6-18-2019

Effects of dietary taurine level on visual function in European sea bass (Dicentrarchus labrax)

Richard Brill
Virginia Institute of Marine Science, rbrill@vims.edu

Andrij Z. Horodysky

Allen R. Place

Mary E. Larkin

Renate Reimschuessel

Effects of dietary taurine level on visual function in European sea bass (*Dicentrarchus labrax*)

Richard W. Brill[1]*, **Andrij Z. Horodysky**[2], **Allen R. Place**[3], **Mary E. M. Larkin**[3], **Renate Reimschuessel**[4]

1 Department of Fisheries Science, Virginia Institute of Marine Science, Gloucester Point, Virginia, United States of America, **2** Department of Marine and Environmental Science, Hampton University, Hampton, Virginia, United States of America, **3** Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, Maryland, United States of America, **4** Center for Veterinary Medicine, U.S. Food and Drug Administration, Rockville, Maryland, United States of America

* rbrill@vims.edu

Abstract

Dietary insufficiencies have been well documented to decrease growth rates and survival (and therefore overall production) in fish aquaculture. By contrast, the effects of dietary insufficiencies on the sensory biology of cultured fish remains largely unstudied. Diets based solely on plant protein sources could have advantages over fish-based diets because of the cost and ecological effects of the latter, but plant proteins lack the amino acid taurine. Adequate levels of taurine are, however, necessary for the development of a fully functional visual system in mammals. As part of ongoing studies to determine the suitability of plant-based diets, we investigated the effects of normal and reduced taurine dietary levels on retinal anatomy and function in European sea bass (*Dicentrarchus labrax*). We could not demonstrate any effects of dietary taurine level on retinal anatomy, nor the functional properties of luminous sensitivity and temporal resolution (measured as flicker fusion frequency). We did, however, find an effect on spectral sensitivity. The peak of spectral sensitivity of individuals fed a 5% taurine diet was rightward shifted (i.e., towards longer wavelengths) relative to that of fish fed a 0% or 1.5% taurine diet. This difference in in spectral sensitivity was due to a relatively lower level of middle wavelength pigment (maximum absorbance .500 nm) in fish fed a 5% taurine diet. Changes in spectral sensitivity resulting from diets containing different taurine levels are unlikely to be detrimental to fish destined for market, but could be in fishes that are being reared for stock enhancement programs.

Introduction

Carnivorous fishes cannot synthesize many essential amino and fatty acids and must receive them through their diet [1,2]. Consequently, the aquaculture of these species requires diets based on, or at least supplemented with, protein sources from wild-caught fish. Up to two to five times more fish is, however, required to culture a product than is provided by it [3]. This

increases the cost of production and can have ecological consequences as capture of wild-caught fish may impact forage fish populations and the higher trophic level species that feed on them [4,5]. An equally important concern is the potential for fishmeal to contain xenobiotic compounds such as polychlorinated biphenyls (PCB's) and mercury. These undergo biomagnification leading to elevated levels of contaminates in the endproduct. In brief, fishmeal diets for the aquaculture of carnivorous fishes are costly and potentially both unsustainable as well as unsafe. Purely plant-based diets circumvent these issues, but generally lack essential and semi-essential amino acids (e.g., taurine, methionine, lysine), as well as many vitamins and minerals needed in microquantities [6]. Plant-based diets are, therefore, currently supplemented with fishmeal or fish oil [7–9]. But we argue, as have others [10], that there remains an exigent need to determine if purely plant-based diets can support survival rates, growth rates, and feeding efficiencies (i.e., the ratio of the mass of fish produced per mass of feed) necessary for the successful aquaculture of carnivorous fishes.

Omnivorous species have been the easiest to convert to low or no fishmeal diets whereas marine carnivorous species have been the most difficult [11]. But it is the latter that are produced by aquaculture for restocking programs (i.e., to augment wild populations) [12]. Diets differing in fatty acid composition can have significant impacts on the growth performance, energetics, cardiorespiratory physiology, hypoxia tolerances, as well as exercise and recovery performance of fishes [13–17]. It is therefore plausible that the metabolic performance and hypoxia tolerances of fishes fed plant-based diets may be significantly different–with major implications for the suitability of fishes for re-stocking programs.

More specific to our project, plant protein sources lack taurine. Taurine is a sulfur containing amino acid that is found in higher concentrations than any other free amino acid (i.e., amino acids not incorporated into any known proteins) and its roles in the proper development and function in a variety of vertebrate tissues have received considerable attention [18–20]. Taurine is considered a conditionally indispensable amino acid for humans and non-human primates, and an essential amino acid in some mammalian carnivores (e.g., felines) [21], but little attention has been paid to the required levels of this amino acid or its roles in fishes. We hypothesize that most marine carnivorous fishes lack the little ability to synthesize taurine (due to the large quantities found in their natural prey items) and therefore require it to be supplied in the diet. We also hypothesize that plant-based diets may not allow for development of fully functional visual system as a low taurine diet, or treatment with agonists of taurine uptake, have been documented to impede the embryonic development of the retina and maintenance of normal retinal function in mammals; the latter because of taurine's role as an antioxidant and osmolyte (i.e., a compound maintaining intracellular osmotic balance) [22–24]. High concentrations of taurine have, moreover, been found in the photoreceptor cells (i.e., rod and cone cells) and retinal pigment epithelium in several teleost fish species [25–30]. Although it has been suggested that taurine is only an osmolyte in retinal cells of fishes [26], other investigators have concluded that the role of taurine in development and maintenance of retinal function is conserved throughout the vertebrate order [21,31]. We therefore posit that diets containing inadequate levels of taurine could negatively impact visual function in carnivorous fishes. We recognize that less than fully functional visual systems are unlikely to be detrimental to fish destined for market, but we contend that a diminished visual function in fishes being cultured for stock enhancement programs would decrease their survival and fitness (e.g., growth and reproduction) relative to wild individuals. More specifically, we contend that programs rearing fish for restocking to sizes where they require formulated feeds prior to release would be less able to meet their ultimate objective. We do acknowledge, however, that restocking programs rearing fish to be released at small body sizes, such that individuals can be reared using feeds such as live copepods, will not be subject to this issue. Nonetheless, in conjunction

with our ongoing studies examining the overall efficacy of plant-based diets for generating acceptable growth rates in European sea bass (*Dicentrarchus labrax*), we expanded our efforts to examine the effects of dietary taurine level on visual function in this carnivorous species.

