment x Open Field Treatment	1,56	3.494	0.067

* indicates a significant result at $p < 0.05$.

Table 5-1b. Results of type II sums of squares analyses of Total Squares Travelled by female fish in the open field task. Emerge Time (time to emerge from the boldness task), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as independent variables.

Source of Variation	df	*F*	*p*
Emerge Time	1,56	1.812	0.183
Stress Treatment	1,56	4.641	0.035*
Open Field Treatment	1,56	0.015	0.903
Emerge Time x Stress Treatment	1,56	2.159	0.147
Emerge Time x Open Field Treatment	1,56	0.487	0.488
Stress Treatment x Open Field Treatment	1,56	0.004	0.949

* indicates a significant result at $p < 0.05$.

Table 5-2a. Results of type II sums of squares analyses of Corner Time in the open field task for male fish. Emerge Time (time to emerge from the boldness task), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as independent variables.

Source of Variation	df	*F*	*P*
Emerge Time	1,56	7.584	0.008**
Stress Treatment	1,56	4.958	0.030*
Open Field Treatment	1,56	0.243	0.624
Emerge Time x Stress Treatment	1,56	3.268	0.076
Emerge Time x Open Field Treatment	1,56	1.069	0.306
Stress Treatment x Open Field Treatment	1,56	0.803	0.374

* indicates a significant result at $p < 0.05$.

** indicates a significant result at $p < 0.01$.

Table 5-2b. Results of type III sums of squares analyses of Corner Time in the open field task for female fish. Emerge Time (time to emerge from the boldness task), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as independent variables.

Source of Variation	df	*F*	*P*
Emerge Time	1,56	2.273	0.137
Stress Treatment	1,56	1.075	0.304
Open Field Treatment	1,56	0.807	0.372
Emerge Time x Stress Treatment	1,56	2.846	0.097
Emerge Time x Open Field Treatment	1,56	7.826	0.007**
Stress Treatment x Open Field Treatment	1,56	0.323	0.572

** indicates a significant result at $p < 0.01$.

Table 5-3a. Results of type II sums of squares analyses of Total Squares Travelled in the open field task. Habenular LI (directional asymmetry in the habenula), Sex (male or female), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as independent variables.

Source of Variation	df	*F*	*p*
Habenular LI	1,112	0.409	0.524
Sex	1,112	0.037	0.848
Stress Treatment	1,112	8.078	0.005**
Open Field Treatment	1,112	0.166	0.684
Habenular LI x Sex	1,112	0.001	0.991
Habenular LI x Stress Treatment	1,112	1.249	0.266
Sex x Stress Treatment	1,112	0.006	0.938
Habenular LI x Open Field Treatment	1,112	0.585	0.446
Sex x Open Field Treatment	1,112	0.670	0.405
Stress Treatment x Open Field Treatment	1,112	1.944	0.166

** indicates a significant result at $p < 0.01$.

Table 5-3b. Results of type II sums of squares analyses of Total Squares Travelled in the open field task. Habenular ALI (strength of asymmetry in the habenula), Sex (male or female), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as independent variables.

Source of Variation	df	*F*	*p*
Habenular ALI	1,112	1.657	0.201
Sex	1,112	0.001	0.990
Stress Treatment	1,112	7.590	0.007**
Open Field Treatment	1,112	0.331	0.566
Habenular ALI x Sex	1,112	0.045	0.832
Habenular ALI x Stress Treatment	1,112	2.169	0.144
Sex x Stress Treatment	1,112	0.001	0.990
Habenular ALI x Open Field Treatment	1,112	0.581	0.447
Sex x Open Field Treatment	1,112	0.887	0.348
Stress Treatment x Open Field Treatment	1,112	1.739	0.190

** indicates a significant result at $p < 0.01$.

Table 5-3c. Results of type II sums of squares analyses of Corner Time in the open field task. Habenular LI (directional asymmetry in the habenula), Sex (male or female), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as independent variables.

Source of Variation	df	*F*	*p*
Habenular LI	1,112	0.033	0.855
Sex	1,112	0.276	0.600
Stress Treatment	1,112	0.031	0.861
Open Field Treatment	1,112	0.031	0.862
Habenular LI x Sex	1,112	2.723	0.102
Habenular LI x Stress Treatment	1,112	0.065	0.799
Sex x Stress Treatment	1,112	5.109	0.026*
Habenular LI x Open Field Treatment	1,112	1.051	0.308
Sex x Open Field Treatment	1,112	0.890	0.347
Stress Treatment x Open Field Treatment	1,112	1.264	0.263

* indicates a significant result at $p < 0.05$.

Table 5-3d. Results of type II sums of squares analyses of Corner Time in the open field task. Habenular ALI (strength of asymmetry in the habenula), Sex (male or female), Stress Treatment (control or net stressed), and Open Field Treatment (control or in the presence of alarm cues) were included as independent variables.

Source of Variation	df	*F*	*p*
Habenular ALI	1,112	0.006	0.937
Sex	1,112	0.326	0.569
Stress Treatment	1,112	0.085	0.772
Open Field Treatment	1,112	0.003	0.954
Habenular ALI x Sex	1,112	1.746	0.189
Habenular ALI x Stress Treatment	1,112	0.335	0.564
Sex x Stress Treatment	1,112	4.747	0.031*
Habenular ALI x Open Field Treatment	1,112	3.170	0.078
Sex x Open Field Treatment	1,112	0.858	0.356
Stress Treatment x Open Field Treatment	1,112	1.136	0.289

* indicates a significant result at $p < 0.05$.

5.6 Figures

Figure 5-1. Fish that were chased with a net for two minutes a day for 10 days (dark grey bars) travelled through more total squares in the open field task than fish that had not been net chased (open bars). This pattern was true for both male ($p = 0.050$) and female ($p = 0.035$) fish. Bars show Mean ± SEM. N = 34 for each Control bar and 30 for each Net Stress bar.

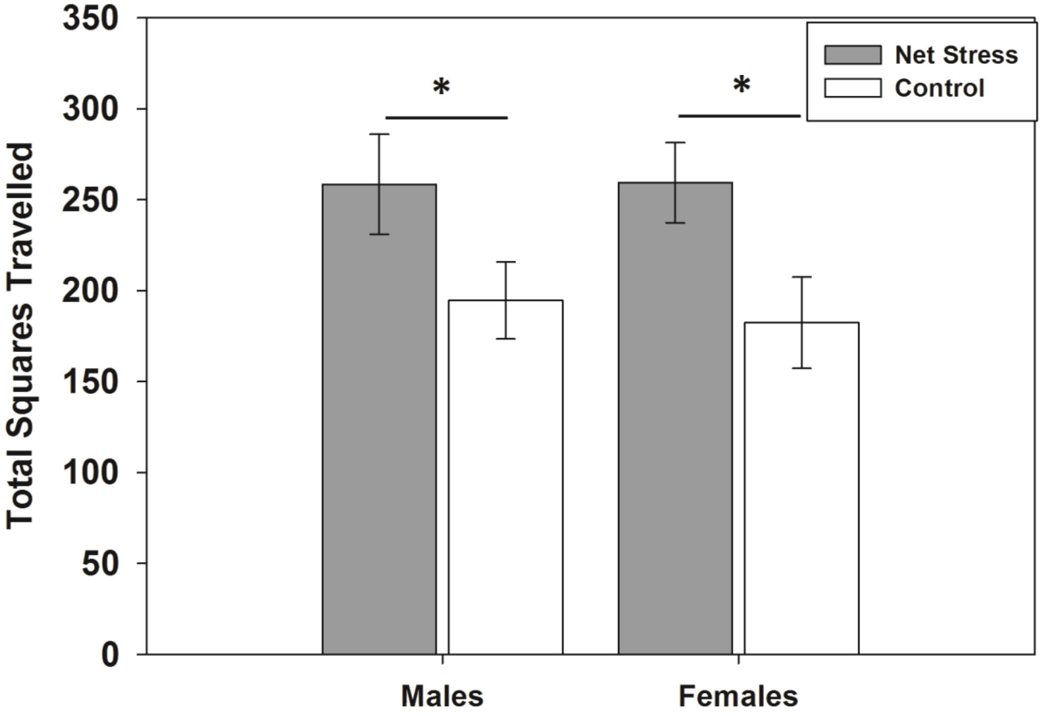


Figure 5-2. Male fish that were chased with a net for two minutes a day for 10 days (dark grey bars) spent significantly less time in corner squares in the open field task than male fish that were not net chased (open bars; *p* = 0.030). This effect was not found in females (*p* = 0.304). Bars show Mean ± SEM. N = 34 for each of the Control bars and 30 for each of the Net Stress bars.

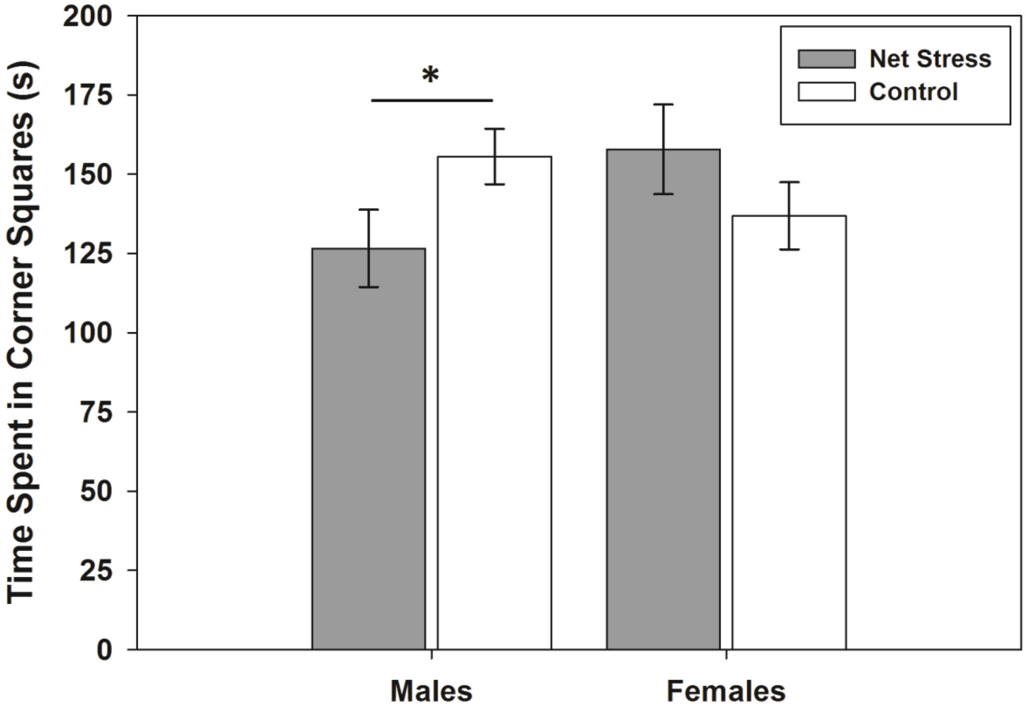


Figure 5-3. I found a significant positive relationship between time to emerge into a novel environment and time spent in corner squares in the open field task in male fish (*p* = 0.008).

