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Brain Size and Brain Organization of the Whale Shark, *Rhincodon typus*, Using Magnetic Resonance Imaging

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Key Words
Comparative brain morphology · Evolution · Chondrichthyan · Ecomorphology · Allometry · Whale shark · Rhincodon

Abstract
Very little is known about the brain organization of the suction filter feeder, *Rhincodon typus*, and how it compares to other orectolobiforms in light of its specialization as a plankton-feeder. Brain size and overall brain organization was assessed in two specimens of *R. typus* in relation to both phylogeny and ecology, using magnetic resonance imaging (MRI). In comparison to over 60 other chondrichthyan species, *R. typus* demonstrated a relatively small brain for its body size (expressed in terms of encephalization quotients and residuals), similar to the lamniforms *Carcharodon carcharias*, *Cetorhinus maximus*, and *Carcharias taurus*. *R. typus* possessed a relatively small telencephalon with some development of the dorsal pallium, which was suggestive of moderate social behavior, in addition to a relatively large diencephalon and a relatively reduced mesencephalon. The most notable characteristic of the brain of *Rhincodon* was a large and highly foliated cerebellum, one of the largest cerebellums within the chondrichthyan clade. Early development of the brain was qualitatively assessed using an in situ MRI scan of the brain and chondrocranium of a neonate specimen of *R. typus*. There was evidence that folding of the cerebellar corpus appeared in early development, although the depth and number of folds might vary ontogenetically in this species. Hierarchical cluster analysis and multidimensional scaling ordinations showed evidence of convergent evolution with the basking shark, *Cetorhinus maximus*, another large-bodied filter feeding elasmobranch, supporting the claim that organization of the brain is more similar in species with analogous but independently evolved lifestyles than those that share taxonomic classification.

Introduction

Despite their basal place in vertebrate evolution, little more than qualitative data is available on the variations in chondrichthyan brain organization and the implications these variations have for evolutionary adaptations in sensory/motor function in vertebrate nervous systems [Masai, 1969; Bauchot et al., 1976; Northcutt, 1977; Graeber, 1978]. The cartilaginous fishes are comprised of approximately 1,100 extant species [Compagno, 1999], 10 of which grow up to lengths greater than 4 m [Freedman and Noakes, 2002]. The largest fish in the world's ocean [Compagno, 2001], the whale shark *(Rhincodon typus)* [Smith, 1828], is a migratory [Eckert and Stewart, 2001;

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Wilson et al., 2006; Hueter et al., 2008], epipelagic filter-feeding member of the Orectolobiform order, with a circumglobal distribution, growing up to total lengths of at least 12 m [Last and Stevens, 1994; Compagno, 2001; Stevens, 2007]. *R. typus* is one of three sharks to have evolved a specialized planktivorous lifestyle, along with the megamouth shark, *Megachasma pelagios* [Taylor et al., 1983], and the basking shark, *Cetorhinus maximus* [Gunnerus, 1765]. Until recently, very little has been known about the biology, ecology, and behavior of *R. typus* [Norman, 2000; Compagno, 2001; Martin, 2007; Stevens, 2007], and even less about their neural characteristics [Sato, 1986].

The allometric scaling of brain mass with body mass has demonstrated that chondrichthyans have relatively large brains in comparison to other vertebrates [Bauchot et al., 1976; Northcutt, 1977, 1978; Striedter, 2005; Yopak et al., 2007; Lisney et al., 2008]. Detailed and descriptive illustrations of brain morphology from a number of chondrichthyan species have provided evidence of substantial interspecific variation of component parts [e.g., Garman, 1913; Northcutt, 1977, 1978; Kruska, 1988; Narenda, 1991; Smeets, 1998] and more recent work has begun to quantify the phylogenetic component of variation in brain size in chondrichthyans [Yopak et al., 2007; Lisney et al., 2008; Yopak and Montgomery, 2008]. It has been suggested that brain organization and the relative development of major brain structures (telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata) reflects an animal's ecology [Yopak et al., 2007; Lisney et al., 2008], even in phylogenetically unrelated species that share certain lifestyle characteristics [Yopak and Montgomery, 2008]. Although studies have begun to quantify neural organization in large filter-feeding elasmobranchs species [Kruska, 1988; Ito et al., 1999], little work has been done to quantitatively assess the brain of *R. typus* [Sato, 1986], likely due to its rarity and high vulnerability [Norman, 2000; Cheung et al., 2005]. There are large quantitative data sets on brain organization of other vertebrate groups such as teleost fishes, birds, and mammals. Strong correlations have been found between brain patterns and various ecological factors, such as diet and feeding habits, habitat complexity, and increased sociality and/or cognitive capability in these vertebrate clades [e.g., Barton et al., 1995; Kotrschal et al., 1998; Hutcheon et al., 2002; Lefebvre et al., 2002; Pollen et al., 2007; Lefebvre and Sol, 2008; Shumway, 2008]. What is lacking is an understanding of how highly specialized lifestyles of some chondrichthyans, such as that of the whale shark, might be related to brain organization [Sato, 1986; Yopak and Montgomery, 2008]. This paper will explore the brain organization of *R. typus* in comparison to other orectolobiforms, other pelagic sharks [Yopak et al., 2007], and other filter-feeding species [Kruska, 1988; Ito et al., 1999].

Gross dissections have provided imperative building blocks for the quantification of brain size and the measurement of structural properties in extant cartilaginous fishes [Yopak et al., 2007; Lisney et al., 2008; Yopak and Montgomery, 2008] and histological sections are invaluable for micro-structural description and analysis [e.g., Northcutt, 1978; Smeets et al., 1983]; however, both methodologies are highly invasive and are thus impractical for the study of valuable specimens [Corfield et al., 2008a]. Further, they typically destroy the precise relative positions of various structures, and resolution is often lost during attempts to reconstruct brain volumes from 2D slides. Magnetic resonance imaging (MRI) offers a solution to these current drawbacks. MRI is unique in its ability to non-invasively acquire high-resolution, 3D digital data from soft tissue structures. Although these cutting-edge technologies and methods are extensively developed for applications in human brain research, their utility in comparative neurobiology remains largely unexplored. MRI technology has recently emerged as an effective investigative tool for non-invasive visualization and quantification of the internal anatomy of fishes [Perry et al., 2007; Sepulveda et al., 2007; Rogers et al., 2008], as well as studies on comparative brain anatomy of vertebrates [Marino, 2001a, b, 2003; Yopak and Frank, 2007; Montie et al., 2008; Corfield et al., 2008a]. These approaches can potentially transform the way other researchers view, quantify, and disseminate neuroanatomical data, allowing for analyses of rare and/or extinct specimens [Corfield et al., 2008a] and the analysis of 3D surface structures that were once impossible to derive from 2D sections [Yopak and Frank, 2007]. These methods also create a permanent 3D digital record of these samples, which serve as a platform on which new technologies and methodologies can be applied in the future.

The aim of this study was to assess the brain organization of *R. typus* by comparing relative brain mass (encephalization), the relative size of the five major brain areas, telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata [relative deviation, θ; Wagner, 2001a, b], and the foliation of the cerebellar corpus [foliation index score; Yopak et al., 2007] to existing data on 63 other chondrichthyan species [Northcutt, 1978; Kruska, 1988; Ito et al., 1999; Yopak et al., 2007; Yopak and Montgomery, 2008]. This study marks one of the first attempts to gain an evolutionary perspective into

Table 1. Morphometrics from two specimens of the whale shark, *Rhincodon typus*. Brain mass was calculated from digital reconstructions

Specimen	Species	Measurement				
		TL, cm	FL, cm	PCL, cm	body mass, kg	brain mass, g
RT_1	*Rhincodon typus*	592.5	542	472	1,398.5	36.12
RT_2	*Rhincodon typus*	652	537	478	1,377.73	34.75

the brain of *R. typus*, in comparison to both phylogenetically related species as well as those that share certain lifestyle characteristics.

Materials and Methods

Specimen Collection and Preparation

Brains from two specimens of *R. typus* (RT_1 and RT_2) were acquired from the Georgia Aquarium on January 12, 2007 (RT_1) and June 13, 2007 (RT_2). Both specimens were juvenile males ranging from 592.5 to 692.0 cm total length with body mass ranging from 1,377.7 to 1,398.5 kg (table 1). Although some allometric bias was introduced when including data from juvenile specimens [Brandstätter and Kotrschal, 1990], these data were used due to the rarity of these samples and limited morphological information available on these animals.

Qualitative brain data for a third juvenile specimen of *R. typus* (RT_3) were acquired [E. Clark, personal communications]; sketches of dorsal and lateral views of the brain were provided from a 700-cm female specimen caught on May 14, 1973. Additionally, a neonate specimen of *R. typus* (RT_4) was borrowed from the Marine Vertebrates Collection of the Scripps Institution of Oceanography. The specimen (60.1 cm total length) was caught on October 8, 1984 from the Guatemala Basin and preserved in isopropyl alcohol. Both of these specimens (RT_3 and RT_4) were used for anatomical comparison only.

For RT_1 and RT_2, the brain was excised from each specimen and was hemisected by the Georgia Aquarium's marine veterinarian prior to this study, with the right hemisected side utilized for neural autopsy. The left side (without the olfactory bulbs, but including the cranial nerves) was preserved in 10% formalin in 0.1 M phosphate buffer at 4°C for further analyses. After fixation, the meninges, blood vessels, choroid plexa, and connective tissue were dissected away and each hemisected brain was blotted (including draining the ventricles of fluid) and weighed to the nearest 0.01 g (table 2). Whole brain mass was estimated from the hemisected samples using the methods described below. Brain mass was not corrected for fixation, but was acquired with the cranial nerves digitally removed. Body mass information was recorded on fresh, unfixed samples.

After fixation, brains were transferred to 1× PBS + 0.01% sodium azide for at least 14 days to remove excess fixative before transferring to fresh 1× PBS + 0.01% sodium azide with the addition of 5 mM of the contrast agent Prohance® (Bracco Diagnostics Inc., Princeton, N.J., USA) for a further 7 days at 4°C. Equilibrating the tissue in this contrast agent achieves a significant reduction in the longitudinal relaxation time (T1) of the sample and a corresponding increase in the SNR efficiency of the data acquisition. MR image data was acquired from contrast-enhanced, fixed brains.

High-Resolution Anatomical Imaging

Brains were removed from the contrast agent solution and secured in a 50 ml Falcon tube for imaging. Experiments were performed on a Bruker 7Tesla small animal scanner, with a 22-cm bore and gradient strengths of 46 Gauss/cm. MR imaging consisted of a high-resolution (100–150 μm), T1-weighted anatomical acquisition using a gradient recalled echo with no RF spoiling [well-established for brain imaging; Edelman et al., 1996] (fig. 1A). High-resolution, T1-weighted anatomical imaging produces high contrast between gray and white matter in chondrichthyan brains [Yopak and Frank, 2007], from which structural characteristics were derived. The pulse sequence parameters used for this study are shown in table 3.