Materials and methods

Our study was carried out under protocols approved by the Institutional Animal Care and Use Committees of the University of Maryland Baltimore Medical School and the College of William and Mary and followed all applicable laws and regulations. European sea bass were obtained from the Les Poissons du Soleil's hatchery (Balaruc Les Bains, France) as 0.22g fry. They were raised at the Aquaculture Research Center, Institute of Marine and Environmental Technology (Baltimore, MD, USA) on a commercial diet until an average starting weight of ~15 g for fish subsequently reared on the 0% and 5% taurine diets and ~25 g for fish subsequently reared on the 1.5% taurine diet. Fish were separated by diet into three groups (150 fish in each) and reared in eight-foot diameter, four cubic meter recirculating systems. Fish reared on the 0% and 5% taurine diets were in tanks with shared mechanical and life support systems. Fish reared on the 1.5% taurine diet were housed in tanks with a separate mechanical and life support systems. Both systems included a protein skimmer, ozonation, mechanical filtration (in the form of bubble-bead filters), and biological filtration. Water quality (measured two to three times per week) was not significantly different between the systems (ANOVA, $p > 0.05$). Mean ($\pm$ SEM) water quality values in the tanks were: dissolved oxygen 5.7 ± 1.6 mg L^{-1}, temperature $27 \pm 2^{\circ}$ C, pH 7.6 ± 0.3, total ammonia nitrogen (NH_3) 0.06 ± 0.06 mg L^{-1}, nitrite (NO_2^-) 0.12 ± 0.08 mg L^{-1}, nitrate (NO_3^-) 49 ± 9 mg L^{-1}, alkalinity 96 ± 23 meq L^{-1}, and salinity 25 ± 2 ppt. Fish were fed 3.5% of their body weight per day and maintained on each specific diet for five to six months. The amount of feed was adjusted monthly as needed. Weight gain was calculated using the formula: ((FBW-IBW x 1000)/IBW + FBW)/2 x days); where FBW = final body weight and IBW = initial body weight. Feed efficiency was calculated as the ratio of wet weight gain per dry feed intake (g/kg/day).

Diet preparation

The three diet formulations (Table 1) were prepared by Zeigler Bros. (Gardners, PA, USA) and analysis of their proximate composition (Table 2) was performed by New Jersey Feed Laboratory, Inc. (Ewing Township, NJ, USA).

Tissue sampling for analysis retinal anatomy

Eyes were harvested from fish not used in the experiments to measure retinal responses to light stimuli. Food was withheld for 24 hours and 10–12 fish from each diet were anesthetized in a bath containing 25 mg L^{-1} MS-222 (Syndel, Ferndale, WA, USA) buffered with 50 mg L^{-1} sodium bicarbonate (Sigma-Aldrich, St. Louis, MO, USA). Upon removal from the anesthesia bath, the spinal cord was immediately severed, and eyes harvested. Retinal tissue was subsequently embedded, sectioned, mounted on glass slides, and stained with Hematoxylin-Eosin using standard histological procedures. Images were captured using a BX53 microscope outfitted with a DP73 camera and visualized with the cellsSens software (version 510, Olympus, Center Valley, PA, USA).

Retinal responses to light stimuli

A total of 27 fish were transferred to the Virginia Institute of Marine Science (Gloucester Point, VA, USA) where whole-animal corneal electroretinography (ERG) was used to assess

Table 1. Formulations for the three experimental diets on a dry-matter-basis.

Constituent	0% taurine	1.5% taurine	5% taurine
Profine VF (Soy bean concentrate)	28.75	28.75	28.75
Soybean meal, 47.5%	23.33	23.33	23.33
Wheat flour, bagged	16.54	15.04	11.54
Corn gluten, 60%	15.34	15.34	15.34
Menhaden gold oil, top-dressed	5.96	5.96	5.96
Monocalcium phosphate FG	3.95	3.95	3.95
Lecithin FG	3	3	3
L-Lysine, 98.5%	0.75	0.75	0.75
Choline chloride, 70%	0.6	0.6	0.6
Potassium chloride FG	0.56	0.56	0.56
DL-Methionine, 99%	0.45	0.45	0.45
Sodium chloride	0.28	0.28	0.28
Vitamin C	0.2	0.2	0.2
Premix AquaVit	0.12	0.12	0.12
Premix Aquamin Fish	0.12	0.12	0.12
Magnesium oxide FG	0.05	0.05	0.05
Taurine FG	0	1.5	5

FG = food grade

three standard metrics of retinal function: (1) luminous sensitivity (V log I response), (2) flicker fusion frequency (FFF, a measure of temporal resolution), and (3) spectral sensitivity. Anesthesia and handling methodologies were as described previously (32–35). Teflon-coated, silver–silver chloride, 0.5 mm diameter wire electrodes were used to measure ERG responses to light stimuli. The active electrode was placed on the corneal surface and the reference electrode in the nasal cavity. All subjects were dark-adapted for a minimum of 60 minutes prior to visual trials. Electrode placements, as well as any further modifications to the experimental setup, were conducted under a dim red LED light source (peak wavelength of 660 nm) which we presumed would be well beyond the spectral sensitivity of European sea bass, as it is in a variety of inshore temperate teleost fishes [32–35].

Luminous sensitivity was assessed using stimulus intensities covering six orders of magnitude using a collimated white LED source and neutral density filters progressing from subthreshold to saturation intensity levels in 0.2 log unit steps. FFF was assessed by measuring the ability of the retinal responses to track sinusoidally modulated white light stimuli ranging in frequency from 1 Hz (0 log units) to 100 Hz (2.0 log units), presented in increments of 0.2 log unit frequency steps. FFF was measured at stimulus intensities required to produce responses 25%, 50% and 100% of the maximum response, as well as fixed light levels (log I = 1.9, 2.7, and

Table 2. Proximate composition (%) of three experimental diets as fed.

