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Mallory L. Wiper

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AN EXPLORATION OF DRIVERS OF BEHAVIOURAL AND MORPHOLOGICAL LATERALITY AND EXPENSIVE TISSUES IN CHINOOK SALMON
(ONCORHYNCHUS TSHAWYTSCHA)

by
Mallory L. Wiper

A Dissertation
Submitted to the Faculty of Graduate Studies through the
Department of Biological Sciences
in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy at the University of Windsor

Windsor, Ontario, Canada

2018

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AN EXPLORATION OF DRIVERS OF BEHAVIOURAL AND MORPHOLOGICAL LATERALITY AND EXPENSIVE TISSUES IN CHINOOK SALMON
(ONCORHYNCHUS TSHAWYTSCHA)

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8 December, 2017
DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship

I hereby declare that this dissertation incorporates material that is the result of joint research, as follows:

Outside of the introductory and conclusions chapters, this dissertation incorporates the result of several joint research collaborations. Chapter 2 of the dissertation was co-authored with Dr. Sarah Lehnert, and Dr. Daniel Heath; all work was carried out under the supervision of Dr. Dennis Higgs. The primary contributions, data analysis, interpretation, and writing were performed by the author. The main contribution of Dr. Sarah Lehnert was through genetic analyses to obtain heterozygosity estimates for groups of fish, as well as providing helpful feedback on drafts of the manuscript. Dr. Daniel Heath also provided feedback on early versions of the second data chapter prior to submission for publication. Chapter 3 was designed, analyzed and written by the author under the supervision of and in collaboration with Dr. Dennis Higgs. Chapter 4 was a collaboration between the author, Ms. Shawna L. Semple and Dr. Dennis Higgs. All collaborators worked on processing of the fish used for analysis in this chapter. Ms. Semple’s primary contribution was through running genetic analyses on fin clips to determine heterozygosity estimates for each fish population. Analysis and interpretation of data on laterality and expensive tissue output, as well as writing of the manuscript were done by the author. Finally, chapter 5 was a joint collaboration of Ms. Jessica Mayrand, Dr. Christina Semeniuk, and Dr. Dennis Higgs. Ms. Mayrand and Dr. Semeniuk provided some of the fish from the 2014 and all fish from the 2015 year, including on site processing and transport to the University of Windsor. Ms. Mayrand helped with brain
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I certify that, with the above qualification, this dissertation, and the research to which it refers, is the product of my own work.

II. Previous Publication

This dissertation includes two original papers that have been previously published/submitted for publication in peer reviewed journals, as follows:

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I declare that, to the best of my knowledge, my dissertation does not infringe upon anyone’s copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my dissertation, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my dissertation.

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ABSTRACT

Specific hypotheses have been put forth to help explain and guide further studies of patterns of brain or body growth, as well as lateralized outcomes in fishes. In terms of laterality, what I refer to as the genetic variation and laterality hypothesis has been proposed, stating that there is an inverse relationship between the genetic variation of an organism and measured laterality. The expensive tissue hypothesis, on the other hand, has been proposed as an explanation of differences in brain size, stating that for increased brain growth there must be a compensatory trade-off with other ‘expensive’ tissues. In the present dissertation I have used a salmonid species, Chinook salmon (Oncorhynchus tshawytscha), to explore both the differential investment into brain and body growth, and drivers behind morphological and behavioural laterality. In the first data chapter, as an examination of the genetic variation and laterality hypothesis, I investigate how four different ‘inbreeding levels’ affect morphological laterality of the hemispheres of two main brain regions, the optic tectum and cerebellum. As well, I examine how fish in the ‘inbreeding levels’ differ on a brain-to-body ratio measure as a test of how genetic background might affect expensive tissue investment. In the second data chapter, I use juvenile salmon of six different genetic backgrounds, three domestic and three outcrosses, as well as a manipulation of flow direction (clockwise or counter-clockwise) in the rearing barrels of the fish, to investigate the genetic background, environmental, and gene-by-environment (GxE) interaction effects on both behavioural (C-start; mirror inspection) and morphological (brain; whole eye) laterality. The third chapter examines the effect of population differentiation of seven Chinook populations on morphological brain laterality, again, as an examination of the genetic variation and laterality hypothesis.
In this chapter I also looked at the differences between populations on the expensive tissue trade-offs of the brain and the body, the brain and the gut and the gut and the body. Finally, in the fourth data chapter I examine the brain-to-body trade-off on six populations of Chinook salmon over three years: 2014, 2015 and 2016. As a whole, the laterality results demonstrate that there is some genetic effect on morphological laterality of the brain hemispheres, but not following the pattern suggested by the genetic variation and laterality hypothesis. From behavioural examinations I note that the manipulation of flow direction and the GxE interaction show the most significant effects on laterality. Results of expensive tissue trade-offs show that there is differential investment into the brain versus the body in Chinook salmon, and this investment also shows differences between populations examined, indicating that there are drivers to expensive tissue trade-offs which require more exploration. Investigating these areas may hold important information for aquaculture facilities, especially with regards to differential tissue investment, where often times a larger body is the end goal. However, investment into the brain may be a reflection of cognitive ability which would be of greater importance for those hatcheries rearing fish for conservation purposes: higher cognitive ability may very likely equate to higher overall survival. In regard to lateralization, the further exploration of laterality, both morphological and behavioural, can help us to better understand how and why laterality developed, its advantages, and how, and perhaps why, it has been maintained throughout the evolution of vertebrates.
DEDICATION

Dedicated to my mom.
Thank you for showing me what a woman is capable of and for always making me feel like I was smart enough.
ACKNOWLEDGEMENTS

First and foremost I owe a BIG thank you to my advisor, Dr. Dennis Higgs. Dennis, you were the best advisor I could have asked for! You helped me to believe in my own knowledge and to be more comfortable with not always knowing the answer. The support and guidance that you gave was truly unique, but I wouldn’t have it any other way. I appreciate you trying to teach me to “go with the flow” and learn more about “teachable moments”, but some lessons just don’t stick. Most of all, though, I appreciate you being willing to take a chance on someone you didn’t know who wanted to work on a topic you weren’t familiar with. You provided me an opportunity for which I am forever grateful.

A huge thanks also goes to the members of the Higgs lab: Dr. Kirsten Poling, Ms. Megan Mickle, Ms. Melissa Macksoud, and Mr. Christian Ieraci. First, Kirsten, thank you for understanding many of my particular neuroses! I’ve appreciated your advice and friendship, and will always be concerned that one day you will drop one of your thousand coffee cups you carry with you. Megan, we’ve known each other for quite a while during our respective time in the Higgs lab and I’m so happy we’ve been able to grow together as science-y friends! You’ve always been a wonderful sounding board to bounce ideas off of and you were always, always willing to help, no matter what, if I needed it. You are one of the most supportive and encouraging friends I’ve ever had the pleasure to meet and have in my life. You are a force to be reckoned with and I hope the world is ready for you! Melissa, you and I have a lot in common with respect to our introverted personalities and it’s been wonderful to have someone who just “gets” the introverted thing. You, too, have been a great person to discuss ideas with and I feel privileged to have gotten to
know you over the past couple of years. You are so clever and capable that there’s no
doubt in my mind that you will end up exactly where you want to be. There’s a fire inside
of you that is waiting to burst; don’t be afraid to let it shine bright! Finally, Christian. You
have been integral to much of the research in this dissertation. As an undergrad you spent
time analyzing videos and measuring brains for which I am eternally grateful; without
your help as one of my volunteers I never would have completed any of this. Now, as a
grad student, I’ve been able to get to know you more and it’s been fun! You’re
lighthearted and don’t take yourself too seriously, and those are such wonderful qualities
to have. You’re a talented, intelligent guy and I know that you will be successful in
whatever you do! To all four of you, I feel honoured that I’ve been able to count you as
colleagues during my academic career. It wouldn’t have been the same without you!

Thanks also goes out to all of the volunteers I’ve had over the years that have
helped me in some way with brain measuring or video analysis: Jessica Garabon, Nina
Milidrag, Natalie Kindiak, Lisa Truong, Jessica Montaleone, Allana Belsito, Angela
Teoderescu, Daniel Dubovan and Vivian Vegh. Without these people this project never
would have come together.

To my committee members, Dr. Barbara Zielinski, Dr. Christina Semeniuk, and
Dr. Daniel Heath, I owe gratitude for your insightful comments, suggestions and guidance
throughout my academic career. You have been very important to bringing this
dissertation together and getting it to its final point. In addition, I’d like to extend a huge
thank you to my external examiner, Dr. Gabrielle Nevitt, for being a part of the defence
committee and being so supportive and just a wonderful person in general. Thank you!

As always, I extend my thanks to the people who run the Biology Building. To the
ladies in the office, Nancy Barkley, Judy Janzen, Paula Tetreault, and Christine Côté, all
of you have been so kind and helpful whenever I’ve been in need of an answer. Thank you for keeping things running smoothly! To the Stockroom staff, especially Ingrid Churchill and Rodica Leu, thank you for helping me any time I needed it, advising me on protocols, and helping me get any items I needed, especially when they were for my salmon brains! To Bob Hodge, let’s be real: you keep this building afloat. Without you, Bob, this building would 110% fall apart. And also, thanks for bringing the mint Girl Guide cookies! Finally, to the custodial staff, Jane and Dan: Thank you for keeping the building clean for everyone who comes through every day. Your efforts are really appreciated!

Now to those who have been with me the longest, my family. Thank you, thank you, thank you for always being supportive of my academic path. You may not have always been totally clear on what I was studying or why it was important but you’ve all been so supportive and made me feel like I was in the right place, and I’ve really, truly appreciated that!

My final and biggest thanks goes to my husband, Frazier Fathers. I feel like “thank you” isn’t quite enough to say for all that you’ve done for me. You’ve supported me from the start and believed I was capable of anything—even when I didn’t believe in myself. You’ve helped me to become the woman I am today. You’ve made me stronger, happier, and a better person than I ever thought I could be. You’ve made a world of difference to me and I feel so privileged and grateful to have you by my side. Thank you for choosing me and for sticking with me during this adventure!
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Laterality as a field of study

Laterality as a field of study has advanced greatly since its broadening into non-human organisms, with several reviews laying out the progress of this field and how it has evolved. Bisazza et al. (1998) and Vallortigara and Rogers (2005) have done an excellent service outlining the now well-accepted origins of cerebral asymmetry and addressed, in detail, the rise of individual and population level laterality as well as the connection between the two. A missing feature of these reviews, though, is a detailed explanation of the formulas used to calculate measures of laterality and the mechanistic and evolutionary drivers behind laterality. In the current review I endeavour to address these missing pieces in an effort to synthesize what we know about mechanisms and to call for a standardization of laterality calculations. We must continue to study behavioural laterality but begin to incorporate further questions and research that help determine what genetic, environmental, and gene-by-environment interaction effects there may be on the development and maintenance of laterality to help us better understand this biological characteristic that has been widely found among vertebrate species.

Laterality defined and measured

The argument for lateralized differences of brain hemispheres was historically held as applicable only to humans, beginning largely with the landmark discovery of Broca’s area in 1861 (Keller et al., 2009; Rogers et al., 2013). This uncovering of what was deemed the speech and language control centre in the left hemisphere of the brain, along with population-level bias for right-hand dominance in humans, was enough for
some to hold to the belief of functional and structural “human only” hemisphere differences (MacNeilage et al., 2009). Evidence from Finch’s (1941) work with chimpanzees, however, began to move the idea of functional laterality beyond the confines of the human brain, and from that time forward, confirmation of laterality in nonhuman species continued. Evidence was put forth that hemispheric specialization of vocal control existed in canaries (Serinus canarius; Nottebohm, 1977) that lateralization of function for visually guided behaviours was present in domestic chickens (Gallus gallus domesticus; Rogers & Anson, 1979); that differential hemispheric control of behaviour in rats was evident (Denenberg, 1981); and that asymmetry of hand use could be identified in non-human primates (MacNeilage et al., 1987).

Asymmetrical differences in morphological characteristics have been identified in a variety of features (Sheridan & Pomiankowski, 1997; Bryden & Heath, 2000; Gutiérrez-Ibáñez et al., 2011; Takeuchi & Hori, 2013) and fall into one of three categories of asymmetry: fluctuating asymmetry, antisymmetry or directional asymmetry. Fluctuating asymmetry, a maladaptive form of asymmetry, has been defined as the “inability [of an organism] to undergo identical development of a bilateral trait on both sides of the body” (Swaddle et al., 1994), and is assessed based on measurements of both halves of the trait. The suggestion with fluctuating asymmetry is that there has been random deviation from identical bilateral symmetry since the expected developmental path of perfect symmetry has been perturbed (van Valen, 1962; Møller & Swaddle, 1997). Antisymmetry is a form of physical development of an organism wherein asymmetry is the norm, but whether the larger character is on the right or left side varies and can happen with equal frequency (Leary & Allendorf, 1989; Møller & Swaddle, 1997; Van Valen, 1962). For example, fiddler crabs (Uca pugilator) will always have one larger
Figure 1.1: Graphical depictions of the three types of asymmetry at the population level. (A) indicates **Fluctuating Asymmetry**, where there is an equal number of individuals who are left or right biased, resulting in a normal distribution, centered around perfect symmetry of development. (B) shows the pattern of asymmetry in those populations where **Antisymmetry** is present. Here a population will have a bimodal distribution, having many individuals with a left bias, many with a right bias, and very few exhibiting perfect symmetry of development. Generally, perfect symmetry in populations where asymmetrical development is the norm would be detrimental. Finally, (C) represents **Directional Asymmetry** of a population wherein measurement of a characteristic is skewed to one side of perfect symmetrical development. Here, the majority of members within a population will show the same asymmetrical growth of a feature or behavioural side preference.
signalling claw but whether it is the left or the right claw is variable (Pratt & McLain, 2002). Finally, directional asymmetries occur when greater than 50% of a population exhibit an asymmetry where one half of a bilateral trait has a larger measured value, indicating increased growth on that side (van Valen, 1962; Leary & Allendorf, 1989; Møller & Swaddle, 1997). The term directional asymmetry largely applies to the typically skewed asymmetry of physical characteristics, the norm for the developmental trajectory (Leary & Allendorf, 1989), wherein one half of a bilateral characteristic always (predictably) shows greater overall development (Van Valen, 1962). Little work, however, continues to investigate the physical directional asymmetries of organisms and instead focuses on ‘laterality’, or ‘lateralization’; it is this type of asymmetry upon which I will focus in the present review. In more recent research, laterality has become synonymous with measures of cognitive and behavioural asymmetries and seems to be extending to neuroanatomical asymmetries as well. Laterality then is best understood as the phenomenon of differential structural specializations or differential processing of specific stimuli in the left and right hemispheres of the brain (Frasnelli et al., 2012; Rogers et al., 2013; Dadda et al., 2015). This differential processing has been argued to be displayed through specific behavioural inclinations, wherein there is a preferential use of one half of a bilateral characteristic (e.g. Facchin et al., 1999; Braccini & Caine, 2009) or movement in a preferred direction (e.g. Bisazza et al., 2000a; Dadda et al., 2010). Overall, laterality can be suggested to provide a relative advantage, or benefit, to those organisms that express this characteristic.

No matter the cognitive, behavioural or neuroanatomical asymmetry under investigation there are specific formulae used to evaluate laterality but there is as yet little consistency between investigators as to the specific formula used. In these formulae ‘L’ is
the measure of the left side or preference, and ‘R’ is the value for the right. The first
formula, the ‘laterality index’ (LI), is most commonly calculated as $LI = \frac{(L-R)}{(L+R)}$,
where positive values indicate a leftward preference and negative values indicate a
rightward preference (Alonso et al., 1997; Shoblock et al., 2013; Broder & Angeloni,
2014). This formulation of the laterality index, which can be multiplied by 100 to aid in
interpretation, generally ranges from -1 (or -100) to +1 (or +100) and is the strongest
indicator of the direction of asymmetry (Batt et al., 2007; Barnard et al., 2016). The
second laterality index equation commonly used focuses on one side or directional
preference: $LI = \left[\frac{R}{L+R}\right] \times 100$ (e.g. Sovrano & Andrew, 2006; Reddon et al., 2009;
Hopkins et al., 2016). When this formula is utilized, a cut-off value of 50% is used to
determine left or right dominance. Generally, values above 50% will indicate a rightward
preference—the greater the number is away from 50, the stronger the preference—and
any value below 50% indicates a leftward preference (Sovrano, 2004; Sovrano &
Andrew, 2006). The third commonly used measure of lateralization is absolute laterality
(ALI). This index is used to gauge the strength of laterality irrespective of direction and is
often used to assess individual asymmetry (Brown et al., 2007; Barnard et al., 2016). The
common way of calculating absolute laterality is by taking the absolute, or unsigned,
value of the laterality index: $|LI|$. In cases where the formula $LI = \frac{R}{L+R}$ has been
used, the calculation of absolute laterality is: $ALI = |LI – 0.5|$ (Dadda & Bisazza, 2012;
Bibost et al., 2013). Since this laterality index is centred around 50%, the value of 50%
(or 0.5 if the LI equation was not multiplied by 100) must be subtracted to establish
deviation from random (C. Brown, personal communication, July 6, 2016). Values of 0
would denote individuals who are ambidextrous or show no directional preference, and a
score of 50 (or 0.5) indicates a completely lateralized individual (Brown & Magat,
Of the three formulae, the absolute laterality index has previously been argued to be the “more functionally important dimension” (Gutiérrez-Ibáñez et al., 2011 and references therein) to measure asymmetry of an organism since the laterality index alone may lead to a loss of overall information on individual variation in laterality (Brown et al., 2007; Reddon & Hurd, 2008; Reddon et al., 2009). In general, many studies have shown a preference for the proportional measure of laterality as opposed to the absolute (e.g. Cantalupo et al., 1995; De Santi et al., 2001; Sovrano, 2004), yet using both measures provides more information on the measured laterality of an organism.

Both invertebrate and vertebrate species have been assessed for the presence of laterality, and while the majority of studies have been carried out in vertebrate species, strong evidence does exist for asymmetry in invertebrates (see Frasnelli et al., 2012 for review). For example, giant water bugs (Belostoma flumineum) have shown a significant left-turn bias when tested in a T-maze (Kight et al., 2008) and honeybees (Apis mellifera) have been found to show a lateral shift between right and left antenna use when tested for short and long-term olfactory memory recall (Rogers & Vallortigara, 2008). Cuttlefish (Sepia lycidas) exhibit morphological asymmetry of the curvature of their cuttlebone, and behavioural asymmetry of prey capture is related to this curvature (i.e. right curvature, leftward turn) (Lucky et al., 2012).

Among vertebrate species, all major classes have been investigated for lateralized tendencies or preferences, with at least one representative study of lateralization from each class (Table 1). For instance, mammals have been widely studied for lateralized preference or control of a wide-range of behaviours, including, for example, the preferred side on which an orca (Orcinus orca) calf stays next to its mother (Karenina et al., 2013); lateralized eye and nostril use in domestic horses (Equus caballus) (Des Roches et al.,
2008); lateralized eye use in *feral* horses (Austin & Rogers, 2012); and hand use preference in pig-tailed macaques (*Macaca nemestrina*) (Regaiolli et al., 2016). Both bony and cartilaginous fishes have been investigated for lateralized eye use preferences (e.g. Sovrano, 2004), and for asymmetry of escape or turning behaviour (e.g. Green & Jutfelt, 2014). Eye use preference, “footedness”, and task ability or efficiency related to level of lateralization has been investigated in bird species, including domestic chickens (Rogers et al., 2004), parrots (Brown & Magat, 2011a) and Gouldian finches (*Erythrura gouldiae*; Templeton et al., 2012). Amphibians and reptiles have been studied less often, but there is evidence for lateralization of escape or attack direction (e.g. toads, *Bufo* spp.: Vallortigara et al., 1998; Lippolis et al., 2002) and eye use (wall lizards, *Podarcis muralis*, Bonati et al., 2013; tree lizards, *Urosaurus ornatus*, Hews & Worthington, 2002). Since much support exists in the literature for the presence of lateralization in all classes of vertebrate species the remainder of this review will focus on non-human vertebrate organisms.

**Laterality in history**

*Asymmetrical neural structures in non-human animals*

One of the earliest studies to focus on bilateral neuroanatomy investigated the size difference between the Mauthner cell neurons, which control a fish’s quick escape (C-start) response (Fetcho, 1992; Vallortigara & Bisazza, 2002), in a small sample of goldfish (*n* = 3; Moulton & Barron, 1967) finding that the left Mauthner cell was three times the size of that of the right (Moulton & Barron, 1967). The avian visual system also shows clear asymmetry of neural structures. Injection of a retrograde tracer into the left or right visual hyperstriatum of chickens provided some of the first neuroanatomical
evidence for the development of lateralization of the visual pathways, and also demonstrated that lateralization is dependent upon asymmetrical light stimulation pre-hatch (Rogers & Sink, 1988). Tracer studies in pigeons also show clear contralateral projections of neurons from the right tectum to the left tectorotundus that are twice as numerous as the reverse projection (Güntürkün et al., 1998). Additionally, the development of the visual system of the pigeon, an altricial species, is much slower than that of the chick, which is precocial, yet lateralization in both species is affected by asymmetrical light stimulation during incubation (Güntürkün, 2002). Morphological brain asymmetries in non-human primates, too, have been examined: magnetic resonance images (MRI) from chimpanzees (Pan troglodytes), bonobos (Pan paniscus), and gorillas (Gorilla gorilla) show that Broadman’s area 44, part of Broca’s area in the human brain, has similar left hemisphere asymmetries to those found in the brains of humans (Cantalupo & Hopkins, 2001). The habenular nuclei, a highly conserved pair of bilateral neural structures within the limbic system (Bianco & Wilson, 2009; Reddon et al., 2009), have often been compared for asymmetries, especially in fish, reptiles, and amphibians (see Concha & Wilson, 2001 for review). Across these taxa there is differential size between the habenular nuclei (e.g. Rana esculenta, Kemali et al., 1990; Petromyzon marinus, Vallortigara & Bisazza, 2002; Amatitlania nigrofasciata, Gutiérrez-Ibáñez et al., 2011), and in at least one species (pearl cichlids, Geophagus brasiliensis) the asymmetry is dependent on body size – with the direction of asymmetry changing as the fish grow (Reddon et al., 2009). With studies focusing on bilateral neural structures in vertebrates that inhabit both land and water, it is clear that there is a slowly growing interest in the idea that physical differences between bilateral neural features, not just asymmetrical behavioural displays, may be present in a wide range of vertebrate species. However,
Table 1.1

Examples of lateralized morphology and behaviour, as well as mechanistic drivers of laterality, in major classes of vertebrates. This table is not meant as an exhaustive collection of all studies conducted to date on vertebrate organisms but rather as a sample summary demonstrating evidence for laterality found in each vertebrate class, and that laterality is evolutionarily conserved in many cases (e.g. handedness in amphibians and mammals). The table also indicates areas for future research.

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<th>Type of Laterality Tested</th>
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<td>Quaranta <em>et al.</em>, 2007</td>
<td>Regaiolli <em>et al.</em>, 2016</td>
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<td>Bonati et al., 2013</td>
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<td>Prey or food Detection, inspection, Handling, avoidance</td>
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<td>Brown &amp; Magat, 2011a*</td>
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<td>Vallortigara et al., 1998*</td>
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*Note*: References investigating multiple species are indicated with an asterisk (*).
the literature thus far has left a gap between the physical differences of bilateral neural structures and their potential connection to cognitive processing and expressed behaviour.