An in situ scan was also performed on a neonate specimen of *R. typus* (RT_4) using a T1-weighted, 3D fast-spoiled gradient recalled echo (FSPGR) sequence. Due to the specimen size, images were acquired on a GE Signa 3Tesla whole-body scanner using a custom-built rectangular surface coil (76 × 41 mm) placed over the brain case to increase the signal-to-noise ratio; however, the spatial resolution attainable on this scanner (~500 μm) was significantly less than on the small animal scanner. Moreover, gray-white matter contrast was reduced because of its preservation in isopropyl alcohol. Consequently, the quality of this brain image data was insufficient to be used for analysis and was used only as a reference for digitally reconstructing the hemisected brains of RT_1 and RT_2.

Segmentation of MR Images

Three-dimensional data, acquired from high-resolution MRI, were digitally segmented using ITK-SNAP. This program is a cross-platform, open-source application that includes a toolbox for manual delineation and a simple intuitive interface for user-guided automatic segmentation using an active contour (level set) algorithm [Yushkevich et al., 2006]. The five major brain structures (telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata) were individually segmented from the 3D data. The volume of each brain region as a proportion of the total brain volume was calculated from these digital segmentations and compared across species. The five brain areas for both methods were identified using the criteria of Northcutt [1977, 1978]

Table 2. Body mass, brain mass, and residual values for 15 orectolobiforms and lamniforms, including *Rhincodon typus*, as well as the planktivorous pelagic sharks, *Megachasma pelagios* and *Cetorhinus maximus*. Standard deviations (SD) provided where more than three specimens were available

Source	Species abbrev	Order	Family	Species	n	Body mass kg ± SD	Brain mass g ± SD	EQ	Standardized residuals	Foliation index
Yopak et al., 2007	BW	Orectolobiformes	Brachaeluridae	*Brachaelurus waddi*	1	0.69	1.39	0.68	–0.17	1
Yopak et al., 2007	OM		Orectolobidae	*Orectolobus maculatus*	1	12.3	3.23	0.40	–0.30	1
Yopak et al., 2007	OO			*Orectolobus ornatus*	5	3.41 ± 1.2	2.11 ± 0.34	0.48	–0.26	1
Yopak et al., 2007	CP		Hemiscyllidae	*Chiloscyllium punctatum*	2	0.7	2.1	1.04	0.02	2
Yopak et al., 2007	HO			*Hemiscyllium ocellatum*	3	0.6 ± 0.10	1.96 ± 0.17	1.02	0.00	2
Yopak et al., 2007	NF		Ginglymostomatidae	*Nebrius ferrugineus*	1	32.2	15.16	1.17	0.21	4
This study	RT		Rhincodontidae	*Rhincodon typus*	2	1,388.12	35.43	0.45	–0.06	5
Yopak et al., 2007	CT	Lamniformes	Odontaspidae	*Carcharias taurus*	1	152.4	14.25	0.53	–0.08	3
Yopak et al., 2007	PK		Pseudocharidae	*Pseudocarcharias kamoharai*	1	3.9	4.8	1.02	0.07	2
Ito et al., 1999	MP		Megachasmidae	*Megachasma pelagios*	1	1,040	19.8	0.29	–0.27	3
Yopak et al., 2007	AS		Alopiidae	*Alopias superciliosus*	1	62.73	30.2	1.70	0.40	5
Yopak et al., 2007	AV			*Alopias vulpunis*	1	5.83	11.13	1.83	0.35	5
Kruska, 1988	CMa		Cetorhinidae	*Cetorhinus maximus*	1	385	20.7	0.49	–0.08	4
Yopak et al., 2007	CC		Lamnidae	*Carcharodon carcharias*	3	727.27	29.53	0.44	–0.07	4
Yopak et al., 2007	IO			*Isurus oxyrhinchus*	3	186.53 ± 8.24	25.59 ± 3.91	0.86	0.14	4

EQ values and residuals based on analysis of 60 species, including RT.

and Yopak et al. [2007] (fig. 1B). Delineation of major neural structures from MR data was compared to previous methods, which employed gross dissection and/or structural identification from cross sections [Yopak et al., 2007]. The average variation found between identifying major structures using either method in the mako shark, *Isurus oxyrinchus*, was less than 2.8%, which was within the range of the interspecific variation in the relative size of the five brain areas in similarly-sized individuals [Yopak et al., 2007], indicating that MR was a viable method for visualizing these boundaries.

To obtain both absolute and relative sizes of individual brain structures for *R. typus*, the right hemisected portion of the brain from each specimen (which had been dissected to determine cause of death prior to this study) was inspected, and major brain areas were identified and weighed to the nearest 0.01 g. As tissue from the right hemisected telencephalon of RT_2 was missing, the mass of this structure was estimated using the method described below for morphological reconstruction (by digitally mirroring one complete half of the telencephalon). Although this was not ideal, in the case of rare specimens it is often necessary to work from samples that are damaged. The variation between estimated relative mass of the telencephalon of RT_2 and the calculated relative mass of the telencephalon of RT_1 was less than 0.1% and was therefore considered acceptable and used for analyses.

In both cases of neural hemisection for RT_1 and RT_2, the procedure did not split the brain into two perfectly equal halves. This situation was rectified by mirroring the complete half of the brain from each specimen along its true hemisection point. As RT_2 was sagittally sliced beyond the brain midline, central markers on this specimen were used to estimate the brain's true bilateral hemisection point, in particular the median longitudinal sulcus separating the two hemispheres of the telencephalon, the lateral plane dividing either lobe of the optic tectum, the bisection point of the cerebellar auricles, and the dorso-central fold of the fossa rhomboidea extending down the medulla. All segmented tissue beyond this plane was digitally removed prior to mirroring to obtain an estimate of overall brain morphology and an approximate mass of the telencephalon for RT_2 (fig. 1B, 2).

The approximated overall shape of the brain and its components were illustrated based on anatomical and digital data (fig. 2). Reconstruction was verified based on sketches of an *R. typus* brain [RT_3; E. Clark, personal communications] and digital renderings of a neonate whale shark brain scanned in situ (RT_4; fig. 3). As there have been records of cerebellar asymmetry in highly foliated brains [e.g. Yopak et al., 2007; Lisney et al., 2008], symmetry was not assumed when reconstructing the hemisected samples (dorsal; fig. 2).

The brain mass data and volumetric data of *R. typus*, derived from segmentation of MR images, were combined with similar data on 63 additional species from Northcutt [1977, 1978], Kruska [1988], Ito et al. [1999], Yopak et al. [2007], and Yopak and Montgomery [2008] (for a total of 64 species), in order to compare *R. typus* with phylogenetically related species, those in similar ecological habitats, and those with similar lifestyles.

Encephalization

A total of 60 shark and holocephalan species [brain mass data on *Etmopterus hillianus*, *Scyliorhinus canicula*, *Mustelus canis*, and *Hydrolagus colliei* from Northcutt, 1978 were not available], including data on *R. typus*, were analyzed using both raw species

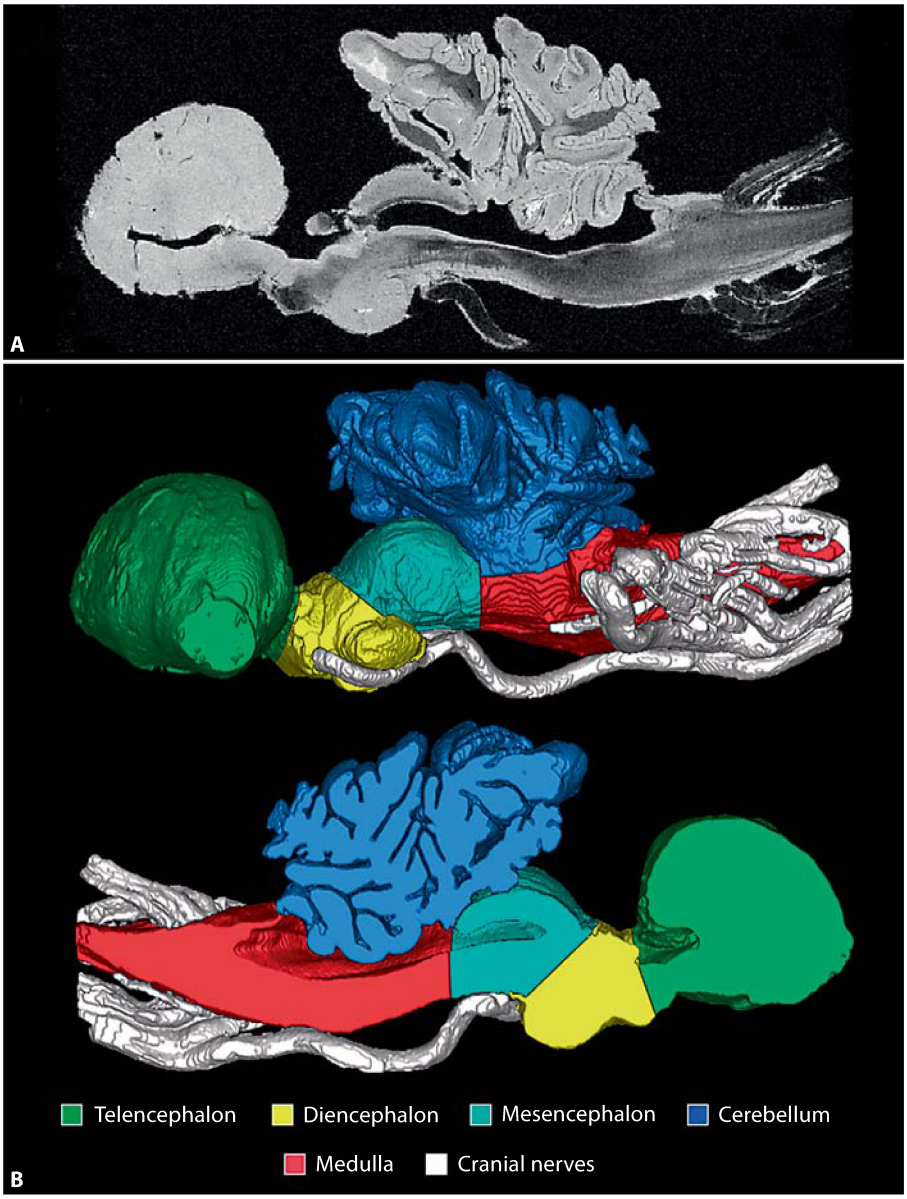


Fig. 1. Brain images of one whale shark specimen, *Rhincodon typus* (RT_2), acquired using MRI. (**A**) Sagittal slice of the brain. (**B**) Digital segmentation of the major structures of the brain and cranial nerves using ITK-SNAP, in inner and outer lateral views.