Constituent	0% taurine	1.5% taurine	5% taurine
Moisture	8.05	6.97	9.5
Protein	43.82	45.02	46.65
Fat	5.94	6.19	6.94
Fiber	3.06	1.95	2.25
Ash	7.08	7.75	7.71
Taurine	0.07	1.48	4.85

3.7; with I in units of candela per m^2). Spectral sensitivity was assessed using stimuli over wavelengths from the ultraviolet (300 nm) to the near infrared (700 nm) presented sequentially in 10 nm steps. Monochromatic light flashes (50% bandwidth = 5 nm) were made approximately equally quantal through a series of neutral density filters and subsequently corrected to predict isoquantal responses as described previously (32–35). To form hypotheses regarding the number and spectral distribution of visual pigments present, and the effects of dietary taurine levels on the distribution of pigments contributing to spectral sensitivity, we fitted the SSH [36] and GFRKD [37] vitamin A1 rhodopsin absorbance templates separately to the photopic spectral sensitivity data. Estimates of the unknown model parameters (λ_{max} values and their respective weighting proportions) were derived by fitting the summed curves to the ERG data using maximum likelihood. We objectively selected the appropriate template (SSH or GFRKD) and number of contributing pigments using an information theoretic approach following Akaike's information criterion that is a parsimonious measure which strikes a balance between model simplicity and complex overparameterization [38]. All parameter optimization, template fitting and model selection was conducted using the software package R version 3.2.2 (R Development Core Team).

Statistical tests comparing the effects of dietary taurine levels on body mass and the magnitude of responses to various light levels were done using Sigmaplot (version 11.2, Systat Software, San Jose, CA, USA). Both employed a Kruskal-Wallis one-way analysis of variance on ranks because the former failed the equal variance test and the latter the Shapiro-Wilk test for normality. We limited comparisons to the magnitude of responses to various light levels (i.e., luminous sensitivity) to the effects of diet within a given light level. Statistical tests comparing the effects of dietary taurine levels on FFF were preformed using the two-way repeated measures ANOVA procedure in Sigmaplot, with the Holm-Sidak method to conduct all pairwise comparisons.

Results and discussion

The taurine requirement for growth and nitrogen accretion for juvenile European sea bass has recently been determined to be 0.47–0.50% dry matter [39]. Our diets covered this range as reflected in the weight gain (7.2 vs 9.2 g/kg/day) and the feed efficiency (0.5 vs 0.86) of fish reared on 0% taurine vs 5% taurine diets. Mortality among diets was less than 3%. The median body masses of the fishes from the three dietary taurine levels used in the vision experiments were not significantly different (Fig 1).

Retinal morphology

There were no obvious effects of dietary taurine level on the morphology of identifiable layers in European sea bass retina (Fig 2), nor retinal cell layer thickness ratios (Fig 3). These results are in direct contrast to the massive disruption seen in retinal tissue of domestic cats fed low taurine diets [40].