**Lateralized Behaviour in Non-Human Animals**

Contrary to the early belief that humans were the only species capable of showing hand preference, Finch (1941) found that in 800 handedness trials 25 out of 30 chimps in the sample displayed preferential hand use in 80% or more of the trials. The early exploration into handedness of chimpanzees continued (e.g. Hopkins & Bennet, 1994; Hopkins, 1996; McGrew & Marchant, 1999; Llorente et al., 2011), with an upsurge in the exploration of this trait in other primate species, including marmosets (*Callithrix spp.*) (Hook & Rogers, 2000; Braccini & Caine, 2009), orangutans (*Pongo pygmaeus*; Peters & Rogers, 2008), gorillas (Tennie et al., 2008), and capuchins (*Cebus paella*) and Rhesus macaques (*Macaca mulatta*) (Westergaard & Sumoi, 1996), all finding strong evidence of handedness. Other surveys of functional laterality have included analyses of detour behaviour in fish (Bisazza et al., 1997b; Facchin et al., 1999; Heuts, 1999), domestic chickens (Regolin et al., 1994; Vallortigara et al., 1999a), and dogs (e.g. Siniscalchi et al., 2013). Lateralized eye use investigations in vertebrates have found differential eye use preferences in fish, wherein the right eye is preferred when investigating a dummy predator (Facchin et al., 1999), and the left eye is more often used when inspecting familiar, social stimuli (Sovrano et al., 1999; Sovrano, 2004; Sovrano & Andrew, 2006). In Gouldian finches right eye use is critical to choosing a genetically compatible mate (Templeton et al., 2012). In Australian magpies (*Gymnorhina tibicen*) left eye inspection of a predator indicates that the bird will withdraw, whereas right eye investigation precedes approach and mobbing behaviour (Koboroff et al., 2008). Toads (*Bufo spp.*)
have also shown very strong preferences for viewing stimuli: the right eye is more
commonly used when viewing prey items (Vallortigara et al., 1998), whereas the left eye
is preferred for predator inspection (Lippolis et al., 2002); lizards (Podarcis muralis) too
have shown a left eye preference when viewing a predator (Bonati et al., 2013).
Examinations of lateralized footedness in parrots have found that more strongly
lateralized individuals exhibit better problem-solving skills (Magat & Brown, 2009), and
that eye preferences for food inspection correlate highly with foot use (i.e. left eye, left
foot) (Brown & Magat, 2011a). Behavioural laterality has also been found in the
“flippered-ness” of female sea turtles (Dermochelys coriacea) who more often use their
right flipper to bury their eggs in the sand (Sieg et al., 2010).

Experimental work has also shown defined links between bilateral brain structures
and expressed behaviour. Nottebohm and Nottebohm (1976), for example, explored the
hemispheric control of vocalization in canaries and white-crowned sparrows (Zonotrichia
leucophrys) through surgically severing the right or left hypoglossal nerve
(tracheosyringealis) that innervates the muscles of the syrinx responsible for song
production. Those birds whose right hypoglossal nerve was severed experienced little
change to their song production; those birds that had received left nerve severance,
however, experienced disturbed song production ability, indicating left brain control of
vocalization (Nottebohm & Nottebohm, 1976). Similarly, in frogs (Rana pipiens), when
lesions are made to the left pretrigeminal area (PTA), the area housing neurons that
project to and innervate vocal control muscles, release call vocalization is disrupted, but
not when lesions are made to the right PTA neurons (Bauer, 1993). When domestic
chicks were injected with a glutamate solution in either the left or right visual
hyperstriatum area of the brain, only those chicks that had received glutamate in the left
hemisphere showed disruption in their visual discrimination ability, as well as exhibiting increased attack and copulation behaviours (Deng & Rogers, 1997). From the research of laterality in non-human vertebrates, commonalities of functional lateralization across vertebrate species have been identified, yet there is clearly still a need to examine laterality in a diverse array of species, especially regarding ecologically relevant behaviours controlled by the left and right brain hemispheres (see Rogers et al., 2013 for a review).

**Laterality and evolution**

*Hemispheric specialization*

Two main hypotheses have been advanced to explain the evolution of vertebrate hemispheric specialization: asymmetry driven by specializations in feeding structures (reviewed by Andrew, 2002; Rogers et al., 2013), or driven by the evolution of two eyes (Andrew, 2002; Rogers et al., 2013). Evidence for the first hypothesis has been modelled by the single-eyed chordate, the lancelet (*Amphioxus*) (Andrew, 2002; Rogers et al., 2013). In the larval stage, the lancelet is asymmetrical in its feeding anatomy with its mouth located on the left side of the body which permits stationary substrate exploration for food particles (Stokes, 1997). In the adult stage, however, the lancelet’s mouth shifts to a central position, but the neural connections controlling feeding behaviour remain delegated to the left side of the nervous system (Rogers et al., 2013). This left hemisphere control of the feeding response has endured over the course of vertebrate evolution and has been demonstrated in species such as toads (Vallortigara et al., 1998) and chickens (Deng & Rogers, 1997). The hypothesized role of two eyes relies on the supposition that with two eyes organisms were able to remain in a stable position and make directed
movements toward or away from stimuli (Rogers et al., 2013). Additionally, two eyes permitted an organism to take in more of its surroundings, meaning there were a greater number of stimuli to process at one time. This increased need for processing of multiple stimuli may have been largely responsible for the differentiation of the processing specialities of the hemispheres over time. The left hemisphere became responsible for categorizing familiar experiences and stimuli, such as food and conspecifics, and for regulating routine behaviour (Rogers, 2000; Lippolis et al., 2002; MacNeilage et al., 2009; Rogers et al., 2013). Toads, for example, have demonstrated more efficient prey capture ability when a prey item is presented in their right versus their left visual field (Vallortigara et al., 1998; Robins & Rogers, 2004; Robins & Rogers, 2006), and harpy eagles (*Harpia harpyja*; Palleroni & Hauser, 2003) and Japanese macaques (*Macaca fuscata*; Petersen et al., 1978) both show a left hemisphere bias for recognition of conspecific vocalizations. The right hemisphere, on the other hand, manages detection of, and decisive action in response to, novelty and unexpected stimuli (MacNeilage et al., 2009; Rogers et al., 2013). Shorter reaction times and more efficient escape behaviour have been found in toads (Lippolis et al., 2002), fish (Cantalupo et al., 1995), and stripe-face dunnarts (*Sminthopsis macroura*; Lippolis et al., 2005) when a predator, an unexpected stimulus, is viewed in the left visual field. The evolutionary differentiation of the left and right hemispheres resulting in hemispheric specialization across vertebrates was likely out of necessity permitting quick processing of multiple forms of ecologically relevant stimuli in environments with increasing complexity.
**Costs and benefits of laterality**

Having a distinct cerebral asymmetry may hold an ecological cost: there may be a *decrease* in efficiency of processing if a given task necessitates interhemispheric communication (Dadda et al., 2009). For example, in fish choosing between high- and low-quality shoals, highly lateralized individuals make more errors in choice for quality due to decreased interhemispheric communication (Dadda et al., 2009). For those individuals that are highly lateralized in behavioural output it may become difficult to overcome stereotyped responses to external stimuli leading to response errors. In a radial maze, highly lateralized fish (*Brachyraphis episcopi*) consistently turned in their preferred direction, instead of following a visual cue which signified an immediate food reward (Brown & Braithwaite, 2004). Further, predators and prey can learn and exploit behavioural biases, since with repeated exposure response biases may become predictable (Ghirlanda & Vallortigara, 2004). A final cost of cerebral lateralization is that the natural environment within which species live is unbiased; prey and predators do not consistently present themselves on an organism’s “preferred” side, which can lead to increased vulnerability if responses are strongly biased (Lippolis et al., 2002; Ventolini et al., 2005); however, animals can still react to stimuli in the “wrong” visual field but the reaction is less efficient. Taken together, the problem with these costs is plain: a highly lateralized organism can become vulnerable to predation, as well as to missing feeding opportunities. In addition, strict adherence to cerebral asymmetry can affect the efficiency and speed of response (Rogers et al., 2004).

The benefits of laterality, on the other hand, are largely focused on the cognitive advantages of cerebral lateralization. First, with a lateralized brain an organism may increase its neural capacity (Levy, 1977; Vallortigara, 2006). Secondly, lateralization of
the brain may spare the need for increased neural tissue volume, which is energetically costly to produce (Aiello & Wheeler, 1995; Tsuboi et al., 2015). Finally, lateralization helps avoid duplication of function in both hemispheres (Ghirlanda & Vallortigara, 2004; Vallortigara, 2006) facilitating simultaneous processing of multiple types of stimuli. This last benefit has been directly demonstrated in pigeons (Güntürkün et al., 2000) and domestic chicks (Deng & Rogers, 1997) that showed improved visual discrimination ability between food and non-food items when using the right eye; in lateralized chicks better able to find food in the presence of a predator as compared to their non-lateralized counterparts (Rogers et al., 2004); and, similarly, in highly lateralized topminnows, which were quicker at capturing prey when in the presence of a predator (Dadda & Bisazza, 2006a) or a harassing male (Dadda & Bisazza, 2006b). While there are significant costs, laterality must provide a greater benefit or relative advantage to the overall fitness of organisms as we see this characteristic throughout vertebrate evolution. The advantages discussed here are of clear benefit to the individual but do not explain population-level laterality (Vallortigara, 2006). Population-level laterality, therefore, should be investigated from an evolutionary standpoint to understand the importance of this strategy to survival and fitness (Vallortigara, 2006).

Laterality and population ecology

When considering animal populations, laterality has often been discussed as an evolutionarily stable strategy (ESS) (e.g.; Ghirlanda & Vallortigara, 2004; Vallortigara, 2006; Rogers et al., 2013; Barnard et al., 2016): a strategy (i.e. behavioural phenotype) that, once adopted by the majority of a population, cannot be usurped by any other strategy (Maynard Smith, 1982). In the case of laterality, even if an individual’s
directional bias differs from the group majority the most advantageous course is to align individual behavioural action with the majority bias (Vallortigara, 2006; Rogers et al., 2013). Laterality has been modelled as an ESS through game theory modelling by Ghirlanda and Vallortigara (2004), the first to outline how laterality can be exhibited in the context of decision making during predator-prey interactions, suggesting that population-level laterality can, in many conditions, be a beneficial course of action. Laterality then can be considered a strategy that dictates how an animal may respond in a given situation (Maynard Smith, 1982). In this case, it means choosing to escape in a leftward or rightward direction, effectively increasing the probability of escape if each member of the group is lateralized in the same direction (Ghirlanda & Vallortigara, 2004). Overall, there is no assumption of a greater benefit of a leftward or rightward escape bias, but from a population-level point of view, the assumption for greater probability of success lies with whichever directional preference is in the majority (Ghirlanda & Vallortigara, 2004). In other words, population-level laterality may be under the influence of frequency dependent selection, an evolutionary selection process wherein the fitness of one phenotype (i.e. behavioural strategy) is dependent upon its frequency in relation to other phenotypes within a given population (Conner & Hartl, 2004). In cases of positive frequency dependent selection, there is a positive correlation between phenotype and fitness with the opposite being the case for negative frequency dependent selection (Conner & Hartl, 2004). From a behavioural lateralization standpoint, both positive and negative frequency-dependent selection can provide a benefit to those individuals in the majority or minority, respectively. Frequency-dependent selection may, therefore, work as an explanation to why majority and minority biases exist with respect to laterality (Conner & Hartl, 2004; Ghirlanda & Vallortigara, 2004). Therefore, it is
necessary to investigate vertebrate lateralization from the point of view of evolutionary game theory, evolutionarily stable strategies, and frequency dependent selection (Vallortigara, 2006) as these concepts provide a potential explanatory pathway to population level lateralization. Investigating laterality from the standpoint of an ESS may provide the necessary link required to bring together neuroanatomical, neuropsychological and evolutionary approaches to the study of vertebrate lateralization (Vallortigara, 2006; Rogers et al., 2013).

**Mechanistic drivers of laterality**

While laterality has been tested in species from each of the major classes of vertebrates, the mechanisms controlling lateralized growth and/or behaviour have been studied in only a handful of organisms. For example, canaries, (Nottebohm & Nottebohm, 1976), frogs (Bauer, 1993), and domestic chicks (Deng & Rogers, 1997), have all shown disrupted behaviour when bilateral neuroanatomical structures have been manipulated. It is clear that research must continue on mechanistic drivers and in many more species, but the evidence gathered thus far in this area has provided valuable insight and direction to further discussion and discovery of the driving forces behind the mechanistic driving forces of laterality.

**Genetic mechanisms**

While it is unlikely that one gene controls laterality in all species, evidence is slowly coming to light through genetic commonalities found within species groups, leading to the idea of key genetic mechanisms responsible for lateralization. In mice there is evidence for a single gene with alternate alleles affecting laterality of paw use (Biddle
et al., 1993; Collins et al., 1993), and more recently, directional asymmetry has been found in the genetic expression of the ASE neurons—a bilateral pair of gustatory neurons—of all *Caenorhabditis elegans* worms (Sagasti, 2007). In *C. elegans* there is a reciprocal repression of the microRNAs and transcription factors in the genetic circuit that determines asymmetry of the ASE neurons (Sagasti, 2007, and references therein). Specifically, for the left ASE neuron the DIE-1 transcription factor promotes the expression of the microRNA *lsy-6*, whereas in the case of the right ASE neuron the COG-1 transcription factor activates expression of the microRNA *mir-273* (Sagasti, 2007). This reciprocal repression circuit in *C. elegans* is a well-studied and understood genetic circuit that is responsible for structural or functional laterality, but the genetic keystones to lateralized brains are, overall, not as well understood in the majority of species requiring further investigation.

While a genetic mechanism is an important part of the laterality determination process, this mechanism often requires *environmental stimulation* at a given time in development to allow for the gene(s) to be more effectively “turned on” and for laterality to emerge (Cowell & Denenberg, 2002). In fact, research with domestic chickens (Deng & Rogers, 1997; Rogers et al., 2004), rats (e.g. Denenberg et al., 1978; Garbanati et al., 1983), goldbelly topminnows (Dadda & Bisazza, 2012) and zebrafish (*Danio rerio*; Sovrano et al., 2016) has shown that this may be the case since lateralization of visual behaviour develops only if the embryo is exposed to light. However, more work needs to be carried out with clearly defined genetic mechanisms upon which the environmental manipulation is acting if we are to make strong conclusions on the overall gene-by-environment interaction effect on lateralization across species.
Rather than implicating a single gene as a driver of lateralization, an alternative hypothesis suggests that the level of measured asymmetry is related to the overall genetic variation of an organism, with increased asymmetry predicted in conjunction with decreasing genetic variation (Leary et al., 1985; Leary & Allendorf, 1989; Bisazza et al., 1998). What little evidence exists for this hypothesized relationship remains inconclusive: while some studies have found an effect of level of inbreeding (e.g. Leary et al., 1985), others have found no such relationship (e.g. Collins et al., 1993; Sheridan & Pomiankowski, 1997). It may be that there is a connection between measured laterality and genetic variation, but this relationship could be species-specific and trait dependent. If so, the level of genetic variation could be used as a determining factor for some species as to whether laterality is likely to be present and how strongly it may be expressed. The connection between genetic variation and laterality needs to be more rigorously explored to better understand what true relationship, if any, exists.

**Parental effects**

There may be heritability of laterality from parent to offspring, but there is conflicting evidence on this topic. In mice some studies have found no evidence of heritability of laterality of paw preference (e.g. Collins, 1968), whereas studies of handedness in chimpanzees have proposed heritability of the trait (Hopkins et al., 1994), suggesting, perhaps, that the likelihood of heritability of laterality may differ among species. One of the best examples of the potential heritability of laterality is in the topminnow, *Girardinus falcatus*. Bisazza et al. (2000a; 2007) used artificial selection over five generations of fish to create preferential turning lines (right, left and no preference) using only the most strongly behaviourally-biased males and females as the
parental fish. When tested in a T-maze, the offspring of the right and left preference lines exhibited the same behavioural biases as their parental fish, whereas the offspring of the ‘no preference’ line showed an even distribution of directional preferences (Bisazza et al., 2007), showing clear evidence of a heritable genetic element maintaining lateralization, since no opportunities for learning the parental preferences were available to the offspring. Correspondingly, motor asymmetries may be rooted in asymmetries of neuroanatomy. While conservation of the nervous system can be argued to persist across generations, and potentially across species (Tierney, 1996; Katz & Harris-Warrick, 1999), the question remains: if there is heritability or conservation of neuroanatomy, are lateralized preferences inevitably retained? Could conservation of neuroanatomy, perhaps determined by particular gene sequences, be enough to maintain laterality across the evolutionary time scale? Questions such as these have only really begun to be answered in detail but with more research on lateralized behaviour and neuroanatomy, and incorporating measures of heritability and maternal and paternal effects, the role of parental effects on lateralization will become clearer.

The effects of rearing environment

In recent years, the effect of the environment on overall brain development has garnered particular interest (e.g. Gonzalez-Voyer et al., 2009; Näslund et al., 2012; Kotrschal et al., 2013) with evidence for differences in brain size corresponding to differences in rearing environment (Marchetti & Nevitt, 2003; Kihslinger & Nevitt, 2006; Kihslinger et al., 2006; Burns et al. 2009; Mayer et al., 2011). More recently, environmental effects have been extended to the study of how features of the environment may influence functional lateralization. In 23 species of wild and captive parrots and
cockatoos there was no difference in strength or direction of laterality between captive birds and their wild counterparts (Brown & Magat, 2011b), but in the crimson-spotted rainbow fish (Melanotaenia duboulayi), enrichment or impoverishment of the rearing environment did affect brain lateralization, wherein males from impoverished environments and females from enriched environments were more strongly lateralized in a test for eye preference (Bibost et al., 2013). In mammals, domestication may be responsible for limb preferences as observed in horses (Austin & Rogers, 2012) and yet does not appear to have any effect on eye use preference, as similar patterns have been found in domestic (Farmer et al., 2010) and feral (Austin & Rogers, 2012) horses indicating that environmental influences may affect certain forms of laterality but not all. Predation level can also act as an environmental driver to laterality, with fish (Brachyraphis episcopi) reared in high predation environments showing different patterns of laterality compared to their low predation conspecifics (Brown et al., 2004), and male Trinidadian guppies (Poecilia reticulate) reared with olfactory predator cues having higher degrees of laterality than those reared without predator cues (Broder & Angeloni, 2014). Light exposure, too, influences visual and motor laterality in both chicks (Deng & Rogers, 1997; Rogers et al., 2004), and fish (G. falcatus; Dadda & Bisazza, 2012). Finally, some environmental aspects can negatively affect (i.e. reduce) lateralized behaviour. Increased levels of CO₂ experienced by some fish species (Neopomacentrus azysron, Gasterosteus aculeatus, and Amphirion percula) have resulted in a disruption of lateralized behaviour, which could present a danger to shoaling fishes (Domenici et al., 2012; Nilsson et al., 2012; Jutfelt et al., 2013). While the evidence investigating environmental effects on functional lateralization is intriguing, we must keep in mind the point discussed by Cowell and Denenberg (2002): laterality of an organism is likely the
result of the *interaction* of a genetic predisposition for laterality and specific environmental conditions occurring during a sensitive period. The development of laterality, therefore, can be considered from a genetic standpoint and, separately, from an environmental standpoint, but the strongest explanation providing a greater understanding of lateralization of an organism will come from the discussion of the interaction between genetic and environmental drivers.

**Species differences**

Rogers et al. (2013) eloquently outlined the relationships between many organisms that display laterality in some form, but the main commonality with respect to laterality among vertebrate species studied to date is that so many of them have demonstrated this phenomenon, with similarities for certain traits being quite comparable between species. For example, canaries, white-crowned sparrows (Nottebohm & Nottebohm, 1976), and frogs (Bauer, 1993) have shown left hemisphere control of vocalization, and magpies (Koboroff et al., 2008) and some fish (Facchin et al., 1999) demonstrate a right eye preference when investigating a model predator in close proximity. Yet even with the general similarities present across species, there are examples demonstrating that the adage speaks true: differences within groups are often greater than differences between groups.

**Within species differences**

In studies investigating larger groups of species for lateralized behaviour, differences *within* species groups have been found, where a smaller group of individuals makes up a minority with respect to directional behavioural preference. In handedness
studies of chimps, uneven distributions of the preferred hand are often found within the group, with the majority using their right hand over their left (e.g. Hopkins & Bennett, 1994; Llorente et al., 2011). In some instances, physical development is asymmetrical within a species group but there remains a small minority that develops the opposite asymmetry to the majority, such as with the left and right habenular nuclei in both pearl cichlids (Reddon et al., 2009) and convict cichlids, *Amatitlania nigrofasciata* (Gutiérrez-Ibáñez et al., 2011), and with the left- or right-facing “mouth turn” development in scale-eating cichlids, *Perissodus microlepis* (Hori, 1993). Where there is polymorphism of lateral types within a group, it may be that laterality is under the influence of frequency-dependent selection (discussed above). Frequency-dependent selection helps to explain why minority groups of differing lateralized preferences from the majority not only exist, but persist: in certain circumstances, those organisms expressing the minority phenotype may experience greater fitness advantages, as the rare directional bias may be the more beneficial.

*Individual vs. population differences*

While most often studied from the population point of view, laterality is found at both the population and individual levels (Vallortigara, 2006). Laterality at the individual level means that each individual within a population will exhibit its own directional bias or preference, with the population made up of an even number of left and right lateral forms (i.e. fluctuating asymmetry), whereas population-level laterality means that the majority of individuals within a population are significantly lateralized in the same direction, sharing the same directional bias (i.e. laterality) (Takeuchi & Hori, 2008). Most often studies of lateralization focus on population-level laterality with individual variation
discussed as an interesting footnote but individuals from a larger group do often show significant and individual lateralized preferences. For instance, on measures of behavioural lateralization, individual, but not population, biases have been found in pearl cichlids (Reddon et al., 2009), in onesided livebearers, *Jenynsia lineata*, (Bisazza et al., 1997a), and in Siamese fighting fish, *Betta splendens* (Takeuchi et al., 2010). It has been hypothesized that findings of individual level laterality are representative of the typical behaviour of animals from solitary species, whereas population level laterality is more likely among animals from more “social” species groups (Bisazza et al., 2000b), but from where might these individual preferences arise and how do they relate to population level laterality?

The need for increased brain efficiency has been posited as one explanation of why individual level lateralization developed (Rogers et al., 2004; Vallortigara & Rogers, 2005; Vallortigara, 2006) and it is suggested that individuals within a group may share the same *pattern* of laterality but that any two individuals may show the opposite directional preference with respect to specific behaviours due to differential processing of stimuli and to differing levels of arousal (Ventolini et al., 2005). Further, individual-level lateralization may be a reflection of asymmetrical morphology of an individual (Vallortigara & Bisazza, 2002). Conjecture has been that individual asymmetries may have risen from, and indicate, fluctuating asymmetry—random deviations from perfect symmetrical development—which, some suggest, can lead to differential behavioural biases at the individual level (Bisazza et al., 1997a; Vallortigara & Bisazza, 2002). In the case of a group of organisms exhibiting population-level laterality, however, the direction of an individual’s behavioural biases are of little consequence, but the individual’s ability to align with the asymmetries of the group may affect social interaction and group
structure (Rogers, 1989). Along this line, population level laterality may be an evolutionarily stable strategy (Vallortigara, 2006) wherein all members experience greater fitness benefits when performing the same behavioural tactic (Vallortigara & Rogers, 2005). Overall, aligning individual behaviour with that of the majority may not just be for cohesiveness and safety, but may reflect the typical and expected social organization of the group.

**Future directions**

While the study of laterality has moved far beyond the argument of “humans only”, there is still much ground to cover. There are gaps in the research that would benefit from greater attention to both neuroanatomy and to the effect of the interaction between genetics and rearing environment. We need to have an all-inclusive exploration of the roots of laterality – not just focusing on genetic, morphological, or environmental drivers separately, but on how all aspects may interact to affect the development of physical and behavioural laterality. Below are suggested areas of future research that I feel would move the field forward in valuable directions.

**Phylogenetic approach**

Investigation into phylogenetic relationships between and among species with regards to lateralization (physical and behavioural) must continue if we are to more conclusively answer the question of, “where did it come from?”. Has the development of laterality been a straight shot from the early ancestors of vertebrates, or did it evolve on several occasions in different ancestral species? Questions along these lines have begun to be investigated in some species, including parrots (Brown & Magat, 2011b) and
anabantoid fishes (Clotfelter & Kuperberg, 2007). Brown and Magat (2011b) examined lateralized foot use during feeding of both wild and captive parrots and cockatoos of 23 different species. Direction and strength of laterality were shown to be linked to phylogeny, with strength of laterality closely related to body size. There was an evolutionary divergence of lateralization of limb use between large- and small-bodied birds related to their main dietary source. To the contrary, Clotfelter and Kuperberg (2007) found that while there were differences in cerebral laterality and aggression between six anabantoid fish species investigated none of these differences could be attributed to phylogenetic distance.

