Journal of the Marine Biological Association of the United Kingdom

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Ontogenetic shifts and sexual dimorphism in the brain organization of the small-spotted catshark *Scyliorhinus canicula*

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Original Article

Cite this article: Roussos I, Megalofonou P (2021). Ontogenetic shifts and sexual dimorphism in the brain organization of the small-spotted catshark *Scyliorhinus canicula*. *Journal of the Marine Biological Association of the United Kingdom* **101**, 189–198. https://doi.org/10.1017/S0025315420001368

Received: 13 January 2020
Revised: 20 November 2020
Accepted: 30 December 2020
First published online: 11 February 2021

Key words:
Brain size; brain somatic index; elasmobranchs; Mediterranean Sea; neuromorphology; ontogeny; *Scyliorhinus canicula*; sexual dimorphism

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Abstract

In this study, we investigated ontogenetic and sexual changes of the brain scaling as well as the scaling and the relative size of six major brain areas in the small-spotted catshark *Scyliorhinus canicula* from the Mediterranean Sea. The brain somatic index (0.31–1.25%) did not differ significantly between sexes but was significantly affected by size with smaller specimens exhibiting higher values. Brain growth exhibited negative allometry (allometric coefficient 0.634), not affected by sex or maturity status. The brain growth rate was found to be higher compared with a previous study from the Atlantic Ocean. Regarding the scaling of the brain areas, the olfactory bulbs scaled with positive allometry, the telencephalon and the diencephalon scaled with the same rate of negative allometry, the mesencephalon exhibited even higher negative allometry, while the cerebellum and the medulla oblongata both followed a close-to-isometric growth pattern. Immature *S. canicula* possessed a larger mesencephalon and diencephalon, highlighting the importance of vision in this life period, while mature specimens had enlarged olfactory bulbs, indicating that olfaction may be more important after the animal attains sexual maturity. In respect of sexual dimorphism, males had a larger cerebellum and medulla oblongata, while females had enlarged telencephalon and olfactory bulbs.

Introduction

Fish brains and their architecture vary greatly between species (Kotrschal *et al.*, 1998), exhibiting a higher degree of divergent differentiation than in any other group of vertebrates. Overall brain size and the size of the main brain areas is one of the simplest and most frequently used proxies for the cognitive ability of species in the studies of brain development, while the sizes of brain areas involved in sensory processing are used as measures of the development and relative importance of different sensory systems (Striedter, 2005). Besides the traditional interest in interspecific brain variation, an increasing number of studies focus on within-population brain plasticity patterns (Gonda *et al.*, 2013) in relation to a number of factors such as lifestyle, diet, habitat and behaviour (Kotrschal *et al.*, 1998; Kolm *et al.*, 2009; Yopak & Frank, 2009; Lisney *et al.*, 2017).

Teleost fishes experience many ontogenetic shifts in morphology, physiology or behaviour, corresponding to changes in habitat and resource use that force the organism to cope with a new range of biotic and abiotic factors (Snover, 2008). These ontogenetic shifts relate to adaptations, such as modifications in the structure and the function of the peripheral sensory organs (Shand *et al.*, 2000), that are reflected in changes in the structure and the relative size of the sensory brain areas including the olfactory bulbs, the optic tectum and the anterior and posterior lateral line lobes (Cadwallader, 1975; Brandstätter & Kotrschal, 1989; Montgomery *et al.*, 1997; Kotrschal *et al.*, 1998; Wagner, 2003). The central changes are not only reflected in the sensory areas but also in the multimodal integrative areas, for instance the telencephalon and the cerebellum (Ogawa, 1968; Cadwallader, 1975; Brandstätter & Kotrschal, 1989; Masuda, 2009). The above observations have also been documented in lampreys (Salas *et al.*, 2015).

Despite exhibiting a range of conservative biological traits, such as slow growth, late maturity and low fecundity (Hoenig & Gruber, 1990), elasmobranch life histories very often include ontogenetic shifts in habitat, such as the shift from nursery areas to adult grounds (Castro, 1993), and diet, concerning the prey species and size (Lowe *et al.*, 1996; Heupel & Bennett, 1998). Following sexual maturation there are also changes in behaviour and habitat associated with reproductive activities, such as mating (Grubbs, 2010). As in the case of teleosts, several studies have elaborated the adaptation of the elasmobranch sensory systems and the relevant brain areas in the ontogenetic shifts, but not in extensively (Sisneros & Tricas, 2002; Lisney *et al.*, 2007, 2017; Yopak & Frank, 2009). Brain growth follows similar patterns in elasmobranchs and teleosts, with rapid early growth that slows down in larger individuals resulting in a negative allometric relationship (Kearney, 1914; Bauchot *et al.*, 1976). Besides the existing comparative studies of fish brain size and structure, little is known about the extent of variation in brain size and structure within species and between sexes, although distinguishable patterns have been documented (Ridet *et al.*, 1973; Bauchot *et al.*, 1976; Gonzalez-Voyer *et al.*, 2009; Kolm *et al.*, 2009; Kotrschal *et al.*, 2012; Lisney *et al.*, 2017). In the present study, we

investigated ontogenetic and sexual changes of brain size scaling as well as the scaling and the relative size of the major brain areas (telencephalon, olfactory bulbs, diencephalon, mesencephalon, cerebellum, medulla oblongata) in a catshark of the family Scyliorhinidae.

