Life histories and brain evolution of sharks, rays, and chimaeras

by

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Abstract

The brain is perhaps one of the most fundamental organs in all vertebrates. It determines not only an individual's ability to sense and process stimuli from the environment, but is also crucial in maintaining internal homeostatic processes as well as determining an individual's cognitive abilities. Brains come at a steep energetic cost however, with neural tissue requiring ~20 times the energy of muscle tissue. With such an important role to play, the 'expensive brain hypothesis' was been established to understand the evolutionary correlates of brain size. Maternal investment, defined as energetic investment during development, is a strong underlying factor in brain size evolution where higher energy investment from mothers is associated with increased brain size. However, much of what we know about brains comes from studying birds and mammals, while generally overlooking other vertebrate classes. Despite their diversity, all jawed vertebrate brains are comprised of similar components, a pattern that first appeared in sharks, rays, and chimaeras (Chondrichthyans). Chondrichthyans are often disregarded as unremarkable from a comparative perspective, which overlooks their true diversity of life histories and ecological niches. This thesis seeks to understand the evolution of brain size and organization in relation to life history and maternal investment using chondrichthyans as a model system. First, I reveal the sequence of reproductive evolution, finding that egg-laying is ancestral and that live-bearing and additional maternal investment (matrotrophy) have evolved independently several times, and are correlated with increasing body size. Second, I find that the evolution of reproductive mode and ecological lifestyle underlie the evolution of both brain size and brain organization, such that shallowwater matrotrophic species have large brains that are predominantly composed of regions related to enhanced cognitive abilities, the telencephalon and cerebellum. Conversely, deepwater lecithotrophic species have small brains composed predominantly of medulla oblongata. Lastly, I find that similar patterns of regional scaling in mammals, birds and chondrichthyans differ from those of teleosts, agnathans, and amphibians, and I propose that differing reproductive strategies may underlie this variation.

Keywords: Chondrichthyan; Reproductive Mode Evolution; Maternal Investment; Brain Evolution; Brain Organization; Phylogenetic Comparative

Dedication

This thesis is dedicated to my dad, for always pushing me to be curious.

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Chapter 1. General Introduction

The evolution of the brain is perhaps, one of the key innovations in the evolution of animals. First appearing more than 500 million years ago, brains have diversified into a range of size, morphologies, and patterns of organization (Striedter 2005). Brains maintain complex homeostatic processes, receive and process stimuli from other components of the central nervous system, and ultimately determine the behavior of individuals. The suite of physiological and behavioral innovations that enthralls biologists is due to the evolution of the brain and central nervous system. With such a crucial role to play, it is a wonder why brain size and organization varies so dramatically between lineages. The evolution of brains has become a key question in understanding the diversification of life.

1.1. Brain Size Evolution

The evolution of brain size has often been examined in terms of ecology and cognitive benefits, and more recently in terms of energetic costs (Figure 1.1). Hypotheses of brain evolution have frequently centered on the constraints and trade-offs of evolving a large brain. Constraints can come in many forms (e.g. phylogenetic, evolutionary, morphological) but when dealing with brain evolution the strictest constraint is energetic, as neural tissue is energetically expensive to produce and maintain (Martin 1996; Striedter 2005). This energetic constraint is imposed as an individual can only acquire so much energy through foraging, and implies a trade-off, typically by reducing the energy available for other important biological processes like growth and reproduction. While there is considerable evidence of the cognitive benefits on increased brain size (Sol et al. 2005, 2007; Sayol et al. 2016*b*), what is unclear is how species can afford the metabolic cost of a larger brain.

Two broad hypotheses have been forward to explain: the 'cognitive-buffer' hypothesis (Sol 2009) and the 'expensive brain' hypothesis (Isler and Schaik 2009), though these are not mutually exclusive and can both relate to life history (Barton and Capellini 2011). The 'cognitive-buffer' hypothesis predicts that the cognitive benefits of increased brain size increase survival and longevity in the case of environmental unpredictability, and has found limited support in lemurs (van Woerden et al. 2010). The 'expensive brain' hypothesis predicts that the cost of increased brain size imposes on energetic constraint on organisms that should trade-off with other structures ('expensive tissue' hypothesis) or processes ('developmental constraints' hypothesis). The 'expensive tissue' hypothesis predicts trade-offs between brain size and the size of other energetically expensive tissues, for example the gut, and has found weak support in groups with diet variability (e.g. herbivores and omnivores) (Aiello and Wheeler 2010; Tsuboi et al. 2014). The 'developmental constraints' hypothesis predicts trade-offs with production (e.g. somatic growth and offspring production) and has been supported in mammals (Barton and Capellini 2011), birds (Iwaniuk and Nelson 2003), and teleosts (Kotrschal et al. 2013). The idea of 'developmental constraints' is rooted in the 'maternal energy' hypothesis, where energetic allocation from the mother influences the brain size of offspring as a large proportion of energetically costly neurogenesis occurs during early development (Martin 1981, 1996). Notably, these hypotheses have not been tested in regards to varying strategies of parity and embryonic trophic mode, and chondrichthyans represent and ideal test group due to their numerous reproductive modes (Wourms and Demski 1993; Dulvy and Reynolds 1997; Musick and Ellis 2005) and range of relative brain sizes (Northcutt 2002*a*; Yopak 2012) (Figure 1.2).

1.2. Brain Organization

With increased interest in brain size, there has been substantial debate surrounding the evolution of brain organization (the relative size of individual brain components). In gnathostome vertebrates, these broadly defined regions are comprised of the telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata.

The debate has centered around two traditionally ascribed modes of evolution: concerted and mosaic (Striedter 2005). Under the concerted model of brain evolution, regions evolve in a coordinated fashion due to developmental constraints, such as a highly conserved sequence of neurogenesis (Finlay and Darlington 1995). Convergent patterns of regional scaling between the divergent lineages of chondrichthyan and primates (Yopak et al. 2010*b*) potentially support this, though the it has not been explicitly tested. Strictly speaking, this can only be tested by measuring neurogenesis and comparative analyses of the same dataset have been used to support both hypotheses. Under the mosaic model of brain evolution, regions or modules can evolve independent of absolute brain size (Iwaniuk et al. 2004), likely related to selection on functionally constrained units associated with niche-specific tasks (Striedter 2005). While it has been increasingly accepted that brain organization likely evolves by a combination of both modes (Striedter 2005; Gutiérrez-Ibáñez et al. 2014), there are limited studies on the relative influence of each mode within a group (Gutiérrez-Ibáñez et al. 2014) or working hypotheses about what may drive the relative influence of developmental and functional constraints.

1.3. Why study shark brains?

The focus of this thesis is the evolution of life history and ecology and exploring links with brain evolution using chondrichthyans (sharks, rays, and chimaeras). The influence of life history on the evolution of brain size and organization (the relative size of major structures) has received increasing attention in the past two decades with development of hypotheses centered on the costs and benefits of increasing relative brain size (i.e. brain size as a proportion of body size). In particular, increasing the amount of time and energy allocated to developing offspring can increase relative brain size (Iwaniuk and Nelson 2003; Barton and Capellini 2011). However, much of the work has focused on mammals and birds while other vertebrate groups have received relatively little attention. Chondrichthyans present an ideal group for testing theories about brain evolution for three reasons. First, chondrichthyans exhibit a diversity of life history traits, particularly reproductive mode, and inhabit nearly every aquatic habitat (Compagno

1990). Second, relative brain size in chondrichthyans are more diverse than in any other vertebrate group, overlapping ranges of amphibians, teleosts, birds, and mammals (Northcutt 2002*a*; Yopak 2012). Third, chondrichthyans are sister to all other jawed vertebrates and mark the emergence of the vertebrate brain *bauplan* (conserved organization composed of five major regions), characterized by the first true cerebellum, found in all subsequent vertebrate lineages. Chondrichthyans have largely been overlooked due to the logistical difficulties of studying and acquiring specimens, though they are an important group for testing broad hypotheses about vertebrate brain evolution.

1.4. Chondrichthyan Diversity and Reproduction

One major advantage of using chondrichthyans as a model system is the diversity of life histories, particularly reproductive modes. Sharks, rays and chimaeras (Class Chondrichthyes) comprise ~1200 species among three main lineages: holocephali, batoidea, and neoselachii comprised of 14 orders, 60 families, and 198 genera (Figure 1.3). Chimaeras (subclass Holocephali) are sister to all elasmobranchs, and are predominantly deep-water, small to moderately sized benthic species. Elasmobranchs (subclass Elasmobranchii) consists of two major lineage: batoids (division Batoidea) and sharks (division Neoselachii). Batoids are characterized by their dorso-ventrally flattened body plan, predominantly benthic or benthopelagic lifestyle, and includes four main orders: deepwater and temperate coastal skates (order Rajiformes); deepwater and coastal electric rays (order Torpediniformes); coastal guitarfish, wedgefish, and sawfish (order Rhinopristiformes), and the diverse stingrays (order Myliobatiformes) which inhabit deepwater, coastal, oceanic, and freshwater habitats. Sharks consist of two sister groups squalomorph sharks (superorder Squalomorphii) and galeomorph sharks (superorder Galeomorphii). Squalomorph sharks and typically deepwater continental shelf dwelling species in five main orders: the deepwater bramble sharks (order Echinorhinformes), the large bodied cowsharks (order Hexanchiformes), the dorso-ventrally flattened and benthic angel sharks (order Squatiniformes), the small bodied sawsharks (order Pritstiophoriformes), and the diverse deepwater and coastal gulper sharks, lantern sharks,

sleeper sharks, and dogfish (order Squaliformes). Galeomorphs sharks are notable for their diversity in body sizes, lifestyles, and habitats and consist of four main orders: the small coastal and temperate reef bullhead sharks (order Heterodontiformes); the coastal-benthic and oceanic-pelagic bamboo sharks, carpet sharks, nurse sharks, and whale shark (order Orectolobiformes); the large-bodied oceanic-pelagic mackerel sharks (order Lamniformes) most of which exhibit regional endothermy; and the diverse ground sharks (order Carcharhinformes) which encompass and range of body size, reproductive modes, and inhabit deepwater, coastal, oceanic, and freshwater habitats.

Vertebrate reproductive modes can be broadly classified according parity mode (oviparity versus viviparity), and trophic mode. Live-bearing species can be classified as lecithotrophic (where nutrients for embryonic development come solely from yolk-sac) or matrotrophic (where maternal provisioning to developing embryos is provided beyond the initial yolk-sac) (Blackburn 2015). Chondrichthyans exhibit a wide range of reproductive modes from the dominant mode of egg-laying (found in 43% of species) to lecithotrophic live-bearing (27% of species) to several distinct forms of matrotrophy (30% of species) (Wourms 1977; Compagno 1990; Wourms and Demski 1993) (Table 1.1). The exact sequence of reproductive evolution in chondrichthyans remains unclear mostly due to previous uncertainties in phylogenetic relationships between major orders, particularly the placement of batoids, and interpretations of the fossil record. Reproductive modes appear to be both taxonomically and ecologically distributed, with egg-laying predominant in deep-water chimaeras (Holocephali), skates (Rajiformes) and catsharks (Scyliorhindae) while substantial matrotrophy (e.g. lipid histotrophy and placentotrophy) is found predominantly in shallow tropically distributed stingrays (Myliobatiformes) and requiem sharks (Carcharhinidae). Despite this apparent pattern, correlation of these traits has not been carried out in a comparative framework.

1.5. New Tools for Phylogenetic Comparative Inference

In the past decade there has been an explosion in the variety and use of phylogenetic comparative methods (PCM) (Pennell and Harmon 2013). These tools primarily address violating of assumptions independence when using traditional statistical tools, and the fitting of varying models of trait evolution. Ignoring the underlying phylogenetic relatedness between species when using traditional statistical tools, such as ordinary least squares regression, can lead to inflated Type I Error rates (i.e. erroneously ascribing significance, a false-positive result) and biased parameter estimates (Freckleton 2009; Revell 2010). As a result, association between variable may be due to patterns of shared inheritance rather than true functional or adaptive relationships. Thus all analyses throughout this thesis incorporate a novel molecular phylogeny (Stein et al. *In Review*) to correct account for the underlying phylogenetic relationships of species within my dataset. I implement novel methods to account for heterogeneity in rates of diversification (Alfaro et al. 2009; Pennell et al. 2014), and incomplete sampling when examining reproductive evolution (Fitzjohn 2012), novel methods for modeling the evolution of discrete characters and the evolutionary correlation with continuous traits (Hadfield 2015), and phylogenetic correction for the analysis of linear regression of brain-body allometry (Freckleton et al. 2002; Revell 2010) and for multivariate analysis (Revell 2009) of brain organization.

The increasing availability of life history and biogeographical data (Last and Stevens 2009; Ebert et al. 2013; Rigby and Simpfendorfer 2013; IUCN 2014), the first time-calibrated molecular phylogeny covering over half of all extant chondrichthyans (Stein et al. *In Review*), and the development of novel statistical tools have been crucial to the development of this thesis. I have used these tools to address uncertainties and test hypotheses about the evolution of reproductive mode and brain size and organization.

1.6. Main objectives of Thesis

The main objectives of this thesis are:

1. To clarify the evolutionary history of reproductive mode in chondrichthyans. Specifically, I aim to determine the sequence of reproductive evolution, measure the strength of support for ecological relationships underlying reproductive evolution, and how this evolution is associated with patterns of diversification, and (Chapter 1).

2. To evaluate the relationship between reproductive evolution and the evolution of brain size (Chapter 2). Specifically, I aim to test how maternal investment, in offspring, is related to relative brain size in accordance with the Maternal Energy Hypothesis (Martin 1996).

3. To understand how brain organization is related to the evolution of brain size. Specifically, I look at the patterns of allometric scaling, covariation, and axes of variation between major brain regions in association with brain size, body size, reproductive mode, and ecological lifestyle (Chapter 3).

4. To place the evolution of brain size and organization within the broader vertebrate context. Specifically I look across major vertebrate classes for commonalities and variation in patterns of brain organization with respect to varying life history strategies (Chapter 4).

Figure 1.1 Path diagram of brain evolution

Path Diagram of the costs and benefits of evolving a large relative brain size against a backdrop of differing environments and habitats. This thesis mainly focuses on the energetic costs and how sharks and rays afford larger brains.

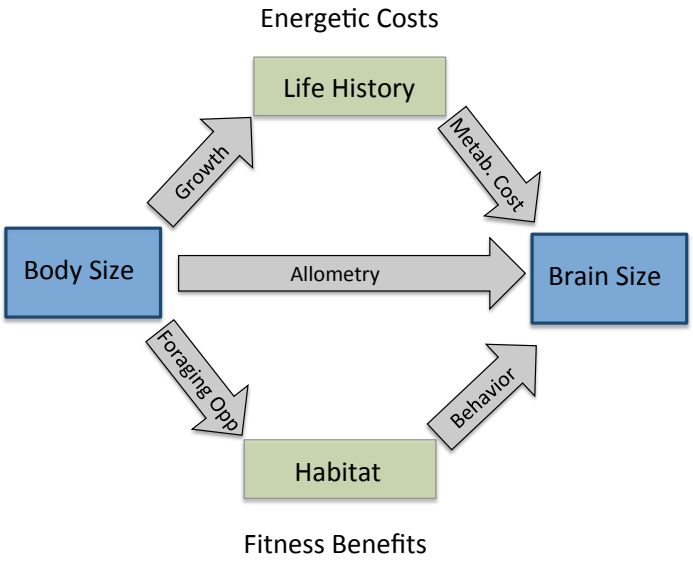


Figure 1.2 Vertebrate Brain-body allometries

Allometric scaling of brain body allometries for major vertebrate lineages. Silhouettes and colors denote lineages, recreated from Yopak (2012).

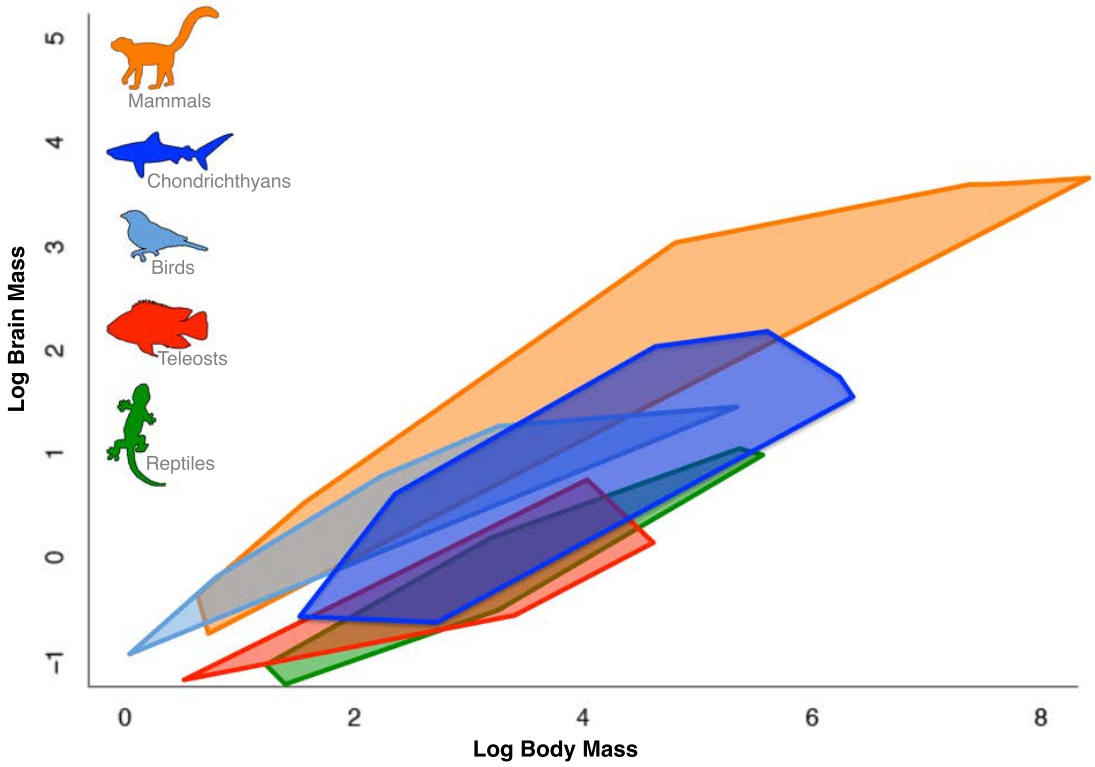


Figure 1.3 Chondrichthyan Phylogeny

Phylogenetic tree from Stein et al (In Review). Phylogenetic tree is representative of 500 trees generated and covers 1,192 species from 14 major orders.

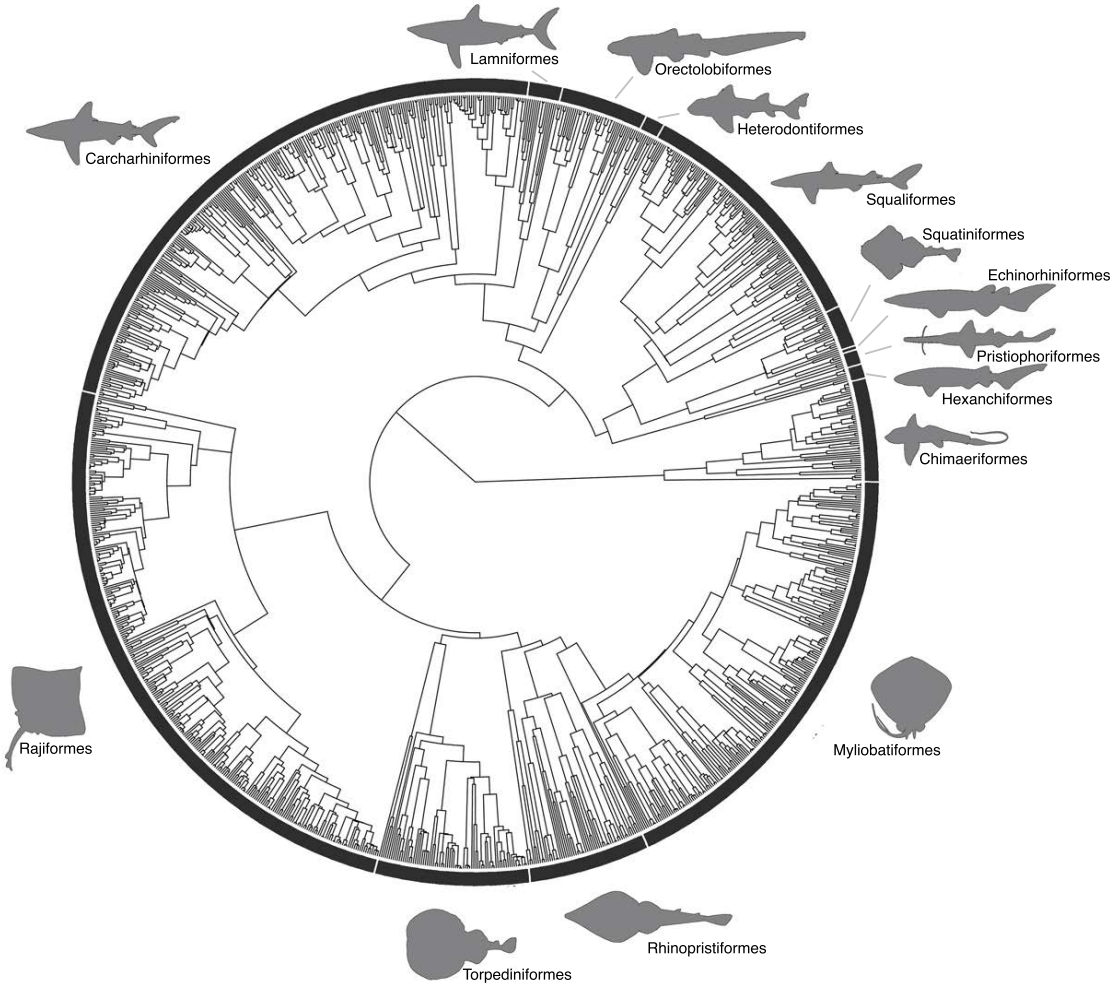


Table 1.1 Reproductive Modes in Chondrichthyans

Parity	Trophic Mode	Reproductive Mode	Description
egg-laying	lecithotrophy	egg-laying	Eggs in a collagen case laid singly, in pairs or sequentially, sometimes with prolonged retention. Found in all Chimaeriformes, Rajifomres, Heterdontiformes, Hemiscyillidae, Stegostomatidae, most Scyliorhinidae
live-bearing	lecithotrophy	yolk-sac live-bearing	Embryo retained until birth and feed solely on yolk-sac. Found in all Torpediniformes, Hexanchiformes, Pristiophoriformes, Squatiniformes, most Rhinopristiformes, Squaliformes, Orectolobiformes, and some Triakidae
live-bearing	matrotrophy	histotrophy	Embryo initially feeds on yolk-sac, supplemented by lipid, protein, or muccus rich uterine secretions. Lipid histotrophy found in all Myliobatiformes. Muccoid histotrophy found in some Rhinopristiformes, Squaliformes, Triakidae and *Galeocerdo cuvier*
live-bearing	matrotrophy	oophagy	Embryo initially feeds on yolk-sac, and consume subsequently ovulated ova. Found in most Lamniformes and Pseudotriakidae. An extension in seen in *Carcharias taurus* where siblings are cannibalized in utero
live-bearing	matrotrophy	placentotrohy	nutrition supplied by yolk-sac and via subsequently established yolk-sac placenta. Some species also supplement with uterine secretions. Found in Triakidae, Hemigaleidae, Sphyrnidae, and Carcharhinidae.

Chapter 2. Diversification and the ecology of live-bearing and maternal investment in sharks, rays and chimaeras

2.1. Abstract

Across, vertebrates, live-bearing has evolved at least 150 times from the ancestral state of egg-laying with a diverse array of forms and degrees of prepartum maternal investment. A key question is how this diversity of reproductive modes arose and whether reproductive diversification underlies species diversification? To test these questions I evaluate the most basal jawed vertebrates – sharks, rays, and chimaeras – which have one of the greatest ranges of reproductive and ecological diversity. Here, I reconstruct the ancestral state and transitions in reproductive mode across a time calibrated molecular phylogeny of 610 extant chondrichthyans. I show that the ancestral state of chondrichthyans was egg-laying, live-bearing evolved at least seven times and matrotrophy evolved 15 times with evidence of one reversal. Focusing only on sharks, transitions to live-bearing and matrotrophy are more prevalent in larger-bodied species in the tropics, suggesting a connection between life histories and biogeographic patterns of species diversity. The evolution of live-bearing is associated with a near-doubling of the diversification rate, but there is only a small increase in diversification associated with the appearance of matrotrophy. In summary, the chondrichthyan diversification and radiation particularly throughout the shallow shelf seas and oceanic pelagic habitats in appears to be associated with the evolution of live-bearing and the proliferation of a wide range of forms of maternal investment in developing offspring.

2.2. Introduction

Patterns of biodiversity vary with life history and ecological opportunities, which determine the niches available for species to persist and the variation that can drive diversification. Life histories, or the probabilities of survival and rates of growth and reproduction throughout life, can evolve in response to different environments (Partridge and Harvey 1988; Wellborn and Langerhans 2015). A key aspect of vertebrate life history strategies is reproductive mode, varying from egg-laying to live-bearing with differing amounts of maternal energetic input, which influences fecundity and offspring development and survival, and is in turn shaped by body size, environmental conditions, and maternal-fetal interactions.

A key transition in the evolution of vertebrate life is the appearance live-bearing from egg-laying. Live-bearing is presumed to have evolved to increase offspring survival in the face of environmental and biological challenges. Deposited eggs are potentially vulnerable to environmental extremes and predation, and hence the retention of eggs provides a "safe harbor" for developing embryos. For example, in montane reptiles the transition to live-bearing potentially protects eggs from freezing (Shine 1995) or predation (Guillette 1993). While offspring may benefit from greater survival, mothers may suffer from reduced fecundity or greater predation risk resulting from lower mobility. In live-bearers, the tradeoff in offspring number and offspring size is potentially shaped by maternal body size (Smith and Fretwell 1974) as small-bodied females have reduced internal body cavity space sufficient to accommodate many large embryos through gestation. This body size effect has been observed in elasmobranchs (Goodwin et al. 2002), though there is no clear evidence of this in teleosts (Goodwin et al. 2002) or reptiles (Pyron and Burbrink 2014). Compared to viviparity little is known of the evolution of prepartum maternal investment or matrotrophy. In sharks and rays, however, reproductive transitions are hypothesized follow an evolutionary trajectory from deep water to shallow habitats (Compagno 1990), such that egg-laying and lecithotrophy are predominantly found in colder habitats of deep water and high latitude seas with matrotrophy more prevalent in shallow tropical habitats, potentially reflecting resource

availability and temperature effects on the rate and efficiency of development (Dulvy 1998). This temperature – reproductive investment hypothesis remains untested largely due to the limitation of available phylogenies that have relied heavily on morphological characters and a small number of taxa (Dulvy and Reynolds 1997; Musick and Ellis 2005).

