

Article

Right-Biassed Crystalline Lens Asymmetry in the Thornback Ray (Rajiformes: Rajidae: *Raja clavata*): Implications for Ocular Lateralisation in Cartilaginous Fish

Giorgio Fedele ^{1,*}, Patrizia C. Rima ¹, Samira Gallo ¹, Chiara Carpino ¹, Claudia Valerioti ¹, Gianni Giglio ¹, Francesco L. Leonetti ¹, Concetta Milazzo ¹, Laura Piredda ², Annalisa Zaccaroni ², Giacomo Sardo ³, Sergio Vitale ³, Vita Gancitano ³ and Emilio Sperone ¹

¹ Department of Biology, Ecology and Earth Sciences, University of Calabria, 87036 Rende, Italy; patrizia.rima@unical.it (P.C.R.)

² Department of Veterinary Medical Sciences, University of Bologna, 47032 Bologna, Italy

³ National Research Council—Institute of Marine Biological Resources and Biotechnologies (CNR IRBIM), 91026 Mazara del Vallo, Italy

* Correspondence: giorgio.fedele@unical.it

Abstract

Directional asymmetry (DA) is a widespread yet often overlooked feature of animal morphology. Here, we report a consistent right-biassed asymmetry in the crystalline lenses of the thornback ray *Raja clavata*. Across 71 individuals sampled from the Strait of Sicily, 24% exhibited lens asymmetry, and in all cases, the right lens was larger. This pattern, supported by binomial and distributional tests, represents the first evidence of ocular DA in this species. Body size and age emerged as the primary correlates of asymmetry: the odds of exhibiting DA increased significantly with body weight, whereas the effects of sex and sampling site were not significant. The prevalence of asymmetry thus appears to rise with age and ontogenetic growth. Two non-exclusive mechanisms may account for this pattern. First, the association with positive allometry ($b = 3.33$) suggests that right-lens enlargement could reflect a functional or developmental lateralisation, potentially conferring a visual or ecological advantage to larger individuals. Alternatively, the same right-lens bias could arise through an age-related pathological process, such as oxidative or osmotic lens swelling preceding cataract formation, consistent with asymmetric physiological wear. These findings reveal a novel case of morphological lateralisation in an elasmobranch and highlight the need for comparative, histological, and functional approaches to disentangle adaptive asymmetry from lateralised senescence in the visual system of *R. clavata*.

Keywords: *Raja clavata*; crystalline lens; directional asymmetry; adaptation; pathology; lateralisation



Academic Editor: Bror Jonsson

Received: 21 November 2025

Revised: 22 December 2025

Accepted: 24 December 2025

Published: 25 December 2025

Copyright: © 2025 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

Key Contribution: A consistent right-biassed asymmetry of the crystalline lens is documented in *Raja clavata*. Its prevalence, directionality, and association with body size are quantified, and potential functional, developmental, and pathological explanations for this directional lateralisation are evaluated.

1. Introduction

Symmetry is a pervasive pattern in nature, often associated with beauty and a fundamental hallmark of animal body organisation [1]. Bilateral symmetry, the most common

form, involves a reflection across the left–right axis [2]. Departures from this ideal plan are termed asymmetry. They are typically grouped into three categories: fluctuating asymmetry (small, random deviations with a mean of approximately zero), antisymmetry (bimodal variation centred around zero), and directional asymmetry (DA; a systematic mean shift away from zero) [2,3]. This tripartite scheme carries distinct developmental and evolutionary implications and motivates explicit tests for both direction and shape of variation [4]. Increasing evidence highlights how widespread asymmetries are actually across animals and the importance of distinguishing their causes and consequences [5].

Laterality frameworks further clarify that directional asymmetry represents population-level alignment rather than individual noise [4,6–8]. This population-level alignment of biases poses an evolutionary question that individual efficiency alone cannot fully resolve. Game-theoretic treatments demonstrate that shared directionality can arise as an evolutionarily stable strategy under social or coordination pressures, with frequency-dependent selection helping to maintain consistent bias within populations [4,9]. In fishes, for instance, structural laterality can be biologically informative: directional bilateral asymmetry in otolith shape has been documented across large samples and proposed as a tool for stock discrimination [10]. In vertebrates, the assumption of strict bilateral symmetry is particularly strong in sensory systems, where structural equivalence is thought to ensure balanced perception and function. Consequently, departures from this balance are of special interest, as they may reveal developmental instability, functional lateralisation, or pathological degeneration [5,11]. However, while ocular structures are generally assumed to be bilaterally symmetric in position and morphology in most species, notable exceptions exist. The most extreme examples are found in flatfish (*Pleuronectiformes*), where one eye physically migrates to the other side of the head during development as an adaptation to a benthic lifestyle, resulting in inherent, massive skeletal and positional asymmetry [12]. Similarly, some deep-sea molluscs, such as cock-eyed squids (*Histioteuthidae*), exhibit a significant morphological size difference between their two eyes, an adaptation for asymmetric visual fields in low light [13,14]. Despite these specialised, adaptive cases, for the vast majority of bilaterally symmetrical animals, including rays, the eyes themselves are expected to be morphologically similar. The crystalline lens, in particular, is among the most geometrically conservative components of the vertebrate eye, as even minor deviations in curvature or volume can disrupt refractive balance and binocular alignment [15]. Yet, despite this presumed equivalence, many vertebrates exhibit pronounced functional visual lateralisation, asymmetries in eye use or neural processing that enhance behavioural efficiency [6,7,16]. This functional laterality is well-documented in fish [17,18], but it has rarely been linked to consistent morphological correlates within ocular anatomy.

During routine dissections for ocular ectoparasite inspection in the thornback ray (*Raja clavata*), a consistent right-biased difference in crystalline-lens size was serendipitously observed. To date, crystalline lens asymmetry has not been reported in this species, within the genus *Raja*, or more broadly in elasmobranchs, and the present study therefore represents the first systematic description of this phenomenon.

Because such observations may arise from individual anomalies, sampling artefacts, or measurement bias, a formal evaluation was required to determine whether the observed lens difference was statistically supported and biologically meaningful. Accordingly, lens variation was first examined to assess whether asymmetry departed from random variation and, if so, to determine its distributional form. Distinguishing directional asymmetry from fluctuating asymmetry or antisymmetry is essential, as these patterns reflect distinct developmental and evolutionary processes and constrain subsequent interpretation [2–5,9,19,20].

In addition, predictors of asymmetry were examined exploratorily, with basic biological and sampling-related variables (e.g., body size, sex, and sampling site) evaluated as plausible correlates of the observed variation.

Finally, guided by the most informative predictors, volumetric measurements were used to assess how asymmetry was expressed morphologically, specifically whether lens size differences were driven by enlargement of one lens rather than by reduction in the other. Establishing this structural manifestation provides a basis for subsequent consideration of alternative functional or pathological interpretations [6,21,22].

Overall, the study tested the hypothesis that apparent crystalline lens size differences represent a genuine biological asymmetry rather than random variation, and that any such asymmetry would exhibit a structured form with a consistent morphological basis.

2. Materials and Methods

Study species, sampling, and morphometrics. Thornback rays (*Raja clavata*) individuals were sampled in the Central Mediterranean (Strait of Sicily and adjacent coasts; Figure 1), using standard commercial trawling gear deployed at depths typical of local demersal fisheries [23]. Specimens were obtained as by-catch of the scientific trawling operations and were not alive upon retrieval, with mortality occurring during the fishing process. Following collection, individuals were maintained under a continuous cold chain until laboratory processing. For each, the date/time, capture site (GPS coordinates), and sex were recorded. Body weight (W) was measured to the nearest gram, and total length (TL) to the nearest millimetre; missing weights were estimated from the pooled length–weight relationship (Equation (2)). The dataset comprised 71 individuals.