Table 3. Pulse sequence parameters for the T1-weighted scans of two hemisected brains of *Rhincodon typus*

Specimen	Pulse sequence	Flip angle °	TR ms	TE ms	FOV, mm	Matrix size	Voxel dim. mm	NEX	Acq. time
RT_1, RT_2	FLASH 3D	15	23.3	11.5	90 × 40 × 20	1,024 × 267 × 100	200 × 200 × 200	10	1 h 43 m 32 s

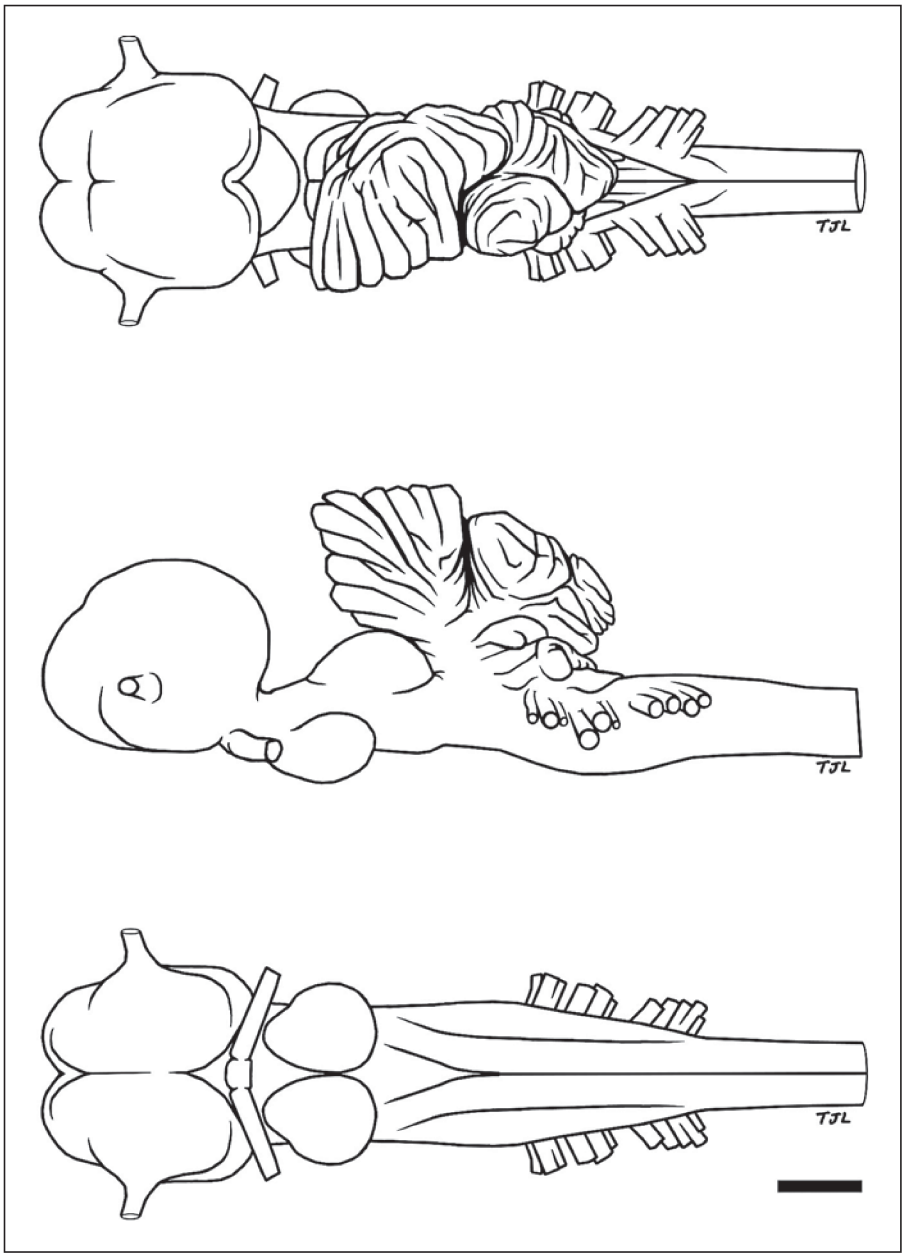


Fig. 2. Estimated dorsal, ventral, and lateral drawings of the brain of *Rhincodon typus*. The stylized composition was based on both anatomical and digital data from hemisected adult samples (RT_1 and RT_2), a neonate brain scanned in situ (RT_4), as well as sketches of the brain of a 700-cm female specimen (RT_3). Drawings were composed by T. Lisney, with guidance from original sketches by E. Clark. Scale bar corresponds to 1 cm.

data (where the species were treated as independent data points) and phylogenetically independent contrasts [Felsenstein, 1985]. Independent contrasts were calculated using the PDAP software package [Garland et al., 1999; Garland and Ives, 2000] and Shirai's [1992, 1996] elasmobranch phylogeny, supplemented with additional information taken from Goto [2001], Martin et al. [1992], Compagno [1988], Naylor [1992], and Yopak et al. [2007] (fig. 4). Because the branch lengths for many taxa were unknown, arbitrary branch lengths were assumed [Pagel, 1992].

The raw species data were plotted on logarithmic coordinates and the regression line describing the allometric relationship was calculated using the generalized least squares (GLS) regression [Garland and Ives, 2000] with the equation: $\log(y) = \log(\beta) + \alpha \log(x)$, where y = brain mass, and x = body mass. One standard method for comparing brain mass between species is by calculating encephalization quotients (EQs). Although this method has been more recently described as problematic as it does not control for phylogeny [e.g., Felsenstein, 1985; Harvey and Pagel, 1991; van Dongen, 1998], it is useful when comparing newer and older datasets. EQs [Jerison, 1973] were calculated using the formula: $EQ = E_a/E_e$, where E_a = actual brain mass and E_e = expected brain mass. The expected brain mass for a species was calculated using the allometric equation for the brain mass to body mass relationship. EQs of >1.0, 1.0, and <1.0 indicated that the species of inter-

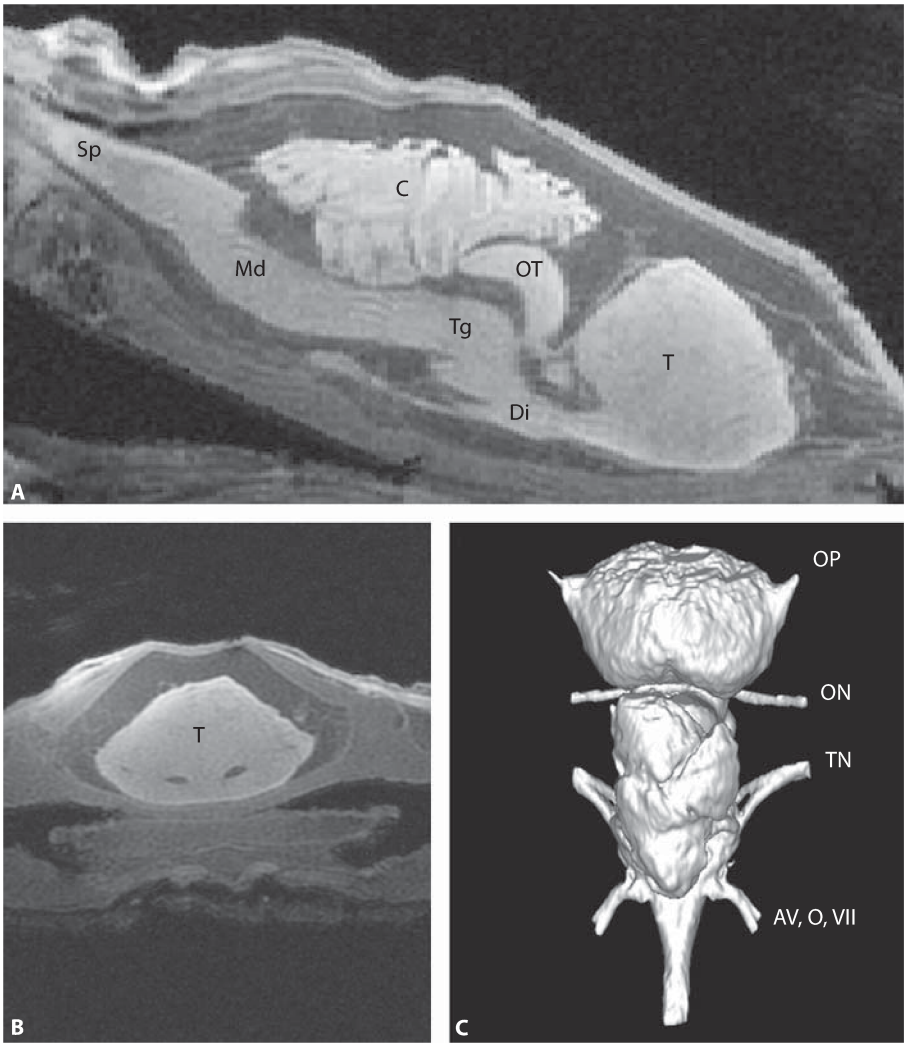
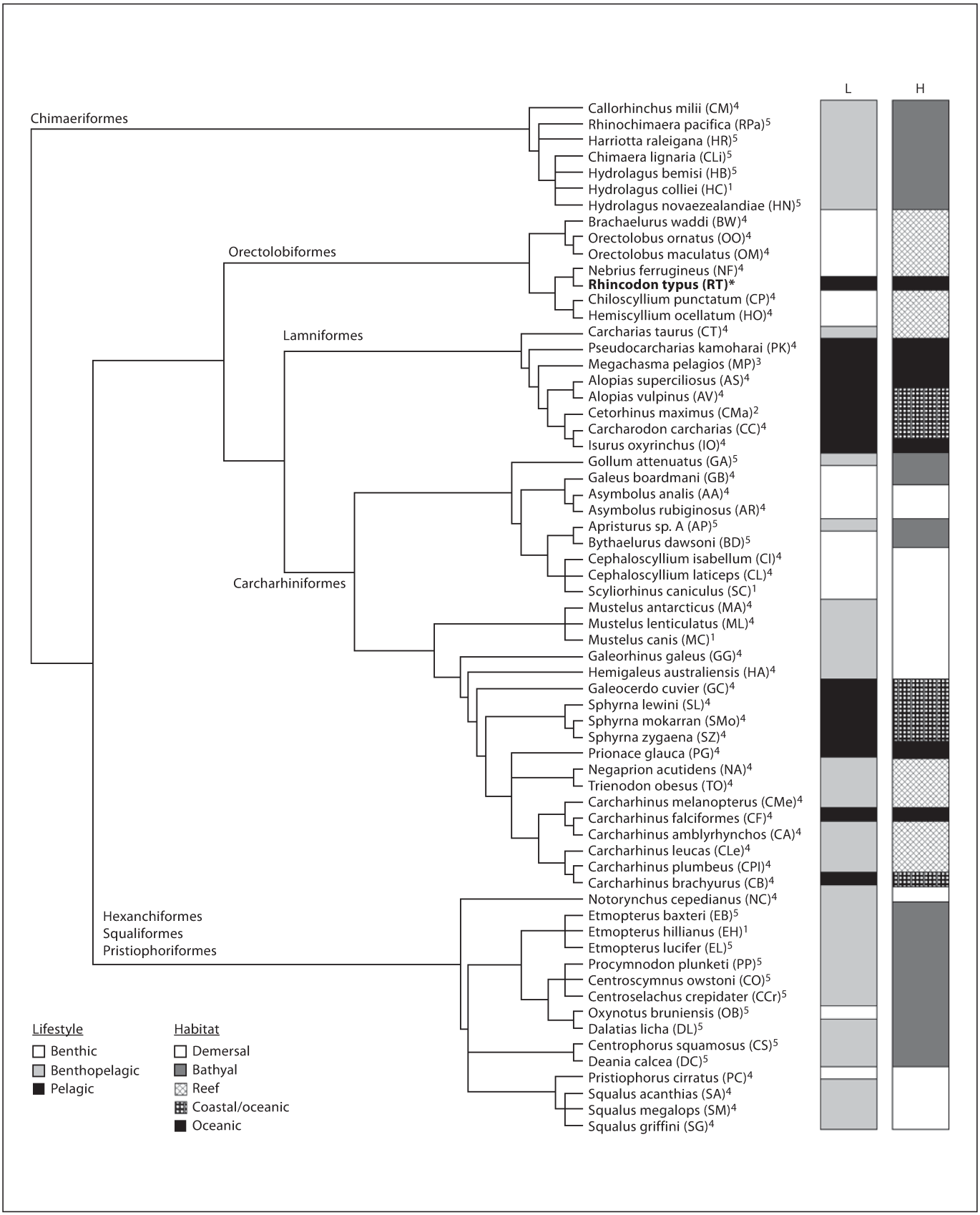


Fig. 3. The brain of a neonate specimen of *Rhincodon typus* (RT_4), scanned in situ using T1-weighted 3D FSPGR in sagittal (**A**) and coronal (**B**) views. Brain structures were identified as follows: T = telencephalon; Di = diencephalon; OT = mesencephalic optic tectum; Tg = mesencephalic tegmentum; C = cerebellum; Md = medulla oblongata; Sp = spinal cord. The brain was then digitally segmented (**C**) using ITK-SNAP. Although the spatial resolution was not high enough to identify each individual cranial nerve, those which were identified are labeled as follows: OP = origin of the olfactory peduncle (which leads to the olfactory bulb); ON = optic nerve; TN = trigeminal nerve (including the mandibular and maxillary ramus); AV = anteroventral lateral-line nerve; O = octaval nerve (both anterior and posterior ramus); VII = facial nerve.

est had a relative brain mass that was greater than, average, or less than expected for its body mass, respectively.