Focusing on the phylogenetic relationships among species, and studying the underlying genetic mechanisms guiding laterality are important pieces to solving the puzzle of lateralization. It should be noted that morphology, especially neuroanatomy, may be more useful than behaviour in tracking phylogenetic relationships of laterality between species, since behaviour is more amenable to change via selection or other factors (e.g. ESS) (Tierney, 1996). With a continued understanding of morphology, especially of bilateral structures, and its effect on lateralized behaviour we will come closer to discovering the phylogentic path of laterality through vertebrate evolution, but a greater knowledge of phylogenetic relationships will require the “painstaking collection” of data on many species to better understand the biological characteristic of brain lateralization (Vallortigara et al., 1999b).

Genetics and heritability

Studies of the effect of genes on laterality and the heritability of laterality must continue, especially in cases where the genetic background of an organism can be
controlled; this will help with learning how and why laterality develops and how big of a role is played by genetic mechanisms. Ideally, it would be most beneficial to study a species whose genetic makeup is well known via reference to breeding history, or whose genetic makeup, specifically heterozygosity, can be controlled in some manner. It may even be possible to begin with what we know about heterozygosity at certain loci in other well studied species and find comparable genes in species not yet studied in this way to strengthen investigations of the genetic effect on development of lateralization (Geschwind & Miller, 2001). Admittedly, this is a large job and would require meticulous research, but it may be invaluable. Genetic investigations will not only allow a better understanding of phylogenetic relationships, but will permit increased comprehension of what specific mechanisms work in the brain to turn laterality on and off in multiple species. In addition, organisms of different genetic backgrounds must be tested in differing environments to further explore the gene-by-environment interaction effect on the emergence and persistence of laterality. We must keep in mind, however, that some genetic relationships may be species-specific. Genetic variation and potential for heritability may play a bigger role for some, whereas for other species the propensity to exhibit laterality may rely more on their genetic foundation interacting with the right environmental factors at the right time; however, further research is necessary to illuminate the workings of genetic mechanisms on laterality.

*Staying the course and beyond*

In addition to behavioural studies, work must continue on the examination of hypotheses describing the genetic component of laterality in non-human vertebrates. Only with repeated studies in a multitude of organisms can we better understand the effect of
genetic variation (heterozygosity), the strength of the gene-by-environment interactions, and how heritable laterality may be. At present, evidence is scant in this area with some suggestion of heritability (e.g. Hopkins et al., 1994; Bisazza et al., 2000a) and some work backing the idea of environmental effects, with potential for gene-by-environment interactions (e.g. Denenberg et al., 1978; Deng & Rogers, 1997). Yet very little work has investigated the often discussed hypothesis of a decrease in genetic variation coinciding with an increase in measured laterality of an organism (Leary & Allendorf, 1989; Bisazza et al., 1998). The work that does exist focuses largely on physical measures of asymmetry (Leary et al., 1985; Sheridan & Pomiankowski, 1997) with no extension to the area of behavioural laterality, leaving a gap in the research. It would be ideal to be able to move toward tests of different genetic variants of a species on multiple measures of laterality—physical, cognitive, and behavioural—to more accurately describe laterality’s development and effect on an organism’s life history. Gaining knowledge of the genetic basis for lateralization will lead to increased insight into the evolutionary path of laterality, perhaps helping to determine why it has been such a resilient trait in non-human vertebrates, and maybe even allowing greater insight into why, in humans, a lateralized brain is strongly correlated with neurodegeneration (Geschwind & Miller, 2001).

The far reach of behavioural studies has permitted many species to be investigated and has shown that animals from every major species group exhibit lateralization, suggesting deep evolutionary roots. But, to better appreciate cerebral and functional lateralization across species, it has been suggested that a standardized system of measures be adopted (Forrester et al., 2011). In creating a standardized system however, we must remember that context of the behaviour in question may influence directional preference
in different species (Forrester et al., 2011); therefore the creation of a standardized system should be carried out with caution. If a standardized system for measuring laterality can be developed and accepted, we will be able to make stronger conclusions about the costs and benefits of laterality for specific species, as well as conclusions regarding differences and similarities within and between species.

**Hypotheses**

More than 25 years ago, Rogers (1989) said that “laterality is a dynamic phenomenon varying with age, experience and the particular situation in which [an] animal finds itself”. This statement is a perfect summation of how intricate the study of laterality can be: it is not a static, unchanging characteristic, but one that ebbs and flows with different stages of life, genetic makeup, and experiences of an organism. It is not just about turning left or right or using the right or left paw, but the reasons behind the directional choice: What is driving that preference? What benefit does the organism gain from lateralized biases in a given situation? And, where did it all begin? Only continued work incorporating multiple drivers to laterality and explorations into its benefits will enlighten us of the answers to these questions. Research must move forward with investigations considering genetic contributions to laterality, the heritability of laterality of the brain and behaviour, and further environmental and gene-by-environment interaction effects. We must explore as many avenues as we can to more fully understand the biological characteristic of laterality.

In the current dissertation I seek to provide the groundwork for filling the gaps in some areas of laterality research using juvenile and adult Chinook salmon (*Oncorhynchus tshawytscha*) as my study species. For this work, Chinook salmon provide an ideal study
system first because of their relatively short, approximately 3 to 4 year, lifespan allowing me to inspect tissue investment at different ages or life stages. In addition, the salmon used here are from several different genetic crosses, created at an organic salmon farm and hatchery, Yellow Island Aquaculture (YIAL). These crosses were ideal for the current work as I am able to begin to expand into a deeper examination of how genetic background relates to, and may affect, different tissue investment and laterality in this fish species. More specifically, I will explore potential effects of genetic variation (i.e. cross) on lateralized behaviour and brain morphology. In exploring the effect of genetic background I am adapting and reframing the genetic variation and asymmetry hypothesis, developed and outlined in the fluctuating asymmetry literature (Leary et al., 1985; Leary & Allendorf, 1989), and applying it to the beneficial characteristic of laterality. In its original form this hypothesis suggests a difference on measured asymmetry between organisms of differing genetic background. Specifically, the predicted direction of this relationship is that the less genetic variation an organism has (i.e. more inbred), the more asymmetrical they would be in terms of external morphology. As fluctuating asymmetry is a measure of environmental or, more specifically, genetic perturbations in the expected developmental track (Van Valen, 1962; Leary & Allendorf, 1989; Møller & Swaddle, 1997) this predicted relationship makes sense. In my reframing of this hypothesis, however, the presence of asymmetry is not a negative feature but instead a beneficial one, providing a relative advantage to organisms. As such, the more genetically variable organisms should show greater asymmetry on measured characteristics.

In addition to exploring the positive relationship between genetic variation and laterality, I will be examining the potential effect of the interaction between genetic background and environment (GxE) on lateralized behaviour and morphology through the
use of directional water flow manipulation (i.e. clockwise or counter-clockwise). It is hypothesized here that there will be a differential effect of genetic background and flow direction, however, specific relationships are difficult to predict.

Finally, as a further investigation into differences of brain development, I will be exploring the effect of genetic variation (cross) on differential investment into what are deemed ‘expensive’ tissues (i.e. brain and gut) as an investigation of the expensive tissue hypothesis (Aiello & Wheeler, 1995) in Chinook salmon. In terms of trade-offs between expensive tissues, here it is predicted that I will see the greatest relative investment into brain mass from those crosses that result from artificial spawning between a hatchery female and milt from males from wild river locations, as previous work has indicated greater relative brain size in wild, likely more genetically variable, fish in comparison to their hatchery counterparts (e.g. Kihslinger et al., 2006; Mayer et al., 2011). Taken together, my findings will first provide an innovative examination into potential genetic differences behind expensive brain tissue investment. More notably, my work is not only addressing the understudied GxE effects on behavioural and morphological laterality, but it is reframing the previously suggested negative relationship between genetic variation and measured asymmetry, exploring it from a positive relationship point of view, and bringing this idea into the realm of behavioural laterality and morphological laterality of the brain itself.
References


CHAPTER 2: NEUTRAL GENETIC VARIATION IN ADULT CHINOOK SALMON 
(ONCORHYNCHUS TSHAWYTSCHA) AFFECTS BRAIN-TO-BODY 
TRADE-OFF AND BRAIN LATERALITY

Introduction

The brain is responsible for the direction of body movements, decision making, and hormone production, which directs somatic growth (Hazon & Balment, 1998; Kolm et al., 2009), and it is also one of the most costly vertebrate organs to produce and maintain (Mink et al., 1981). The expensive-tissue hypothesis previously suggested a trade-off in growth of gut size to compensate for larger brain size (Aiello & Wheeler, 1995), but this formulation of somatic trade-offs has been met with some scepticism (Navarrete et al., 2011) leading to an extension of this hypothesized relationship, known as the energy trade-off hypothesis. The energy trade-off hypothesis suggests that increases in brain size are associated with corresponding decreases in energy consumption from “flexible functions”, such as reproduction, digestion, and locomotion (Isler & Van Schaik, 2006; Tsuboi et al., 2015). The energy trade-off hypothesis, therefore, may be an evolutionary mechanism to explain constraints on brain and body function.

A decrease in size of some expensive-tissues (e.g. brain, gut, reproductive tissue) or the reduction in energy consumption of ‘flexible functions’ could allow for an increase in brain size without increasing net metabolic costs. However it is possible that there are other drivers, thus far overlooked, that are responsible for the size of the brain and other organs. Inbreeding, or mating between closely related individuals, will often lead to inbreeding depression: a decrease in an individual’s fitness due to increased genetic homozygosity and the expression of recessive deleterious alleles (Connor & Hartl, 2004;
Wang et al., 2002). While life history traits related to fitness may experience the highest inbreeding effects (DeRose & Roff, 1999), morphological traits can also be significantly impacted. Inbreeding has led to body weight reductions in rainbow trout (*Oncorhynchus mykiss*), where the consequences of inbreeding became more pronounced with increasing age, and resulted in significantly decreased female reproductive fitness (i.e. egg production) (Su et al., 1996; Pante et al., 2001). Thus, inbreeding effects on body size, which may be a characteristic crucial to some flexible functions, would also be expected to affect brain size with the energetic trade-offs outlined above.

In addition to energetic trade-offs at the whole brain level, inbreeding may also affect differential investment of the right and left brain hemispheres. Differential responses of brain hemispheres, also known as directional asymmetry or lateralization, has been proposed as a mechanism for increased efficiency of neural processing (Levy, 1977; Rogers et al., 2004) and therefore may respond to differing “flexible functions”. In many vertebrate species, it has become apparent that the right and left hemispheres of the brain are responsible for different and specific tasks (Rogers & Andrew, 2002; Rogers et al., 2004; Vallortigara & Rogers, 2005; MacNeilage et al., 2009; Rogers et al., 2013), and while most studies of laterality have focused on lateralization of behaviour (e.g. Bauer, 1993; Sovrano, 2004; Reddon & Hurd, 2008; Braccini & Caine, 2009; Templeton et al., 2012), there is increasing evidence of the asymmetry or differential contributions of underlying bilateral neural structures that underpin the roots of asymmetry (Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011). It has been hypothesized in literature outlining fluctuating asymmetry (FA), a maladaptive form of development (Leary et al., 1985; Leary & Allendorf, 1989; Bisazza et al., 1998; discussed in Wiper, 2017), that there is a link between levels of genetic variation and directional asymmetry in vertebrates but
support for this hypothesis remains inconclusive, with some studies finding a positive relationship between asymmetry of meristic characteristics and inbreeding (Leary et al., 1985) while others found no association (Sheridan & Pomiankowski, 1997). FA is used to indicate an environmental or genetic perturbation of symmetrical development (Leary & Allendorf, 1989; Møller & Swaddle, 1997), thus, the previously predicted relationship makes intuitive sense. However, here I am suggesting that laterality is a positive and beneficial characteristic for an organism to possess, thus, with this information I suggest a reframing of the hypothesis where the more genetically variable an organism is, the more likely they are to show laterality.

The purpose of the current chapter is to examine effects of genetic variation on potential trade-offs between brain and somatic growth as hypothesized in the energy trade-off framework, as well as inbreeding effects on brain laterality, as both have been postulated to fluctuate with genetic variation (Leary & Allendorf, 1989; Su et al., 1996; Bisazza et al., 1998; DeRose & Roff, 1999). In addition, lateralization and brain growth have shown evidence of being passed on through some, as yet unidentified, heritable component. Artificial selection on turning behaviour (i.e. greater left or right turning preference) in minnows (Girardinus falcatus) over five generations results in offspring showing the same turning preferences as the parental fish (Bisazza et al., 2007). In that study, there might be an underlying lateralized brain component leading to the specific lateralized behavioural output. Indeed brain morphology can be inherited from parents, as demonstrated in guppy (Poecilia reticulata) offspring, who were artificially selected for large or small brain size (Kotrschal et al., 2013), and showed brain size comparable to their parental fish overall. Therefore, it would be expected that morphological lateralization of the brain would be inherited from generation to generation. By using
offspring from previously created lines of Chinook salmon with different levels of inbreeding, assessed as percent heterozygosity using fin clips from representative fish of the same genetic lines but different cohorts, I was able to begin to test the potential role of genetic variation on both energetic trade-offs as well as on lateralized differences in gross brain morphology, which has not yet been rigorously investigated. Here I hypothesize that the group deemed to have the highest level of inbreeding (“Very High”) will show the lowest investment into energetic trade-offs (i.e. brain-to-body ratio), and that the group with the lowest level of inbreeding (“Low”) will show the greatest laterality, based on the above suggested relationship between genetic variation and measured laterality.

Methods

Sample Collection

Study Species

All measures were collected from seven different crosses of three year old Chinook salmon in the fall of 2012 to 2014 from Yellow Island Aquaculture, Ltd. (YIAL) (Quadra Island, British Columbia, Canada), where distinct genetic crosses have been created and maintained since the late 1990s (Docker & Heath, 2002). The seven genetic crosses consisted first of offspring from self-crossed hermaphrodites which originated at YIAL in 2009 as the result of the incomplete sex-reversal of a female broodstock fish (see Komsa, 2012, for further breeding details). Secondly, I used offspring from crosses maintained as YIAL’s broodstock; their “high performance” (HHxHH) and “low performance” (LLxLL) purebred lines. These HHxHH and LLxLL lines were created from fish chosen for high or low performance based on gene markers related to growth and survival (see Docker & Heath, 2002 and Lehnert et al., 2014 for detailed breeding
information) rather than from crosses specifically designed to test inbreeding effects. I also used offspring from crosses involving a hermaphrodite fish (H₁ or H₃) and high performance line (HH) fish (H₁ x HH and H₃ x HH); and my final crosses were made up of hybrid performance offspring (HHxLL and LLxHH fish) from crosses of the purebred genetic lines (see Lehnert et al., 2014 for detailed breeding information). The first letters of the notation for all crosses indicate the maternal line and the second letters indicate the paternal line.

Fin clips were collected from fish from each of the above outlined crosses (see Table 1; hybrid performance crosses pooled) of Chinook salmon at YIAL at different times and different stages of development. First, fin clips were collected and preserved in June 2009 from offspring of hybrid performance crosses (HHxLL and LLxHH) when fish were approximately 7 months post-fertilization. Fin clips were also collected and preserved from fish from hermaphrodite crosses (self-crossed hermaphrodite offspring; hermaphrodite offspring x normal fish crosses) at approximately 1.5 years post-fertilization in April 2011. Finally, in the fall of 2011, fin clips were collected and preserved from sexually mature individuals from purebred crosses (HHxHH and LLxLL), where individuals ranged from 4 to 5 years in age. It should be noted here that fin clips were not collected from the fish that were sampled for brain and body measurements; instead fin clips were collected from fish in the same genetic lines as the study fish; however, some samples may represent groups of different cohorts. Therefore, different samples were used for genotyping to infer heterozygosity of the sample groups.

Prior to all analyses, genetic crosses were separated into groups based on parental lineage to better test the hypothesized relationship between laterality and genetic variation. Thus, all offspring derived from self-fertilization (i.e. hermaphrodites)
composed the first group, all offspring derived from hermaphrodite x HH [and the reciprocal] crosses made up the second group, all purebred cross fish (both HHxHH and LLxLL) were a third group, and all hybrid performance fish (HHxLL and LLxHH crosses) constituted the fourth group.

Somatic and Brain Measurements

To address the energy trade-off hypothesis, two absolute somatic measures, brain mass and body mass, were collected from all fish. After sacrifice and prior to brain removal, the weight of all fish was measured on site in kilograms to two decimal places (Marel M1100, Marel, Gardabaer, Iceland). A small section of the head containing the brain was removed from each fish and preserved in a 50mL Falcon tube (Corning, Inc., https://www.fishersci.com/) containing 30mL of 10% buffered formalin for 48-72 hours. The formalin was removed and the head sections were transported to the laboratory at the University of Windsor where the brains were dissected from the head section and placed in 70% ethanol. Total brain mass, in grams, was obtained in the laboratory using a two decimal standard scale (Ohaus Scout Pro SP202, Ohaus Corporation, New Jersey, USA). To estimate the growth energy invested into brain versus body growth, a brain-to-body ratio measure was obtained using the two absolute measurements of brain and body mass (Wiper et al., 2014).

Following brain removal and weighing, dorsal images of all brains were taken with a digital camera (Q-imaging Q1 Cam Fast 1394) connected to a dissecting microscope (Leica L2 10445930). Area and perimeter measurements were collected for the right and left hemispheres of the optic tectum and cerebellum (Figure 2.1) from dorsal brain images using Northern Eclipse imaging software (Empix Inc.,
Similar to Marchetti and Nevitt (2003), whole brain mounts were used in place of histological sectioning which, while useful for illuminating internal detail of brain structures, may lead to irregularities of fixation which can cause differential shrinkage of brain regions following tissue dehydration and embedding (e.g. Kihslinger & Nevitt, 2006). As the aim here was to obtain ‘larger-scale’ measurements of the Chinook brain as a whole and not focus on internal detail, whole brain mounts were deemed appropriate for the present work. To obtain left and right hemisphere measurements from the single-lobed cerebellum, this region received a superficial bisection. The midline between the right and left optic tecta was used as an anchoring point of reference for the superficial bisection line through the cerebellum (Figure 2.1), as the tectal ventricle and rhombencephalic ventricle within the brain make up the internal midline of the optic tectum lobes, continuing through the cerebellum providing an internal left-right division (Wullimann et al., 1996).

**Genetic analyses of heterozygosity**

DNA was extracted from fin clips following an automated plate-based extraction protocol (Elphinstone et al., 2003). Individual genotypes were determined through polymerase chain reactions (PCR) using 10 previously described microsatellite loci, specifically OtsG68, OtsG432, OtsG78b (Williamson et al., 2002), RT212, RT36 (Spies et al., 2005), Ots 211, Ots213 (Greig et al., 2003), Ots1 (Banks et al., 1999), Ots107 (Nelson & Beacham, 1999) and Omy325 (O’Connell et al., 1997). All primers were fluorescently dye-labeled thus PCR products could be visualized using a LiCor 4300
Figure 2.1: The two salmonid brain regions of interest measured in the present study: the optic tectum (OT), and the cerebellum (CB). The black line indicates where the cerebellum was divided into a right and left hemisphere using the midline of the optic tectum lobes as an anchoring point.
DNA analyzer (LiCor Biosciences, Inc.). Fragment sizes (alleles) were then scored using GENE IMAGIR 4.05 software (Scanalytics Inc.).

Using the heterozygosity estimates I was able to assign each of the previously organized genetic crosses a “level of inbreeding” group, ranging from low to very high, allowing me to more readily hypothesize about where each group may fall according to the genetic variation and laterality hypothesis. The four “levels” defined from the analyses of heterozygosity were as follows: The “Very High” inbreeding level label was given to the offspring derived from self-fertilization (i.e. from self-crossed hermaphrodites); these were the fish with the lowest average genetic variation (average heterozygosity: 46%). The label of “High” inbreeding (average heterozygosity: 68%) was for those fish whose parentage consisted of a hermaphrodite parent (H₁ or H₃) and normal stock (HH) fish (denoted as H₁ x HH or H₃ x HH crosses, and the reciprocals; see Table 2.1). Fish from purebred crosses (HHxHH and LLxLL) (average heterozygosity: 77%) were given the label of “Medium” inbreeding level, and the “Low” inbreeding level label was given to the hybrid performance offspring (average heterozygosity: 84%).

**Statistical Analyses**

*Somatic and Brain Measurements*

Prior to analyses, assumptions of normality, homogeneity of variance and lack of outliers were assessed. For assumptions to be met, 15 cases were removed due to incomplete dissection and damage to key brain regions, leaving us with a total sample size of 118 fish.

The brain-to-body ratio was used as a measure of the energy trade-off hypothesis, calculated using the formula: brain mass (g) / body mass (g). Differences between the
Table 2.1: Heterozygosity (observed, Ho and expected, He) and number of individuals genotyped (N) for six groups of captive Chinook salmon.

<table>
<thead>
<tr>
<th>Genetic Crosses</th>
<th>N</th>
<th>Ho</th>
<th>He</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-crossed hermaphrodite offspring</td>
<td>29</td>
<td>0.456&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.451&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hermaphrodite 1 x High 1 and reciprocal cross (H&lt;sub&gt;1&lt;/sub&gt; x HH; HH x H&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>27</td>
<td>0.676&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.660&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hermaphrodite 3 x High 3 and reciprocal cross (T&lt;sub&gt;3&lt;/sub&gt; x HH; HH x H&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>27</td>
<td>0.677&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.619&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>CRD purebred (LLxLL)</td>
<td>31</td>
<td>0.765&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.683&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>CRD purebred (HHxHH)</td>
<td>34</td>
<td>0.787&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.766&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CRD hybrid (HHxLL and LLxHH)</td>
<td>29</td>
<td>0.835&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.811&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters represent significant differences between groups (alpha level = 0.0083).
inbreeding level groups were investigated using a univariate ANOVA. Tukey’s post-hoc analyses provided clarification of significant effects of inbreeding level.

Left and right hemisphere measurements of perimeter and area were collected from dorsal images of all brains extracted and were used to obtain the ‘laterality index’, LI = (L-R) / (L+R), where ‘L’ indicates the left side measurements and ‘R’ indicates right side measurements (Gutiérrez-Ibáñez et al., 2011). This formula allows for a determination of side dominance and a consideration of asymmetry of each region independent of overall brain size. Positive values (from 0 to +1) are indicative of greater left hemisphere size whereas negative values (from 0 to -1) are indicative of greater right hemisphere size. In addition, the absolute (unsigned) value of the LI was taken (i.e. |LI|) as a measure of the strength of asymmetry irrespective of direction (Brown et al., 2007; Barnard et al., 2016). Because of a strong correlation between the laterality index and absolute index values of the OT (r = 0.215, p = 0.019), and between laterality index and absolute index values of the CB (r = 0.531, p < 0.001)—but no correlation between the laterality and absolute index values across regions—two separate MANOVAs were run: one for the laterality index measures and one for the absolute index measures. Analyses were carried out in this way as results are more reliable when the dependent variables being investigated in a MANOVA are not, themselves, related (French et al., 2008; Field, 2013). Because two separate tests were run, I used a Bonferroni corrected alpha value of p = 0.025 (0.05/2) for the brain morphology results. Perimeter values are reported here as patterns for differences between groups were similar with respect to area and perimeter measurements.
Heterozygosity

Individuals that were genotyped at fewer than 6 loci were removed from subsequent analyses. All genetic analyses therefore included 27 to 34 individuals for each of the six groups (see Table 2.1). Significant deviations from Hardy-Weinberg Equilibrium (HWE) were tested at all loci using GenePop version 4.2 (Rousset, 2008). Significant linkage disequilibrium using GenePop version 4.2 (Rousset, 2008) was also tested, with an adjusted alpha level of 0.005 (p = 0.05/10) given multiple pairwise comparisons among the 10 loci. Mean observed (H₀) and expected (Hₑ) heterozygosity across all loci were calculated using GenAlEx version 6.5 (Peakall & Smouse, 2012). Heterozygosity estimates were compared among groups using the Kruskal-Wallis test, and if significant differences were detected then Tukey’s post-hoc tests were performed. Given that multiple comparisons were conducted among the six groups, an adjusted alpha level of 0.0083 (p = 0.05/6) was used for the analyses.