The small-spotted catshark, *Scyliorhinus canicula* (Linnaeus, 1758), is a very common species in the North-eastern Atlantic Ocean and the Mediterranean Sea (Compagno, 1984; Ellis & Shackley, 1997; Kousteni *et al*., 2010). It is found mainly over sandy, gravelly or muddy bottoms at depths from a few metres up to 550 metres, more frequently on the continental shelf between 50 and 250 m (Compagno, 1984; Abella *et al*., 2017). Variations on the life history traits between the Atlantic and the Mediterranean populations have been detected, namely, the maximum body size and the size of first maturity (Ellis & Shackley, 1997; Kousteni *et al*., 2010), along with genetic differentiations (Kousteni *et al*., 2015). The maximum length of small-spotted catshark in the Atlantic Ocean has been documented as 1000 mm (Compagno, 1984), but specimens exceeding 800 mm are rarely observed (Ivory *et al*., 2004). In the Mediterranean Sea, the highest values were in Northern Tunisia, where males and females reached 580 and 560 mm in length, respectively (Capapé, 1977). Females generally mature at a greater length than males (Ellis & Shackley, 1997; Ivory *et al*., 2004; Kousteni & Megalofonou, 2019) and sexual dimorphism in the head, mouth, body morphology and dentition occurs on the onset of sexual maturity (Arthur, 1950; Bas, 1964; Jardas, 1979; Erdogan *et al*., 2004; Filiz & Taskavak, 2006). In a previous study, the scaling of brain size was investigated in the small-spotted catshark from the Atlantic Ocean (Ridet *et al*., 1973) and it was revealed that the species exhibits the same basic pattern of brain growth as described in other fishes. Nevertheless, no information on the relative size of the major brain areas and differences between juveniles and adults was provided. Here, we present a more comprehensive assessment of ontogenetic shifts in the relative brain size in specimens of *S. canicula* from the Mediterranean Sea. The main goals of the study were (a) to test the hypothesis whether *S. canicula* from the Mediterranean Sea would show different patterns of brain development in relation to *S. canicula* from the Atlantic Ocean, considering the differences that exist in life history traits and genetic variations between the populations in these areas and (b) to investigate the brain organization by means of the relative size of the main brain areas between sexes and between immature and mature (adult) specimens in order to identify ontogenetic shifts.

Materials and methods

Specimen collection and measurements

A total of 65 specimens of *S. canicula* (Table 1) were sampled from commercial catches of the bottom trawl fishery near the Cyclades Islands in the Aegean Sea and were kept frozen at −20°C. Each specimen was defrosted and sex, body mass, total length and head length were recorded (Table 1). Sex was determined externally by the presence of claspers in males (ICES, 2013). Total length (TL) was measured to the nearest millimetre (mm) from the tip of the snout to the tip of the upper lobe of the caudal fin and head length (HL) was measured to the nearest millimetre (mm) from the tip of the snout to the last gill slit (Compagno, 1984). Body mass (round weight, RW) was measured to the nearest gram (g). In order to preserve the brain intact, once the initial measures had been recorded, the head of each specimen was removed and immersed in 4% formalin solution in 0.1 phosphate buffer for 3–5 days with the brain partially exposed. Afterwards, the brain was removed and immersed in 4% formalin

Table 1. Morphometrics and brain characteristics of the 65 specimens of *Scyliorhinus canicula*

		N	TL (mm)	HL (mm)	RW (g)	BW (g)	% BSI	TEL (mm^3)	OLF (mm^3)	DIE (mm^3)	MES (mm^3)	CER (mm^3)	MED (mm^3)
Male	Immature	15	287 ± 43	45 ± 8	67 ± 40	0.44 ± 0.14	0.778 ± 0.197	62.73 ± 32.61	10.50 ± 5.79	5.52 ± 3.06	5.59 ± 2.60	33.53 ± 15.78	14.73 ± 5.18
	Mature	23	438 ± 35	76 ± 8	245 ± 61	1.14 ± 0.18	0.477 ± 0.072	160.65 ± 39.54	31.03 ± 7.96	11.83 ± 3.90	13.20 ± 4.76	82.41 ± 24.25	38.56 ± 13.71
	Total	38	378 ± 84	64 ± 17	178 ± 105	0.87 ± 0.39	0.595 ± 0.200	122.00 ± 60.70	22.71 ± 12.43	9.12 ± 4.73	10.20 ± 5.50	62.59 ± 32.12	29.54 ± 16.18
Female	Immature	20	285 ± 67	46 ± 13	77 ± 60	0.57 ± 0.28	0.825 ± 0.255	77.05 ± 41.58	15.47 ± 11.71	3.95 ± 1.27	6.31 ± 2.44	36.24 ± 27.04	13.03 ± 8.59
	Mature	7	425 ± 15	68 ± 2	238 ± 41	1.10 ± 0.11	0.483 ± 0.089	156.65 ± 37.79	30.81 ± 8.58	8.52 ± 1.53	11.95 ± 2.22	67.50 ± 20.25	26.33 ± 11.42
	Total	27	321 ± 85	52 ± 15	119 ± 90	0.70 ± 0.34	0.725 ± 0.269	97.69 ± 53.45	19.45 ± 12.81	5.38 ± 2.55	7.83 ± 3.45	44.66 ± 28.72	16.61 ± 10.99
Male & Female	Immature	35	286 ± 57	45 ± 11	73 ± 52	0.51 ± 0.24	0.803 ± 0.227	70.91 ± 38.15	13.34 ± 9.83	4.86 ± 2.55	5.99 ± 2.50	35.05 ± 22.50	13.75 ± 7.29
	Mature	30	435 ± 32	74 ± 8	247 ± 57	1.13 ± 0.17	0.478 ± 0.074	159.72 ± 38.52	30.98 ± 7.96	11.17 ± 3.78	12.91 ± 4.30	78.81 ± 23.90	35.71 ± 14.05
	Total	65	355 ± 88	59 ± 17	153 ± 103	0.80 ± 0.38	0.646 ± 0.236	111.90 ± 58.62	21.33 ± 12.59	7.95 ± 4.50	9.23 ± 4.89	55.19 ± 31.80	24.20 ± 15.55

N, sample size; TL, Total length; HL, head length; RW, round weight; BW, brain weight; % BSI, % Brain Somatic Index; TEL, Telencephalon volume; OLF, Olfactory bulbs volume; DIE, Diencephalon volume; MES, Mesencephalon volume; CER, Cerebellum volume; MED, Medulla oblongata volume.
The specimens were divided by sex (Male – Female) and maturity status (Immature – Mature). Mean ± standard deviation (SD) of each group is presented