Retinal function

Retinal responses to increasing light levels showed the expected steep increases up to those levels producing a maximum response (Fig 4, panel A). When light levels are expressed in log units, retinal response curves were the expected sigmoidal shape (Fig 4, panel B). There was only one significant effect of dietary taurine levels on luminous sensitivity in European sea bass, and only at light levels needed to produce a response 75% of maximum (Fig 5). In other words, significantly higher light levels were required to achieve responses above approximately 50% of maximum in fish fed a diet lacking taurine. Flicker fusion frequency data showed that
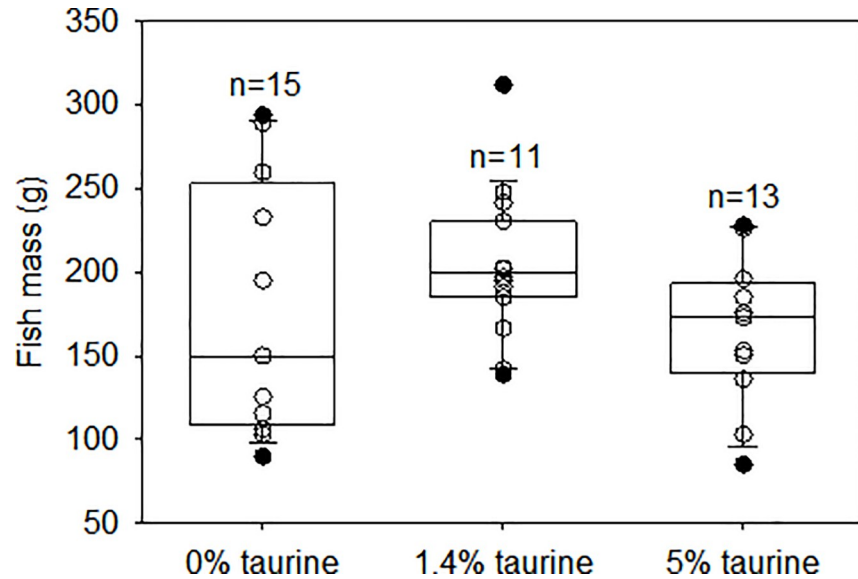
Fig 1. Body masses of the fishes from the three dietary taurine levels used in the vision experiments. The solid lines within the boxes mark the median values, the boundaries of the boxes the 25th and 75th percentiles, and the whiskers (error bars) above and below the boxes the 90th and 10th percentiles. Data points between the 90th and 10th percentiles are shown as open circles and those outside this interval as filled circles. The number of individuals from which data were obtained ("n") are shown above each bar.
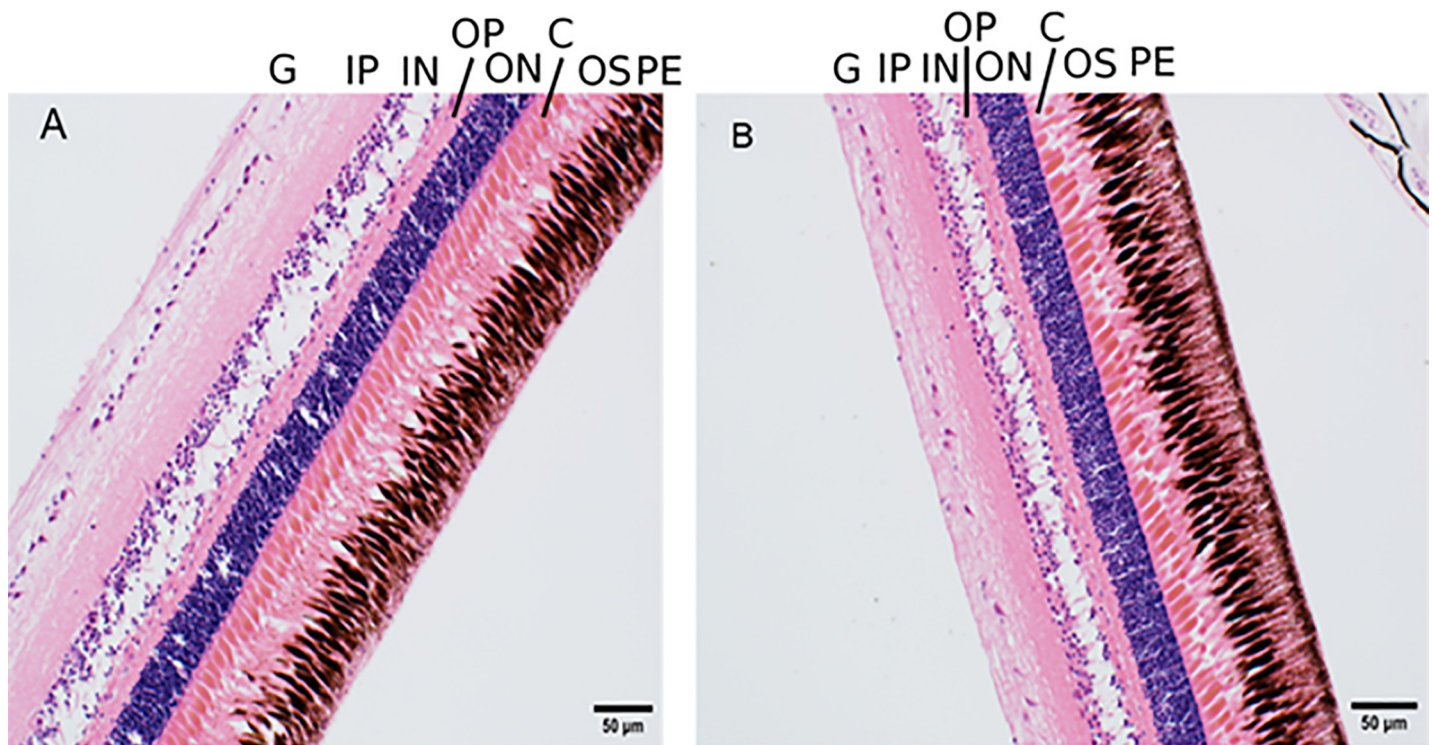
Fig 2. Identifiable layers in European sea bass retinas. Panels A and B are representative retina from fish fed a 0% and 5% taurine diet, respectively. G = ganglion cell layer, IP = inner plexiform layer, IN = inner nuclear layer, OP = outer plexiform layer; ON = outer nuclear layer, C = cone photoreceptors, OS = outer segments of the photoreceptor layer, PE = pigmented epithelium.
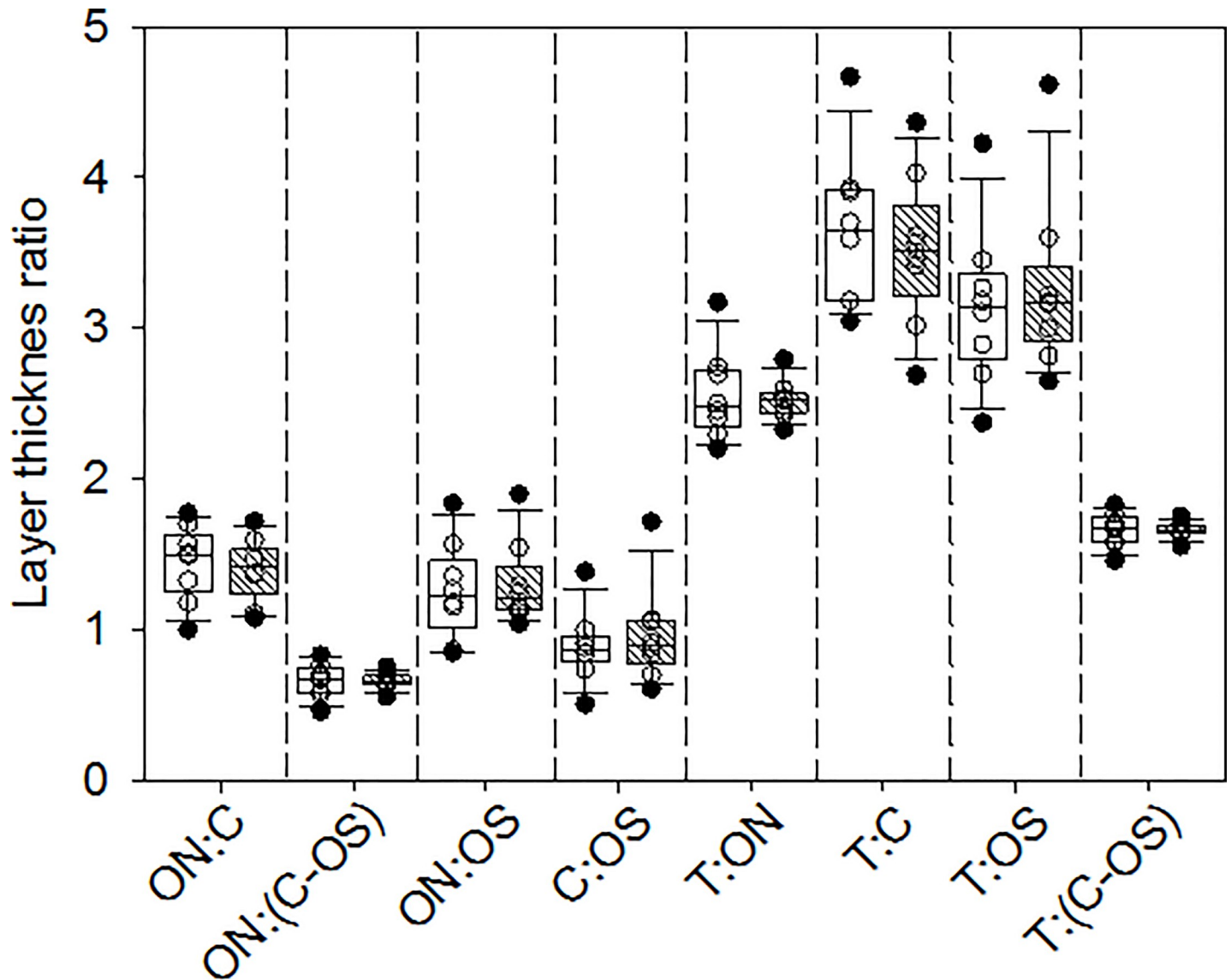
Fig 3. Retinal cell layer thickness ratios in European sea bass fed a 0 or 5% taurine diet (open and cross hatched boxes, respectively). The solid lines within the boxes mark the median values, the boundaries of the boxes the 25th and 75th percentiles, and the whiskers (error bars) above and below the boxes the 90th and 10th percentiles. Data points between the 90th and 10th percentiles are shown as open circles and those outside this interval as filled circles. T = total retina thickness, C = cone photoreceptors layer thickness, ON = outer nuclear layer thickness, OS = thickness of the outer segments of photoreceptor layer, IN = inner nuclear layer thickness (μm). Measurements were taken on retinas from eight individuals.