Results

Heterozygosity Estimates

No loci showed significant deviations from HWE in any of the six groups, and no pairs of loci showed significant linkage disequilibrium (p < 0.005) in more than two of the six groups. Observed heterozygosity ranged from 45.6 to 83.5%, and was significantly different among groups (Table 2.1; p = 0.0005). Post-hoc tests revealed that self-crossed hermaphrodite offspring experienced statistically significantly lower heterozygosity compared to both HHxHH (p = 0.006) and hybrid groups (p = 0.0003). Expected heterozygosity was also statistically significantly different between the groups (Table 2.1; p < 0.001), where self-crossed hermaphrodite offspring showed significantly lower
Figure 2.2: As a measure of the energy trade-off hypothesis, the mean brain-to-body ratio values across inbreeding levels indicate that those fish with the lowest inbreeding level, and thus highest percent of heterozygosity, show the greatest investment into brain mass when body mass is taken into account. Error bars represent mean ± 1 standard error.
expected heterozygosity relative to both HHxHH and hybrid groups ($p$ values < 0.001).

Somatic Trade-Offs

There was a statistically significant effect of inbreeding level on the brain-to-body ratio measure, indicating differential investment of growth energy into the brain versus the body ($F_{3, 114} = 5.140, p = 0.002$ (Figure 2.2)). The Low inbreeding level group showed an overall greater investment into brain growth when body growth was taken into account, whereas the Very High inbreeding group showed the lowest brain versus body investment. A Tukey’s post-hoc analysis revealed that these differences were greatest between the Low and Very High ($p = 0.004$), and Low and High ($p = 0.029$) groups (Figure 2.2).

Laterality Measures

Multivariate tests indicated that there was no effect of inbreeding level on the absolute asymmetry values (Wilks’ lambda, $\Lambda = 0.950$, $F_{6, 226} = 0.988$, $p = 0.434$). Multivariate tests on the laterality index values showed that, while not significant, the effect of inbreeding level was close to the threshold for statistical significance ($\Lambda = 0.890$, $F_{6, 226} = 2.261$, $p = 0.039$). Despite the overall non-significant effect for the laterality index [at the corrected alpha value] the between-subjects effects of inbreeding level were examined. These tests showed that there was no significant effect of inbreeding level on the directionality of the OT ($F_{3, 114} = 1.566$, $p = 0.202$) but there was an effect on the CB ($F_{3, 114} = 3.005$, $p = 0.033$), and while all four groups showed a larger left cerebellar hemisphere as indicated by the positive laterality index values (Figure 2.3), the Low inbreeding level had the highest laterality index (see Table 2.2 for all values).
Figure 2.3: Representation of the laterality index (LI) of the cerebellum. Note that the values are all positive, indicating a larger left side of the cerebellum in fish of all inbreeding levels. Error bars represent mean ± 1 standard error.
Table 2.2: Mean (M), standard error (SE) and confidence intervals (95% CI) for the effect of inbreeding level on four measures of morphology.

<table>
<thead>
<tr>
<th>Morphology Measure</th>
<th>Inbreeding Level</th>
<th>M</th>
<th>SE</th>
<th>95% CI (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic tectum, Laterality Index</td>
<td>Very High</td>
<td>-0.00297</td>
<td>0.00415</td>
<td>-0.01170, 0.00575</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.00822</td>
<td>0.00520</td>
<td>-0.00255, 0.01898</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.01305</td>
<td>0.00408</td>
<td>-0.00470, 0.02139</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.00579</td>
<td>0.00446</td>
<td>-0.00320, 0.01478</td>
</tr>
<tr>
<td>Optic tectum, Absolute Index</td>
<td>Very High</td>
<td>0.01625</td>
<td>0.00176</td>
<td>0.01256, 0.01993</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.02149</td>
<td>0.00315</td>
<td>0.01497, 0.02801</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.02053</td>
<td>0.00282</td>
<td>0.01476, 0.02631</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.02397</td>
<td>0.00276</td>
<td>0.01841, 0.02953</td>
</tr>
<tr>
<td>Cerebellum, Laterality Index</td>
<td>Very High</td>
<td>0.00132</td>
<td>0.00299</td>
<td>-0.00496, 0.00760</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.00151</td>
<td>0.00245</td>
<td>-0.00355, 0.00657</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.00324</td>
<td>0.00236</td>
<td>-0.00158, 0.00806</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.00934</td>
<td>0.00197</td>
<td>0.00538, 0.01331</td>
</tr>
<tr>
<td>Cerebellum Absolute Index</td>
<td>Very High</td>
<td>0.01091</td>
<td>0.00155</td>
<td>0.00765, 0.01417</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.01052</td>
<td>0.00113</td>
<td>0.00820, 0.01285</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.00998</td>
<td>0.00158</td>
<td>0.00675, 0.01320</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.01305</td>
<td>0.00141</td>
<td>0.01022, 0.01589</td>
</tr>
</tbody>
</table>
Post-hoc tests were not statistically significant between all groups and only the difference between the Low and High inbreeding groups approached marginal significance ($p = 0.081$) (Figure 2.3), but these differences appear to be driven mainly by the Low inbreeding level group.

Discussion

Somatic Trade-offs

The energy trade-off hypothesis has not explicitly been investigated with reference to genetic variation and its effects on potential differences between the brain and the body, but the results presented here begin to suggest that there may be differential effects of genetic variation on a brain-to-body trade-off measure, although this would need to be confirmed with a breeding design specifically setup to test inbreeding effects. Other work on the energy trade-off hypothesis has examined the relationship between the brain and the gonads in bats (Pitnick et al., 2006), pectoral muscle mass in multiple bird species (Isler & Van Schaik, 2006), the number of offspring produced in guppies (Kotrschal et al., 2013), and egg size and duration of parental care in cichlids, which both showed a positive correlation with brain size (Tsuboi et al., 2015). In this chapter, when the brain and body mass were considered together as a reflection of the energy trade-off hypothesis the Very High inbreeding group showed the lowest brain-to-body ratio, and differed significantly from the most genetically variable group (Low inbreeding) that showed the highest ratio. This relative measure of brain to body mass is a reliable proxy for investigating patterns of somatic investment (Wiper et al., 2014) and here I noted divergence in brain size as a function of body size between groups of differing estimated heterozygositieS (Gonda et al., 2011). Because there may be other uncontrolled for
genetic differences among the groups, it is possible that differences in heterozygosity are not solely responsible for the differences observed so a follow-up study could use a controlled breeding design. A higher relative investment in brain size coupled with a higher level of heterozygosity may be a potent combination for overall fitness and survival given that genetic history has shown important influences on body size (e.g. Su et al., 1996; Pante et al., 2001; Fessehaye et al., 2007; Falica et al., 2017) and on gonadosomatic index (i.e. a trade-off between body size and gonad size) (Heath et al., 2002) in fish, and that inbred (i.e. low heterozygosity) individuals have, overall, shown decreases in growth, fitness and survival rates (Wang et al., 2002; Hutchings & Fraser, 2008). Enhanced investment in brain over body, then, may indicate enhanced sensory or behavioural abilities (Kotrschal et al., 2013). Fish in aquaculture facilities are often highly inbred (e.g. Kincaid, 1983) and optimized for high growth rates (e.g. Chavanne et al., 2016) but when aquaculture fish are released into the wild for restocking purposes they often experience high levels of mortality due to predation (e.g. Dellefors & Johnsson, 1995; Hawkins et al., 2007). One option to enhance post-release survival when restocking may be to focus on increasing genetic diversity in offspring to enhance relative brain size and, perhaps, cognitive abilities since the results showed a potential linkage between heterozygosity and relative brain size.

Genetic Effects on Laterality

As a test of the beneficial relationship between genetic variation and measured laterality, my results indicated that this relationship appeared to hold true but only for one brain region measured. Only the cerebellum showed any indication of differences of measured laterality between groups. In line with the suggested relationship between
genetic variation and the beneficial characteristic of laterality, I found that the Low inbreeding level group had the highest measured morphological asymmetry between cerebellar hemispheres, and appeared to be driving the significant effects observed. These results reflect the opposite pattern to that outlined in the fluctuating asymmetry literature, which suggests that lower genetic variation (i.e. increased inbreeding) is equated with asymmetrical developmental outcomes (Leary et al., 1985; Leary & Allendorf, 1989).

Previous studies investigating brain differences in fish have done so using brain size as a function of environmental rearing conditions generally focusing on each brain region as a whole (Mink et al., 1981; Marchetti & Nevitt, 2003; Kihslinger et al., 2006; Burns et al., 2009; Peakall & Smouse, 2012). Fewer studies, however, have actually investigated asymmetrical differences of brain regions and, when looking at neuroanatomy, have been more likely to focus on smaller neuroanatomical features like the habenular nuclei (see Concha & Wilson, 2001 for review). Here I have presented one of the first studies to investigate morphological differences between hemispheres of the salmonid brain, whose growth is continuous throughout life (Kihslinger & Nevitt, 2006; Näslund et al., 2012) responding to both external stimuli, such as environmental rearing conditions, and internal physiological status. Previous work has shown that larger overall brain size is related to increased cognitive ability in a fish species, *Poecilia reticulata* (Kotrschal et al., 2013), suggesting perhaps a greater number of or larger neurons within the brain. Differential growth of the left vs. right hemispheres in a fish, then, may be related to greater reliance on and use of one hemisphere of a given region due to increased dependence for stimulus processing. Having a lateralized brain has been hypothesized to be beneficial (Wiper, 2017 and references therein) but there has been little connection to how this benefit might correlate with or be explained by larger brain
regions. In fish, the cerebellum is responsible for motor control, muscle coordination and general movement (Butler & Hodos, 1996), therefore if a greater number of synaptic connections, or larger or more numerous neurons are found delegated to the left hemisphere, for example, fish may show a propensity for and efficiency of escape, random turns, or general movement in a rightward direction. However the connection between motor asymmetries and hemispheric differences of the cerebellum has yet to be rigorously investigated.

**Conclusion**

Here I have presented one of the first studies to use crosses with different levels of genetic diversity, likely caused by inbreeding to examine the potential effect of genetic variation on somatic trade-offs and, using measures of lateralization of brain morphology, to assess the speculation that the higher the genetic variation of an organism the more likely they are to show beneficial laterality of measured characteristics, in this case, the brain. While the lines used were not specifically bred to control for inbreeding and there may be other genetic differences involved in the response, the results suggest that a reduction in genetic variation does lead to a reduced brain-to-body ratio. This study is only beginning to examine patterns that may exist with respect to genetic variation but because I did not carry out a specific and controlled inbreeding design I can only suggest potential effects of genetic variation. In the future, more controlled studies of inbreeding will need to be carried out to get at the true effect that genetic variation has on somatic trade-offs and lateralized brain morphology. Investigating through controlled breeding how genetic makeup may influence the division of energy to certain tissues could hold potential for aquaculture facilities and restocking programs aiming to ensure the
healthiest fish possible with the greatest chance of survival (Heath et al., 2002). In investigating differences in laterality as an effect of the grouping variables of “inbreeding level”, I found some evidence that greater heterozygosity may lead to greater laterality and this study is the first, to my knowledge, to address this finding in detail with respect to brain hemisphere differences. Further work on relationship between genetic variation and measured laterality must be done to gain a better understanding of how genetic variation may affect measures of lateralization. Studies of lateralized morphology and behaviour to date have largely left out the component of genetic background of the study organisms but moving forward studies wishing to test the genetic variation and laterality hypothesis must incorporate breeding designs necessary to test inbreeding effects to truly understand the nature of the suggested relationship. Based on the inbreeding level groups estimated herein, I have shown that there may be effects of genetic background in that higher genetic variation may be suggested to promote laterality.
References


CHAPTER 3: GENETIC, ENVIRONMENTAL AND INTERACTION EFFECTS ON BEHAVIOURAL AND MORPHOLOGICAL LATERALITY IN JUVENILE CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA)

Introduction

The term laterality is commonly used to refer to the processing specializations of, or structural differences between, the brain hemispheres of vertebrates (Frasnelli et al., 2012; Rogers et al., 2013; Dadda et al., 2015). Processing specializations of differential stimuli have often been assessed through behavioural output, especially with studies of hand or paw use (e.g. Braccini & Caine, 2009; Regaiolli et al., 2016), eye use (Facchin et al., 1999; Des Roches et al., 2008), and turning preferences (e.g. Dadda et al., 2010). Through this behavioural work distinct processing responsibilities of the right and left hemispheres of the brain have become apparent in several vertebrate species (see Rogers & Andrew, 2002; Vallortigara & Rogers, 2005; and, Rogers et al., 2013 for review), especially in response to social stimuli. Gouldian finches (Erythrura gouldiae), for example, are better able to recognize a potential mate when using the right over the left eye (Templeton et al., 2012). On the other hand, some teleost fish species have been found to rely significantly more on their left eye when investigating a mirror image or live conspecific (Sovrano et al., 1999; Sovrano, 2004; Sovrano & Andrew, 2006). Even with the evidence strongly supporting lateralized behaviour, the driving force behind these differences is still not agreed upon with possible suggested drivers including neuroanatomical (e.g. Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011), genetic (e.g. Biddle et al., 1993; Bisazza et al., 2007), and environmental components (e.g. Brown et al., 2004; Austin & Rogers, 2012).
Little work has been done, overall, to examine lateralized neuroanatomical drivers that may underlie the behavioural output of an organism. The greatest neuroanatomical focus in recent years has been on the habenular nuclei in cichlid fishes. These nuclei are a pair of highly conserved neural structures in the limbic system which connect the forebrain and midbrain (Bianco & Wilson, 2009; Reddon et al., 2009; Gutiérrez-Ibáñez et al., 2011). In pearl cichlids (*Geophagus brasiliensis*) smaller-bodied fish tended to have larger left habenula and larger fish had a larger right habenula (Reddon et al., 2009). More importantly, pearl cichlids exhibit a positive correlation between strength of asymmetry of their habenular nuclei and strength of behavioural asymmetry in a detour task. Such work begins to illuminate well the point that morphological lateralities within the brain have the potential to be expressed through behaviour (Rogers et al., 2013); however, the brain as a whole has been overlooked for similar comparisons and an examination of how the left and right hemispheres of the brain may be related to differences in lateralized behavioural output is necessary.

A further driver to the development of laterality that has been relatively understudied is the connection between an organism’s genetic makeup and lateralization of a given measured characteristic (Wiper, 2017). Some work has investigated the key genetic mechanism responsible for laterality of paw use in mice (*Mus spp.*; Biddle, 1993; Collins et al., 1993), and there is some evidence for directional gene expression in *Caenorhabditis elegans* (Sagasti, 2007). There is a hypothesis, however, that is based in the fluctuating asymmetry literature which suggests that the lower the genetic variation of an organism the more asymmetry that organism should display on measured characteristics (Leary & Allendorf, 1989; Bisazza et al., 1998). The idea behind this hypothesis was born out of morphological measures of fluctuating asymmetry, which is a
measure of disturbed development (Van Valen, 1962; Leary & Allendorf, 1989), but what results do exist are overall inconsistent, with the relationship being found in some species (e.g. rainbow trout, *Oncorhynchus mykiss*; Leary et al., 1985) but not in others (e.g. Trinidadian guppies, *Poecilia reticulata*; Sheridan & Pomiankowski, 1997). However, because laterality is argued to be a beneficial characteristic, here it is suggested that the relationship between measured laterality, both behavioural and morphological, would increase with increasing genetic variation. Further investigation into this hypothesis is necessary to allow us a better understanding of the role that genetic variation may have with respect to morphological and behavioural laterality.

Since genetic variation may play a role in explaining both neural and behavioural laterality, it is essential to explore laterality in the context of the interaction of an organism’s genetic background and environment (Cowell & Denenberg, 2002). Gene-by-environment (i.e. G x E) studies of laterality are virtually non-existent but there has been some strong support of environment alone as a driver to development of behavioural laterality. For example, strength of eye use was differentially affected in crimson-spotted rainbow fish (*Melanotaenia duboulayi*) dependent upon enrichment or impoverishment of the environment and sex of the fish (Bibost et al., 2013). In addition, male Trinidadian guppies reared with olfactory predator cues have been found to show stronger laterality (irrespective of direction) compared to their counterparts reared without predator cues (Broder & Angeloni, 2014). Light exposure during incubation has also led to significant effects on visual laterality in zebrafish (*Danio rerio*; Sovrano et al., 2016), and in both visual and motor laterality in Trinidadian guppies (Dadda & Bisazza, 2012). Clearly, aspects of the environment can play a role in the emergence of laterality in fish species, yet how genetic background and components of the environment interact to affect
laterality has not been rigorously studied in any system to date. There is more than likely a genetic predisposition for laterality but the switch for laterality will not be “turned on”, so to speak, unless particular environmental conditions (e.g. light, enrichment) are present during a sensitive developmental period (Cowell & Denenberg, 2002). One of the strongest explanations for the development of laterality, then, will come from considering the interaction of genetic and environmental factors.

Using purposely-crossed lines of juvenile Chinook salmon, three domestic and three outcross, I will provide one of the first tests to more closely begin to investigate what relationship exists between genetic background and measured morphological and behavioural laterality. To do so, I investigate morphological laterality of brain hemispheres and eyes in intact fish to determine if juvenile Chinook salmon show any pattern between cross and measured laterality. Here, too, I provide one of the first examinations of how genetic background may be related to behavioural morphology. Because it is suggested within this chapter that those organisms with greater genetic variation will express higher measures of laterality, it is hypothesized that the outcross stocks will show higher laterality than the domestic stocks tested.

**Methods**

**Subjects**

Six genetic crosses, consisting of 5 to 6-month-old juvenile Chinook salmon (*Oncorhynchus tshawytscha*), were used for the present research. All crosses were created through artificial spawning at Yellow Island Aquaculture, Ltd. (YIAL) hatchery (Quadra Island, British Columbia, Canada) in October of 2013. Three crosses were deemed domestic and were denoted as “HH”, “LL” and “H3”. Juveniles of the H3 line were the
resulting offspring of a self-fertilized hermaphrodite (see Komsa, 2012 for breeding information); the HH and LL juveniles were the offspring of eggs from hermaphrodite dams and milt from males from the “high-high” and “low-low” performance crosses, respectively, which are the broodstock maintained at YIAL characterized, correspondingly, by high or low rates of growth and survival (see Docker & Heath, 2002 and Lehnert et al., 2014). Juveniles from the remaining three crosses used were offspring which resulted from artificial outcrossing between a YIAL created cross (hermaphrodite) and milt of males from three different river locations within British Columbia: the Robertson Creek (RC), the Nitnat (Nit), and Quinsam (Q) rivers. The RC, Nit and Q crosses represented the three outcross variants.

**Housing**

Fish from all six crosses were moved at approximately four months of age from their egg trays to housing barrels (200L) (120 fish/barrel for domestic crosses; 50 fish/barrel for outcrosses) where they remained for the duration of behaviour trials and before sacrifice. Four barrels were assigned to each genetic cross, resulting in a total of 24 barrels. In two of the four barrels for each cross the inflow tube, threaded through aluminum piping to keep it stable, was placed so as to create a clockwise (CW, n = 12) flow of water, and in the remaining two barrels the flow was counter-clockwise (CCW, n = 12). This simple manipulation allowed for a test of the effect of water flow on direction and strength of laterality of the behavioural and morphological measures.
**Behaviour Trials**

**C-Start Behaviour**

C-start behaviour trials were conducted from June 1 to June 3, 2014. Four tanks (62.5cm x 45cm x 18cm; water height 11cm) were set up beneath a ball drop mechanism (Figure 3.1), which allowed the simultaneous release of the startling stimulus (a golf ball) to all tanks. A GoPro Hero 3 camera (GoPro, Inc.) was setup 160 cm above the tanks for video recording; all four tanks were visible in one video frame. Five fish from each barrel were tested together and four barrels were tested per video. Upon startle, I examined only the first c-start turn direction of each fish in each tank. A c-start, the characteristic response of fish when startled, is the contraction of muscles on one side of the body, bending the body into a ‘c’ shape (Heuts, 1999). A total of right c-start turns per behaviour tank were noted and these scores were pooled for each tank in each trial. Since every barrel (4 per cross) was tested a total of 8 times, 32 scores per cross were collected (N = 192). It should be noted that because fish were not tagged or otherwise marked after use in one behaviour trial there is a possibility that some fish were tested multiple times; however, to avoid habituation to the stimulus with repeated testing there was a minimum time of 1 ½ to 2 hours between the testing of fish from each barrel (Jain et al., 1998).

At the start of every trial, fish were placed in one of the four designated testing tanks and allowed to acclimate for 10 minutes with video recording starting in the latter five minutes of the acclimation period. Immediately following the acclimation period the golf ball stimulus was remotely released toward but not into the tank, and then removed. Golf balls were not allowed to hit the water so as to not activate mechanoreceptors and to avoid problems with video analysis due to water disruption. Videos were analysed using
**Figure 3.1:** Simple schematic of the ball drop mechanism used during the C-start trials (golf balls darkened in black for clarity). Note that when the golf balls are fully extended they do not drop into the water.
VLC media player (Version 2.2.1) where the first c-start direction (left or right) made by each individual fish (5 per tank) was noted and the laterality index (LI), indicating the proportion of rightward escapes, was calculated (per tank) following Cantalupo et al. (1995):\( ((\text{total rightward escapes})/ (\text{total rightward escapes} + \text{total leftward escapes})) \times 100 \). In addition, absolute laterality using the formula, \( AI = |\text{LI} - 50| \), where maximum scores of 50 represent the strongest lateralized individuals (Brown & Magat, 2011), was obtained as a measure of the strength of laterality irrespective of direction.

*Inspection Behaviour*

Mirror inspection behaviour was tested from June 4 to June 7, 2014. Twenty-four fish from each of the 24 barrels were tested, with a total of 96 fish tested from each cross. All fish were tested individually in matte grey tanks (approximately 26cm x 20cm x 18cm) with a mirror replacing the front wall. Two GoPro Hero 3 cameras were used for overhead recording of all trials. Timing of each 20 minute trial began when all fish were placed in their respective tanks and the researcher had exited the testing area. Upon completion of the trial all fish were removed from their individual tanks and returned to their respective housing barrels.

Videos were played back using VLC Media Player to determine fish body position with respect to the mirror (Figure 3.2). Body position and location in the tank was noted every 2 seconds over the 20-minute trial, which was broken into 4 blocks of 5 minutes each, to allow for an analysis of eye use preference over time (Sovrano et al., 1999; De Santi et al., 2001). Eye use preference was noted only for fish which positioned themselves within approximately 5cm of the mirror. Eye use preference was determined
Figure 3.2: Acceptable fish body positions used for right or left eye use during the mirror inspection behaviour test.
using the following formula for the laterality index (Sovrano et al., 1999): 

\[ LI = \left( \frac{\# \text{ right eye uses}}{\# \text{ of right eye uses} + \# \text{ of left eye uses}} \right) \times 100 \]

I also calculated the absolute laterality index (AI = |LI – 50|) to determine the strength, irrespective of direction, of asymmetrical eye use over time for each genetic cross.

**Morphology**

One day following completion of the inspection behaviour trials, 20 fish from each cross were sacrificed. After sacrifice, all fish were fixed for 48 to 72 hours in 10% buffered formalin before transport back to the laboratory at the University of Windsor where they were then placed in 70% ethanol for further fixation. Prior to dissection, the body mass of each fish was taken (in grams) using a two-decimal standard scale (Ohaus Scout Pro SP202, Ohaus Corporation, New Jersey, USA). With a digital camera (Q-imaging Q1 Cam Fast 1394) connected to a dissecting microscope (Leica L2 10445930) I captured images of both the left and right eyes of each fish and measured the perimeter of the eye as a whole in the intact fish, using Northern Eclipse Imaging Software (Empix Inc., [http://www.empix.com](http://www.empix.com)). Following brain removal, dorsal images of each brain were taken and all brains were weighed using a four-decimal precision scale (Sartorius Extend ED124S, Sartorius AG, Goettingen, Germany).