Matrotrophy should evolve in response to increased availability of food resources (Trexler and Deangelis 2003), coupled with a change from "capital" to "income" breeding strategies (Bonnet et al. 1998). In capital breeders all the resources required for reproduction are acquired and allocated prior to fertilization – thus all egg-laying and lecithotrophic live-bearing species are obligate capital breeders. This strategy should be optimal when food available to mothers is scarce or patchily distributed. Conversely, income breeders continuously acquire resources for developing offspring throughout gestation, necessitating regular and reliable access to food (Trexler and Deangelis 2003). This strategy should be optimal when resources are sufficient to support offspring development through to birth, but only if females have the ability to regulate offspring numbers (e.g. through diapause, selective abortion and absorption of resources). Increased provisioning has been shown to affect the reproductive success (through increased offspring size, litter size, and rate of nutrient transfer) in matrotrophic teleosts (Marsh-Matthews and Deaton 2006) and lizards (Van Dyke et al. 2014).

Live-bearing and matrotrophy may influence speciation and diversification dynamics through colonization of novel habitats (Yoder et al. 2010) or parent-offspring conflicts (Zeh and Zeh 2000; Crespi and Semeniuk 2004). Live-bearing sharks and rays tend to have larger range size compared to smaller species, potentially spanning a greater range of habitats (Goodwin et al. 2005). Larger range sizes coupled with egg retention can potentially facilitate colonization of novel habitats and speciation with the new ecological opportunities. Speciation and diversification can also be driven by conflict between maternal and offspring genomes in live-bearing species (Zeh and Zeh 2000), whereby conflict over resource allocation during gestation drives antagonistic coevolution between maternal and paternal genomes increasing the rate of species

divergence. It is in the father's best interest to have large well provisioned offspring, whereas it is in the mother's best interest to weigh current offspring against her survival and future reproductive success (Zeh and Zeh 2000). Live-bearing provides an arena in which this conflict can occur, and morphological adaptations for matrotrophic nutrient transfer also provide an opportunity for embryos to influence maternal input during gestation (Crespi and Semeniuk 2004). This resulting conflict over resources could increase the rate of genetic divergence within populations ultimately resulting in speciation (Zeh and Zeh 2000). Hence, the potential for parent-offspring conflict in live-bearing species may drive higher rates of diversification and vary over the range of maternal investment. Increased rates of diversification associated with live-bearing have been noted in squamates (Pyron and Burbrink 2014) and teleosts (Helmstetter et al. 2016), although within livebearers the degree to which matrotrophy versus lecithotrophy is related to species diversification has yet to be tested.

Sharks, rays, and chimaeras (Class Chondrichthyes, hereafter 'sharks and rays') are an ideal group for studying the evolution of reproductive mode and maternal investment. Sharks and rays exhibit egg-laying, live-bearing, and multiple forms of matrotrophy along a continuum of maternal post-fertilization input with yolk-sac live-bearing (lecithotrophy) at one end in contrast with lipid histotrophy, oophagy, and placentotrophy representing the extreme forms of matrotrophy. There is considerable debate on whether the first shark lay eggs (Dulvy and Reynolds 1997) or gave birth to live young (Musick and Ellis 2005).

Here I use a new molecular phylogeny of 610 chondrichthyan species with complete information on reproductive mode and habitat to reconstruct the evolutionary history of reproductive mode in chondrichthyans. I test the following hypotheses: (1) that the first shark laid eggs based on the basal position of Chimaeriformes and increasing evidence of batoids as a sister group to sharks (Naylor et al. 2012*a*, 2012*b*); the evolution of live-bearing and varying forms of matrotrophy is related to (2) larger body size, and (3) a radiation into shallow water tropical habitats (Compagno 1990). Finally, (4) that the

evolution of live-bearing and matrotrophy is associated with increasing rates of diversification.

2.3. Methods

I describe, first, the collection of trait data and the development of a molecular phylogeny for comparative analysis. Second, I describe the sequence of reproductive mode evolution and estimation of evolutionary correlation with ecological variables in sharks using reduced animal mixed models. Finally, I describe the estimation of diversification, speciation, and extinction rates.

2.3.1. Trait Data and Phylogeny

Data on the reproductive mode and habitat type were collected for the 610 species of chondrichthyans present in our phylogeny from primary literature and species catalogues (Ebert et al. 2013; Dulvy et al. 2014; IUCN 2014). Chondrichthyans exhibit eight distinct reproductive modes (Dulvy and Reynolds 1997), though we focus the evolution of live bearing and maternal investment, therefore species were broadly categorized into three distinct modes: egg-laying, lecithotrophic live-bearing (yolk-sac viviparity – previously known as ovoviparity – where internally developing embryos are nourished solely via yolk sac), and matrotrophic live-bearing where embryos are nourished via the initial yolk-sac investment and additional maternal contributions during gestation (oophagy, histotrophy, and placentotrophy). Data on maximum size and depth ranges (minimum, mean, median, and maximum) was collected from species field guides and catalogues and primary literature. Minimum and maximum latitudinal range was collected from species Extent Of Occurrence range maps from the International Union for the Conservation of Nature (IUCN) Redlist database (IUCN 2014). Median latitude was calculated as the midpoint between minimum and maximum, and was expressed as absolute value to represent distance north or south from the equator. All continuous trait values were standardized, centered and divided by two standard deviations, using the

`rescale` function in the `arm` (version 1.9-3) (Gelman and Su 2016) package prior to analyses to facilitate comparison of coefficients. I conducted all analyses on a novel 610 species chondrichthyan molecular phylogeny (Stein et al. *In Review*). Phylogenetic trees were inferred using sequence data from 13 mitochondrial and 2 nuclear loci using RaxML (Stamatakis 2006). Temporal calibration was conducted using 10 fossil calibration points (log-normally distributed root node and 9 hard minimum internal nodes) in treePL (Smith and O'Meara 2012) to generate a distribution of 500 dated trees. I sequentially selected 21 trees, every 25th tree from one to 500, to account for the full range of temporal calibrations, and all results were pooled across all trees.

2.3.2. Ancestral State Reconstruction and Diversification

I reconstructed the evolutionary sequence of reproductive mode and habitat while estimating state dependent diversification rates using the multistate speciation and extinction (MuSSE) method with maximum likelihood implemented with `musse` (Pagel 1994) in the `diversitree` (version 0.9-9) package in R (Fitzjohn 2012). MuSSE models are known to suffer from issues associated with incomplete sampling and rate heterogeneity across the tree (Rabosky and Goldberg 2015). Firstly, state dependent speciation and extinction (SSE) models assume homogenous variation in diversification rate across the entire phylogeny, though this assumption is often violated especially with large phylogenies (Rabosky and Goldberg 2015). I tested for rate heterogeneity using `medusa` (Alfaro et al. 2009) implemented in the `Geiger` (version 2.0.6) package (Pennell et al. 2014). Species missing from the molecular phylogeny (n = 582) were added to their respective genera or families. Medusa analysis identified three clades with consistent shifts in diversification rates across all trees: skates (Rajiformes), South American freshwater stingrays (Potamotrygonidae), and ground sharks (Carcharhiniformes). To account for higher diversification rates independent of reproductive mode or habitat in these three clades, a split tree was created with partitions at the root node of each of the three monophyletic clades. SSE models also assume complete taxonomic sampling across a phylogeny, so sampling corrections factors (i.e.

the proportion of sampled to all valid taxa for each reproductive mode) for each trait state within each partition. Speciation (λ), extinction (μ), and transition rates (*q*) were estimated for each partition separately. Because estimations of extinction rates from molecular phylogenies can be difficult and inferences problematic (Rabosky 2010), two models were run: (1) with state dependent extinction rates unconstrained ($\mu_{\text{egg-laying}} \neq \mu_{\text{live-bearing}} \neq \mu_{\text{matrotrophic}}$) and (2) constrained to be equal ($\mu_{\text{egg-laying}} = \mu_{\text{live-bearing}} = \mu_{\text{matrotrophic}}$). I report only findings from the unconstrained model as there was no significant difference between models (Log likelihood = -2946.3 unconstrained model vs -2965.6 constrained model). Additionally the main issue arises from bias due to potential diversification rate heterogeneity, which should be accounted for with a partitioned tree. I only report speciation (λ), extinction (μ), net diversification ($r = \lambda - \mu$), and transition rates (*q*). I treated reproductive mode as an ordinal multistate character and did not allow transitions directly between egg-laying and matrotrophic live-bearing. Models were run for 10,000 generations with the first 1,000 generations discarded as burn-in, using an exponential prior with a rate of $1/(2r)$ where *r* is the character state independent diversification rate (Fitzjohn 2012).

2.3.3. Evolutionary Covariation with Ecological Traits

I used a threshold model adapted from quantitative genetics to test for the evolutionary covariation between reproductive mode and continuous ecological traits (body size, depth, and latitude). The threshold model assumes that state changes in a discrete variable occur when a threshold value of an underlying latent variable has been reached, and can be used to model the correlation between two traits that vary with regards to this underlying liability (Felsenstein 2012). Accurate estimation of evolutionary covariance requires a suitable number of transitions and distribution of traits across the phylogeny (Maddison and Fitzjohn 2015). Because there is only one transition in parity and few appearances of matrotrophy within Chimaeriformes and the rays (Batoideii), I focus on sharks (superorders Galeomorphii and Squalomorphii) to evaluate evolutionary covariance between reproductive transitions and three ecological traits

(body size, median depth, and latitude) across 292 species. I estimated covariance using Bayesian methods, sampling from the posterior distribution using a special reduced animal model implemented in a mixed effects modeling framework, while accounting for phylogeny using the package `MCMCglmmRAM` (version 2.24) in R (Hadfield 2015). These models are a special case of generalized linear mixed effects models where heritability, akin to Pagel's λ, is set to a value of one corresponding to Brownian motion with respect to the phylogenetic tree (Pagel 1999). Twenty chains were run for 2 million generations with the first 200,000 iterations discarded as burn-in, using priors with an inverse-Wishart distribution and the residual covariance matrix set to zero (Hadfield 2015). Samples were drawn every 500 iterations to avoid temporal autocorrelation in parameter estimates. Chains were visually inspected to ensure convergence using `coda` (version 0.19-4) (Plummer et al. 2006), and posterior samples were summarized to generate mean and 95% highest posterior densities (HPD) with samples sizes greater than 1000. Models were run using three different treatments of reproductive mode with the threshold family: binary parity mode (egg-laying versus live-bearing), binary embryo trophic mode (lecithotrophic versus matrotrophic), and ordinal reproductive mode (egg-laying, lecithotrophic live-bearing, and matrotrophic live-bearing).

2.4. Results

2.4.1. Did the first shark lay eggs?

The first chondrichthyans most likely laid eggs as there is a high level of support for egg-laying as the ancestral state of reproductive mode in chondrichthyans (>99% probability; Figure 2.1). There are multiple independent origins of live-bearing and matrotrophy, from the superordinal to subgeneric level, with few instances of reversals. Live-bearing appears to have evolved from egg-laying seven times: (a) base of Rhinopristiformes and Myliobatiformes, (b) base of Squalomorphii, (c) base of clade encompassing Brachaeluridae, Orectolobidae, and Rhincodontidae, (d) base of Ginglymostomatidae, (e) within the genus *Bythaelurus*, (f) in *Galeus polli*, and (g) basal

to clade encompassing Pseudotriakidae, Triakidae, Hemigaleidae, and Carcharhinidae (Figure 2.2A). I found no evidence of reversals from live-bearing to egg-laying. Matrotrophy appears to have evolved independently from lecithotrophic live-bearing 15 times with one reversal: (a-c) one to three origins within guitarfish and wedgefish (Rhinopristiformes), (d) basal to stingrays (Myliobatiformes), (e-f) one to three origins within sleeper sharks (Somniosidae), (g) great lanternsharks (*Etmopterus princeps*), (h) tawny nurse shark (*Nebrius ferrugineus*), (i) mackerel sharks (Lamniformes), (j) Pseudotriakidae, (k-m) one to three origins within houndsharks (Triakidae), and (n) base of requiem sharks (Carcharhinidae). There was evidence of a single instance of reversal from matrotrophy to lecithotrophic live-bearing in the sharptooth houndshark (*Triakis megalopterus*). Overall transitions from egg-laying to live-bearing and to matrotrophy occurred at higher rates then reversals across all partitions that contained multiple reproductive modes (Figure 2.2B,C).

2.4.2. Are live-bearers and matrotrophs larger?

Reproductive mode was related to body size such that larger body species had a higher probability of live-bearing and matrotrophic investment. Using the threshold model to test for evolutionary covariance between discrete values of reproductive mode and continuous ecological traits, I found positive covariation with body size (median = 0.16, 95% CI = 0.09 – 0.23; Effective Sample Size = 3812) indicating transitions in reproductive mode are more prevalent in lineages with larger body size (Figure 2.3A). The relationship with larger body size was slightly stronger for the transition from egg-laying to live-bearing (0.23 [0.11 – 0.35]; ESS = 3306) than for lecithotrophy to matrotrophy (0.19 [0.07 – 0.29]; ESS = 3600).

2.4.3. Is the evolution of live-bearing and matrotrophy associated with shallow-tropical habitats?

Live bearing species are more prevalent in the tropics, specifically transitions from egg-laying to live-bearing are more prevalent in lineages at lower latitudes (-12.66

[-24.22 – 1.09]; ESS = 3003; Figure 2.3B). However, there was little evidence that transitions in reproductive mode are related to either median latitude (-2.62 [-11.75 - 7.03], ESS = 3062) or median depth (0.07 [-0.05 – 0.17]; ESS = 3600; Figure 2.3C).

2.4.4. Does diversification differ between reproductive modes?

Overall, the evolution of live-bearing and matrotrophy is associated with greater diversification (Figure 2.4A-C), mainly due to high species turnover in egg-laying species resulting from high extinction relative to speciation (Figure 2.4A,B). The evolution of matrotrophy is associated with marginally greater diversification compared to lecithotrophic live-bearing and egg-laying, 1.27 and 2.4 times respectively. This is likely due to elevated rates of extinction in egg-laying lineages relative to others as speciation rate is highest in egg-laying lineages (mean 0.046 lineages/MY) compared with lecithotrophic live-bearing (mean 0.03 lineages/MY) and matrotrophic lineages (0.026 lineages/MY), compared to egg-laying species (mean 0.046 lineages/MY) (Figure 2.4A-C). Of the three radiations, the connection between reproductive mode and diversification is more nuanced. Within the ground sharks (Carcharhiniformes), egg-laying lineages have a higher diversification rate than lecithotrophic live-bearing lineages (Figure 2.4D-F), mainly driven by high speciation in egg-laying cat sharks. Speciation is particularly high in both skates (0.078 lineages/MY; Figure 2.4G) and South American freshwater stingrays (0.070 lineages/MY; Figure 2.4J), though this likely reflects colonization of novel deep-water benthic and freshwater habitats.

2.5. Discussion

Here I reveal the first chondrichthyan was an egg-layer and there have been numerous transitions toward live-bearing and matrotrophy. The evolution of live-bearing and matrotrophy covaries with increasing body size and is more prevalent in tropical latitudes. The evolution of live-bearing, and to a lesser extent matrotrophy, appears to have resulted in greater diversification. Next I consider three questions: What is the

sequence of reproductive mode evolution? What ecological factors have driven the evolution of live-bearing and matrotrophy? Is chondrichthyan speciation and diversification explained by viviparity driven conflict or by novel ecological opportunity?

2.5.1. The sequence of reproductive mode evolution

I find support of egg-laying as the ancestral state with numerous independent origins of live-bearing and matrotrophy with few instances of reversals. Previous analyses of reproductive evolution in chondrichthyans have been limited by phylogenetic information, particularly on the position of batoids (Shirai 1992; Naylor et al. 2012*b*). I find transitions in reproductive mode are generally toward live-bearing or matrotrophy, though this not a strictly linear progression as reversals are possible, if rare; though increased sampling, particularly in groups displaying subgeneric transitions (e.g. within catsharks, Scyliorhindae) may yield examples. This supports the general consensus of egg-laying being ancestral in elasmobranchs (Dulvy and Reynolds 1997; Blackburn 2015) despite a previous study that found live-bearing to be ancestral with a high rate of reversals (Musick and Ellis 2005). With this new phylogeny I find little support for reversals to egg-laying from live-bearing. Interestingly similar controversy has occurred in squamates, a group with highly labile reproductive modes. The most parsimonious model suggested an early origin of viviparity with a high rate of reversals (Pyron and Burbrink 2014), though this has be questioned based on morphological features of live-bearing lineages, highlighting that statistical models should be grounded in our biological understanding (Griffith et al. 2015), and the current consensus supports egg-laying as ancestral. Live-bearing chondrichthyans, particularly lecithotrophic live-bearers develop within an egg envelope or candle, though it is thinner than in egg-laying species (Castro 2009; Conrath and Musick 2012). This retention of morphological requirements for egg production could make reversals to egg-laying feasible, and may allow for some of the subgeneric diversity seen within catsharks (Scyliorhinidae). Transitions between matrotrophic modes can also occur, for example in hound sharks (Family Triakidae) muccoid histotrophy can be used in lieu of or in concert with placentotrophy (Hamlett et

al. 2005), resulting in intergeneric diversity (López et al. 2006). Similarly, lecithotrophic live-bearing and some forms of matrotrophy, particularly muccoid histotrophy, may not represent discrete character states but rather a continuum. Muccoid histotrophy can be difficult to distinguish due to a lack of uterine morphological specializations without accurate measurements of ash free dry weight of embryos to ovum. In several species of squaliform sharks this has been used to identify muccoid histotrophy (Paiva et al. 2011; Cotton et al. 2014), though there is uncertainty given the exact threshold value that should be used to distinguish between modes (Frazer et al. 2012). Thus large groups predominantly composed of lecithotrophic live-bearing species (i.e. Rhinopristiformes, Squalimorphii) may actually contain a greater diversity of maternal investment that currently measurable. Despite this uncertainty, chondrichthyans still exhibit a remarkably labile reproductive modes compared with other vertebrate groups, similar to the rate seen in the much larger clade Squamata (~10,000 species with >150 origins of live-bearing and 6 origins of matrotrophy) (Blackburn 2015).

2.5.2. Evolutionary correlates of reproductive mode

In sharks, reproductive mode has evolved in association with body size and latitude, while there is no evidence of association with depth. Of those considered, body size is the only trait correlated with the evolution of reproductive mode in sharks, where origins of live-bearing and matrotrophy are associated with increasing body size. Body size may in effect be capturing differences in predation pressure and access to food resources, predicted drivers of reproductive evolution. The origin of live-bearing necessitates an increase in body size to accommodate retained embryos throughout gestation in limited internal body space (Blackburn 2015). As a result lecithotrophic species typically have fewer, but larger pups that are subject to less predation pressure (Conrath and Musick 2012) presumably resulting in reduced juvenile mortality (Kindsvater et al. 2016). The origin of matrotrophy requires abundant energetic resources, which for a predatory species may be patchily distributed. The number of potential prey species with relaxed gape size limitation (Lucifora et al. 2009), home range

size (Tamburello et al. 2015), and potentially access to varied resources increase with body size (Hussey et al. 2015) suggest that increasing body size can be associated with increased resource acquisition.

Despite correlations between reproductive mode and body size, depth, and latitude in extant chondrichthyans (Rigby and Simpfendorfer 2013), the ability to test for evolutionary correlations is hindered by the clustering of traits phylogenetically and the use of depth and latitude as proxies for environmental temperature and resource availability. As a result is can be difficult to disentangle evolutionary hypotheses, as large groups with similar character states may reflect a single origin and subsequent coinheritance rather than a functional relationship (Maddison and Fitzjohn 2015). Batoids present an interesting transition from deep egg-laying skates, to shelf and coastal live-bearing electric rays (Torpediniformes) and guitarfish, wedgefish, and sawfish (Rhinopristiformes), and shallow coastal and pelagic matrotrophic stingrays (Myliobatiformes). However, because this groups contains a single origin of live-bearing and one certain origin of matrotrophy our power to test for plausible correlations between this reproductive evolution and depth or latitude due to thermal physiology (Dulvy 1998), predation (Harper and Peck 2016), and productivity are limited.

2.5.3. Viviparity-driven conflict versus novel ecological opportunity

Drivers of diversification potentially differ between groups, with evidence of radiations into novel or fragmented habitats rather than of reproductive mode affecting diversification rates across all chondrichthyans. While there is weak support (i.e. different mean estimates but with overlapping 95% CI) that live-bearing and matrotrophy increases diversification across the main partition of the tree, genomic conflict may be a weak driver of diversification compared to ecological forces in chondrichthyans. Increases in diversification seen in skates (Rajiformes), South American Freshwater stingrays (Potamotrygonidae), and ground sharks (Carcharhiniformes) appear to be related to colonization of new ecological niches. However, in each case the niche is different – skates radiated into deepwater habitats with the opening up of the Atlantic

Ocean (McEachran and Miyake 1990), Potamotrygonid stingrays colonized of freshwater with the closure of the isthmus of Panama (Lovejoy 1996, 1997; Lovejoy et al. 1998; de Carvalho and Lovejoy 2011). High species diversity of deepwater skates was maintained following deepwater colonization by subsequent isolation of ocean basins (Long 1994), limited dispersal (McEachran and Miyake 1990), and high spatial niche differentiation (Bizzarro et al. 2014; Humphries et al. 2016). Neotropical freshwater stingray diversity has followed the availability of novel and highly fragmented habitat and resources, and the emergence of unique morphological adaptation (Kolmann et al. 2016). The increased diversification in ground sharks is driven by both reinvasion of deepwater by catsharks (Scyliorhinidae) and the colonization of coral reefs by requiem sharks (Carcharhinidae) (Sorenson et al. 2014). Patterns of cladogensis in chondrichthyans may be driven by particular colonization events or character states within particular lineages. Future investigations may require refined measurements of ecological (Burin et al. 2016) or life history (Marki et al. 2015) drivers of diversification with tests restricted to smaller groups.

2.5.4. Conclusion

The evolution of chondrichthyan reproductive modes from egg-laying to live-bearing and matrotrophy is a rich pattern correlated with ecology and patterns of diversification. While patterns of diversification appear to be more strongly driven by novel habitat colonization, the evolution of reproductive mode diversity remains a fruitful area of investigation. Parent-offspring conflict over resources during development and subsequent antagonistic coevolution is an intriguing potential driver of reproductive mode evolution. Chondrichthyans are an ideal group to test for this given the diversity of reproductive modes and the frequency of polyandry, though this requires a better understanding of maternal-fetal interactions across a wide range of species. Future research should focus on the refinement of maternal investment, particularly for identifying the continuum on which lecithotrophic live-bearing and histotrophic matrotrophy may be expressed. Combined with further refinement of phylogenetic

hypotheses with more extensive taxon sampling will help to clarify patterns of energetic investment, the degree of income breeding in live-bearing species, and inter and intra specific plasticity.

Figure 2.1 Reproductive Mode Ancestral State Reconstruction

Ancestral state reconstruction reproductive mode on a representative tree of 610 species of chondrichthyans. Pie symbols represent the likelihood of the character state for each node being egg-laying (black), live-bearing (blue), or matrotrophic (red). Dark grey symbols denote the partitions encompassing diversification rate shifts. Silhouettes depict representative species from the major orders.

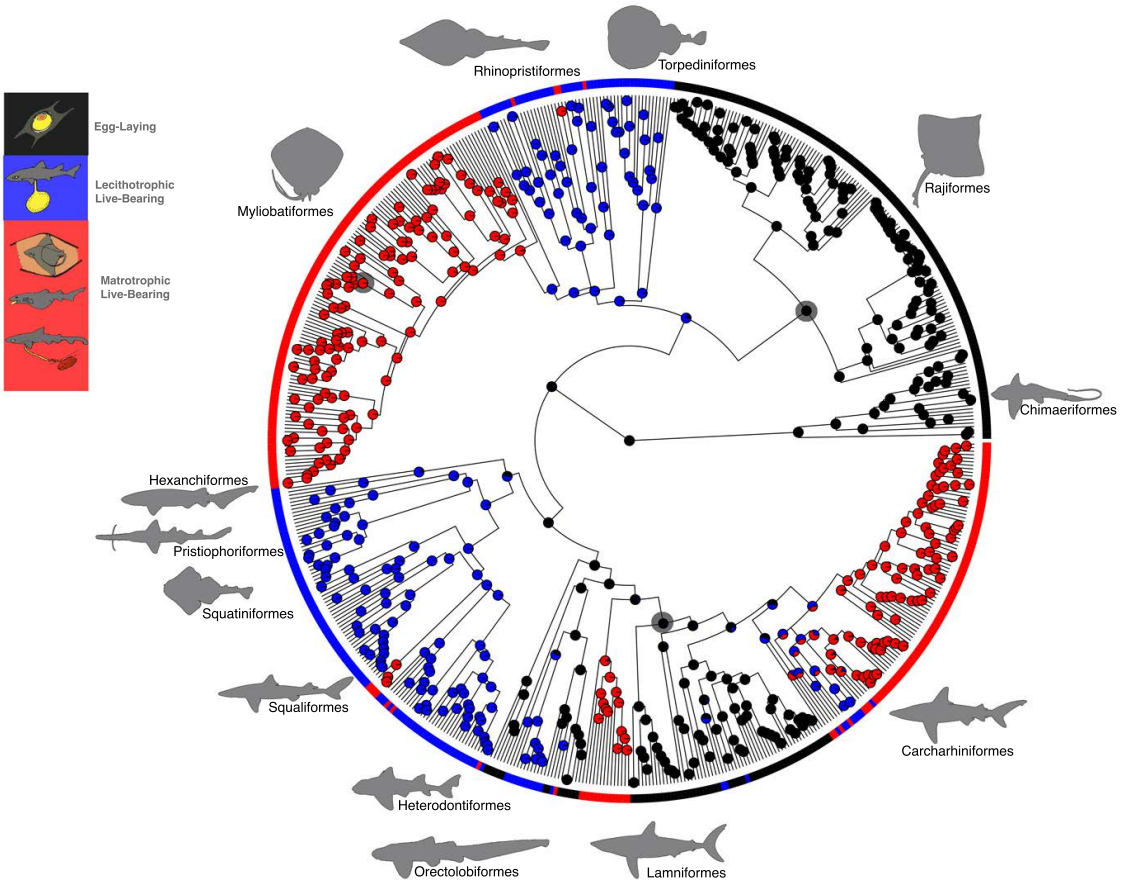


Figure 2.2 Reproductive Mode Transitions

The number (A) evolutionary transitions in reproductive mode across chondricnthyans and transition rates between modes in (B) main partition of the tree and (C) within Carcharhiniformes. Origins of live-bearing from egg-laying are depicted in blue with reversals in black, and origins of matrotrophy from lecithotrophic live-bearing are depicted in red with reversals in green. Bars and shaded regions in represent the 95% posterior density of transition rate estimates.