only light intensity (log I) had a significant effect (Table 3). Flicker fusion frequencies generally showed the expected increases with increasing light intensities, whereas dietary taurine level had no influence on FFF when comparisons are made at the same light level (Fig 6). For comparisons made within the 0% taurine diet, p-values (in the order listed above) were: 0.002, <0.001, and 0.003; for comparisons made with 1.5% taurine diet p-values were: 0.013, <0.001, and <0.001; and for comparisons made with 5% taurine diet p-values were: 0.024, <0.001, and 0.003.

The spectral sensitivity of fish fed diets with reduced taurine levels (i.e., 0 and 1.5%) were not significantly different from each other (Fig 7, panels A and B) and these data were
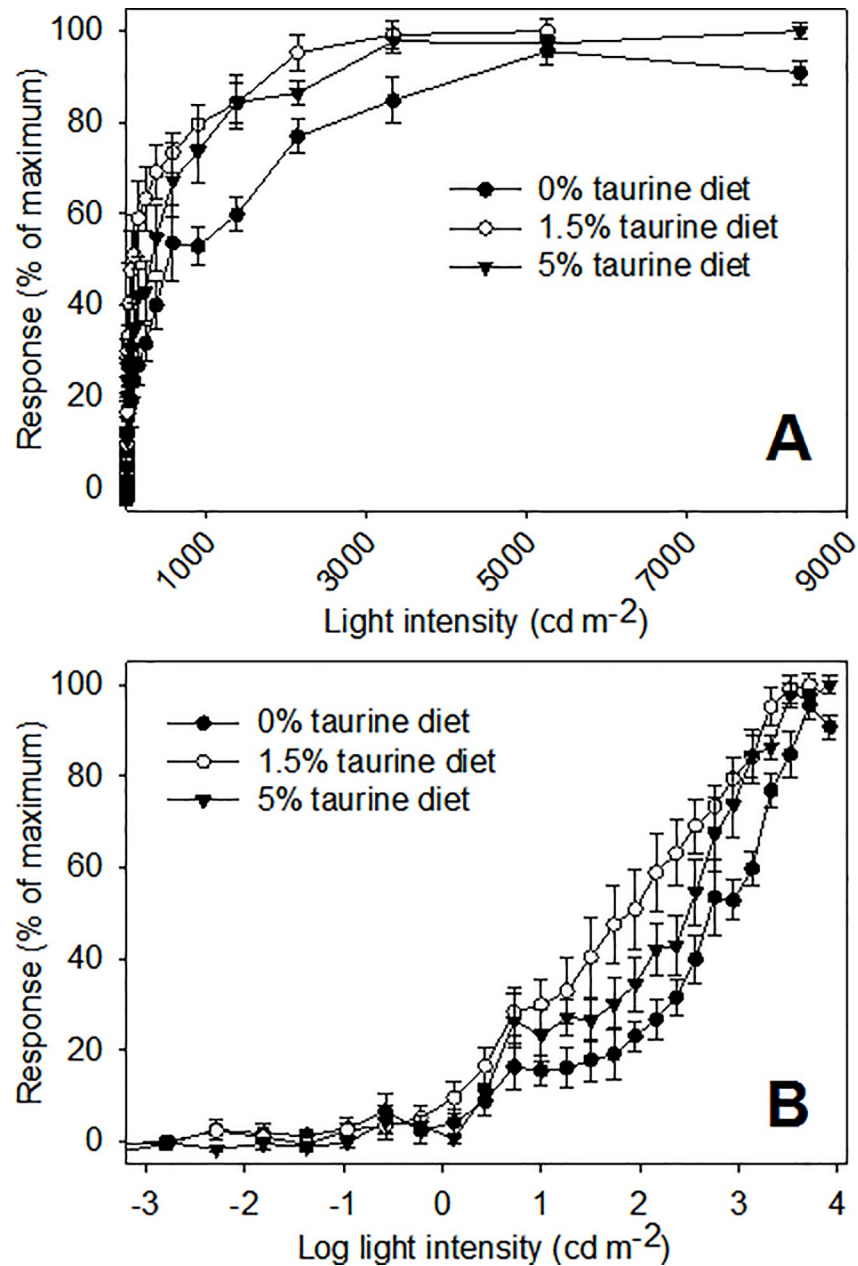
Fig 4. Luminous sensitivity (i.e., intensity–response curves) of European sea bass. Data are mean values (± SEM). Response values for individuals were normalized to 0–100%, the values averaged, and the mean values rescaled to 0–100%. Panel A shows luminous sensitivity with light intensities expressed in candela per square meter. Panel B shows the same data, but with light intensities expressed in log units. Increases in light intensity were in 0.2 log unit steps.