In Northern Eclipse, perimeter values were measured from the brain images of the left and right hemispheres of the four main brain regions: the olfactory bulb, telencephalon, optic tectum, and cerebellum. To obtain a right and left hemisphere measurement from the cerebellum, which is a single-lobed structure, I artificially bisected this region in Northern Eclipse using the midline of the optic tectum lobes as an anchoring point of reference (Figure 3.3). Since the internal midline of the optic tectum
Figure 3.3: Image of juvenile Chinook salmon brain indicating the major brain regions measured in the current study: the olfactory bulb (OB), the telencephalon (TC), the optic tectum (OT) and the cerebellum (CB). The line through the CB indicates where it was artificially divided to measures a right and left ‘hemisphere’.
lobes, made up of the tectal and rhombencephalic ventricles, continues back through the
cerebellum (see Wullimann et al., 1996) this method of bisecting the cerebellum was
deemed an appropriate proxy for hemisphere division (Wiper et al., 2017). All differences
between left and right morphological measures were assessed using the laterality index
(LI) formula: \( \frac{\text{size of left} - \text{size of right}}{\text{size of left} + \text{size of right}} \times 100 \). The strength
of lateralized differences in morphology was assessed as well using the absolute index
(\( |LI| \)). Due to damage during dissection and missing hemispheres, the olfactory bulb
region of the brain was not used for neuroanatomical investigations.

**Statistical Analysis**

**Tests for Normality**

Prior to analysis all datasets were assessed for normality. Across all datasets, cases
were removed as outliers if they had two or more standardized Z-scores which exceeded
\( +/- 2.58 \). No outliers were removed for the C-start (N = 192) or inspection behaviour data
(N = 576), but 27 cases were removed as outliers from the morphology dataset (N = 457).
For all, skewness and kurtosis values were generally within acceptable ranges, but,
Shapiro-Wilk tests of normality indicated that there was a violation (i.e. all \( p < 0.05 \)) of
the assumption of data from normally distributed populations. However, upon visual
inspection (histograms; Q-Q plots) of all datasets and with the relatively large sample
sizes for all datasets (Field, 2013) it was determined that no other corrections needed to
be made or alternative test methods (i.e. non-parametric tests) used. It should be noted
that all tests were initially run with the covariate of rearing barrel as part of the model.
Unless otherwise stated, barrel was found to be a non-significant factor and was removed
from each model, provided its removal did not change the pattern of the effects of all other factors.

**Behaviour**

*C-Start*

Following the tests for normality, a MANOVA was conducted on the outcome variables of the laterality and absolute indexes to determine differences in right turn behaviour and strength of asymmetry, respectively, between the six crosses, between flow directions, and the interaction of these two variables to investigate gene-by-environment (GxE) interaction effects.

*Inspection Behaviour*

To examine mirror inspection behaviour in juvenile Chinook salmon, a repeated measures ANOVA was conducted. I was interested in the main effect of time on the direction and strength of eye use, as well as the main effects of, and interaction between, genetic cross and flow direction. Two separate repeated measures tests were run, one to investigate right eye use preference (LI) and the second to assess the absolute laterality (strength of asymmetrical eye use) regardless of direction. Due to multiple tests on the same dataset, I used a Bonferroni corrected alpha value of $p = .025$ (0.05/2) for all tests. Mauchly’s test for sphericity for both the laterality ($\chi^2 (5) = 194.101, p < 0.001$) and absolute index ($\chi^2 (5) = 136.86, p < 0.001$) outcome variables showed that this assumption had been violated, therefore lower-bound corrected tests are reported throughout the results section (both $\varepsilon = 0.333$) (Field, 2013).
Morphological Analysis

Differences of the absolute and laterality indexes of brain hemispheres and eyes as an effect of genetic cross, flow direction, and the interaction of cross and flow (i.e. GxE) were examined using a MANOVA.

Behaviour and Morphology Correlations

Because there was no significant effect of time on eye use preference for either the laterality or absolute index I used only the instances of eye use from the first five minutes of the trials. I examined the relationship between behaviour and morphology using a behavioural index of inspection behaviour, (right eye uses – left eye uses / right eye uses + left eye uses) x 100 (Jozet-Alves et al., 2012), which I correlated with both an eye size ratio (perimeter of right eye / perimeter of left eye), and an optic tectum hemisphere ratio (perimeter of right OT / perimeter of left OT) (Jozet-Alves et al., 2012). Twenty-three cases were removed as outliers from the correlation dataset (standardized scores greater than +/- 2.58) prior to analyses. In addition, for the correlation analysis, because the number of fish from which morphological measurements were obtained (n = 20/cross) was not equal to the number of fish tested for behavioural trials (n = 24/cross), four fish from each barrel were removed from the behavioural dataset using a random number generator.
Results

Behaviour

C-Start (Startle Behaviour)

There was no significant effect of cross (Wilks’ Lambda, Λ = 0.968, 
\( F_{10,358} = 0.578, p = 0.832 \)) on proportion of right turns or strength of laterality, but when
comparisons of behaviour of each cross were pooled, there was a significant effect of
flow direction (\( Λ = 0.951, F_{2,179} = 4.633, p = 0.011 \)). The effect of flow was significant
for the laterality index (\( F_{1,180} = 7.768, p = 0.006 \); Figure 3.4), but not for the absolute
index. The clockwise flow (M = 54.77, SE = 2.28, 95% CI [50.2, 59.30]) showed a
higher average value of right turns than the counter-clockwise flow (M = 46.09, SE =
2.09, 95% CI [41.95, 50.24]). There was also a significant effect on the interaction of
cross x flow direction (\( Λ = 0.894, F_{10,358} = 2.066, p = 0.026 \)). This effect was only for the
absolute (strength) measure (\( F_{5,180} = 2.933, p = 0.014 \)) (Figure 3.5; Table 3.1 for all
values).

Inspection Behaviour

Right Eye Use Preference. There was no significant difference between crosses on
right eye use preference (\( F_{5,483} = 1.61, p = .155 \)), but there was a significant effect of flow
direction (\( F_{1,483} = 32.25, p < .001 \); clockwise: M = 49.18, SE = 0.755, 95% CI [47.70,
50.66]; counter-clockwise: M = 57.51, SE = 0.679, 95% CI [56.18, 58.85]) (Figure 3.6).
There was also a significant G x E interaction of flow direction and cross (\( F_{5,483} = 5.10, p
< .001 \)) (Figure 3.7; Table 3.2). In addition, there was no significant interaction between
time and flow direction (\( F_{1,483} = 0.62, p = .433 \), or time and cross (\( F_{5,483} = 2.15, p =
.058 \) on right eye use.

90
Figure 3.4: The effect of flow direction on average number of rightward escapes (C-starts) upon startle of all fish in either a clockwise or counter-clockwise flow barrel. Values above 50 indicate greater preference for rightward escapes. Error bars represent +/- 1 SE.
Figure 3.5: The effect of the gene-by-environment interaction on the strength of asymmetrical C-starts, which helps to indicate individual variation in asymmetrical escape behaviour when startled. Overlapping error bars indicate no difference between crosses when reared in the same flow direction (error bars represent +/- 1 SE).
Table 3.1: Mean (M), standard error (SE) and confidence intervals (95% CI) for the interaction effect of flow-by-cross on C-start escapes. Highest and lowest values in bold.

<table>
<thead>
<tr>
<th>Flow Direction</th>
<th>Cross</th>
<th>M</th>
<th>SE</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Lower, Upper)</td>
</tr>
<tr>
<td>Clockwise</td>
<td>HH</td>
<td>15.94</td>
<td>2.991</td>
<td>22.03, 26.58</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>15.63</td>
<td>2.991</td>
<td>17.79, 22.35</td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td><strong>25.94</strong></td>
<td>2.991</td>
<td>15.55, 19.98</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>24.69</td>
<td>2.991</td>
<td>13.69, 18.03</td>
</tr>
<tr>
<td></td>
<td>Nit</td>
<td>15.63</td>
<td>2.991</td>
<td>15.31, 19.67</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td><strong>15.42</strong></td>
<td>2.991</td>
<td>15.41, 19.87</td>
</tr>
<tr>
<td>Counter-Clockwise</td>
<td>HH</td>
<td>16.35</td>
<td>2.991</td>
<td>10.45, 22.26</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>18.44</td>
<td>2.991</td>
<td>12.54, 24.34</td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td>17.19</td>
<td>2.991</td>
<td>11.29, 23.09</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td><strong>11.88</strong></td>
<td>2.991</td>
<td>5.98, 17.78</td>
</tr>
<tr>
<td></td>
<td>Nit</td>
<td><strong>20.00</strong></td>
<td>2.991</td>
<td>14.10, 25.90</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>18.96</td>
<td>2.991</td>
<td>13.06, 24.86</td>
</tr>
</tbody>
</table>
Figure 3.6: The effect of flow direction on the laterality index measure to determine significance of right eye use preference during inspection behaviour trials. Values above 50 indicate greater preference for right eye use. Error bars represent +/- 1 SE.
Figure 3.7: The effect of the gene-by-environment interaction on the laterality index (directional measure) of right eye use in the inspection behaviour trials. The interaction is being driven by the H₃ cross.
Table 3.2: Mean (M), standard error (SE) and confidence intervals (95% CI) for the interaction effect of flow-by-cross on right eye use preference (laterality index). Highest and lowest values in **bold**.

<table>
<thead>
<tr>
<th>Flow Direction</th>
<th>Cross</th>
<th>M</th>
<th>SE</th>
<th>95% CI (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clockwise</td>
<td>HH</td>
<td>47.00</td>
<td>1.767</td>
<td>43.54, 50.47</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>48.48</td>
<td>1.772</td>
<td>45.00, 51.96</td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td><strong>59.34</strong></td>
<td>1.723</td>
<td>55.97, 62.72</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>45.83</td>
<td>1.690</td>
<td>42.51, 49.14</td>
</tr>
<tr>
<td></td>
<td>Nit</td>
<td>44.60</td>
<td>1.699</td>
<td>41.26, 47.93</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>50.20</td>
<td>1.735</td>
<td>46.80, 53.60</td>
</tr>
<tr>
<td>Counter-Clockwise</td>
<td>HH</td>
<td><strong>66.15</strong></td>
<td>1.789</td>
<td>62.64, 69.66</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>62.23</td>
<td>1.762</td>
<td>58.78, 65.69</td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td><strong>51.16</strong></td>
<td>1.704</td>
<td>47.82, 54.51</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>55.59</td>
<td>1.690</td>
<td>52.27, 58.90</td>
</tr>
<tr>
<td></td>
<td>Nit</td>
<td>56.00</td>
<td>1.708</td>
<td>52.65, 59.35</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>55.06</td>
<td>1.690</td>
<td>51.75, 58.38</td>
</tr>
</tbody>
</table>
**Absolute Laterality.** In the case of the strength of asymmetry there was a statistically significant effect of the covariate, rearing barrel, \((F_{1, 482} = 7.81, p = .005)\) thus it remained in the model. There was a significant effect of flow direction on the absolute index \((F_{1, 482} = 6.76, p = .01)\); clockwise: \(M = 18.68, SE = 0.497, 95\% CI [17.71, 19.66]\); counter-clockwise: \(M = 17.66, SE = 0.476, 95\% CI [16.73, 18.60]\) (Figure 3.8). In addition, there was a statistically significant difference between the genetic crosses on the measure of absolute laterality, \((F_{5, 482} = 3.01, p = .011; \text{Table 3.3})\) and on the interaction of flow-by-cross \((F_{5, 482} = 3.29, p = .006)\) (Figure 3.9; Table 3.4). There was no significant interaction of time and flow \((F_{1, 482} = 2.15, p = .144)\) or time and cross \((F_{5, 482} = 1.998, p = .078)\) on the overall strength of asymmetry of eye use.

**Brain and Whole Eye Morphology, and Behaviour and Morphology Correlation**

While there was no significant overall effect of flow on morphology \((\Lambda = 0.977, F_{8, 438} = 1.295, p = 0.244)\), and no significant effect of the GxE interaction of cross and flow \((\Lambda = 0.893, F_{40,1911.99} = 1.258, p = 0.130)\), a MANOVA indicated that there was a significant effect of cross on morphology measures \((\Lambda = 0.753, F_{40,1911.99} = 3.218, p < 0.001)\). This effect was significant for four main measures (See Table 3.5 for all values). First, the strength of asymmetry (absolute index) of the OT \((F_{5, 445} = 5.896, p = 0.001)\) (Figure 3.10 A) was significantly affected by cross, with a Tukey’s post-hoc analysis revealing that the HH cross differed significantly from all other crosses \((p < 0.014)\).

The laterality index of the CB \((F_{5, 445} = 9.875, p < .001)\) (Figure 3.10 B) was also significantly affected by cross, with no discernible pattern differentiating the domestic from the outcrosses. However, the HH cross differed significantly from both LL and RC crosses (Tukey’s post-hoc, both \(p = 0.022\)), LL differed significantly from Nit \((p = 0.008)\)
Figure 3.8: The effect of flow direction on the absolute index of right eye use during inspection behaviour trials. The absolute index is a measure of the strength of lateralized preference irrespective of direction; the higher the value, the stronger the lateralized preference. Error bars represent +/- 1 SE.
Table 3.3: Mean (M), standard error (SE) and confidence intervals (95% CI) for the effect of cross on the strength of asymmetrical eye use (absolute index). Highest and lowest values in bold.

<table>
<thead>
<tr>
<th>Cross</th>
<th>M</th>
<th>SE</th>
<th>95% CI (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH</td>
<td>25.58</td>
<td>.989</td>
<td>23.63, 27.52</td>
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<tr>
<td>LL</td>
<td>21.55</td>
<td>.922</td>
<td>19.74, 23.37</td>
</tr>
<tr>
<td>H3</td>
<td>17.19</td>
<td>.809</td>
<td>15.59, 17.78</td>
</tr>
<tr>
<td>RC</td>
<td>15.32</td>
<td>.757</td>
<td>13.83, 16.81</td>
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<td>Nit</td>
<td>15.34</td>
<td>.740</td>
<td>13.88, 16.79</td>
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<tr>
<td>Q</td>
<td><strong>14.89</strong></td>
<td>.694</td>
<td><strong>13.53, 16.26</strong></td>
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</table>
Figure 3.9: The effect of the gene-by-environment interaction on the strength of asymmetrical eye use in the inspection behaviour trials. The absolute index is a measure of the strength of lateralized preference irrespective of direction; the higher the value, the stronger the lateralized preference.
**Table 3.4:** Mean (M), standard error (SE) and confidence intervals (95% CI) for the interaction effect of flow-by-cross on the strength of asymmetrical eye use (absolute index). Highest and lowest values in **bold**.

<table>
<thead>
<tr>
<th>Flow Direction</th>
<th>Cross</th>
<th>M</th>
<th>SE</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Lower, Upper)</td>
</tr>
<tr>
<td>Clockwise</td>
<td>HH</td>
<td><strong>24.30</strong></td>
<td>1.158</td>
<td>22.03, 26.58</td>
</tr>
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<td></td>
<td>LL</td>
<td>20.07</td>
<td>1.162</td>
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<td>H3</td>
<td>17.76</td>
<td>1.130</td>
<td>15.55, 19.98</td>
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<tr>
<td></td>
<td>RC</td>
<td><strong>15.86</strong></td>
<td>1.108</td>
<td>13.69, 18.03</td>
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<td></td>
<td>Nit</td>
<td>17.49</td>
<td>1.114</td>
<td>15.31, 19.67</td>
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<td></td>
<td>Q</td>
<td>17.64</td>
<td>1.138</td>
<td>15.41, 19.87</td>
</tr>
<tr>
<td>Counter-Clockwise</td>
<td>HH</td>
<td><strong>26.93</strong></td>
<td>1.173</td>
<td>24.63, 29.23</td>
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<td>Clockwise</td>
<td>LL</td>
<td>23.10</td>
<td>1.155</td>
<td>20.83, 25.36</td>
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<td></td>
<td>H3</td>
<td>16.89</td>
<td>1.117</td>
<td>14.69, 19.08</td>
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<td>14.79</td>
<td>1.108</td>
<td>12.62, 16.96</td>
</tr>
<tr>
<td></td>
<td>Nit</td>
<td>13.19</td>
<td>1.120</td>
<td>10.995, 15.39</td>
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<td></td>
<td>Q</td>
<td><strong>12.41</strong></td>
<td>1.108</td>
<td>10.24, 14.58</td>
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</table>
Table 3.5: Mean (M), standard error (SE) and confidence intervals (95% CI) for the effect of cross on four measures of morphology. Highest and lowest values in **bold**.

<table>
<thead>
<tr>
<th>Morphology Measure</th>
<th>Cross</th>
<th>M</th>
<th>SE</th>
<th>95% CI (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic tectum, Abs. Index</td>
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<td>0.230</td>
<td>2.35, 3.27</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>1.94</td>
<td>0.172</td>
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<td></td>
<td>H3</td>
<td>1.98</td>
<td>0.196</td>
<td>1.59, 2.37</td>
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<tr>
<td></td>
<td>RC</td>
<td>1.93</td>
<td>0.178</td>
<td>1.57, 2.28</td>
</tr>
<tr>
<td></td>
<td>Nit</td>
<td><strong>1.56</strong></td>
<td>0.136</td>
<td>1.29, 1.83</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>1.72</td>
<td>0.129</td>
<td>1.47, 1.98</td>
</tr>
<tr>
<td>Cerebellum, Laterality Index</td>
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</tr>
<tr>
<td></td>
<td>LL</td>
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<td>0.327</td>
<td>-1.38, -0.08</td>
</tr>
<tr>
<td></td>
<td>H3</td>
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<td>0.318</td>
<td>-0.95, 0.32</td>
</tr>
<tr>
<td></td>
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<td>0.221</td>
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<tr>
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<tr>
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<td>LL</td>
<td><strong>2.43</strong></td>
<td>0.194</td>
<td>2.04, 2.81</td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td>2.18</td>
<td>0.191</td>
<td>1.80, 2.56</td>
</tr>
<tr>
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<td>RC</td>
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<td>0.168</td>
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<tr>
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<td>Nit</td>
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<td>0.137</td>
<td>1.40, 1.95</td>
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<td></td>
<td>Q</td>
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</tr>
<tr>
<td>Whole Eye, Abs. Index</td>
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<td><strong>2.76</strong></td>
<td>0.243</td>
<td>2.27, 3.25</td>
</tr>
<tr>
<td></td>
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<td>2.43</td>
<td>0.245</td>
<td>1.94, 2.92</td>
</tr>
<tr>
<td></td>
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<td>2.46</td>
<td>0.217</td>
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<td>Q</td>
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<td>1.42, 2.16</td>
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</table>
Figure 3.10: Significant effects of cross on four main morphology measures; absolute index of the optic tectum (A); the laterality index for the cerebellum (B); the absolute index for the cerebellum (C); and the absolute index for the whole eye (D).
and Q \((p < 0.001)\), and Q additionally differed significantly from H3 and RC (both \(p < 0.001\)). Between cross differences were also found for the absolute index for the CB (\(F_5, 445 = 2.785, p = .017\)) (Figure 3.10 C) where a Tukey’s post-hoc showed that the main difference could be seen between LL and Nit crosses \((p = 0.03)\). Finally, there was a significant cross effect on the absolute laterality of the whole eye perimeter (\(F_5, 445 = 3.539, p = 0.004\)) where the HH cross differed marginally from both RC (Tukey’s post-hoc, \(p = 0.052\)) and Nit (\(p = 0.048\)), as well as from the Q cross (\(p = 0.013\)) (Figure 3.10 D).

When comparing the size (mm) of the optic tectum hemispheres to their respective contralateral eyes, there was a significant correlation between the right OT and left eye \((r = 0.388, p < 0.001; \text{Figure } 3.11 \text{ A})\), and between the left OT and right eye \((r = 0.338, p < 0.001; \text{Figure } 3.11 \text{ B})\). However, when examining the relationship between inspection behaviour and OT size ratio there was no significant relationship \((r = -0.035, p = 0.474)\), nor was there a significant relationship between the inspection behaviour and the eye size ratio \((r = -0.037, p = 0.450)\).

**Discussion**

Laterality is a characteristic that has been shown to be driven by many factors, including parental direction of escape behaviour in golden topminnows (Bisazza et al., 2000), light exposure before hatching in domestic chickens (\textit{Gallus gallus domesticus}; Deng & Rogers, 1997), and even domestication in horses (Farmer et al., 2010; Austin & Rogers, 2012). Here, I have found strong evidence that a simple environmental manipulation of flow direction can affect behavioural outcomes in Chinook salmon. In addition, this environmental effect can be mediated by genetic cross. Genetic cross itself
Figure 3.11: Correlation of optic tectum and contralateral eye size. A: correlation of right optic tectum and left eye; B: left optic tectum and right eye.
is more likely, at least in this study, to show significant effects on morphological measures of laterality, and very little effect on behaviour.

**Effect of Flow**

Environmental enrichment has been shown in some studies as a driver of brain growth (Näslund et al., 2012; Toli et al., 2017; Kotrschal et al., 2017; Naslund et al., 2017). In the current chapter, I used a manipulation of flow direction (clockwise or counter-clockwise) to determine whether differential effects on behavioural and morphological measures could be found. When investigating the C-start, or startle responses, of fish I found that the manipulation of flow direction was enough to significantly affect the direction of escape. At least one other study investigating directional preference of escape behaviour has found that shiner perch (*Cymatogaster aggregata*) show no significant difference of directionality in escape responses when startled (Dadda et al. 2010). Thus, while it is a ballistic response to a startling stimulus, specific features of the environment may be a potential driver to C-start turns. Flow direction also showed a significant and differential effect on both direction and strength of eye use preference. The CCW flow direction appeared to increase the overall directional preference of eye use but the *strength* of asymmetrical eye use was affected more by the CW flow. In a CCW flow barrel the fish were swimming against the current created, meaning that their left eyes were facing the outer barrel and right eyes were looking inward toward other fish. This may indicate that in juvenile Chinook salmon a CCW flow direction will increase the likelihood of right eye use when viewing conspecifics, and the left eye will be used for scanning the environment. The reverse pattern for lateralized eye use (i.e. left eye preference) when viewing a mirror-image
conspecific has been found in several other teleost species (Sovrano et al., 1999; De Santi et al., 2001; Sovrano & Andrew, 2006), however, at least one fish species (*Poecilia reticulata*) use their right eye when viewing familiar, and their left eye when viewing unfamiliar, conspecifics (Kaarthigeyan & Dharmaretnam, 2005). It may be that Chinook salmon share the left eye preference for viewing conspecific fish but here the flow direction could have manipulated this typical pattern. Alternatively, eye use preference for viewing a conspecific may be species specific, but more work would need to take place with this species to further investigate patterns typical for them with regards to visual inspection.

At the juvenile life stage there was no significant effect of flow direction on the eye and brain morphology measures. There may be no effect of environmental factors on brain morphology over a fish’s lifetime, however, it is more likely that the fish were too young for there to have been any notable differences. Alternatively, I may have been investigating the wrong environmental manipulation to see effects on brain morphology, as other studies (Kihslinger & Nevitt, 2006; Näslund et al., 2012) have shown initial significant effects on brain size early on in fish with environmental enrichment. On the other hand, the environmental manipulation used here may be sufficient but may not have been experienced early enough or for a long enough time to have a significant affect. Work will need to continue with this species to determine what environmental manipulations truly affect morphological measures, and during many life stages, to see when these effects are most important.

An additional environmental factor that could have some influence on outcomes, while not directly tested here, is the effect of density in each rearing barrel: three crosses were barreled at 120 fish/barrel (HH, LL, H3) and three crosses were barreled at 50
fish/barrel (RC, Nit, Q). High density has been equated with decreased growth in some fish species, including brown trout (*Salmo trutta*; Jenkins et al., 1999; Bohlin et al., 2002), but not in others, like the Artic charr (*Salvelinus alpinus*; Brown et al., 1992). Density has also been found to affect behaviour of fish, namely in agonistic interactions (Kaiser et al., 1995), shoaling behaviour (Brown et al., 1992) and feeding behaviour (Jørgensen et al., 1993; Alanärä & Brännäs, 1996), but has not been directly applied to lateralized behaviour. Fish in higher density barrels may have greater familiarity with conspecifics and show a higher likelihood for lateralized visual inspection behaviour when presented with a mirror but no support exists for this supposition and further work would be necessary to begin to make conclusions. I do not feel that density effects were of relevance in the current study however because my fish were 5 to 6 months of age, averaging a fork length of 64.7 mm and body mass of 3.5g. In the 200L barrels used to rear the fish at this early life stage, even with 120 fish per barrel, there was at least 1 litre of water per fish present - a level that would count as “low density” in previous work so density was likely not a limiting factor here but it could be an interesting avenue to pursue in future work.