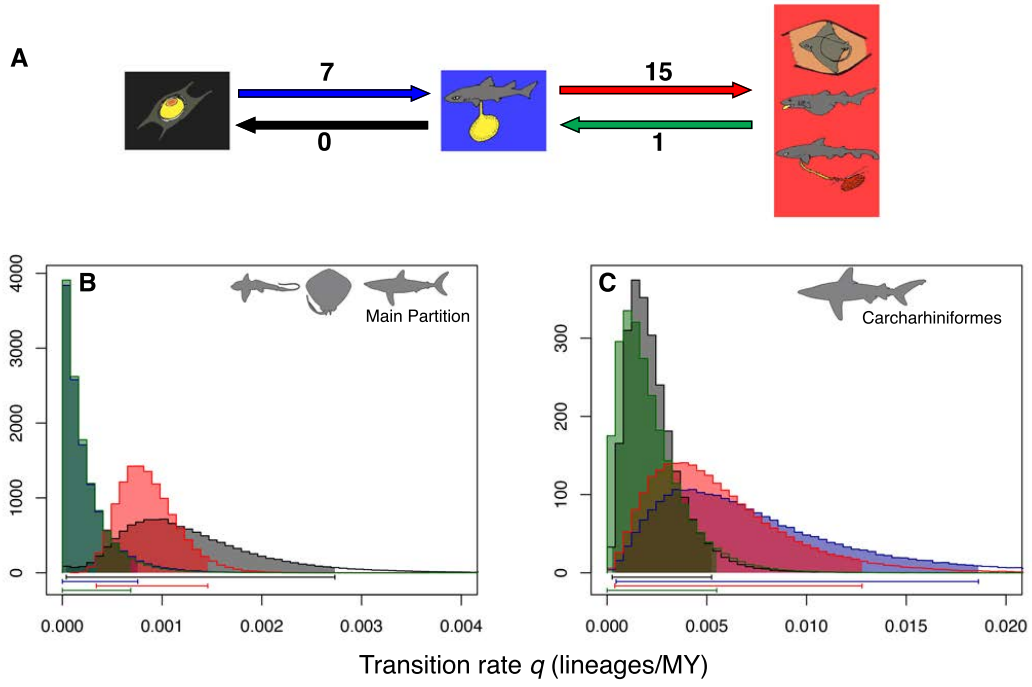


Figure 2.3 Evolutionary covariation with ecological traits

Coefficient plots of evolutionary covariation between (A) body size, (b) median depth, and (c) median latitude from MCMCglmm models. Black circles denotes egg-laying vs live-bearing, grey circles denote live-bearing vs matrotrophy, and open circles denote reproductive mode as an ordinal variable with all three character states. Horizontal bars represent the 95% confidence intervals of the mean posterior estimate.

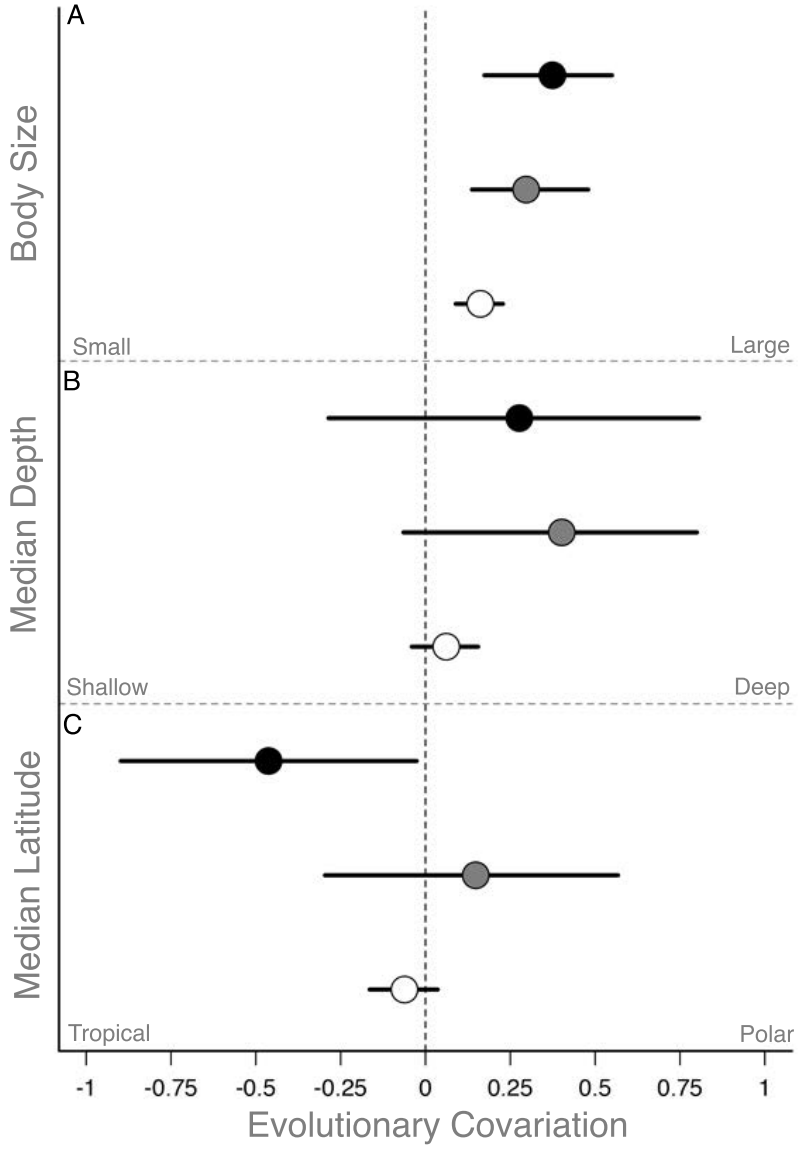
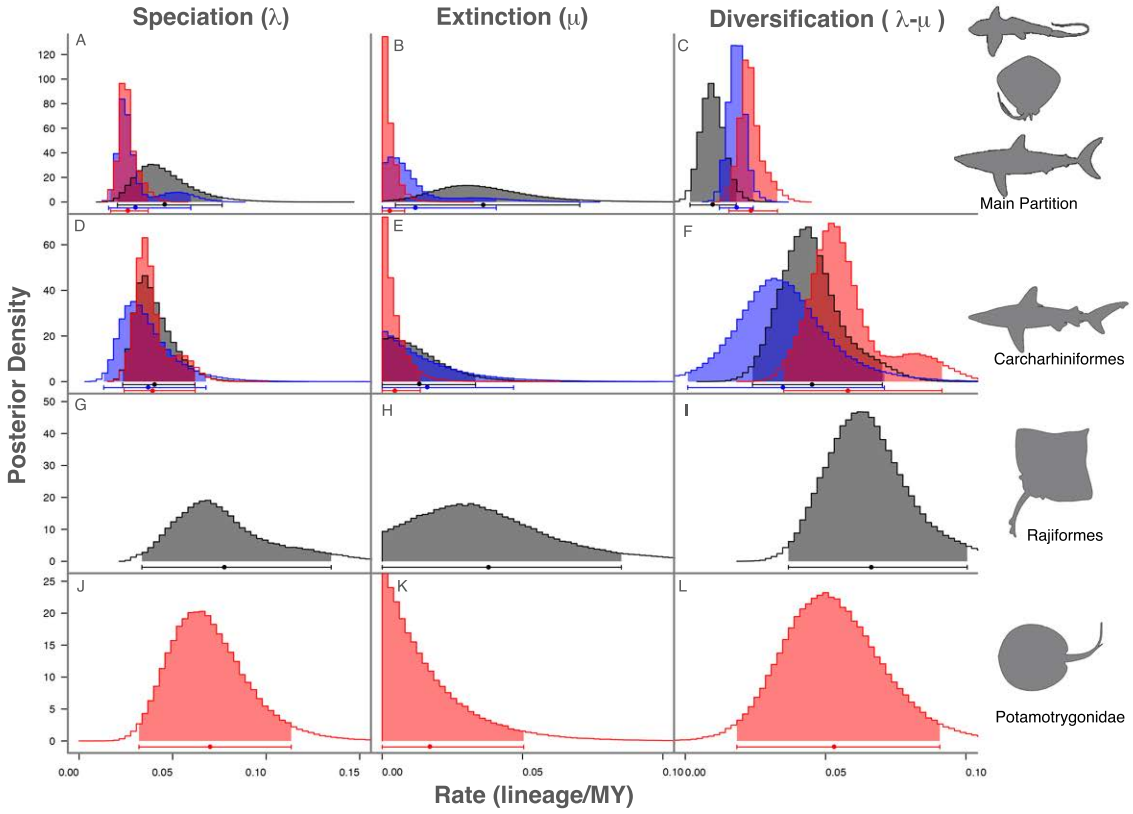


Figure 2.4 Diversification between reproductive modes

Posterior densities of parameter estimates from MuSSE model showing state dependent speciation (λ) and net diversification rate (*r*) from the main partition of the tree (A,B), within the order Carcharhiniformes (C,D), within the order Rajiformes (E,F), and within the family Potamotrygonidae (G,H). Egg-laying is depicted in black, live-bearing in blue, and matrotrophy in red. Bars and circles represent the mean and 95% confidence interval of the posterior mean estimate.



Chapter 3. Does more maternal investment mean a larger brain? Evolutionary relationship between reproductive mode and brain size in chondrichthyans[1]

3.1. Abstract

Chondrichthyans have the most diverse array of reproductive strategies of any vertebrate group, ranging from egg-laying to live-bearing with placental matrotrophy. Matrotrophy is defined as additional maternal provisioning beyond the yolk to the developing neonate; in chondrichthyans, this occurs through a range of mechanisms including uterine milk, oophagy, uterine cannibalism and placentotrophy. Chondrichthyans also exhibit a wide range of relative brain sizes and highly diverse patterns of brain organisation. Brains are energetically expensive to produce and maintain, and represent a major energetic constraint during early life in vertebrates. In mammals, more direct maternal–fetal placental connections have been associated with larger brains (steeper brain–body allometric scaling relationships). We test for a relationship between reproductive mode and relative brain size across 85 species from six major orders of chondrichthyans by using several phylogenetic comparative analyses. Ordinary least-squares (OLS) and reduced major axis (RMA) regression of body mass versus brain mass suggest that increased maternal investment results in a larger relative brain size. Our findings were supported by phylogenetic generalised least-squares models (pGLS), which also highlighted that these results vary with evolutionary tempo, as described by different branch-length assumptions. Across all analyses, maximum body size had a significant influence on the relative brain size, with large-bodied species (body mass >100 kg) having relatively smaller brains. The present study suggests that there may be a link between reproductive investment and relative brain size in chondrichthyans; however, a more definitive test requires a better-resolved phylogeny and a more nuanced categorisation of the level of maternal investment in chondrichthyans.

[1] Modified from Mull, C.G., Yopak, K.E., Dulvy, N.K. Does more maternal investment mean a larger brain: Evolutionary relationships between reproductive mode and brain size in chondrichthyans. 2011. *Marine and Freshwater Research* 62: 567-575

3.2. Introduction

The evolution of additional parental investment in offspring, such as through live-bearing and parental care, is thought to occur when the increased survival of offspring outweighs the parental costs of reduced fecundity and mobility (Clutton-Brock and Godfrey 1991; Reynolds et al. 2002). Though there have been numerous origins of parental care, there have been very few transitions to higher levels of maternal investment, such as placentation, in vertebrates (Wourms 1977, 1981; Dulvy and Reynolds 1997). Live-bearing is thought to have evolved more than 150 times in vertebrates (Shine 1989; Clutton-Brock and Godfrey 1991; Blackburn 2015), with most transitions occurring in squamate reptiles and , 9 to 15 transitions occurring in chondrichthyans (Dulvy and Reynolds 1997; Reynolds et al. 2002, Chapter 2). The greatest number of gains and losses of maternal investment (matrotrophy) in vertebrates has occurred in the chondrichthyans, where matrotrophy has evolved 15 times with only a single loss (Chapter 2). In contrast, matrotrophy has evolved only four times in teleosts and appears never to have been lost (Wourms 1994; Goodwin et al. 2002). In fishes, including chondrichthyans, there appear to be few ecological correlates with reproductive mode; for example, there are no differences in the biogeographic distributions of live-bearers and egg-layers that cannot be explained by the differences in body size (Goodwin et al. 2005). However, there are life-history correlates with reproductive mode, with live-bearers having a large maternal body size, larger offspring size and lower fecundity (Goodwin et al. 2002). It still remains an open question as to why complex forms of reproductive investment have evolved in vertebrates (Shine 1989, 2005; Reznick et al. 2007), particularly chondrichthyans.

The brain is perhaps the most energetically expensive organ to produce and maintain, and thus has been the subject of many allometric studies in mammals, birds and reptiles (Martin 1981; Iwaniuk and Nelson 2003; Elliot and Crespi 2008; Isler and Schaik 2009). Most studies have focussed on functional implications of brain size and the development of major brain regions in vertebrates, examining ecological or behavioural correlates with brain organisation (Barton et al. 1995; Kotrschal et al. 1998; Lefebvre et

al. 2002; Yopak et al. 2007; Shumway 2008), whereas few have examined any potential physiological mechanisms that drive this diversity (Elliot and Crespi 2008). Much of neural development occurs during gestation and brain tissue accounts for a disproportionate amount of metabolic costs in early life (Martin 1996; Elliot and Crespi 2008), raising questions about the role of reproductive diversity, and the level of maternal investment in the evolution of relative brain sizes.

Until recently, there has been little investigation into the diversity of reproductive strategies and modes of maternal investment in chondrichthyans and how they may correlate with the diverse brain morphologies documented across this group (Pagel and Harvey 1988; Martin 1996; Yopak et al. 2007). Previous studies of placental (eutherian) mammals suggest that it is worth searching for such a link; more 'invasive' forms of placentation (haemochorial), with the closest connection between maternal and fetal blood supply, exhibit a steeper brain–body allometric relationship than does less invasive placentation (endochorial and epithiliochorial) (Elliot and Crespi 2008). Hence, larger haemochorial mammals have larger brains for a given body size than do those with other less tightly connected placentas. Elliot and Crespi (2008) proposed that the development of larger brains is facilitated by better embryonic access to maternally derived fatty acids, which are essential for the development of brain tissue; however, whether this is true of cartilaginous fishes remains unknown.

Chondrichthyans, comprising more than 1100 extant sharks, skates, rays and chimaeras (Last 2007), offer an ideal taxon in which to study the potential links between reproduction and the brain size and morphology. Chondrichthyans have the most diverse array of reproductive strategies of any vertebrate taxa, ranging from single oviparity to placental viviparity, with many intermediate forms of matrotrophy (i.e. maternal nourishment provided to developing embryos) (Wourms 1977; Wourms and Demski 1993). The exact pattern of reproductive evolution in chondrichthyans remains open to debate, with older studies finding oviparity to be the ancestral form (Dulvy and Reynolds 1997), whereas studies using more recent phylogenetic hypotheses argue that viviparity is ancestral (Musick and Ellis 2005). Ultimately, the ancestral reproductive mode depends

largely on the underlying evolutionary tree, which is still subject to considerable debate (Naylor et al. 2005; Vélez-Zuazo and Agnarsson 2011). Both Dulvy and Reynolds (1997) and Musick and Ellis (2005) yielded a relatively high amount of transitions between oviparity and viviparity; the former estimated 9 or 10 transitions from oviparity to viviparity, with two reversals, whereas the latter study estimated six transitions from viviparity to oviparity, with one reversal. The high number of transitions and reversals suggests that reproductive mode is evolutionarily labile. It is possible that this plasticity of reproductive mode has also developed as a result of changes in ecological roles, geographic distribution and the trade-offs associated with increased maternal investment (Goodwin et al. 2002, 2005; Crespi and Semeniuk 2004).

The relative brain size of chondrichthyans has been shown to be comparable to other vertebrates, with allometric-scaling ranges overlapping with reptiles, teleosts, birds and mammals (Bauchot et al. 1976; Northcutt 1977, 1989). Much of the variation in brain organisation in chondrichthyans can be predicted by the overall brain size, with some major brain regions (e.g. telencephalon, cerebellum) enlarging disproportionately as the brain size increases (Yopak et al. 2010*b*). Relative brain size and the relative development of major brain areas have been correlated with ecology (i.e. habitat type, feeding mode), and these patterns do not necessarily follow phylogenetic groupings (Yopak et al. 2007; Lisney et al. 2008; Yopak and Montgomery 2008; Yopak and Frank 2009), similar to patterns seen in other vertebrates, such as teleosts, birds and mammals (Huber et al. 1997; Kotrschal et al. 1998; de Winter and Oxnard 2001). There is some evidence that reproductive mode may be correlated with variation in brain size and organisation; the sharks with placental viviparity, such as the Carcharhinidae and Sphyrnidae, have among the largest relative brain sizes within chondrichthyans (Yopak et al. 2007), although this has not as yet been statistically tested.

Comparative analyses in mammals (Elliot and Crespi 2008; Barton and Capellini 2011) and birds (Iwaniuk and Nelson 2003) suggests that higher levels of maternal investment will increase nutrient allocation to developing embryos, allowing increased growth of energetically expensive brain tissue. Our primary hypothesis is that variation in

the relative brain size among chondrichthyans is directly related to reproductive mode. Specifically, chondrichthyans with matrotrophy (additional maternal contribution beyond yolk sac, including the presence of histotroph, oophagy, or the formation of a yolk sac placenta), will exhibit larger relative brain sizes than do lecithotrophic (yolk-only nourishment) species.

3.3. Materials and Methods

3.3.1. Data Collection

Data on brain weight and bodyweight for 85 chondrichthyan species were obtained from previously published studies (Northcutt 1977, 1978; Kruska 1988; Ito et al. 1999; Lisney et al. 2008; Yopak and Montgomery 2008; Yopak and Frank 2009). Data on reproductive mode were collected from published literature (Dulvy and Reynolds 1997; Last and Stevens 2009) and each species was categorised into one of two reproductive modes, namely lecithotrophic or matrotrophic (Figure 3.1).

3.3.2. Statistical Analyses

To examine whether there was a difference in brain–body allometric scaling among reproductive modes, several general linear modelling methods were applied, including ordinary least-squares (OLS) and reduced major axis (RMA) regression. OLS regression is often used to examine changes in brain mass with body mass, and allometric-scaling relationships conform to the equation:

$$y = ax^b$$

where y = brain mass, x = body mass, a is the allometric coefficient and b is the allometric component. OLS regression assumes no error in the measurement of the independent variable (Ives et al. 2007), although this is often not the case with body-mass data. To account for inherent measurement error in the independent variable (i.e. body

mass), we used reduced major axis (RMA) regression (Smith 2009). Despite the inherent statistical violations (particularly in the OLS method), we include them here to enable comparisons with previous research. For both regression methods, brain and body mass were $\log_{10}$- transformed and normality of the variables was confirmed with the Shapiro–Wilk test.

Because of shared evolutionary history, species are hierarchically autocorrelated and thus cannot be treated as independent samples drawn from a normal distribution (Freckleton 2000, 2009). Species are more likely to be similar to other species in the same genus because of their shared evolutionary history rather than because of convergent evolution to shared selective pressures. The use of OLS or RMA regression, without consideration of phylogenetic relationships, will often overestimate the extent of a correlation and result in Type I errors (Garland et al. 1992; Smith 2009). One way to account for this issue is to use a phylogeny to account for the relatedness of species by nesting the data. I tested the allometric relationship between brain mass and body mass by using phylogenetic generalised least-squares (pGLS) (Freckleton et al. 2002) as implemented in the APE package for R (Paradis et al. 2004). This method accounts for the relatedness and hence correlation among species within families and families within order by accounting for the hypothesised phylogenetic relationships as a variance–covariance matrix in the generalised least-squares modelling framework.

A phylogenetic hypothesis of our study species was redrawn with the Mesquite phylogenetic analysis package (Maddison and Maddison 2011), using parsimony reconstruction. The tree used was primarily based on Shirai's phylogeny (Shirai 1992, 1996) with additional information (Compagno 1988; Martin et al. 1992; Goto 2001; Rosenberger 2001; Didier 2004; McEachran and Aschliman 2004; Naylor et al. 2005) (Figure 3.1). This parsimony tree provides only the topology (shape) of the relationships among species and as such does not provide any information on the evolutionary tempo or distance (as measured by branch lengths) among species. Hence, we considered two models of evolutionary distance among species. First, we considered two extreme transformations of a similar form, by setting branch lengths to either zero or one. Second,

we considered a non-parametric rate-smoothing model, wherein rates change smoothly between connected branches of the tree (Sanderson 2002). This method was implemented using the 'chronopl' function, which produces longer branch-tip lengths, in the APE package in R (Paradis et al. 2004). We tested the relative importance of reproductive mode for explaining the relative brain size by comparing two models using Akaike information criteria (AIC): 'brain size ~ body mass' and 'brain size ~ body mass + reproductive mode' (Hilborn and Mangel 1997; Burnham and Anderson 2002).

Following a preliminary analysis, data were reanalysed after the removal of large-bodied species (body mass > $\log_{10}$ 5 or 100 kg: *Prionace glauca, Sphyrna mokorran, Galeocerdo cuvier, Carcharias taurus, Isurus oxyrhinchus, Centorhinus maximus, Megachasma pelagios, Rhincodon typus, Carcharodon carcharias*) to examine the potential effect of an extremely large body size on the relative brain size and the influence on the allometric coefficient (Striedter 2005).

3.4. Results

There is a positive relationship between brain mass and body mass, which appears to differ between the reproductive modes in a manner that suggests that increased maternal investment is associated with a larger relative brain size, but only in the smaller species (Figure 3.2A). Matrotrophy results in brains that are 20–70% larger than those with lecithotrophy for chondrichthyans between 3 kg ($\log_{10}$ 3.5) and 100 kg ($\log_{10}$ 5). This marked difference in relative brains mass at small body mass (significant difference in intercept, $p < 0.05$) decays with increasing body size (there is no significant difference between slopes; $p = 0.3382$) (Table 3.1). This pattern is more striking if the largest chondrichthyans (>100 kg, $\log_{10}$ body mass of >5) are removed from the statistical and graphical analysis (Figure 3.2B). The smallest matrotrophic species have brain masses that are on average 70% larger (difference between intercepts significant at $p = 0.0225$) and this advantage increases for sharks up to 100 kg, where relative brain sizes are 87% larger for matrotrophic species (a significant difference between slopes; $p < 0.0001$)

(Table 3.1). This marked difference with the exclusion of large-bodied species indicates that the mode of body-size evolution may have a significant effect on relative brain sizes, a phenomenon known as 'gigantism' (Striedter 2005). This pattern is robust to statistical method and the incorporation of phylogenetic information.

Reduced major axis regression of all body sizes yielded similar results, with no significant ($p = 0.962$) difference between the slopes, and a significant difference between the intercepts ($p < 0.001$) (Table 3.1). When all body sizes were included, matrotrophic species exhibited brains that were 6–26% larger than those of lecithotrophic species (Figure 3.2C). When large body sizes (body mass > 100 kg) were removed, there was a significant difference in both slope ($p = 0.007$) and intercept ($p < 0.001$) (Table 3.1). Similar to OLS regression, differences in relative brain size were more pronounced when large body sizes were excluded, with matrotrophy resulting in brains that were 55–68% larger than those of lecithotrophic species (Figure 3.2D).

The phylogenetic GLS results were highly dependent on the branch-length assumptions of the available tree. The uniform branch-length transformation (zero or one) suggests that the inclusion of reproductive mode was not significant when all body sizes were included (Table 3.2). The use of a smoothed branch-length transformation yielded a more parsimonious model that included reproductive mode as a significant factor for all body sizes (Table 3.2). When large-bodied species (body mass > 100 kg) were removed, the inclusion of reproductive mode improved all models irrespective of tree topology (Table 3.2). The finding of a robust signal despite tree topology suggests that although increased forms of maternal investment are associated with a larger relative brain size, body-size evolutionary trends may exert a significant influence on the brain development.

3.5. Discussion

The present study represents the first test of whether maternal investment beyond the yolk may contribute significantly to the relative brain size of chondrichthyans. My

results provide intriguing evidence that increased levels of maternal input during gestation through matrotrophy may facilitate the development of relatively larger brains in these species, particularly for smaller species (body mass < 100 kg). This pattern is consistent across statistical methods; however, it is most pronounced at smaller body sizes, suggesting that the reproductive mode and maximum body size exert a significant influence on the relative brain size.

3.5.1. Encephalisation and reproduction

Across vertebrate taxa, small-bodied species tend to have larger relative brain sizes than do larger-bodied species, potentially driven by metabolic constraints and body-size evolution (Striedter 2005). Relative brain sizes of small-bodied (body mass < 100 kg) matrotrophic species are larger than those of small-bodied lecithotrophic species, although this difference breaks down for larger body sizes (body mass > 100 kg). This observed pattern is potentially explained by 'gigantism', where dramatic evolutionary increases in body size often do not have concurrent increases in the absolute brain size, resulting in smaller relative brains for larger-bodied species (Striedter 2005). This is especially pronounced in matrotrophic species (7 of 9 large-bodied species in the present study), where relative brain size appears to decline rapidly at body mass > 100 kg. It is important to note, however, that the neonatal body size is not always correlated with maximum adult body size. The lecithotrophic whale shark (*R. typus*) is the largest extant fish, reaching a maximum size of 20m total length (TL), and has pups of ~0.5m TL, whereas the matrotrophic white shark (*C. carcharias*) has pups >1m TL. These were the two largest species included in the present dataset, and individuals had very similar brain and body masses, yet data may have been collected from very different life-history stages. Interspecific differences in body size can be reversed between neonate and adult life stages in chondrichthyans and this must be accounted for in data collection. One confounding factor of the present analysis is the varying life stages of samples used. To tease out the effect of maternal investment on brain development, future studies will need to examine differences, specifically in neonatal brain–body allometry.