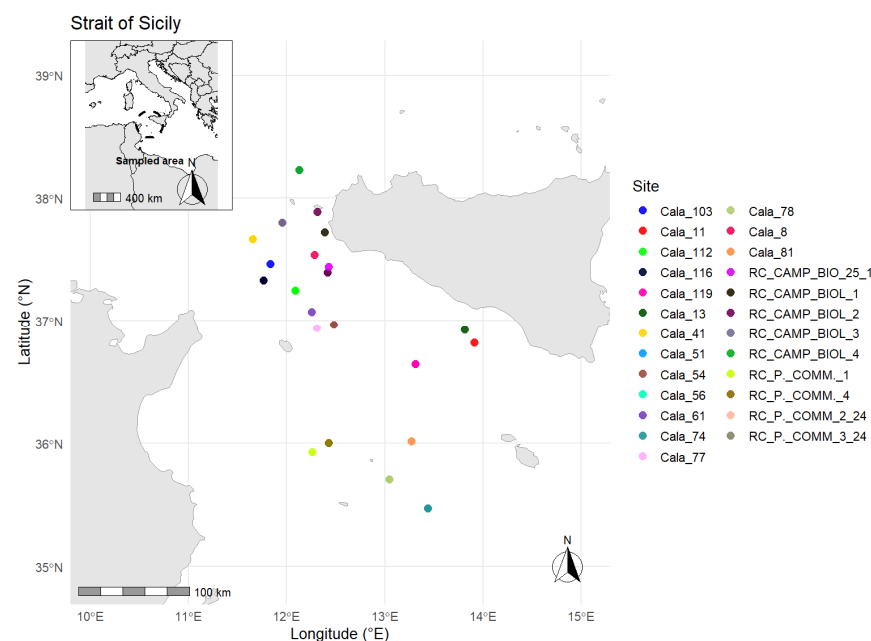


Figure 1. Sampling locations of *Raja clavata* in the Central Mediterranean Sea. (Inset) Overview map showing the position of the sampled area within the Central Mediterranean. (Large map) Zoomed map of the Strait of Sicily with capture sites obtained by bottom trawling. Colours indicate sampling sites.

Georeferencing and mapping. Geographic coordinates provided as packed DMS integers were converted to decimal degrees. Sampling locations were stored as WGS84 (EPSG:4326) sf geometries. To visualise the sampling footprint, the centroid of all points was computed, buffered by 250 km in Web Mercator (EPSG:3857), and re-projected to WGS84 for plotting. Basemaps were sourced from Natural Earth [24].

Estimated age Based on [25] age of the specimen was back-calculated using the three-parameter von Bertalanffy growth function (3VBGF, [26]):

$$L = L_{\infty} \left(1 - e^{-K(t-t_0)} \right) \quad (1)$$

Dissection Specimens previously stored at -20°C were thawed at room temperature for approximately 24 h prior to dissection. Each specimen was dissected under laboratory conditions using surgical stainless-steel scissors, tweezers, and disposable scalpels (number 22). A dorsal incision was made above the optic capsule, after which the optic nerves were severed and the entire capsule carefully excised. The capsule was then transferred to a Petri dish containing sterile physiological saline (0.9% NaCl, Galenica Senese, Monteroni D'Arbia, Siena Italy) and opened to expose the crystalline lenses. Both lenses were visually inspected and compared for relative size (left vs. right). When possible, extracted lenses were placed in 1.5 mL cryogenic vials filled with physiological solution and stored at 4°C until volumetric measurements were performed.

Volumetric calculation of the lens. A randomly selected subset ($n = 29$ out of the total 71) of crystalline lenses was volumetrically measured. The small sample size reflects logistical constraints during dissection, which prevented measurements from being taken on all specimens. Each lens was immersed in a 5 mL graduated cylinder containing 4 mL of physiological solution (0.9% NaCl). Following submersion, the displaced solution was withdrawn with a handheld pipette (LLG Labware, Meckenheim, Germany) until the meniscus returned to the 4 mL mark. The aspirated volume was recorded as the volumetric measure of the lens (in μL).

Lens asymmetry scoring. Binary lens asymmetry was scored from field notes as: "DX" (right lens larger), "SX" (left lens larger), or "n.a" (no difference). These were recoded as a three-level factor ($R > L$, $L > R$, $R = L$) and as a binary outcome (1 for any asymmetry; 0 for $R = L$). For the volumetric subset ($n = 29$), the laterality index (D) was defined as [27]:

$$D = \frac{V_R - V_L}{V_R + V_L} \quad (2)$$

where V_R is the volume of the right lens and V_L is the volume of the left. Such that positive values indicate a larger right lens.

Paired right-left differences in lens volume were assessed separately for symmetric and asymmetric individuals using Wilcoxon signed-rank tests, given their non-normal distribution of the data. Mean lens volume was analysed using analysis of covariance (ANCOVA) with asymmetry status and body weight as fixed effects. Separate linear models were fitted for right and left lenses to test whether asymmetry was associated with changes in a specific eye while controlling for weight. All variables were inspected for normality and homoscedasticity prior to analysis.

Length-weight relationship. The relationship between body weight (W) and total length (TL) was described using the allometric growth equation [28]:

$$W = a \times TL^b \quad (3)$$

where a is the intercept (coefficient) and b is the allometric exponent. The parameter b indicates the type of growth, with $b = 3$ corresponding to isometric growth, $b < 3$ to negative allometric growth (slimmer with size), and $b > 3$ to positive allometric growth (stouter with size). The coefficients a and b of the allometric weight-length equation were estimated by

log-transforming the measurements to linearise the exponential relationship, followed by fitting the data using simple linear regression [29]:

$$\log_{10}(W) = \log_{10}(a) + b \log_{10}(L) \quad (4)$$

Prevalence and direction of asymmetry. Counts across $R = L$, $R > L$, and $L > R$ were summarised, and the overall proportion asymmetric was estimated. Observed counts of asymmetric individuals were compared with null expectations of 5%, 10%, 15% and 20% using one-sided exact binomial tests (H_0 : observed $\leq$ null). For presentation, Clopper–Pearson 95% confidence intervals for the observed proportion were reported from a two-sided exact binomial calculation. For the volumetric subset ($n = 29$), departures of D (Equation (2)) from zero were assessed using a two-sided one-sample t -test and a two-sided Wilcoxon signed-rank test. Distributional shape was evaluated by comparing univariate Gaussian mixtures via BIC, and by Hartigan’s dip test for unimodality. A single shifted normal with non-zero mean was interpreted as directional asymmetry (DA); a symmetric bimodal mixture centred on zero as antisymmetry (AS); and a zero-mean unimodal distribution with excess variance as fluctuating asymmetry (FA, [19,20]).

Predictors of asymmetry (regression modelling). The binary outcome of presence/absence of asymmetry was modelled using Firth logistic regression (bias-reduced penalised likelihood) to mitigate small-sample bias and potential (quasi)-separation. Candidate models included:

- Single-predictor models: W, Age, TL, Sex, and Site (fixed factor).
- Additive two-predictor models: W + TL, W + Sex, TL + Sex.
- Full additive: W + TL + Sex.
- Interaction: $W \times TL$,

Where W is total weight, TL is total length.

Models were ranked by Akaike Information Criterion (AIC) computed from the (penalised) log-likelihood, and Akaike weights were used to quantify relative support. Odds ratios (OR) and 95% CIs were obtained by exponentiation of coefficient estimates and their profile (or Wald) intervals.

Software. All analyses were conducted in R (version 4.2). Key packages included *sf*, *ggplot2*, *ggpubr*, *ggspatial*, *rnaturalearth*, *mclust*, *diptest*, *logistf*, *pROC*, and *flextable*.

3. Results

Seventy-one *Raja clavata* individuals were examined from the Central Mediterranean (Figure 1). The relationship between total length (TL) and body weight (W) in *Raja clavata* followed the expected allometric growth function (Equation (3)). The fitted exponent was $b = 3.33$, indicating positive allometric growth (fish becoming proportionally heavier with increasing size). No significant differences were observed between sexes in the TL–weight relationship (Figure 2).