**Effect of Genetic Cross**

This study was one of the first to test the directional c-start response and visual inspection behaviour of fish for a potential effect of genetic background. The c-start behaviour observed in my study was not affected by the genetic background of the fish being tested, which is not surprising considering that this is a ballistic movement, used to avoid startling stimuli or other obstructions, and is not likely to be highly determined by
genetic makeup. C-starts are largely controlled by a pair of neurons, known as the Mauthner cells, which are housed in the medulla oblongata and innervate the contralateral body musculature in fish (Zottoli, 1977; Fetcho, 1992). Some work in golden topminnows (Girardinus falcatus) from lines artificially selected for right or left turning preferences has suggested that the startle response in highly lateralized versus non-lateralized individuals does not differ, with all lines showing comparable speed and efficiency of escape (Agrillo et al., 2009). As I only tested juvenile fish it is possible that genetic background might still have a greater effect in adults but more work would need to be done to truly test this possibility.

With respect to the inspection behaviour of a mirror image conspecific, the pattern of differences appeared to somewhat follow the suggested relationship between genetic background and asymmetry but I was curious as to why the H$_3$ cross, which were self-fertilized hermaphrodite offspring, were not the most asymmetrical as would be predicted by the genetic variation and laterality hypothesis. A likely explanation for this finding is that there is a significant effect of the specific combination of parental genetic material. Bisazza et al. (2007) demonstrated that lateralized turning behaviour in offspring can be affected by their parents. Offspring were more likely to show a right turn behaviour bias when both parents had the same right turn direction preference (Bisazza et al., 2007), but when the parental turning preference directions differed from one another the offspring did not significantly turn more in one direction. In my case I did not specifically partition the genetic variance components (Neff & Pitcher, 2005; Evans et al., 2007; Janhunen et al., 2011) but I did see similarities in lines based on their maternal lineage so it may be the maternal contributions that affect lateralized behaviours, although a more comprehensive breeding design is needed to fully test this hypothesis.
Mine is the first study, to my knowledge, to examine the lateralized side differences of brain regions as a whole as opposed to more minute investigations of other neural features like the Mauthner cells (Moulton & Barron, 1967) or the habenular nuclei (Gutiérrez-Ibáñez et al., 2011). I found that for the optic tectum, responsible for visual input, there was no effect of genetic cross on the differences between the left and right hemispheres of this region, but a clear pattern emerged for the strength of asymmetry, wherein being from a domestic cross led to greater strength of laterality. On this measure there was a striking difference between the crosses: the HH cross differed significantly from all others, which may be an artefact of the selective breeding of this line for an increased growth rate (Docker & Heath, 2002), and body size may impact morphological laterality. In cichlids (Geophagus brasiliensis), in whom the laterality of the habenular nuclei have been investigated, fish with a greater standard length tended to have larger right habenulae (Reddon et al., 2009). However, no relationship with size was found for the absolute measure of laterality in these cichlids. In my fish, it may be that the higher growth rate of the HH fish is driving the differences on the absolute measure of the optic tectum. More work would need to be carried out in these lines of fish, and at different life stages accounting for and incorporating body size, to better determine if this pattern is persistent over time.

For the side differences of the cerebellum I saw mixed effects but I noted that it was one of the outcrosses that showed the highest mean laterality index. When I considered the absolute index of the cerebellum there was again no obviously discernible pattern between the domestic crosses and outcrosses. The lack of pattern with the absolute measure may be a reflection of higher individual variation on neuroanatomy measures in juvenile salmon. With greater age perhaps I would see a more obvious pattern displayed
between the genetic crosses. In addition the method of measurement must be considered. Perhaps artificially bisecting the cerebellum, a naturally single-lobed brain region, is not appropriate at the juvenile life stage when brains may not yet be fully developed and may be in a period of greater fluctuation (Näslund et al., 2012) in response to internal and external environments. I am left to wonder, however, whether there would be a greater effect of lateralized differences if both parents were from the RC, Nit or Q populations, instead of only the paternal line supplying the “outcross” genes, since previous work has shown that ‘strain specific’ brain morphology in fish of the same species (medaka, *Oryzias latipes*) can occur (Ishikawa et al., 1999). In this case, an H3 fish was used for the maternal line so there may have been a dampening effect on lateralized differentiation. Continued examinations with several combinations of parental genes will need to be carried out for firmer conclusions on cross-specific differentiation of brain morphology.

Finally, when measuring the differences between the right and left eyes there was no side difference but there was a significant effect of strength of asymmetry, perhaps indicating more individual variation in eye growth within crosses. In this measure I saw a clearer pattern of differences where the domestic crosses, especially the HH cross, showed higher strength of asymmetry of eye size than the outcrosses. In work with Moorish geckos (*Tarentola mauritanica*) eye size differences have been investigated but this has largely been examined between sexes (e.g. Zuffi et al., 2011; Massetti et al., 2017) and genetic cross has not been considered, and only one study investigated asymmetry of eye size—finding one of 62 species that showed side differences (Werner & Seifan, 2006). Thus, we can see that there is a beginning interest in investigating lateralized differences of eye size, but continued examinations are needed in fish and may show that differences between eye size may reflect overall sensitivity and acuity of the
eyes (Walls, 1942 as cited in Werner & Seifan, 2006) within or between groups of a species.

An additional potential source of variation in the current study is outbreeding depression—the reduction in fitness due to mating between crosses that are distantly related (Conner & Hartl, 2004)—particularly for the outcross fish. While I was unable to fully characterize it in the current work, outbreeding depression has been shown to affect growth and survival (Gharret et al., 1999; Tymchuk et al., 2007) as well as increase asymmetry of meristic characteristics reflecting fluctuating asymmetry (Gharrett & Smoker, 1991). While fluctuating asymmetry and laterality are not one-and-the-same, how outbreeding might relate to the beneficial characteristic of laterality will require more examination so that we can either rule out outbreeding depression as a factor of variation, or be better able to understand how the relationship between genetic background and development (and maintenance) of laterality might work. Future work will need to make use of specific breeding programs implementing crosses designed to test outbreeding over several generations to fully address this possibility.

**Gene-by-Environment Interaction**

In the present research, the directional measure of lateralized eye use was significantly affected by the interaction of genetic cross and flow direction, however, not all crosses were affected the same. In previous research, decreased genetic variation (i.e. inbreeding) has been suggested to negatively impact traits related to fitness, including a decrease in overall growth (Pante et al., 2001) and a decrease in the number of eggs produced by females (Su et al., 1996), and recently has shown some negative effects on mating behaviour in fish (van Oosterhout et al., 2003; Ala-Honkola et al., 2009) and fruit
flies (*Drosophila melanogaster*; Miller et al., 1993). In my investigation of right eye use I noted an incidence of decreased genetic variation differentially, but not negatively, affecting viewing behaviour. The different effect I was seeing in the H₃ fish may indicate that there is something unique about the combination of this environmental intervention (flow) and the genetic makeup of the H₃ fish that may be “turning on” a different switch responsible for eye use preference (Cowell & Denenberg, 2002). When considering the strength of lateralized eye use (the absolute measure) I saw two different patterns emerge within the crosses tested. The HH and LL crosses appear to show a greater increase in asymmetry of eye use when reared in a CCW barrel; and the remaining crosses showed overall moderate increases in strength of asymmetry when reared in the CW flow. At least one study (Bibost et al., 2013) has investigated environmental manipulation (enrichment vs. impoverishment) and its effect on lateralized eye use in male and female rainbowfish (*Melanotaenia duboulayi*) finding that the sex of the fish modified the effect of the environment on strength of laterality: males from impoverished rearing environments were more lateralized than “enriched” males, and females from enriched environments were more lateralized than “impoverished” females (Bibost et al., 2013). Here, too, I was seeing differential effects of flow direction manipulation modified by cross.

The differences in response we saw between the different flow environments is likely due to GxE interactions. Flow effects may show differential responses depending on the genetic background of the responding fish and GxE effects have been previously shown to be important for salmon growth (e.g. Hanke et al. 1989; Forest et al. 2017) and disease resistance (e.g. Becker et al. 2014), although it is difficult to ascertain exactly what is driving these effects when they are observed (Becker et al. 2014). It is possible
that other unmeasured environmental differences (e.g. small differences in lighting due to barrel location in the hatchery) could have driven additional GxE effects but I would expect these more subtle effects to be less important than the large flow differences.

Correlation of Lateralized Behaviour and Morphology

When I compared the lateralized inspection behaviour to the ratio of the optic tectum lobes as a comparison of behaviour and morphology I found no relationship between these measures. This type of comparison is not often carried out, but some results contrary to my own have been reported. For example, the strength of asymmetry of the habenular nuclei in cichlids was positively related to the strength of asymmetrical differences on a detour task (Reddon et al., 2009). In cuttlefish (Sepia officinalis) there was a positive relationship between a larger right optic tectum lobe and the propensity for left turning (Jozet-Alves et al., 2012). In the case of my study I may not have seen a relationship between our morphology and behaviour because the fish were too young for a strong connection to be formed, or, more likely, I may have needed to use a different behavioural measure, like a turning preference, or needed to consider other brain regions to correlate with behaviour, in addition to the optic tectum.

Conclusions

Through my tests of the genetic effect on behavioural and morphological laterality I have provided one of the first strong examinations into the genetic variation and laterality hypothesis, which has previously received mixed support (Leary et al., 1985; Sheridan & Pomiankowski, 1997). In my case, it seems that this hypothesis cannot be
extended to behavioural measures, but this may be an issue with age of the fish, where greater age may show stronger relationships when considering genetic cross. Or, more likely, it may be that the hypothesis does not explain well the measures that I used in regard to genetic cross and may be better suited to a different behavioural test, such as a detour or mating behaviour investigation. It will be important to continue work such as this on both morphological and behavioural measures of laterality as the information that we can obtain will not only allow us to better understand the drivers to laterality in more and more fish species, but will also promote a better understanding of the evolution of this characteristic from early vertebrate species to humans. Laterality is not static and changes with life stages (Rogers, 1989) and we, too, must continue to adapt our methods of investigation, considering as many potential driving factors as we can to gain the clearest picture of the roots of laterality.
References


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


CHAPTER 4: AN INVESTIGATION OF THE EFFECTS OF POPULATION DIFFERENTIATION ON EXPENSIVE TISSUE TRADE-OFFS AND BRAIN LATERALITY IN ADULT CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*)

**Introduction**

Subpopulations of species can be widespread across large geographical areas meaning that each subpopulation will experience a different environment from other subgroups. These varying environments can result in meaningful differences between populations of the same species thus reflecting population differentiation among groups. The evolutionary process leading to differentiation between populations in separate habitats is the result of the interaction between an organism’s genotype and their environment, where natural selection pressures help to shape dominant phenotypes and thus the animals’ genotypes (Conner & Hartl, 2004). Male *Phylloscopus* warblers, for example, can have differing plumage colouration based on the light environment of their habitat (Marchetti, 1993). In more recent years brain size divergence among populations of the same species has been of interest, with most studies involving hatchery vs wild comparisons of fish. An examination of differences in the forebrain regions of Chinook salmon (*Oncorhynchus tshawytscha*) reared in a conventional hatchery, an enriched hatchery, and in a natural river habitat found that the wild fish showed larger olfactory and telencephalic brain regions than the fish reared in the conventional hatchery treatment (Kihlslinger et al., 2006). Populations of wild-caught female guppies (*Poecilia reticulata*), too, show larger brain regions than populations of their female offspring who have been reared in a laboratory setting (Burns et al., 2009). Marchetti and Nevitt (2003) found no difference in brain size between two hatchery populations of rainbow trout (*Oncorhynchus mykiss*) but did uncover a difference between fish from two wild...
populations on measures of brain size, indicating a clear population differentiation that led to the divergence of brain size between the two groups. Differences in brain size between geographically isolated populations of nine-spined sticklebacks (*Pungitius pungitius*) (Gonda et al., 2009; Gonda et al., 2011), and shortfin mollies (*Poecilia mexicana*; Eifert et al., 2015) have also been found.

Brain size divergence can be further investigated from the perspective of laterality, better known as the examination of the differential processing of information between, or investment into, the right and left brain hemispheres (Frasnelli et al., 2012; Rogers et al., 2013; Dadda et al., 2015). Laterality studies are often discussed from a “population- vs. individual-level” point of view, where results outline whether the laterality measures of interest are particular to the population; that is, whether the majority of individuals show a significant preference for the same lateralized direction, or if the patterns are seen only in individuals, meaning that the population under study will show an even number of left and right lateral preferences (Takeuchi & Hori, 2008). While population-level discussions of laterality are more common, there is little to no comparison *between* populations measured for laterality because most studies focus on one, lab-controlled population. Some manipulations of lab rearing environments in fish, however, have indicated that if groups of the same species are exposed to key differences within their habitats there will be differentiation between the groups on at least behavioural laterality. For example, enrichment or impoverishment of the rearing environment can have significant effects on eye use preference in crimson-spotted rainbow fish (*Melanotaenia duboulayi*) (Bibost et al., 2013). Light exposure during incubation has also shown significant effects on behavioural laterality in topminnows (*Girardinus falcatus*) (Dadda & Bisazza, 2012) and zebrafish (*Danio rerio*) (Sovrano et
al., 2016), and guppies (*Poecilia reticulata*) reared with or without olfactory predator cues have shown differentiation in lateralized behaviour as well: those fish reared with predator cues show higher measured degrees of behavioural laterality (Broder & Angeloni, 2014). While these studies are enlightening and show that differences between groups of organisms of the same species do occur with environmental manipulations, we still require comparison between populations from differing habitats, or at least from offspring whose parents were from markedly different habitats, that allow a strong examination of the breadth of population differentiation with respect to laterality measures.

The brain is an expensive tissue to produce (Mink et al., 1981) so investigating differences as an effect of population differentiation can help to clarify the drivers most responsible for brain size as a whole and for differences between hemispheres. The expensive tissue and energy trade-off hypotheses suggest that the brain “trades off” with other tissues or metabolically expensive behavioural processes to allow for increase in brain size (e.g. Aiello & Wheeler, 1995; Pitnick et al., 2006). For example, migratory birds that have to deal with the energetic costs of long-distance migration have been shown to have smaller brains, but larger pectoral muscle mass, than non-migrants (Isler & van Schaik, 2006). Guppies (*Poecilia reticulata*) have shown a trade-off between brain and gut size and between brain size and number of produced offspring, where the larger brained fish had smaller guts and would produce fewer offspring (Kotrschal et al., 2013). Similarly, across 71 species of cichlids, a negative relationship between brain size and gut size has been found (Tsuboi et al., 2015), but a positive correlation was found between brain size and reproductive investment in these cichlids: the bigger the brain, the longer the duration of parental care (Tsuboi et al., 2015). None of the expensive tissue or energy
trade-off research to my knowledge has incorporated the concept of population
differentiation as a potential factor driving trade-offs within a species. There may be
trade-offs between tissues or expensive behavioural outputs that can lead to a larger or
smaller brain but how might these trade-offs be differentially affected if we consider
differences between populations? Some work has suggested differences of body size
growth between studies of hatchery fish and their wild counterparts (Marchetti & Nevitt, 2003) but often only one wild population is involved, providing little opportunity for comparison between different wild habitats. Here, I present an investigation into energetic trade-offs between the offspring of seven populations (one hatchery and six wild outcross) of Chinook salmon. In addition, I present an examination into the size differences between brain hemispheres of Chinook to begin to assess the extent of the effect of different populations on a morphological measure of laterality.

Methods

Subjects

All brains used in the present study came from 3-year-old adult Chinook salmon from Yellow Island Aquaculture, Ltd. (YIAL; Quadra Island, British Columbia, Canada). The fish used were the offspring of artificial spawning between a YIAL stock dam (hermaphrodite cross), and milt from males of YIAL broodstock crosses (the ‘high’ (HH) and ‘low’ (LL) performance crosses) resulting in the ‘YIAL’ cross, as well as from males of seven different river locations around British Columbia: Robertson Creek, Big Qualicum, Quinsam, Chilliwack, Capilano, Puntledge, and Nitinat.
**Genetic analyses**

To further explore the differences between the single hatchery (YIAL) and seven outcross populations, estimates of percent heterozygosity were obtained using DNA extracted from fin clips. Fin clips were collected and preserved in August 2015 and November 2016 from the seven outbred populations and the YIAL population. DNA was extracted from fin clips using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer’s instructions. Individual genotypes were determined through polymerase chain reactions (PCR) at 9 previously described microsatellite loci, specifically Oneu3, Oneu8 (Scribner et al. 1996), Omm1135 (Rexroad et al. 2001), Omy325 (O’Connell et al. 1997), OtsG432, OtsG474 (Williamson et al. 2002), Ots1, Ots4 (Banks et al. 1999) and Ogo4 (Olsen et al., 1998). PCR conditions included: a 5-min denaturation step (94°C), followed by 30 cycles of a 20-s denaturation step (94°C), a 20-s annealing step (64.6°C – Omy325, Ots4; 63.5°C – Oneu8, Ogo4; 54.3°C – Oneu3; 58.3°C – Omm1135, Ots1; 60.2°C – OtsG474, OtsG432) and a 30-s extension step (72 °C), followed by a final extension step of 3 min. PCR products at all 9 loci were pooled by individual, cleaned by precipitation with isopropanol, resuspended in milliQ water and then individually barcoded. The barcoding PCR included: a 2-min denaturation step (94 °C), followed by 8 cycles of a 30-s denaturation step (94 °C), a 30-s annealing step (60 °C) and a 1-min extension step (72 °C), followed by a final extension of 5 min. The barcoded amplicons were then pooled and gel-extracted using a Qiaquick Gel Purification Kit (Qiagen).

The library was assessed and quantified using the High Sensitivity DNA Reagents Kit (Agilent) on a Bioanalyser 2100 (Agilent Technologies) and was then diluted to 60 pM for template preparation using the 400 bp Hi-Q View Kit (Life Technologies). Sequencing of the library was performed on the Ion Torrent Personal Genome Machine.
(Life Technologies) using a 318 v2™ chip (Life Technologies). Truncated and low-quality sequences were removed from the data and the remaining sequences were separated based on individual and microsatellite loci using Mothur (Schloss et al. 2009). Fragment sizes (alleles) were then identified using an R based bioinformatics framework developed to score microsatellites generated from next generation sequencing platforms (R Core Team 2017, Roy et al. 2017).

**Tissue Processing and Measurement**

During fish processing, the body weight of each fish was measured on a scale in grams and rounded to the nearest whole number (Marel M1100, Marel, Gardabaer, Iceland). The mass of the gastrointestinal tract (stomach and intestine) was collected (in grams) as well. These somatic measures, in addition to brain weight, were used for three calculations of the energy trade-off hypothesis: a brain-to-gut ratio, a brain-to-body ratio, and a gut-to-body ratio.

After sacrifice, a small section of the head containing the brain of each fish was removed and put into a 50 mL Falcon tube containing 25 to 30 mL of 10% buffered formalin for 8 to 24 hours. When all brains were brought back to the laboratory at the University of Windsor they were placed into 70% ethanol for further fixation. Using a Leica dissecting microscope (Leica L2 10445930) each brain was carefully extracted from the head section and was photographed (Q-imaging Q1 Cam Fast 1394) for measurement. Perimeter measurements were collected using Northern Eclipse imaging software (Empix Inc., [http://www.empix.com](http://www.empix.com)). The right and left hemispheres of four main brain regions of interest were measured: the olfactory bulbs, the telencephalon, the
Figure 4.1: Image of Chinook salmon brain indicating the major brain regions measured in the current study: the olfactory bulb (OB), the telencephalon (TC), the optic tectum (OT) and the cerebellum (CB). The line through the CB indicates where it was artificially divided to measure a right and left ‘hemisphere’.
optic tectum, and the cerebellum (Figure 4.1). Because the cerebellum is a single-lobed region I chose to artificially bisect it in the photos using the midline of the optic tectum lobes as an anchoring point for the bisection line (Wiper et al., 2017). Because the tectal ventricle and rhombencephalic ventricle within the brain provide an internal left-right divide, which reaches from the middle of the optic tectum lobes back through the cerebellum (Wullimann et al., 1996), this artificial division was deemed a sufficient proxy for bisection of the single-lobed brain region. After brains were dissected and photographed, brains were weighed for absolute [wet] mass (in grams) using a four-decimal digital scale (Sartorius Extend ED124S, Sartorius AG, Goettingen, Germany).

**Statistical Analysis**

*Heterozygosity*

Individuals that were genotyped at fewer than 6 loci were removed from subsequent analyses. As a result, all genetic analyses included 19-28 individuals for each of the eight populations (see Table 4.1). At all loci, significant deviations from Hardy-Weinberg Equilibrium (HWE) were tested using Genepop version 4.2 (Raymond & Rousset, 1995; Rousset, 2008). Significant linkage disequilibrium was also analyzed using Genepop version 4.2 (Raymond & Rousset, 1995; Rousset, 2008) with an adjusted alpha level of 0.006 (p = 0.05/9) given multiple pairwise comparisons among the 9 loci. The mean observed (H₀) and mean expected (Hₑ) heterozygosity across all loci were calculated using GenAlEx version 6.5 (Peakall & Smouse 2012). After confirming normal distributions in each of the populations and homogeneity of variance,
Table 4.1: Observed (Ho) and expected (He) heterozygosity, and number of individuals genotyped (N) for seven groups of Chinook salmon.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>Ho</th>
<th>He</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big Qualicum</td>
<td>27</td>
<td>0.636</td>
<td>0.624</td>
</tr>
<tr>
<td>Chilliwack</td>
<td>28</td>
<td>0.614</td>
<td>0.600</td>
</tr>
<tr>
<td>Nitinat</td>
<td>27</td>
<td>0.580</td>
<td>0.561</td>
</tr>
<tr>
<td>Puntledge</td>
<td>29</td>
<td>0.631</td>
<td>0.613</td>
</tr>
<tr>
<td>Quinsam</td>
<td>27</td>
<td>0.602</td>
<td>0.591</td>
</tr>
<tr>
<td>Robertson Creek</td>
<td>24</td>
<td>0.634</td>
<td>0.609</td>
</tr>
<tr>
<td>YIAL</td>
<td>22</td>
<td>0.539</td>
<td>0.566</td>
</tr>
</tbody>
</table>
heterozygosity estimates were then compared among the eight groups using a One-Way ANOVA in the statistical software, Statistica (StatSoft, Tulsa, OK).

**Measures of Morphology**

Three ratio values were applied in the analyses as a measure of energetic trade-offs. Specifically, brain-to-body, brain-to-gut, and gut-to-body ratios were calculated using the following formulas (respectively): brain mass (g) / body mass (g); brain mass (g) / gut mass (g); and, gut mass (g) / body mass (g). Because the absolute and trade-off measures were, overall, highly correlated, two separate MANOVAs were carried out, one for the effect of population on absolute variables and one for the effect of population on trade-off measures. It has been suggested that when dependent variables are highly correlated results from a MANOVA will be less reliable (French et al., 2008; Field, 2013). With running two separate MANOVAs, I used a Bonferroni corrected alpha value of $p = 0.025$ (0.05/2) to determine statistical significance.

To examine morphological laterality of the brain, perimeter measurements of the left and right hemispheres of the four brain regions of interest were collected from dorsal images of each brain (Figure 4.1). These values were used to calculate the ‘laterality index’ (LI), following the formula: $LI = [(L – R) / (L + R)] \times 100$ (Gutiérrez-Ibáñez et al., 2011), where ‘L’ indicates perimeter values for left hemisphere measurements and ‘R’ indicates perimeter values for right hemisphere measurements. The values for the laterality index provide information on the directional biases in growth or size of the region of interest. As a measure of the strength of the side bias irrespective of direction the absolute value of the laterality index was taken ($|LI|$) (Brown et al., 2007; Barnard et al., 2016). This absolute measure is used as it has been suggested that the absolute
measure may be more informative, accounting for individual variation which may be lost if only the directional measure is considered (Brown et al., 2007; Reddon & Hurd, 2008; Reddon et al., 2009). Finally, a one-way MANOVA was used to determine the differential effect of population on the trade-off measures of morphology, as well as on the laterality index and absolute laterality values. Tukey’s post-hoc analyses were used, where applicable, to further explore relationships between the populations.