The large relative brain sizes of small-bodied matrotrophic species is of note from a reproductive stand point, as many of these species are from the Order Carcharhiniformes and exhibit placental viviparity. Species from Carcharhiniformes, especially those from Sphyrnidae and Carcharhinidae, exhibit larger brains than expected, on the basis of allometric scaling of raw species data, significantly larger than do lecithotrophic species of a similar body size. With a few exceptions, members of Carcharhinidae and Sphyrnidae have among the most highly encephalised brains (Yopak et al. 2007), suggesting an influence of a more direct maternal fetal connection that has been associated with increased relative brain size in mammals (Martin 1996; Elliot and Crespi 2008). Developing embryos of placental species have more direct access to maternal resources, specifically to long-chain fatty acids, which are important for the development of neural tissue (Elliot and Crespi 2008). Thus, whereas lecithotrophic species are limited in their supply of resources during development (i.e. yolk sac), matrotrophic species have access to resources limited only by the mother's energetic resources.

Variation in brain size is likely to be better explained with a more nuanced measuring of maternal investment and inclusion of other reproductive parameters (i.e. litter size, gestation or incubation length). In chondrichthyans, the relative size of most major brain areas, including the telencephalon and cerebellum, are highly predictable from the overall brain size (Yopak et al. 2007, 2010*b*; Lisney et al. 2008; Yopak and Montgomery 2008), potentially owing to a conserved order of neurogenesis (Yopak et al. 2010*b*), as documented in other vertebrates (Finlay and Darlington 1995; Finlay et al. 1998, 2001). The telencephalon and cerebellum, in particular, enlarge disproportionately as the absolute brain size increases in chondrichthyans and scale similarly to the neocortex and cerebellum of mammals (Yopak et al. 2010*b*), brain areas that have been shown to continue neurogenesis longest through early development in mammals (Finlay and Darlington 1995; Yopak et al. 2010*b*). This suggests that maternal investment may not be as simple as the amount of energy allocated during development, but instead may include the amount of time over which resources are allocated, and the window of time

over which neurogenesis occurs. With gestation lengths ranging from 3 to 22 months (Wourms 1977; Wourms and Demski 1993), chondrichthyans are an ideal taxon for examining these hypotheses.

3.5.2. Life-history strategies and relative investment

There are other life-history parameters correlated with body size in chondrichthyans, which could exert some influence on the relative offspring brain size, such as litter size and gestation period, a pattern similarly observed in mammals (Pagel and Harvey 1988). In sharks, litter size is positively correlated with the maximum body size of the species, a relationship that varies with the reproductive mode (Cortés 2000; Goodwin et al. 2002). There is a trade-off between the size and number of offspring, and larger sharks tend to have smaller offspring than do smaller bodied species, after litter size is accounted for (Smith and Fretwell 1974; Cortés 2000; Goodwin et al. 2002). With fewer embryos to nourish, smaller-bodied sharks can potentially allocate proportionally more resources to each individual embryo, enhancing brain growth per individual at the cost of overall fecundity for the mother. The benefits of per-offspring investment, such as larger neonate brain sizes, may well be greater for mothers of small-bodied species (Smith and Fretwell 1974) and, indeed, there may be a greater selection for precociality in the neonates of smaller species because of the elevated risk of predation on small individuals (Branstetter 1990).

The trade-off between the litter size and pup size can also be significant, and sharks show an inverse correlation between the litter size and offspring size (Cortés 2000). Lecithotrophic species exhibit a wide range of fecundities, from 2 to 300 pups per litter (Cortés 2000), and species with the largest litter sizes tended to have the lowest relative brain sizes (i.e. *Rhincodon typus, Notorhynchus cepedianus*) (Yopak et al. 2007; Yopak and Frank 2009). Indeed, in the construction of the pGLS models to explain apparent differences in the relative brain size, the best-fit models according to AIC criterion included both gestation length and maximum litter size (C. Mull, unpubl. data), although neither of these factors exerted a significant effect alone, which suggests a

potentially fruitful avenue for further research. Although both lecithotrophic and matrotrophic species exhibited significant allometric-scaling coefficients, more variability in these linear models could potentially be explained with the inclusion of other life-history and reproductive parameters, although these are not yet available for all species.

I used a simple binary categorisation of reproductive mode into lecithotrophy and matrotrophy, which does not account for variation in the degree of maternal investment among species (Wourms and Lombardi 1992). One way of measuring the degree of relative investment in offspring is to consider the relative mass increase between ovum and neonate size. Many lecithotrophic species produce offspring that are considerably lighter than the ovum mass, likely as a result of the loss of energy because of metabolic conversion during development (Hou et al. 2008). For example, there is a 21% and 40% reduction in dry mass between the ovum and the neonate stage in the egg-laying (oviparous) lesser-spotted dogfish (*Scyliorhinus canicula*) and the lecithotrophic piked dogfish (*Squalus acanthias*) (Wourms 1993). By contrast, in matrotrophic species, there is a 1,286% and 6,806,169% increase in neonate dry mass compared with the dry ovum mass in the pelagic stingray (*Pteroplatytrygon violacea*), which exhibits uterine milk and embryonic vilification, and the sandtiger shark (*Odontaspis taurus*) which exhibits oophagy and intra-uterine cannibalism (adelphophagy), respectively (Wourms 1993). One of the greatest levels of degree of placentotrophic maternal investment is exhibited by a smallbodied spadenose shark (*Scoliodon laticaudus*), which exhibits as 5,833,845% mass increases from ovum to neonate (Wourms 1993).

3.5.3. Future directions

The tentative conclusions we draw from the pGLS analysis highlight the need for a more highly resolved phylogeny of chondrichthyans that includes branch lengths. The use of trees with different branch lengths yielded different results, with some including the reproductive mode as a significant factor affecting brain mass. The development of

broad chondrichthyan molecular phylogenies will contribute significantly to more powerful tests for potential linkages between maternal investment and brain size.

Aside from awaiting better phylogenetic hypotheses, we suggest the following three directions for future research in the area of maternal investment, brain size and brain morphology: (1) an increased sample size is needed to adequately represent and provide statistical rigour across every reproductive mode, (2) future models of allometric scaling should consider other reproductive parameters (i.e. fecundity, gestation length) as those data become available, and (3) finally, future sampling must also focus on neonatal and juvenile individuals to adequately measure any potential effects of maternal investment.

3.5.4. Conclusions

There are allometric-scaling differences between reproductive modes in chondrichthyans, with matrotrophic species exhibiting a positive grade shift in regard to their allometric-scaling relationship. Smaller-bodied species with matrotrophic reproductive modes tend to have larger relative brain sizes, whereas larger-bodied species show no differences between the modes. This suggests that additional maternal investment may have evolved to provide offspring with a 'head-start' in brain development, particularly for small-bodied species, but that, ultimately, the maximum body size may be a limiting factor on relative adult brain size. Further data collection and analysis will allow researchers to examine the energetic relationship of the maternal–fetal conflict in greater detail, and estimate the amount of caloric input per pup and how this correlates with the relative brain size, as well as accounting for other influencing factors such as litter size and gestation. The present study represents a first examination of the evolutionary linkages between the level of maternal investment and relative brain size in chondrichthyan fishes, and will hopefully provide a platform on which new studies can be built in the future.

Figure 3.1 Phylogeny of chondrichthyan brain size data

A phylogenetic tree of the 85 species used in the study, with reproductive mode indicated. White bars represent lecithotrophic species and black bars represent matrotrophic species. The relationships between the species are based on Shirai's (1992, 1996) phylogeny, with additional information from Compagno (1988), Martin et al. (1992), Naylor (1992), Didier (1995), Goto (2001), Rosenberger (2001) and McEachran and Aschliman (2004). The letters on branches represent the major chondrichthyans orders, as follows: Chimaerifomres (Ch), Rajiformes (R), Squaliformes (Sq), Orectilobifomres (O), Lamniformes (L) and Carcharhiniformes (C).

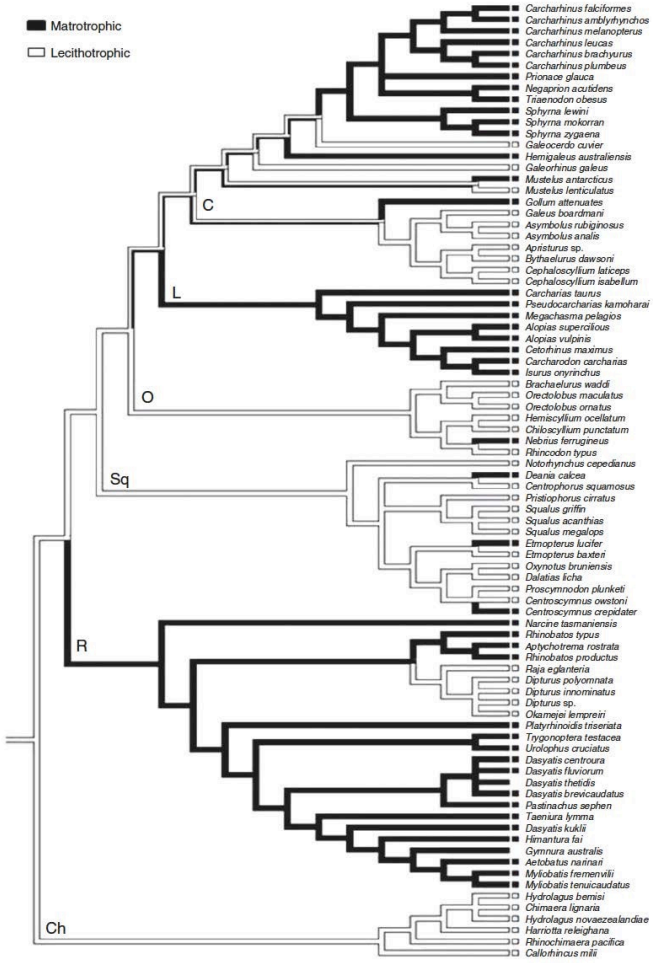


Figure 3.2 Chondrichthyan Brain-Body Allometries

Ordinary least-squares regression of the log brain mass (g) against the log body mass (g) of (a) all and (b) small-bodied species with lecithotrophy (open circles) and matrotrophy (closed circles). Reduced major axis regression of raw data of brain mass against body mass for (c) all and (d) small-bodied lecithotrophic (open circles) and matrotrophic species (closed circles). In all graphs, the solid line represents regression for lecithotrophic species and the broken line represents regression for matrotrophic species. P-values represent the results of ANCOVA tests between lecithotrophic and matrotrophic species for each regression analysis.

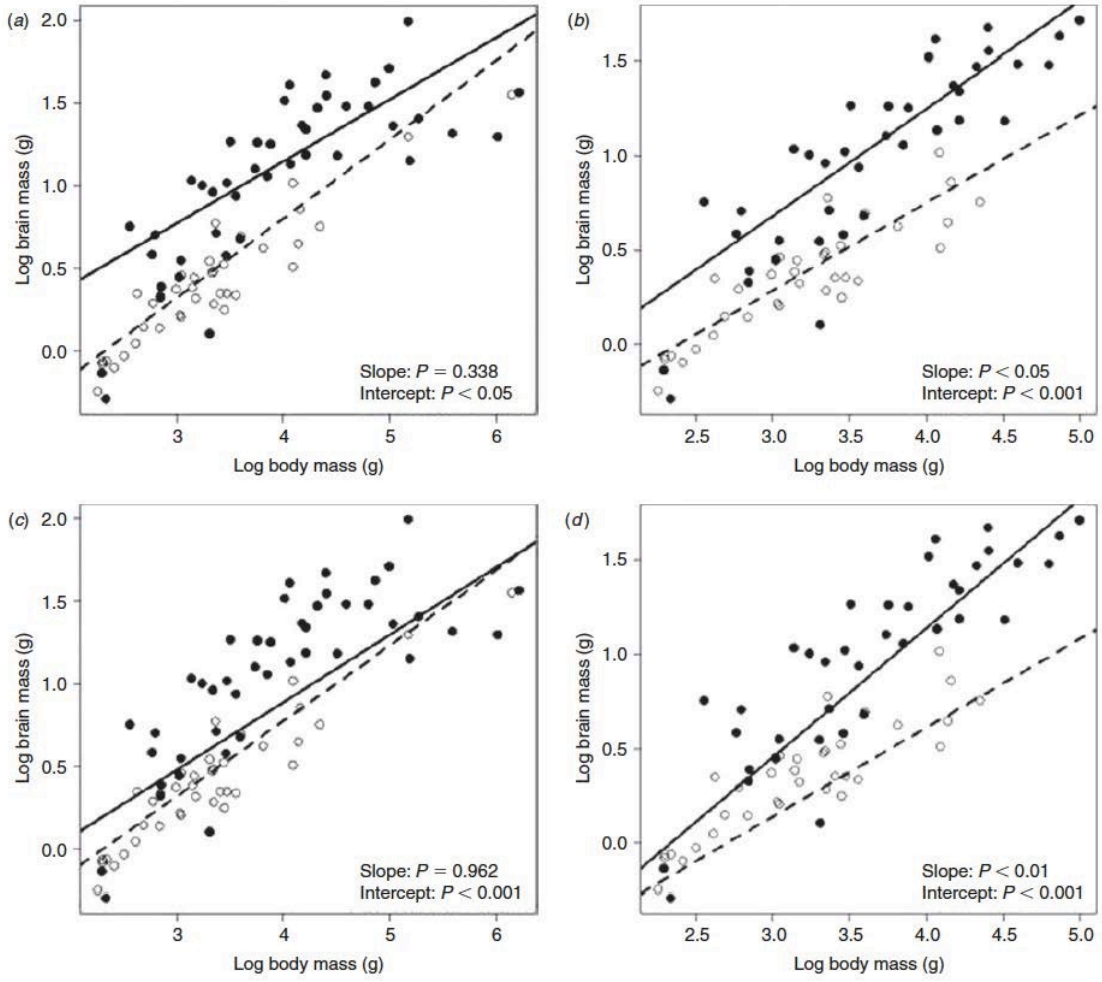


Table 3.1 Allometric scaling between reproductive modes

Parameter estimates (s.e.) of ordinary least-squares (OLS) and reduced major axis (RMA) regression of brain:body allometric scaling, using all and small-bodied (body mass <100 kg) species.

Regression	Species	Mode	Slope	Intercept	F	d.f.	r-sq	P
OLS	All	YO	0.478 (0.04)	-1.109 (0.16)	122.8	36	0.773	<0.001
		Y+	0.374 (0.05)	-0.349 (0.2)	62.9	44	0.588	<0.001
	Small	YO	0.465 (0.05)	-1.109 (0.16)	96.6	30	0.763	<0.001
		Y+	0.568 (0.05)	-1.02 (0.18)	141.9	38	0.789	<0.001
RMA	All	YO	0.456 (0.04)	-1.05 (0.16)	120.8	36	0.77	<0.0001
		Y+	0.408 (0.06)	-0.74 (0.27)	15.38	44	0.26	0.0003
	Small	YO	0.472 (0.05)	-1.07 (0.17)	79.26	30	0.725	<0.0001
		Y+	0.685 (0.05)	-1.42 (0.2)	109	38	0.734	<0.0001

Table 3.2 Brain size model selection

Generalised least-squares model (pGLS) results, with branch-length transformations used for models. The lowest Akaike information criterion (AIC) scores indicate the best model. Typically AIC values differing by 2 or more units are significantly better and are indicated with an asterisk.

Species	Branch	Model	d.f.	AIC
All	0	logbrain ~ logbody	82	-6.31*
		logbrain ~ logbody + repro	81	-2.42
	1	logbrain ~ logbody	80	-8.33*
		logbrain ~ logbody + repro	79	-4.81
	Chronopl	logbrain ~ logbody	78	8.02
		logbrain ~ logbody + repro	77	7.55
Small-bodied (body mass < 100kg)	0	logbrain ~ logbody	74	-16.95
		logbrain ~ logbody + repro	73	-18.06
	1	logbrain ~ logbody	74	-15.75
		logbrain ~ logbody + repro	73	-18.88*
	Chronopl	logbrain ~ logbody	74	-14.43
		logbrain ~ logbody + repro	73	-22.46*

Chapter 4. Matrotrophy, ecological lifestyle, and the evolution of brain size and structure in sharks, rays, and chimaeras

4.1. Abstract

The gnathostome vertebrate brain is comprised of five major regions, ordered from fore- to hindbrain: telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata. Despite similarities in the overall brain plan, it has been shown that different species have brains comprised of differing proportions of these primary structures, with little consensus as to the factors underlying this variation. Patterns of brain organization are reflect both phylogeny and ecology across a range of vertebrate groups, suggesting a combination of allometric, developmental and adaptive forces are contributing to variation in brain organization. From a developmental point of view maternal investment may be an important contributing factor in both brain size and organization. In mammals and chondrichthyans, increased maternal investment in the fetus and neonate (via increased pre and postnatal provisioning) is associated with larger relative brain size, though little is known about the links between maternal investment and the relative size of brain regions. Here, I test to what degree maternal investment and ecological lifestyle are related to variation in brain organization across 100 species and 12 of orders sharks, rays, and chimaeras (chondrichthyans). Brain size and structure varies along four principal component axes. Allometry accounts for the majority of variation, while both maternal investment and ecological lifestyle explain some of the remaining variation. Ultimately, deepwater chondrichthyans have lower levels of maternal investment and possess a relatively small brain with a large medulla. By contrast, matrotrophic chondrichthyans found in coastal reef or shallow oceanic habitats have the largest relative brain sizes, predominantly composed of telencephalon. Different

axes of brain organization vary with allometry, life history and ecology, suggesting brain organization may vary along a gradient rather than as distinct 'cerebrotypes' associated with specific ecological niches, which may broadly mirror patterns documented across vertebrates.

4.2. Introduction

Gnathostome brains are complex organs composed of five distinct regions (telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata). Correlative evidence exists between the relative size of these major regions (referred to as brain organization) and environmental parameters, suggestive of niche specific selection for cognitive, sensory, or behavioral specialization. The vast majority of variation in brain organization, however, is attributable to absolute brain size, which is closely related to body size and life history. Specifically, relative brain size is correlated with the level and duration of energetic investment and parental care (collectively referred to as maternal investment) (Iwaniuk and Nelson 2003; Elliot and Crespi 2008; Barton and Capellini 2011; Mull et al. 2011, Chapter 3). Increased maternal investment during early development can potentially influence neurogenesis, whereby brain organization is determined (Charvet and Striedter 2009; Charvet et al. 2011). Despite these lines of evidence, with few notable exceptions, little attention has been paid to the role of life history in shaping brain organization (Northcutt 2002*b*; Iwaniuk and Hurd 2005).

Consistent patterns of regional brain scaling have been observed in both chondrichthyans (sharks, rays and chimaeras) and mammals, including primates (Yopak et al. 2010*b*). As brains increase in absolute size in mammals, they become disproportionately composed of neocortex and cerebellum, the last major regions to differentiate during pre-natal brain development (Finlay and Darlington 1995; Finlay et al. 2001). Similarly, the telencephalon and cerebellum of chondrichthyans, key brain areas associated with higher cognitive function and complex motor coordination, also show a positive allometry with brain size (Yopak et al. 2010*b*; Yopak 2012). Increased

relative brain size is associated with prenatal placental and postnatal lactotrophic investment in mammals, with precociality in birds, and with matrotrophic reproduction in chondrichthyans (Iwaniuk and Nelson 2003; Elliot and Crespi 2008; Barton and Capellini 2011; Mull et al. 2011, Chapter 3). While correlation between maternal investment and brain size combined with consistent regional scaling across vertebrate groups supports a significant role of life history on brain organization, a low degree of pairwise regional covariation within birds and mammals has been suggested as evidence for the role of ecology and behavior within these groups (Barton and Harvey 2000; Iwaniuk et al. 2004).

Patterns of vertebrate brain organization have frequently been examined in light of ecology or behavior. Similar patterns of brain organization, termed 'cerebrotypes', have typically been associated with ecological and behavioral specializations across a range of taxa (Clark et al. 2001; Iwaniuk and Hurd 2005; Yopak et al. 2007). Within mammalian orders, brain organization reflects locomotion and forelimb morphology, habitat type, and diet (de Winter and Oxnard 2001). In birds, ecological correlates of brain organization have not been as clear cut, though patterns of brain organization associated with locomotion, tasks requiring enhanced cognition (e.g. vocalizations, problem solving, tool use, and social behavior), prey capture mode, and developmental mode (e.g. altricial versus precocial) (Iwaniuk and Hurd 2005; Iwaniuk et al. 2010) have been observed. (de Winter and Oxnard 2001; Iwaniuk et al. 2004). In teleosts, variation in telenephalon and optic tectum size have been correlated with habitat complexity and depth (Huber et al. 1997; Wagner 2001*a*, 2001*b*, Shumway 2008, 2010; Gonzalez-Voyer et al. 2009*a*; Gonzalez-Voyer and Kolm 2010), prey size and agility (Huber et al. 1997), sociality (Shumway 2008; Gonzalez-Voyer et al. 2009*a*), and mating system (Gonzalez-Voyer et al. 2009*a*; Gonzalez-Voyer and Kolm 2010). Within mammals and birds, weak covariation in regional size between species, has been used to support the inference of ecological or behavioral specialization not accounted for by allometric scaling

Chondrichthyan brain organization has similarly been linked to niche specialization, as specific regions may be associated with sensory perception or higher cognitive functions (Bodznick and Northcutt 1984; Bodznick 1991; Mehlhorn et al.

2010). Previous studies across a range of chondrichthyan taxa have suggested the existence two 'cerebrotypes' that vary across habitat and behavior (Yopak et al. 2007; Lisney et al. 2008; Yopak and Montgomery 2008; Yopak 2012). The reef-associated cerebrotype is characterized by a relatively enlarged telencephalon (Yopak et al. 2007), a relatively enlarged optic tectum (Yopak and Lisney 2012), and relatively reduced olfactory bulbs (Yopak et al. 2014), while the deep-sea cerebrotype is characterized by reduced telencephalon, enlargement of the medulla, specifically the termination sites of primary projections of the lateral line senses (Yopak and Montgomery 2008; Kajiura et al. 2010), a relatively reduced optic tectum (Yopak and Lisney 2012), and relatively enlarged olfactory bulbs (Yopak et al. 2014). Hypertrophy of the telencephalon is also associated with larger brains (Yopak et al. 2010*b*), and while many of these species inhabit shallow reef-associated or pelagic environments they also tend to be matrotrophic, suggesting this is not solely the result of ecological niche specialization.

In addition to commonalities in the relative development of major brain regions in certain groups of cartilaginous fishes (Yopak et al. 2007; Lisney et al. 2008; Yopak and Montgomery 2008), chondrichthyans exhibit the greatest diversity of reproductive strategies of any vertebrates taxa. Furthermore, significant differences in the intercepts of brain:body allometric slopes have been linked to differences in reproductive mode (Mull et al. 2011). Across vertebrates, chondrichthyans have the greatest diversity of reproductive modes, but they can be separated into two distinct classes of investment (Shine and Bull 1979; Dulvy and Reynolds 1997; Musick and Ellis 2005). Lecithotrophic species provide investment only in the form the yolk sac, where as matrotrophic species provide additional maternal contribution beyond the initial yolk-sac, through a range of mechanisms including placentation, oophagy and uterine milk (histotrophy) (Wourms 1977; Wourms and Demski 1993; Hamlett et al. 2005). The influence of maternal investment is striking: matrotrophic sharks and rays have brains that are 20-70% larger than lecithotrophic species (Mull et al. 2011, Chapter 3). The key question is whether trophic investment in developing chondrichthyan neonates influences not only relative size but the organization and relative size of major brain regions.

Here, I test the degree to which chondrichthyan brain organization is explained by ecological lifestyle and reproductive mode using a phylogenetic comparative model selection framework. I ask three key questions: (1) does brain organization differ between sharks and batoids, which exhibit marked differences in allometric brain scaling (Lisney et al. 2008; Yopak 2012), (2) what is the strength of pairwise regional covariation, and (3) to what degree is variation in brain organization (i.e. the relative proportion of five major components: telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata) related to brain size, maternal investment, and ecological lifestyle? If allometry and reproductive mode have a large influence, I expect absolute brain size to account for a large proportion of organizational variation, a high degree of correlation between regional sizes, and for maternal investment to be an important predictor. Conversely, if ecological lifestyle has a larger influence, I expect a lack of inter-regional correlation and that organizational variation will be associated with ecological lifestyle and associated distinctive cerebrotypes. I predict that chondrichthyan brain organization is influenced by an interplay between allometry, reproductive mode, and ecological lifestyle, and will show both covariation between major regions, with regionally independent evolution associated with ecological lifestyle.

4.3. Methods

4.3.1. Data Collection

Absolute brain mass (g), and absolute mass of the forebrain (telencephalon, diencephalon), midbrain (mesencephalon), and hindbrain (cerebellum and medulla oblongata) were collected for 100 chondrichthyan species spanning 12 orders (Naylor et al. 2012*b*; Yopak 2012) (Figure 4.1). In addition to data from 82 species collated from the literature (Yopak et al. 2007; Lisney et al. 2008; Yopak and Frank 2009; Yopak 2012), data was collected from an additional 18 species (*Carcharhinus dussumieri*, *Carcharhinus limbatus*, *Carcharhinus sorrah*, *Carcharhinus tilstoni*, *Rhizoprionodon taylori*, *Scoliodon laticaudus*, *Triakis semifasciata*, *Cephaloscyllium albipinnum*,

Heterodontus portusjacksoni, *Heptranchias perlo*, *Pristiophorus nudipinnis*, *Centrophorus harrisoni*, *Cirrhigaleus australis*, *Urolophus bucculentus*, *Urolophus paucimaculatus*, *Urobatis halleri*, *Dipturus canutus*, and *Dipturus gudgeri*).Samples were processed according to protocols from previous studies of brain organization within chondrichthyan superorders (Yopak et al. 2007). Reproductive mode was collected from the literature (Dulvy and Reynolds 1997; Last and Stevens 2009) due to known effects on relative brain mass (Mull et al. 2011, Chapter 3), and species were categorized as lecithotrophic or matrotrophic,. Lecithotrophic species nourish embryos solely by yolk-sac and can be either egg-laying (n=23) or yolk-sac live-bearing (n=26) (Wourms 1977; Dulvy and Reynolds 1997). In matrotrophic species, embryos are nourished initially via the yolk-sac followed by additional maternal contributions *in utero* being muccoid histotrophic (n=6), lipid histotrophic (n=19), oophagous (n=8), adelphophagous (intrauterine cannibalism), or placental live-bearing (placentotrophy) (n=19) (Wourms and Demski 1993; Hamlett et al. 2005) (Figure 4.1). Ecological lifestyle was characterized into four categories according to both primary habitat and lifestyle (Compagno 1990; Yopak 2012): two shallow water lifestyles (pelagic, n= 17 species; shelf, 32), deepwater (32), and reef-associated (17) (Figure 4.1). Ecological lifestyles were based on defined 'ecomorphotypes' (Compagno 1990). Deep species are benthic and benthopelagic species found along the continental slope. Shelf species are benthic, benthopelagic, or pelagic and typically found from the intertidal down to 200 meters depth. Pelagic species are found generally in depth less than 200 meters above continental slope and plain. Reef-associated species are benthic or benthopelagic and typically found in association with reef structures (coral, rocky, or deepwater).