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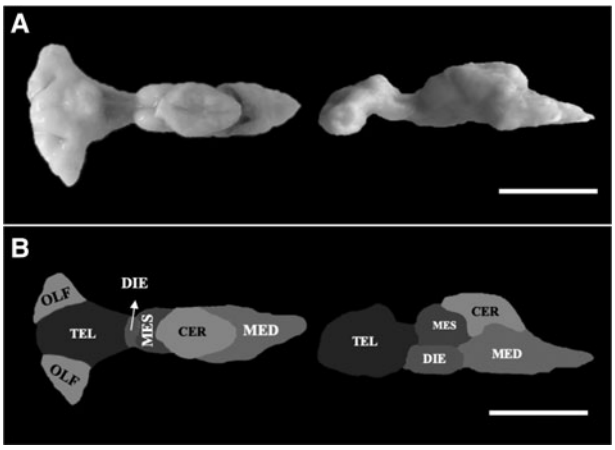

Fig. 1. Dorsal and lateral views of *Scyliorhinus canicula* brain from a representative adult male individual (A) and diagram of the six brain areas that were identified (B). TEL, telencephalon; OLF, olfactory bulbs; DIE, diencephalon; MES, mesencephalon; CER, cerebellum; MED, medulla oblongata. Scale bar: 1 cm.

solution in 0.1 M phosphate buffer for fixation for about 2 weeks until the analysis was done. The specimens were divided into immature/young and mature/adult individuals based on their total length in accordance with the published total length at 50% maturity for the species in the Aegean Sea (382 mm for males and 397 mm for females) by Kousteni & Megalofonou (2019) and/or macroscopic examination of their reproductive organs (Kousteni *et al.*, 2010).

Brain measurements

Brain mass

Each brain was detached from the spinal cord in the region of the first cervical spinal nerve. The meninges, blood vessels, choroid plexus, the connective tissue and the sensory and cranial nerves were dissected away (Yopak *et al.*, 2007). The brains were then weighed to the nearest 0.01 g (Table 1). Additionally, the brain somatic index (BSI) was calculated using the following formula:

$$\text{Brain somatic index (BSI)} = \frac{\text{Brain mass (g)}}{\text{Body mass (g)}} \times 100$$

Brain masses were not corrected for shrinkage due to fixation.

Brain areas

Six major brain areas (telencephalon, olfactory bulbs, diencephalon, mesencephalon, cerebellum, medulla oblongata) were identified according to Northcutt (1977, 1978), Smeets *et al.* (1983) and Lisney *et al.* (2017). In *S. canicula* the olfactory peduncles are very short, so the olfactory bulbs are not separated from the hemispheres (Figure 1). The boundaries between each area were set according to Smeets *et al.* (1983).

The size of the six brain areas was assessed volumetrically using the ellipsoid method, which approximates the volume of each brain structure by assuming that it takes the shape of an idealized ellipsoid or a fraction of it (Wagner, 2001; Lisney & Collin, 2006; Lisney *et al.*, 2007; Salas *et al.*, 2015). Linear measurements of the length (a), width (b) and depth (c) of each brain structure were taken from digital photographs using the ImageJ software (Abramoff *et al.*, 2004) and were translated into volumes using the formula:

$$V = \frac{1}{6}\pi^* a^* b^* c$$

Because no lateralization was found, in the case of the paired structures, the volumes were doubled. Each brain area was assessed in terms of its proportion to the total brain volume, the combined volume of all six areas (Wagner, 2003; Lisney *et al.*, 2007).

Statistical analysis

For the scaling analysis of the brain mass we fitted least squares linear regression on the $\log_{10}$-transformed data with the brain mass as the dependent variable (y) and the body mass as the independent variable (x). For the scaling of the brain areas we also fitted least squares linear regressions to the $\log_{10}$-transformed data. The dependent variable was the volume of the area in question and the independent variable was the total brain volume minus the volume of the brain area (Deacon, 1990; Iwaniuk *et al.*, 2010; Salas *et al.*, 2015). We used one-way ANOVA followed by Tukey's multiple comparison to test whether the slopes of the regressions for the six brain areas were significantly different from each other (Zar, 2010). To test whether sex or ontogenetic stages (maturity status) influence the scaling of the overall brain size and the size of the brain areas we used univariate general linear models (GLM) (Lisney *et al.*, 2017). Sex and maturity status were included as fixed factors, $\log_{10}$-transformed brain mass or brain area volume were considered as dependent variables while $\log_{10}$-transformed body mass or total brain volume minus brain area volume were considered the covariates in each case. We also included the interaction terms between the factors (sex × maturity status) and between the factors and the covariates (sex × $\log_{10}$-body mass & maturity status × $\log_{10}$-body mass/ sex × $\log_{10}$-brain volume & maturity status × $\log_{10}$ brain volume). However, neither of the interaction terms between the factors and the covariates were significant ($P > 0.05$) so they were excluded from the final models (Lisney *et al.*, 2017). Finally, we used a two-way ANOVA to test whether sex or maturity influence the relative size of each brain area (per cent of total brain volume). All data sets were tested for normality distribution using the Kolmogorov–Smirnoff test and for homogeneity of variances using the Levene's test. All statistical analyses were performed in SPSS 21 software (IBM Corp., Armonyk, NY, USA) and Prism 7 (GraphPad Software, San Diego, CA, USA).

Results

Gross morphology

A representative brain from an adult individual is shown in Figure 1 as well as the main areas that were measured. The morphometrics and brain characteristics of the studied specimens by sex and maturity status are shown in Table 1. The total length and round weight of the specimens ranged from 195–495 mm and 16–385 g respectively and no statistical difference was found between males and females. Head length was found to be longer in males than females, but only in mature specimens (two-way ANOVA; $P < 0.001$).

Regarding the brain measurements, brain weight ranged from 0.25–1.5 g with a mean value of 0.8 g and no statistical difference was found between sexes. Brain somatic index (BSI) ranged from 0.31–1.25% and, although it did not differ significantly between sexes, it was significantly affected by maturity status with immature animals exhibiting a higher BSI (two-way ANOVA; $P <$ 0.001).With regard to brain organization, no statistical differences were found between sexes for either absolute brain area volumes.