Prior to all analyses, absolute, trade-off and brain morphology scores were standardized and assessed for extreme outliers, defined as any case with two or more scores that exceeded +/- 2.58 (Field, 2013). Sixteen cases in total were removed as extreme outliers. In addition, six cases were removed because they did not have an assigned population, and three cases were removed as they were the only members of the Capilano outcross, thus removing this population from the analysis (leaving six outcrosses in total). With these 25 cases removed from the dataset I had a sample size of 192 fish. Through tests of normality and visual inspection of the data, assumptions of normality were deemed to have been met and no further alteration to the dataset was carried out.

**Results**

*Heterozygosity*

None of the loci showed significant deviations from HWE in all eight groups and no pairs of loci showed significant linkage disequilibrium. Observed heterozygosity ranged from 53.9% to 63.6% but there were no significant differences between any of the eight populations (Table 4.1; p = 0.8137). Expected heterozygosity was also not significantly different between the groups (Table 4.1, p = 0.9883).
**Absolute Measures and Energy Trade-Offs**

Population significantly affected the absolute somatic measures (Wilk’s Lambda, \(\Lambda = 0.835, F_{18, 518.09} = 1.890, p = 0.015\)), however, separate univariate ANOVAs indicated that there was no significant difference among populations on brain mass (\(F_{6, 185} = 1.719, p = 0.119\)), body mass (\(F_{6, 185} = 1.918, p = 0.080\)), or gut mass (\(F_{6, 185} = 0.753, p = 0.608\)). There was also a significant effect of population on the trade-off measures (\(\Lambda = 0.795, F_{18, 515.26} = 2.412, p = 0.001\)). While there was no significant effect of population on the brain-to-gut-ratio (\(F_{6, 184} = 0.719, p = 0.635\)), population had a statistically significant effect on the brain-to-body mass ratio (\(F_{6, 184} = 2.813, p = 0.012\)). Tukey’s post-hoc analyses showed that these differences were most significant between Chilliwack, which showed the highest brain investment, and the YIAL (\(p = 0.003\)), Big Qualicum (\(p = 0.026\)), and Quinsam (\(p = 0.017\)) populations (Figure 4.2). Population also statistically significantly affected the gut-to-body ratio measure (\(F_{6, 184} = 3.702, p = 0.002\)). Through a Tukey’s post-hoc analysis, the Chilliwack population, having the highest investment into gut tissue, showed significant differences with both the YIAL (\(p = 0.001\)) and Roberston Creek (\(p = 0.021\)) populations, with a marginally non-significant difference between Chilliwack and Quinsam (\(p = 0.052\)) (Figure 4.3).

**Brain lateralization**

The MANOVA for laterality index and absolute laterality showed that there was no statistically significant effect of population on either of the laterality measures (\(\Lambda = 0.786, F_{48, 806.1} = 0.843, p = 0.767\)).
Figure 4.2: Mean brain-to-body mass ratio between crosses. Letters indicate significant differences and error bars represent +/- 1 SE.
**Figure 4.3:** Mean gut-to-body mass ratio between crosses. Letters indicate significant differences and error bars represent +/- 1 SE.
Discussion

Measurements of brain size in fish have been studied from the effect of environmental rearing conditions (e.g. Marchetti & Nevitt, 2003; Kihslinger & Nevitt, 2006; Kihslinger et al., 2006; Mayer et al., 2011), and reproductive tactic (e.g. Bass & Baker, 1990; Bass, 1992; Wiper et al., 2014), but only recently have investigations been concerned with how brain size might differ between fish of different genetic backgrounds under the same rearing conditions (e.g. Ishikawa et al., 1999). Herein I have examined fish from seven variant crosses to test the genetic effect on the expensive tissue hypothesis (Aiello & Wheeler, 1995), as well as what relationship there may be between genetic background and measured laterality.

Somatic Trade-Offs

Aiello and Wheeler (1995) were the first to suggest that there was an energetic trade-off between the expensive tissues of the brain and the gut which allowed for an overall larger brain size in primates, with the same trade-off later being found in fishes (Kaufman et al., 2003; Kotrschal et al., 2013). While my results did not show a significant trade-off of brain and gut size between populations, these tissues did show significant population differentiation when the body size of the fish was accounted for. For both the brain-to-body and gut-to-body ratios the differences were most pronounced for the Chilliwack population, which incidentally had the overall lowest mean body mass (mean = 1260.83g) of all seven crosses examined in this study. Thus, fish from the Chilliwack population appear to be showing compensatory growth through increased gut and brain size while sacrificing energy devoted to body size.
It has previously been suggested that an increase in gut size is inversely related to the food quality of an organism’s diet, especially when the diet consists mainly of vegetation (Aiello & Wheeler, 1995; Kaufman et al., 2003; Couture et al., 2016). Additionally, there is some evidence in grasshoppers (Yang & Joern, 1994) and tadpoles (Liess et al., 2015) that gut size can change in response to surrounding environmental factors like food quality and habitat temperature. In fact, gut lengths differ significantly between Arctic and Boreal populations of *Rana temporaria* tadpoles (Liess et al., 2015). The longer guts of Arctic tadpoles are proposed to be an adaptation to their higher latitude environment where consistent access to nutrient dense food cannot be guaranteed. An overall larger or longer gut may help to circumvent the problem of lower quality food by increasing the total amount of nutrient extraction from the food available (Yang & Joern, 1994), but this requires further direct testing, especially in fish (Stevens & Devlin, 2005).

Predation pressure may also play a role in body versus gut size. In *R. temporaria*, tadpoles from higher latitudes have been observed to have larger guts (Liess et al., 2015), which coincides with the finding that predation is often lower at higher latitudes where tadpoles show greater morphological growth of characteristics conducive to higher activity (i.e. tail depth; Laurila et al., 2008). With lower predation, tadpoles are able to be more active foragers and thus invest more energy from nutritional resources into growth of the gut (Lindgren & Laurila, 2005, Laurila et al., 2008; Liess et al., 2015). In the case of Chinook salmon, especially the Chilliwack outcross, body and gut size may be evolutionarily constrained by environmental conditions and food quality experienced by the paternal fish, who are from different wild river locations at different latitudes, and so may have experienced different selection pressures due to experiencing different environmental conditions with which to cope. Thus, even though all of the fish used here
were reared in a common environment, the Chilliwack population may still be
constrained by their selective history to increase their relative gut size even though in the
current experiment they had ample high-quality food. If such adaptations have been
ingrained in the natural parental population over many generations, one generation of
outcrossing with a hatchery stock may not necessarily be enough to reverse a potentially
heritable effect which has persisted in the F1 generation, however, a more controlled
breeding design and investigation of additive and non-additive genetic effects would be
required in future work to determine if this could be the case.

With an increase in brain size there could be an increase in connectivity between
neurons, an increase in processing efficiency and information gathering, and memory
storage, but may not make an overall qualitative difference to behaviour (Näslund et al.,
2012). A larger body size with greater muscle mass and speed is admittedly useful for
escape from predators, and better prey capture ability; however, these characteristics may
be more efficiently executed with a relatively larger brain in comparison to body size
since greater cognitive ability, not body size, may be what is truly required to escape
dangers and to find food (e.g. Brown et al., 2003; Kotrschal et al., 2015). As brain size
relative to body size has been shown to predict problem solving ability in mammals
(Benson-Amram et al., 2016) and has also been implicated in greater cognitive abilities in
at least one species of fish (guppies; Kotrschal et al., 2013) it would not be a stretch to
suggest that with an overall larger brain, a fish may be a better “problem solver” which
can translate to increased survival ability. While an enhanced brain-to-body ratio might
not be desirable for commercial aquaculture with an emphasis on high biomass, it may be
advantageous for conservation hatcheries that are rearing fish to augment dwindling
populations. Some evidence exists which suggests that brain size may be an inherited trait
and predict enhanced cognitive ability (Kotrschal et al., 2013); if so, aquaculture facilities could essentially “predict” brain size in offspring from that of parental fish, however, behaviour testing would be required to additionally ensure improved behavioural outcomes (e.g. appropriate response to predators) to ideally enhance overall survival. A major benefit in this regard is that, while released fish often die in the wild, the use of hatchery enrichments (e.g. Brown et al., 2003) may reverse this pattern, producing fish better able to survive due to increased brain capacity.

Laterality

Very little work has investigated the effect of genetic background of fishes on their behavioural or morphological laterality. The strongest example of genetic differences in the literature is through the work in golden topminnows (Girardinus falcatus) (Bisazza et al., 2000; Bisazza et al., 2007) where several generations of different ‘turning lines’ have been created based on the behavioural turning preferences of the parental fish. In this case it is likely that there is a genetic mechanism, as yet unknown, at work which helps to determine the brain morphology of a fish. If there are lateralized differences in morphology at the neuroanatomical level, these brain differences may have been conserved across generations (Katz & Harris-Warrick, 1999; Tierney, 1996) and may in turn be responsible for behavioural output. This work, however, aimed to look more closely at the potential effect of genetic background on the often-overlooked characteristic of morphological laterality. Through the estimates of heterozygosity, I noted that there was very little difference between the crosses in this regard, especially between the wild river outcross populations whose heterozygosity estimates ranged from around 58% to 64%. The biggest difference appeared to be with the YIAL domestic cross,
whose heterozygosity was lowest, at about 54% (Table 4.1). Overall, though, explorations on the genetic effects on laterality are little explored. Some evidence exists that there may be a genetic mechanism at work with respect to behavioural laterality (Bisazza et al., 2007), but no studies to my knowledge have begun to investigate the effect of genetic background on morphological laterality. The results presented here indicate that, in adult Chinook salmon, there was no significant difference between the crosses on measured laterality of brain hemispheres. Work in juveniles of some of the same genetic lines (chapter 3), however, has indicated some lateralized brain differences in these fish. Thus, morphological brain lateralization may differ with age, being present mainly in the earlier life stages and disappearing as a fish grows older. Alternatively, lateralized development of the brain may not only require a genetic propensity toward lateralization but also the presence of specific environmental factors which are important to the development and maintenance of laterality (Cowell & Denenberg, 2002). This latter hypothesis requires much more extensive study in fishes to uncover changes in lateralized brain growth during progressive life stages. Incorporating different genetic crosses and environmental features that have previously been shown to lead to the expression of laterality in fishes—such as enrichment of the environment (Bibost et al., 2013), light levels (Dadda & Bisazza, 2012; Sovrano et al., 2016), and actual (Brown et al., 2004) or perceived (Broder & Angeloni, 2014) predation levels—will be necessary if we wish to better understand the drivers to morphological lateralization.

Conclusions

Here I have shown that population differentiation exists between the populations studied when considering the expensive tissues of the brain and the gut mass compared,
respectively, to the body mass. Most studies on the expensive tissue hypothesis determine trade-offs at the species level (e.g. Tsuboi et al., 2015), sometimes determining differences between males and females (e.g. Kotrschal et al., 2013), but no other work, to my knowledge, has investigated expensive tissue trade-off differences between fish from lines of differing genetic backgrounds. From the results presented here I am able to suggest that genetically isolated populations may show differentiation with respect to their investment into the growth of expensive tissues. However, much more work will be necessary to better determine how genetic differences are driving these trade-offs, or if factors outside of the animal itself, such as differences in wild river habitats, are responsible. While not done here, future work should be carried out using controlled breeding designs to be able to suggest additive, non-additive and maternal effects on body, brain, and other somatic trade-offs. Furthermore, research should consider making comparisons between fish directly from the different environmental backgrounds used here. Environments of fish from which milt came are likely to differ on important factors, including complexity of environment, resource availability, temperature, or turbidity, all of which may affect growth and development. Testing fish from environments differing on such factors could more accurately reflect local adaptation between populations.

My laterality measures, on the other hand, indicated that in three-year-old Chinook salmon there were no notable differences between the left and right hemispheres of the telencephalon, optic tectum, or cerebellum brain regions, either on the directional or the strength measures of laterality. Other work in salmonids has indicated that changes to neuroanatomy can be observed in the early life stages, but that these changes fade over time (e.g. Kihslinger & Nevitt, 2006; Näslund et al., 2012). We may be seeing a similar occurrence with the study sample, wherein lateralized differences of the brain between
the genetic crosses may have been evident in the juvenile stage of life but dissipate over time. As outlined above, studies using controlled breeding designs to better estimate additive and non-additive effects would be useful in the measure of laterality, especially as pertains to measuring the potential maternal effect on laterality. One study thus far has found no such maternal effect (Bisazza et al., 2007), but more work must be carried out to further investigate this possible driver. Examing laterality in this way could help to provide more information on the overall pattern of inheritance, or heritability, of laterality.

Alternatively, differences may have been more pronounced if fish were reared in a more enriched habitat throughout their life cycle. Fish from wild habitats have been shown to have differences in neuroanatomy as compared to their hatchery conspecifics (e.g. Marchetti & Nevitt, 2003; Kihslinger et al., 2006; Wiper et al., 2014). In the case of the present chapter, if fish were reared in their respective habitats perhaps differences of brain morphology would persist through to adulthood because of a more cognitively stimulating environment requiring greater and more efficient processing ability, which has been touted as a hallmark of the benefits of a lateralized brain (Vallortigara, 2006).

Overall, future work will need to investigate further not only the environmental effects on lateralized brain morphology, but the potential genetic effects, and, perhaps most importantly, the genetic-by-environment interaction of a given genetic background and specific features of a fish’s habitat.
References


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CHAPTER 5: MIND OVER MATTER: DIFFERENTIAL INVESTMENT INTO BRAIN VS. BODY GROWTH IN CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) ACROSS LIFE STAGES

Introduction

The brain is a metabolically expensive organ to produce and maintain (Mink et al., 1981), so increases in brain size are often discussed in light of the accompanying costs or trade-offs to account for larger brains (Aiello & Wheeler, 1995; Isler & van Schaik, 2006a; 2009; Pitnick et al., 2006). An early explanation for large brain size in mammals, the expensive tissue hypothesis, suggested that larger brains occur because of compensatory decreases in the size of other expensive tissues or organs in the body, specifically the gut (Aiello & Wheeler, 1995). Testing of this hypothesis has begun to include other ‘expensive’ tissues and organs beyond the gut. For example, Pitnick et al. (2006) have demonstrated a relationship between brain size and testes size in several species of bats. Trade-offs between the brain and muscle tissue, like the pectoral muscle mass important for flight in birds (Isler & van Schaik, 2006b), has also been found, and some extensions of the expensive tissue hypothesis have begun to investigate the trade-off between brain size and expensive aspects other than tissues, including reproductive investment (Tsuboi et al., 2015).

Such explanations of large brain size, and its accompanying costs, have been put forth to best explain larger brains in homeothermic vertebrates; however, it has been suggested that for ectothermic organisms the overall brain maintenance costs will be higher (Liao et al., 2016). As such, fish are becoming more widely used for research on
energetic trade-offs. For example, Wiper et al. (2014) found that in Chinook salmon (*Oncorhynchus tshawytscha*) jacks—early maturing males with a significantly smaller adult body size (Gross, 1985; 1991)—had a significantly higher brain mass than hooknoses, the later-maturing, larger males (Gross, 1985; 1991), when body size was accounted for. Similarly, precocial, early maturing brown trout (*Salmo trutta*) that do not migrate during their life cycle had larger brains (when body size was accounted for) than their anadromous conspecifics (Kolm et al., 2009), indicating a trade-off of brain size and migration behaviour. Between males and females, Kotrschal et al., (2013) found that larger brained guppies (*Poecilia reticulata*), especially males, developed smaller guts and produced fewer offspring. A larger brain also has an effect on predator avoidance in guppies, where possessing a larger brain confers survival benefits to females but makes no difference to the survival of males (Kotrschal et al., 2015). In this case, there is no negative trade-off between predator evasion and a large brain but instead a positive relationship. Thus, a large brain may not always incur costs for some behavioural aspects of life history, but may instead be of benefit. Where the costs or true trade-offs seem to lie, at least with studies of fish, is in the differential investment into growth of the brain as compared to other expensive tissues or the body as a whole.

One area of effect that remains to be rigorously studied is the effect of genetic background on investment into brain growth and what costs may be incurred or what relationships, positive or negative, exist. The work in guppies (e.g. Kotrschal et al., 2013; Kotrschal et al. 2016) has begun to explore this area, having artificially selected large- and small-brained lines of fish (Kotrschal et al., 2013), showing that features received from particular parental crosses may affect specific trade-offs in the offspring. In addition, the guppy work has demonstrated that brain size is heritable in fish (Kotrschal et
al., 2013), but more work is needed to better understand how genetic background affects brain investment and trade-offs. We also need to begin to determine how brain features or differential investment may change over time. Again, the work focusing on guppies and brain size has begun to look into this area, with breeding several generations of artificially selected fish (Kotrschal et al., 2013), but there has been little specific mention of brain trade-offs in different life stages. Here, I used artificially spawned crosses of Chinook salmon from eight different genetic backgrounds from three different years (2014, 2015, and 2016) to assess whether there are specific differences between the genetic crosses on growth of body and brain tissues, and whether there is a consistent pattern over time within and between crosses on absolute brain and body measures as well as on the brain-to-body trade-off.

**Methods**

**Subjects**

All Chinook salmon used for the present study came from Yellow Island Aquaculture, Ltd. (YIAL), an organic salmon farm and hatchery located on Quadra Island, British Columbia, Canada. Seven crosses were artificially spawned at YIAL in 2013 and the subsequent offspring raised to maturation at YIAL. The offspring were the result of the breeding of hatchery dams from a self-crossed hermaphrodite line (Komsa, 2012) and milt from males of the seven populations being examined. Milt from males of six wild river locations were used creating six wild outcross populations, as well as milt from YIAL broodstock males, resulting in a domestic stock. The six river locations from whence the males originated, resulting in corresponding outcrosses, were: the Robertson
Creek (RC), Big Qualicum (BQ), Quinsam (Q), Puntledge (Punt), Chilliwack (Chill), and Nitinat (Nit) rivers.

Somatic Measurements

In June 2014, all YIAL cross fish and 10 fish from each of the RC, Nit and Q crosses were sacrificed on site and placed into a 50mL Falcon tube containing 30 mL of 10% buffered formalin for 48 hours. The formalin was removed and the fish were transported to the laboratory at the University of Windsor where they were placed in 25mL of 70% ethanol. Body mass of all fish was weighed using a standard scale (Ohaus Scout Pro SP202, Ohaus Corporation, New Jersey, USA) before brain extraction. For the remaining crosses in 2014, BQ, Punt, and Chill, and 12 fish each from RC, Nit and Q, fish were sacrificed and weighed on site (Ohaus Adventurer Pro Model AV4101) in grams to two decimal places. After weighing, fish were placed into 50 mL Falcon tubes containing RNALater (Thermofisher Scientific Inc., Mississauga) and stored at -20 °C. Prior to brain removal, the RNALater stored samples were thawed overnight. Once the brain was removed from the fish and photographed it was placed into a falcon tube containing 70% ethanol.

In May of 2015, were anesthetized and the weight of each fish (g) from that year was taken on site using a scale (Marel M1100, Marel, Gardabaer, Iceland). In June 2015, fish were sacrificed and small pieces of the skull containing the brain were preserved in RNALater (Thermofisher Scientific Inc., Mississauga) and stored at -20 °C prior to brain removal. Following brain extraction and photographing, the brains were placed in 70% ethanol. In the following year (2016), fish were euthanized and weighed on site, via a scale (Marel M1100, Marel, Gardabaer, Iceland), to the nearest two decimal places.
Sections of the head containing the brains were removed and placed in 10% buffered formalin before being brought back to the laboratory at the University of Windsor and being placed in 70% ethanol for further fixation. Brains from all three years were weighed on a four-decimal point scale (Sartorius Extend ED124S, Sartorius AG, Goettingen, Germany) for greatest precision. It should be noted here for clarity: the fish sacrificed in 2014 are the 1-year old fish, those sacrificed in 2015 are 2-year old fish, and those sacrificed in 2016 are the 3-year old fish.

*Statistical Analyses*

To ensure that the tissues of interest (brain and body) were appropriately comparable I needed to adjust the mass values of the tissue that had not been preserved in a fixative. The majority of comparative shrinkage studies that exist are most concerned with body length shrinkage in different preservatives (Fowler & Smith, 1983; Hjorleifsson & Klein-MacPhee, 1992; Buchheister & Wilson, 2005), but at least one estimate of body *mass* shrinkage in two fish species showed that, when preserved in 80% ethanol, there is an approximate 30-35% decrease in body mass in the first 50 days of preservation (Kristoffersen & Vea Salvanes, 1998). Brain tissue in this study was fixed in 70% ethanol and for no more than 20 to 25 days. Using the estimates from Kristoffersen and Vea Salvanes’s (1998) work as a template I adjusted the ‘fresh’ body weights so that they were akin to the fixed brain weights. Therefore, all fish in the study whose body weights were taken immediately after sacrifice had body mass decreased by 10%, referred to hereafter as the ‘absolute body mass’ measure. Following body mass adjustments, a brain-to-body ratio was calculated using the following formula: absolute brain mass (g) / absolute body mass (g) (Wiper et al., 2014). This ratio was used as a method for assessing
the energy investment that fish put in to their brain tissue while controlling for overall body mass.

Body mass, brain mass, and the brain-to-body ratio values were highly correlated, thus between year differences on each dependent variable were run in a separate ANOVA. I chose to avoid the use of a single MANOVA because it has been suggested that highly correlated dependent variables can reduce the reliability of results (French et al., 2008; Field, 2013). With running three separate ANOVAs, I used an adjusted alpha-value of \( p = 0.017 \) (0.05/3). Seven cases were removed as outliers prior to analysis (having Z-score values greater than +/- 2.58).

**Results**

Age had a statistically significant effect on absolute body mass (\( F_{2,420} = 1704.95, p < 0.001 \)) (Figure 5.1A), where, as expected, mean body mass increased each year with increasing age, with the greatest increase in body mass occurring between 2015 (age 2) and 2016 (age 3) (Table 5.1). A Tukey’s post-hoc analysis indicated that all three ages differed significantly from each other (all \( p < 0.001 \)). Brain mass was also significantly affected by age (\( F_{2,418} = 4859.94, p < 0.001 \)) (Figure 5.1B), with all three years differing significantly from one another (Tukey’s, all \( p < 0.001 \)). The greatest mean value of absolute brain mass was in the 3-year old fish from 2016, which aligns with the greatest absolute body mass. Finally, age also significantly affected the brain-to-body ratio (\( F_{2,418} = 1051.98, p < 0.001 \)) (Figure 5.2); however, with body size being taken into account, it was the 1-year old fish from 2014 which showed the greatest investment in brain size as compared to the 2- or 3-year old fish (Table 5.1), but a Tukey’s post-hoc showed that all
ages were significantly different from one another (all $p < 0.001$) on the brain-to-body ratio measure.

Figure 5.1: Mean absolute body mass (A) and mean absolute brain mass (B) in grams between 2014, 2015 and 2016. Error bars represent +/- 1 SE.
Table 5.1: Mean (M), standard error (SE) and confidence intervals (95% CI) for the effect of year (2014, 2015 and 2016) on brain mass, body mass and the brain-to-body ratio.