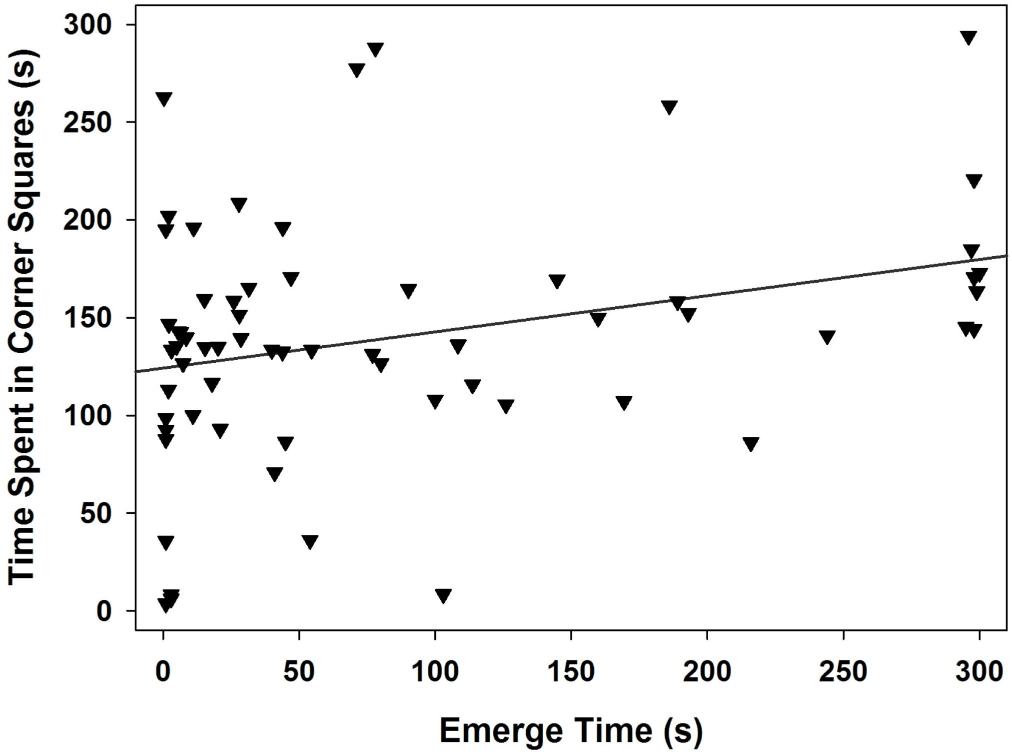


Figure 5-4. The relationship between time to emerge into a novel environment and time spent in corner squares of an open field tank in female fish in 4 different stress conditions. (1) Not pre-exposed to net chasing and alarm cues not present in the open field tank (Ctl, Ctl; filled circles, solid line); (2) Not pre-exposed to net chasing and alarm cues present in the open field tank (Ctl, AC; open inverted triangles, long dashed line); (3) Chased with a net and alarm cues not present in the open field tank (Net, Ctl; filled squares, medium dashed line); (4) Chased with a net and alarm cues present in the open field tank (Net, AC; open diamonds, short dashed line). The relationship between emerge time and time spent in corner squares was modulated by the presence of alarm cues in the open field tank in female fish that were not pre-exposed to the net chasing stressor (solid line vs. long dashed line, $p = 0.007$).

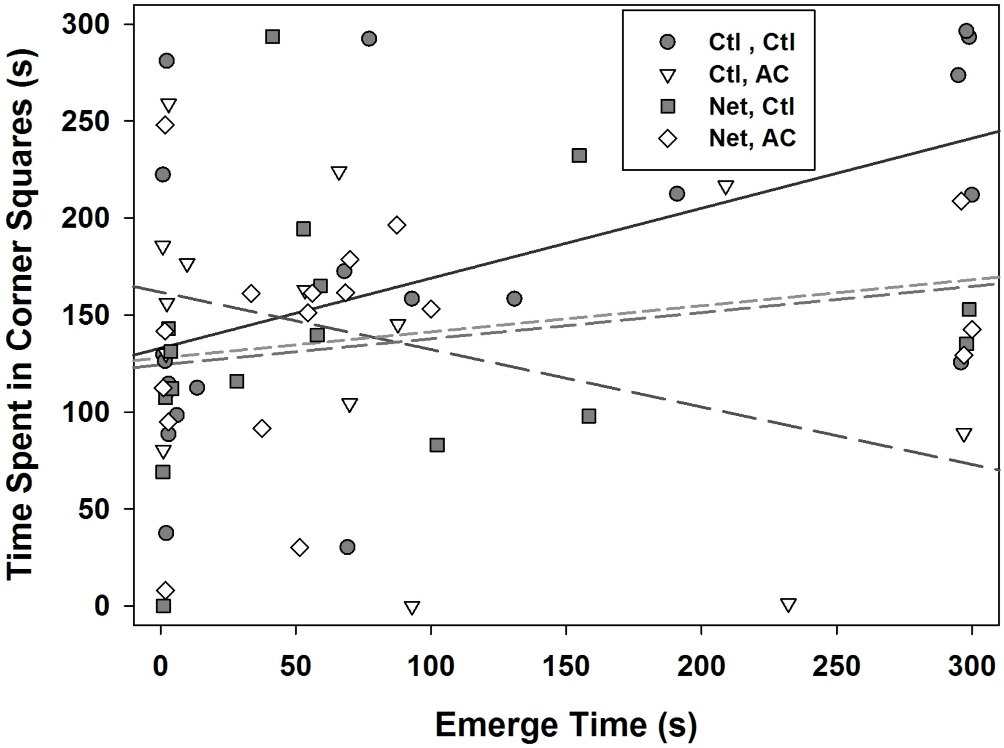
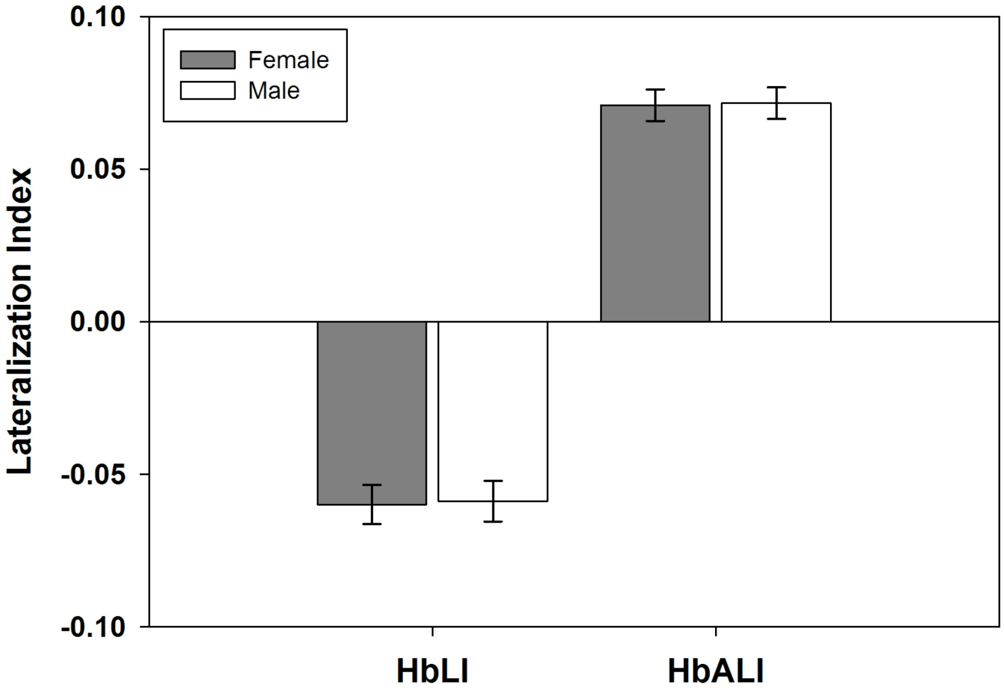


Figure 5-5. Both female and male fish had significantly left-biased habenulae at the population level (HbLI: Females = dark grey bars, $p < 0.001$; Males = open bars, $p < 0.001$). There was no difference between the sexes in either direction or strength of lateralization of the habenula. Both females and males showed significant asymmetry of the habenula at the individual level (HbALI: $p < 0.001$ for both). Bars show Mean ± SEM. N = 64 for each sex.



5.7 References

Amat, J., Sparks, P.D., Matus-Amat, P., Griggs, J., Watkins, L.R., & Maier, S.F. (2001). The role of the habenular complex in the elevation of dorsal raphe nucleus serotonin and the changes in the behavioral responses produced by uncontrollable stress. *Brain Research, 917,* 118–126.

Andrew, R.J., & Rogers, L.J. (2002). The nature of lateralization in tetrapods. In L. J. Rogers, & R.J. Andrew (Eds.), *Comparative Vertebrate Lateralization* (pp. 94-125). Cambridge: University Press.

Archard, G.A., Earley, R.L., Hanninen, A.F., & Braithwaite, V.A. (2012). Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. *Functional Ecology, 26,* 637-645.

Assis, P. (2008). guvcview v.1.2.0 [computer program]. http://guvcview.sourceforge.net/index.html. Accessed 26 August 2013.

Barth, K.A., Miklosi, A., Watkins, J., Bianco, I.H., Wilson, S.W., & Andrew, R.J. (2005). *fsi* zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. *Current Biology, 15,* 844-850.

Bianco, I.H., & Wilson, S.W. (2009). The habenular nuclei: A conserved asymmetric relay station in the vertebrate brain. *Philosophical Transactions of the Royal Society of London. Series B - Biological Sciences, 364,* 1005-1020.

Blumstein DT, Daniel JC, Evans CS (2010) JWatcher Software, http://www.jwatcher.ucla.edu/.

Boyce, W.T., & Ellis, B.J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and function of stress reactivity. *Development and Psychopathology, 17,* 271-301.

Brown, C., Burgess, F. & Braithwaite, V.A. (2007). Heritable and experiential effects on boldness in a tropical poeciliid. *Behavioural Ecology and Sociobiology, 62*, 237-243.

Brown, C., Gardner, C., & Braithwaite, V.A. (2005b). Differential stress responses in fish from areas of high- and low-predation pressure. *Journal of Comparative Physiology. Part B. Biochemical, Systematic, and Environmental Physiology, 175,* 305-312.

Brown, C., Jones, F., & Braithwaite, V.A. (2005a) In situ examination of boldness-shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Animal Behaviour, 70,* 1003–1009.

Brown, G.E., Ferrari, M.C.O., & Chivers, D. (2011). Learning about danger: Chemical alarm cues and threat-sensitive assessment of predation risk by fishes. In J. Krause, K. Laland, & C. Brown (Eds.), *Fish Cognition and Behavior* (pp. 59-80). Iowa: Wiley-Blackwell.

Brown, G.E., Foam, P.E., Cowell, H.E., Guevara Fiore, P., & Chivers, D.P. (2004). Production of chemical alarm cues in convict cichlids: the effects of diet, body condition and ontogeny. *Annales Zoologici Fennici, 41,* 487-499.

Brydges, N.M., Colegrave, N., Heathcote, R.J.P., & Braithwaite, V.A. (2008). Habitat stability and predation pressure affect temperament behaviours in populations of three-spined sticklebacks. *Journal of Animal Ecology, 77*, 229–235.

Budaev, S., & Brown, C. (2011). Personality traits and behaviour. In J. Krause, K. Laland, & C. Brown (Eds.), *Fish Cognition and Behavior* (pp. 135-165). Iowa: Wiley-Blackwell.

Burns, J. (2008) The validity of three tests of temperament in guppies (*Poecilia reticulata*). *Journal of Comparative Psychology, 122,* 344–356.

Conrad, J.L., Weinersmith, K.L., Brodin, T., Saltz, J.B., & Sih, A. (2011). Behavioural syndromes in fishes: A review with implications for ecology and fisheries management. *Journal of Fish Biology, 78,* 395-435.

Dadda, M., Domenichini, A., Piffer, L., Argenton, F., & Bisazza, A. (2010) Early differences in epithalamic left-right asymmetry influence lateralization and personality of adult zebrafish. *Behavioural Brain Research, 206,* 208–215.

Dall, S.R.X., Houston, A.I., & McNamara, J.M. (2004). The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters, 7,* 734-739.

Dingemanse, N.J., Van Der Plas, F., Wright, J., Réale, D., Schrama, M., Roff, D.A., Van Der Zee, E., & Barber, I. (2009). Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proceedings of the Royal Society B, 276,* 1285–1293.

Fox, J., & Weisberg, S. (2011). An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Gainotti, G. (2012). Unconscious processing of emotions and the right hemisphere. *Neuropsychologia, 50,* 205-218.

Gosling, S.D. (2001). From mice to men: What can we learn about personality from animal research. *Psychological Bulletin, 127,* 45-86.

Gunnar, M.R., Frenn, K., Wewerka, S.S., & Van Ryzin, M.J. (2009). Moderate versus severe early life stress: associations with stress reactivity and regulation in 10–12-year-old children. *Psychoneuroendocrinology, 34,* 62–75.

Gutiérrez-Ibáñez, C., Reddon, A.R., Kreuzer, M.B., Wylie, D.R., & Hurd, P.L. (2011). Variation in the asymmetry of the habenular nucleus correlates with behavioural asymmetry in a cichlid fish. *Behavioural Brain Research, 221,* 189-196.

Harris, S., Ramnarine, I.W., Smith, H.G., & Pettersson, L.B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos, 119,* 1711-1718.

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nature Reviews Neuroscience, 11,* 503-513.

Jones, K.A., & Godin, J-G.J. (2010). Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. *Proceedings of the Royal Society B – Biological Sciences, 277,* 625-632.

Koolhaas, J.M., Korte, S.M., de Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., & Blokhuis, H.J. (1999). Coping styles in animals: Current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews, 23,* 925-935.

Lee, A., Mathuru, A.S., Teh, C., Kibat, C., Korzh, V., Penney, T.B., & Jesuthasan, S. (2010). The habenula prevents helpless behavior in larval zebrafish. *Current*

Biology, 20, 2211-2216.

Leliveld, L.M.C., Langbein, J., & Puppe, B. (2013). The emergence of emotional lateralization: Evidence in non-human vertebrates and implications for farm animals. *Applied Animal Behaviour Science, 145,* 1-14.