Independent contrasts were obtained by $\log_{10}$ transforming the data and analyzing brain mass and body mass together using the PDTREE algorithm within PDAP [Garland et al., 1993, 1999; Garland and Ives, 2000], with body mass as the independent variable. Unstandardized contrasts for both the dependent and independent variable were divided by the standard deviation (square root of the sum of the corrected branch lengths) to generate standardized contrasts. To reduce ambiguity resulting from the indeterminate signs of the independent contrasts, any negative contrasts for the independent variable (brain mass contrasts) were made positive, and any corresponding signs for the dependent variable (body mass contrasts) were correspondingly flipped [Garland et al., 1992; Garland and Janis, 1993]. The dependent variable (y) was then regressed on the 'positivized' independent variable (x) using LS regression forced through the origin [Garland et al., 1992].

Vertical deviations from the predicted slope were then calculated from independent contrasts, termed residuals, but the original LS regression line assumed no error associated with the independent variable [Ives et al., 2007]. This is problematic, as a standard protocol for measuring body size is often difficult to achieve. Body mass in large vertebrates is calculated with varying post-mortem times and animal conditions, and is often estimated from length-weight relationships. To reduce this error and best describe the relationship between the dependent and independent variable, the reduced major axis (RMA) was calculated. The RMA is equal to the LS regression slope α (forced through the origin) divided by the correlation coefficient R [Garland et al., 1992]. A phylogeny-weighted estimate of the mean of the dependent and independent variables, derived from the independent contrasts root node value, was used to anchor the RMA and determine the y-intercept (β). The resultant regression equation was then fitted back onto the raw species data (which were $\log_{10}$ transformed) and the 'RMA residuals' were calculated as the deviation from the phylogenetically reduced major axis, mapped back onto



the original data space (table 2). These 'RMA residuals' reflected interspecific variation in relative brain size, using a slope (α_{RMA}) that was independent of phylogenetic constraints [Purvis and Rambaut, 1995a, b] and were comparable to the EQs calculated using the raw species data for each species. Because the RMA was mapped back onto the original data space to calculate these vertical deviations, 'RMA residuals' do not sum to zero [Garland et al., 1992].

With the exception of *R. typus* [this study], *Cetorhinus maximus* [Kruska, 1988], and *Megachasma pelagios* [Ito et al., 1999], both EQ and/or residual values (using both raw data and independent contrasts) for 57 other chondrichthyans have been previously published [e.g., Northcutt, 1978; Yopak et al., 2007; Yopak and Montgomery, 2008], but these values have been altered slightly here following similar analyses, which included the addition of the three planktivorous pelagic species.

Brain Organization

Brain organization in the whale shark, *R. typus*, was investigated by assessing the relative size of each of the five brain areas and comparing it across 62 other chondrichthyans from Northcutt [1977, 1978], Kruska [1988], Yopak et al. [2007], and Yopak and Montgomery [2008] (relative structure size data for *Megachasmas pelagios* were not available). As the variation between the relative mass of all major brain structures for RT_1 and RT_2 was between 0.1–3.9%, means were used.

All relative structure size data were normalized with a weighted factor analysis (θ), which was calculated by dividing the relative proportion of each brain area by the average across all species [Wagner, 2001a, b], adjusted to center at zero. The weighted factor (θ) shows the deviation from the average relative size of each brain area. Each brain area was considered relatively large if $\theta > 0.1$, relatively small if $\theta < -0.1$, and relatively average-sized if $0.1 \leq \theta \leq -0.1$. Data on lifestyle and habitat for *R. typus* was compiled [Compagno, 2001; Duffy, 2002; Musick et al., 2004; Stevens, 2007], alongside ecological information on 62 other chondrichthyans [Eschmeyer et al., 1983; Compagno, 1984a, b; Last and Stevens, 1994; Cox and Francis, 1997; Compagno and Niem, 1998a, b; Didier, 2002, 2004; Musick et al., 2004; Kyne and Simpfendorfer, 2007; Yopak et al., 2007; Yopak and Montgomery, 2008]. With the exception of *R. typus* and *Cetorhinus maximus* [Kruska, 1988], weighted factor (θ) values for 60 other chondrichthyans have been previously published [Yopak et al., 2007; Yopak and Montgomery, 2008], but these values have been altered slightly here following similar analyses, which included the addition of the two aforementioned species.

Comparison between *R. typus* and another filter feeding elasmobranch, *Cetorhinus maximus*, was critical due to their similarities in primary habitat, locomotory mode, and feeding style. Brain structure divisions for *C. maximus* [Kruska, 1988] did not conform to those used in this study, nor to Northcutt [1978] and Yopak et al. [2007]. For this reason, and the critical nature of this sample, a method to normalize this data to current structural delineation methods was devised. Drawings and photographs of the brain [Kruska, 1988; D. Kruska, personal communications] in 3 orthogonal views were imported into SigmaScan® image analysis software (Systat Software Inc., Richmond, Calif., USA) and calibrated for distance. Lateral images of the brain were divided into the five major brain areas (telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata) using the current structural delineation method, and a Cartesian coordinate grid pattern was superimposed over each individual structure. Within each 5-mm^2 grid section, the outline of visible brain tissue was drawn to create a 2D polygonal shape using SigmaScan. The area of this polygonal shape was then multiplied by the width of the structure obtained from dorsal/ventral views to obtain a 3D volume, assuming uniform shape in the sagittal direction. Volumes were summed from each component to calculate a measure of total structure volume. For structures with clearly defined hemispheres (i.e., the mesencephalon), this method was applied to each half of the brain, so as not to result in an overestimation of volume. This measure was repeated five times for each brain structure, with a maximum variance of 0.05. For validation purposes, this method was then applied to photographs of a species with known structural volumes, *Isurus oxyrinchus* [Yopak et al., 2007]. Estimates of brain structure volume were within 3.73% of published values, which is within the range of interspecific variation in this species. Because it was just at the boundary of the range of interspecific variation for *Isurus* [Yopak et al., 2007], these values must be used cautiously; this type of structure volume estimation was not a precise method for quantifying brain organization in elasmobranchs. It was concluded, though, that this method provided reasonable but conservative assumptions about relative brain structure volume in *Cetorhinus*, derived from our structural delineations, and was a practical procedure given that this specimen was highly vulnerable and therefore not readily available [Cheung et al., 2005]. This estimate of brain structure volume could not be used for *Megachasma pelagios*, as there were no photographic records of the sample, and the lateral drawings available [Ito et al., 1999] were not ideal for this type of numerical structure estimation.

Cerebellar Foliation

The level of folding in the cerebellar corpus of *R. typus* was assessed using a visual grading index from Yopak et al. [2007]. This method involved assigning a quantitative score (1–5) to the length, depth, and number of folds in the cerebellum. A grade of 1 corresponded to a smooth cerebellar surface with no folding, increasing in complexity to a grade of 5, which corresponded to extreme foliation with deep, branched grooves [Yopak et al., 2007]. This method has been successfully applied to a wide range of chondrichthyan species in previous studies [Yopak et al., 2007; Lisney et al., 2008; Yopak and Montgomery, 2008].

Fig. 4. A phylogenetic tree of 60 species, showing the evolutionary relationships of *Rhincodon typus* to other chondrichthyans for which brain mass and body mass data were available in the literature, coded on the tree from: [1]Northcutt [1978], [2]Kruska [1988], [3]Ito et al. [1999], [4]Yopak et al. [2007], and [5]Yopak and Montgomery [2008]. The relationships between species were primarily based on Shirai's [1992, 1996] elasmobranch phylogeny, supplemented with additional information taken from Goto [2001], Martin et al. [1992], Compagno [1988], Naylor [1992], Yopak et al. [2007], and Yopak and Montgomery, [2008]. Information on lifestyle (L) and habitat (H) was compiled, and coded alongside the phylogenetic tree. The species specifically examined in this study (*) is in boldface. Species abbreviations are provided in parentheses.

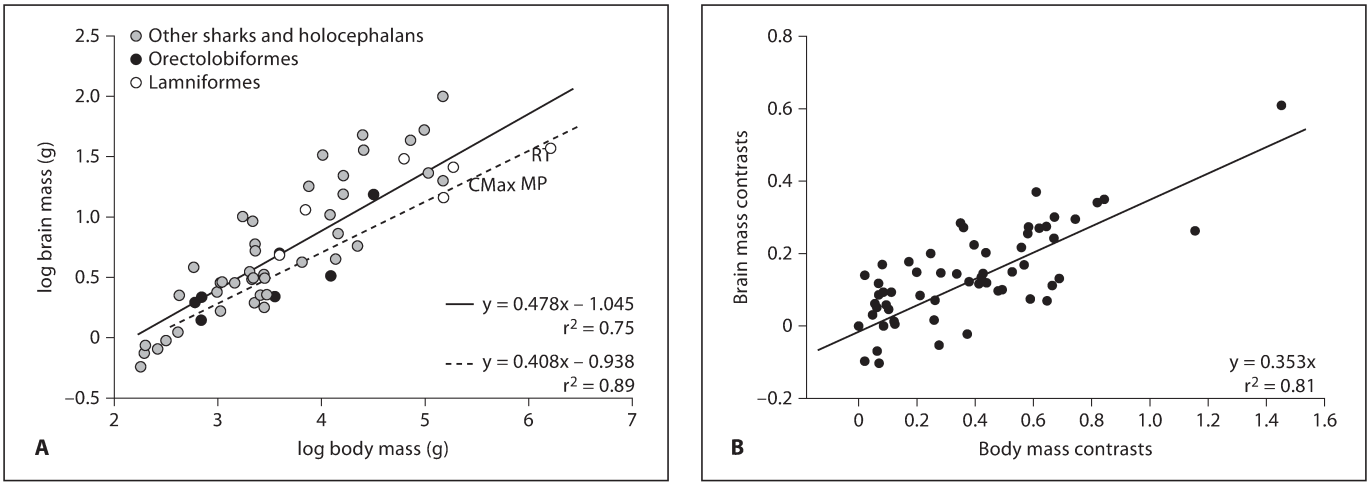


Fig. 5. Scaling of brain mass with body mass in 60 species of sharks and holocephalans from Northcutt [1978], Yopak et al. [2007], and Yopak and Montgomery [2008] using (**A**) species as independent data points, showing the position of the species examined in this study, *Rhincodon typus* (RT), as well as the basking shark, *Cetorhinus maximus* (CMa) [Kruska, 1988], and the megamouth shark, *Megachasma pelagios* (MP) [Ito et al., 1999]. The solid line indicates the regression equation for all 60 species. The hatched line represents the regression equation for members of the Orectolobiformes order only (n = 7). Brain mass was then scaled against body mass using (**B**) phylogenetically independent contrasts, with positivized body mass contrasts.