Brain scaling

n Figure 2A, the scaling pattern of brain mass against body mass for logarithmic transformed data is shown. The linear regression

https://doi.org/10.1017/S0025315420001368 Published online by Cambridge University Press

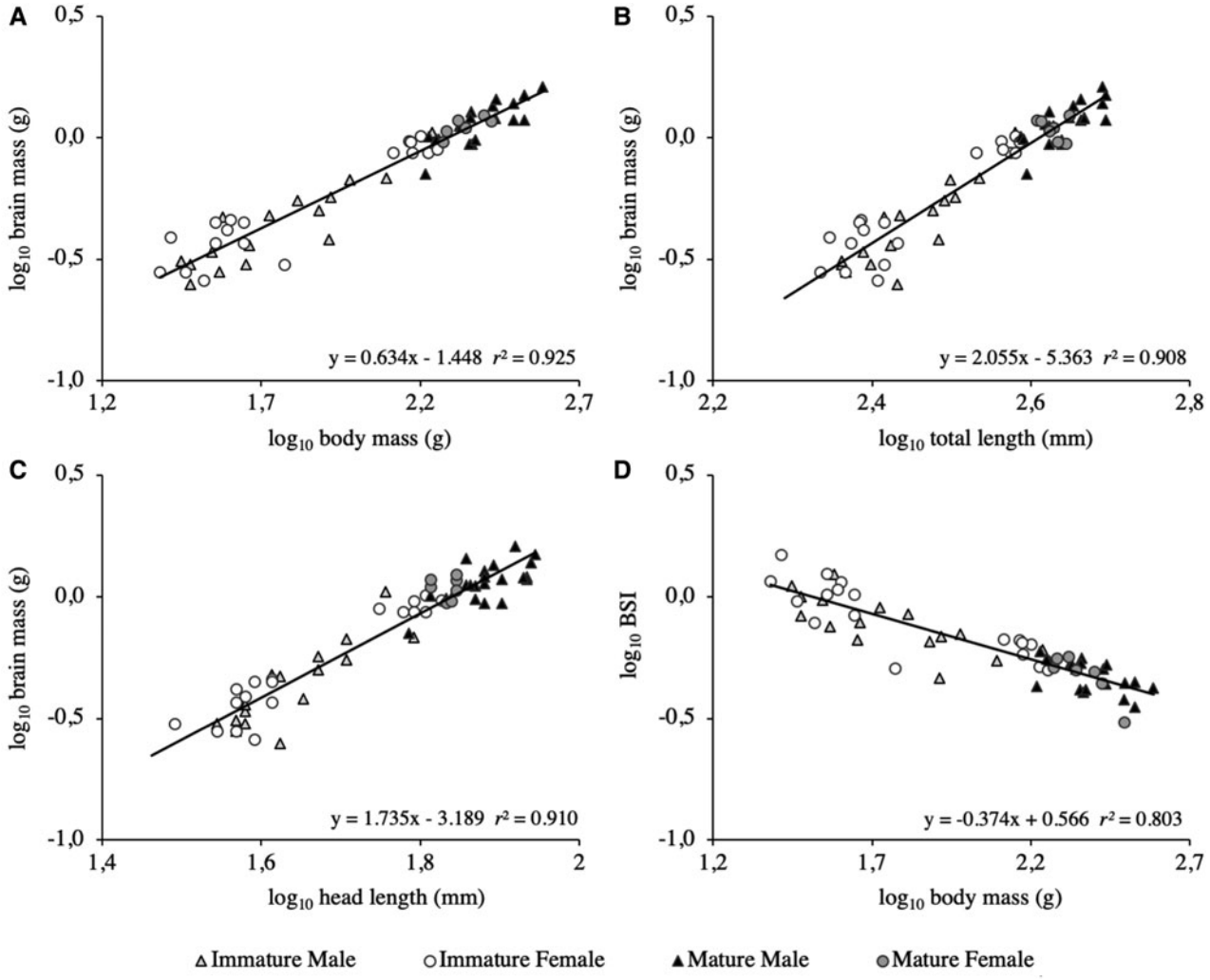

Fig. 2. Scaling of the brain mass of *Scyliorhinus canicula*. Scaling relationship between brain and (A) body mass, (B) total length, (C) head length. (D) Scatterplot of brain somatic index (BSI) and body mass. All data are $\log_{10}$ transformed. The solid lines represent the least squares linear regression lines. The equations and the coefficient of determination (r^2) are given in each graph.

for this relationship was $y = 0.634x - 1.448$ ($r^2 = 0.925$). The slope of the equation (0.634) was <1, thus indicating a negative allometric relationship of brain mass against body mass. This was more evident in the negative trend between BSI and body mass (Figure 2D). We see that the brain represented a higher percentage of body mass in small animals, and this percentage decreased as the body size increased. In Table 2, the results from the univariate general linear model indicate that only body mass was a significant predictor of brain mass, whereas sex, maturity status and their interaction (sex × maturity) had no significant effect.

To test if total length and head length could be significant predictors of brain mass, $\log_{10}$-transformed brain mass was scaled against $\log_{10}$-transformed total length and head length in Figure 2B, C respectively. The equations for the relationships were $y = 2.055x - 5.363$ ($r^2 = 0.908$) and $y = 1.735x - 3.189$ ($r^2 = 0.909$) indicating strong trends in both cases.

Scaling of brain structures

The scatterplots of the volume of each brain area against the total brain volume minus the volume of the brain area ($\log_{10}$-transformed data) are shown in Figure 3. As with the brain mass scaling, least squares linear regressions were used to describe the scaling patterns (Table 3). A one-way ANOVA was used to compare the slopes of the regression lines which were found to be significantly different ($F_{(5,30)} = 39.59$, $P < 0.0001$). Tukey post-hoc tests showed that the slopes of the telencephalon and the diencephalon were not statistically different, as were the slopes of the cerebellum and the medulla oblongata. The slopes of the olfactory bulbs and the mesencephalon were different from the slopes of all other brain areas. As such, the brain areas were grouped in four groups based on their slope (Table 3): Group A (telencephalon and diencephalon), Group B (olfactory bulbs), Group C (mesencephalon) and Group D (cerebellum and medulla oblongata). In Groups A and C, the slopes were <1 which indicated negative allometry, meaning that these areas grew at a lower rate compared with the rest of the brain. In Group D, the slopes were very close to 1, meaning that these areas scaled with isometry, i.e. they grew with the same rate as the rest of the brain. Finally, only in Group B (olfactory bulbs), the slope was higher than 1, which indicated positive allometry, i.e. this area grew at a higher rate compared to the rest of the brain.