<table>
<thead>
<tr>
<th>Morphology Measure</th>
<th>Year</th>
<th>M</th>
<th>SE</th>
<th>95% CI (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain Mass</td>
<td>2014</td>
<td>0.0339</td>
<td>0.00121</td>
<td>0.0315, 0.0363</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>0.2924</td>
<td>0.0044</td>
<td>0.2837, 0.3011</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>0.8542</td>
<td>0.0098</td>
<td>0.8349, 0.8736</td>
</tr>
<tr>
<td>Body Mass</td>
<td>2014</td>
<td>2.8683</td>
<td>0.0827</td>
<td>2.7048, 3.0318</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>165.339</td>
<td>4.3760</td>
<td>156.6910, 173.9888</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>1246.5346</td>
<td>29.7041</td>
<td>1187.7643, 1305.3049</td>
</tr>
<tr>
<td>Brain-to-Body Ratio</td>
<td>2014</td>
<td>0.01206</td>
<td>0.000304</td>
<td>0.01145, 0.01266</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>0.00195</td>
<td>0.000057</td>
<td>0.00183, 0.00206</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>0.00075</td>
<td>0.000025</td>
<td>0.000701, 0.000801</td>
</tr>
</tbody>
</table>
Figure 5.2: Mean brain-to-body ratio between three years of 2014, 2015 and 2016. Fish in their earliest year of life invest the most into their brain when body size is taken into account. Error bars represent +/- 1 SE.
To further explore the significant between-age effects outlined above, I examined each year, accounting for each age group, separately to determine cross effects within each year. Again, all dependent variables were run separately, thus the adjusted alpha value cut-off of \( p = 0.017 \) was used for determination of significance. In 2014 there was a significant effect of genetic cross on absolute body mass (\( F_{6, 139} = 15.3, \ p < 0.001 \)) (Figure 5.3A), where only the YIAL cross differed most significantly from the six outcrosses (Tukey’s, all \( p < 0.001 \); see Table 5.2 for mean values). The absolute brain mass was also significantly affected by cross in 2014 (\( F_{6, 137} = 33.71, \ p < 0.001 \)). Post-hoc tests revealed that the YIAL cross differed from all other crosses (all \( p < 0.001 \)); Q differed significantly from BQ, Chill, and Punt (all \( p < 0.009 \)); and RC differed from BQ (0.004) (Figure 5.3B). The brain-to-body ratio showed significant differences between genetic crosses within the 2014 year (\( F_{6, 138} = 3.74, \ p = 0.002 \)). The most significant differences were between the BQ cross, who showed the overall lowest relative brain investment, and Q (\( p = 0.006 \), the cross with the highest brain investment, and marginally from the YIAL cross (\( p = 0.015 \)), which showed the second highest brain investment (Figure 5.4; see Table 5.2 for all mean values).

In the 2015 year there was no effect of cross on absolute body mass (\( F_{6, 140} = 1.09, \ p = 0.373 \)), absolute brain mass (\( F_{6, 140} = 2.53, \ p = 0.024 \)), or on the brain-to-body ratio (\( F_{6, 139} = 1.02, \ p = 0.418 \)). In 2016, no significant differences between crosses were seen on body mass (\( F_{6, 123} = 1.62, \ p = 0.146 \)), brain mass (\( F_{6, 123} = 1.97, \ p = 0.075 \)), or on the brain-to-body measure (\( F_{6, 123} = 2.18, \ p = 0.049 \)).
Within this year was the only instance of between cross differences. Error bars represent +/- 1 SE.
Table 5.2: Mean (M), standard error (SE) and confidence intervals (95% CI) for the effect of cross on brain mass, body mass, and the brain-to-body ratio for Chinook from the 2014 year cohort.

<table>
<thead>
<tr>
<th>Morphology Measure</th>
<th>Cross</th>
<th>M</th>
<th>SE</th>
<th>95% CI (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain Mass</td>
<td>YIAL</td>
<td>0.05772</td>
<td>0.00164</td>
<td>0.054313, 0.061123</td>
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<tr>
<td></td>
<td>BQ</td>
<td>0.02324</td>
<td>0.00085</td>
<td>0.02145, 0.025020</td>
</tr>
<tr>
<td></td>
<td>Chill</td>
<td>0.02579</td>
<td>0.00077</td>
<td>0.024186, 0.027396</td>
</tr>
<tr>
<td></td>
<td>Nit</td>
<td>0.3317</td>
<td>0.00367</td>
<td>0.25495, 0.40845</td>
</tr>
<tr>
<td></td>
<td>Punt</td>
<td>0.02555</td>
<td>0.00076</td>
<td>0.023958, 0.027133</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>0.03609</td>
<td>0.00243</td>
<td>0.030997, 0.041173</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>0.03495</td>
<td>0.00296</td>
<td>0.028699, 0.041201</td>
</tr>
<tr>
<td>Body Mass</td>
<td>YIAL</td>
<td>4.2827</td>
<td>0.12329</td>
<td>4.0263, 4.5391</td>
</tr>
<tr>
<td></td>
<td>BQ</td>
<td>2.5965</td>
<td>0.18975</td>
<td>2.1993, 2.9937</td>
</tr>
<tr>
<td></td>
<td>Chill</td>
<td>2.2295</td>
<td>0.12815</td>
<td>1.9630, 2.4960</td>
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<tr>
<td></td>
<td>Nit</td>
<td>2.6733</td>
<td>0.23717</td>
<td>2.1769, 3.1697</td>
</tr>
<tr>
<td></td>
<td>Punt</td>
<td>2.5417</td>
<td>0.13585</td>
<td>2.2592, 2.8242</td>
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<tr>
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<td>Q</td>
<td>2.7922</td>
<td>0.17435</td>
<td>2.4272, 3.1571</td>
</tr>
<tr>
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<td>RC</td>
<td>2.9228</td>
<td>0.20902</td>
<td>2.4818, 3.3638</td>
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<td>Brain-to-Body Ratio</td>
<td>YIAL</td>
<td>0.01309</td>
<td>0.00072</td>
<td>0.011594, 0.014580</td>
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<tr>
<td></td>
<td>BQ</td>
<td>0.00985</td>
<td>0.00073</td>
<td>0.008310, 0.011382</td>
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<tr>
<td></td>
<td>Chill</td>
<td>0.01224</td>
<td>0.00062</td>
<td>0.010947, 0.013523</td>
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<td></td>
<td>Nit</td>
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<td>0.00109</td>
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<tr>
<td></td>
<td>Punt</td>
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<td>0.009489, 0.011561</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>0.01350</td>
<td>0.00088</td>
<td>0.011665, 0.015341</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>0.01217</td>
<td>0.00081</td>
<td>0.010460, 0.013881</td>
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</tbody>
</table>
Figure 5.4: Mean brain-to-body ratio between crosses within the 2014 year. Error bars represent +/- 1 SE.
**Discussion**

Differential patterns of selection on brain size growth and evolution have been found in several vertebrate species (Smaers et al., 2012), with specific differences between investment into brain growth in comparison to expensive tissues being uncovered, such as the comparisons of brain and testes mass in bats (Pictnick et al., 2006) and other mammals (Lemaitre et al., 2009), and of the brain and gut size comparisons in fish (Kotrschal et al., 2013; Tsuboi et al., 2015). In the present chapter, I show that the measures of absolute brain and body mass increase predictably with age between the years of 2014 and 2016. Body mass differences in 1-year old fish in 2014 were driven largely by the domestic hatchery stock. Hatchery fish do not experience the same conditions as wild conspecifics (Marchetti & Nevitt, 2003; Kihslinger et al., 2006), who must contend with other fish species for resources, may experience scarcity of resources, and who have to work to avoid predators; thus, fish from purely domestic stocks are more likely, on average, to show a larger body size (e.g. Kihslinger et al., 2006; Mayer et al., 2011). Absolute brain mass between years (i.e. across ages) showed the same pattern as absolute body mass: that is, brains were the heaviest in the 3-year old fish 2016. This is not an altogether unexpected result; as a fish grows, so too do its internal organs, including the brain. In addition, larger bodies are more likely to need a larger brain to control bodily movements and internal physiological functioning (Striedter 2005; Gonda et al. 2011).

When I considered the ratio measure used to help determine the energy invested into brain vs. body growth, the previously seen patterns were reversed. When fish had the lowest *absolute* measures, at age 1 in 2014, is when they showed the greatest investment into brain over body growth. Therefore, for the present sample, Chinook salmon are
putting much more energy into brain growth early in life and it is later in life, at least after their first year, that body mass appears to become more important. A possible explanation for the differentiation of brain investment over body with increasing age, then, may have to do with the importance of life history factors at different life stages for Chinook salmon. Conceivably, within the first year of life there may be an information acquisition period where the majority of the necessary “cognitive work” is done: in this time fish will explore and learn how to respond to the biotic and abiotic factors of their environment, importantly, learning how to obtain food, avoid predators, and respond to conspecifics. In addition, during the first year of life fish may be too small to outgrow their predators, making cognitive behavioural decisions potentially more important to survival (e.g. Kotrschal et al., 2015). Previous work has demonstrated that in steelhead (Oncorhynchus mykiss) (Kihslinger & Nevitt, 2006) and Atlantic salmon (Salmo salar) (Näslund et al., 2012) early environmental enrichment significantly increased brain size measures, but these changes disappeared with age. However, in Chinook salmon the effects of early environmental enrichment, leading to increases in brain size, appeared to persist into later life stages (Wiper et al., 2014). Thus, there may be species specific differences in how long the effects of environmental enrichment last, but more work will need to be carried out, and on several populations within a species, to determine why there are changes in some fish species but not others.

My results show investment in brain size in the first year but that body size becomes more important in subsequent years. The significant population effects in brain investment also dissipated after the first year, again supporting the overriding importance of investment into somatic growth in adult Chinook. The lack of differences between crosses in the two latter years of the study may also be explained by the environmental
conditions within which fish were reared. Namely, the fish in this study were all reared under identical environmental conditions in a common garden approach, yet half of the genetic material for the offspring used here came from males who were reared in wild river habitats, which likely differed on important features, including complexity of the overall environment, predation pressures, and resource availability. Thus, the offspring may be expressing some genetically inherited characteristic reflecting specific environmental differences which developed in their paternal forebears over several generations. If fish were tested over three year timeframe used here but were reared in their natural habitats, and especially with both parents being of wild stock, I would expect that greater population differentiation would be observed on the measures of absolute body mass and, more likely, the brain-to-body trade-off measure indicating differential investment into tissues.

My examination of the expensive tissue hypothesis in Chinook salmon over three years has indicated that while differences in brain versus body growth are evident in early life, these differences do not appear to carry-over into adulthood, at least in this species. This pattern is likely typical of those fish who spend their lives in a hatchery setting, regardless of genetic differences, but genetic background and how it affects the growth of the brain versus the body requires more study. While most work on brain size differences of fish is carried out between hatchery and wild fish (Marchetti & Nevitt, 2003; Kihslinger & Nevitt, 2006; Kihslinger et al., 2006; Burns et al., 2009; Wiper et al., 2014) little attention has been given to what genetic differences there may be and how these may be driving brain and body size differences between groups. Some work on the medaka (Oryzias latipes), however, has provided evidence of “strain-specific morphology” of brain differences between different populations (Ishikawa et al., 1999).
And at least one study has compared differences between geographically and genetically isolated populations of nine-spined sticklebacks (*Pungitius pungitius*) finding that relative brain size as a whole did differ between these genetically distinct populations (Gonda et al., 2009). In the Chinook salmon populations tested here, milt from wild male salmon was used to fertilize the eggs of a hatchery female, thus, the potential for the occurrence of either hybrid vigor or outbreeding depression, neither of which were assessed here, could be considered. There may be a potentially beneficial or detrimental effect on the growth and development of the offspring produced, or on their investment into expensive tissues. However, specific breeding designs would need to be employed to best assess the potential influence of hybrid vigor or outbreeding depression.

The results presented here lend support to the expensive tissue hypothesis, but this support seems to be relegated to the early life stages in this sample of Chinook salmon. No other work, to my knowledge, compares the investment into the brain versus the body mass with increasing age, however, examinations of these differences can be of great importance for those hatcheries which rear fish for conservation efforts, supporting dwindling populations in the wild. In cases of the conservation of at-risk populations, understanding differential brain investment of different strains of hatchery-reared fish may hold a key for the greater success of restocking programs. If hatcheries notice that particular crosses show gains in brain vs. body investment early in life, steps should be taken to explore what factors may assist in the maintenance of greater brain investment, as it has previously been suggested that an increase in brain size, as compared to other expensive tissues, is related to an increase in cognitive ability (Kotrschal et al., 2013). Hatchery-reared fish often exhibit high mortality upon release for reintroduction, largely due to the inability to successfully avoid predators (e.g. Dellefors & Johnsson, 1995;
Álvarez & Nicieza, 2003; Hawkins et al., 2007). Improving management practices to emphasize brain growth — with its accompanying increases in cognitive capacities (Kotrschal et al., 2013; 2015) — over simply large size may lead to vastly improved predator avoidance capabilities and improved outcomes for conservation release.
References


CHAPTER 6: CONCLUSIONS AND FUTURE WORK

In the preceding four chapters I have endeavoured to explore two main research areas: expensive tissue trade-offs and laterality in a teleost fish, the Chinook salmon (*Oncorhynchus tshawytscha*), which has been little studied in either regard. While fish are an ideal model for many types of research due to their high species diversity (Nelson, 2006) and the ease with which they can be studied (Cossins & Crawford, 2005), salmonids have proven most useful in investigations of inbreeding effects (e.g. Rye & Mao, 1998), swimming performance (e.g. Falica & Higgs, 2013), fitness (e.g. Heath et al., 2002) and brain size (e.g. Kihslnger & Nevitt, 2006; Kihslnger et al., 2006; Marchetti & Nevitt, 2003), among countless other areas. Yet little to no work to date exists presenting information on the differential investment into expensive tissues, or the behavioural and/or morphological laterality of any salmonid species. Chinook salmon were a prime study species for the current work as they provided an opportunity for me to investigate aspects of laterality, as well as differential tissue investment, at different life stages over their relatively short lifespan (i.e. 3 to 4 years). What is particularly important about the salmon from Yellow Island Aquaculture is that they are from specific, artificially selected genetic backgrounds, allowing me to explore the areas of expensive tissue investment and laterality from a point of view that is little considered.

Many drivers to tissue investment and laterality (behavioural and morphological) have yet to be explored thoroughly. Herein, I define a ‘driver’ as an environmental or genetic mechanism experienced by an organism which may be said to affect the development of a given characteristic. For example, environmental drivers have been shown to affect the phenotype of *Phylloscopus* warblers, dependent upon bright or dark
light conditions within their environment (Marchetti, 1993), and genetic mechanisms have shown effects on brain morphology in five different genetic strains of the medaka (*Oryzias latipes*; Ishikawa et al., 1999).

For the previously discussed research, I investigated the driver of genetic variation, or cross/population differences, and how this affected differential tissue investment, as well as behavioural and morphological laterality. With respect to morphological laterality of brain hemispheres in juvenile and adult fish of differing genetic backgrounds, it was found that, in general, there were significant differences among crosses, especially for the visual (OT) and motor (CB) brain regions, where most often those crosses considered to have more genetic variation showed greater side differences of hemispheres. Behaviourally, tests of C-start (startle) and mirror inspection behaviour indicated that there was no effect of cross on first turn direction upon startle, and there was a mixed effect of cross on eye use preference for inspection: cross affected the strength of asymmetrical eye use but not the specific eye used. In terms of differential tissue investment it can be concluded from the present work that the outcrossed groups tended to show greater investment into expensive tissues (i.e. brain and gut) than the domestic, hatchery crosses. In addition to genetic background differences, chapter 3 investigated an environmental driver through the manipulation of water flow direction in the rearing barrels of juvenile fish. This additional driver allowed me to explore the suggested but largely untested interaction effect of the genetic background and the environment (GxE) on morphological and behavioural laterality. The GxE driver showed the most significant outcome for the inspection behaviour test, affecting both eye use preference and strength of asymmetry irrespective of direction.
Overall these findings can hold importance for two main areas—rear and release hatcheries and our evolutionary understanding—and can also provide a firm starting ground for future investigations in the laterality and tissue trade-off literature. Aquaculture facilities rearing fish to release them in an attempt to support dwindling populations may be interested in factors that lead to increased brain size, as increased brain size has been shown to lead to better cognitive ability in some fish (Kotrschal et al., 2013; Dadda et al., 2015). The assumption in this case would be that having a higher cognitive ability may serve a fish well when faced with predators and prey; they may be better able to respond to these factors and would be more likely to experience greater survival success. Laterality, too, especially behavioural, may be particularly crucial for such aquaculture facilities to consider. Studies have demonstrated that organisms show enhanced responses to predators, prey and conspecifics when they are lateralized (Deng & Rogers, 1997; Vallortigara et al., 1998; Lippolis et al. 2002; Rogers et al., 2004; Lippolis et al., 2005; Templeton et al., 2012). Inspection studies have brought to light typical patterns of eye use in many species with regards to specific ecologically relevant stimuli (i.e. predators, prey, and conspecifics) and this lateralization of eye use has been observed in fishes (e.g. Facchin et al., 1999; Sovrano et al., 1999; De Santi et al., 2001; Sovrano, 2004; Sovrano & Andrew, 2006). Having lateralization of eye use very likely reflects differential processing of stimuli between the two hemispheres of the brain, as studies on differential eye use have suggested (e.g. Templeton et al, 2012). With distinction of hemisphere processing of varying stimuli we can suppose that it would be of benefit for hatchery fish to exhibit similar lateralized differences when viewing predators or prey. Thus it may be crucial to determine the responses of hatchery fish to predators, prey and conspecifics prior to release into the wild. Ensuring that there are
consistent eye use preferences, which will allow for more efficient responses, could potentially result in an increase in survival of fish reared in a hatchery and released in the wild. However, a greater, more detailed understanding of the drivers which promote brain and behavioural laterality would be required for an overall improvement to survival of released fish. This more detailed appreciation of drivers could be achieved through particular aspects of future research, discussed below.

With respect to our evolutionary understanding of both topics, further explorations of brain growth and its trade-off(s) with other tissues or metabolically expensive characteristics (e.g. reproductive investment) may provide a better understanding of the importance of specific tissues (or life history characteristics) to a given species group, which would provide improved knowledge on their evolutionary development. It is important, too, to continue the study of behavioural and morphological laterality in fish and to consider as many potential drivers to the development and maintenance of lateralization as we can, so that we can better understand the characteristic of laterality as a whole. In gaining a better understanding of what drives laterality in fish, one of the earliest vertebrate species, and how it is maintained, we will end up learning more about this characteristic and how it has stood the test of time and been preserved as an important characteristic throughout the evolutionary history of vertebrates.

**Future Work**

While future work will need to continue the exploration of potential drivers to tissue trade-offs and laterality there are some specific avenues down which both areas should be directed to better expand the research and move forward in a meaningful way. In the realm of expensive tissue trade-offs, what constitutes an ‘expensive’ tissue very
likely has a species-specific element to it, however, certain environmental characteristics (e.g. complexity; predation pressure) may show similar effects on brain development across species. In terms of brain development, I believe we need to push for investigations looking not only at which tissue(s) the brain may trade-off with, but we need to begin to look at what is happening with the brain itself. That is, investigating the occurrence of neural proliferation, either via increased cell numbers or increased synaptic connections. As previously stated, increased brain size has been equated in the fish literature with increased cognitive ability (Kotrschal et al., 2013; Dadda et al., 2015) but incorporating neural proliferation would allow researchers to investigate what is happening in terms of cell number in brain regions crucial to a given cognitive task. In addition, we can begin to explore gene expression for growth and development of brain tissue compared to that of other tissues with which the brain may be said to “trade-off” to examine whether, in some organisms, specific development of one tissue type as compared to another is upregulated. I would also suggest that gene expression explorations focus on the different regions of the brain to determine if brain region growth is differentially affected.

Following from the idea of investigating neural proliferation in brain regions as a whole, to gain a better understanding of how differential processing may work in the brain, researchers should also begin to explore how neural proliferation might differ between hemispheres. In determining which hemisphere, if either, shows a greater number of neurons we may then be led to greater knowledge as to why some organisms are more highly lateralized than others, especially if there is greater proliferation of the visual regions of the brain, since behavioural laterality is largely visually controlled. To that end, investigations of feature detectors (i.e. ganglion cells) in each retina may hold
information that has been suggested about the viewing of specific stimuli in one eye over
the other but that has not been thoroughly explored. Research on toads and some birds,
for example, has led to conclusions that organisms more readily respond to a prey item
when it is in the right visual field (e.g. Vallortigara et al., 1998) but little explanation, has
been given as to why there is a stronger response overall except to suggest that the
contralateral hemisphere is responsible for processing of a given stimulus. Explorations of
feature detectors in each eye and the strength of synaptic responses with regard to signals
from the feature detector may help to better explain, at a more ‘neural’ level, why
responses to one stimulus are more readily responded to when in one visual field over the
other. At the very least we would be able to see if visual lateralities are driven by
differences in neural activation. Research can also begin to examine gene expression
between hemispheres. No work, to my knowledge, exists on such differences in fish, but
we may see that expression is upregulated in one hemisphere over the other, leading to
greater reliance by an organism on the “upregulated” hemisphere in terms of processing
or behavioural response.

Future work should also move more toward further investigating the effects of
hormones and neurotransmitters on lateralized brain development. There is some
suggestion in the literature that the neurotransmitter dopamine and the hormone
testosterone are related to behavioural lateralization in some species. Many hypotheses
surrounding the effect of testosterone on brain organization and behaviour have been
suggested in humans and other mammals (see Pfannkuche et al., 2009 for review), but in
at least one species of fish, *Aequidens rivulatus*, testosterone has shown a significant
behavioural effect. Prenatal testosterone was found to affect the direction of eye use
preference in female but not male fish, the opposite to the preference used by control
females (Schaafsma & Groothuis, 2012). Therefore, testosterone appears to have reversed the typical pattern of eye use for predator inspection. With respect to dopamine, striatal dopamine asymmetry in the brain of adult rats has been shown to be related to tail posture during infancy (e.g. Rosen et al., 1984). Rotational behaviour has also been associated with dopamine, such that increased dopamine is measured in the hemisphere contralateral to the rotational direction (e.g. Robinson et al., 1980). Thus dopamine does show an effect on potentially important behavioural lateralization but the work in this area must be updated and applied to many more species before we can say we truly comprehend how the relationship works. Further work should continue examining the extent of the effects of the neurotransmitters and hormones known thus far, but should be expanded. How might estrogen affect laterality in comparison to testosterone? How do other excitatory neurotransmitters compare to inhibitory ones with respect to their effect on brain and behavioural lateralization? Questions such as these may lead to potentially crucial, but certainly interesting, results with respect to how neurotransmitters and hormones may affect brain laterality.

Finally, brain trade-off and laterality research, especially, would benefit from more investigation into environmental effects and potential gene-by-environment (GxE) interactions. Resource availability has shown effects on expensive tissue (e.g. gut size) in tadpoles (Liess et al., 2015) and grasshoppers (Yang & Joern, 1994), and variation in rearing environment (i.e. hatchery vs. wild; marine vs. pond) has resulted in differences in overall brain size in several fish species, including Atlantic cod (Gadus morhua; Mayer et al., 2011), guppies (Poecilia reticulata; Burns et al., 2009), and nine-spined sticklebacks (Pungitius pungitius; Gonda et al., 2009). Environment has also shown effects on behavioural laterality in goldbelly topminnows (Dadda & Bisazza, 2012), zebrafish
(Sovrano et al., 2016) and chickens (Deng & Rogers, 1997; Rogers et al., 2004) who were exposed to light during incubation, rainbowfish (*Melanotaenia duboulayi*; Bibost et al., 2013) who were reared in enriched or impoverished environments, and even guppies who were reared with or without olfactory predator cues (Broder & Angeloni, 2014). It is clear that environment is quite well studied with respect to its effects on tissue investment and behavioural laterality but these studies generally do not take into account a genetic component that may be a crucial factor in the outcomes observed. The genetic background of an organism is intricately connected with the environment within which it lives and functions, thus both drivers should be considered in some way to provide the strongest description of brain trade-offs and expressed laterality.

**Bringing It All Together**

Expensive tissue trade-offs and morphological and behavioural laterality are generally not explored together but future work should consider merging these two topic areas to glean more detailed information about organisms under study, specifically when considering investment into brain growth and size. It may be of benefit to look at the brain as a whole, considering trade-offs with other ‘expensive’ tissues or aspects, then look at it in more detail by considering lateralization of hemispheres. This could potentially reflect differences in gene expression or neural proliferation differences between hemispheres, demonstrating greater investment into one side over the other—a form of differential investment ‘within’ itself, perhaps. We can then also compare brain size as a whole *and* the differential hemispheric investment to metabolically expensive behavioural characteristics, which could, for example, include courtship (Ventolini et al., 2005) or escape behaviours (Dadda et al., 2010), that have been related to lateralization in
some regard. No work has investigated lateralization in conjunction with brain investment but future work may be able to uncover interesting or unique relationships that are currently unknown. Perhaps we would be better able to answer questions of why laterality developed and learn more about its evolutionary path in concert with the evolution of brain size.
References


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