Lima, S.L., & Dill, L.M. (1990). Behavioral decisions made under the risk of predation – a review and prospectus. *Canadian Journal of Zoology, 68,* 619-640.

Liu, D., Caldji, C., Sharma, S., Plotsky, P.M., & Meaney, M.J. (2000) The effects of early life events on in vivo release of norepinepherine in the paraventricular nucleus of the hypothalamus and hypothalamic-pituitary-adrenal responses during stress. *Journal of Neuroendocrinology, 12,* 5–12.

Lopez, L.P., Hawlena, D., Polo, V., Amo, L., & Mart, J. (2005). Sources of individual shy–bold variations in antipredator behaviour of male Iberian rock lizards. *Animal Behaviour, 69,* 1–9.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10,* 434–445.

Lyons, D.M., & Parker, K.J. (2007). Stress inoculation-induced indications of resilience in monkeys. *Journal of Trauma Stress, 20,* 423–33.

Magnhagen, C., & Borcherding, J. (2008). Risk-taking behaviour in foraging perch: does predation pressure influence age-specific boldness? *Animal Behaviour, 75,* 509-517.

Miklosi, A., & Andrew, R.J. (1999). Right eye use associated with decision to bite in zebrafish. *Behavioural Brain Research, 105,* 199–205.

Niemela, P.T., DiRienzo, N., & Hedrick, A.V. (2012). Predator-induced changes in the boldness of naïve field crickets, *Gryllus integer*, depends on behavioural type. *Animal Behaviour, 84,* 129-135.

Øverli, Ø., Sorensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W.J., Summers, C.H., & Nilsson, G.E. (2007). Evolutionary background for stress-coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neuroscience and Biobehavioral Reviews, 31,* 396–412.

Overmier, J.B., & Seligman, M.E. (1967). Effects of inescapable shock upon subsequent escape and avoidance responding. *Journal of Comparative and Physiological Psychology, 63,* 28–33.

Plotsky, P.M., & Meaney, M.J. (1993). Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Molecular Brain Research, 18,* 195–200.

Pollock, M.S., Zhao, X., Brown, G.E., Kusch, R.C., Pollock, R., & Chivers, D.P. (2005). The response of convict cichlids to chemical alarm cues: an integrated study of behaviour, growth and reproduction. *Annales Zoologici Fennici., 42,* 485-495.

Pryce, C.R., & Feldon, J. (2003). Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms. *Neuroscience & Biobehavioral Reviews, 27,* 57–71.

R Core Development Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. url:http://www.R-project.org/.

Raoult, V., Brown, C., Zuberi, A., & Williamson, J.E. (2012). Blood cortisol concentrations predict boldness in juvenile mulloway (*Argyosomus japonicus*). *Journal of Ethology, 30,* 225-232.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., & Dingemanse, N.J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82,* 291–318.

Reddon, A.R., Gutiérrez-Ibáñez, C., Wylie, D.R., & Hurd, P.L. (2009). The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. *Behavioural Brain Research, 201,* 223-228.

Reddon, A.R., & Hurd, P.L. (2009a). Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. *Behavioural Processes, 82,* 25-29.

Reddon, A.R., & Hurd, P.L. (2009b). Individual differences in cerebral lateralization are associated with shy-bold variation in the convict cichlid. *Animal Behaviour, 77,* 189-193.

Ricon, T., Toth, E., Leshem, M., Braun, K., & Richter-Levin, G. (2012). Unpredictable chronic stress in juvenile or adult rats has opposite effects, respectively, promoting and impairing resilience. *Stress, 15,* 11-20.

Rogers, L.J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Applied Animal Behaviour Science, 127,* 1-11.

Roh, E., Mirza, R.S., & Brown, G.E. (2004). Quality or quantity? The role of donor condition in the production of chemical alarm cues in juvenile convict cichlids. *Behaviour, 141,* 1235-1248.

Scharf, S.H., & Schmidt, M.V. (2012). Animal models of stress vulnerability and resilience in translational research. *Current Psychiatry Reports, 14,* 159-165.

Sih, A., & Bell, A.M. (2008). Insights for behavioural ecology from behavioural syndromes. *Advances in the Study of Behavior, 38,* 227-281.

Sih, A., Bell, A.M., & Johnson, J.C. (2004a). Behavioural syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution, 19,* 372-378.

Sih, A., Bell, A.M., Johnson, J.C., & Ziemba, R.E. (2004b). Behavioral syndromes: An integrative overview. *Quarterly Review of Biology, 79,* 241-277.

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecology Letters, 15,* 278-289.

Thomson, J.S., Watts, P.C., Pottinger, T.G., & Sneddon, L.U. (2012). Plasticity of boldness in rainbow trout, *Oncorhynchus mykiss*: do hunger and predation influence risk-tasking behaviour? *Hormones and Behavior, 61,* 750-757.

Toms, C.N., Echevarria, D.J., & Jouandot, D.J. (2010). A methodological review of personality-related studies in fish: Focus on the shy-bold axis of behavior. *International Journal of Comparative Psychology, 23,* 1-25.

Weiss, J.M., & Glazer, H.I. (1975). Effects of acute exposure to stressors on subsequent avoidance-escape behavior. *Psychosomatic Medicine, 37,* 499–521.

Wisenden, B.D. (1995). Reproductive behaviour in free-ranging convict cichlids, *Cichlasoma nigrofasciatum*. *Environmental Biology of Fishes, 43,* 121– 134.

Wisenden, B.D., & Sargent, R.C. (1997). Antipredator behaviour and suppressed aggression by convict cichlids in response to injury-released chemical cues of conspecifics but not to those of an allopatric heterospecific. *Ethology, 103,* 283-291.

6. GENERAL DISCUSSION

The main goal of this thesis was to investigate the influences of size, sex, stimuli, and stressors on lateralized and boldness behaviour in the convict cichlid. Recently, research attention has focussed on the convict cichlid, and indeed many fish species, as model species to study lateralization and personality. I sought to extend the work on convict cichlids, and fish species in general, in order to better understand how both development and experience, particularly experience with stressors, affects lateralization and the personality trait of boldness. I initially hypothesized these factors would have an effect for two reasons: 1) previous research has shown that in wild populations experience with predators affects the strength of lateralized behaviour (Brown et al. 2007b); and 2) Rogers (2010) has presented convincing evidence that lateralized behaviour may be related to the stress response, which could be affected by the developmental experiences of an organism. To carry out my aims I investigated four different questions, which are detailed below.

6.1 Size and Behavioural Lateralization in Convict Cichlid Sibling Cohorts

In experiment 1 (described in chapter 2), I examined the relationship between body size and behavioural lateralization in response to simulated social stimuli in sibling cohorts of juvenile convict cichlids. I conducted this study to determine if growth rate in these fish played a role in how they viewed simulated conspecifics in a lateralized manner. Growth rate during development may very likely play a role in forming adult lateralized preferences and personality characteristics, as fish that grow faster earlier may be employing a different life history strategy than fish that delay growth (e.g., Stamps, 2007). I specifically attempted to ascertain whether juvenile convict cichlids displayed the same relationship between growth rate and behavioural lateralization as that found in another species of cichlid fish, *G. brasiliensis*. In *G. brasiliensis,* larger fish in a cohort were more strongly lateralized (Reddon et al., 2009). I also sought to establish whether juvenile convict cichlids viewed social stimuli preferentially with the left eye, as seen in many adult fish species (e.g., Sovrano et al., 1999, 2001; De Santi et al., 2001; Sovrano, 2004; Sovrano & Andrew, 2006; Andrew et al., 2009; Karenina et al., 2013). I wanted to investigate these lateralized preferences in juveniles to determine if these preferences were innate or if they developed gradually during the juvenile stage. If the preferences were innate, then growth rate should have no effect; however, if the preferences developed gradually, then growth rate, and size within a cohort as a juvenile, may have an impact on later adult lateralized viewing behaviour.

I did not find a population-level preference for my subjects to view simulated social stimuli with the left eye. I did, however, find that larger fish viewed these stimuli preferentially with the left eye while smaller fish did so with the right eye. I did not find a relationship between size and strength of behavioural asymmetry in juvenile convict cichlids, as was found in *G. brasiliensis*. In *G. brasiliensis* there were sex differences in this relationship; males exhibited a positive relationship, with larger fish showing stronger behavioural lateralization, while females showed no significant relationship. *G. brasiliensis* also exhibited a relationship between size and sex, such that larger fish in the cohort were males (Reddon et al., 2009). The fish in this study were too young to sex individuals prior to behavioural testing. It has been demonstrated in convict cichlids that size at 195 days is unrelated to sex (Espinoza, 2006). Sex differences in eye preference have been found in adult convict cichlids when detouring around a barrier; however, lateralized viewing of social stimuli had not yet been investigated in adults of this species. It is possible that sex differences in viewing social stimuli exist in this species and that sex may affect the general relationship between size and eye preference.

I am currently performing a study to determine the sex of juvenile convict cichlids post-behaviour testing to investigate whether gender influences the relationship between size and behavioural lateralization in juveniles of this species. Although size and sex are confounded in *G. brasiliensis*, I believe it is still reasonable to expect that sex may influence lateralized behaviour in convict cichlids regardless of size. I expect this because convict cichlids have extended parental care of the young. The sexes take on different roles in this parental care, with females spending more time directly caring for the young and males spending more time defending the young against attack. Much like humans, these different parental care roles could lead to a difference both in how male and female convict cichlids perceive stressors and in how they view conspecifics. Experiment 2 was conducted in part to address the question of whether sex differences exist in eye preference for viewing social stimuli in adult convict cichlids.

6.2 Sex Differences in Lateralized Behaviour when Viewing Different Stimuli

In experiment 2 (described in chapter 3), I examined sex differences in behavioural lateralization in adult convict cichlids when viewing different stimuli. As mentioned, I expected sex would affect lateralized viewing of different stimuli because of the different roles of the sexes in parental care and how these roles may cause a different interpretation of conspecifics and stressors. I investigated whether the direction and strength of eye preference was consistent in individual fish when viewing social vs. non-social stimuli. I also examined the relationship between quantitative asymmetry in the habenula and behavioural lateralization, which had not been previously examined in this species when viewing social stimuli. Convict cichlids have recently been used to investigate the links between habenular asymmetry and lateralized behaviour (Gutiérrez-Ibáñez et al., 2011) and personality and lateralized behaviour (Reddon & Hurd, 2008, 2009a). In these studies, the only lateralized behaviour tested was turning preference in a detour task; these results were then used to make general statements about lateralized behaviour. In contrast, many studies have shown that behavioural lateralization measured via eye preference is highly dependent on the stimuli being viewed (Bisazza et al., 1997, 1998; Dadda et al., 2010; De Santi et al., 2001). I sought to investigate whether stimulus type influenced lateralized behaviour in the convict cichlid, and the relationship that behaviour may have with asymmetry in the habenula.

I found that both males and females showed a population-level preference to view non-social stimuli in the form of empty space in the detour task with the right eye. I found a sex difference for social stimuli viewing; females showed a population-level preference for left eye viewing while males showed no eye preference. I found no significant relationship between the two stimulus types for either direction or strength of lateralized behaviour. I also found no significant relationship between habenular asymmetry and behavioural asymmetry when viewing social stimuli for either sex.

My results suggest that reports linking behavioural lateralization and other factors such as personality and brain asymmetry in the convict cichlid should be treated with caution. I found no significant relationship between eye preferences when viewing two different stimuli. I also found no significant relationship between eye preference and brain asymmetry when fish were viewing social stimuli, whereas this relationship has previously been found in this species for a non-social stimulus (Gutiérrez-Ibáñez et al., 2011). Bisazza and colleagues (2001) tested lines of fish selected for either right- or left-eye preference to view a model predator in the detour task. These lines subsequently differed in eye preference on 5 separate tests of lateralized behaviour, which led the authors to suggest that eye preferences in the detour task are indicative of eye preferences for other behavioural tasks. There is an important distinction between this study and the convict cichlid studies linking behavioural lateralization to other individual factors. Bisazza and colleagues (2001) looked at eye preference at the group level; no data were reported regarding the consistency in eye preference within individuals across the 5 tasks. Individual-level data is desired if one is making claims regarding the relationship between behavioural lateralization and individual-level factors like quantitative habenular asymmetry and personality characteristics. There is a burgeoning field investigating the relationship between behavioural asymmetry and personality (see section 6.5 below). My work underlines that researchers interested in more individual-level factors like personality characteristics should investigate lateralized behaviour in response to a variety of stimuli and report data for individual fish in addition to group-level data.