The results of the univariate general linear models for each brain area are shown in Table 2. The results indicated that $\log_{10}$ brain volume (minus the brain area) was a significant predictor of the volume of all brain areas ($P < 0.0001$). Sex was also a significant indicator for the telencephalon ($P = 0.025$) and the medulla oblongata ($P = 0.001$) volume, while maturity had a significant effect on the scaling of the diencephalon volume. In each of

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Table 2. Results from the univariate general linear model analysis for the brain mass and the volume of the six brain areas

	$\log_{10}$ body mass			Sex			Maturity			Sex × maturity		
	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
Brain mass	1.097	149.660	**<0.0001**	0.018	2.517	0.118	0.019	2.560	0.115	0.004	0.532	0.469
	$\log_{10}$ brain volume – brain area			Sex			Maturity			Sex × maturity		
	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
Telencephalon	1.413	153.914	**<0.0001**	0.049	5.341	**0.025**	0.035	0.384	0.055	0.001	0.089	0.767
Olfactory bulbs	2.744	182.970	**<0.0001**	0.045	3.013	0.088	0.019	1.237	0.270	0.003	0.183	0.670
Diencephalon	2.096	134.145	**<0.0001**	0.001	0.082	0.776	0.078	4.97	**0.030**	0.045	2.912	0.094
Mesencephalon	1.098	108.379	**<0.0001**	<0.0001	0	0.991	0005	0.476	0493	0.001	0.114	0.737
Cerebellum	1.825	125.602	**<0.0001**	0.054	3.711	0.059	0.010	0.686	0.411	0.008	0.574	0.452
Medulla oblongata	1.418	113.125	**<0.0001**	0.159	12.641	**0.001**	0.002	0.199	0.658	0.001	0.103	0.749

SS, Sum of Squares; *F*, F statistic.
Sex, maturity and their interaction were included in all models. For the scaling of brain mass, body mass was used as a covariate, while for the scaling of the brain areas, total brain volume minus the brain area was used. All data were $\log_{10}$ transformed. Statistically significant results are shown in bold.

these three cases, the slopes of the equations were not significantly different between males/females or immature/mature animals, but the intercepts of the equations were, as confirmed by an ANOVA analysis.

Brain organization

The volume of each of the six brain areas was expressed as percentage of the total brain volume (Table 1), and the mean percentage for each brain area is shown in Figure 4, divided by sex (a), maturity status (b) or both (c). The results of the two-way ANOVA, which was used to compare the means of the relative volumes with sex and maturity as fixed factors, are shown in Table 4. We observe that the interaction term (sex × maturity) was not significant in all cases, so we can interpret the main effects. The telencephalon was larger in females than males ($P = 0.023$) and the same was applied to the olfactory bulbs ($P = 0.018$). On the other hand, the cerebellum ($P = 0.016$) and the medulla oblongata ($P = 0.005$) were larger in males than females. Moving on to maturity, the olfactory bulbs were larger in mature animals ($P = 0.003$), while the diencephalon ($P = 0.014$) and the mesencephalon ($P = 0.005$) were larger in immature individuals.

Discussion

In this study we assessed the overall brain scaling patterns, as well as the scaling patterns of six brain areas (telencephalon, olfactory bulbs, diencephalon, mesencephalon, cerebellum, medulla oblongata), and we studied the differences of brain organization between sexes and throughout ontogeny in a shark species, the small-spotted catshark, *S. canicula*. Previous studies have described ontogenetic shifts in brain organization of teleosts (Cadwallader, 1975; Brandstätter & Kotrschal, 1989; Montgomery *et al*., 1997; Kotrschal *et al*., 1998; Wagner, 2003; Masuda, 2009), chondrichthyans (Lisney & Collin, 2006; Lisney *et al*., 2007, 2017), and lampreys (Salas *et al*., 2015). However, in respect to chondrichthyans, the studies have only focused on large pelagic sharks or rays and not on small bottom dwelling fishes such as the small-spotted catshark. Additionally, the study of Ridet *et al*. (1973) on *S. canicula* did not focus on the relative size of the brain areas and was based on animals from the Atlantic Ocean. Many studies have shown that *S. canicula* attains a smaller size and mature at a smaller length in the Mediterranean Sea, than in the Atlantic Ocean (Capapé, 1977; Ivory *et al*., 2004; Kousteni *et al*., 2010) and this could also affect the brain growth patterns.

Brain scaling

The brain of *S. canicula* grows with a pattern similar to other teleosts and elasmobranchs (Bauchot *et al*., 1976; Lisney *et al*., 2017). The brain continues to grow throughout the lifespan of the animal, displaying indeterminate growth. However, the initial growth is fast in comparison to body mass, and gradually the rate decreases, resulting in a negative allometric relationship. The univariate general linear model analysis showed that males and females exhibit the same brain growth pattern and that the onset of sexual maturity does not affect this pattern, confirming the previous results on *S. canicula* in the Atlantic Ocean by Ridet *et al*. (1973), and similar results on other species such as *Mustellus canis* in the Atlantic Ocean (Kellicott, 1908), *Torpedo marmorata* in the Atlantic and Indian Ocean (Bauchot *et al*., 1976) and *Neotrygon kuhlii* in Australia (Lisney *et al*., 2017).