3.1. Prevalence of Lens Asymmetry

During gross examination and dissection of the eyes, an asymmetry in crystalline lens size was noted (Figure 3). This observation motivated a focused assessment of its prevalence, direction, and potential correlates. Of the 71 specimens examined, 54 (76%) were bilaterally symmetric in crystalline lens size, while 17 (24%) showed asymmetry. Binomial tests showed that this prevalence was significantly greater than rare-asymmetry expectations of 5% ($p < 5.867 \times 10^{-8}$), 10% ($p < 5.174 \times 10^{-4}$), and 15% ($p = 0.0314$), but not significantly greater than 20% ($p = 0.242$, Figure 4A). Yet, asymmetry was clearly present within the cohort at a non-trivial frequency.

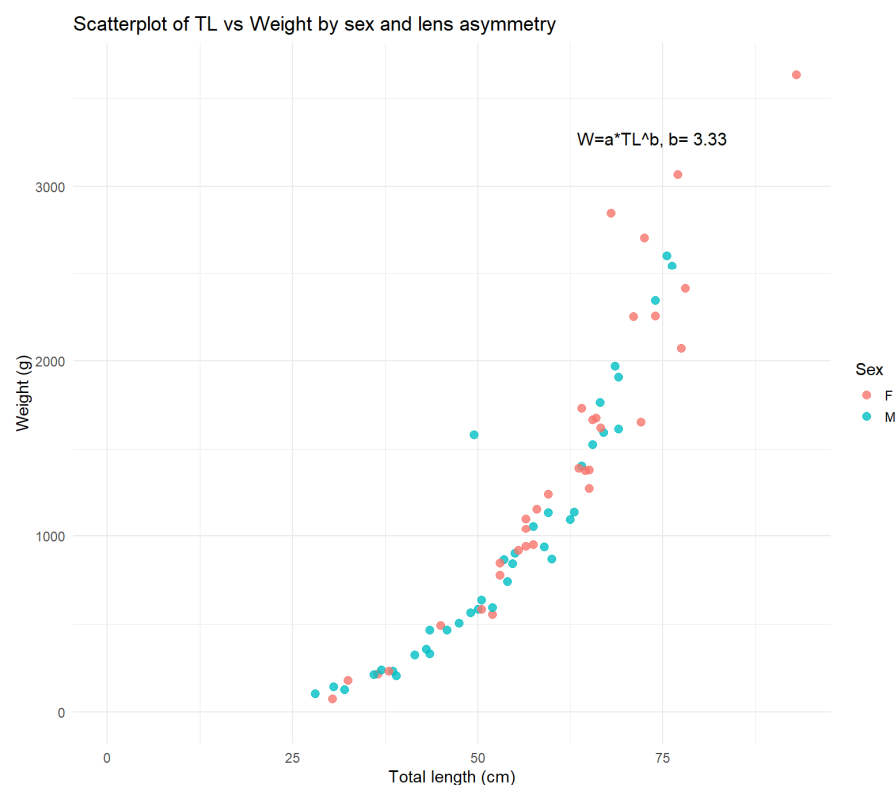


Figure 2. Relationship between total length (TL) and body weight (W) in *Raja clavata*. Scatterplot of TL (cm) versus W (g) for males (blue) and females (red). The fitted length–weight relationship is $W = a \cdot TL^b$, with $b > 3$, consistent with positive allometric growth.

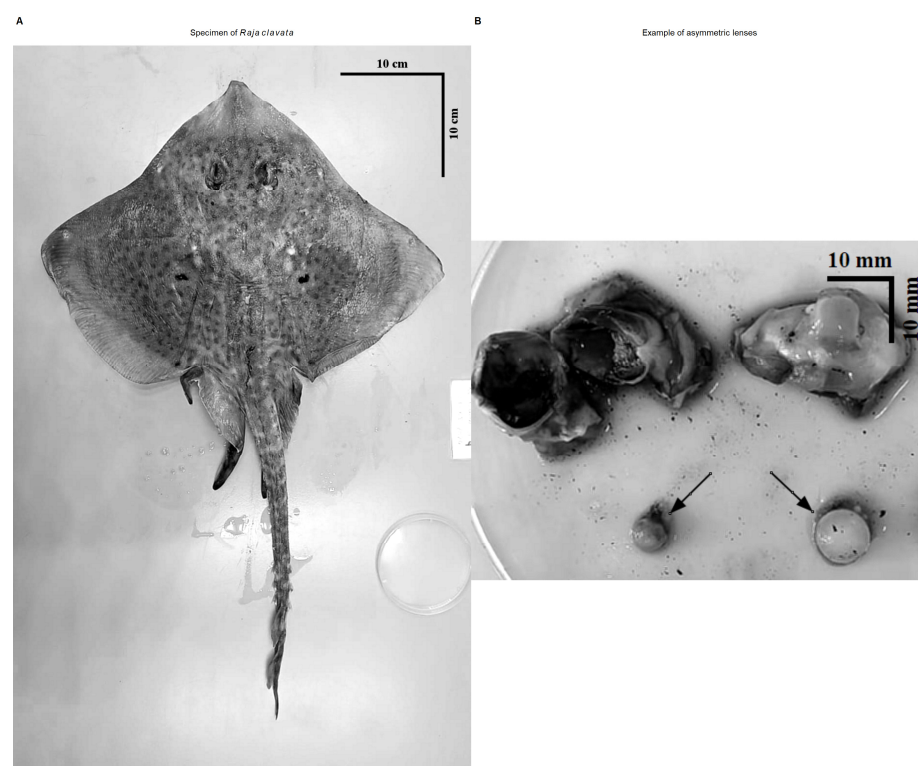


Figure 3. Example of *Raja clavata* male specimen (A) and its asymmetric crystalline lenses (arrows, (B)).