https://doi.org/10.1371/journal.pone.0214347.g004

subsequently combined. The results from fitting the data to both SSH and GFRKD rhodopsin templates suggested that European seabass are trichromats (i.e., have three visual pigments) (Tables 4 and 5). The SSH rhodopsin template was clearly best fitting (i.e., had the lowest AIC value) for the combined data from fish fed diets with reduced taurine levels (Table 4). In the case of data from fish fed 5% taurine diet (Table 5), the AIC values for SSH rhodopsin templates for dichromats (i.e., two retinal pigments) with secondary absorbing peaks (i.e., ∃
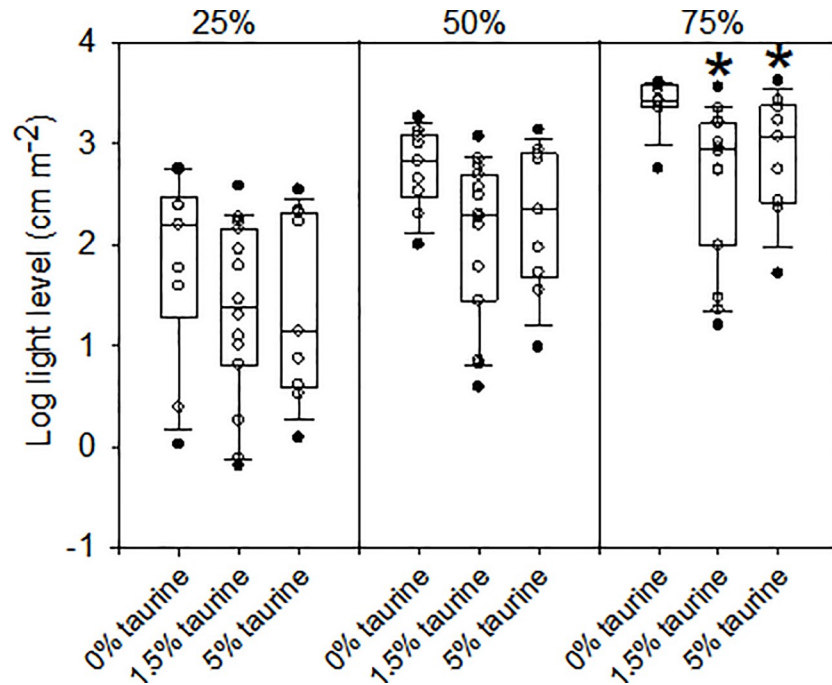
Fig 5. Light levels needed to produce a given response relative to that of the maximin response in intensity–response curves (Fig 4). The solid lines within the boxes mark the median values, the boundaries of the boxes the 25th and 75th percentiles, and the whiskers (error bars) above and below the boxes the 90th and 10th percentiles. Data points between the 90th and 10th percentiles are shown as open circles and those outside this interval as filled circles. Significant differences between median values from fish fed 0% and 1.5% taurine diets and those between median values from fish fed 0% and 5% taurine diets are indicated by the * symbol (p-values were 0.002 and 0.044, respectively).

bands) on both the visual pigments, and the SSH and GFRKD rhodopsin templates for trichromats, were indistinguishable ()AIC < 5). The SSH template for a dichromat with secondary absorbing peaks on both pigments predicted, however, the short wavelength pigment to have maximum absorbance in the UV wavelength (376 nm), which we consider unreasonable given the results from ERG data fitted similarly from a variety of inshore fishes (32–35). We therefore based our subsequent conclusions on the effects diet on SSH rhodopsin templates for trichromats.

Our results suggested that the visual pigments of European seabass fed 0 or 1.5% taurine diets have maximal absorbance peaks (λ_{max}) of 389, 512, 611 nm (P_1, P_2, P_3, respectively; Fig 7,

Table 3. Results from a two-way repeated measures ANOVA procedure comparing the effects of dietary taurine levels and light intensity (log I) on flicker fusion frequencies. Only the latter had a significant effect.

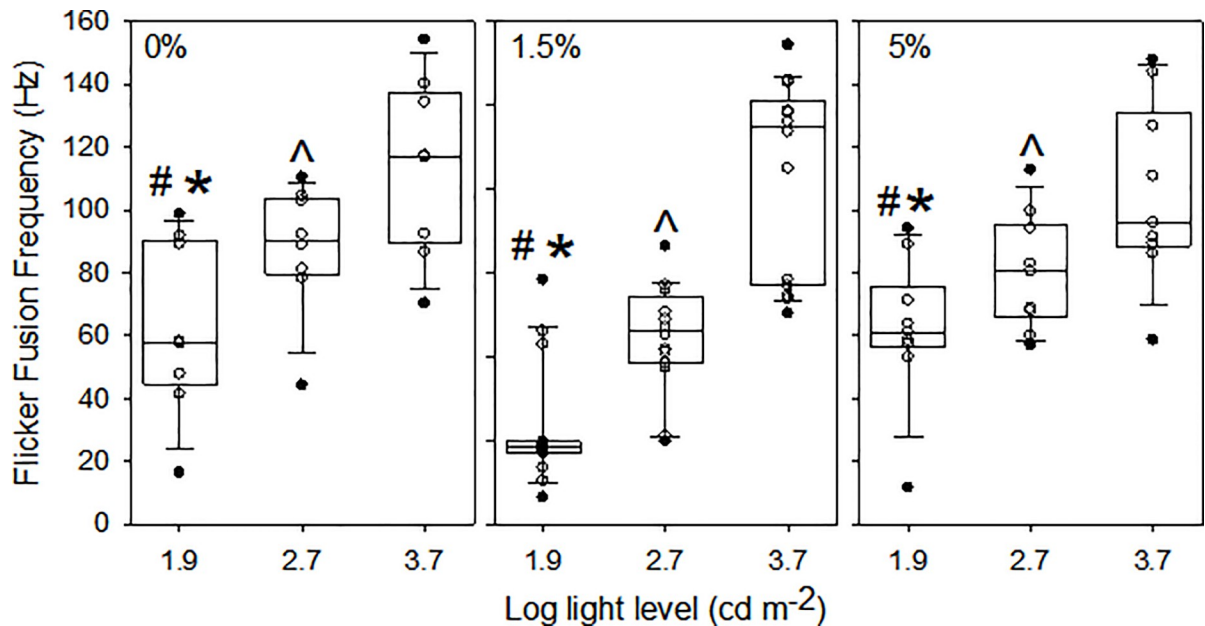| Source of variation	Degrees of freedom	Sums of squares	Mean square	F	P
subject	7	10210	1458	1.167	0.372
diet	2	2037	1018	0.943	0.413
diet x subject	14	15110	1079		
log I	2	31868	15934	45.80	<0.001
log I x subject	14	4871	348		
diet x log I	4	554	138	.0781	0.547
residual	28	4963	177		
total	71	6961	980		