Moreover, the brain-weight relationship (Figure 2) displayed the highest r^2 (0.925), but the brain-total length and brain-head

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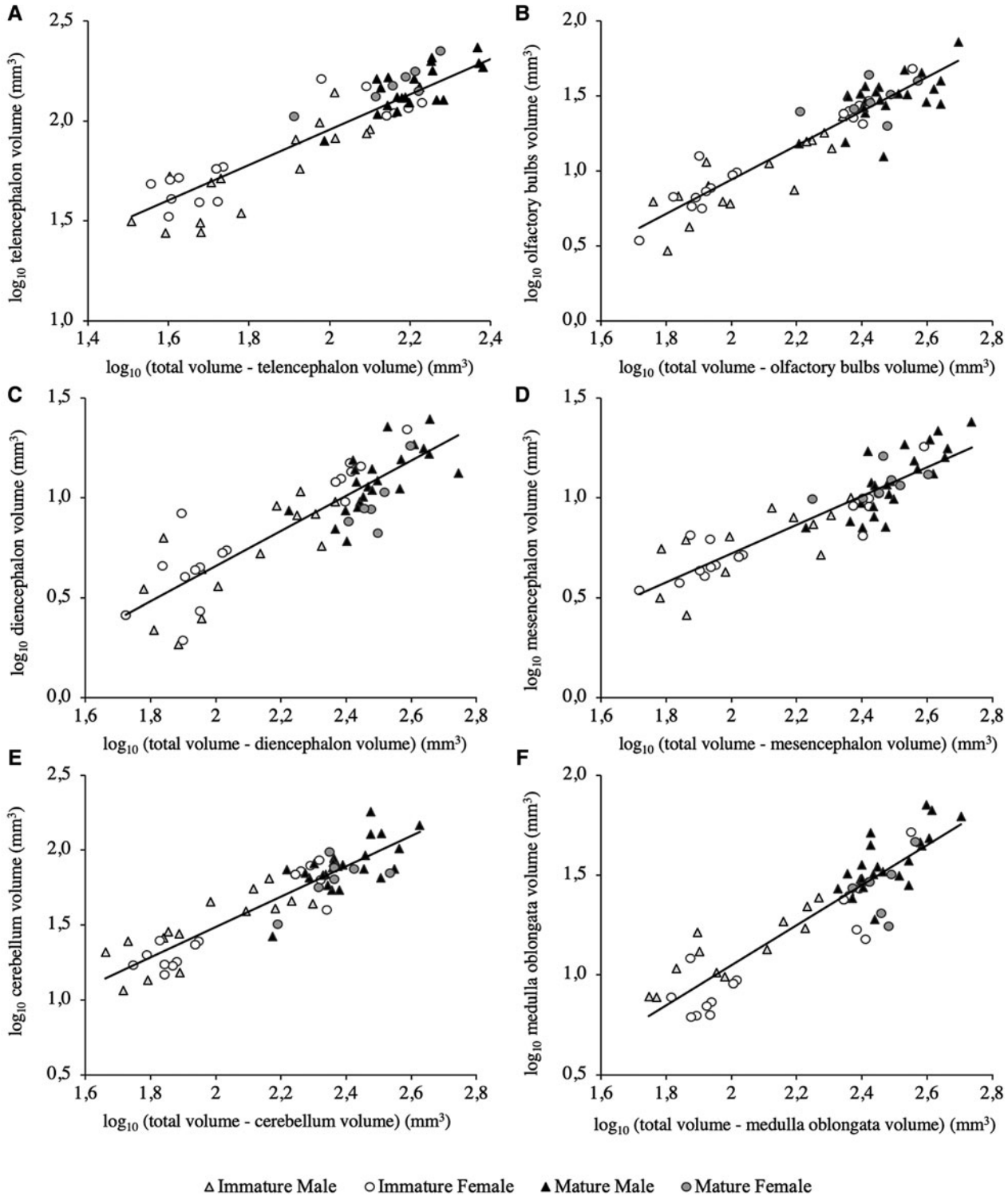

Fig. 3. Scaling relationship of the volume of each brain area against the total brain volume minus the brain area volume. (A) telencephalon, (B) olfactory bulbs, (C) diencephalon, (D) mesencephalon, (E) cerebellum, (F) medulla oblongata. All data are $\log_{10}$ transformed. The solid lines represent the least squares linear regression lines. The equations and the coefficient of determination (r^2) are shown on each chart.

length relationships also exhibited very good fit with $r^2 = 0.908$ and $r^2 = 0.909$ respectively. The results are in accordance with the results of Ridet *et al.* (1973) from the Atlantic Ocean, however there were significant differences between the coefficients of the equations we calculated and the equivalents from their results. Specifically, regarding the brain mass–body mass equation, the allometric coefficient we calculated was 0.634 while Ridet *et al.* (1973) found it equal to 0.533. Regarding the brain mass–total length relationship and the body mass–head length relationship, we found the coefficients equal to 2.055 and 1.735 respectively, in contrast to the values of 1.725 and 1.617 calculated by Ridet *et al.* (1973). A one-way ANOVA showed that the difference was significant for the brain–body mass relationship ($P < 0.01$) and the brain–total length relationship ($P < 0.01$). These results indicate that the brain growth rate of *S. canicula* is higher in the Mediterranean Sea than in the Atlantic Ocean. In addition, the brain somatic index (BSI) of mature individuals that we calculated (0.478) was significantly higher (one-way ANOVA, $P <$

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Table 3. Slope (a), intercept (b) and coefficient of determination (r^2) of linear regression lines for the six brain areas in *Scyliorhinus canicula*

Groups	Brain areas	$a \pm$ SE	$b \pm$ SE	r^2
A	Telencephalon	0.884 ± 0.053	0.190 ± 0.108	0.820
	Diencephalon	0.879 ± 0.063	−1.098 ± 0.145	0.864
B	Olfactory bulbs	1.141 ± 0.057	−1.340 ± 0.129	0.763
C	Mesencephalon	0.721 ± 0.045	−0.719 ± 0.104	0.810
D	Cerebellum	1.012 ± 0.062	−0.535 ± 0.136	0.817
	Medulla oblongata	0.999 ± 0.060	−0.949 ± 0.137	0.830

The equations were grouped according to their slope, based on an one-way ANOVA and Tukey post hoc tests. Group A, Telencephalon and Diencephalon; Group B, Olfactory bulbs; Group C, Mesencephalon; Group D, Cerebellum and Medulla oblongata.