Cluster Analyses and Multidimensional Scaling

Multivariate hierarchical cluster analysis, using Euclidean distances, was used to determine the connectivity among *R. typus* and other chondrichthyan species, using PRIMER 6 software (PRIMER-E Ltd., Plymouth, UK). The cluster was based on foliation index scores and arcsine-transformed, relative brain structure mass data for 63 chondrichthyan species, including *R. typus* (data for *Megachasma pelagios* was unavailable). All data were normalized prior to analysis and were subjected to the SIMPROF test within PRIMER 6 to test for statistical significance of individual clusters.

Interpretation of cluster analyses was best used in conjunction with non-metric multidimensional scaling (MDS) ordinations [e.g., Kruskal and Wish, 1978]. An MDS plot was constructed based on the parameters described above and stress, or 'goodness of fit', was calculated within PRIMER 6 [Kruskal, 1964]. Although computationally demanding, MDS offered advantages over other multivariate analyses (such as principal components analysis or correspondence analysis) [Clarke and Warwick, 2001], with better conversions of dissimilarity to distances in small-dimensional space and fewer assumptions about the pre-existing relationships between data points [e.g., Everitt, 1978; Kenkel and Orloci, 1986].

Results

Encephalization Relative to Other Elasmobranchs

The size of the brain of the whale shark, *R. typus*, was compared to 59 other cartilaginous fishes from Northcutt [1978], Kruska [1988], Ito et al. [1999], Yopak et al. [2007], and Yopak and Montgomery [2008]. Total brain mass was estimated based on hemisected samples from two juvenile specimens of *R. typus* that were scanned and digital segmented using MRI. Using 60 chondrichthyans (including *R. typus*) as independent data points, the dependent variable (brain mass) increased with the independent variable (body mass) according to the allometric relationship $y = 0.478x - 1.045$ ($r^2 = 0.75$, n = 60; fig. 5A, solid line). When seven orectolobiform species were plotted separately, the allometric relationship became $y = 0.408x - 0.938$ ($r^2 = 0.89$, n = 7; fig. 5A, hatched line). As the former was more similar to previously published values [Northcutt, 1978; Myagkov, 1991; Yopak et al., 2007; Yopak and Montgomery, 2008], this equation was used to calculate encephalization quotients (EQs) (table 2). Within the orectolobiforms, EQs ranged from 0.40 *(Orectolobus maculatus)* to 1.17 *(Nebrius ferrugineus)*. The EQ value for *R. typus* was 0.45, one of the lowest EQ values for the order. Species with comparable EQ values to *R. typus* included the lamniforms, *Carcharodon carcharias* (EQ = 0.44) and *Cetorhinus maximus* (EQ = 0.49), as well as the orectolobiform *O. ornatus* (EQ = 0.48).

Brain mass was then scaled against body mass using phylogenetically independent contrasts, with positivized body mass contrasts. The allometric relationship based on these analyses was $y = 0.353x$ ($r^2 = 0.81$, n = 59; fig. 5B).

To assess the relationship between the size of the brain of *R. typus* and other cartilaginous fishes, vertical deviations from the predicted slope were then calculated from independent contrasts, termed 'RMA residuals', using the phylogenetically reduced major axis [Ives et al., 2007], where brain mass was found to increase with body mass according to the relationship $y = 0.393x - 0.7998$ ($R^2 = 0.81$, $n = 59$). The pattern of results was significantly similar to those obtained from EQs ($R = 0.932$, $n = 60$, $p < 0.0001$, Rank Correlation). Within the orectolobiforms, as with the EQs, *O. maculatus* had the lowest RMA residual value (–0.30) and *N. ferrugineus* had the highest (0.21). *R. typus*, with an RMA residual value of –0.06, had a small brain for its large body size, a pattern similarly seen in other large elasmobranchs such as *C. maximus* (RMA residual = –0.8) and *C. carcharias* (RMA residual = –0.7) (table 2).

Quantitative Brain Organization

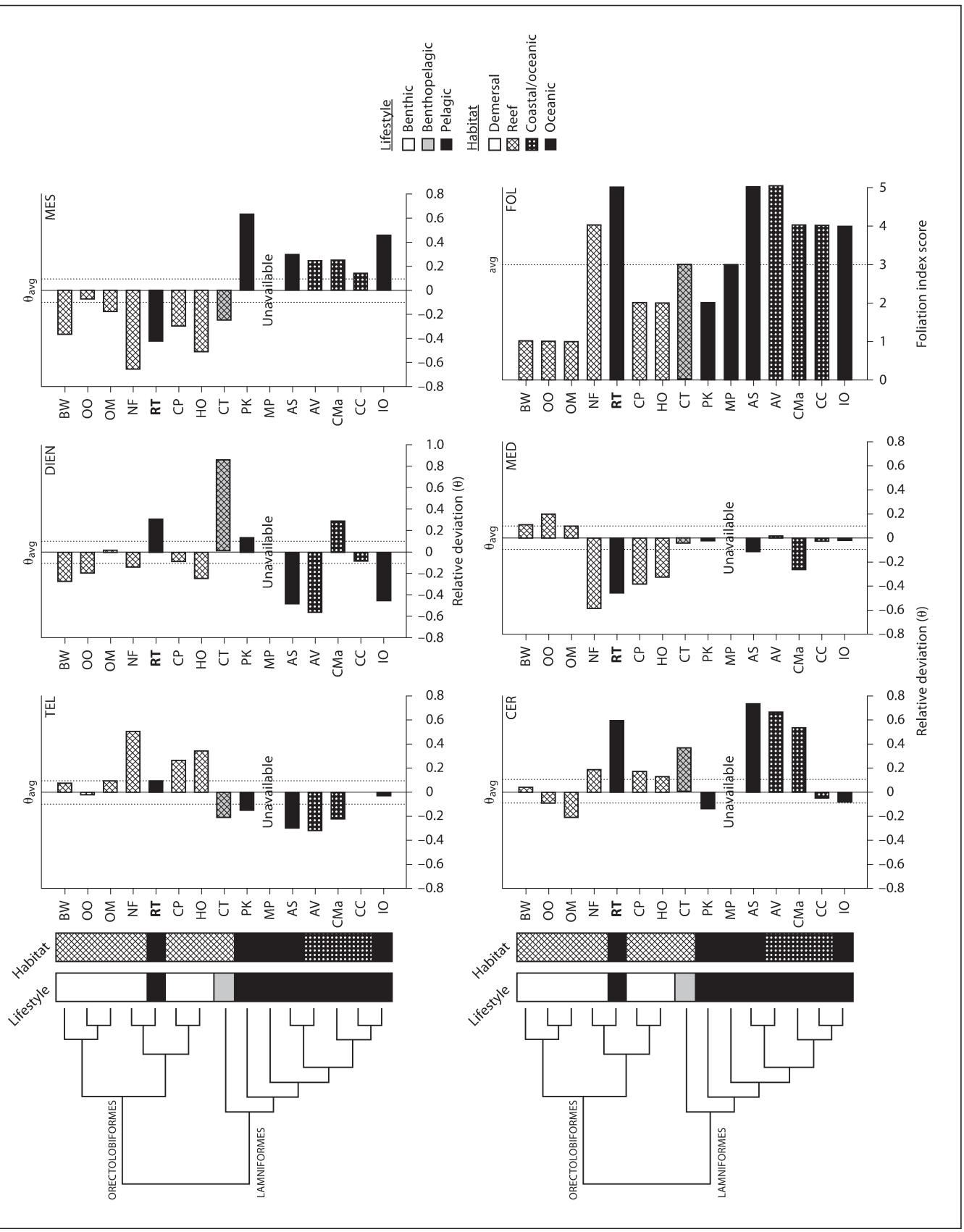
The relative size of the major brain areas (telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata) was assessed in 63 sharks and holocephalans, including *R. typus*. For comparative purposes, relative development (θ) for *R. typus* was presented alongside data for seven orectolobiforms and seven lamniforms from Northcutt [1978], Yopak et al. [2007], and Yopak and Montgomery [2008] (fig. 6). For orectolobiforms, the telencephalon occupied between 38% (θ = –0.02; *Orectolobus ornatus*) and 58% of total brain mass (θ = 0.50; *Nebrius ferrugineus*). The telencephalon of *R. typus* accounted for 42% of total brain (θ = 0.09), showing relatively average development, similar to other members of its order such as *O. maculatus* and *Brachaelurus waddi* (fig. 6). The diencephalon of *R. typus* was relatively enlarged (θ = 0.30), occupying 7.9% of the brain. Orectolobiforms generally had relatively average to reduced diencephalons occupying between 6.2% in *O. maculatus* (θ = 0.01) and 4.4% in *B. waddi* (θ = –0.28) of the brain. In contrast, the planktivorous lamniform *C. maximus* had a diencephalon that accounted for approximately 7.8% of the brain (θ = 0.29), similar to the diencephalon of *R. typus*. Mesencephalic development in the whale shark (θ = –0.42), which was 7.2% of total brain mass, closely matched that of other orectolobiforms, which showed relative reduction of this structure [-0.07 *(O. ornatus)* $\leq \theta \leq -0.65$ *(N. ferrugineus)*], whereas lamniforms showed relative enlargement of the mesencephalon [0.14 *(C. carcharias)* $\leq \theta \leq 0.63$ *(Pseudocarcharias kamoharai)*] (fig. 6).

Relative size of the cerebellum varied across both the Orectolobiformes and the Lamniformes orders (fig. 6). *R. typus* showed relative enlargement of this structure, occupying 29% of the total brain. Extreme hypertrophy of the cerebellum is not seen in any other orectolobiform, but is found in lamniforms such as *Alopias superciliosus* (θ = 0.73), *A. vulpinus* (θ = 0.66), and *C. maximus* (0.53) (fig. 6). Similarly, foliation of the cerebellar corpus was generally not found in orectolobiforms, with the exception of *N. ferrugineus* and *R. typus*. According to the classification schema of Yopak et al. [2007], which ranges from 1–5 in increasing complexity, the foliation of the cerebellum in the whale shark had a score of 5. High foliation was seen in the majority of lamniforms, particularly the alopiids (fig. 6). Interestingly, all three large-bodied planktivorous pelagic sharks *(R. typus*, *C. maximus*, *M. pelagios)* had medium to high levels of foliation of the cerebellar corpus (fig. 6).