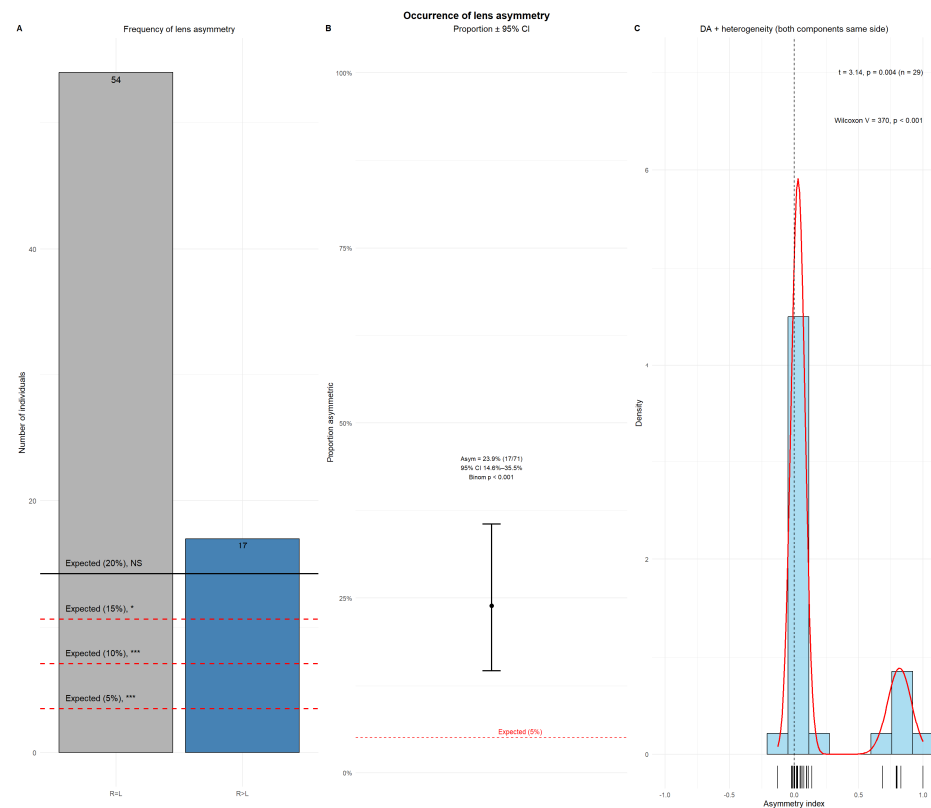


Figure 4. Evidence for directional asymmetry in crystalline lens volume of *Raja clavata*. **(A)** Frequency of asymmetry in the full sample ($n = 71$). Seventeen fish (24%) were asymmetric, all $R > L$. Horizontal dashed lines indicate reference prevalences (5–25%). Exact binomial tests: observed prevalence $> 5\%$ and $> 10\%$ (both $p < 0.001$), $> 15\%$ ($p < 0.05$), but not $> 20\%$ (ns). Statistical significance is denoted as * $p < 0.05$, *** $p < 0.001$. **(B)** Proportion asymmetric with Clopper–Pearson 95% CI (point = 17/54). The dashed red line marks the 5% expectation used for the one-sided test. **(C)** Normalised volumetric asymmetry index (D) for the measured subset ($n = 29$). The distribution of D is shifted towards positive values, indicating a rightward bias. One-sample tests confirmed a shift > 0 ($t = 3.14$, $p = 0.004$; Wilcoxon, $V = 370$, $p < 0.001$). Red curve: best-fitting Gaussian density over the histogram; vertical dashed line: symmetry reference at $D = 0$; rug ticks: individual observed.

3.2. Directionality of Asymmetry

All asymmetric cases had the right lens larger than the left (17/17; $p < 0.001$ vs. 50:50), indicating strong directional asymmetry rather than fluctuating asymmetry or antisymmetry. The estimated prevalence and its Clopper–Pearson 95% CI are shown in Figure 4B. To further corroborate these results, a subset of specimens underwent volumetric analysis. This subset of lenses was randomly selected from the full sample of 71 individuals. It included both specimens classified as symmetric and a subset of those classified as asymmetric based on gross inspection ($n = 29$). The normalised asymmetry index Directional asymmetry was evident: the normalised asymmetry index (D , Equation (2)) showed a positive central tendency (mean = 0.19, SD = 0.33; median = 0.02; range = -0.12 to 1), indicating a rightward bias in the cohort. One-sample tests against zero confirmed a non-zero central tendency (t -test and Wilcoxon signed-rank, both $p < 0.05$), and a sign test indicated universal right bias (23/29, $p = 0.002$). Gaussian mixture modelling occasionally supported a two-component fit; however, both component means were greater than zero, and Hartigan’s dip test indicated non-unimodality ($D = 0.068$, $p = 0.334$). Taken together, these results point towards directional asymmetry with heterogeneous magnitude rather than antisymmetry (Figure 4C).

3.3. Predictors of Asymmetry

Given these results, we next investigated which potential drivers could account for the asymmetry. Across the Firth logistic candidate set (Table 1), model selection identified age as the best single predictor of crystalline lens asymmetry (AIC = 72.37), with body weight providing nearly equivalent support ($\Delta\text{AIC} = 0.55$; weight = 0.15). Both predictors showed a positive association with the likelihood of asymmetry; the odds of asymmetry increased by approximately 1.2-fold per year of age ($p = 0.08$) and 1.7-fold per standard deviation in weight (W , $p = 0.043$). Because age and body weight are highly correlated and describe the same growth trajectory, capturing ontogenetic development, making their effects statistically redundant, the simpler W -only model was retained for interpretation (Figure 5). Body weight is a direct, easily measurable proxy of somatic development and condition, avoiding uncertainties associated with age estimation from von Bertalanffy growth parameters. Among multi-term models, only $W + \text{Sex}$, $W + \text{TL}$, and the interaction model $W \times \text{TL}$ achieved $\Delta\text{AIC} \leq 2$ (≤ 1.67); however, additional coefficients were non-significant and did not improve interpretability, so single-predictor models were favoured by parsimony. Total length alone sat right at the conventional threshold ($\Delta\text{AIC} = 2.00$), while full and $\text{TL} + \text{sex}$ models were clearly worse ($\Delta\text{AIC} \geq 2.20$). Sex, the null, and especially site performed poorly ($\Delta\text{AIC} \geq 4.72$ and $\gg 2$ for site). Taken together, the evidence suggests that ontogenetic scaling, captured by age (best AIC) or body weight (near-tie), explains asymmetry, whereas TL, sex, and site do not significantly improve the fit.

Table 1. Candidate Firth logistic regression models for the presence of crystalline lens asymmetry in *Raja clavata* (outcome: asymmetric = 1, symmetric = 0). Predictors include body weight (W), total length (TL), Age (A), sex (Sex), and their combinations; “INT” includes the $W \times \text{TL}$ interaction; “SITE” is a fixed-effect site model. Effect sizes are reported as odds ratios (OR) with 95% confidence intervals for each predictor (ORs for continuous predictors are per 1-SD increase). Models are ranked by Akaike Information Criterion (AIC); ΔAIC is relative to the best model; Akaike weights quantify relative support.

Model	Predictor	Effect Size	<i>p</i> -Value	AIC	ΔAIC	Akaike Weight
A	Age	1.23 [0.98, 1.59]	0.0803	72.37	0.00	0.201
W	Weight	1.71 [1.02, 2.99]	0.0425	72.92	0.55	0.153
INT	Weight	2.46 [0.47, 13.02]	0.2730	73.43	1.06	0.118
	Total length	0.64 [0.13, 3.15]	0.5690	73.43	1.06	0.118
	Weight $\times$ Total length	1.00 [0.63, 1.71]	0.9960	73.43	1.06	0.118
WS	Weight	1.62 [0.95, 2.87]	0.0773	73.46	1.09	0.117
	Sex: male vs. female	0.69 [0.22, 2.16]	0.5240	73.46	1.09	0.117
WTL	Weight	2.47 [0.64, 10.02]	0.1880	74.03	1.67	0.087
	Total length	0.65 [0.16, 2.81]	0.5500	74.03	1.67	0.087
TL	Total length	1.59 [0.91, 2.94]	0.1020	74.37	2.00	0.074
FULL	Weight	2.35 [0.6, 9.61]	0.2160	74.56	2.20	0.067
	Total length	0.64 [0.16, 2.79]	0.5460	74.56	2.20	0.067
	Sex: male vs. female	0.69 [0.22, 2.15]	0.5210	74.56	2.20	0.067
TLS	Total length	1.49 [0.85, 2.79]	0.1690	74.70	2.34	0.063
	Sex: male vs. female	0.65 [0.21, 1.99]	0.4470	74.70	2.34	0.063
S	Sex: male vs. female	0.53 [0.18, 1.55]	0.2490	77.09	4.72	0.019
NULL				77.60	5.23	0.015

Table 1. Cont.

Model	Predictor	Effect Size	p-Value	AIC	ΔAIC	Akaike Weight
SITE	Cala_11	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000
	Cala_112	1.29 [0.04, 234.63]	0.8940	139.83	67.46	0.000
	Cala_116	0.27 [0, 61.13]	0.5620	139.83	67.46	0.000
	Cala_119	3.00 [0.07, 612.54]	0.5700	139.83	67.46	0.000
	Cala_13	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000
	Cala_41	1.80 [0.05, 339.13]	0.7560	139.83	67.46	0.000
	Cala_51	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000
	Cala_54	0.23 [0, 51.35]	0.5130	139.83	67.46	0.000
	Cala_56	0.60 [0, 142.76]	0.8210	139.83	67.46	0.000
	Cala_61	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000
	Cala_74	1.59 [0.07, 251.77]	0.7840	139.83	67.46	0.000
	Cala_77	9.00 [0.15, 3674.77]	0.3060	139.83	67.46	0.000
	Cala_78	0.88 [0.03, 145.57]	0.9440	139.83	67.46	0.000
	Cala_8	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000
	Cala_81	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000
	RC_CAMP_BIO_25_1	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000
	RC_CAMP_BIOL_1	2.14 [0.07, 369.65]	0.6670	139.83	67.46	0.000
	RC_CAMP_BIOL_2	3.00 [0.1, 534.3]	0.5380	139.83	67.46	0.000
	RC_CAMP_BIOL_3	3.00 [0.07, 612.54]	0.5700	139.83	67.46	0.000
	RC_CAMP_BIOL_4	9.00 [0.15, 3674.77]	0.3060	139.83	67.46	0.000
	RC_P_COMM_1	3.00 [0.07, 612.54]	0.5700	139.83	67.46	0.000
	RC_P_COMM_4	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000
	SiteRC_P_COMM_2_24	0.60 [0, 142.76]	0.8210	139.83	67.46	0.000
	SiteRC_P_COMM_3_24	1.00 [0, 257.8]	1.0000	139.83	67.46	0.000

