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DETERMINATION OF AGE AND GROWTH OF *Galeus melastomus, RAFINESQUE, 1810, A DEEPWATER SHARK, USING A MODIFICATION TO THE COBALT NITRATE TECHNIQUE*

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INTRODUCTION & AIMS

Galeus melastomus Rafinesque, 1810, is a deepwater bottom-dwelling Scyliorhinidae shark widely distributed in the northeastern Atlantic Ocean

Size ranges between 9 cm - 64 cm (males) and 9 - 81 cm (females) (Figueiredo et al., 1995). Size at first maturity is 48-52 cm (males) and 56-59 cm (females; Costa et al., 2005)

Considerable abundance/biomass make this species important to the marine ecosystem (Tursi et al., 1993)

Limited commercial interest to portuguese fisheries (Figueiredo et al., 1994) but there was an increase in landings over the past 25 years

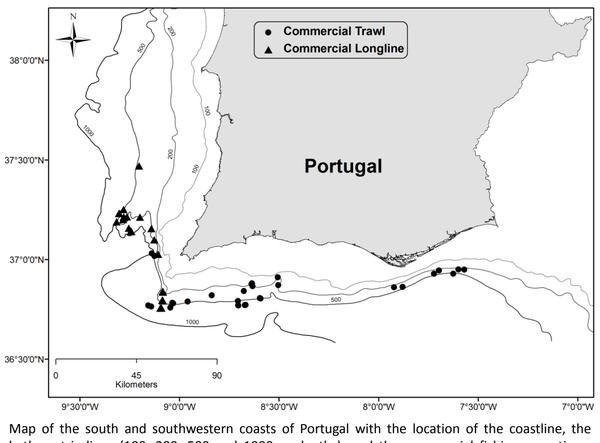
G. melastomus exhibits deep coned vertebrae, very small and poorly calcified which render common band enhancement techniques useless (Correia and Figueiredo, 1997)

Galeus melastomus individual. http://www-tc.pbs.org/wgbh/nova/sharks/world/images/lablackmouthcat.jpeg

In Portugal (and to our knowledge, worldwide) only one study on age and growth was performed, which used nitric acid (Correia and Figueiredo, 1997) resulting in an over decalcification of the vertebrae margin

AIMS ⟹ To test for the most suitable band enhancing technique for estimating age on vertebrae of *G. melastomus* and estimate growth parameters through adjustment of different growth models

MATERIALS AND METHODS



Map of the south and southwestern coasts of Portugal with the location of the coastline, the bathymetric lines (100, 200, 500 and 1000 m depths), and the commercial fishing operations. Bathymetric lines and coastline adapted from "Atlas do Ambiente Digital – Instituto do Ambiente".

Monthly sample (July 03 – June 04, except September 04) from commercial trawlers and from the fish market

Total length (TL, cm) was measured for each sampled individual

Vertebrae were removed from trunk region since these are bigger and probably better suited for ageing studies. Vertebral cone length (VCL) was determined

Relationships between TL-VCL, for males and females, were assessed by linear regression analysis

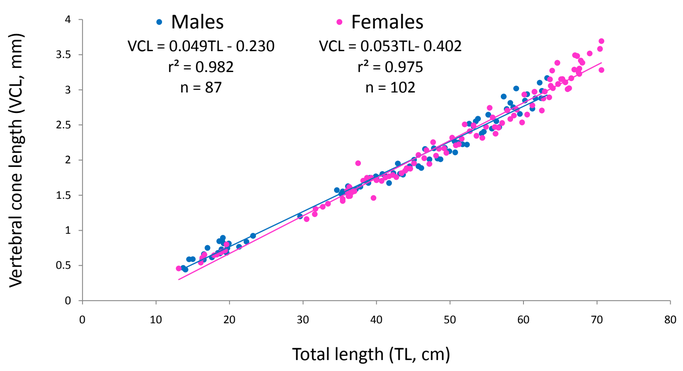
In order to obtain visualization of growth bands, 3 sectioning methods were essayed against 5 band enhancement techniques following immersion periods proposed by other authors and new extended ones for cobalt nitrate and haematoxylin

500 µm vertebrae sections immersed in cobalt nitrate for 18 h were used for age estimation, assuming a yearly deposition of one band pair

Four growth models were fitted for length-at-age data: VBGF, VBGF $_{\text{fixed } L_0}$, Gompertz, Logistic

RESULTS AND DISCUSSION

INDIVIDUAL GROWTH – VERTEBRAL GROWTH



Relationship between total length (TL) and vertebral cone length (VCL) for *Galeus melastomus* individuals. The regression equations with the respective coefficients of determination (r^2) and the sample sizes (n) are given.

Statistically significant relationship between individual growth and vertebral growth (♂+♀)

→ vertebrae suitable for age determination

VERTEBRAE PROCESSING AND BAND ENHANCEMENT TECHNIQUES

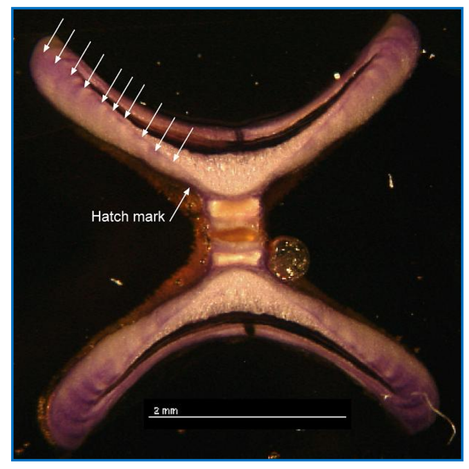
Summary of the effectiveness for band enhancement of the combination of different sectioning method and band enhancement techniques. Each combination was classified accordingly to this scale: (-) combination that did not provide band differentiation; (-+) combination that provided a weak band differentiation; (+) combination that provided clear band differentiation. Numbers in red indicate extended immersion periods.