4.3.2. Data Transformation

An inherent issue when dealing with brain regional masses is the underlying allometric relationships with total brain mass (Freckleton 2002; Revell 2009). Two main methods are commonly used to account for the underlying covariation with total brain mass, including: (1) analysing the absolute mass of regions, while using total brain mass

as a covariate in subsequent analyses (García-Berthou 2001; Freckleton 2002), and (2) the calculation of size-corrected residuals for subsequent analysis (Revell 2009). We present the analysis of absolute regional mass (method 1), because residual analysis (method 2) yields biased parameter estimates (Freckleton 2002). Both methods provide broadly consistent results, thus only results of absolute regional size are presented here. Further results are provided in the supplementary information (Section 4.6).

4.3.3. Regional Covariation

To compare the degree to which brain regions covary, I tested for pairwise covariation between regions and for grade shifts (i.e changes in intercept or regional allometries associated with taxonomy) in regional size. Pairwise covariation between regions was examined by regressing regions against each other while accounting for total body size and phylogenetic relatedness using phylogenetic Generalized Least Squares (pGLS) in the `ape` package in R (Paradis et al. 2004). This analysis allowed for direct comparison with patterns of covariation previously reported for mammals (Barton and Harvey 2000) and birds (Iwaniuk et al. 2004). I tested for grade shifts (i.e. differences in mean regional size after accounting for allometric scaling (Barton and Harvey 2000)) between taxonomic orders by regressing regional mass over total brain mass using pGLS with pairwise comparisons of order intercepts with parallel slopes using the `lsmeans` package. Grade shifts in regional size relative to total brain size, such as that seen in neocortex volume between primates and insectivores (Barton and Harvey 2000), support distinct cerebrotypes within particular lineages.

4.3.4. Phylogenetic Multivariate Analyses

I analysed absolute and regional brain mass by phylogenetic principal component analysis (pPCA) using the `phytools` package (Revell 2012) in R (version 3.1.1) (R Core Team 2013). The number of principal component axes explaining significant variation can then be used to infer: (1) the number of dimensions or axes of brain organization, (2) the relative importance of the total brain and individual regions for

explaining each axis, and (3) covariation between brain regions according to the sign of their loading on each axis. The scores for the principal components accounting for the majority of variation (i.e. up to 99%) were then examined in a model selection framework, as described next.

4.3.5. Phylogenetic Generalized Least Squares Modeling and Model Selection

I used model selection to evaluate the relative importance of ecological lifestyle and reproductive mode when explaining variation in brain organization, while controlling for evolutionary relationships using pGLS. The phylogeny for the taxa set was created by pruning a larger 610 species molecular tree (Stein et al. *In Review*) to the desired taxa set (Figure 4.1). I used a maximum likelihood approach to simultaneously estimate the phylogenetic signal in the model parameters and error structure (Freckleton et al. 2002; Revell 2010) using Pagel's λ statistic (Pagel 1999). A λ value of one indicates correlation between species reflecting Brownian motion while a λ of zero indicates no correlation between species (Pagel 1999).

I used Akaike Information Criteria (AIC) to identify the models that best explain our data from the suite of candidate models (Hilborn and Mangel 1997; Burnham and Anderson 2002). Four candidate models explaining variation in principal components were tested: (1) body mass, brain mass, reproductive mode and ecological lifestyle, (2) body mass, brain mass and reproductive mode, (3) body mass, brain mass and ecological lifestyle, and (4) body mass and brain mass. Because brain mass loaded heavily on the first principal component, as expected with multivariate allometry (Klingenberg 1996), explanatory models for PC1 only contained total body mass as a covariate. Model significance was based on a ΔAIC of 2, with the model yielding the lowest AIC value being the most supported. Post hoc testing of reproductive modes and ecological lifestyles was done by least squares means using the `lsmeans` package in R (Lenth 2016). LS means are useful to describe patterns associated with a specific variable holding other factors constant (Vélez et al. 2015). Due to limitations of sample size

within taxonomic groups model selection could only be carried out on the complete data set. To test for differences between sharks and batoids a factor tribe factor was included in the best-fit model and tribes (i.e. chimaeras, sharks, batoids) were compared using `lsmeans`.

4.4. Results

4.4.1. The strength of regional pairwise covariation

There was a high degree of pairwise covariation among chondrichthyan brain regions, after accounting for total body size across all species, as well as sharks and rays independently (Figure 4.2). Regional covariation in all chondrichthyans (Figure 4.2A) reflected those of sharks (Figure 4.2B), which is not surprising given that a large proportion of the overall dataset is comprised of sharks. Most regions covaried with the exception of cerebellum with diencephalon or mesencephalon (Figure 4.2 A,B). Negative covariation between the telencephaon and medulla likely reflects their divergent allometries with total brain size Negative covariation between the diencephalon and medulla may reflect variation between ecological lifestyles, though this is unclear due to the multifunctional nature of both regions. Batoids exhibited a higher degree of positive covariation amongst most brain regions than sharks (Figure 4.2C), though the patterns varied. Specifically in contrast to sharks, batoids did not exhibit significant covariation between the telencephalon and diencephalon, and exhibited negative covariation between the cerebellum and medulla (Figure 4.2C). Additionally the cerebellum covaried positively with both the diencephalon and mesencephalon. Regional allometries were consistent across taxonomic groups, with no evidence of grade shifts between superorders (Holocephali, Batoidea, Squalomorphii, and Galeomorphii) or orders.

4.4.2. The importance of brain size, reproductive rode, and ecological lifestyle

Regional brain organization varies across four main axes across all chondrichthyans and within sharks and batoids (Table 4.1). These axes reflect variation in regional allometries with brain mass and axes of negative regional covariation (e.g. diencephalon:mesencephalon, telencephalon:medulla; Figure 4.2). Due to statistical power limitations, we were only able to test the effect of reproductive mode and ecological lifestyle across all chondrichthyans, while testing for taxonomic differences as a factor. There was no difference between sharks or batoids across any of the 4 axes of variation (PC1 $t = -0.35$, $p = 0.94$; PC2 $t = 0.24$, $p = 0.97$; PC3 $t = 0.65$, $p = 0.79$; and PC4 $t = 0.48$, $p = 0.88$), and including tribe (i.e. chimaeras, sharks, and batoids) eroded support for all models.

The first axis was related primarily to brain size, seen by large loadings for total brain mass and all brain regions, and explained the vast majority (95%) of variance (Table 4.1). Body mass had the largest effect on total brain mass (Figure 4.3A) with reproductive mode being an important additional predictor (Table 4.2). Matrotrophic species have significantly enlarged brains relative to lecithotrophic species ($t = 3.004$, $p < 0.01$) (Figure 4.3A). While there was a trend of increasing relative brain size moving from deep to shallow ecological lifestyles (Figure 4.3A), this is driven by matrotrophic species (Figure 4.4A), as there were no difference between ecological lifestyles (Table 4.3). Lecithotrophic species exhibit similar relative brain sizes across all ecological lifestyles, while the relative brain size of matrotrophic species is significantly larger, particularly in pelagic and reef systems (Figure 4.4A).

The second axis accounts for half of the remaining variation (2.3%) and reflects independent variation between mesencephalon (loading = 0.264) and diencephalon (loading = -0.27) (Table 4.1, Figure 4.3B). There was no clear pattern of differences between reproductive modes ($t = -0.20$, $p = 0.84$), ecological lifestyles (Table 4.3) or evidence of allometric effects (Figure 4.3B, 4.4B; Table 4.2), though reef-associated

species were characterized by relatively small mesencephala. Variation in this axis may be driven by some independent evolution associated with some latent variable associated with processing of sensory information (optic tectum and tegmentum of mesencephalon) or sensory and homeostatic processes (epithalamus, dorsal thalamus, ventral thalamus, and hypothalamus of the diencephalon). Notably, the only taxonomic group to exhibit significantly enlarged diencephala relative to all other groups was the torpediniformes (electric rays), unique among chondrichthyans in their ability to generate electricity to deter predators or stun prey.

The third axis (1.5%) reflects the negative covariation between the telencephalon (0.177) and the medulla (-0.201), associated with their varying allometries. Brain mass had the largest effect on relative telencephalon and medulla size (Figure 4.3C) and ecological lifestyle was informative (Table 4.2), while there was no effect of reproductive mode ($t = -0.51$, $p = 0.61$). The strong effect of total brain size reflects the opposing allometries of these two regions. The effect of ecological lifestyle (Figure 4.4C) may reflect varying sensory and cognitive requirements. Reef-associated species are characterized by significantly larger telencephalons, while shelf and deepwater species are characterized by significantly larger medullas, irrespective of total brain mass or reproductive mode (Table 4.3). Deepwater species may be more reliant on electro and mechanoreception in the absence of light (medulla) while reef-associated species are likely more reliant on visual processing and spatial learning in complex habitats (telencephalon) (Yopak 2012).

The fourth axis (1%) reflects differences in cerebellum size relative to all other regions. There was no clear pattern of variation with brain mass or reproductive mode ($t = -0.63$, $p = 0.53$) (Figure 4.3D), though there was weak evidence of variation associated with ecological lifestyle (Table 4.2; 4.3). The largest relative cerebellum sizes were seen in pelagic species (Figure 4.3D, 4.4D), especially relative to shelf species.

4.5. Discussion

I show that brain organization in chondrichthyans varies across four main axes, rather than as a distinct clustering of cerebrotypes. The majority of variation in brain organization is attributed to absolute brain size, with subsequent axes reflecting varying allometries of distinct regions strongly related to reproductive mode and habitat. I show that smaller-bodied lecithotrophic species found in deep and bathyal waters tend to have small brains comprised of large medullas. In contrast, large-bodied matrotrophic species found in shallow water tend to have larger brains dominated by large telencephalons, with small medullas. The gradient in brain organization mirrors evolutionary transitions in both reproductive mode and the expansion of ecological niches over time. Next we examine three issues more closely: (1) patterns of regional covariation between sharks and batoids, (2) the role of maternal investment on brain size and structure, (3) the evolutionary trajectory of ecological lifestyle and reproduction, and (4) contrasting cerebrotypes with gradients in regional brain organization.

While both sharks and batoids exhibited high degrees of interregional covariation, there were marked differences between the patterns, particularly with regards to the cerebellum. In both groups, the telencephalon exhibits the steepest positive allometry with total brain size, while the medulla exhibits the shallowest negative allometry (Yopak 2012). In other words, as brain size increases they become disproportionately composed of telencephalon (Yopak 2012). Previous studies have shown a correlation between an enlarged telencephalon and species occupying complex reef habitats in both sharks and batoids (Yopak et al. 2007; Lisney et al. 2008), potentially reflecting a link between telencephalon size, habitat complexity and "social intelligence" (Gonzalez-Voyer et al. 2009*a*; Gonzalez-Voyer and Kolm 2010), a pattern similarly documented in bony fishes (Kotrschal et al. 1998). Negative covariation between the diencephalon and mesencephalon may reflect cognitive differences between deep benthic species and other ecological lifestyles (Yopak 2012), as shallowwater species more reliant on vision exhibit enlarged optic tecta, a major component of the mesencephalon (Yopak and Lisney 2012). Sharks and batoids both exhibited a high degree of variation in relative size of the

cerebellum, though patterns of covariation vary between these two groups. The enlargement of cerebellum is found in the typically large-bodied and highly mobile pelagic species (e.g. whale shark, *Rhincodon typus*, thresher sharks, *Alopias* spp., and hammerhead sharks, *Sphyrna* spp.; Figure 3) (Yopak et al. 2007; Lisney et al. 2008). Previously, larger and more structurally complex cerebellums (in terms of degree of folding of the corpus, termed foliation) have been found in highly agile species (e.g. species example), and/or large bodied species orienting in open ocean habitats (e.g. manta rays, whale shark). These organizational differences may reflect active prey capture, target tracking, and proprioception (Yopak and Frank 2009), suggestive of a link between cerebellum size and complex motor repertoires (Yopak 2012). While cerebellum varies more independently in sharks (Figure 4.2), patterns of covariation between the cerebellum and other regions within batoids may reflect differing patterns of organization among orders. Stingrays (myliobatiformes, n = 17) are characterized by large relative brain sizes, with large and highly foliated cerebellums, in contrast with electric rays (torpediniformes, n = 2), guitarfish (rhinopristiformes, n = 3), and skates (rajiformes, n = 7), species all characterized by smaller relative brain sizes and a small, smooth cerebellar corpus (Lisney et al. 2008). While we were unable to test for ecological lifestyle correlates of covariation in batoids alone due to sample size, this covariation with the cerebellum may reflect the unique array of locomotory forms of myliobatiformes stingrays, which maximizes speed and maneuverability, traits that have been previously correlated with cerebellar complexity in chondrichthyans (Lisney et al. 2008; Yopak 2012). Overall, batoids are characterized by more significant regional covariation, despite displaying similar axes of variation and diversity of ecological lifestyles and reproductive modes, although this may be an effect of smaller sample size.

In addition to correlations between ecological parameters and an increase in brain size, evidence is accumulating that pre- and postpartum maternal investment in offspring is associated with larger brain sizes (Iwaniuk and Nelson 2003; Barton and Capellini 2011; Mull et al. 2011; Tsuboi et al. 2014). My key contribution is to provide evidence that this may be a cross-vertebrate phenomenon, with new evidence from

chondrichthyans. This connection appears to be irrespective of the nature of maternal investment, whether it is indirect, via duration of parental care in cichlid fishes and birds (Iwaniuk and Nelson 2003; Weisbecker and Goswami 2010; Isler 2011; Tsuboi et al. 2014), or more direct, via energetic investment during lactation (Weisbecker and Goswami 2010), or during embryonic development in mammals and chondrichthyans (Barton and Capellini 2011; Mull et al. 2011). Underlying this connection between maternal investment and encephalization (or a larger than expected brain for a given body size), is the association between total brain size and brain organization (Yopak et al. 2010*b*). While most regions scale predictably with total brain size, evolutionary transitions in the timing of neurogenesis, size of initial cellular founder pool, or progenitor cell cycle rates, can alter patterns of brain organization (Charvet et al. 2011). Though not yet empirically shown in this group, the timing of neurogenesis and cell cycle rates could vary dramatically in chondrichthyans, as periods of embryonic development range from 4 months to 3 years (Wourms 1977; Wourms and Demski 1993) possibly 'stretching' the period of neurogenesis resulting in variation in regional allometries (Striedter 2005). Additionally, chondrichthyans exhibit indeterminate growth, whereby neurogenesis continues throughout life (Zupanc 2006), and metabolic rates vary with ambient temperatures over a large depth range (Childress 1995). Although not yet measured in chondrichthyans, these unique developmental differences could provide a mechanism to alter patterns of brain organization both within and across species, enabling an evolvable architecture in relation to both ecology and life history characteristics.

In terms of life history, there are clear differences in brain organization between species with differing life history strategies. Large-bodied matrotrophic species found in shallow water tend to have larger brains dominated by large telencephalons, with small medullas. Conversely, smaller-bodied lecithotrophic species found in deep and bathyal waters tend to have small brains comprised of large medullas (Figure 4.3). The dichotomy in brain size and organization of shallow matrotrophic chondrichthyans versus deepwater lecithotrophs appears consistent with the hypothesized evolutionary trajectory

of chondrichthyan lifestyles (Compagno 1990). Compagno hypothesized that ancestral chondrichthyans were deepwater lecithotrophs, from which the matrotrophs evolved to radiate into shallow shelf, reef, and oceanic ecological lifestyles (Compagno 1990; Sorenson et al. 2014). We suggest that this evolutionary sequence may coincide with an increase in relative brain sizes that are predominantly comprised of telencephalon and cerebellum (Compagno 1990; Dulvy and Reynolds 1997). Deepwater species are almost exclusively lecithotrophic (egg-laying and yolk-sac live-bearing) (Dulvy and Reynolds 1997; Musick and Ellis 2005), with relatively small brains (Lisney et al. 2008; Yopak and Montgomery 2008; Yopak et al. 2010*a*) with relatively larger medullas (Montgomery et al. 2012*a*) and olfactory bulbs (Yopak et al. 2014), but relatively small optic tecta (Yopak and Lisney 2012). It has been suggested that these patterns of brain organization reflect a specialization of non-visual senses in bathyal habitats, where there is a scarcity of both prey and conspecifics (Yopak and Montgomery 2008). In contrast, shallow water species employ every reproductive mode and exhibit relatively enlarged brains, with increased telencephalon and enlarged optic tecta (Yopak 2012; Yopak and Lisney 2012), suggestive of a shift to a more visual lifestyle. The pattern of brain evolution across the diversity of shallow water habitats and reproductive modes, and the ancestral state of chondrichthyans, deserves greater attention once more continuous measures of maternal investment can be developed.

While the majority of variation in chondrichthyan brain organization is associated with total brain size, half of the remaining variation is associated with deep-uniform and shallow-complex ecological lifestyles, which suggests differences in sensory and cognitive requirements. However, despite a wide range of correlative evidence between patterns of brain organization and ecology, the degree to which this pattern reflects developmental constraints (e.g. a conserved schedule of neural development), functional constraints (e.g. selection acting on functional systems spanning multiple regions such as the visual system) remains unclear. To further clarify these patterns, future work could consider variation in size of the the olfactory bulbs (Yopak et al. 2014), relative proportions of subregions of major brain structures (e.g. telencephalic pallia, optic

tectum, tegmentum), and integrated functional and sensory systems, which have shown correlations to functional or behavioral specializations in chondrichthyans (Hofmann and Northcutt 2012; Montgomery et al. 2012*b*; Yopak and Lisney 2012; Yopak et al. 2014) and other vertebrate taxa (Iwaniuk and Hurd 2005; Barton 2007; Iwaniuk et al. 2010; Gutiérrez-Ibáñez et al. 2014; Corfield et al. 2015). Likewise, the coarse aggregate ecological lifestyle categories we used may not reflect the full range of ecological or behavioral specializations within chondrichthyans. In birds, cerebrotypes are associated with tool use or complex vocalizations, though the strongest grouping is seen for feeding mode (Iwaniuk and Hurd 2005). While all chondrichthyans are predatory, feeding mode ranges from passive planktonic filtration, location and consumption of sessile invertebrates, lie-and-wait predation, or active hunting of mobile prey (Wetherbee and Cortés 2004). Each feeding mode requires specific sensory and motor repertoires, which may be reflected in brain organization (Yopak and Frank 2009). Two planktivorous filter feeders, the basking shark (*Cetorhinus maximus*) and the whale shark (*Rhincodon typus*) exhibit similar brain organization as two species of thresher sharks (*Alopias* spp.), similarly characterized by enlarged, highly foliated cerebellums; however, given the stark difference in feeding strategy between planktivorous sharks and thresher sharks, who use the elongated upper lobe of their caudal fin to stun and capture prey, much more work is required to understand the link between brain form and function (Yopak and Frank 2009). Increased research into the suite of behaviors in chondrichthyans may inform future analyses of chondrichthyan brain structure evolution.

4.5.1. Conclusions

Chondrichthyan brain organization varies across several axes, each reflecting varying influences of allometry, reproductive mode, or ecological lifestyle. The concurrent increases in brain regions with total size, particularly in the disproportionate addition of regions correlated with higher cognitive functions (e.g. telencephalon and cerebellum) may have allowed for the capitalization of new niche opportunities at the onset of and throughout the diversification of the chondrichthyan radiation. The exact

mechanisms of brain evolution remain subject to debate, yet I hypothesize that the evolution of increased maternal investment allowed for the evolution of relatively larger brains in chondrichthyans, and perhaps more generally across vertebrates. Brain evolution in chondrichthyans likely consists of a push-pull between developmental constraints (e.g. conserved sequence of neurogenesis) and ecological and behavioral specializations. The habit of defining broad taxonomic groups as conforming to one mode of evolution or another may obfuscate more nuanced patterns of brain organization. Examining where upon this gradient groups fall may help to elucidate the mechanisms and selective pressures that have driven vertebrate brain evolution, though this will require much more refined data than currently available.

Figure 4.1 Phylogeny of chondrichthyan brain organization

Phylogenetic tree of 100 chondrichthyans species across 12 orders and distribution of (A) reproductive mode and (B) ecological lifestyle. The phylogeny was obtained from Stein et al *In Review*. Reproductive modes can be broadly defined into two categories: lecithotroph and matrotroph. Lecithotrophic species nourish embryos only via an initial yolk and includes egg-laying (dark blue) and yolk-sac live-bearing (light blue). Matrotrophic species nourish embryos via additional maternal contribution beyond the yolk-sac, including uterine secretions in histotrophy (green), ovulation of unfertilized ova for *in* utero consumption in oophagy (yellow), or the development of a yolk-sac placenta following uterine implantation (red). Ecological lifestyles were based on defined 'ecomorphotypes' (Compagno 1990). Deep species are benthic and benthopelagic species found along the continental slope. Shelf species are benthic, benthopelagic, or pelagic and typically found from the intertidal down to 200 meters depth. Pelagic species are found generally in depth less than 200 meters above continental slope and plain. Reef-associated species are benthic or benthopelagic and typically found in association with reef structures (coral, rocky, or deepwater).

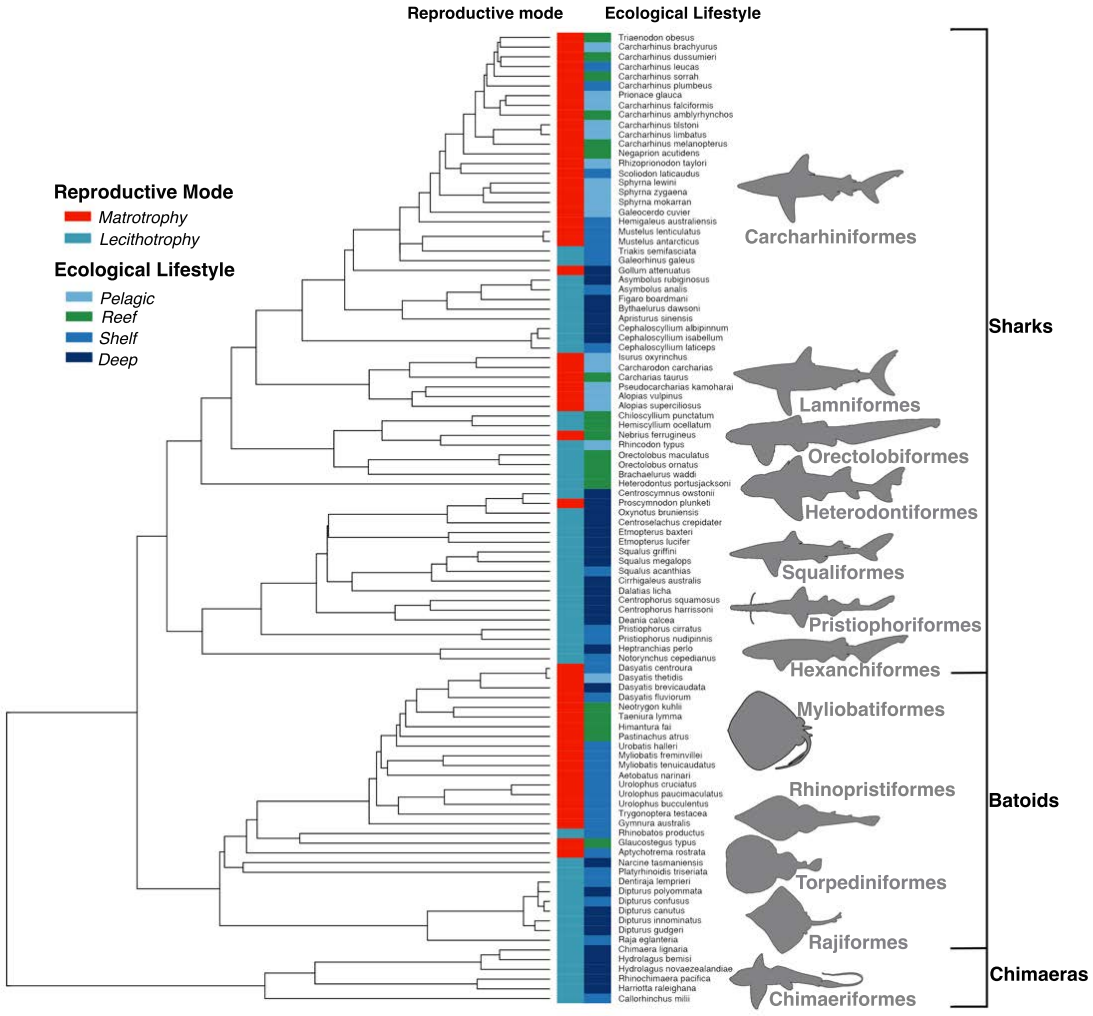

Reproductive mode
Ecological Lifestyle
Reproductive Mode
Matrotrophy
Lecithotrophy
Ecological Lifestyle
Pelagic
Reef
Shelf
Deep
Carcharhiniformes
Lamniformes
Orectolobiformes
Heterodontiformes
Squaliformes
Pristiophoriformes
Hexanchiformes
Myliobatiformes
Rhinopristiformes
Torpediniformes
Rajiformes
Chimaeriformes
Sharks
Batoids
Chimaeras

Figure 4.2 Regional covariation

Covariation between brain regions for (A) all chondrichthyans, (B) sharks, and (C) batoids. Dark lines represent significant positive covariation between regions, while grey lines represent significant negative variation between regions. Numbers in parentheses denote sample sizes for each group.