In general, both the orectolobiforms and the lamniforms had relatively average to small medullas in comparison to other chondrichthyans, with the exception of *O. ornatus*, ranging from 29 to 10% of total brain (fig. 6). The medulla of *R. typus* (θ = –0.45), along with *N. ferrugineus* (θ = –0.59), *Chiloscyllium punctatum* (θ = –0.38), *Hemiscyllium ocellatum* (θ = –0.32), and *C. maximus* (θ = –0.26), were the most relatively reduced across the two orders (fig. 6).

Cluster Analyses and Multidimensional Scaling

Interspecific differences between the brain organization of *R. typus* and the relative proportions of five major brain areas in 62 other chondrichthyans from Northcutt [1978], Yopak et al. [2007], and Yopak and Montgomery [2008] were assessed statistically using cluster analysis, on which the SIMPROF test was performed to determine significance of individual clusters. The analysis produced a dendrogram with eight significant clusters (A–H; fig. 7). Although there were some instances of closely related species clustering together, *R. typus* did not cluster with other orectolobiforms, but rather was grouped into Cluster A with three lamniforms: the two alopiids and the other plantivorous pelagic shark, *Cetorhinus maximus* (fig. 7), likely due to the relative size of the cerebellum across these four species (fig. 7). The cerebellum of all four species in Cluster A accounted for between 28 and 32% of the brain [0.53 *(C. maximus)* $\leq \theta \leq 0.73$ *(A. superciliosus)*], with foliation scores of 4 or 5 (fig. 6). Other members of the Oretolobiformes and Lamniformes orders were widespread across the eight clusters, grouping with species that shared similar habitats and/or lifestyle



characteristics as opposed to phylogenetic relatedness. The orectolobiforms occupied four of the eight clusters presented (Clusters A, B, C, G), whereas the lamniforms were found across five of the eight total clusters (Clusters A, D, E, F, G; fig. 7).

To check the consistency of both representations, nonmetric multidimensional scaling (MDS) was also used. The result of MDS (fig. 8) was a two-dimensional (2D) plot where the position of each species was indirectly proportional to the similarity of each species, with shorter distance corresponding to a greater similarly (stress = 0.15). A stress value less than 0.2 indicated the plot was useful and not due to randomness [Clarke, 1993]. The first representation of the plot depicted the groupings derived from cluster analysis (fig. 7) at the 95% confidence level (fig. 8A) and the second depicted taxonomic order, with Cluster A (fig. 7) superimposed onto the 2D MDS with a solid line (fig. 8B). Although it was clear that chondrichthyans, in general, shared similarities in terms of their brain, as the groupings within the plot were not all naturally defined. *R. typus*, *C. maximus*, and the alopiids were markedly separated from the other specimens in terms of the relative proportion of their five major brain areas and foliation of the cerebellar corpus. This visual representation of the structured grouping of species supported the argument that the brain organization *R. typus* was more similar to species that lived in a pelagic environment and, in the case of *C. maximus*, those that had similar locomotory and prey capture strategies, than other members of the Orectolobiformes order.

Discussion

Whale sharks *(R. typus)*, along with megamouth and basking sharks, represent an enormous lifestyle change in chondrichthyan evolution, with extremely large body sizes and unique feeding mechanisms. Distributed circumglobally, little is known about the biology, behavior, and biogeography of *R. typus*, and even less is known about their neural development. Although some data is available on brain organization in planktivorous pelagic sharks [Sato, 1986; Kruska, 1988; Ito et al., 1999], this paper takes into account the underlying influence of phylogeny and explores some of the neuromorphological adaptations in *Rhincodon* that might have accompanied adaptations in primary habitat, locomotory mode, and prey capture strategy. The brain organization of *R. typus* was assessed by comparing relative brain mass (encephalization), the relative size of the telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata [relative deviation, θ; Wagner, 2001a, b], and the foliation of the cerebellar corpus [foliation index score; Yopak et al., 2007] to data on 63 other chondrichthyan species from Northcutt [1978], Kruska [1988], Ito et al. [1999], Yopak et al. [2007], and Yopak and Montgomery [2008]. This study marks one of the first attempts to gain an evolutionary perspective into the brain of *R. typus*, in comparison to both phylogenetically related species as well as those that share certain lifestyle characteristics.

The study of species with unique behavioral and morphological specializations is critical when teasing apart evolutionary trends, yet becomes difficult as often these specimens are rare and/or difficult to obtain. The use of MRI in comparative neuroanatomical studies facilitates the acquisition of non-invasive, high-resolution 3D data of soft tissue structures, which is particularly important in rare samples where destructive gross dissection is impossible. MRI technology has been recently emerging as an effective investigative tool for non-invasive visualization and quantification of the internal anatomy of teleost fishes [Forbes et al., 2006; Perry et al., 2007; Sepulveda et al., 2007; Rogers et al., 2008], as well as for the comparative brain anatomy of birds [Corfield et al., 2008a, b], chondrichthyans [Yopak and Frank, 2007; Yopak et al., 2009], and mammals [e.g., Marino et al., 2001a, b, 2003; Hakeem et al., 2005; Montie et al., 2008]. Although histological techniques provide higher spatial resolution (up to the cellular level) than MRI data, these more traditional methods are highly manual and constrained to two dimensions. MRI is able to efficiently and non-invasively identify and delineate major brain areas to produce volumetric data [Yopak and Frank, 2007; Yopak et al., 2009] that is comparable to existing data on chondrichthyan brain morphology and is similar to traditional methods (such as histology) [e.g., Marino et al., 2003; Kaufman et al., 2005; Oelschlager et al., 2008], and thus has the po-

Fig. 6. Weighted factors (θ) for the five major brain structures, the telencephalon (TEL), diencephalon (DIEN), mesencephalon (MES), cerebellum (CER), and medulla (MED), as well as foliation index scores (FOL), for seven Orectolobiformes and eight Lamniformes. Weighted factors show the deviation from the average relative volume for each brain structure [Wagner, 2001a, b]. Species are grouped phylogenetically and coded according to their lifestyle and primary habitat. For species abbreviations, see table 2 and/or figure 4. Note that only foliation data was available for *Megachasma pelagios*.

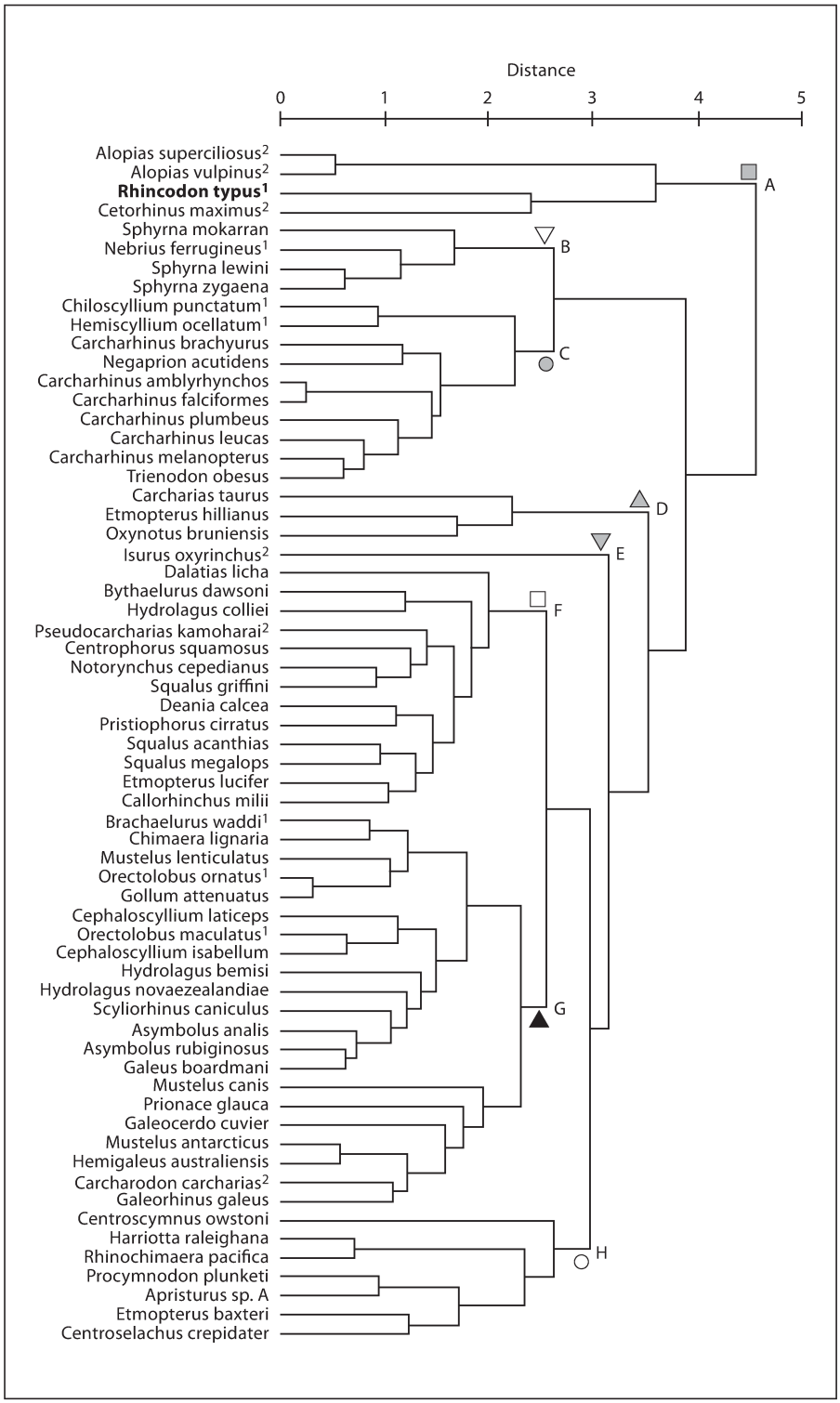


Fig. 7. Cluster analysis dendrogram based on the relative size of each of the five brain areas as a proportion of total brain and foliation index score for 63 chondrichthyan species, including *Rhincodon typus*. Dotted lines indicate clusters that were not statistically significant (SIMPROF, Primer 6, $p < 0.05$, $n = 63$). Orectolobiforms and lamniforms are indicated by superscripts 1 and 2, respectively.