		Band enhancement technique																
		Nitric acid			Alizarin red s			Haematoxylin				Modified cobalt nitrate				Crystal violet		
		15 min	20 min	30 min	3 days	4 days	5 days	½ min	5 min	15 min	12, 16, 18, 24 h	1 min	5 min	15 min	12, 16, 18, 24 h	10 min	15 min	12 , 24, 36 h
Sectioning method	Half vertebrae (micrometer)	-	-	-	-+	-+	-+	-	-	-	-	-	-	-	++	-	-	-+
	60 µm section (micrometer)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	500 µm section (high speed)	-	-	-	-+	-+	-+	-	-	-	-	-	-	-	-+	-	-	-+

All evaluated band enhancement techniques failed when following immersion periods previously used

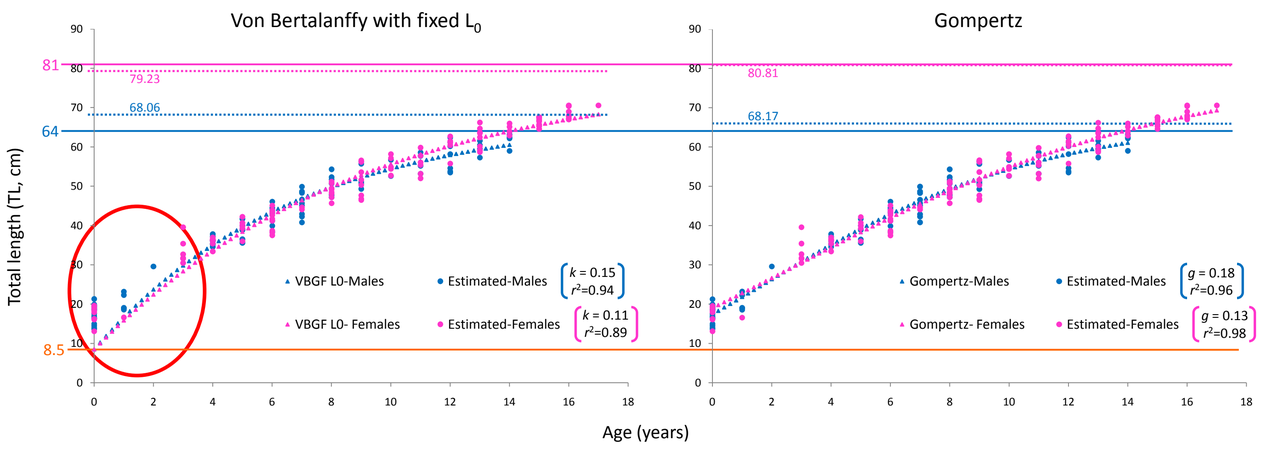
→ obtaining 500 µm section while increasing immersion time in cobalt nitrate to 12-24h provided the best band differentiation

AGE ESTIMATION



Microphotograh of a 500 µm vertebrae section stained with cobalt nitrate. This vertebrae was extracted from a *Galeus melastomus* female with 56.6 cm TL estimated to be 10 years old. It is possible to distinguish 10 translucid bands deposited following the hatch mark.

GROWTH MODELING



Length at age data for males and females of *Galeus melastomus*, with the fitted Von Bertalanffy with fixed L_0 and Gompertz growth models. Blue and pink lines indicate maximum TL (cm) reported for males and females, respectively. Orange line indicates estimation of size at hatching. Blue and pink dotted lines indicate maximum TL (cm) calculate for males and females, respectively, by each growth model. The red ovaloid is used to highlight the low fit to data of the $VBGF_{fixedL_0}$ curve for the first 3 years. The growth coefficients and r^2 values for each curve are also presented.

VBGF and Logistic models produced values for L_∞ which are not biologically reasonable

Gompertz exhibits higher r^2 values than $VBGF_{fixedL_0}$ but calculates an L_0 which is not biologically reasonable

Lower fit of the $VBGF_{fixed\ L0}$ model should derive from the low fit to data existing for the first 3 years → Age estimation inaccuracy and/or L_0 incorrectly presumed are regarded as possible causes

Growth rates on all models were smaller for females

→ as for other elasmobranch species (Coelho and Erzini, 2007), females exhibit a different growth related strategy, growing slower but reaching larger sizes

Estimated longevities of 14 years (♂) and 17 years (♀) (not validated) ≠ 8 years previously estimated (not validated; Correia and Figueiredo, 1997)

→ Validation remains crucial for determining the length-age relationship describing this species growth

CONCLUSION

⟹ **Newly estimated relatively high longevity and late maturity [7-9 years (♂) and 9-13 years (♀)] (deduced from Costa et al., 2005) render this species vulnerable to exploitation even if not commercially targeted**

⟹ **This study resulted in a new, rapid, simple and cheap methodology for growth band enhancement appropriate for usage on *G. melastomus* and possibly for other elasmobranch species exhibiting poorly calcified vertebrae**

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