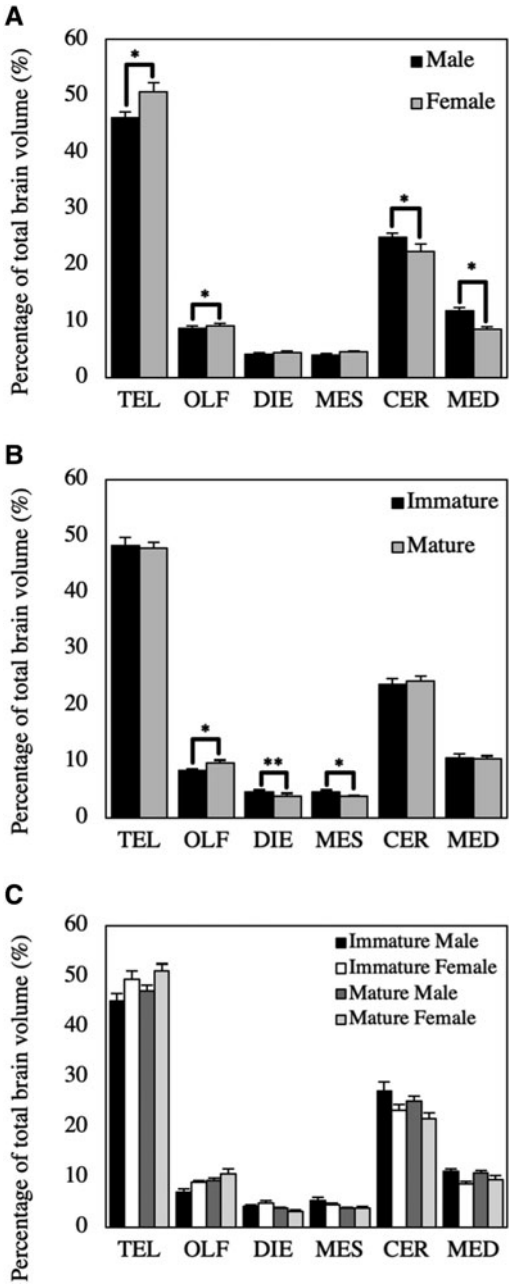

Fig. 4. Bar charts of the relative volume (%) of each brain area for (A) males and females, (B) immature and mature animals, (C) immature and mature males and immature and mature females. No interaction effect was found so the main effects were assessed (A & B). *, $P < 0.05$; **, $P < 0.01$. Bars represent mean ± SE. TEL, telencephalon; OLF, olfactory bulbs; DIE, diencephalon; MES, mesencephalon; CER, cerebellum; MED, medulla oblongata.

0.0001) from the respective measure (0.241) in the Atlantic Ocean from Ridet *et al.* (1973). This indicates that the brain occupies a higher percentage of the body in the Mediterranean than in the Atlantic, possibly due to the lower body size of specimens in the warm Mediterranean waters (Ivory *et al.*, 2004). However, analyses of larger sample sizes from *S. canicula* populations both in the Atlantic and the Mediterranean are required in order to undisputedly verify these findings.

Brain areas scaling and organization

The telencephalon, the diencephalon and the mesencephalon scaled negatively to the rest of the brain (with the mesencephalon exhibiting an even lower slope), the olfactory bulbs scaled with positive allometry while the cerebellum and the medulla oblongata followed the brain growth pattern (isometry) (Figure 3). The univariate general linear model analysis showed that sex had a significant effect on the scaling of the telencephalon and medulla oblongata, while maturity affected the scaling of the diencephalon (Table 2). When comparing the scaling equations of the telencephalon and the medulla oblongata for males and females separately, and the scaling equation for the diencephalon for immature and mature animals separately with a one-way ANOVA, we found that for each brain area the slopes of the equations were the same and only the intercepts were significantly different. This means that the rate of growth of the telencephalon and the medulla oblongata was the same between males and females, and the growth rate of the diencephalon was the same in mature and immature animals. However, females always exhibited bigger telencephalon, males exhibited bigger medulla oblongata and the diencephalon was bigger in immature animals regardless of sex. For the brain area comparisons between immature and mature fish of both sexes, the sample sizes were large and fairly well balanced. However, a larger sample size of mature females would add more weight to our findings relevant to differences between sexes.

To interpret the differences found in brain organization, we have to consider both the differences in behavioural patterns of male/female and immature/mature animals, and the suggested ecological function of these brain areas. In previous studies, the higher telencephalon size has been correlated with behaviours that are associated with cognitive abilities, such as social and spatial complexity, and the need for versatile social behaviours (Kotrschal *et al.*, 1998; Striedter, 2005), while higher medulla oblongata size has been documented in benthopelagic species with feeding strategies that require movement off the substrate and the use of specialized non-visual senses (Yopak & Montgomery, 2008). The scaling of diencephalon has not been extensively studied due to the multisensory nature of this area (Yopak & Montgomery, 2008), although we have to notice the

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Table 4. Results of the two-way ANOVA analysis on the relative volume of the six brain areas with sex and maturity status as fixed factors

	Sex			Maturity			Sex × maturity		
	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
Telencephalon	170.345	5.549	**0.023**	30.234	0.985	0.326	0.512	0.017	0.898
Olfactory bulbs	25.353	5.982	**0.018**	40.119	9.466	**0.003**	1.081	0.255	0.616
Diencephalon	0.049	0.034	0.854	9.412	6.539	**0.014**	4.274	2.969	0.091
Mesencephalon	1.530	1.010	0.320	13.069	8.624	**0.005**	2.134	1.408	0.241
Cerebellum	129.609	6.268	**0.016**	36.267	1.754	0.192	0.335	0.016	0.899
Medulla oblongata	32.336	8.685	**0.005**	0.762	0.205	0.653	0.762	0.205	0.653

SS, Sum of Squares; *F*, F statistic.
Statistically significant results are shown in bold.

poorer fit of the diencephalon scaling equation compared with the other equations.

Regarding the behaviour of *S. canicula*, different patterns have been documented in different geographic areas, with studies in the Atlantic suggesting segregation by sex and the occurrence of immature animals in shallower waters (Compagno, 1984; Sims *et al.*, 2001), while studies in the Mediterranean vary, with some suggesting that no segregation of sexes and sizes occurs in the population (D' Onghia *et al.*, 1995) while others suggest that there is segregation (Baino & Serena, 2000). In the Northern Aegean Sea, spawning seems to take place in deeper waters, contrary to other areas, where mature animals move to shallower waters to spawn and juveniles are found on the edge of the shelf and the upper slope (D' Onghia *et al.*, 1995; Abella *et al.*, 2017).