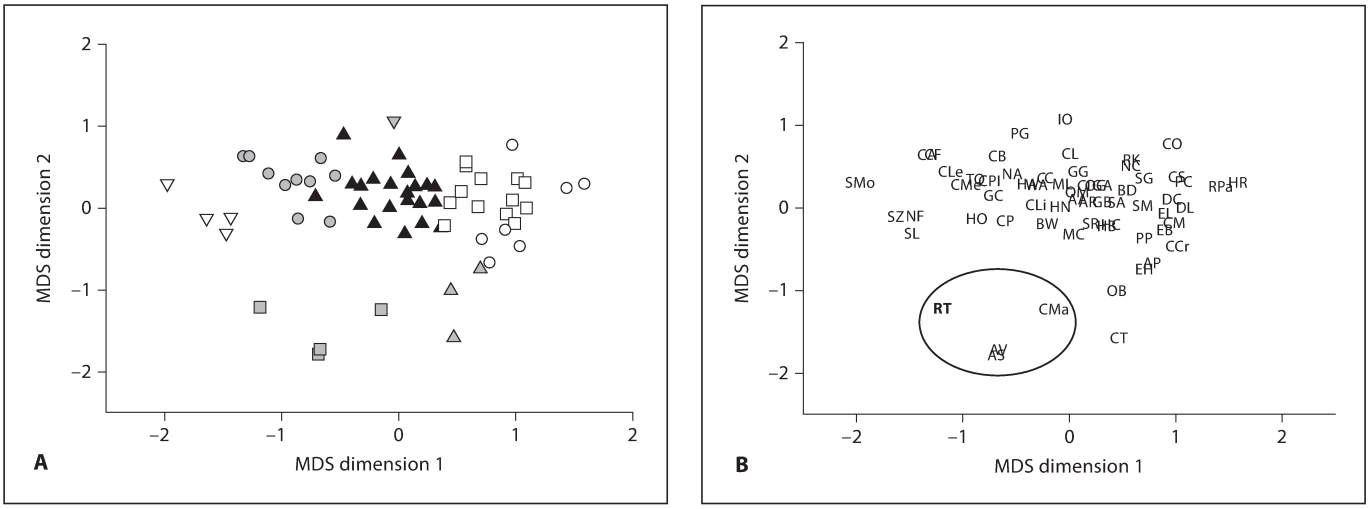


Fig. 8. Two-dimensional non-metric multidimensional scaling (MDS) plots of 63 chondrichthyans (2D stress = 0.15), based on Euclidean distances. MDS configurations show both species coded according to cluster group (**A**), as well as individual species data (**B**). The cluster containing the species specifically examined in this study (*Rhincodon typus,* boldface) was superimposed onto the 2D MDS at the 95% confidence level with a solid line. For species abbreviations, see table 2 and figure 4.

tential to revolutionize the way we study disparate types of neuroanatomical information.

When dealing with rare specimens, it is often the case that allowances must be made for parameters that could bias data. This study quantified the brain organization of two aquarium specimens of *R. typus*, whose brains were hemisected to determine cause of death. In this instance, cause of death was not verified and although no neural anomalies were observed, it must still be taken into account that overall brain mass and brain structure mass might have been affected by an unknown illness. Both brain mass and brain structure mass values between the two samples were comparable, however; therefore it was determined that the data was sufficiently representative of the brain organization for this unique species.

Encephalization

Encephalization of the brain of cartilaginous fishes has been addressed in previous studies [Bauchot et al., 1976; Northcutt, 1977, 1978; Myagkov, 1991; Striedter, 2005]. More recent studies have augmented this data and quantified the phylogenetic component of brain size in sharks [Yopak et al., 2007; Yopak and Montgomery, 2008] and batoids [Lisney et al., 2008], and chondrichthyans have been found to have brain weight to body weight ratios that are comparable to birds and mammals [Northcutt, 1977, 1978; Striedter, 2005; Yopak et al., 2007].

The coefficient of allometry (α) for the Orectolobiformes order, calculated using species as independent data points ($\alpha = 0.41$, n = 7), was lower than the value calculated across 60 sharks and holocephalans ($\alpha = 0.48$, n = 64) (fig. 5), indicating that orectolobiforms have small brains relative to their body size in comparison to other chondrichthyans. Previous coefficients of allometry recorded for sharks have been higher than those reported here, as documented in recent studies by Myagkov [1991; $\alpha = 0.54$, n = 38], Demski and Northcutt [1996; $\alpha = 0.543$, n = 27], Yopak et al. [2007; $\alpha = 0.542$, n = 42]; Yopak and Montgomery [2008; $\alpha = 0.54$, n = 57]. Although allometric relationships across large datasets of sharks and holocephalans have been documented [e.g., Myagkov, 1991; Demski and Northcutt, 1996; Yopak et al., 2007; Yopak and Montgomery, 2008], this is the first study that has included large-bodied filter-feeding elasmobranchs. The incorporation of species such as *R. typus* [this study], *Cetorhinus maximus* [Kruska, 1988], and *Megachasma pelagios* [Ito et al., 1999] has clearly influenced the allometric scaling of brain mass to body mass in this clade. The coefficient of allometry obtained from the independent contrasts analysis ($\alpha = 0.353$; fig. 5B) was lower than those reported when using species as independent data points (fig. 5A), indicating that previous estimates have been biased by phylogenetic contrasts. When the phylogenetically reduced major axis (RMA) was calculated ($\alpha_{RMA} = 0.39$), the LS regression slope that

was obtained was independent of both phylogenetic constraints and the error associated with the variability inherent in estimating body mass [Ives et al., 2007]. These values were more comparable to those reported in the literature from independent contrasts analysis [$\alpha = 0.38$, $n = 37$: Yopak et al., 2007; $\alpha = 0.37$, $n = 57$: Yopak and Montgomery, 2008].

Encephalization quotients (EQs) and 'RMA residuals' were calculated with all available raw species data in order to assess the relative brain size in *R. typus* compared to other orectolobiforms and those species living in similar primary habitats. In comparison to data for 59 other cartilaginous fishes from the literature [Northcutt, 1978; Yopak et al., 2007; Yopak and Montgomery, 2008], *R. typus* demonstrated a small brain for its body size, similar to the relative brain sizes of other large-bodied planktivorous pelagic sharks (table 2; fig. 5A). Although *R. typus* was one of only seven shark species to have an EQ value <0.50, indicating an extremely small brain for its body size, its RMA residual value (–0.06: table 2) indicated that, although the brain of *R. typus* was relatively reduced, other species, such as *M. pelagios* (RMA residual = –0.27) and *Orectolobus* spp. (residual ≤ –0.26) showed far greater decreases in relative brain size. These data corroborated the view that EQs were, in fact, highly flawed [e.g., Felsenstein, 1985; Harvey and Pagel, 1991; van Dongen, 1998] and that when comparing brain mass to body mass in chondrichthyans, it was critical to assess these relationships independent of phylogenetic constraints [Felsenstein, 1985].

Independent decreases in relative brain size have occurred several times in the chondrichthyan radiation, observed in the large-bodied basking shark *(Cetorhinus)* [Kruska, 1988] and megamouth shark *(Megachasma)* [Ito et al., 1999], as well as other small-bodied species, such as the cookie cutter shark *(Istitius)* and the angel shark *(Squatina)* [Bauchot et al., 1976; Striedter, 2005]. The whale shark was clearly another example of a phylogenetic decrease in relative brain size (fig. 5), coinciding with an enormous increase in body size, reaching total body lengths of 12 m or more [Compagno, 2001]. In contrast, the closest phylogenetic relative to *R. typus* examined here, the orectolobiform *Nebrius ferrugineus*, had a relatively enlarged brain (table 2) [Yopak et al., 2007] and was known to reach a maximum size of only 320 cm [Compagno, 1984a]. Larger brain sizes have been correlated with habitat, lifestyle, and cognitive capabilities in a variety of vertebrate groups [e.g., Budeau and Verts, 1986; Kotrschal et al., 1998; Striedter, 2005; Pollen et al., 2007; Shumway, 2008], including chondrichthyans [Bauchot et al., 1977; Northcutt, 1977, 1978; Yopak et al., 2007; Yopak and Montgomery, 2008; Lisney et al., 2008]. It has been proposed that the relatively small brains of planktivorous predators were related to their opportunistic passive predation strategies [Nilsson et al., 2000], which might be less cognitively demanding in terms of sensory and/or motor requirements in comparison to more agile hunters [Kruska, 1988; Ito et al., 1999; Nilsson et al., 2000; Yopak et al., 2007]. Diet, foraging strategies, and strategic hunting have been linked to brain size [reviewed by Striedter, 2005] in various vertebrate groups, such as teleosts [Bauchot et al., 1977; Kotrschal et al., 1998], birds [Bennet and Harvey, 1985; Lefebvre et al., 1997; Hunt et al., 2001], and mammals [Eisenberg and Wilson, 1978; Clutton-Brock and Harvey, 1980; Pirlot and Jolicoeur, 1982; Bennet and Harvey, 1985; Harvey and Krebs, 1990; Hutcheon et al., 2002]. An exception has been noted, however, in the planktivorous mobulids, species that have the most encephalized brains of all chondrichthyans [Striedter, 2005; K. Yopak, unpublished observations], a trait that is likely linked to its complex behaviors and 'social intelligence' as opposed to its foraging strategy [Kotrschal et al., 1998; Striedter, 2005].

In amniotes, it has been suggested that brains increase in size up to a certain plateau, whereas body size continues to increase [reviewed by Striedter, 2005]. Indeed, even various components of the brain itself might develop at different rates, with the hindbrain developing more slowly than the forebrain [Finlay and Darlington, 1995; Finlay et al., 1998]. Ito et al. [1999] proposed that the brain of the megamouth shark might approach maximum size at birth, as evidenced by its extremely large endocranial cavity relative to the brain in its adult form [Ito et al., 1999], although all that can be definitively inferred from this observation was that the brain and the cranium grow at different rates. Results on other large-bodied elasmobranchs, including *Carcharodon carcharias* [Demski and Northcutt, 1996] and *Cetorhinus maximus*, supported this assertion, where the brain occupied merely 1/16 of the cranial cavity in some cases [Kruska, 1988]. Although only observed qualitatively, the size of the brain relative to the cranial space in the two juvenile whale sharks (RT_1, RT_2) was similar, in that it only occupied a small portion of the existing endocranial cavity [R. Hueter, unpublished observations]. Cranial endocasts have often been used to make assumptions about the size of the brain in extinct and/or rare specimens [e.g., Jerison, 1973; Tobias, 2001; Smith, 2002], although these studies were prone to error as the cranial space to brain ratio varied amongst

different vertebrate groups [Hopson, 1979; Striedter, 2005] and, in the case of large planktivorous elasmobranchs [Kruska, 1988; Ito et al., 1999], would have lead to gross overestimations of relative brain size. The extent of unoccupied cranial space in *R. typus* appeared to vary with ontogeny, as the brain of the neonate specimen of this species nearly filled the cranial cavity (fig. 3), which suggested that the brain of these animals was larger relative to their body size in early developmental stages and that the body (and by extension, the cranium) grew at a more rapid rate than the neural tissue throughout its lifespan.

General Brain Organization

The brain organization of *R. typus*, in terms of the relative proportions of the five major brain structures and foliation of the cerebellum, was compared across 63 chondrichthyans from Northcutt [1978], Yopak et al. [2007], and Yopak and Montgomery [2008]. Cluster analysis and MDS were used to compare the brain of *Rhincodon typus* with members of its taxonomic order (Orectolobiformes), as well as with those that occupied a similar pelagic habitat and/or employed similar predation strategies. The majority of orectolobiforms were clustered with species that shared habitat and lifestyle similarities as opposed to taxonomic classification (fig. 7). Although some of the brain characteristics of *R. typus*, including relative reduction of the mesencephalon (fig. 6), were similar to other orectolobiforms, this species formed a cluster (Cluster A) with three members of the Lamniformes order, *Alopias superciliosus*, *A. vulpinus*, and *Cetorhinus maximus*, and were in close proximity in the MDS ordination (fig. 8). These four species shared the common characteristics of a relatively average to reduced telencephalon and medulla, and an exceptionally large cerebellum with high levels of foliation.