Though I did not find a significant relationship between lateralized eye preferences when viewing different stimuli in experiment 2, I have reason to believe this type of correlation could be present under certain circumstances. Recent evidence suggests that stress in the form of predation pressure can lead to more correlated behaviours in animals (e.g., Bell & Stamps, 2004; Dingemanse et al., 2007; Bell & Sih, 2007; Archard & Braithwaite, 2011). Stressful conditions or stimuli have also been linked to increased right hemisphere processing (reviewed in Rogers, 2010). The habenula has been implicated in functions related to stress, pain, and anxiety (reviewed in Hikosaka, 2010). Based on these data, I postulated that the presence of a stressor would increase right hemisphere processing of any type of stimulus. I further hypothesized that an increase in right-hemisphere processing, combined with the known effect of stressors on behavioural correlations, would result in a correlation in eye preference between stimulus types in the convict cichlid. Lastly, due to the function of the habenula in stress responses, I predicted a relationship between habenular asymmetry and behavioural asymmetry when viewing stimuli in the presence of a stressor. These hypotheses were the motivation for conducting experiment 3.

6.3 Sex Differences in Lateralized Behaviour in the Presence of a Stressor

In experiment 3 (described in chapter 4), I examined the same variables as experiment 2. Namely, I investigated the relationship between eye preferences when viewing social and non-social stimuli, as well as the relationship between eye preference and habenular asymmetry, in adult convict cichlids. The addition to experiment 3 was the presence of damage-induced alarm cues to the testing apparatus while fish were making behavioural decisions. My rationale for this experiment is discussed above (section 6.2). Researchers interested in the effects of stress or predation on lateralized behaviour typically use actual predators and examine which eye the focal animal uses to investigate the predator (e.g., Bisazza et al., 1999, 2005; De Santi et al., 2000, 2001, 2002) or compare populations of the same species that come from high vs. low predation sites (Brown et al., 2004, 2007a). This is the first study to my knowledge to use alarm cues to increase fear or stress while animals are making lateralized decisions to view non-fearful stimuli.

In experiment 3, in line with my hypotheses that alarm cues would increase both right-hemisphere processing of stimuli and strength of lateralized behaviour, I found that females preferentially used their right eye (left hemisphere) to view solitary stimuli when alarm cues were absent and their left eye (right hemisphere) when alarm cues were present. I found this same trend for males. I also found that females, regardless of the type of stimulus they were viewing, showed stronger eye preferences when alarm cues were present vs. absent. I did not find this same effect in males. Contrary to my hypothesis that stress in the form of alarm cues would lead to a stronger correlation between lateralized eye preferences when viewing different types of stimuli, neither sex showed an increase in eye preference correlation between the two stimuli in the presence of alarm cues.

I did find a significant positive relationship in males between direction of habenular asymmetry and eye preference for solitary stimuli in the form of an empty space. Fish with larger left habenulae used their left eye more to view solitary stimuli in the presence of alarm cues. This is the opposite of the relationship found by Gutiérrez-Ibáñez and colleagues (2011), which showed that males with right-biased or more symmetrical habenulae tended to view an empty space with their left eye; however, the males in that study were not tested in the presence of alarm cues.

Alarm cues may differentially affect the motivation of fish to view stimuli based on different factors, such as stimulus type and sex. Fish viewing social stimuli may be less motivated by fear in the presence of alarm cues than when viewing an empty space. When viewing social stimuli, if fish do indeed treat mirror images as other fish, there are more fish present to diffuse a potential predation threat. When viewing an empty space, the focal fish is the only animal present that a potential predator may target; thus, that fish may be more motivated by fear in the presence of alarm cues than when that fish is viewing simulated conspecifics. Motivation has been shown to influence eye preference in the detour task (Bisazza et al., 1998). In this experiment, I showed a sex difference in how eye preference changes when in the presence or absence of alarm cues. Sex may be another factor differentially affecting motivation in the presence of alarm cues in this species (as discussed in sections 6.1 and 6.2).

The habenula has been implicated in processing motivational information. In a study using macaque monkeys (Matsumoto & Hikosaka, 2009), it was shown that lateral habenula neurons were strongly excited by either the certainty of not receiving a positive stimulus (i.e., juice) or the certainty of receiving a negative stimulus (i.e., an air puff). These results suggest the habenula responds to the negative value of a stimulus. The negative value of alarm cues may differ between males and females in convict cichlids, perhaps based on the interpretation of, or ability to sense, these cues. This difference in value could relate to a difference in motivation to respond to alarm cues. This potential value difference may then translate into a differential relationship between habenular asymmetry and behaviour between the sexes in this species. Additionally, motivational, or negative-value differences, may explain why the relationship I found between habenular asymmetry and behavioural lateralization in males in the presence of alarm cues was different from that found in males when alarm cues were absent.

Another potential factor that may differentially influence motivation in response to alarm cues is individual personality. For example, a bold fish may be more motivated to find and fend off a predator whose presence is indicated by alarm cues, whereas a shy fish may instead be motivated to hide from a potential predator. In addition to its involvement in processing motivational information, the habenula may contribute to inhibition of behaviour based on information regarding the negative value of a stimulus. In primates, excitation of lateral habenula neurons inhibits downstream dopamine neurons which can then inhibit motor behaviours (reviewed in Hikosaka, 2010). It is possible that alarm cues have differential negative value based on an individual's personality. If this is the case, then individuals would show differential inhibition of behaviour in the presence of alarm cues.

Sex differences in lateralized behavioural response to stimuli conditioned to have different value (i.e., positive by being paired with food or negative by being paired with alarm cues) have been reported in convict cichlids (Reddon & Hurd, 2009b). Experiment 3 also showed sex differences in lateralized responding when in the presence of alarm cues in this species. I know of no studies, however, that have examined sex differences in non-lateralized responding to alarm cues in the convict cichlid. The data described above, combined with the hypothesized potential for individual personality to differentially affect the negative value of alarm cues (described above), and the potential sex differences in the interpretation of, or ability to sense, alarm cues (also described above), were the inspiration for conducting experiment 4.

6.4 Sex and Stress Affect Boldness in Convict Cichlids

In experiment 4 (described in chapter 5), I tested the effects of prior and current exposure to stressors on the behaviour of convict cichlids in an open field task. The open field assay is a popular behavioural task for measuring anxiety and fear behaviours in mammals (see Prut & Belzung, 2003 for review) and fish (e.g., Champagne et al., 2010). I was also interested in how an initial assessment of boldness, as measured by time to emerge into a novel environment, would relate to boldness in the open field both in the presence and absence of a stressor. I hypothesized that individual fish personality (i.e., placement along the bold-shy continuum) would affect both the response to a prior stressor and to a current stressor. As mentioned above, I suggest this effect may be based on the negative value that fish associate with the stressor. Due to the involvement of the habenula in responses to the negative value of stimuli, and inhibition of motor behaviour based on that negative value assessment, I expected to see a relationship between asymmetry in the habenula and motor-based boldness behaviour in the open field task. Based on my findings in experiment 3, and the sex differences previously reported for lateralized responses of convict cichlids to positive and negative stimuli, I expected to find sex differences in non-lateralized behaviour in response to stressors in convict cichlids.

I did find sex differences in response to alarm cues in the convict cichlid in this experiment. Most notably, I found that the presence of alarm cues in the open field task disrupted the relationship between boldness in a novel environment and in an open field task in females but not in males. This effect only occurred in females not previously exposed to the stress of net chasing. When females were chased with a net before testing in the open field task, they showed the same relationship between boldness in both tasks regardless of whether alarm cues were present in the open field. This behaviour was more similar to the behaviour of males of this species.

Sex differences in the response of convict cichlids to alarm cues have now been shown in both lateralized and boldness behaviour. It remains unclear if these differences are due to a differential ability of the sexes to detect alarm cues. It is possible that females may detect alarm cues at a lower concentration than males, as is the case in zebrafish (Gandolfi et al., 1968). It is also possible that males and females can detect alarm cues equally well but assess those cues differently. In guppies, females are thought to be more risk-averse than males due to different life-history strategies. Females are typically surrounded by potential mates and are able to store sperm; therefore, females can produce a new brood without a new mating with a male. To increase female fitness, it is most important to survive and grow as fecundity is correlated with body size in this species (Reznick et al., 1990). Male guppies increase fitness by continuing to seek mating opportunities throughout life (Magurran & Seghers, 1994). These different life history strategies suggest that male guppies are exposed to more risk in seeking mating opportunities than females (Harris et al., 2010).

Convict cichlids, on the other hand, are monogamous and biparental, with both parents caring for the young for 4-6 weeks after hatching (Noakes, 1991). Although both sexes provide parental care in this species, the sexes typically differ in the roles of this care. Females spend more time with the offspring while males spend more time protecting the nest and brood (Itzkowitz et al., 2001, 2003). Females will also often aim attacks at male partners to limit their access to offspring (Itzkowitz et al., 2003). Sex differences in parental behaviour have also been observed in relation to the risk associated with those behaviours. Snekser and Itzkowitz (2009) showed that females generally retrieve displaced altricial young when they are closer to the nest, and the risk of retrieval is lower, while males retrieve the young when they are displaced further from the nest, and the retrieval risk is higher. In a field component of Snekser and Itzkowitz's (2009) experiment, displaced wrigglers were retrieved by the female in an area where predation pressure was low and by the male in an area where predation pressure was high. These results suggest that female convict cichlids may be more risk-averse than males. This difference in risk aversion is a possible explanation for the sex differences I found in behavioural responses to alarm cues in the convict cichlid.

Although as a group females may be more risk averse than male convict cichlids, there is still variation within each sex in the amount of risk-aversion or risk-taking propensity for each individual. The variation in boldness scores in the first behavioural task supports this claim. The degree of risk-aversion may relate to the negative value that an individual associates with the presence of alarm cues; a more risk-prone fish may associate a lower negative value with alarm cues than a more risk-averse fish. I previously suggested that this link between risk-taking propensity, or boldness, may be associated with the habenula, which is involved in processing negative value information (Hikosaka, 2010). I found no significant relationship between habenular asymmetry and boldness in either the time to emerge into a novel environment or behaviour in the open field task. This lack of relationship does not necessarily imply that the habenula is not involved in risk-taking behaviour; however, asymmetry in the volume of the habenula may be unrelated to boldness behaviour and risk-taking.

Research on zebrafish has shown that there are additional asymmetries in the habenula beyond asymmetries in volume. In zebrafish, the unpaired parapineal organ typically lies to the left of the pineal stalk adjacent to the left habenula. Early in development, parapineal precursor cells innervate the left habenula and ultimately lead to left-right habenular asymmetry. This asymmetry is characterized by the left habenula being larger, containing denser neuropil, exhibiting a different pattern of gene expression, and projecting to different regions of the midbrain (Gamse et al., 2003, 2005). The habenula has not been studied as extensively in the convict cichlid but it is clear from zebrafish studies that there are other potential asymmetries in the habenula besides volume that may relate to boldness behaviour and risk taking.

6.5 Lateralization and Personality

Throughout this thesis I have suggested that there may be a link between cerebral and behavioural lateralization and personality. This relationship has been studied in the convict cichlid with respect to the personality traits of boldness and aggression. In fish that were familiar with the detour task (i.e., had spent 24 hours in the apparatus prior to testing), those with stronger eye preferences were also bolder (Reddon & Hurd, 2009a). In a separate experiment, sex differences were observed in the relationship between aggression and behavioural lateralization. Non-aggressive females and aggressive males tended to use their right eye to view around a barrier while aggressive females and non-aggressive males tended to use their left eye. Non-aggressive females were more strongly lateralized than their aggressive counterparts while the opposite was true for males (Reddon & Hurd, 2008).

In zebrafish, shyness has been associated with left-eye viewing of a novel stimulus. In this same study, fish reared in darkness were shyer and displayed reduced behavioural asymmetry compared to zebrafish exposed to light during rearing (Budaev & Andrew, 2009). In a separate study, fish with atypical right-sided parapineal position, which showed a different pattern of behavioural lateralization than the more common left-parapineal position fish, were bolder when inspecting a predator and spent less time in the periphery of an open field task (Dadda et al., 2010). Irving and Brown (2013) examined a sample of feral guppies and found no significant relationship between behavioural lateralization and any of boldness, activity, or sociability in males. Females, however, showed a strong trend towards a positive relationship between strength of behavioural lateralization when viewing social stimuli and boldness.