It is worth noting that the same models were also fitted on the subset of 56 rays for which trawl depth was recorded. In this reduced dataset, depth emerged as the strongest single predictor of asymmetry: the depth-only model (m_depth) was overwhelmingly supported (AIC = 42.05; ΔAIC = 0; Akaike weight = 0.94), far outperforming all other predictors (all ΔAIC > 8, model weights < 0.02). The estimated effect size was small (OR = 1.00, 95% CI: 1.00–1.01) and not statistically significant ($p = 0.145$), yet depth nonetheless provided the most important explanatory power within this restricted dataset. However, because depth was unavailable for many individuals and reflects trawl deployment location rather than actual individual habitat use, this effect is interpreted cautiously, and depth was not included in the final model comparison.

3.4. Lens Size Differences in Function of Body Weight

Among the 29 individuals for which both crystalline lenses were measured ($n = 29$; 15 symmetric, 14 asymmetric), paired right–left differences in lens volume were first examined separately for symmetric and asymmetric individuals. In both groups, paired differences deviated significantly from normality (Shapiro–Wilk tests; symmetric: $n = 15$, $W = 0.88$, $p = 0.049$; asymmetric: $n = 14$, $W = 0.84$, $p = 0.017$).

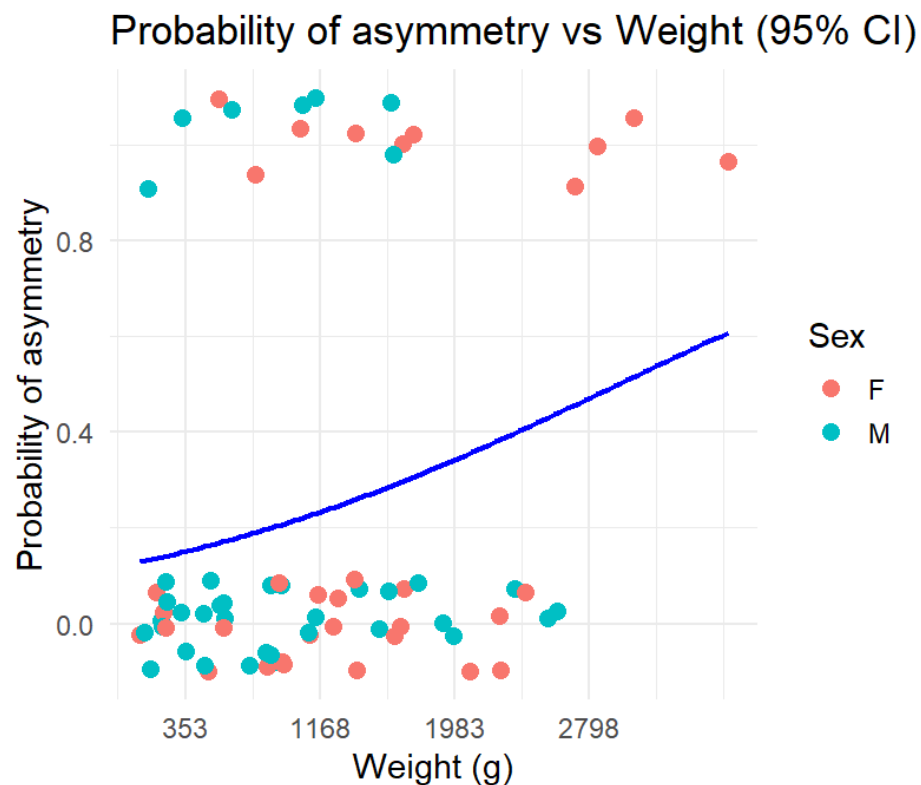


Figure 5. Predicted probability of directional asymmetry (DA) in crystalline lens size of *Raja clavata* as a function of body size, using Weight (g) as a predictor. Blue line shows fitted probabilities from Firth logistic regression. Points represent individual observations (pink = females, blue = males), jittered vertically for clarity (0 = symmetric, 1 = asymmetric).

Accordingly, paired comparisons were conducted using Wilcoxon signed-rank tests. In symmetric individuals, no significant difference between right and left lenses was detected (Wilcoxon signed-rank test: $n = 15$, $V = 75.5$, $p = 0.15$), whereas asymmetric individuals showed a pronounced right–left difference, with the right lens significantly larger than the left (Wilcoxon signed-rank test: $n = 14$, $V = 103$, $p = 0.0017$). Mean lens volume scaled weakly with body weight but differed significantly between symmetric and asymmetric rays (ANCOVA, $F_{1,26} = 8.06$, $p = 0.0087$). The interaction between weight and asymmetry status was non-significant ($p = 0.34$), indicating that the relationship between lens size and body weight did not differ between groups. When analysed separately, the right lens was markedly larger in asymmetric individuals, even after accounting for body weight (difference $\approx 107 \mu\text{L}$, $p = 0.0005$). In contrast, the left lens showed no detectable difference between groups ($p = 0.60$, Figure 6). Given the weak scaling of lens volume with body weight, the observed asymmetry primarily reflects right-lens enlargement rather than left-lens reduction.

Such consistent, unidirectional bias further strengthens the hypothesis of a directional asymmetry rather than random or fluctuating variation, possibly reflecting lateralised developmental processes or functional visual specialisation.

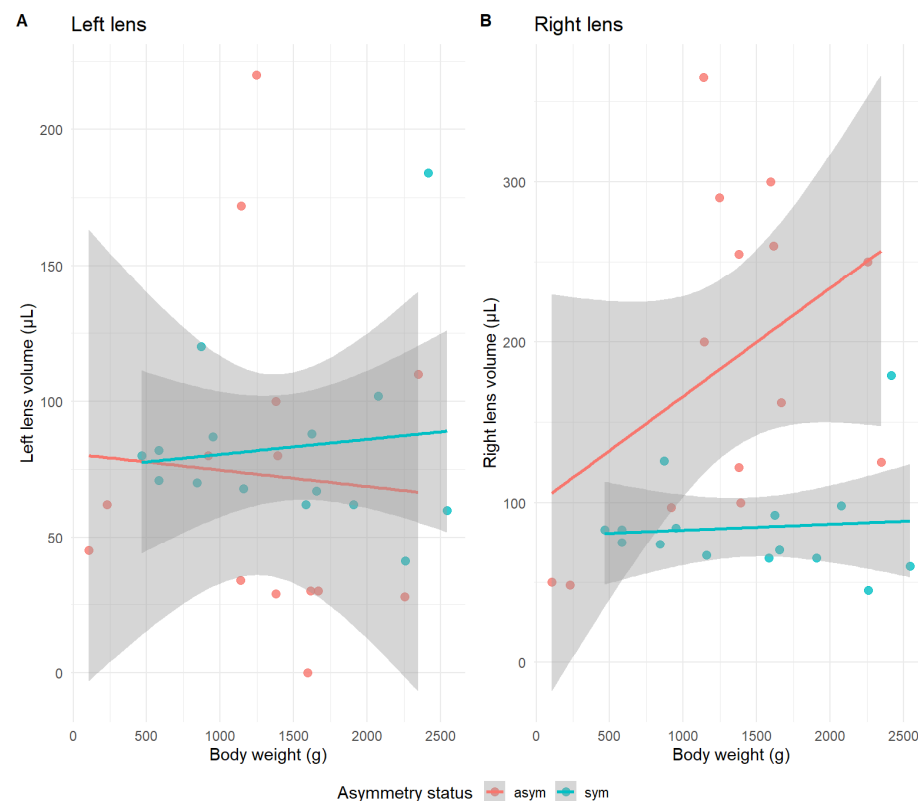


Figure 6. Relationship between body weight and crystalline lens volume in *Raja clavata*. (A) Left and (B) right lenses are shown for individuals classified as symmetric (blue) or asymmetric (red). Points represent individual rays, and shaded ribbons indicate 95% confidence intervals around fitted linear regressions. Left-lens volume did not differ between groups, whereas right-lens volume was significantly larger in asymmetric individuals after accounting for body weight (ANCOVA, $p = 0.0005$). This pattern suggests that asymmetry primarily arises through right-lens enlargement rather than left-lens reduction.