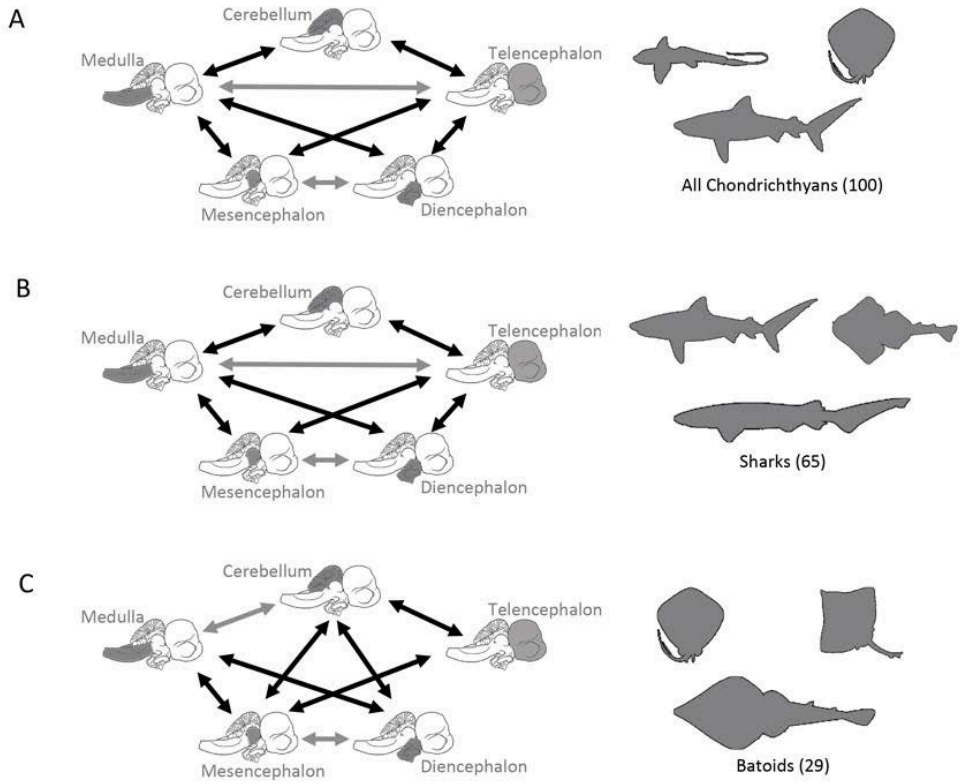


Figure 4.3 Regional variation with reproductive mode and habitat

Mean effects (± standard error) for all significant principal components across chondrichthyans (n=100). Shaded diagrams highlight the brain regions loading heavily on each component. Numbers in parentheses represent the cumulative proportion of variance explained by each component. Note brain mass was not included as a predictor variable of PC1 which characterizes variation in brain mass.

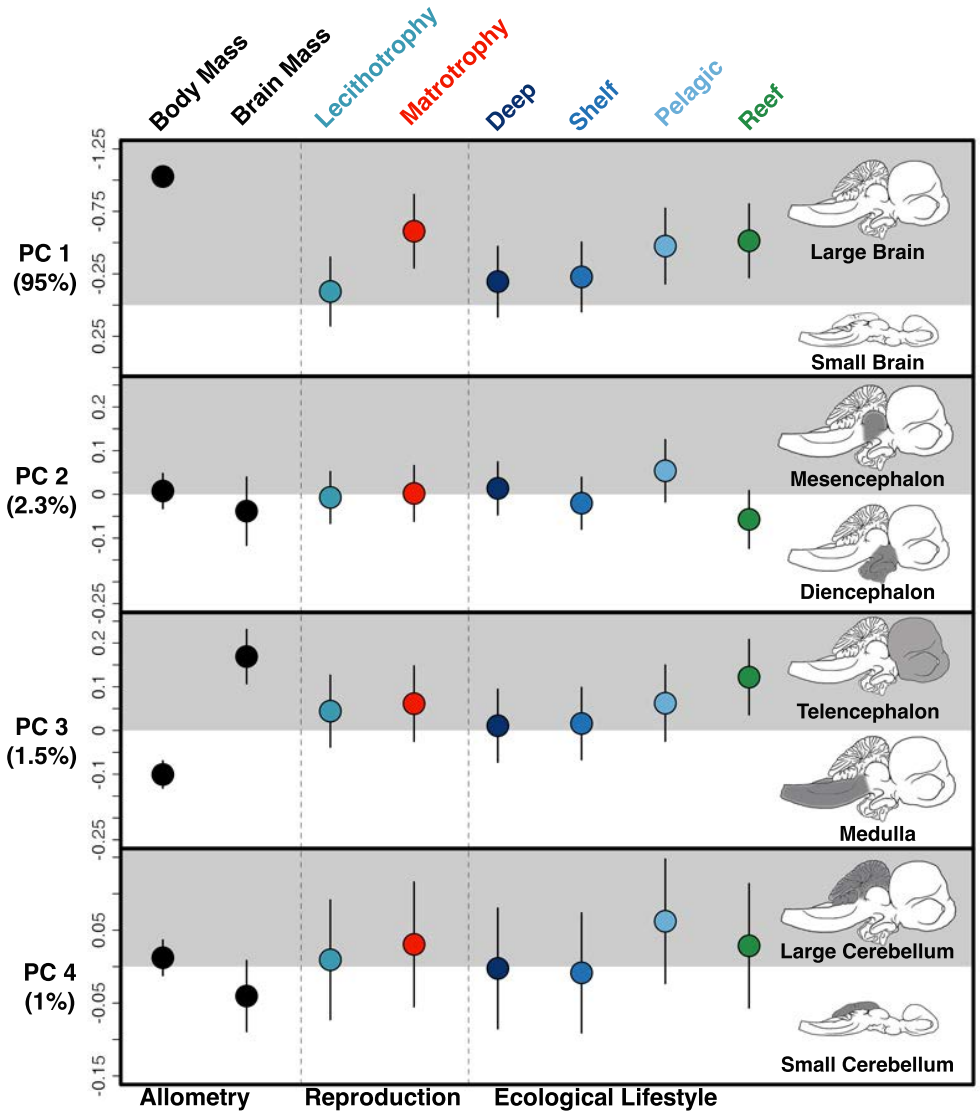


Figure 4.4 Effect of reproduction within ecological lifestyles

Least squares means (± standard error) for all principal components for lecithotrophic (blue circles) and matrotrophic (red circles) species for each ecological lifestyle. Shaded diagrams represent the regions that load most heavily on each principal component.

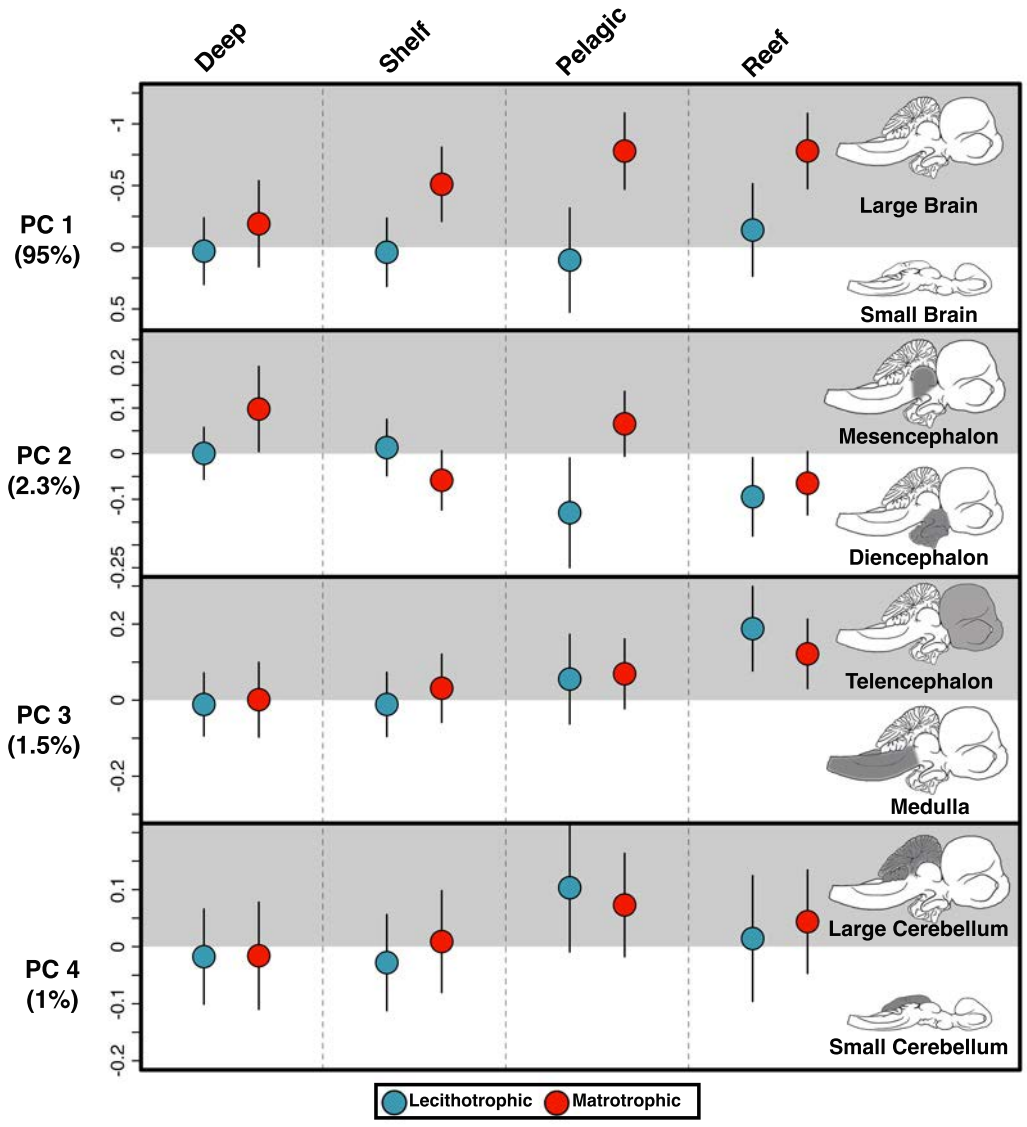


Table 4.1 Phylogenetic principal components

Eigenvector values of each brain region and total brain mass across the four main principal component axes.

	Structure	PC loadings			
		PC1	PC2	PC3	PC4
All Chondrichthyans	Telencephalon	-0.979	-0.032	0.177	0.077
	Diencephalon	-0.943	-0.296	-0.126	0.067
	Mesencephalon	-0.954	0.253	-0.061	0.115
	Cerebellum	-0.983	0.002	0.055	-0.164
	Medulla	-0.966	0.089	-0.201	-0.052
	Brain Mass	-0.998	0.013	0.036	0.003
	Standard deviation	0.081	0.013	0.010	0.008
	Proportion of variation	0.946	0.023	0.015	0.010
	Cumulative variation	0.946	0.969	0.985	0.994
	λ	0.893			
	Structure	PC loadings			
		PC1	PC2	PC3	PC4
Sharks	Telencephalon	-0.979	-0.068	0.149	-0.103
	Diencephalon	-0.944	-0.270	-0.174	-0.055
	Mesencephalon	-0.950	0.264	-0.063	-0.131
	Cerebellum	-0.981	-0.005	0.083	0.156
	Medulla	-0.967	0.114	-0.154	0.108
	Brain Mass	-0.998	-0.001	0.043	-0.005
	Standard deviation	0.087	0.014	0.011	0.010
	Proportion of variation	0.945	0.023	0.014	0.012
	Cumulative variation	0.945	0.968	0.983	0.994
	λ	0.867			

	Structure	PC loadings			
		PC1	PC2	PC3	PC4
Batoids	Telencephalon	-0.986	-0.068	0.117	-0.094
	Diencephalon	-0.949	0.311	0.001	-0.014
	Mesencephalon	-0.969	-0.207	-0.097	0.006
	Cerebellum	-0.989	-0.021	0.071	0.126
	Medulla	-0.971	0.022	-0.229	-0.014
	Brain Mass	-0.999	-0.034	0.010	-0.032
	Standard deviation	0.079	0.012	0.009	0.006
	Proportion of variation	0.959	0.022	0.012	0.005
	Cumulative variation	0.959	0.981	0.993	0.998
	λ	0.672			

Table 4.2 Model Selection

Model selection table for the major principal components across all chondrichthyans. The models with significantly greater support than all others, based on AICc, are presented in bold. M_B denotes total body mass, M_{Br} denotes total brain mass, RM denotes reproductive mode, and EL denotes ecological lifestyle. The ΔAIC is calculated relative to best model, hence models with greatest support have a ΔAIC <2 and are shown in bold and ordered by relative support. λ indicates the phylogenetic signal in the residual error of the model; a value of one indicates correlation between species reflecting Brownian motion while a λ of zero indicates no correlation between species.

Response	Candidate Model Variables	logLik	r^2	AICc	delta	weight	λ
PC1	**~M_B + RM**	**-59.06**	**0.79**	**128.12**	**0.00**	**0.89**	**0.82**
	~M_B + RM + EL	-58.28	0.79	132.57	0.00	0.10	0.78
	~M_B	-65.41	0.06	138.83	6.26	0.00	0.89
	~M_B + EL	-62.43	0.77	138.87	6.30	0.00	0.85
PC2	**~M_B + M_{Br}**	**52.51**	**0.00**	**-95.03**	**0.00**	**0.40**	**0.50**
	~M_B + M_{Br} + EL	**55.31**	**0.01**	**-94.62**	**0.00**	**0.33**	**0.44**
	~M_B + M_{Br} + RM	**52.53**	**0.00**	**-93.07**	**1.55**	**0.15**	**0.48**
	~M_B + M_{Br} + RM + EL	55.38	0.00	-92.77	1.85	0.13	0.4
PC3	**~M_B + M_{Br} + EL**	**82.62**	**0.20**	**-149.24**	**0.00**	**0.60**	**0.91**
	~M_B + M_{Br} + RM + EL	**82.69**	**0.19**	**-147.52**	**0.00**	**0.25**	**0.91**
	~M_B + M_{Br}	77.82	0.14	-145.65	1.87	0.10	0.91
	~M_B + M_{Br} + RM	78.00	0.14	-144.38	3.14	0.05	0.91
PC4	**~M_B + M_{Br} + EL**	**96.11**	**0.01**	**-176.23**	**0.00**	**0.34**	**0.97**
	~M_B + M_{Br}	**93.06**	**0.00**	**-176.12**	**0.00**	**0.33**	**0.96**
	~M_B + M_{Br} + RM	**96.16**	**0.00**	**-174.85**	**1.27**	**0.17**	**0.97**
	~M_B + M_{Br} + RM + EL	**93.13**	**0.00**	**-174.65**	**1.47**	**0.16**	**0.96**

Table 4.3 Comparison between ecological lifestyles

Post hoc testing of ecological lifestyles across the four main principal components that explain variation in chondrichthyan brain organisation. Post hoc testing was performed by pairwise comparison of least squares means for reproductive mode and ecological lifestyle. Significant pairwise tests are indicated in bold.

PC1				
	Reef	Pelagic	Shelf	Deep
Reef	-	0.28 (0.99)	-1.95 (0.21)	1.81 (0.27)
Pelagic	0.04	-	-1.51 (0.43)	1.49 (0.45)
Shelf	-0.28	-0.24	-	0.29 (0.99)
Deep	0.32	0.28	0.04	-
PC2				
	Reef	Pelagic	Shelf	Deep
Reef	-	2.18 (0.14)	-0.84 (0.83)	1.32 (0.56)
Pelagic	0.11	-	1.33 (0.55)	-0.62 (0.92)
Shelf	-0.04	0.07	-	0.78 (0.85)
Deep	0.07	-0.04	0.03	-
PC3				
	Reef	Pelagic	Shelf	Deep
Reef	-	-1.67 (0.35)	**2.91 (0.02)**	**-2.59 (0.05)**
Pelagic	-0.06	-	1.23 (0.61)	-1.20 (0.63)
Shelf	**0.11**	0.05	-	-0.25 (0.99)
Deep	**-0.11**	-0.05	-0.01	-
PC4				
	Reef	Pelagic	Shelf	Deep
Reef	-	1.12 (0.68)	1.19 (0.64)	-0.85 (0.83)
Pelagic	0.034	-	2.41 (0.08)	-1.89 (0.25)
Shelf	0.037	0.07	-	0.26 (0.99)
Deep	-0.031	-0.064	0.006	-

4.6. Supporting Information

Table 4.4 Regional Covariation Coefficients

Correlated evolution of major brain regions by multiple regressions on absolute mass, proportional mass, and size-corrected residuals. Standardized regression coefficients are reported with associated t-values in parentheses. Asterisks denote significance (*, $p < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

All Chondrichthyans				
	Diencephalon	Mesencephalon	Cerebellum	Medulla
Telencephalon	0.311 (4.07***)	0.407 (4.21***)	0.558 (6.23***)	-0.170 (-2.40*)
Diencephalon		-0.290 (-2.56*)	0.161 (1.39)	0.443 (3.19**)
Mesencephalon			0.092 (0.89)	0.599 (5.70***)
Cerebellum				0.413 (3.68**)
Sharks				
	Diencephalon	Mesencephalon	Cerebellum	Medulla
Telencephalon	0.361 (3.82**)	0.312 (3.06**)	0.523 (4.94***)	-0.166 (-1.76*)
Diencephalon		-0.289 (-2.29*)	0.090 (0.65)	0.162 (1070*)
Mesencephalon			0.009 (0.07)	0.590 (4.10***)
Cerebellum				0.497 (3.73**)
Batoids				
	Diencephalon	Mesencephalon	Cerebellum	Medulla
Telencephalon	0.043 (0.23)	1.077 (4.82***)	0.172 (0.97)	-0.675 (-2.90**)
Diencephalon		-0.957 (-4.66***)	0.803 (4.60***)	1.22 (5.86***)
Mesencephalon			0.617 (2.63*)	0.658 (10.99***)
Cerebellum				-0.299 (-2.16*)

4.6.1. Grade Shifts

We tested for grade shifts, defined as an increase in mean regional scaling associated with a taxonomically associated shift in ecological lifestyle. There was no clear evidence of grade shifts of regional size between orders (Table 4.5), superorders (Holocephali, Batoidea, Galeomorphs, Squalomorphs), or tribes (Holocephali, Batoidea, Selachii). The lack of grade shifts does not necessarily represent evidence against mosaic evolution as there are not clear transitions in ecological lifestyle associated with distinct taxonomic groups (Figure 4.1) like those seen in mammals and birds (Barton and Harvey 2000; Iwaniuk and Hurd 2005).

Table 4.5 Taxonomic Grade Shift in Brain Organization

Taxonomic grade shifts in regional mass between orders by ANCOVA. Numbers represent pairwise t-ratios and associated p-values of the intercepts for regression of absolute regional mass over total brain mass. Significant comparisons are represented in bold.

Telencephalon	Lamniformes	Orectolobiformes	Squaliformes	Myliobatiformes	Rajiformes	Rhinopristiformes	Chimaeriformes
Carcharhiniformes	-1.43 (0.16)	0.27 (0.77)	-1.58 (0.12)	0.26 (0.79)	-0.71 (0.48)	-0.71 (0.48)	-1.56 (0.12)
Lamniformes	-	1.48 (0.14)	-0.46 (0.65)	1.16 (0.25)	0.08 (0.94)	0.24 (0.81)	0.61 (0.14)
Orectolobiformes		-	-1.84 (0.07)	-0.04 (0.97)	-1.01 (0.31)	-0.92 (0.36)	1.71 (0.09)
Squaliformes			-	-1.64 (0.11)	-0.48 (0.63)	-0.67 (0.50)	0.25 (0.81)
Myliobatiformes				-	-1.43 (0.16)	-1.60 (0.12)	1.77 (0.08)
Rajiformes					-	0.26 (0.80)	0.68 (0.50)
Rhinopristiformes						-	0.85 (0.40)
Diencephalon	Lamniformes	Orectolobiformes	Squaliformes	Myliobatiformes	Rajiformes	Rhinopristiformes	Chimaeriformes
Carcharhiniformes	0.08 (0.93)	-0.11 (0.92)	1.07 (0.29)	-0.93 (0.35)	0.79 (0.43)	1.01 (0.36)	0.69 (0.49)
Lamniformes	-	-0.16 (0.87)	0.82 (0.42)	-0.88 (0.38)	0.63 (0.53)	0.83 (0.41)	-0.54 (0.59)
Orectolobiformes		-	1.08 (0.28)	0.77 (0.45)	0.82 (0.41)	1.02 (0.31)	-0.71 (0.48)
Squaliformes			-	1.80 (0.08)	0.04 (0.97)	-0.15 (0.88)	0.17 (0.86)
Myliobatiformes				-	1.74 (0.09)	**2.14 (0.04)**	-1.45 (0.15)
Rajiformes					-	0.21 (0.83)	0.12 (0.91)
Rhinopristiformes						-	0.29 (0.77)
Mesencephalon	Lamniformes	Orectolobiformes	Squaliformes	Myliobatiformes	Rajiformes	Rhinopristiformes	Chimaeriformes
Carcharhiniformes	1.85 (0.07)	-1.73 (0.09)	0.6 (0.55)	0.37 (0.71)	-0.30 (0.76)	-0.17 (0.87)	0.14 (0.89)
Lamniformes	-	**-3.16 (0.002)**	-0.86 (0.39)	-0.93 (0.36)	-1.43 (0.18)	-1.37 (0.17)	1.04 (0.31)
Orectolobiformes		-	2.11 (0.04)	-1.66 (0.10)	1.11 (0.27)	1.11 (0.27)	-1.34 (0.18)
Squaliformes			-	0.16 (0.88)	0.79 (0.43)	0.68 (0.5)	0.35 (0.73)
Myliobatiformes				-	-0.79 (0.44)	-0.73 (0.47)	0.20 (0.84)
Rajiformes					-	0.21 (0.84)	-0.40 (0.69)
Rhinopristiformes						-	-0.28 (0.78)
Cerebellum	Lamniformes	Orectolobiformes	Squaliformes	Myliobatiformes	Rajiformes	Rhinopristiformes	Chimaeriformes
Carcharhiniformes	0.74 (0.46)	0.74 (0.46)	0.47 (0.64)	0.06 (0.95)	0.25 (0.80)	0.25 (0.80)	0.52 (0.60)
Lamniformes	-	0.07 (0.94)	-0.10 (0.92)	-0.43 (0.67)	-0.18 (0.86)	-0.24 (0.81)	-0.04 (0.97)
Orectolobiformes		-	-0.17 (0.86)	0.50 (0.62)	-0.31 (0.76)	-0.31 (0.76)	0.02 (0.99)
Squaliformes			-	0.35 (0.72)	0.11 (0.91)	0.16 (0.87)	-0.13 (0.90)
Myliobatiformes				-	0.30 (0.77)	0.30 (0.76)	-0.45 (0.65)
Rajiformes					-	-0.07 (0.94)	-0.22 (0.83)
Rhinopristiformes						-	-0.27 (0.79)
Medulla	Lamniformes	Orectolobiformes	Squaliformes	Myliobatiformes	Rajiformes	Rhinopristiformes	Chimaeriformes
Carcharhiniformes	1.16 (0.25)	-0.32 (0.75)	1.45 (0.15)	-0.19 (0.85)	1.02 (0.31)	0.95 (0.35)	1.44 (0.15)
Lamniformes	16 (0.25)	-1.29 (0.20)	0.47 (0.64)	-0.95 (0.35)	0.23 (0.82)	0.45 (0.64)	-0.61 (0.54)
Orectolobiformes		-	1.73 (0.09)	-0.06 (0.95)	1.22 (0.23)	1.16 (0.25)	-1.16 (0.11)
Squaliformes			-	1.44 (0.15)	0.17 (0.87)	0.30 (0.76)	-0.24 (0.81)
Myliobatiformes				-	1.50 (0.14)	1.67 (0.10)	-1.57 (0.12)
Rajiformes					-	-0.19 (0.85)	-0.38 (0.71)
Rhinopristiformes						-	-0.51 (0.61)

Table 4.6 Brain organization between ecological lifestyles

Post hoc testing of ecological lifestyles across the four main principal components that explain variation in chondrichthyan brain organisation. Post hoc testing was performed by pairwise compaison of least squares means for reproductive mode and ecological lifestyle. Significant pairwise tests are indicated in bold.

PC1				
	Reef	Pelagic	Shelf	Deep
Reef	-	0.28 (0.99)	-1.95 (0.21)	1.81 (0.27)
Pelagic	0.04	-	-1.51 (0.43)	1.49 (0.45)
Shelf	-0.28	-0.24	-	0.29 (0.99)
Deep	0.32	0.28	0.04	-
PC2				
	Reef	Pelagic	Shelf	Deep
Reef	-	2.18 (0.14)	-0.84 (0.83)	1.32 (0.56)
Pelagic	0.11	-	1.33 (0.55)	-0.62 (0.92)
Shelf	-0.04	0.07	-	0.78 (0.85)
Deep	0.07	-0.04	0.03	-
PC3				
	Reef	Pelagic	Shelf	Deep
Reef	-	-1.67 (0.35)	**2.91 (0.02)**	**-2.59 (0.05)**
Pelagic	-0.06	-	1.23 (0.61)	-1.20 (0.63)
Shelf	**0.11**	0.05	-	-0.25 (0.99)
Deep	**-0.11**	-0.05	-0.01	-
PC4				
	Reef	Pelagic	Shelf	Deep
Reef	-	1.12 (0.68)	1.19 (0.64)	-0.85 (0.83)
Pelagic	0.034	-	2.41 (0.08)	-1.89 (0.25)
Shelf	0.037	0.07	-	0.26 (0.99)
Deep	-0.031	-0.064	0.006	-

Table 4.7 Brain organization between lifestyles and modes

Post hoc testing of ecological lifestyles and reproductive mode across the four main principal components that explain variation in chondrichthyan brain organisation. Post hoc testing was performed by pairwise compaison of least squares means for reproductive mode and ecological lifestyle combined . Significant pairwise tests are indicated in bold.