It has been proposed that individual variation in behaviour may help maintain variation in the strength of lateralization (Reddon & Hurd, 2009c). Reddon and Hurd (2009c) suggest that more strongly lateralized individuals behave in a bolder, more risk-prone manner than weakly lateralized individuals. Evidence from a variety of animal species supports this claim. For example, human males showing strong preference for one hand are more aggressive than ambidextrous males (Dinsdale et al., 2011), strongly lateralized chicks give fewer alarm calls and resume feeding more quickly after disturbance by a model predator than weakly lateralized chicks (Dharmaretnam & Rogers, 2005), and strongly lateralized convict cichlids are bolder, regardless of sex, than their weakly lateralized conspecifics (Reddon & Hurd, 2009c). Reddon and Hurd (2009c) suggest that in individuals with strongly lateralized brains, one hemisphere is better able to inhibit the other, a trait that would not hold true for individuals with weakly lateralized brains. These authors further suggest that in individuals with weakly lateralized brains, any action will result from a consensus between the two hemispheres, which will typically be less extreme than the action resulting from contributions of a single hemisphere. Ultimately, in weakly lateralized brains, the time taken for consensus to be reached between the two hemispheres may manifest as behavioural hesitancy. In contrast, individuals with strongly lateralized brains would arrive at decisions and take actions more quickly, resulting in potentially more risk-prone behaviour (Reddon & Hurd, 2009c).

Another hypothesis linking variation in lateralization to variation in behaviour, previously discussed in this thesis, focuses more on the direction of lateralization as opposed to Reddon and Hurd's (2009c) hypothesis regarding the strength of lateralization. This hypothesis was proposed by Rogers (2010) and suggests that predominant control of behaviour by the right hemisphere can result in a negative cognitive bias, or a tendency to process neutral or ambiguous stimuli as negative (e.g., threatening or aversive). This tendency may then produce, or at least be related to, anxious or avoidance-related behaviour. For example, chicks exposed to isolation stress, which according to Rogers (2010) would lead to more right hemisphere processing, subsequently treated ambiguous cues (morphed versions or either chick (appetitive) or owl (aversive) silhouettes) as more aversive than chicks that had not been subjected to isolation stress (Salmeto et al. 2011). Further, rats that were moved from unenriched cages to enriched cages treated an ambiguous cue as more appetitive than animals that remained in unenriched cages, showing that living conditions can have an effect on cognitive bias (Brydges et al. 2011), as previously suggested by Rogers (2010). An intriguing area for future research would be to assess cognitive bias in fish (i.e., the tendency to interpret neutral or ambiguous stimuli as positive/appetitive or as negative/threatening/aversive) and determine if such a bias is related to more typically-studied aspects of personality such as boldness, activity, or exploration, as well as how, or if, this bias is affected by either previous or current stressors. Cognitive bias could also be studied in relation to lateralized behaviour in fish to determine whether there is support for Rogers' (2010) hypothesis. For example, determining if fish that display a negative cognitive bias tend to view neutral stimuli predominantly with their left eye (thus processing those stimuli with their right hemisphere and supposedly as more aversive) and subsequently exhibit more risk-averse behaviour. Roger's (2010) cognitive bias hypothesis could very well be a missing link to help explain relationships between lateralized behaviour, personality characteristics, and experience with stressors.

The data presented in this thesis appear to support part of Rogers' (2010) hypothesis, such that, when in the presence of a stressful cue (i.e., damage-induced alarm cues), neutral stimuli were increasingly processed with the right hemisphere by convict cichlids (chapter 4). Data from chapter 4 may also indirectly support the hypothesis put forth by Reddon and Hurd (2009c) regarding strength of lateralization. Females in the presence of a stressor showed increased strength of lateralization, regardless of stimulus type. This increased preference for using one eye may be due to the need to make decisions more quickly in the presence of a danger cue. Perhaps in fish more sensitive to risk (i.e., females in this species), when in a potentially risky situation the need to make decisions quickly is more important than for individuals less sensitive to risk. Studies that have previously examined the relationship between personality traits and lateralization typically use lateralization tasks that involve neutral stimuli or viewing confined predators. A predator confined behind a barrier may not invoke the same level of risk as damage-induced alarm cues, which could indicate a potential unseen predator, especially in predator-naïve individuals. A fruitful area for future research, driven by the results of this thesis, is to investigate the link between boldness and lateralization in a risky context. Additionally, it will be informative to determine if patterns of lateralization in a risky context change differentially based on initial propensity for boldness and prior experience with stressors. If mechanistic links between lateralized behaviour, personality, and experience with stressors can be established, this knowledge could be used to both increase animal welfare by rearing individuals in environments that promote a positive cognitive bias, as proposed by Rogers (2010), and to potentially influence research into the links between stress exposure, hemispheric processing, cognitive bias, and personality characteristics in humans.

6.6 References

Andrew, R.J., Dharmaretnam, M., Gyori, B., Miklosi, A., Watkins, J.A.S., & Sovrano, V.A. (2009). Precise endogenous control of involvement of right and left visual structures in assessment by zebrafish. *Behavioural Brain Research, 196,* 99-105.

Archard, G.A., & Braithwaite, V.A. (2011). Increased exposure to predators increases both exploration and activity level in *Brachyrhaphis episcopi*. *Journal of Fish Biology, 78,* 593-601.

Bell, A.M., & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters, 10,* 828-834.

Bell, A.M., & Stamps, J.A. (2004). Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. *Animal Behaviour, 68,* 1339-1348.

Bisazza, A., Dadda, M., & Cantalupo, C. (2005). Further evidence for mirror-reversed laterality in lines of fish selected for leftward or rightward turning when facing a predator model. *Behavioural Brain Research, 156,* 165-171.

Bisazza, A., De Santi, A., & Vallortigara, G. (1999). Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side. *Animal Behaviour, 57,* 1145-1149.

Bisazza, A., Facchin, L., Pignatti, R., & Vallortigara, G. (1998). Lateralization of detour behaviour in poeciliid fish: The effect of species, gender and sexual motivation. *Behavioural Brain Research, 91,* 157-164.

Bisazza, A., Pignatti, R., & Vallortigara, G. (1997). Detour tests reveal task- and stimulus-specific behavioural lateralization in mosquitofish (*Gambusia holbrooki*). *Behavioural Brain Research, 89,* 237-242.

Bisazza, A., Sovrano, V.A., & Vallortigara, G. (2001). Consistency among different tasks of left-right asymmetries in line of fish originally selected for opposite direction of lateralization in a detour task. *Neuropsychologia, 39,* 1077-1085.

Brown, C., Burgess, F. & Braithwaite, V.A. (2007a). Heritable and experiential effects on boldness in a tropical poeciliid. *Behavioural Ecology and Sociobiology, 62(2*), 237-243.

Brown, C., Western, J., & Braithwaite, V.A. (2007b). The influence of early experience on, and inheritance of, cerebral lateralization. Animal Behaviour, 74, 231-238.

Brown, C., Gardner, C., & Braithwaite, V.A. (2004). Population variation in lateralized eye use in the poeciliid *Brachyraphis episcopi*. *Proceedings of the Royal Society B – Biological Sciences, 271,* S455-S457.

Brydges, N.M., Leach, M., Nicol, K., Wright, R., & Bateson, M. (2011). Environmental enrichment induces optimistic cognitive bias in rats. *Animal Behaviour*, 81, 169-175.

Budaev, S., & Andrew, R.J. (2009). Shyness and behavioural asymmetries in larval zebrafish (*Brachydanio rerio*) developed in light and dark. *Behaviour, 146,* 1037-1052.

Champagne, D.L., Hoefnagels, C.C.M., de Kloet, R.E., & Richardson, M.K. (2010). Translating rodent behavioral repertoire to zebrafish (*Danio rerio*): Relevance for stress research. *Behavioural Brain Research, 214,* 332-342.

Dadda, M., Domenichini, A., Piffer, L., Argenton, F., & Bisazza, A. (2010). Early differences in epithalamic left-right asymmetry influence lateralization and personality of adult zebrafish. *Behavioural Brain Research, 206,* 208-215.

De Santi, A., Bisazza, A., Cappelletti, M., & Vallortigara, G. (2000). Prior exposure to a predator influences lateralization of cooperative predator inspection in the guppy, *Poecilia reticulata*. *Italian Journal of Zoology, 67,* 175-178.

De Santi, A., Bisazza, A., & Vallortigara, G. (2002). Complementary left and right eye use during predator inspection and shoal-mate scrutiny in minnows. *Journal of Fish Biology, 60,* 1116-1125.

De Santi, A., Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Animal Behavior, 61,* 305-310.

Dharmaretnam, M., & Rogers, L.J. (2005). Hemispheric specialization and dual processing in strongly versus weakly lateralized chicks. *Behavioural Brain Research, 162,* 62–70.

Dingemanse, N.J., Wright, J., Kazem, A.J.N., Thomas, D.K., Hickling, R., & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology, 76,* 1128-1138.

Dinsdale, N.L., Reddon, A.R., & Hurd, P.L. (2011). Sex differences in the relationship between aggressiveness and the strength of handedness in humans. *Laterality: Asymmetries of Body, Brain and Cognition, 16,* 385–400.

Espinoza, W. (2006). Sexual differentiation, neurobiology, and behaviour in the convict cichlid (*Cryptoheros nigrofasciatus*). Unpublished M.Sc thesis.

Gamse, J.T., Thisse, C., Thisse, B., Halpern, M.E. (2003). The parapineal mediates left–right asymmetry in the zebrafish diencephalon. *Development, 130,* 1059–1068.

Gamse, J.T., Kuan, Y.S., Macurak, M., Brosamle, C., Thisse, B., Thisse, C., & Halpern, M.E. (2005). Directional asymmetry of the zebrafish epithalamus guides dorsoventral innervation of the midbrain target. *Development, 132,* 4869–4880.

Gandolfi, G., Mainardi, D., & Rossi, A.C. (1968). The fright reaction of zebra fish. *Atti della Societa Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 107,* 74–88.

Gutiérrez-Ibáñez, C., Reddon, A.R., Kreuzer, M.B., Wylie, D.R., & Hurd, P.L. (2011). Variation in the asymmetry of the habenular nucleus correlates with behavioural asymmetry in a cichlid fish. *Behavioural Brain Research, 221,* 189-196.

Harris, S., Ramnarine, I.W., Smith, H.G., & Pettersson, L.B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos, 119,* 1711-1718.

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nature Reviews Neuroscience, 11,* 503-513.

Irving, E., & Brown, C. (2013). Examining the link between personality and laterality in a feral guppy *Poecilia reticulata* population. *Journal of Fish Biology, 83,* 311-325.

Itzkowitz, M., Santangelo, N., & Richter, M. (2001) Parental division of labour and the shift from minimal to maximal role specializations: an examination using a biparental fish. *Animal Behaviour, 61,* 237-245.

Itzkowitz, M., Santangelo, N., & Richter, M. (2003) How does a parent respond when its mate emphasizes the wrong role? A test using a monogamous fish. *Animal Behaviour, 66,* 863-869.

Karenina, K.A., Giljov, A.N., & Malashichev, Y.B. (2013). Eye as a key element of conspecific image eliciting lateralized response in a fish. *Animal Cognition, 16,* 287-300.

Magurran, A.E., & Seghers, B.H. (1994). Sexual conflict as a consequence of ecology – evidence from guppy, *Poecilia reticulata*, populations in Trinidad. – *Proceedings of the Royal Society B – Biological Sciences, 255,* 31–36.

Matsumoto, M., & Hikosaka, O. (2009). Representation of negative motivational value in the primate lateral habenula. *Nature Neuroscience, 12,* 77–84.

Noakes, D.L.G. (1991). Ontogeny of behaviour in cichlids. In M.H.A. Keenleyside (Ed.), *Cichlid Fishes: Behaviour, Ecology and Evolution* (pp. 209-224). London: Chapman and Hall.

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European Journal of Pharmacology, 463,* 3-33.

Reddon, A.R., Gutiérrez-Ibáñez, C., Wylie, D.R., & Hurd, P.L. (2009). The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. *Behavioural Brain Research, 201,* 223-228.

Reddon, A.R., & Hurd, P.L. (2008). Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology Letters, 4,* 338-340.

Reddon, A.R., & Hurd, P.L. (2009a). Individual differences in cerebral lateralization are associated with shy-bold variation in the convict cichlid. *Animal Behaviour, 77,* 189-193.

Reddon, A.R., & Hurd, P.L. (2009b). Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. *Behavioural Processes, 82,* 25-29.

Reddon, A.R., & Hurd, P.L. (2009c). Acting unilaterally: Why do animals with strongly lateralized brains behave differently than those with weakly lateralized brains? *Bioscience Hypotheses, 2,* 383-387.

Reznick, D.A., Bryga, H., & Endler, J.A. (1990). Experimentally induced life-history evolution in a natural population. *Nature, 346,* 357–359.

Rogers, L.J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Applied Animal Behaviour Science, 127,* 1-11.