4. Discussion

This study provides the first evidence of a consistent, right-biased crystalline lens asymmetry in *Raja clavata*. Multiple analytical approaches—prevalence tests, distributional analyses, volumetric measurements, and regression modelling—were used to evaluate its occurrence, directionality, and structural basis. To our knowledge, no published studies have reported consistent left–right morphological asymmetries in the crystalline lens of elasmobranchs. While functional visual lateralisation is well documented in fishes and other vertebrates, corresponding structural asymmetries within ocular components such as the lens appear to be rare or undocumented. This contrast underscores the novelty of the present findings.

4.1. Characterisation of Crystalline Lens Asymmetry

A consistent directional asymmetry (DA) of crystalline lens size, a previously unreported morphological feature, was observed. Lens asymmetry occurred in 24% of individuals (17/71), all of which had a larger right lens. Together with the right-shifted distribution of volumetric differences in the measured subset, these patterns are consistent with directional asymmetry rather than fluctuating asymmetry or antisymmetry [19,20]. This interpretation is further supported by exact binomial tests of prevalence and by concordant one-sample parametric and nonparametric tests of the volumetric data, while acknowledging the limited size of the measured subset.

4.2. Structural and Directional Constraints on Possible Mechanisms

The strong right-biased direction of crystalline lens asymmetry argues against purely random developmental variation and is difficult to reconcile with simple mechanical or exposure-based explanations. In this dorsoventrally flattened species with apical eye placement, both eyes are similarly exposed during both resting and swimming, reducing the plausibility that unilateral abrasion or contact alone could account for the observed pattern.

The apparent bimodality of the normalised index (D) is more parsimoniously interpreted as heterogeneity in the magnitude of a shared right-biased lateralisation (i.e., milder vs. more pronounced DA) rather than the presence of opposing morphs. The distribution of D was overall shifted towards positive values, with a positive mean, including contributions from individuals classified as symmetric by gross inspection, indicating that variation reflects differences in effect size within a single directional process rather than left–right reversals. Importantly, this does not imply that all individuals exhibited macroscopic asymmetry, as D captures continuous variation, including minor right-biased deviations that remain below the threshold of macroscopic detection in otherwise symmetric individuals. The departure of D from zero is therefore primarily driven by the subgroup exhibiting larger asymmetries, whereas the remaining individuals cluster close to zero but retain a consistent rightward bias. This pattern is consistent with a single directional asymmetry expressed with variable intensity across individuals [5,30].

4.3. Predictors of Asymmetry and Ontogenetic Scaling

Body weight (and age) emerged as the primary correlate of asymmetry. In the best-supported Firth logistic model, the odds of asymmetry increased with weight, whereas sex effects were non-significant. Models incorporating spatial structure (sampling site) performed poorly, likely due to over-parameterisation relative to sample size. Together, these results indicate that asymmetry becomes more likely as individuals grow, although additional intrinsic or environmental factors could be implicated. No latitudinal or longitudinal clines were detected.

Bathymetric variation emerged as a predictor only for a restricted subset of individuals with depth data. However, because trawl depth reflects sampling location rather than the actual depth occupied by individuals, and because depth data were incomplete across the dataset, this association must be interpreted with caution. Depth likely serves as a proxy for body size or age, with larger individuals more frequently occurring at greater depths, so the observed relationship reflects size-structured stratification rather than a direct causal effect of depth on crystalline lens morphology [31].

Moreover, the length–weight relationship exhibited positive allometry ($b = 3.33$), indicating that larger individuals are proportionally heavier, matching similar reported data [32]. The co-occurrence of both positive allometry and the size-dependence of asymmetry prevalence suggests that the trait is unlikely to be deleterious and may reflect neutral or advantageous allometric scaling [33]. However, these patterns alone do not discriminate between adaptive and non-adaptive processes [34].

4.4. Functional Lateralisation Versus Asymmetric Degeneration

After accounting for body weight, asymmetric individuals showed a significant enlargement of the right crystalline lens rather than a reduction in the left. In contrast, no right–left difference was detected in symmetric individuals.

One interpretation is functional or developmental lateralisation of the visual system. Functional visual lateralisation is widespread across vertebrates, including fishes, and is often associated with consistent eye preferences, hemispheric specialisation, or asymmetric processing of visual information. In this context, enlargement of the right crystalline lens

could enhance visual sensitivity or performance along a preferred sensory axis, potentially conferring advantages in tasks such as prey detection, spatial orientation, or habitat use [5,7,11,15,16,35]. The association with larger bodies is compatible with such a fitness benefit [33]. Testing this hypothesis will require targeted behavioural assays, optical modelling, and neuroanatomical investigations to determine whether right-lens enlargement translates into asymmetric visual performance.

An alternative, but equally plausible, explanation is that the right-lens enlargement reflects a pathological or degenerative process, such as incipient cataract formation or localised osmotic lens swelling. Cataractogenesis often follows oxidative stress, protein oxidation, and osmotic dysregulation of lens fibres, leading to fluid retention and lens hypertrophy before opacity develops [21,22,36,37]. Such mechanisms align well with the observed increase in asymmetry prevalence with age and body mass, since oxidative load and metabolic wear accumulate over time, particularly in larger, older individuals [38]. The phenomenon's directionality, consistently affecting the right lens, could arise if systemic ageing processes interact with subtle lateral differences in ocular physiology or use. For instance, anatomical or neurovascular asymmetries in ocular perfusion, microcirculatory resistance, or antioxidant capacity could render one eye more prone to metabolic stress. Similarly, behavioural asymmetries might expose the right eye to marginally greater oxidative demand over extensive use [17]. Under this view, the observed pattern would represent a lateralised, age-related pathology, in which degeneration follows an asymmetric physiological bias. This scenario is not mutually exclusive with adaptive interpretations: a pre-existing functional lateralisation could predispose the right eye to greater metabolic demand, thereby coupling a functional asymmetry with asymmetric senescence.

Parasitic infection represents an additional, non-exclusive possibility. Ocular parasites have been shown to cause localised damage in elasmobranchs, occasionally resulting in lateralised impairment. For example, the copepod *Ommatokoita elongata* causes corneal lesions and blindness in Greenland sharks, although no consistent eye preference has been reported [39,40]. By analogy, the right-lens enlargement observed in *Raja clavata* could similarly result from a localised parasitic process that disproportionately affects one eye while being tolerated at the organismal level. Ongoing parasitological and trace-element analyses may help determine whether asymmetry covaries with infection intensity or contaminant load; such associations would support environmental or health-related drivers, whereas their absence would favour a more stable lateralised trait [19,41,42]. Disentangling these overlapping possibilities will require targeted histological, optical, and molecular analyses of lens integrity and composition.

4.5. Limitations and Future Directions

In addition to the considerations discussed above, several further limitations of the present study warrant explicit mention and help define priorities for future work. First, volumetric confirmation was available for a limited subset of specimens, and larger, more balanced samples will be required to refine effect sizes and distributional assumptions. Second, model selection indicated some uncertainty, with multiple candidate models receiving comparable support, suggesting that unmeasured factors likely contribute to asymmetry expression. Third, inferences regarding sex differences remain tentative due to limited statistical power. Finally, measurement protocols and potential subtle handling artefacts should be evaluated systematically in future work.