The results from the two-way ANOVA regarding the relative size of the brain areas (Table 4) not only confirmed the results from the models but also provided additional information. Males, besides the larger medulla oblongata, also exhibit a larger cerebellum, while females, apart from the larger telencephalon, also exhibit larger olfactory bulbs. Immature animals besides the larger diencephalon also possess a larger mesencephalon, while mature animals possess larger olfactory bulbs than immature ones (Figure 4). In previous studies on the elasmobranch brain organization, a larger cerebellum size has been correlated with finer motor control and the need for complex motor commands to take place for agile prey capture (Northcutt, 1977; 1978; Yopak *et al.*, 2007; Yopak & Montgomery, 2008). In addition, it has been suggested that a larger mesencephalon may indicate the importance of visual orientation for the foraging strategies of the animal (Yopak *et al.*, 2007; Yopak & Montgomery, 2008; Yopak & Frank, 2009), while, on the other hand, larger olfactory bulbs may indicate the importance of olfaction in the life of the organism (Lisney *et al.*, 2007).

Combining these findings with the results from the linear models, we can elaborate the ontogenetic shifts and draw the different behavioural patterns of male and female animals. First of all, immature animals regardless of sex seem to rely heavily on vision (larger mesencephalon) either to evade predation or to forage, so they are expected to be found in shallower waters where the light is sufficient (Hueter, 1980; Lisney *et al.*, 2007). As the animals grow, they likely move to deeper waters, where vision is not so vital, so other senses become more important, such as olfaction (larger olfactory bulbs), which becomes a dominant sense for the search of prey and mates (Kotrschal *et al.*, 1998; Lisney *et al.*, 2007). Our samples came from the Cyclades Islands, in the Southern Aegean Sea, a plateau where depths are greatly restricted, which are highly divergent from the environment of the bathyal Northern Aegean Sea (Sakellariou & Tsampouraki-Kraounaki, 2016). Therefore, we speculate that in the studied area, the populations of *S. canicula* possibly follow life history patterns that resemble relatively closely those of the Atlantic Ocean and West Mediterranean populations, with segregations by size and the presence of nurseries in shallower waters. However, further studies are needed in order to clarify these patterns.

The different brain organization of the two sexes, although cannot be explained with absolute certainty, may derive from differences in the reproductive, social and/or feeding behaviour between the two sexes. Males, bearing larger cerebellum and medulla oblongata, are agile, rapid moving predators that recruit electric, hydrodynamic and/or acoustic stimuli to effectively search for prey close to but off the substrate. On the other hand, females bearing a larger telencephalon, perhaps are not so agile, but exhibit more complex social behaviours. This could be attributed to male avoidance behaviours, so as to preserve energy by limiting multiple mating (Sims *et al.*, 2001; Griffiths *et al.*, 2012), or to social interaction between females, if a segregation by sex scenario is accepted (Compagno, 1984; Ellis & Shackley, 1997; Baino & Serena, 2000). The larger olfactory bulbs in females might indicate a higher reliance on olfaction for hunting, but might also be associated with reproduction, as shown in teleosts (Plenderleith *et al.*, 2005) and elasmobranchs (Lisney *et al.*, 2017). A previous study has documented a difference in the diet of males and females small-spotted catsharks in the Aegean Sea, with the latter consuming mainly teleosts, while males consumed equal proportions of teleosts, crustaceans and cephalopods (Kousteni *et al.*, 2017). The differences in mouth dimensions and tooth size between males and females, as well as between immature and adult males, with males developing longer, narrower mouths and longer teeth, could be due to different feeding habits or adaptations for reproductive behaviour (Ellis & Shackley, 1997; Erdogan *et al.*, 2004) and support our assumptions. Males hunting cephalopods, a very agile and intelligent group, need to enlist more complicated motor controls, hence the presence of an enlarged cerebellum. In addition, cephalopods and crustaceans are more commonly found near the bottom, so males must utilize electric and acoustic stimuli while actively searching for prey near the substrate, thus presenting an enlarged medulla oblongata. Females hunting mainly teleosts lack the same degree of enlargement of these structures. A speculation is that females might also hunt in deep waters further from the bottom (Sims *et al.*, 2001) relying more on olfaction, hence the enlarged olfactory bulbs. However, as stated earlier sexual and reproductive reasons for this brain area hypertrophy cannot be excluded. Given that the current evidence for environmental effects on sensory systems and brain architecture is mainly correlative and does not permit secure assumptions concerning interconnections, the need for experimental investigations is essential.

https://doi.org/10.1017/S0025315420001368 Published online by Cambridge University Press

In conclusion, in this study we assessed the brain growth of the small-spotted catshark (*S. canicula*) from the Central Aegean Sea and found no significant difference between sexes and maturity regarding the negative allometric brain scaling. However, we documented that the brain grows more rapidly compared with the population from the Atlantic Ocean (Ridet *et al*., 1973). Utilizing the ellipsoid volumetric approach (Wagner, 2003; Salas *et al*., 2015) we found distinctive ontogenetic shifts and sexual dimorphism in brain organization. Immature animals possess an enlarged mesencephalon and diencephalon, indicating the importance of vision in their clearer, shallow water habitat (Lisney *et al*., 2007). During ontogeny the olfactory bulbs become larger, possibly because the individual relies more on olfaction for foraging and reproduction while moving in deeper waters. Regarding the sexual dimorphism, males possess enlarged cerebellum and medulla oblongata, while females exhibit enlarged telencephalon and enlarged olfactory bulbs. More extensive studies on the behaviour and the brain organization of both the small-spotted catshark and closely related shark species from different areas of its distribution, including gross morphology as well as the histological patterns of the brain areas, will shed light on the different ontogenetic and behavioural patterns and contribute to the understanding of the neuro-ecology of sharks.

Acknowledgements. This work is part of the first author's thesis in the Department of Biology in NKUA. We would like to express our gratitude to the fishermen for their collaboration, support and assistance during the collection of the specimens. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

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