Salmeto, A.L., Hymel, K.A., Carpenter, E.C., Brilot, B.O., Bateson, M., & Sufka, K.J. (2011). Cognitive bias in the chick anxiety-depression model. *Brain Research*, 1373, 124-130.

Snekser, J.L., & Itzkowitz, M. (2009). Sex differences in retrieval behavior by the biparental convict cichlid. *Ethology, 115,* 457-464.

Sovrano, V.A. (2004). Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behavioural Brain Research, 152,* 385-391.

Sovrano, V.A., & Andrew, R.J. (2006). Eye use during viewing a reflection: Behavioural lateralisation in zebrafish larvae. *Behavioural Brain Research, 167,* 226-231.

Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Lateralization of response to social stimuli in fishes: A comparison between different methods and species. *Physiology and Behavior, 74,* 237-244.

Sovrano, V.A., Rainoldi, C., Bisazza, A., & Vallortigara, G. (1999). Roots of brain specializations: Preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behavioural Brain Research, 106,* 175-180.

Stamps, J.A. (2007). Growth-mortality tradeoffs and ‘personality traits’ in animals. *Ecology Letters*, 10, 355-363.

Works Cited

Amat, J., Sparks, P.D., Matus-Amat, P., Griggs, J., Watkins, L.R., & Maier, S.F. (2001). The role of the habenular complex in the elevation of dorsal raphe nucleus serotonin and the changes in the behavioral responses produced by uncontrollable stress. *Brain Research, 917,* 118–126.

Andrew, R.J. (1991). The nature of behavioural lateralization in the chick. in: Andrew, R.J. (Ed.), *Neural and Behavioural Plasticity. The Use of the Chick as a Model*. Oxford University Press, Oxford, pp. 536–554.

Andrew, R.J. (2002). Behavioral development and lateralization, in: Rogers, L.J., & Andrew, R.J. (Eds.), *Comparative Vertebrate Lateralization.* University Press, Cambridge, pp. 157–205.

Andrew, R.J., Osorio, D., & Budaev, S. (2009). Light during embryonic development modulates patterns of lateralization strongly and similarly in both zebrafish and chick. *Philosophical Transactions of the Royal Society B - Biological Sciences, 364,* 983-989.

Andrew, R.J., & Rogers, L.J. (2002). The nature of lateralization in tetrapods, in: Rogers, L.J., Andrew, R.J. (Eds.), *Comparative Vertebrate Lateralization*. University Press, Cambridge, pp. 94-125.

Andrew, R.J., Dharmaretnam, M., Gyori, B., Miklosi, A., Watkins, J.A.S., & Sovrano, V.A. (2009). Precise endogenous control of involvement of right and left visual structures in assessment by zebrafish. *Behavioural Brain Research, 196,* 99–105.

Anfora, G., Frasnelli, E., Maccagnani, B., Rogers, L.J., & Vallortigara, G. (2010). Behavioural and electrophysiological lateralization in a social (*Apis mellifera*) but not in a non-social (*Osmia cornuta*) species of bee. *Behavioural Brain Research, 206,* 236-239.

Archard, G.A., & Braithwaite, V.A. (2011). Increased exposure to predators increases both exploration and activity level in *Brachyrhaphis episcopi*. *Journal of Fish Biology, 78,* 593-601.

Archard, G.A., Earley, R.L., Hanninen, A.F., & Braithwaite, V.A. (2012). Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. *Functional Ecology, 26,* 637-645.

Arnott, G., Ashton, C., & Elwood, R.W. (2011). Lateralization of lateral displays in convict cichlids. *Biology Letters, 7,* 683-685.

Arnott, G., & Elwood, R. (2009a). Gender differences in aggressive behaviour in convict cichlids. *Animal Behaviour, 78,* 1221-1227.

Arnott, G., & Elwood, R. (2009b). Probing aggressive motivation in a cichlid fish. *Biology Letters, 5,* 762-764.

Assis, P. (2008). guvcview v.1.2.0 [computer program]. http://guvcview.sourceforge.net/index.html. Accessed 26 August 2013.

Barth, K.A., Miklósi, A., Watkins, J., Bianco, I.H., Wilson, S.W., & Andrew, R.J. (2005). *fsi* zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. *Current Biology, 15,* 844–850.

Bateson, M., & Matheson, S.M. (2007). Performance on a categorisation task suggests that removal of environmental enrichment induces ‘pessimism’ in captive European starlings (*Sturnus vulgaris*). *Animal Welfare, 16,* 33–36.

Bell, A.M., & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters, 10,* 828-834.

Bell, A.M., & Stamps, J.A. (2004). Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. *Animal Behaviour, 68,* 1339-1348.

Bianco, I.H., & Wilson, S.W. (2009). The habenular nuclei: A conserved asymmetric relay station in the vertebrate brain. *Philosophical Transactions of the Royal Society of London. Series B - Biological Sciences, 364,* 1005-1020.

Bisazza, A., Cantalupo, C., Capocchiano, M., & Vallortigara, G. (2000). Population lateralisation and social behavior: A study with 16 species of fish. *Laterality, 5,* 269-284.

Bisazza, A., & Dadda, M. (2005). Enhanced schooling performance in lateralized fishes. *Proceedings of the Royal Society B – Biological Sciences, 272,* 1677-1681.

Bisazza, A., Dadda, M., & Cantalupo, C. (2005). Further evidence for mirror-reversed laterality in lines of fish selected for leftward or rightward turning when facing a predator model. *Behavioural Brain Research, 156,* 165-171.

Bisazza, A., De Santi, A., Bonso, S., & Sovrano, V.A. (2002). Frogs and toads in front of a mirror: Lateralisation of response to social stimuli in tadpoles of five anuran species. *Behavioural Brain Research, 134,* 417–424.

Bisazza, A., Facchin, L., Pignatti, R., & Vallortigara, G. (1998). Lateralization of detour behaviour in poeciliid fish: The effect of species, gender and sexual motivation. *Behavioural Brain Research, 91,* 157-164.

Bisazza, A., & De Santi, A. (2003). Lateralization of aggression in fish. *Behavioural Brain Research, 141,* 131–136.

Bisazza, A., De Santi, A., & Vallortigara, G. (1999). Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side. *Animal Behaviour, 57,* 1145-1149.

Bisazza, A., Pignatti, R., & Vallortigara, G. (1997). Detour tests reveal task- and stimulus-specific behavioural lateralization in mosquitofish (*Gambusia holbrooki*). *Behavioural Brain Research, 89,* 237-242.

Bisazza, A., Pignatti, R., & Vallortigara, G. (1997b). Laterality in detour behaviour: Interspecific variation in poeciliid fish. *Animal Behaviour, 54,* 1273–1281.

Bisazza, A., Rogers, L.J., & Vallortigara, G. (1998). The origins of cerebral asymmetry: A review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neuroscience & Biobehavioral Reviews, 22,* 411-426.

Bisazza, A., Sovrano, V.A., & Vallortigara, G. (2001). Consistency among different tasks of left-right asymmetries in lines of fish originally selected for opposite direction of lateralization in a detour task. *Neuropsychologia, 39,* 1077-1085.

Blaxter, J.H.S. (1988). Pattern and variety in development. in: Hoar, W.S., & Randall, D.J. (Eds.), *Fish Physiology. Vol. 11,* Academic Press, San Diego, pp. 1–58.

Blumstein DT, Daniel JC, Evans CS (2010) JWatcher Software, http://www.jwatcher.ucla.edu/.

Bonati, B., Csermely, D., López, P., & Martin, J. (2010). Lateralization in the escape behaviour of the common wall lizard (*Podarcis muralis*). *Behavioural Brain Research, 207,* 1-6.

Boyce, W.T., & Ellis, B.J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and function of stress reactivity. *Development and Psychopathology, 17,* 271-301.

Brown, C., & Braithwaite, V.A. (2004). Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour, 68,* 1325–1329.

Brown, C., Gardner, C., & Braithwaite, V.A. (2004). Population variation in lateralized eye use in the poeciliid *Brachyraphis episcopi*. *Proceedings of the Royal Society B – Biological Sciences, 271,* S455-S457.

Brown, C., Gardner, C., & Braithwaite, V.A. (2005b). Differential stress responses in fish from areas of high- and low-predation pressure. *Journal of Comparative Physiology. Part B. Biochemical, Systematic, and Environmental Physiology, 175,* 305-312.

Brown, C., Jones, F., & Braithwaite, V.A. (2005). In situ examination of boldness-shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Animal Behaviour, 70,* 1003–1009.

Brown, C., Burgess, F., & Braithwaite, V.A. (2007). Heritable and experiential effects on boldness in a tropical poeciliid. *Behavioural Ecology and Sociobiology, 62,* 237–243.

Brown, C., Western, J., & Braithwaite, V.A. (2007). The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour, 74,* 231-238.

Brown, G.E., Ferrari, M.C.O., & Chivers, D. (2011). Learning about danger: Chemical alarm cues and threat-sensitive assessment of predation risk by fishes. In J. Krause, K. Laland, & C. Brown (Eds.), *Fish Cognition and Behavior* (pp. 59-80). Iowa: Wiley-Blackwell.

Brown, G.E., Foam, P.E., Cowell, H.E., Guevara Fiore, P., & Chivers, D.P. (2004). Production of chemical alarm cues in convict cichlids: the effects of diet, body condition and ontogeny. *Annales Zoologici Fennici, 41,* 487-499.

Brown, G.E., Rive, A.C., Ferrari, M.C.O., & Chivers, D.P. (2006). The dynamic nature of antipredator behavior: prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. *Behavioral Ecology and Sociobiology, 61,* 9-16.

Brydges, N.M., Colegrave, N., Heathcote, R.J.P., & Braithwaite, V.A. (2008). Habitat stability and predation pressure affect temperament behaviours in populations of three-spined sticklebacks. *Journal of Animal Ecology, 77*, 229–235.

Brydges, N.M., Leach, M., Nicol, K., Wright, R., & Bateson, M. (2011). Environmental enrichment induces optimistic cognitive bias in rats. *Animal Behaviour*, 81, 169-175.

Budaev, S.V., & Andrew, R.J. (2009). Shyness and behavioural asymmetries in larval zebrafish (*Brachydanio rerio*) developed in light and dark. *Behaviour, 146,* 1037-1052.

Budaev, S., & Brown, C. (2011). Personality traits and behaviour. In J. Krause, K. Laland, & C. Brown (Eds.), *Fish Cognition and Behavior* (pp. 135-165). Iowa: Wiley-Blackwell.

Burns, J. (2008) The validity of three tests of temperament in guppies (*Poecilia reticulata*). *Journal of Comparative Psychology, 122,* 344–356.

Cantalupo, C., Bisazza, A., & Vallortigara, G. (1995). Lateralization of predator-evasion response in a teleost fish (Girardinus falcatus). *Neuropsychologia, 33(12),* 1637-1646.

Champagne, D.L., Hoefnagels, C.C.M., de Kloet, R.E., & Richardson, M.K. (2010). Translating rodent behavioral repertoire to zebrafish (*Danio rerio*): Relevance for stress research. *Behavioural Brain Research, 214,* 332-342.

Clement, T.S., Parikh, V., Schrumpf, M., & Fernald, R.D. (2005). Behavioral coping strategies in a cichlid fish: the role of social status and acute stress response in direct and displaced aggression. *Hormones and Behavior, 47,* 336-342.

Clotfelter, E.D., & Kuperberg, E.S. (2007). Cerebral lateralization and its relationship to phylogeny and aggression in anabantoid fishes. *Brain, Behavior and Evolution, 69,* 169-175.

Concha, M.L., Burdine, R.D., Russell, C., Schier, A.F., & Wilson, S.W. (2000). A nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain. *Neuron, 28,* 399–409.

Concha, M.L., & Wilson, S.W. (2001). Asymmetry in the epithalamus of vertebrates. *Journal of Anatomy, 199,* 63–84.

Concha, M.L. *et al.*, (2003). Local tissue interactions across the dorsal midline of the forebrain establish CNS laterality. *Neuron, 39,* 423–438.

Conrad, J.L., Weinersmith, K.L., Brodin, T., Saltz, J.B., & Sih, A. (2011). Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. *Journal of Fish Biology, 78,* 395-435.

Copeland, D.L., Levay, B., Sivaraman, B., Beebe-Fugloni, C., & Earley, R.L. (2011). Metabolic costs of fighting are driven by contest performance in male convict cichlid fish. *Animal Behaviour, 82,* 271-280.

Corballis, M.C. (2009). The evolution and genetics of cerebral asymmetry. *Philosophical Transactions of the Royal Society B – Biological Sciences, 364,* 867-879.