PC 1		Lecithotrophic				Matrotrophic			
		Reef	Pelagic	Shelf	Deep	Reef	Pelagic	Shelf	Deep
LEC	Reef	-	0.59 (1)	0.59 (1)	0.55 (1)	2.06 (0.45)	2 (0.49)	1.18 (0.94)	0.14 (1)
	Pelagic	0.24	-	0.17 (1)	-0.2 (1)	2.66 (0.15)	2.67 (0.15)	1.79 (0.63)	0.75 (1)
	Shelf	-0.18	0.06	-	-0.06 (1)	**3.91 (0)**	**3.8 (0.01)**	2.75 (0.12)	0.87 (0.99)
	Deep	0.17	-0.07	-0.01	-	**3.87 (0.0001)**	**3.77 (0.01)**	2.7 (0.14)	0.88 (0.99)
MAT	Reef	0.64	0.24	**0.82**	**0.81**	-	0.01 (1)	-1.73 (0.67)	2.2 (0.36)
	Pelagic	0.64	0.88	**0.82**	**0.81**	0	-	-1.62 (0.74)	2.19 (0.37)
	Shelf	0.37	0.22	0.55	0.54	-0.27	-0.27	-	1.23 (0.92)
	Deep	0.05	0.29	0.23	0.22	0.59	0.59	0.32	-
PC 2		Lecithotrophic				Matrotrophic			
		Reef	Pelagic	Shelf	Deep	Reef	Pelagic	Shelf	Deep
LEC	Reef	-	-0.28 (1)	-0.28 (1)	1.20 (0.93)	-0.34 (1)	-1.77 (0.64)	-0.43 (1)	-1.77 (0.64)
	Pelagic	-0.03	-	-1.21 (0.93)	1.12 (0.95)	-0.56 (1)	-1.73 (0.67)	-0.61 (1)	-1.73 (0.66)
	Shelf	-0.11	-0.14	-	-0.28 (1)	1.12 (0.95)	-0.73 (1)	1.17 (0.94)	-0.94 (0.98)
	Deep	0.10	0.13	-0.01	-	0.96 (0.98)	-0.93 (0.98)	0.99 (0.98)	-1.13 (0.95)
MAT	Reef	-0.03	-0.03	0.08	0.07	-	2.46 (0.23)	-0.12 (1)	1.79 (0.63)
	Pelagic	-0.16	-0.19	-0.05	-0.06	0.13	-	2.17 (0.38)	0.35 (1)
	Shelf	-0.04	-0.10	0.07	0.06	-0.01	0.12	-	1.79 (0.63)
	Deep	-0.19	-0.23	-0.08	-0.10	0.16	0.03	0.16	-
PC 3		Lecithotrophic				Matrotrophic			
		Reef	Pelagic	Shelf	Deep	Reef	Pelagic	Shelf	Deep
LEC	Reef	-	-1.22 (0.92)	-1.22 (0.92)	-2.27 (0.32)	0.76 (0.99)	1.32 (0.89)	1.78 (0.63)	1.87 (0.58)
	Pelagic	-0.13	-	0.71 (1)	-0.71 (1)	-0.76 (0.99)	-0.16 (1)	0.27 (1)	0.55 (1)
	Shelf	0.2	0.07	-	0 (1)	-2.23 (0.34)	-1.34 (0.88)	-0.77 (0.99)	-0.19 (1)
	Deep	-0.2	-0.07	0	-	-2.25 (0.33)	-1.35 (0.88)	-0.77 (0.99)	-0.21 (1)
MAT	Reef	0.07	-0.13	-0.13	-0.13	-	-1.39 (0.86)	2.32 (0.3)	-1.75 (0.65)
	Pelagic	0.12	-0.01	-0.08	-0.08	-0.05	-	0.95 (0.98)	-0.99 (0.97)
	Shelf	0.16	-0.01	-0.04	-0.04	0.09	0.04	-	-0.46 (1)
	Deep	0.19	0.05	-0.01	-0.01	-0.12	-0.07	-0.03	-
PC 4		Lecithotrophic				Matrotrophic			

		Reef	Pelagic	Shelf	Deep	Reef	Pelagic	Shelf	Deep
LEC	Reef	-	0.88 (0.99)	0.88 (0.99)	-0.38 (1)	-0.35 (1)	-0.69 (1)	0.06 (1)	0.32 (1)
	Pelagic	0.09	-	1.56 (0.77)	-1.44 (0.83)	0.76 (0.99)	0.39 (1)	1.21 (0.93)	1.36 (0.87)
	Shelf	0.04	0.13	-	0.45 (1)	-1.34 (0.88)	-1.9 (0.56)	-0.74 (1)	-0.23 (1)
	Deep	-0.03	-0.12	0.01	-	-1.16 (0.94)	-1.73 (0.66)	-0.54 (1)	-0.03 (1)
MAT	Reef	-0.03	0.09	-0.07	-0.06	-	0.92 (0.98)	1.07 (0.96)	-1 (0.97)
	Pelagic	-0.06	0.03	-0.1	-0.09	0.03	-	2.09 (0.43)	-1.53 (0.79)
	Shelf	0.01	0	-0.04	-0.03	0.03	0.06	-	-0.44 (1)
	Deep	0.03	0.12	-0.01	0	-0.06	-0.09	-0.02	-

4.6.2. Phylogenetically Size-Corrected Residuals

Phylogenetically size corrected residuals (Revell 2009) were calculated by regressing log transformed absolute regional mass over log transformed total brain mass, and by regressing log transformed total brain mass over log transformed total body mass using the `phyresid` function in the `phytools` package in R and analyzed using the same methods as the data on the absolute mass of brain regions. Because the residuals produced by this method are not phylogenetically corrected (Revell 2009), this was accounted for in subsequent analyses using the same methods as in the absolute mass analysis (e.g. pPCA and pGLS).

Phylogenetically corrected principal component analysis (pPCA) yielded five main axes that explained 99% of the variation in brain organisation and were similar to those from absolute mass (Table S4). As with the analysis of regional mass PC axes of residual regional mass reflected: allometric scaling, variation between diencephalon and mesencephalon, variation between telencephalon and medulla, and variation in cerebellum size, respectively.

The first axis described 46% of the variation and reflected differences in allometric scaling of regions with total brain size (Table S4). Total brain size loaded the most heavily while regions with positive allometry (Telencephalon and Cerebellum) both loaded in the same direction, while regions exhibiting negative allometry (Diencephalon, Mesencephalon, and Medulla) (Yopak 2012) loaded in opposite direction. Both reproductive mode and ecological lifestyle were both important explanatory variables of variation in regional allometries (Table S5).

The second axis explained 23% of the variation and reflected the negative covariation between diencephalon (-0.86) and mesencephalon (0.75), as seen in the analysis of absolute mass. There was weak support for reproductive mode and ecological lifestyle as explanatory variables for variation in the second axis (Table S5).

The third axis explained 13.6% of the variation and reflected the increased allometric scaling of telencephalon (-0.78) relative to all other regions, particularly medulla (0.72). Ecological lifestyle was an important explanatory variable of this variation while there was weak support for reproductive mode. The importance of ecological lifestyle reflects the gradient in telencephalon:medulla variation with depth (Figure 3 main text).

The fourth axis represents 9.6% of variation and reflects variation in residual cerebellum size (-0.86) relative to all other regions. Residual brain size was the most important explanatory variable of residual cerebellum size and there was weak support for ecological lifestyle and reproductive mode as explanatory variables.

Table 4.8 Phylogenetic PCA of size-corrected residuals

Phylogenetic principal component (pPCA) for phylogenetic size-corrected residuals of regional brain mass.

Structure	PC loadings					
	PC1	PC2	PC3	PC4	PC5	PC6
Telencephalon	-0.347	-0.233	-0.781	0.339	-0.006	0.317
Diencephalon	0.264	-0.860	0.342	0.214	-0.168	0.016
Mesencephalon	0.217	0.751	0.279	0.379	-0.407	0.036
Cerebellum	-0.064	-0.072	0.098	-0.860	-0.480	0.106
Medulla	0.447	0.262	0.719	-0.116	0.419	0.160
Brain Mass	-0.984	-0.012	0.175	0.034	0.001	0.004
Standard deviation	0.018	0.012	0.009	0.008	0.007	0.003
Proportion of variation	0.463	0.231	0.136	0.096	0.064	0.010
Cumulative variation	0.463	0.694	0.830	0.926	0.990	1.000
λ	0.876					

Table 4.9 Model Selection of size-corrected residuals

Model selection table for the major principal components of phylogenetic size-corrected residual analysis. The models with significantly greater support than all others, based on AICc, are presented in bold. M_B denotes total body mass, M_{Br} denotes total brain mass, RM denotes reproductive mode, and EL denotes ecological lifestyle. The ΔAIC is calculated relative to best model, hence models with greatest support have a ΔAIC <2 and are shown in bold and ordered by relative support.

Response	Candidate Model Variables	df	logLik	r^2	AICc	delta	weight	λ
PC1	**~M_B + RM + EL**	**94**	**25.19**	**0.19**	**-29.92**	**0.00**	**0.800**	**0.770**
	~M_B + RM	97	20.72	0.13	-27.81	2.11	0.190	0.830
	~M_B + EL	95	18.75	0.06	-23.49	6.43	0.003	0.860
	~M_B	98	14.05	0.00	-20.09	9.83	0.000	0.910
PC2	**~M_B + M_{Br}**	**97**	**52.66**	**0.000**	**-95.3**	**0.00**	**0.400**	**0.420**
	~M_B + M_{Br} + EL	**94**	**55.40**	**0.010**	**-94.80**	**0.52**	**0.310**	**0.370**
	~M_B + M_{Br} + RM	**96**	**52.71**	**0.000**	**-93.41**	**1.91**	**0.160**	**0.400**
	~M_B + M_{Br} + RM + EL	93	55.52	0.001	-93.05	2.27	0.130	0.330
PC3	~M_B + M_{Br} + EL	**94**	**84**	**0.12**	**-152.57**	**0.00**	**0.620**	**0.920**
	~M_B + M_{Br} + RM + EL	**93**	**84.42**	**0.11**	**-150.96**	**1.61**	**0.280**	**0.920**
	~M_B + M_{Br}	97	78.98	0.05	-147.96	4.61	0.060	0.920
	~M_B + M_{Br} + RM	96	79.26	0.05	-146.91	5.66	0.040	0.920
PC4	**~M_B + M_{Br}**	**97**	**93.39**	**0.00**	**-176.77**	**0.00**	**0.430**	**0.960**
	~M_B + M_{Br} + EL	**94**	**96.02**	**0.01**	**-176.05**	**0.72**	**0.300**	**0.970**
	~M_B + M_{Br} + RM	**96**	**93.42**	**0.00**	**-174.85**	**1.92**	**0.160**	**0.960**
	~M_B + M_{Br} + RM + EL	93	96.05	0.00	-174.1	2.67	0.110	0.970

Chapter 5. Regional Scaling and Brain Organization Variation Between Vertebrate Classes

5.1. Abstract

Vertebrate brains exhibit exceptional morphological diversity, despite the similarity in primary components (telencephalon, diencephalon, mesencephalon, cerebellum (in all gnathostomes), and medulla oblongata). This diversity likely arises from selection for key behavioral or cognitive traits unique to individual groups, though it is unclear how this happens in different lineages. Selection is thought to act either through developmental mechanisms (such as a conserved sequence of neurogenesis) to produce concurrent changes throughout the brain, or through functional constraints (such as functionally integrated regions or pathways, like the visual system) to produce regional changes independently from total brain size. While there has been substantial debate about the degree to which particular groups, namely mammals and birds, conform to these models, there has been little work on what factors may determine the relative influence of developmental of functional constraints across vertebrates. Here I analyze all the available data on total body, brain, and regional size from six distinct vertebrate lineages to qualitatively assess differences in brain organization. There is a gradient in the proportion of variance explained by total brain size, with mammals at the upper end (98.5%) and frogs at the lower end (80.1%). Mammals, birds, and chondrichthyans exhibit similar regional scaling characterized by positive allometries of the telencephalon and cerebellum, though the degree of regional covariation varies. Conversely, teleosts, lampreys, and frogs are characterized are characterized by negative allometric scaling of the telencephalon, positive allometric scaling of the medulla, and a low degree of covariation between brain regions. Though correlative, these results hint at a potential influence of life history, and I propose that an interplay of life history strategy,

particularly reproduction and complex life cycles, and varying rates of neurogenesis throughout ontogeny dictate the arena in which selection can act upon developmental and functional constraints.

5.2. Introduction

The mechanisms underpinning vertebrate brain evolution are an area of constant debate, despite recent efforts to understand and explain patterns across a broad range of lineages (Charvet and Striedter 2011). While researchers have studied the evolution of relative brain size in the context of cognitive benefits (Sol et al. 2005, 2008) and associated energetic costs (Iwaniuk and Nelson 2003; Isler and van Schaik 2006; Barton and Capellini 2011; Mull et al. 2011; Gonzalez-Voyer et al. 2016; Sukhum et al. 2016, Chapter 3, 4), there is little consensus on how individual brain regions and overall brain organization (the relative size of individual brain components) has evolved. Despite the diversity of vertebrate forms, life histories, ecologies, and behaviors, there are marked similarities in the primary regions of the vertebrate brain plan, with potentially conserved developmental mechanisms by which they form (Reichert 2009; Charvet et al. 2011). In gnathostome vertebrates, these broadly defined regions are comprised of the telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata. Understanding how the size of these individual brain regions changes with total brain size across a wide range of vertebrate groups has implications for understanding the functional advantages of increased brain size across evolutionary time, as well as the link between brain structure and cognitive or sensory specialization.

The use of relative brain size as a relevant metric for general cognition has been extensively debated (Healy and Rowe 2007; Sayol et al. 2016*a*). Despite the fact that increased relative brain size has been associated with increased cognition (Sol et al. 2005), sociality, tool use (Lefebvre et al. 2002; Mehlhorn et al. 2010), environmental variability (Sayol et al. 2016*b*), and survival (Sol et al. 2007) in birds and mammals, this metric has been criticized because brains are composed of several functionally distinct

areas (Healy and Rowe 2007). These broadly defined regions can vary not only in absolute volume but also in terms of functional subregions, degrees of functional connectivity between regions, and in neuron density (Montgomery et al. 2016). Critics contend that analyses should focus on the regions that functions of interest can be attributed to rather than total brain size (Healy and Rowe 2007; Montgomery et al. 2016). The validity of this critique hinges on whether region size scales predictably with absolute brain size. If regional size does not scale predictably, then total brain size would be an inappropriate metric for general cognition; alternatively if region size does scale predictably, then total brain size presents a useful metric for interspecific comparisons. In chondrichthyans and mammals, a conserved pattern of scaling has been observed, with similar allometric slopes for major brain regions (Yopak et al. 2010*b*). In both groups, the telencephalon and cerebellum (regions associated with higher cognitive and motor functions) exhibited positive allometries, such that larger brains become disproportionately composed of these regions relative to all others. Increased brain size is associated with cognition in mammals (Sol et al. 2008) and birds (Sol et al. 2005, 2007; Sayol et al. 2016*b*), while in chondrichthyans it is associated with more complex feeding, social, and courtship behaviors and habitat complexity (Yopak 2012). While these patterns are purely correlative, in these groups at least it suggests that relative brain size could be a useful metric of cognitive function or specialized motor, sensory, or behavioral repertoires. Despite the intriguing evidence for convergent patterns of brain allometry in these widely divergent clades, whether this pattern is evident in other vertebrate groups is still unknown, but is vital for gaining an understanding of the causes and consequence of behavioral complexity across vertebrates.

While these conserved patterns of scaling exist, further debate remains about what drives patterns of covariation between brain regions. Much of the work has been done in mammals and birds, and has focused on whether the evolution of regional size is a product of developmental constraints (Finlay and Darlington 1995; Finlay et al. 2001, 2010; Charvet et al. 2011) or functional constraints (Barton and Harvey 2000; Iwaniuk et al. 2004; Hager et al. 2012; Montgomery et al. 2016). Surprisingly much of the

controversy centers on the interpretation of two datasets, and the inferences from various treatments of the data. While the usefulness of volumetric or mass data of regional brain organization has been questioned (Montgomery et al. 2016), in favor of more refined measurements such as neuron number (Herculano-Houzel 2011, 2012; Barton 2012), it is still the metric that exists for the largest number of species, and thus the best data to use to test cross-vertebrate predictions.

Differences in life history within and across clades have been proposed as an explanation for deviations in patterns of brain organization from predictable slopes (Northcutt 2002*a*), yet this idea has received relatively little attention in comparison to selection for behavioral or sensory specialization. In mammals (Barton and Capellini 2011), birds (Iwaniuk and Nelson 2003), and chondrichthyans (Mull et al. 2011), increasing time and energy allocated to developing offspring (pre and/or postnatal) is correlated with an increase in relative brain size. Interestingly, these lineages are characterized by slower life history traits, particularly in the production of few large offspring, which after the period of parental care (maternal investment and post-partum parental care), are in most respects small replicas of adults referred to as proportional offspring size (Neuheimer et al. 2015). In contrast, reproduction in teleosts and amphibians is predominantly characterized by the production of numerous small offspring, which often undergo a distinct larval phase before metamorphosing into juveniles referred to as invariant offspring size (Neuheimer et al. 2015). During these larval periods, neurogenesis may be constrained by physical space (Northcutt 2002*a*) and susceptible to environmental influence (Woodley et al. 2015), potentially affecting patterns of brain organization. The varying reproductive strategies exhibited in vertebrates may be an important factor in determining patterns of regional scaling given correlations between maternal investment and brain size (Iwaniuk and Nelson 2003; Capellini et al. 2011; Mull et al. 2011) and demonstrated developmental mechanisms that can affect the evolution of brain organization (Charvet et al. 2011).

In this study I ask three main questions across vertebrates: (1) how reliable is brain size as a predictor of brain organization, (2) is there a universal pattern of brain

region scaling across vertebrates, and (3) do patterns of regional scaling and covariation differ between groups with varying reproductive strategies? I predict that brain organization will differ between vertebrate classes and that life history, in particular reproductive strategies, will influence regional scaling patterns between groups. In groups with proportional offspring size and no metamorphosis I predict that brain organization will be predictable from brain size, but that regional allometries will reflect lineage specific behavioral or cognitive specializations. Conversely I predict that in groups with invariant offspring size and a distinct larval phase, brain size will be a less reliable predictor of brain organization.

5.3. Methods

5.3.1. Data Collection

All available datasets of body size, total brain size, and regional brain size were collated for six major vertebrate clades: mammals (n = 112 species) (Reep et al. 2007), birds (61 species) (Iwaniuk and Hurd 2005), bony fishes (cichlids, 43 species) (Gonzalez-Voyer et al. 2009*b*), amphibians (anuran frogs, 43 species) (Liao et al. 2015), lampreys (15 species) (Salas et al. 2017), chondrichthyans (sharks, rays, and chimaeras, 100 species) (Chapter 4). Brain regions were defined as the five major components that comprise gnathostome vertebrate brains: telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata. Lampreys do not possess a true corpus cerebellum (Northcutt 2002*a*) and total brain mass only included telencephalon, diencephalon, mesencephalon and medulla oblongata. All data were $\log_{10}$ transformed prior to analysis to achieve normality and linear scaling.

An inherent issue when dealing with brain regional masses is the underlying allometric relationships with total brain mass (Freckleton 2002; Revell 2009). Two main methods are commonly used to account for the underlying covariation with total brain mass, including: (1) analysing the absolute mass of regions, while using total brain mass

as a covariate in subsequent analyses (García-Berthou 2001; Freckleton 2002), and (2) the calculation of size-corrected residuals for subsequent analysis (Revell 2009). I present the analysis of absolute regional mass (method 1), because residual analysis (method 2) yields biased parameter estimates (Freckleton 2002).

Analyzing species data using traditional statistical techniques violates assumptions about the independence of data points due to the underlying phylogenetic relationships between species. To account for phylogenetic relatedness of species in each data set, I used published molecular phylogenies and a phylogenetic comparative framework. Recent molecular phylogenies for mammals (Bininda-emonds et al. 2007), birds (Jetz et al. 2012), chondrichthyans (Stein et al. *In Review*), Lake Tanganyika cichlids (Amcoff et al. 2013), lampreys (Lang et al. 2009; Potter et al. 2015), and amphibians (Alexander Pyron and Wiens 2011) were collated and pruned to include only the species in our datasets. Subsequent tests were applied to all groups to examine for commonalities and allow for across clade comparisons, and all tests were performed in the R (version 3.3.2) software package (R Core Team 2013).

5.3.2. Regional Scaling

Individual brain regions do not necessarily scale isometrically with brain size, and patterns may vary between groups. To assess how individual regions scaled with total brain size within each vertebrate group, regional allometric slopes were determined by regressing regional mass or volume over total brain mass or volume using phylogenetic least squares regression (pGLS) as:

$$\text{regional mass} \sim \text{total brain mass}$$

Positive allometry is characterized by regions with scaling slopes larger than one, and as brains become larger in absolute size they become more predominantly composed of these regions. Regions with scaling slopes less than one exhibit negative allometry and

become proportionally smaller as brain increase in absolute size. pGLS analyses were performed using the `caper` (version 0.5.2) (Orme et al. 2012) package in R.

5.3.3. Phylogenetic Multivariate Analyses

I analysed absolute and regional brain mass within each group using phylogenetic principal component analysis (pPCA) implemented in the `phytools` package (version 0.5-64) (Revell 2012). The number of principal component axes explaining significant variation can then be used to infer: (1) the number of dimensions or axes of brain organization, (2) the relative importance of the total brain and individual regions for explaining each axis, and (3) covariation between brain regions according to the sign of their loading on each axis. In principal component analyses where all variable are correlated with a size variable (in this instance absolute brain size), the first principal component corresponds to an isometric size variable, and all subsequent principal components represent variance in the size of different regions independent of brain size (Klingenberg 1996).

5.3.4. Regional Covariation

Pairwise covariation between regions was examined by regressing regions against each other while accounting for variation attributable to other regions, total body size and phylogenetic relatedness using pGLS. Covariation was established between all possible combinations of regions with total brain size. For example covariation between telencephalon and all other regions was calculated as:

$$\log \text{tel} \sim \log \text{die} + \log \text{mes} + \log \text{cer} + \log \text{med} + \log \text{body mass}$$

A cluster analysis was performed on the resulting variance covariance matrix to examine whether variation in organization occurred across all regions of the brain in concert (a single cluster) or within particular modules (distinct clusters of regions).

Cluster analysis was performed on the variance covariance matrix using `igraph` (version 1.0.1) (Csardi and Nepusz 2006).

5.4. Results

5.4.1. Regional Scaling

There is qualitatively similar regional scaling between mammals, chondrichthyans, and birds. In all three groups, only the telencephalon and cerebellum exhibited positive allometry while all other regions exhibited negative allometry (Figure 5.1 B,D,F). While mammalian and avian brains are dominated by telencephalon regardless of total brain size (Figure 5.1 A,E), small brains in chondrichthyans are predominantly composed of medulla while large brains are predominantly composed of telencephalon (Figure 5.1 C). Overall these three groups are characterized by little variation in regional scaling. In contrast cichlids, lampreys, and frogs were all exhibited isometry or negative allometric scaling of telencephalon and isometry or positive allometric scaling of medulla. Notably in cichlids and lampreys, the telencephalon represents a smaller portion of overall brain size in contrast to all other groups. Cichlid brains are dominated by optic tectum, with telencephalon, cerebellum, and diencephalon as moderate components and reduced medulla. Lamprey brains are dominated by medulla and diencephalon, with mesencephalon and telencephalon as minor components of total brain size.

5.4.2. Brain Size and Covariation

There is a gradient in the proportion of variation in brain organization attributable to total brain size with mammals and frogs at opposing ends of the spectrum. In mammals, total brain size accounts for over 98.5% of the total variation in brain organization, while in chondrichthyans and birds total brain size accounts for 94.6 and 93.4% of variation respectively (Figure 2 A,C,E; PC1 Table 5.1). Subsequent variation in

brain organization, after accounting to brain size, differs with mammals being attributed to telencephalon and medulla (PC 2, Table 5.1) and mesencephalon (PC3), while in chondrichthyans subsequent variation is attributable to diencephalon and mesencephalon (PC2), telencephalon and medulla (PC3), and Cerebellum (PC4). In mammals and chondrichthyans, there is a high degree of covariation between regions and brain organization tends to vary with concomitant changes across all regions (Figure 5.2 B,D; Table 5.2). In birds, subsequent variation after accounting to total brain size is attributable to telencephalon and mesencephalon (PC2) and cerebellum and medulla (PC3). However, in birds, regions vary more independently of one another (Figure 5.2C), with a lower degree of covariation between all regions, and organizational variation occurring in three distinct modules: telencephalon and diencephalon, cerebellum and medulla, and mesencephalon). In cichlids and lampreys, brain size accounted for 90.8% of variation in organization (Figure 5.2 G,I), with two distinct clusters observed in each clade. In cichlids, organization varied with associated changes between the telencephalon, diencephalon, and medulla representing one cluster and cerebellum and mesencephalon representing another cluster (Figure 5.2H). Variation after total brain size is attributable to medulla (PC1), optic tectum (PC2), and cerebellum (PC3). In lampreys, the diencephalon varied independently of all other regions, with the rest of the brain covarying concomitantly (Figure 5.2 J). In frogs, brain size accounted for the lowest portion of overall variation in organization (80.9%), and organization variation occurs with three distinct clusters: telencephalon and medulla, cerebellum and mesencephalon, and diencephalon.

5.5. Discussion

For the first time I present a concurrent assessment of brain region scaling and covariation across six major vertebrate clades (jawless, cartilaginous and bony fishes, amphibians, birds, and mammals). I find: (1) that brain size is not a reliable predictor of brain organization across vertebrate classes, but rather a gradient in the proportion of brain organization attributable to allometry; (2) similar scaling in mammals,

chondrichthyans, and birds that differs starkly from teleosts, lamprey, and amphibians; and (3) evidence of regional scaling and covariation differences between groups with different reproductive life history strategies. Regional scaling in mammals, chondrichthyans and birds is characterized by positive allometric scaling of the telencephalon and cerebellum. However, while organizational changes in mammals and chondrichthyans are characterized by a high degree of covariation within the whole brain, covariation in birds occurs between three distinct groupings. Conversely, cichlids, lampreys, and frogs are characterized by lower proportions of brain region size explained by absolute brain size, negative allometric scaling of the telencephalon, positive allometric scaling of the medulla, and a low degree of covariation between brain regions. Differences in scaling and patterns of covariation across these groups suggest that despite broad structural and mechanistic similarities in brains across vertebrates, there may likely be selection for key behavioral or cognitive traits unique to individual groups, which is mediated by the susceptibility to selection and functional connectivity of distinct regions.