Several lines of follow-up appear particularly informative. Mechanistic studies integrating histological, optical, and neuroanatomical approaches could test whether right-lens enlargement has functional consequences. Comparative and ontogenetic surveys across size classes, seasons, and related rajid species would help establish generality and iden-

tify when asymmetry emerges. Finally, investigations of heritability and developmental pathways underlying directional asymmetry may clarify whether genetic or epigenetic mechanisms contribute to the observed pattern. In this context, recent evidence for genetically structured populations of *Raja clavata* across the Mediterranean [43] provides a valuable framework for future comparative studies integrating population genetics or genomics with morphological analyses to test whether patterns of ocular asymmetry covary with population genetic structure.

5. Conclusions

This study identifies a previously undocumented, right-biased directional asymmetry of the crystalline lens in *Raja clavata*. The prevalence, consistent direction, and size dependence of the trait indicate that it is a genuine biological feature rather than random developmental noise. Volumetric evidence shows that asymmetry arises through enlargement of the right lens, pointing to an organised morphological process. Its association with body size is compatible with either an ontogenetically strengthening lateralisation or an age-related physiological change. Distinguishing between functional lateralisation and asymmetric degeneration will require targeted anatomical and physiological work. These findings suggest that directional asymmetry in ocular structures may have evolved repeatedly across vertebrates, offering a potential model for the study of lateralised sensory systems. More broadly, the results suggest that subtle ocular asymmetries may occur in elasmobranchs and warrant further investigation.

Author Contributions: Conceptualization, G.F., P.C.R., S.G. and C.C.; methodology, G.F., P.C.R., S.G., C.C. and C.V.; software, G.F.; validation, G.F.; formal analysis, G.F.; investigation, G.F., P.C.R., S.G., C.C. and C.V.; resources, C.M., L.P., A.Z., G.S., S.V., V.G. and E.S.; data curation, P.C.R., G.S., V.G.; writing—original draft preparation, G.F.; writing—review and editing, G.F., P.C.R., S.G., C.C., C.V., G.G., F.L.L., C.M., L.P., A.Z., G.S., S.V., V.G. and E.S.; visualization, G.F.; supervision, G.F., C.M., A.Z., S.V., V.G. and E.S.; project administration, C.M., A.Z., S.V., V.G. and E.S.; funding acquisition, G.F., C.M., A.Z., S.V., V.G. and E.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Italian Ministry of University and Research (MUR) Young Researchers—Se al of Excellence fellowship under grant number SOE_0000169, awarded to G.F. P.C.R., C.M., L.P., A.Z., G.S., S.V., V.G. and E.S. were supported by the project PRIN 2022 D.D.104 (02/02/2022)—“Deep-sea Elasmobranchs as Environmental Pollution sentinels in the MEDiterranean (DEEP-MED)”, (2022BZJY8J-CUP H53D23003250006)-Piano Nazionale Di Ripresa E Resilienza (Pnrr)—Missione 4 Componente 2 Investimento 1.1—“Fondo per il Programma Nazionale della Ricerca (PNR) e Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN)”. The funders had no role in the study design, data collection and analysis, the decision to publish, or the preparation of the manuscript.

Institutional Review Board Statement: The specimens of the were collected during the international bottom trawl survey in the Mediterranean (MEDITS), and were collected following the Italian law “Decreto Legislativo 4 marzo 2014, n. 26”, which implemented the “European Directive 2010/63/UE” and is recognized as using the guidelines described in [44]. Additionally, the samples involved in the experiment were deceased prior to collection.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data and script for the analysis will be made available upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DA	Directional asymmetry
TL	Total length
W	Weight
3VBGF	Von Bertalanffy Growth Equation

References

- Holló, G. Demystification of Animal Symmetry: Symmetry Is a Response to Mechanical Forces. *Biol. Direct* **2017**, *12*, 11. [\[CrossRef\]](#)
- Graham, J.H.; Raz, S.; Hel-Or, H.; Nevo, E. Fluctuating Asymmetry: Methods, Theory, and Applications. *Symmetry* **2010**, *2*, 466–540. [\[CrossRef\]](#)
- Dongen, S.V. Fluctuating Asymmetry and Developmental Instability in Evolutionary Biology: Past, Present and Future. *J. Evol. Biol.* **2006**, *19*, 1727–1743. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wiper, M.L. Evolutionary and Mechanistic Drivers of Laterality: A Review and New Synthesis. *Laterality* **2017**, *22*, 740–770. [\[CrossRef\]](#)
- Palmer, A.R. Animal Asymmetry. *Curr. Biol.* **2009**, *19*, R473–R477. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sovrano, V.A. Visual Lateralization in Response to Familiar and Unfamiliar Stimuli in Fish. *Behav. Brain Res.* **2004**, *152*, 385–391. [\[CrossRef\]](#)
- Vallortigara, G.; Chiandetti, C.; Sovrano, V.A. Brain Asymmetry (Animal). *Wiley Interdiscip. Rev. Cogn. Sci.* **2011**, *2*, 146–157. [\[CrossRef\]](#)
- Lin, Y.-J.; Chen, H.-M. Directional Asymmetry in Gonad Length Indicates Moray Eels (Teleostei, Anguilliformes, Muraenidae) Are “Right-Gonadal”. *Sci. Rep.* **2023**, *13*, 2963. [\[CrossRef\]](#)
- Ghirlanda, S.; Vallortigara, G. The Evolution of Brain Lateralization: A Game-Theoretical Analysis of Population Structure. *Proc. Biol. Sci.* **2004**, *271*, 853–857. [\[CrossRef\]](#)
- Mahé, K.; MacKenzie, K.; Ider, D.; Massaro, A.; Hamed, O.; Jurado-Ruzafa, A.; Gonçalves, P.; Anastasopoulou, A.; Jadaud, A.; Mytilineou, C.; et al. Directional Bilateral Asymmetry in Fish Otolith: A Potential Tool to Evaluate Stock Boundaries? *Symmetry* **2021**, *13*, 987. [\[CrossRef\]](#)
- Lisney, T.J.; Collin, S.P. Retinal Ganglion Cell Distribution and Spatial Resolving Power in Elasmobranchs. *Brain Behav. Evol.* **2008**, *72*, 59–77. [\[CrossRef\]](#)
- Bao, B. *Flatfish Metamorphosis*, 1st ed.; Springer: Singapore, 2023; ISBN 9789811978586.
- Steffen, J.E. The Optics of Life: A Biologist’s Guide to Light in Nature. Sonke Johnsen. *Integr. Comp. Biol.* **2012**, *52*, 331. [\[CrossRef\]](#)
- Thomas, K.N. Vision and Bioluminescence in Cephalopods. Ph.D. Thesis, Duke University, Durham, NC, USA, 2018.
- Fernandes, V.F.L.; Glaser, Y.; Iwashita, M.; Yoshizawa, M. Evolution of Left-Right Asymmetry in the Sensory System and Foraging Behavior during Adaptation to Food-Sparse Cave Environments. *BMC Biol.* **2022**, *20*, 295. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kendall-Bar, J.M.; Vyssotski, A.L.; Mukhametov, L.M.; Siegel, J.M.; Lyamin, O.I. Eye State Asymmetry during Aquatic Unihemispheric Slow Wave Sleep in Northern Fur Seals (*Callorhinus ursinus*). *PLoS ONE* **2019**, *14*, e0217025. [\[CrossRef\]](#) [\[PubMed\]](#)
- De Russi, G.; Bertolucci, C.; Lucon-Xiccato, T. Artificial Light at Night Impairs Visual Lateralisation in a Fish. *J. Exp. Biol.* **2025**, *228*, JEB249272. [\[CrossRef\]](#)
- Takeuchi, Y.; Higuchi, Y.; Watanabe, T.; Oda, Y. Dominant Eye-Dependent Lateralized Behavior in the Scale-Eating Cichlid Fish, *Perissodus microlepis*. *Sci. Rep.* **2025**, *15*, 24725. [\[CrossRef\]](#)
- Allenbach, D.M. Fluctuating Asymmetry and Exogenous Stress in Fishes: A Review. *Rev. Fish Biol. Fish.* **2011**, *21*, 355–376. [\[CrossRef\]](#)
- Dongen, V.; Lens, M.; Molenberghs, G. Mixture Analysis of Asymmetry: Modelling Directional Asymmetry, Antisymmetry and Heterogeneity in Fluctuating Asymmetry. *Ecol. Lett.* **1999**, *2*, 387–396. [\[CrossRef\]](#)
- Kulbay, M.; Wu, K.Y.; Nirwal, G.K.; Bélanger, P.; Tran, S.D. Oxidative Stress and Cataract Formation: Evaluating the Efficacy of Antioxidant Therapies. *Biomolecules* **2024**, *14*, 1055. [\[CrossRef\]](#)
- Zhang, X.; Liu, B.; Lal, K.; Liu, H.; Tran, M.; Zhou, M.; Ezugwu, C.; Gao, X.; Dang, T.; Au, M.-L.; et al. Antioxidant System and Endoplasmic Reticulum Stress in Cataracts. *Cell. Mol. Neurobiol.* **2023**, *43*, 4041–4058. [\[CrossRef\]](#)
- Gallo, S.; Nania, G.; Caruso, V.; Zicarelli, G.; Leonetti, F.L.; Giglio, G.; Fedele, G.; Romano, C.; Bottaro, M.; Mangoni, O.; et al. Bioaccumulation of Trace Elements in the Muscle of the Blackmouth Catshark *Galeus melastomus* from Mediterranean Waters. *Biology* **2023**, *12*, 951. [\[CrossRef\]](#)
- Massicotte, P.; South, A. Rnaturalearth: World Map Data from Natural Earth, CRAN[Code]. 2025. Available online: <https://github.com/ropensci/rnaturalearth> (accessed on 1 November 2025).