Dadda, M., & Bisazza, A. (2006a). Does brain asymmetry allow efficient performance of simultaneous tasks? *Animal Behaviour, 72,* 523-529.

Dadda, M., & Bisazza, A. (2006b). Lateralized female topminnows can forage and attend to a harassing male simultaneously. *Behavioral Ecology, 17,* 358-363.

Dadda, M., Domenichini, A., Piffer, L., Argenton, F., & Bisazza, A. (2010). Early differences in epithalamic left-right asymmetry influence lateralization and personality of adult zebrafish. *Behavioural Brain Research, 206,* 208-215.

Dadda, M., Zandona, E., & Bisazza, A. (2007). Emotional responsiveness in fish from lines artificially selected for a high or low degree of laterality. *Physiology & Behavior, 92,* 764-772.

Dall, S.R.X., Houston, A.I., & McNamara, J.M. (2004). The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters, 7,* 734-739.

Davidson, R.J. (1995). Cerebral asymmetry, emotion, and affective style. in: Davidson, R.J., & Hugdahl, K. (Eds.), *Brain Asymmetry*. MIT Press, Cambridge, MA, pp. 361–387.

De Santi, A., Bisazza, A., Cappelletti, M., & Vallortigara, G. (2000). Prior exposure to a predator influences lateralization of cooperative predator inspection in the guppy, *Poecilia reticulata*. *Italian Journal of Zoology, 67,* 175-178.

De Santi, A., Bisazza, A., & Vallortigara, G. (2002). Complementary left and right eye use during predator inspection and shoal-mate scrutiny in minnows. *Journal of Fish Biology, 60,* 1116-1125.

De Santi, A., Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Animal Behaviour, 61,* 305-310.

Dharmaretnam, M., & Rogers, L.J. (2005). Hemispheric specialization and dual processing in strongly versus weakly lateralized chicks. *Behavioural Brain Research, 162,* 62–70.

Dingemanse, N.J., Van Der Plas, F., Wright, J., Réale, D., Schrama, M., Roff, D.A., Van Der Zee, E., & Barber, I. (2009). Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proceedings of the Royal Society B, 276,* 1285–1293.

Dingemanse, N.J., Wright, J., Kazem, A.J.N., Thomas, D.K., Hickling, R., & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology, 76,* 1128-1138.

Dinsdale, N.L., Reddon, A.R., & Hurd, P.L. (2011). Sex differences in the relationship between aggressiveness and the strength of handedness in humans. *Laterality: Asymmetries of Body, Brain and Cognition, 16,* 385–400.

Espinoza, W. (2006). Sexual differentiation, neurobiology, and behaviour in the convict cichlid (*Cryptoheros nigrofasciatus*). Unpublished M.Sc thesis.

Fabre-Thorpe, M., Fagot, J., Lorincz, E., Levesque, F., & Vauclair, J. (1993). Laterality in cats: Paw preference and performance in a visuomotor activity. *Cortex, 29,* 15-24.

Facchin, L., Bisazza, A., & Vallortigara, G. (1999). What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behavioural Brain Research, 103,* 229-234.

Facchin, L., Burgess, H.A., Siddiqi, M., Granato, M., & Halpern, M.E. (2009). Determining the function of zebrafish epithalamic asymmetry. *Philosophical Transactions of the Royal Society of London. Series B - Biological Sciences, 364,* 1021-1032.

Fox, J., & Weisberg, S. (2011). An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Frasnelli, E., Vallortigara, G., & Rogers, L.J. (2012). Left-right asymmetries of behaviour and nervous system in invertebrates. *Neuroscience and Biobehavioural Reviews, 36,* 1273-1291.

Gainotti, G. (1972). Emotional behavior and hemispheric side of lesion. *Cortex, 8,* 41–55.

Gainotti, G. (2012). Unconscious processing of emotions and the right hemisphere. *Neuropsychologia, 50,* 205-218.

Gamse, J.T., Thisse, C., Thisse, B., & Halpern, M.E. (2003). The parapineal mediates left–right asymmetry in the zebrafish diencephalon. *Development, 130,* 1059–1068.

Gamse, J.T., Kuan, Y.S., Macurak, M., Brosamle, C., Thisse, B., Thisse, C., & Halpern, M.E. (2005). Directional asymmetry of the zebrafish epithalamus guides dorsoventral innervation of the midbrain target. *Development, 132,* 4869–4880.

Gandolfi, G., Mainardi, D., & Rossi, A.C. (1968). The fright reaction of zebra fish. *Atti della Societa Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 107,* 74–88.

Geschwind, N. (1971). Current Concepts - Aphasia. *The New England Journal of Medicine, 284,* 654-656.

Ghirlanda, S., & Vallortigara, G. (2004). The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proceedings of the Royal Society B – Biological Sciences, 271,* 853-857.

Ghirlanda, S. Frasnelli, E., & Vallortigara, G. (2009). Intraspecific competition and coordination in the evolution of lateralization. *Philosophical Transactions of the Royal Society B – Biological Sciences, 364,* 861-866.

Gordon, D.J., & Rogers, L.J. (2010). Differences in social and vocal behavior between left- and right-handed common marmosets. *Journal of Comparative Psychology, 124,* 402-411.

Gosling, S.D. (2001). From mice to men: What can we learn about personality from animal research. *Psychological Bulletin, 127,* 45-86.

Gunnar, M.R., Frenn, K., Wewerka, S.S., & Van Ryzin, M.J. (2009). Moderate versus severe early life stress: associations with stress reactivity and regulation in 10–12-year-old children. *Psychoneuroendocrinology, 34,* 62–75.

Gutiérrez-Ibáñez, C., Reddon, A.R., Kreuzer, M.B., Wylie, D.R., & Hurd, P.L. (2011). Variation in the asymmetry of the habenular nucleus correlates with behavioural asymmetry in a cichlid fish. *Behavioural Brain Research, 221,* 189-196.

Harding, E.J., Paul, E.S., & Mendl, M. (2004). Cognitive bias and affective state. *Nature, 427,* 312.

Harris, S., Ramnarine, I.W., Smith, H.G., & Pettersson, L.B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos, 119,* 1711-1718.

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nature Reviews Neuroscience, 11,* 503-513.

Hori, M. (1993). Frequency-dependent natural selection in handedness of scale-eating cichlid fish. *Science, 260,* 216–219.

Houde, E.D. (1987). Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium, 2,* 17-29.

Hunt, J., & Hodgson, D. (2010). What is fitness, and how do we measure it?, in: Westneat, D.E., Fox, C.W. (Eds.), *Evolutionary Behavioral Ecology*. University Press, Oxford, pp. 46-70.

Irving, E., & Brown, C. (2013). Examining the link between personality and laterality in a feral guppy *Poecilia reticulata* population. *Journal of Fish Biology, 83,* 311-325.

Irving, P.W. (1996). Sexual dimorphism in club cell distribution in the European minnow and immunocompetence signalling. *Journal of Fish Biology, 48,* 80-88.

Itzkowitz, M., Santangelo, N., & Richter, M. (2001) Parental division of labour and the shift from minimal to maximal role specializations: an examination using a biparental fish. *Animal Behaviour, 61,* 237-245.

Itzkowitz, M., Santangelo, N., & Richter, M. (2003) How does a parent respond when its mate emphasizes the wrong role? A test using a monogamous fish. *Animal Behaviour, 66,* 863-869.

Jones, K.A., & Godin, J-G.J. (2010). Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. *Proceedings of the Royal Society B – Biological Sciences, 277,* 625-632.

Kalin, N.H., Larson, C., Shelton, S.E., & Davidson, R.J. (1998). Asymmetric frontal brain activity, cortisol, and behavior associated with fearful temperament in rhesus monkeys. *Behavioral Neuroscience, 112,* 286-292.

Karenina, K.A., Giljov, A.N., & Malashichev, Y.B. (2013). Eye as a key element of conspecific image eliciting lateralized response in a fish. *Animal Cognition, 16,* 287-300.

Klemm, W.R. (2004). Habenular and interpeduncularis nuclei: shared components in multiple-function networks. *Medical Science Monitor, 10,* 261-273.

Koolhaas, J.M., de Boer, S.F., Coppens, C.M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinologi, 31,* 307-321.

Koolhaas, J.M., Korte, S.M., de Boer, S.F., van der Vegt, B.J., van Reenen, C.G., Hopster, H., de Jong, I.C., Ruis, M.A.W., & Blokhuis, H.J. (1999). Coping styles in animals: current status in behaviour and stress-physiology. *Neuroscience and Biobehavioral Reviews, 23,* 925-935.

Koops, M.A., & Grant, J.W.A. (1993). Weight asymmetry and sequential assessment in convict cichlid contests. *Canadian Journal of Zoology, 71,* 475-479.

Lee, A., Mathuru, A.S., Teh, C., Kibat, C., Korzh, V., Penney, T.B., & Jesuthasan, S. (2010). The habenula prevents helpless behavior in larval zebrafish. *Current Biology, 20,* 2211-2216.

Leliveld, L.M.C., Langbein, J., & Puppe, B. (2013). The emergence of emotional lateralization: Evidence in non-human vertebrates and implications for farm animals. *Applied Animal Behaviour Science, 145,* 1-14.

Lima, S.L., & Dill, L.M. (1990). Behavioral decisions made under the risk of predation – a review and prospectus. *Canadian Journal of Zoology, 68,* 619-640.

Lippolis, G., Bisazza, A., Rogers, L.J., & Vallortigara, G. (2002). Lateralization of predator avoidance responses in three species of toads. *Laterality, 7,* 163–183.

Lippolis, G., Joss, J.M.P., & Rogers, L.J. (2009). Australian lungfish (*Neoceratodus forsteri*): a missing link in the evolution of complimentary side biases for predator avoidance and prey capture. *Brain, Behavior and Evolution, 73,* 295-303.

Liu, D., Caldji, C., Sharma, S., Plotsky, P.M., & Meaney, M.J. (2000) The effects of early life events on in vivo release of norepinepherine in the paraventricular nucleus of the hypothalamus and hypothalamic-pituitary-adrenal responses during stress. *Journal of Neuroendocrinology, 12,* 5–12.

Lopez, L.P., Hawlena, D., Polo, V., Amo, L., & Mart, J. (2005). Sources of individual shy–bold variations in antipredator behaviour of male Iberian rock lizards. *Animal Behaviour, 69,* 1–9.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10,* 434–445.

Lyons, D.M., & Parker, K.J. (2007). Stress inoculation-induced indications of resilience in monkeys. *Journal of Trauma Stress, 20,* 423–33.

MacNeilage, P.F., Rogers, L.J., & Vallortigara, G. (2009). Origins of the left and right brain. *Scientific American, July,* 60–67.

Magat, M., & Brown, C. (2009). Laterality enhances cognition in Austrailian parrots. *Proceedings of the Royal Society B – Biological Sciences, 276,* 4155-4162.

Magnhagen, C., & Borcherding, J. (2008). Risk-taking behaviour in foraging perch: does predation pressure influence age-specific boldness? *Animal Behaviour, 75,* 509-517.

Magnuson, J.J. (1962). An analysis of aggressive behavior, growth, and competition for food and space in medaka (*Oryzias latipes* (Pisces, Cyprinodontidae)). *Canadian Journal of Zoology, 40,* 313–363.

Magurran, A.E., & Seghers, B.H. (1994). Sexual conflict as a consequence of ecology – evidence from guppy, *Poecilia reticulata*, populations in Trinidad. – *Proceedings of the Royal Society B – Biological Sciences, 255,* 31–36.

Matsumoto, M., & Hikosaka, O. (2009). Representation of negative motivational value in the primate lateral habenula. *Nature Neuroscience, 12,* 77–84.

McGrew, W.C., & Marchant, L.F. (1999). Laterality of hand use pay off in foraging success for wild chimpanzees. *Primates, 40,* 509-513.

Miklósi, Á., & Andrew, R.J. (1999). Right eye use associated with decision to bite in zebrafish. *Behavioural Brain Research, 105,* 199–205.

Moscicki, M.K., Reddon, A.R., & Hurd, P.L. (2011). Lateralized behaviour of a non-social cichlid fish (*Amatitlania nigrofasciata*) in a social and a non-social environment. *Behavioural Processes, 88,* 27-32.

Niemela, P.T., DiRienzo, N., & Hedrick, A.V. (2012). Predator-induced changes in the boldness of naïve field crickets, *Gryllus integer*, depends on behavioural type. *Animal Behaviour, 84,* 129-135.

Noakes, D.L.G. (1991). Ontogeny of behaviour in cichlids. In M.H.A. Keenleyside (Ed.), *Cichlid Fishes: Behaviour, Ecology and Evolution* (pp. 209-224). London: Chapman and Hall.