An unresolved issue within vertebrate brain evolution is the degree to which selection acts on developmental or functional constraints to shape brain organization. Under the concerted model of brain evolution, regions evolve in a coordinated fashion due to developmental constraints, such as a highly conserved sequence of neurogenesis (Finlay and Darlington 1995). Under the mosaic model of brain evolution, regions or modules can evolve independent of absolute brain size (Iwaniuk et al. 2004), likely related to differential selection pressures associated with niche-specific tasks (Striedter 2005). While it has been increasingly accepted that brain organization likely evolves by a combination of both modes (Striedter 2005; Gutiérrez-Ibáñez et al. 2014), there are no working hypotheses about what may drive the relative influence of developmental and functional constraints. In part this may be due to insufficient data available to address this question across a large number of taxa, as patterns of regional covariation can be attributed to either mode depending on the underlying assumptions about regional integration and regulation (Montgomery et al. 2016). Indeed the relative influences of developmental or functional constraints likely differ between groups, and examining

patterns across divergent vertebrate clades with various lifestyles and behaviors can be useful for identifying correlates and developing future testable predictions. Brain evolution does not occur in isolation, but rather as facet of complex organisms shaped by ecology and life history (Northcutt 2002*a*), and future approaches should reflect this.

Brain organization across vertebrate groups likely reflects the sensory or cognitive requirements to succeed in varying habitats and ecological lifestyles, though information from a single stimulus may be processed in numerous regions. Birds are highly visual, and while up to 90% of projections from the retinal ganglion cells terminate in the optic tectum (mesencephalon, a major proportion of overall brain size (Figure 5. 1E)), the nucleus rotundus in the thalamus (diencephalon) and entopallium (telencephalon) are also important components for the relaying and processing of visual stimuli (Gutiérrez-Ibáñez et al. 2014). Surprisingly, these regions do not consistently covary, and this may be due to differing reliance on visual acuity, depth perception, color discrimination, and detection of UV wavelengths (Iwaniuk et al. 2010). Telencephalic expansion has been proposed as a cause of the observed decrease in the relative size of the tectofugal pathway with increasing brain size (Iwaniuk et al. 2010), and this expansion combined with varying modes of evolution within distinct elements of the pathway (Gutiérrez-Ibáñez et al. 2014) may help explain the lack of covariation. Similarly, teleosts are predominantly visually oriented (Kotrschal et al. 1998), reflected in the enlarged optic tectum relative to all other structures. The varying degrees of connectivity have not been fully resolved across vertebrate groups, and may help to clarify how selection for a particular function can affect the entire brain.

I propose that an interplay of life history strategy, particularly reproduction and complex life cycles, and varying rates of neurogenesis throughout ontogeny dictate the arena in which selection can act upon developmental and functional constraints. Although speculative, given the two distinct reproductive strategies exhibited by these vertebrate groups, proportional and invariant offspring size (Neuheimer et al. 2015), patterns of brain organization may be associated with differences in life history amongst other variables. In mammals with relatively large well-developed offspring and no

metamorphosis, we may expect developmental constraints (such as the sequence of neurogenesis) to play a larger role. In mammals, the bulk of neurogenesis occurs during early development, with proliferative zones in adult brains restricted to only two or three areas, leaving relatively little arena for selection to act and reducing the influence of functional constraints. The tight scaling of regions with total brain size, the vast majority of variation in brain organization being attributable to absolute brain size (Charvet and Finlay 2012), and high degree of regional covariation in mammals are in line with this prediction. Conversely, in cichlids with relatively small offspring, a distinct larval phase, and neurogenesis that continues throughout life, we may expect selection to act on functional constraints though ontogeny. The requirement of visual feeding combined with limited inter-cranial space during the larval phase have been hypothesized to drive optic tectum enlargement and everted cerebral hemisphere unique to teleosts (Northcutt 2002*a*). Further, extended periods of adult neurogenesis and thus neuronal maturation may increase the susceptibility of the brain phenotype to environmental or ecological pressures (Charvet et al. 2011), resulting in the expansion of key sensory brain regions in association with niche (Gonzalez-Voyer and Kolm 2010), potentially reflected in a low degree of covariation in this group. In fact total brain size, telencephalon, cerebellum size, and subsequent increases in visual acuity and spatial memory are all positively correlated with increasing habitat complexity and social complexity (Shumway 2008, 2010). This susceptibility to environmental pressures may not be limited to species with a distinct larval phase, but may extend to other groups with higher rates of post-embryonic neurogenesis (such as fish, amphibians, and some reptiles) (Zupanc 2006) coupled with varying developmental modes. In fact, it has been proposed that in altricial (e.g. requiring extensive post-hatching parental care) parrots and songbirds, extending the period of post-hatching telencephalic maturation maybe be crucial for learning vocalizations during the juvenile phase (Kirn 2010; Charvet et al. 2011). Conversely in precocial waterfowl (e.g. independent almost immediately post-hatching, with the majority of neurogenesis complete by hatching) telencephalic expansion is achieved by enlarging the cellular precursor pool prior to the onset of neurogenesis (Charvet and Striedter 2009). In addition to the understanding of early development, post-natal neurogenesis influences brain

organization, especially in species with indeterminate growth. In sharks inhabiting distinct habitats between their juvenile and adult phases, there are marked changes between visual and olfactory systems throughout ontogeny (Lisney et al. 2007) potentially reflecting selection for varying sensory importance in different environments. Despite apparent homologies with vertebrate developmental mechanisms (Ferreiro-Galve et al. 2008; Rodríguez-Moldes et al. 2008; Pose-Méndez et al. 2014), our understanding of chondrichthyan early neural development is limited to small, deepwater, egg-laying small-spotted catsharks (*Scyliorhinus canicula*) (Quintana-Urzainqui et al. 2012), which does not reflect the full diversity of chondrichthyan brain organization or lifestyles. The similar regional scaling seen in mammals, chondrichthyans, and birds may be due to a conserved sequence of neurogenesis, whereby regions that differentiate later become disproportionately larger (e.g. 'late-equals-large') and exhibit steeper allometries, such as in the telencephalon (Finlay and Darlington 1995; Finlay et al. 2001, 2010; Yopak et al. 2010*b*). Variation around this conserved scaling can be influenced by alteration in the total duration of neurogenesis, and cell cycle and growth rates (Charvet et al. 2011). However, without information on the exact sequence and duration of neurogenesis across vertebrate groups reconciling these patterns remains difficult.

Focusing on species, particularly within classes, may help to clarify the drivers of brain evolution in particular groups. However, future studies of vertebrate brain organization should move beyond regional size scaling and instead focus on subregions, connectivity between regions, and timing and regulation of neurogenesis. Greater understanding of how developmental mechanisms, such as progenitor pool size, cell cycle rates, and the schedule of neurogenesis, and their underlying genetic architecture (Montgomery et al. 2016), varies with life history and indeed how strongly regions are connected can clarify patterns of brain organization across these very different vertebrate groups.

Figure 5.1 Regional scaling across vertebrates

Allometric scaling of individuals brain regions with total brain size (A,C,E,G,I,K) and coefficient plots of scaling slopes for individual brain regions with total brain size (B,D,F,H,J,L) for six major vertebrate lineages. Homologous brain regions are designated by color (telencephalon=green, diencephalon=orange, mesencephalon=turquoise, dark blue=cerebellum, and red= medulla oblongata). Silhouettes in the right hand column designate which regions exhibit positive allometry (above line) and negative allometry (below line) with total brain size.

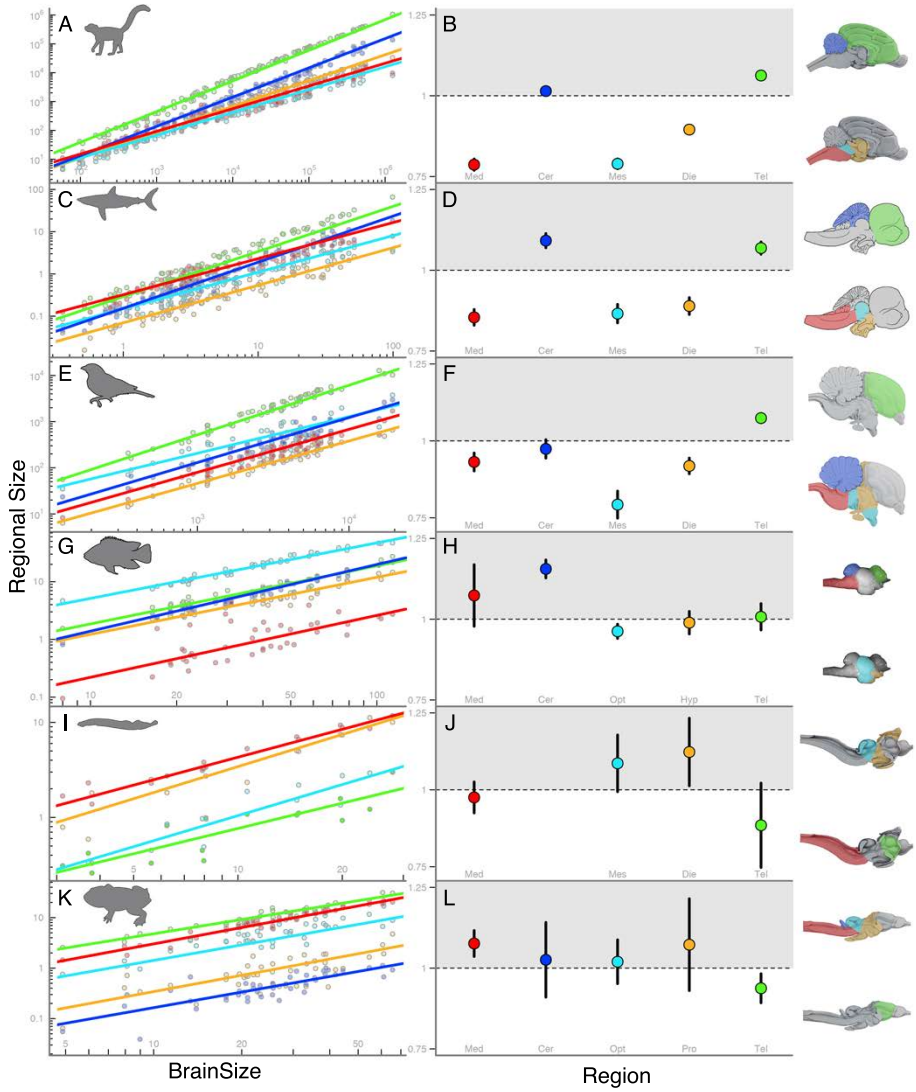


Figure 5.2 Regional covariation across vertebrates

The proportion of variance in brain organization explained by total brain size (A,C,E,G,I,K) and patterns of regional covariation (B,D,F,H,J,L) for six major vertebrate lineages. Black arrows denote positive covariation and grey arrow denote negative covariation, colored clouds denote clusters of regions based on covariation. Homologous brain regions are designated by color (telencephalon=green, diencephalon=orange, mesencephalon=turquoise, dark blue=cerebellum, and red= medulla oblongata).

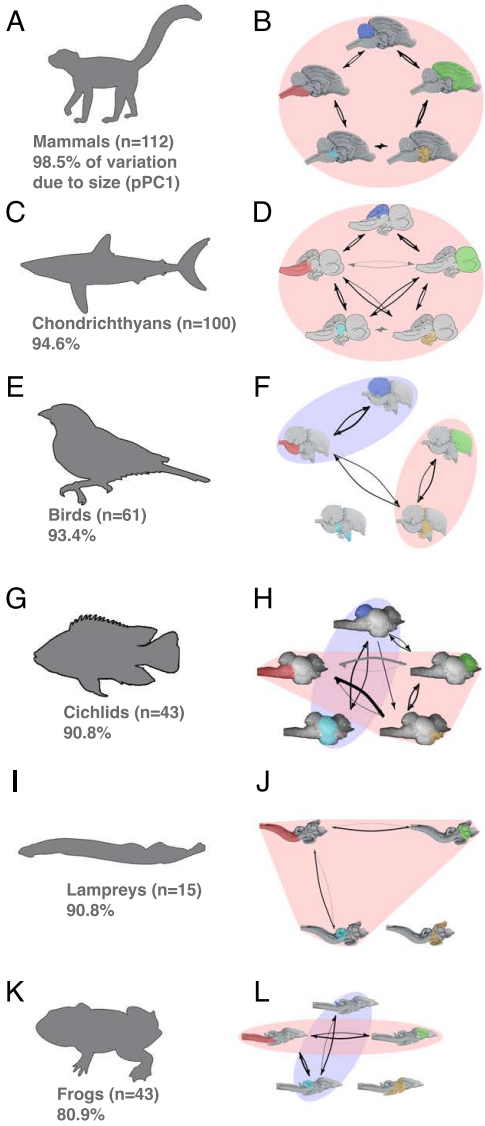


Table 5.1 Axes of variation across vertebrates

Phylogenetically corrected principal components (pPCA) of brain organization across vertebrate lineages.

	Structure	PC loadings					
		PC1	PC2	PC3	PC4	PC5	PC6
All Mammals	Telencephalon	-0.995	0.086	0.013	0.037	-0.028	-0.020
	Diencephalon	-0.994	0.005	0.065	0.023	0.080	0.001
	Mesencephalon	-0.987	-0.078	0.111	-0.076	-0.036	-0.002
	Cerebellum	-0.993	0.019	-0.090	-0.066	0.019	-0.006
	Medulla	-0.981	-0.177	-0.054	0.060	-0.010	-0.005
	Brain Mass	-0.998	0.044	-0.011	0.017	-0.022	0.032
	Standard deviation	0.173	0.014	0.011	0.009	0.007	0.003
	Proportion of variation	0.985	0.007	0.004	0.002	0.001	0.000
	Cumulative variation	0.985	0.992	0.996	0.998	1.000	1.000
	λ	0.975					
	Structure	PC loadings					
		PC1	PC2	PC3	PC4	PC5	PC6
All Chondrichthyans	Telencephalon	-0.979	-0.032	0.177	0.077	-0.058	-0.013
	Diencephalon	-0.943	-0.296	-0.126	0.067	0.052	-0.002
	Mesencephalon	-0.954	0.253	-0.061	0.115	0.098	-0.003
	Cerebellum	-0.983	0.002	0.055	-0.164	0.060	-0.005
	Medulla	-0.966	0.089	-0.201	-0.052	-0.125	-0.010
	Brain Mass	-0.998	0.013	0.036	0.003	-0.032	0.033
	Standard deviation	0.081	0.013	0.010	0.008	0.006	0.001
	Proportion of variation	0.946	0.023	0.015	0.010	0.005	0.000
	Cumulative variation	0.946	0.969	0.985	0.994	1.000	1.000
	λ	0.893					
	Structure	PC loadings					
		PC1	PC2	PC3	PC4	PC5	PC6
Birds	Telencephalon	-0.983	0.115	0.140	-0.028	-0.011	-0.012
	Diencephalon	-0.985	0.031	-0.030	0.158	0.052	-0.001
	Mesencephalon	-0.929	-0.367	0.055	-0.008	-0.004	-0.003
	Cerebellum	-0.984	0.024	-0.133	-0.087	0.077	-0.003
	Medulla	-0.985	0.027	-0.127	0.009	-0.116	-0.001
	Brain Mass	-0.996	0.040	0.073	-0.022	0.000	0.022
	Standard deviation	0.106	0.023	0.012	0.008	0.007	0.001
	Proportion of variation	0.936	0.043	0.011	0.005	0.004	0.000
	Cumulative variation	0.936	0.980	0.991	0.996	1.000	1.000
	λ	0.847					

	Structure	PC loadings					
		PC1	PC2	PC3	PC4	PC5	PC6
Cichlids	Telencephalon	-0.959	-0.237	0.097	-0.085	0.068	0.058
	Hypothalamus	-0.978	-0.039	0.161	0.001	-0.121	-0.018
	Optic Tectum	-0.972	-0.088	-0.196	0.025	-0.064	0.066
	Cerebellum	-0.983	-0.114	0.012	0.132	0.051	-0.028
	Medulla	-0.876	0.482	0.006	-0.015	0.023	0.008
	Total Brain	-0.973	-0.125	-0.121	-0.114	0.009	-0.096
	Standard deviation	0.701	0.189	0.083	0.059	0.048	0.037
	Proportion of variation	0.908	0.066	0.013	0.006	0.004	0.003
	Cumulative variation	0.908	0.974	0.987	0.993	0.997	1.000
	λ	0.805					

	Structure	PC loadings				
		PC1	PC2	PC3	PC4	PC5
Lampreys	Telencephalon	-0.906	-0.376	-0.155	0.122	-0.001
	Diencehalon	-0.927	0.363	-0.048	0.085	-0.005
	Optic Tectum	-0.964	-0.111	0.242	0.021	-0.002
	Medulla	-0.973	-0.055	-0.081	-0.210	-0.010
	Total Brain	-0.995	0.069	-0.036	-0.063	0.019
	Standard deviation	1.943	0.509	0.289	0.228	0.019
	Proportion of variation	0.905	0.062	0.020	0.012	0.000
	Cumulative variation	0.905	0.967	0.987	1.000	1.000
	λ	0				

	Structure	PC loadings					
		PC1	PC2	PC3	PC4	PC5	PC6
Frogs	Telencephalon	-0.909	-0.017	0.241	0.321	-0.109	0.012
	Prosencephalon	-0.845	0.522	-0.102	-0.051	-0.015	0.001
	Optic Tectum	-0.911	-0.261	0.146	-0.255	-0.123	0.005
	Cerebellum	-0.863	-0.262	-0.429	0.054	0.013	0.001
	Medulla	-0.952	-0.081	0.212	-0.037	0.201	0.008
	Total Brain	-0.977	-0.078	0.187	0.067	0.002	-0.028
	Standard deviation	0.043	0.015	0.012	0.007	0.005	0.001
	Proportion of variation	0.810	0.093	0.063	0.024	0.010	0.000
	Cumulative variation	0.810	0.903	0.966	0.990	1.000	1.000
	λ	0.294					

Chapter 6. Discussion

This thesis highlights chondrichthyans as an important group, being sister to osteichthyes including bony fish and tetrapods, for understanding vertebrate evolution. All of the aims set out in the introduction have been achieved, and in this section I will put these findings in a more general context and discuss future research opportunities.

6.1. Life History Evolution

Relative to other vertebrate lineages, chondrichthyan reproductive mode is a particularly labile life history trait. The rate of independent origins of live-bearing and matrotrophy surpasses that of squamate reptiles, a group highlighted for it's large numbers of transitions in reproductive mode (Blackburn 2015). The array of matrotrophic modes of reproduction is unrivaled in vertebrates, and warrants further investigation as to the factors selecting for the emergence of each, particularly lipid histotrophy and placentation. A novel aspect of this work has been demonstrating that the evolution of live-bearing and matrotrophy are associated with increasing body size, though not clearly associated with depth or latitude as predicted. Previously differences in temperature and the associated physiological effects have been proposed as drivers for the evolution of live-bearing and matrotrophy (Dulvy 1998), however this was not supported here.

The uncertainty in ecological correlates of reproductive evolution potentially reflects poorly defined reproductive modes. Reproductive modes based solely on parity and embryonic trophic modes do not capture variability in the agree maternal investment (pre and postfertilization), potential trade-offs in reproductive output (i.e. offspring size versus number), and other aspects of reproductive strategies (e.g. breeding cycle, gestation length). Additionally, discretizing continuous measures of maternal investment

(e.g. ovum size, degree of matrotrophy) reduces the power to test for meaningful correlations with environmental variables (e.g. temperature, depth, and latitude). Future research should seek to refine measures of life history and ecological parameters to more adequately test predictions about the evolution of life histories.

6.2. Brain Evolution

For the first time, I highlight reproductive mode as an important component of the 'expensive brain' framework (Isler and Schaik 2009; Mull et al. 2011, Chapter 3) (Figure 1.1). Matrotrophic species exhibit relative brain sizes 20-70% larger than lecithotrophic species of similar body size (Mull et al. 2011), representing substantial potential benefit to offspring survival associated with the evolution of matrotrophy. As a result I propose the 'head-start' hypothesis, which predicts that relative brain size increases with increasing levels of maternal investment to an individual offspring (Mull et al. 2011). This maternal investment is a function of energy allocated to offspring (Capellini et al. 2011), the trade-off between the duration of investment and offspring size and number (Bueno and López-Urrutia 2012), and the access of embryos to maternal resources (Crespi and Semeniuk 2004; Elliot and Crespi 2008). Chondrichthyans, teleosts, and squamates are all ideal groups for testing the predictions of the 'head-start' hypothesis due to their array of reproductive modes.

I show evidence that rather than distinct 'cerebrotypes' associated with particular niches (Clark et al. 2001; Iwaniuk and Hurd 2005; Yopak et al. 2007), chondrichthyan brain organization may vary along a gradient associated with reproductive mode and ecological lifestyle, using one of the largest compiled datasets in a vertebrate class. While total brain size explains a majority of variation in the relative size of major brain regions, subsequent axes of variation are related to reproductive mode and habitat complexity and depth. As a result shallow-water or reef-associated matrotrophic species (specifically stingrays (Myliobatiformes) and requiem (Carcharhinidae) and hammerhead sharks (Sphyrnidae) tend to have large relative brains that are predominantly composed of

telencephalon and cerebellum. Conversely, deepwater lecithotrophic species (particularly skates (Rajiformes) and dogfish (Squaliformes) tend to have small relative brains that are predominantly composed of medulla oblongata. Whether this gradient is a result of a conserved sequence of neurogenesis, in accordance with 'late-equals-large' (Finlay et al. 1998, 2001) and concerted evolution (Striedter 2005), or selection for niche specific cognitive or sensory specializations (e.g. electroreception versus vision), in accordance with mosaic evolution (Striedter 2005), requires more detailed information about neural development and connectivity. Chondrichthyans, as basal, vertebrates, represent a group where solutions to fundamental adaptive problems may have emerged and consequently been stabilized (Yopak et al. 2010*b*).

I propose that an interplay of life history strategy, particularly reproduction and complex life cycles, and varying rates of neurogenesis throughout ontogeny dictate the arena in which selection can act upon developmental and functional constraints. Despite their wildly different lifestyles, chondrichthyans, birds, and mammals exhibit similar patterns of regional scaling and brain organization. These groups contrast with the regional scaling and brain organization of teleosts, lamprey, and frogs. While there are numerous differences that these lineages face presently, and throughout their evolutionary history, varying reproductive strategies and distinct larval and adult phases present an arena in which selection for functional constraints could be relatively high. To test this prediction more information about developmental patterns, and ontogenetic changes in neurogenesis and brain organization are required.

Future work should focus on the interplay of life history and environmental variables likely to affect ectothermic vertebrates and energy allocation. Much of the work, and hypothesis development, on vertebrate brain evolution has focused on endothermic mammals and birds. The metabolic rate of ectotherms is dictated by environmental temperature, and as a result the allocation of energetic resources is likely to be influenced. Brain size in marine teleosts decreases with depth in accordance with temperature dependence (Iglesias et al. 2015). Since the publication of chapter 3, brain size and ecological data has been collected for ~80 additional species. While metabolic

rate information is extremely sparse in chondrichthyans, future work should focus on the estimation of reliable environmental temperatures to test these predictions.

6.3. The Dangers of Data Deficiency

While large-scale phylogenies and life history datasets are becoming increasingly available (De Magalhães and Costa 2009; Jones et al. 2009; Meiri et al. 2012; Rigby and Simpfendorfer 2013; Madin et al. 2016), an inherent difficulty in comparative analysis is the issue of missing data. This problem of missing data is particularly pressing in chondrichthyans, as almost half of all species are considered 'Data Deficient' by the IUCN (Dulvy et al. 2014), and complete life history information is available for roughly 10% of species (Mull and Dulvy 2016). The typical approach is to ignore missing values and to use only species for which complete information is available. This approach of ignoring missing data reduces statistical power and increases estimation bias (Nakagawa and Freckleton 2008), and can lead to biased estimates of evolutionary parameters (Hadfield 2008). This is particularly problematic if data are not missing completely at random, especially if missing data is distributed in association with another trait (e.g. the difficulty of estimating gestation lengths for large-bodied species) or if there is phylogenetic or taxonomic bias in missing data (Nakagawa and Freckleton 2008; González-Suárez et al. 2012; Pakeman 2014). In chondrichthyans these are relevant issues as missing life history trait information is distributed both taxonomically (i.e. less information for batoids and chimaeras), taxonomically (more research focused on large charismatic mackerel (Lamniformes) and ground sharks (Carcharhiniformes), and for particular traits (more data on body size relative to age/growth and reproductive parameters) (Mull and Dulvy 2016). There has been increased interest in methods to address this issue, and imputation of missing trait values could be a useful tool (Penone et al. 2014). Including phylogenetic information can improve the performance of these imputation techniques, as closely related species tend to be more similar, and many traits exhibit a high phylogenetic signal (Penone et al. 2014). With the recently developed large scale molecular phylogeny of chondrichthyans (Stein et al. *In Review*) imputation of

missing traits could be a useful tool to help address hypotheses about evolutionary ecology and pressing conservation issues.

6.4. Chondrichthyans as a Model System

For too long Chondrichthyans have been overlooked in comparative studies of vertebrate evolution. To the general public the name shark elicits feelings of fear, derision, or morbid curiosity, while amongst biologists it evokes assumptions about behavioral simplicity, slow life histories, and an evolutionary side show with little to offer relative to the speciose bony fishes or the more relatable tetrapods. In this thesis I have shown that chondrichthyans in fact exhibit varied and labile life histories, epitomized by their reproductive modes. I have shown that this life history is related to a diversity in brain sizes and organizations that overlaps with all other major vertebrate lineages. By studying across these groups we can better understand the selective pressures and mechanisms that may have lead to the great diversity among all jawed vertebrates, which all goes back to Chondrichthyans. In the words of Leonard Compagno "*It is time to nail a list of 'chondrichthyan heresies' on the cathedral door of marine biology. As with dinosaurs, recent research on cartilaginous fishes has yielded a very different biological and evolutionary picture from the old mythos of sharks and other cartilaginous fishes being simple, stupid, clumsy, vicious, primitive, harmful, asocial, undiverse, and unimportant animals. It is time to combat the teleost and tetrapod chauvinism shown by researchers and the general public, and to consider chondrichthyans as something other than a minor and morbid sideshow of the anthropocentric circus of more diverse, useful bony fishes, 'lovable' marine mammals and aesthetically pleasing marine birds.*" (Compagno 1990).

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