Fig 6. Flicker fusion frequencies (i.e., the highest frequency of sinusoidal light stimulus detectable) with increasing light intensities of European seabass. The light levels used correspond to approximately those needed to produce responses approximately 25%, 50% and 75% of the maximum response in the intensity–response curves (Fig 3). The solid lines within the boxes mark the median values, the boundaries of the boxes the 25th and 75th percentiles, and the whiskers (error bars) above and below the boxes the 90th and 10th percentiles. Data points between the 90th and 10th percentiles are shown as open circles and those outside this interval as filled circles. The symbols #, *, and ^ indicate differences in flicker fusion frequencies between log light levels 1.9 and 2.7, between log light levels 1.9 and 3.7, and between log light levels 2.7 and 3.7, respectively.

panel C). The maximal absorbance peaks of the visual pigments in fish fed a diet containing 5% taurine had maximal absorbance peaks (λ_{max}) of 380, 497, 598 nm (P_1, P_2, P_3, respectively; Fig 7, panel D). The most significant differences were a significant effect of dietary taurine level on the relative abundance of the three visual pigments (Fig 7, panel C). More specifically, in fish fed 0 and 1.5% taurine diets, the ratio of the middle and long wavelength visual pigments (P_2 and P_3, respectively) were approximately equal (0.91 to 1,) with a reduced abundance of the short wavelength pigment (P_1) relative to the most abundant longwave length pigment (P_3) (0.24 to 1) (Fig 7, panel C). By contrast, in fish fed a 5% taurine diet, there was a reduction in the abundance of the middle wavelength pigment (P_2) relative to the long wavelength pigment (P_3) (0.32 to 1) (Fig 7, panel D), while the ratio of the abundance of the short wavelength pigment (P_1) relative to the most abundant longwave length pigment (P_3) was relatively unchanged (0.17 to 1) (Fig 7, panel D). The overall effect of the changes in abundance of visual pigments was that the peak in overall retinal spectral sensitivity was shifted to longer wavelengths: from .530 nm in fish fed the 0% or 1.5% taurine diets (Fig 7, panel C) to .600 nm in fish fed the 5% taurine diet (Fig 7, panel D).

Replacing fishmeal with plant-based diets is a priority for the aquaculture industry, and the development of optimized diets is underway [41]. Plant-based diets have a high protein content, but commercial feed formulations often vary based on the availability and cost of ingredients, with different batches containing significantly different proportions or quality of ingredients, or different ingredients all together [42]. For this reason, the assessment of multiple ingredients and ingredient combinations is necessary. To address this issue, we formulated a diet based on available and cost-effective plant ingredients which are effective fishmeal replacements in rainbow trout (*Oncorhyncus mykiss*) [43,44] and are highly digestible by cobia
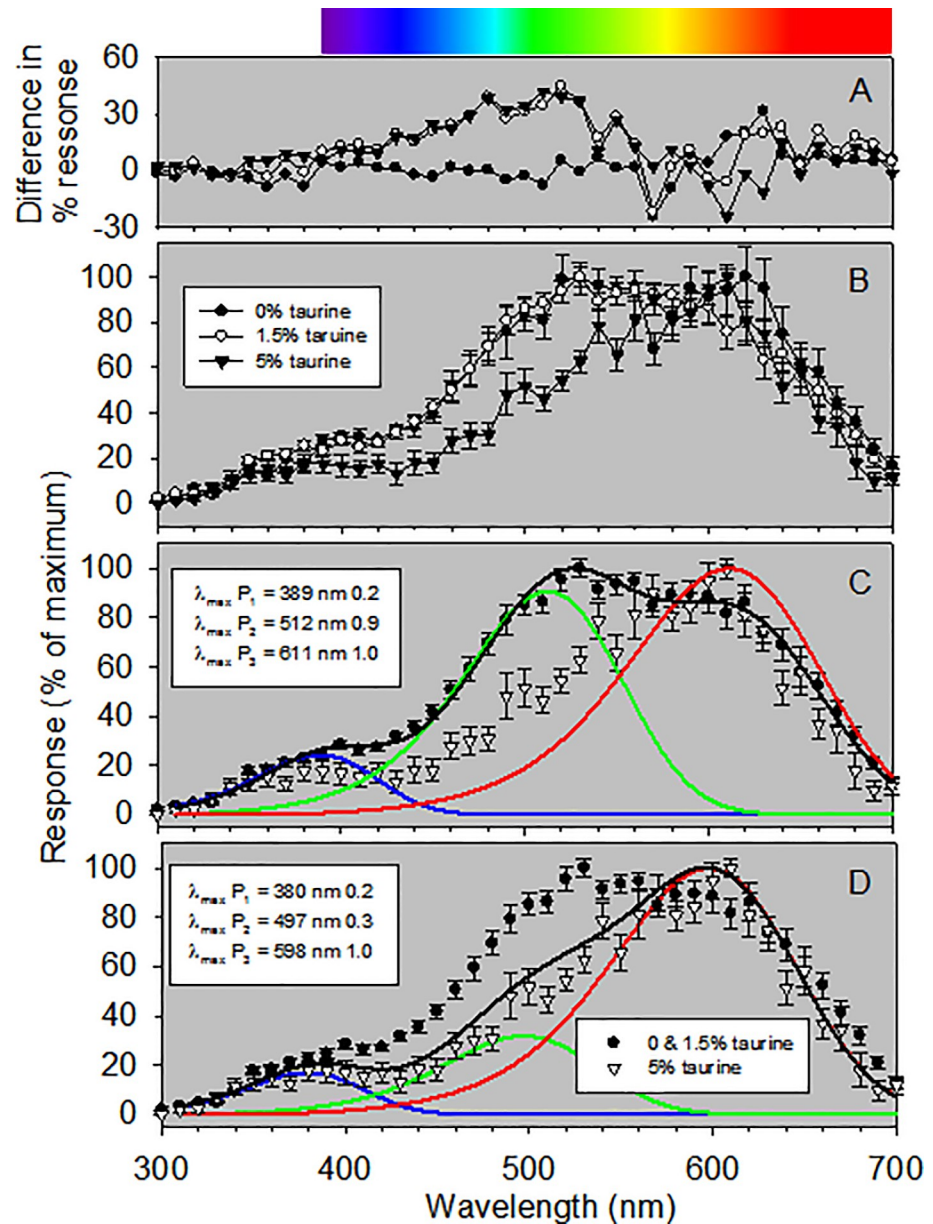
Fig 7. Spectral sensitivity (i.e., responses to monochromatic light) of European sea bass. Data are mean values (± SEM). Response values for individuals were normalized to 0–100%, the values averaged, and the mean values rescaled to 0–100%. Panel A are the differences between mean values of responses of fish fed the three different diets. Panel B are the mean responses of fish feed 0%, 1.5%, and 5% taurine diets. In both panels, fish feed 0%, 1.5%, and 5% taurine diets are indicated by filled circles, open circles and filled triangles, respectively. Because the spectral sensitivities of fish fed 0% or 1.5% taurine diets were largely not different, the data from these individuals were combined and the mean relative responses shown in panels B and C. Wavelengths at maximal absorptions (λ_{max} values) and pigment-specific weight values (to the right of each λ_{max} value) are shown. Black lines are the summed curves of the visual pigment curves multiplied by their respective weighting factors. Color coded lines indicate the absorptive characteristics of individual visual pigments. The color bar at the top of the figure shows the approximate human visual spectrum.