Øverli, O., Korzan, W.J., Hoglund, E., Winberg, S., Bollig, H., Watt, M., Forster, G.L., Øverli, E., Renner, K.J., & Summers, C.H. (2004). Stress coping style predicts aggression and social dominance in rainbow trout. *Hormones and Behavior, 45,* 235-241.

Øverli, Ø., Sorensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W.J., Summers, C.H., & Nilsson, G.E. (2007). Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neuroscience and Biobehavioral Reviews, 31,* 396-412.

Øverli, Ø., Winberg, S., & Pottinger, T.G. (2005). Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout - A review. *Integrative and Comparative Biology, 45,* 463-474.

Overmier, J.B., & Seligman, M.E. (1967). Effects of inescapable shock upon subsequent escape and avoidance responding. *Journal of Comparative and Physiological Psychology, 63,* 28–33.

Perria, L., Rosadini, G., & Rossi, G.F. (1961). Determination of side of cerebral dominance with Amobarbital. *Archives of Neurology, 4,* 173–181.

Plotsky, P.M., & Meaney, M.J. (1993). Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Molecular Brain Research, 18,* 195–200.

Pollock, M.S., Zhao, X., Brown, G.E., Kusch, R.C., Pollock, R., & Chivers, D.P. (2005). The response of convict cichlids to chemical alarm cues: an integrated study of behaviour, growth and reproduction. *Annales Zoologici Fennici., 42,* 485-495.

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European Journal of Pharmacology, 463,* 3-33.

Pryce, C.R., & Feldon, J. (2003). Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms. *Neuroscience & Biobehavioral Reviews, 27,* 57–71.

R Core Development Team. (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Raoult, V., Brown, C., Zuberi, A., & Williamson, J.E. (2012). Blood cortisol concentrations predict boldness in juvenile mulloway (*Argyosomus japonicus*). *Journal of Ethology, 30,* 225-232.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., & Dingemanse, N.J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82,* 291-318.

Reddon, A.R., & Balshine, S. (2010). Lateralization in response to social stimuli in a cooperatively breeding cichlid fish. *Behavioural Processes, 85,* 68-71.

Reddon, A.R., Gutiérrez-Ibáñez, C., Wylie, D.R., & Hurd, P.L. (2009). The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. *Behavioural Brain Research, 201,* 223-228.

Reddon, A.R., & Hurd, P.L. (2008). Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology Letters, 4,* 338-340.

Reddon, A.R., & Hurd, P.L. (2009a). Individual differences in cerebral lateralization are associated with shy-bold variation in the convict cichlid. *Animal Behaviour, 77,* 189-193.

Reddon, A.R., & Hurd, P.L. (2009b). Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. *Behavioural Processes, 82,* 25-29.

Reddon, A.R., & Hurd, P.L. (2009b). Acting unilaterally: Why do animals with strongly lateralized brains behave differently than those with weakly lateralized brains? *Bioscience Hypotheses, 2,* 383-387.

Reznick, D.A., Bryga, H., & Endler, J.A. (1990). Experimentally induced life-history evolution in a natural population. *Nature, 346,* 357–359.

Ricon, T., Toth, E., Leshem, M., Braun, K., & Richter-Levin, G. (2012). Unpredictable chronic stress in juvenile or adult rats has opposite effects, respectively, promoting and impairing resilience. *Stress, 15,* 11-20.

Rogers, L.J. (1989). Laterality in animals. *International Journal of Comparative Psychology, 3,* 5–25.

Rogers, L.J. (2000). Evolution of hemispheric specialization: Advantages and disadvantages. *Brain and Language, 73,* 236-253.

Rogers, L.J. (2002). Advantages and disadvantages of lateralization. in: Rogers, L.J., Andrew, R.J. (Eds.), *Comparative Vertebrate Lateralization*. University Press, Cambridge, pp. 126–153.

Rogers, L.J. (2009). Hand and paw preferences in relation to the lateralized brain. *Philosophical Transactions of the Royal Society of London B – Biological Sciences, 364,* 943–954.

Rogers, L.J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Applied Animal Behaviour Science, 127,* 1-11.

Rogers, L.J., & Andrew, R.J. (2002). *Comparative Vertebrate Lateralization*. University Press, Cambridge.

Rogers, L.J., Zucca, P., & Vallortigara, G. (2004). Advantages of having a lateralized brain. *Proceedings of the Royal Society B – Biological Sciences, 271,* S420-S422.

Roh, E., Mirza, R.S., & Brown, G.E. (2004). Quality or quantity? The role of donor condition in the production of chemical alarm cues in juvenile convict cichlids. *Behaviour, 141,* 1235-1248.

Ross, E.D., Homan, R.W., & Buck, R. (1994). Differential hemispheric lateralization of primary and social emotions. *Neuropsychiatry, Neuropsychology, and Behavioral Neurology, 7,* 1–19.

Roussigne, M., Blader, P., & Wilson, S.W. (2012). Breaking symmetry: The zebrafish as a model for understanding left-right asymmetry in the developing brain. *Developmental Neurobiology, 72,* 269-281.

Ruxton, G.D. (2006). The unequal variance t-test is an underused alternative to Student's t-test and the Mann-Whitney U test. *Behavioral Ecology, 17,* 688-690.

Salmeto, A.L., Hymel, K.A., Carpenter, E.C., Brilot, B.O., Bateson, M., & Sufka, K.J. (2011). Cognitive bias in the chick anxiety-depression model. *Brain Research*, 1373, 124-130.

Schaafsma, S.M., & Groothuis, T.G.G. (2011). Sex specific effects of postnatal testosterone on lateralization in cichlid fish. *Animal Behaviour, 81,* 283-288.

Scharf, S.H., & Schmidt, M.V. (2012). Animal models of stress vulnerability and resilience in translational research. *Current Psychiatry Reports, 14,* 159-165.

Sherry, D.F., & Schachter, D.L. (1987). The evolution of multiple memory systems. *Psychological Review, 94,* 439–454.

Siegel, D.G. (2007). Cheese Webcam Booth v.2.30.1 [computer program]. www.projects.gnome.org/cheese. Accessed 21 March 2011.

Sih, A., & Bell, A.M. (2008). Insights for behavioural ecology from behavioural syndromes. *Advances in the Study of Behavior, 38,* 227-281.

Sih, A., Bell, A.M., & Johnson, J.C. (2004a). Behavioural syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution, 19,* 372-378.

Sih, A., Bell, A.M., Johnson, J.C., & Ziemba, R.E. (2004b). Behavioral syndromes: An integrative overview. *Quarterly Review of Biology, 79,* 241-277.

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecology Letters, 15,* 278-289.

Siniscalchi, M., Quaranta, A., & Rogers, L.J. (2008). Hemispheric specialization in dogs for processing different acoustic stimuli. *PloS ONE, 3,* e3349.

Smith, R.J.F. (1986). Reduced alarm substance cell counts in male and androgen treated zebra danios (*Brachydanio rerio*). *Canadian Journal of Zoology, 64,* 551-553.

Smith, R.J.F. (1992). Alarm signals in fishes. *Reviews in Fish Biology and Fisheries, 2,* 33-63.

Snekser, J.L., & Itzkowitz, M. (2009). Sex differences in retrieval behavior by the biparental convict cichlid. *Ethology, 115,* 457-464.

Sovrano, V.A., Bisazza, A., & Vallortigara, G. (2001). Lateralization of response to social stimuli in fishes: A comparison between different methods and species. *Physiology & Behavior, 74,* 237-244.

Sovrano, V.A., Rainoldi, C., Bisazza, A., & Vallortigara, G. (1999). Roots of brain specializations: Preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behavioural Brain Research, 106,* 175-180.

Sovrano, V.A. (2004). Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behavioural Brain Research, 152,* 385–391.

Sovrano, V.A., & Andrew, R.J. (2006). Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae. *Behavioural Brain Research, 167,* 226–231.

Sovrano, V.A., Dadda, M., & Bisazza, A. (2005). Lateralized fish perform better than non-lateralized fish in spatial reorientation tasks. *Behavioural Brain Research, 163,* 122–127.

Stamps, J.A. (2007). Growth-mortality tradeoffs and 'personality traits' in animals. *Ecology Letters*, 10, 355-363.

Sutherland, R.J. (1982). The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. *Neuroscience and Biobehavioral Reviews, 6,* 1-13.

Thomson, J.S., Watts, P.C., Pottinger, T.G., & Sneddon, L.U. (2012). Plasticity of boldness in rainbow trout, *Oncorhynchus mykiss*: do hunger and predation influence risk-tasking behaviour? *Hormones and Behavior, 61,* 750-757.

Toms, C.N., Echevarria, D.J., & Jouandot, D.J. (2010). A methodological review of personality-related studies in fish: Focus on the shy-bold axis of behavior. *International Journal of Comparative Psychology, 23,* 1-25.

Vallortigara, G. (1992). Right hemisphere advantage for social recognition in the chick. *Neuropsychologia, 30,* 761–8.

Vallortigara, G. (2000). Comparative neuropsychology of the dual brain: A stroll through animals' left and right perceptual worlds. *Brain and Language, 73,* 189-219.

Vallortigara, G. (2006). The evolutionary psychology of left and right: Costs and benefits of lateralization. *Developmental Psychobiology, 48,* 418-427.

Vallortigara, G., & Andrew, R.J. (1991). Lateralization of response by chicks to change in a model partner. *Animal Behaviour, 41,* 187–94.

Vallortigara, G., & Andrew, R.J. (1994). Differential involvement of right and left hemisphere in individual recognition in the domestic chick. *Behavioural Processes, 33,* 41–58.

Vallortigara, G., & Bisazza, A. (2002). How ancient is brain lateralization? in: Rogers, L.J., & Andrew, R.J., (Eds.), *Comparative Vertebrate Lateralization*. University Press, Cambridge, UK, pp. 9–69.

Vallortigara, G., Chiandetti, C., & Sovrano, V.A. (2010). Brain asymmetry (animal). *WIREs Cognitive Science, 2,* 146-157.

Vallortigara, G., & Rogers, L.J. (2005). Survival with an asymmetrical brain: Advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences, 28,* 575-633.

Vallortigara, G., Rogers, L.J., Bisazza, A., Lippolis, G., & Robins, A. (1998). Complementary right and left hemifield use for predatory and agonistic behaviour in toads. *Neuroreport, 9,* 3341-3344.

Vallortigara, G., Rogers, L.J., & Bisazza, A. (1999). Possible evolutionary origins of cognitive brain lateralization. *Brain Research Reviews, 30,* 164-175.

Vavrek, M.A., & Brown, G.E. (2009). Threat-sensitive responses to disturbance cues in juvenile convict cichlids and rainbow trout. *Annales Zoologici Fennici, 46,* 171-180.

Walker, S.F. (1980). Lateralization of functions in the vertebrate brain. *British Journal of Psychology, 71,* 329–367.

Weiss, J.M., & Glazer, H.I. (1975). Effects of acute exposure to stressors on subsequent avoidance-escape behavior. *Psychosomatic Medicine, 37,* 499–521.

Wilson, D. S., Clark, A. B., Coleman, K., & Dearstyne, T. (1994). Shyness and boldness in humans and other animals. *Trends in Ecology and Evolution, 9,* 442–446.

Wilson, D. (1998). Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society B – Biological Sciences, 353,* 199-205.

Wiltschko, W., Freire, R., Munro, U., Ritz, T., Rogers, L., Thalau, P., & Wiltschko, R. (2007). The magnetic compass of domestic chickens *Gallus gallus*. *Journal of Experimental Biology, 210,* 2300–2310.

Wisenden, B.D. (1994). Factors affecting mate desertion by males in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). *Behavioral Ecology, 5,* 439-447.

Wisenden, B.D. (1995). Reproductive behaviour in free-ranging convict cichlids, *Cichlasoma nigrofasciatum*. *Environmental Biology of Fishes, 43,* 121– 134.

Wisenden, B.D., & Sargent, R.C. (1997). Antipredator behavior and suppressed aggression by convict cichlids in response to injury-released chemical cues of conspecifics but not to those of an allopatric heterospecific. *Ethology, 103,* 283–291.

Wittling, W., Block, A., Schweiger, E., & Genzel, S. (1998). Hemisphere asymmetry in sympathetic control of the human myocardium. *Brain and Cognition, 38,* 17–35.

Wittling, W., & Pfluger, M. (1990). Neuroendocrine hemisphere asymmetries: salivary cortisol secretion during lateralized viewing of emotion-related and neutral films. *Brain and Cognition, 14,* 243–265.

Zaidel, D.W. (1987). Hemispheric asymmetry in memory for pictorial semantics in normal subjects. *Neuropsychologia, 25,* 487– 495.