The telencephalon of *R. typus* was relatively average sized, although larger in comparison to the other species in Cluster A (fig. 6, 7). Qualitatively, the telencephalon was a fairly round, bulbous mass (fig. 2) with moderate development of the central nucleus of the dorsal pallium, although this portion of the forebrain was not as pronounced as in other species, such as *Sphyrna* and *Carcharhinus* [Demski and Northcutt, 1996]. A relatively enlarged telencephalon, as reported in members of Carcharhinidae and Sphyrnidae [Yopak et al., 2007], has been associated with species that live in complex habitats and the social interactions that occur in those habitats [Striedter, 2005], similarly documented in other vertebrate groups [Riddell and Corl, 1977; Huber et al., 1997; Pollen et al., 2007; Shumway, 2008]. In particular, development of the central nucleus has been linked to complex social behaviors such as dominance hierarchies and other forms of 'social intelligence' [Cohen et al., 1973; Graeber, 1978; Graeber et al., 1978; Demski and Northcutt, 1996]. An enlarged central nucleus of the dorsal pallium has been recorded in *Cetorhinus maximus* [Kruska, 1988], although was described as being less developed than that of *R. typus* [Sato, 1986]. Moderate development of the dorsal pallium might indicate that *Rhincodon* is a more social species than *Cetorhinus*, although with probably less complex social behaviors than those observed in hammerhead and reef shark species [Springer, 1967; Johnson and Nelson, 1973; Myrberg and Gruber, 1974; Gruber and Myrberg, 1977; Klimley, 1985; Ritter and Godknecht, 2000]. Documented sighting of large numbers of whale sharks have been reported, with oftentimes predictable aggregations occurring throughout their general circumglobal distribution [Colman, 1997; Compagno, 2001; Nelson, 2004; Stewart and Wilson, 2005; Graham and Roberts, 2007; Rowat and Gore, 2007], sometimes associated with reproduction events [Last and Stevens, 1994; Compagno, 2001; Stevens, 2007]. Some social complexity within these aggregations, such as segregation by sex and size, has been reported [Taylor, 1994; Colman, 1997; Eckert and Stewart, 2001], which might have been reflected in the moderate protrusion of the central forebrain of *Rhincodon*.

The mesencephalon of the whale shark was relatively reduced, similar to other orectolobiforms (fig. 6), although this did not seem to affect the clustering pattern (fig. 7) or the MDS ordination (fig. 8). Pelagic predators have been documented as having relatively enlarged mesencephalons, including *Alopias* [Yopak et al., 2007], a characteristic that has been previously attributed to visual predation in elasmobranchs [Yopak et al., 2007; Yopak and Montgomery, 2008], particularly in relation to the size of the optic tectum [e.g., Demski and Northcutt, 1996; Lisney et al., 2007]. Despite its pelagic lifestyle, *Rhincodon* is probably not a visual predator, as the majority of its diet includes the passive suction filter-feeding of planktonic crustaceans in addition to more active and nektonic squid and small fishes [Compagno, 1984a], but the enlarged mesencephalon observed in *Cetorhinus*, a species with similar predation strategies, contrasts with this view. Because this midbrain structure is implicated in processing visual input [Hofmann, 1999], integrating electrosensory and mechanosensory information [Bres, 1993; Tricas et al., 1997], and might be responsible for the

Table 4. The relative sizes (as a proportion of the total brain) and weighted factors (θ) for the five major brain areas (telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata) for *Rhincodon typus* (RT) and *Cetorhinus maximus* (CMa), two planktivorous pelagic shark species. Weighted factors are based on relative size structure data on total of 63 sharks and holocephalans from Northcutt [1978], Yopak et al. [2007], and Yopak and Montgomery [2008]. Note: Relative sizes of the brain areas for CMa were based on a polygonal estimation analysis

Species	Relative structure mass, %									
	Telencephalon		Diencephalon		Mesencephalon		Cerebellum		Medulla	
	mass, %	θ	mass, %	θ	mass, %	θ	mass, %	θ	mass, %	θ
RT	42.19	0.09	7.92	0.30	7.24	–0.42	29.47	0.59	13.17	–0.45
CMa	30.44	–0.22	7.83	0.29	15.60	0.25	28.39	0.53	17.70	–0.26

behavioral response to novel visual stimuli [Bodznick, 1991], an examination of discrete sub-sections of this brain structure would be required to assess functional correlates with relative development in this clade [Yopak and Montgomery, 2008].

The diencephalon of *Rhincodon* was relatively large in comparison to other chondrichthyans, particularly other orectolobiforms. The size of the diencephalon of *R. typus* was most similar to that of *Cetorhinus*, which might have influenced the clustering pattern observed here (fig. 7). The medulla of the four species comprising Cluster A, including *Rhincodon*, was relatively average to reduced, with the medulla of both *Rhincodon* and *Cetorhinus* accounting for less than 18% of the brain (table 4). Reduction of the medulla has been seen primarily in pelagic elasmobranchs and those species that are associated with coral reefs (fig. 6) [Yopak et al., 2007]. Enlargement of the medulla, in contrast, has been found in deep sea chondrichthyans, and has been associated with development of the dorsal and medial octavolateral nuclei [Yopak and Montgomery, 2008], which receive projections from the electroreceptive and lateral line organs [Hofmann, 1999]. Although the vestibulolateral lobe of *Rhincodon* has been previously described as well developed [Sato, 1986], this was not reflected in the relative size of the medulla, and further research is needed in order to assess the peripheral and central anatomy of *Rhincodon* related to the acoustico-lateralis senses.

Cerebellar Development

The cerebellum appeared first in early elasmobranchs [Butler, 2003] and exhibits substantial variation in both its degree of folding and level of symmetry [Northcutt, 1977, 1978; Yopak et al., 2007, 2009; Yopak and Montgomery, 2008; Yopak and Frank, 2007; Lisney et al., 2008]. One of the most notable characteristics of the brain of *R. typus* was a large and highly foliated cerebellum, two traits shared with *Alopias* spp. and *Cetorhinus* (fig. 6). The cerebellum of these four species was the most relatively enlarged to date within the sharks and holocephalans, occupying between 28 and 32% of the brain [Yopak et al., 2007; Yopak and Montgomery, 2008]. Although physiological and behavioral research on chondrichthyan fishes has resulted in a lack of functional consensus, similar to the functional debate for the human cerebellum [Cordo et al., 1997; Highstein and Thach, 2002], there has been compelling evidence for the cerebellum's role in modulation of motor programs [Paul and Roberts, 1979; New, 2001], self-motion error correction [Montgomery et al., 2002], and dynamic state estimation for coordination of target tracking [Paulin, 1993, 1997].

The cerebellum of *Rhincodon* was divided by transverse sulci into at least two lobes, with a noticeably developed anterior lobe (fig. 2) and both prior research [Sato, 1986] and neonate data have suggested that there might be a medial lobe as well (fig. 3). The surface of each lobe was comprised of a multitude of invaginations and folds (fig. 2) that extended deep into the cerebellar tissue (fig. 1A), more similar to members of the Lamniformes order. According to the foliation classification scheme of Yopak et al. [2007], the foliation of the cerebellar corpus of the whale shark was given a score of 5, on a scale of 1–5 (fig. 6). High foliation levels in cartilaginous fishes have been linked to agile prey capture and habitat dimensionality [Northcutt, 1978; Yopak et al., 2007; Yopak and Frank, 2007; Yopak and Montgomery, 2008; Lisney et al., 2008] and the ability to perform more multi-faceted mo-

tor tasks [New, 2001]. Scans of a neonate specimen of *R. typus* demonstrated that corpus foliation appeared in early development (fig. 3), although the depth of folds did not appear to be as pronounced, indicating ontogenetic shifts in cerebellar foliation. The body and fin structure of neonate whale sharks was suggestive of an inefficient anguilliform swimming motion. Although speculative, these morphological characteristics potentially provide a functional basis for the ontogenetic changes in cerebellar foliation levels, as the fin and body morphology of adolescent and adult whale sharks, in contrast, indicate propulsive capabilities [Martin, 2007].

The highest level of foliation (index = 5) has been previously documented in highly active and/or maneuverable predators, such as *Alopias* and *Sphyrna* [Yopak et al., 2007] and some myliobatids [Lisney et al., 2008]. Although *Rhincodon* was not noted for being a highly active or agile predator with a complex motor repertoire, the whale shark has been known to make vertical migrations, sometimes to depths of over one mile [Colman, 1997; Hueter et al., 2008], travel extensive distances [Eckert and Stewart, 2001; Wilson et al., 2006; Hueter et al., 2008], and can suspend vertically in the water column [Colman, 1997; Motta, 2004]. This suggested that foliation of the cerebellar corpus might reflect habitat dimensionality and a level of proprioception in these animals to a greater extent than previously thought. With the exception of *Nebrius ferrugineus*, foliation of the cerebellum for the majority of orectolobiforms was low, with scores of 1–2, indicating a possible independent evolution of this characteristic in the whale shark.

The cerebellum of the basking shark and megamouth shark showed average to high levels of foliation, with a foliation index score of 4 and 3, respectively (fig. 6), although it must be noted that the foliation of *Megachasma* was conservatively assessed from drawings rather than photographs [Ito et al., 1999]. Basking sharks are highly migratory [Compagno, 1984a] and the body morphology of both *Cetorhinus* and *Rhincodon* is comparable to fast-moving pelagic swimmers [that have correspondingly high levels of foliation; Yopak et al., 2007] with a thunniform body shape and heterocercal tail, characteristics that have been attributed to efficient cruising speeds [Wilga and Lauder, 2004]. The average foliation of the cerebellar corpus of *Megachasma* suggested that it was much less active than *Rhincodon* or *Cetorhinus*, which corroborated with its body morphology [Compagno, 1984a]; but a more in-depth investigation into the behavioral ecology of these animals is required. Although it was uncertain whether structural enlargement equated to sensory specialization, similar patterns of brain organization, particularly a large and highly foliated cerebellum, provided evidence for convergent evolution between the lamniform *Cetorhinus maximus* and the orectolobiform *R. typus*, two taxonomically divergent planktivorous pelagic shark species.

Conclusions

The brain of *R. typus* has been described in terms of its relative size (encephalization) and brain organization. High-resolution 3D images of two hemisected samples were obtained using MRI, from which major brain characteristics were assessed. *R. typus* had a small brain relative to its body mass, similar to the lamniforms *Carcharodon carcharias*, *Cetorhinus maximus*, and *Carcharias taurus*. *R. typus* possessed a relatively small telencephalon with some development of the dorsal pallium, suggestive of moderate social behavior, as well as a relatively enlarged diencephalon and a relatively reduced mesencephalon. The most notable characteristic of the brain of *Rhincodon* was a large and highly foliated cerebellum, one of the largest cerebellums within the chondrichthyan clade. Similar development of the cerebellum was seen in the alopiids and the basking shark, *C. maximus*. Hierarchical cluster analysis and multidimensional scaling provided evidence for convergent evolution in the brains of two planktivorous pelagic sharks, *R. typus* and *C. maximus*, wherein organization of the brain was more similar in species with analogous but independently evolved lifestyles than those that shared taxonomic classification.

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