25. Carbonara, P.; Bellodi, A.; Palmisano, M.; Mulas, A.; Porcu, C.; Zupa, W.; Donnaloia, M.; Carlucci, R.; Sion, L.; Follesa, M.C. Growth and Age Validation of the Thornback Ray (*Raja clavata* Linnaeus, 1758) in the South Adriatic Sea (Central Mediterranean). *Front. Mar. Sci.* **2020**, *7*, 586094. [\[CrossRef\]](#)
26. Von Bertalanffy, L. A Quantitative Theory of Organic Growth (Inquiries on Growth Laws. Ii). *Hum. Biol.* **1938**, *10*, 181–213.
27. Dale Broder, E.; Angeloni, L.M. Predator-Induced Phenotypic Plasticity of Laterality. *Anim. Behav.* **2014**, *98*, 125–130. [\[CrossRef\]](#)
28. Gayon, J. History of the Concept of Allometry. *Am. Zool.* **2000**, *40*, 748–758. [\[CrossRef\]](#)
29. De Robertis, A.; Williams, K. Weight-length Relationships in Fisheries Studies: The Standard Allometric Model Should Be Applied with Caution. *Trans. Am. Fish. Soc.* **2008**, *137*, 707–719. [\[CrossRef\]](#)
30. Palmer, A.R. Symmetry Breaking and the Evolution of Development. *Science* **2004**, *306*, 828–833. [\[CrossRef\]](#)
31. Staeudle, T.M.; Parmentier, B.; Poos, J.J. Accounting for Spatio-Temporal Distribution Changes in Size-Structured Abundance Estimates for a Data-Limited Stock of *Raja clavata*. *ICES J. Mar. Sci.* **2024**, *81*, 1607–1619. [\[CrossRef\]](#)
32. Krstulović Šifner, S.; Vrgoč, N.; Dadić, V.; Isajlović, I.; Peharda, M.; Piccinetti, C. Long-Term Changes in Distribution and Demographic Composition of Thornback Ray, *Raja clavata*, in the Northern and Central Adriatic Sea. *J. Appl. Ichthyol.* **2009**, *25*, 40–46. [\[CrossRef\]](#)
33. Ahti, P.A.; Kuparinen, A.; Uusi-Heikkilä, S. Size Does Matter—The Eco-Evolutionary Effects of Changing Body Size in Fish. *Environ. Rev.* **2020**, *28*, 311–324. [\[CrossRef\]](#)
34. Lahti, D.C.; Johnson, N.A.; Ajie, B.C.; Otto, S.P.; Hendry, A.P.; Blumstein, D.T.; Coss, R.G.; Donohue, K.; Foster, S.A. Relaxed Selection in the Wild. *Trends Ecol. Evol.* **2009**, *24*, 487–496. [\[CrossRef\]](#)
35. Mascetti, G.G. Unihemispheric Sleep and Asymmetrical Sleep: Behavioral, Neurophysiological, and Functional Perspectives. *Nat. Sci. Sleep* **2016**, *8*, 221–238. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Hsueh, Y.-J.; Chen, Y.-N.; Tsao, Y.-T.; Cheng, C.-M.; Wu, W.-C.; Chen, H.-C. The Pathomechanism, Antioxidant Biomarkers, and Treatment of Oxidative Stress-Related Eye Diseases. *Int. J. Mol. Sci.* **2022**, *23*, 1255. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Rodella, U.; Honisch, C.; Gatto, C.; Ruzza, P.; D'Amato Tóthová, J. Antioxidant Nutraceutical Strategies in the Prevention of Oxidative Stress Related Eye Diseases. *Nutrients* **2023**, *15*, 2283. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Leyane, T.S.; Jere, S.W.; Houreld, N.N. Oxidative Stress in Ageing and Chronic Degenerative Pathologies: Molecular Mechanisms Involved in Counteracting Oxidative Stress and Chronic Inflammation. *Int. J. Mol. Sci.* **2022**, *23*, 7273. [\[CrossRef\]](#)
39. Borucinska, J.D.; Benz, G.W.; Whiteley, H.E. Ocular Lesions Associated with Attachment of the Parasitic Copepod *Ommatokoita elongata* (Grant) to Corneas of Greenland Sharks, *Somniosus microcephalus* (Bloch & Schneider): Ocular Lesions Associated with Parasitic Copepods. *J. Fish Dis.* **1998**, *21*, 415–422.
40. Williams, D. Ophthalmology of Cartilaginous Fish: Skates, Rays, and Sharks. In *Wild and Exotic Animal Ophthalmology*; Springer International Publishing: Cham, Switzerland, 2022; pp. 47–59, ISBN 9783030713010.
41. Fazio, G.; Lecomte-Finiger, R.; Bartrina, J.; Moné, H.; Sasal, P. Macroparasite Community and Asymmetry of the Yellow Eel *Anguilla anguilla* in Salses-Leucate Lagoon, Southern France. *Bull. Fr. Peche Piscicult.* **2005**, *378–379*, 99–113. [\[CrossRef\]](#)
42. Pečínková, M.; Vøllestad, L.A.; Koubková, B.; Huml, J.; Jurajda, P.; Gelnar, M. The Relationship between Developmental Instability of Gudgeon *Gobio gobio* and Abundance or Morphology of Its Ectoparasite *Paradiplozoon homioion* (Monogenea). *J. Fish Biol.* **2007**, *71*, 1358–1370. [\[CrossRef\]](#)
43. Melis, R.; Vacca, L.; Cariani, A.; Carugati, L.; Charilaou, C.; Di Crescenzo, S.; Ferrari, A.; Follesa, M.C.; Mancusi, C.; Pinna, V.; et al. Baseline Genetic Distinctiveness Supports Structured Populations of Thornback Ray in the Mediterranean Sea. *Aquat. Conserv.* **2023**, *33*, 458–471. [\[CrossRef\]](#)
44. Ebert, D.A.; Dando, M.; Fowler, S. *Sharks of the World: A Complete Guide*; Princeton University Press: Princeton, NJ, USA, 2021.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.