https://doi.org/10.1371/journal.pone.0214347.g007

(*Rachycentron canadum*) [45]. But, as with other plant-based diets, our formulation lacks taurine. There is evidence that, although that taurine can be recycled through the taurine transporter and biliary recycling pathways, constant dietary supply is required to maintain proper function of multiple tissue types [20,21]. We assumed that taurine's role as a photoreceptor

Table 4. The spectral sensitivity of European seabass fed diets with reduced taurine levels (i.e., 0 and 1.5%) where the data from fish in these two groups have been combined.

Condition	Template	$\lambda_{max,1}$	$\lambda_{max,2}$	$\lambda_{max,3}$	$-\log(L)$	p	AIC	ΔAIC	Pigment weights
Mono	GFRKD	574			-32.1	8	-58	169	
	SSH	575			-26.5	8	-47	180	
Di, α	GFRKD	410	510		-91.3	8	-172	55	
	SSH	411	510		-91.0	8	-173	55	
Di, β, S	GFRKD	498	603		-79.7	8	-147	80	
	SSH	504	604		-97.7	8	-183	44	
Di, β, L	GFRKD	439	513		-79.5	8	-147	80	
	SSH	461	513		-96.5	8	-181	46	
Di, β, B	GFRKD	509	601		-67.2	8	-120	107	
	SSH	527	605		-82.0	8	-150	77	
Tri, α	GFRKD	388	501	603	-97.4	8	-181	47	
	SSH	389	512	611	120.7	8	-227	0	0.24, 0.91, 1

https://doi.org/10.1371/journal.pone.0214347.t004

protectant would most likely be as an antioxidant, like the role it plays in the liver of Atlantic salmon (*Salmo salar*) challenged with pro-oxidants such as cadmium chloride [46]. We therefore hypothesized that visual disparities between European seabass fed 0%, 1.5% and 5% taurine diets would be due to differences in photoreceptor and pigment repair.

We did not, however, see a reduction of luminous sensitivity resulting from reduced dietary taurine (Fig 4). In contrast, other investigators have demonstrated a large progressive decrease in ERG amplitudes over a range of light intensities (12–197 cd m^{-2}, or log light level 1.1 to 2.3) in rats fed a soybean-based diet (containing a negligible amount of taurine) and treated with guanidinoethyl sulfonate (a compound that depletes of taurine) over 15 weeks [23]. Similar decreases in ERG amplitudes have been recorded in cats fed a taurine deficient (casein-based) diet (47). These results (in comparison to ours) imply that the loss of luminous sensitivity resulting from a low taurine diet is related to a reduction in the cellular defense mechanisms countering light- and oxygen induced damage in retinal receptor cells [24,47,48], and that taurine deficiency has different effects in mammals and teleost fishes.

Effective visual function at low light levels requires both spatial and temporal summation within the retina [49–50]. The former results from the convergence of photoreceptors onto bipolar cells, as seen in fishes occupying dimly lit environments [51–53]. Spatial summation

Table 5. The spectral sensitivity of European seabass fed a 5% taurine diet.

Condition	Template	$\lambda_{max,1}$	$\lambda_{max,2}$	$\lambda_{max,3}$	$-\log(L)$	p	AIC	ΔAIC	Pigment weights
Mono	GFRKD	586			-52.2	8	-98	92	
	SSH	588			-44.6	8	-83	108	
Di, α	GFRKD	485	597		-83.7	8	-155	35	
	SSH	496	598		-96.9	8	-182	9	
Di, β, S	GFRKD	485	597		-83.7	8	-155	35	
	SSH	496	598		-96.9	8	-182	9	
Di, β, L	GFRKD	439	513		-79.5	8	-147	44	
	SSH	461	513		-96.5	8	-181	10	
Di, β, B	GFRKD	506	597		-76.6	8	-139	52	
	SSH	376	599		-102.4	8	-191	0	
Tri, α	GFRKD	382	492	597	-101.4	8	-189	2	
	SSH	380	497	598	-101.3	8	-189	2	0.17, 0.32, 1

https://doi.org/10.1371/journal.pone.0214347.t005

therefore reduces visual acuity (i.e., the ability to detect the details of an object). Demonstrating anatomical changes in spatial summation (i.e., changes in the convergence of photoreceptors onto bipolar cells) was, however beyond the scope of this study. Temporal summation refers to photoreceptors responding slowly to flashing light as evinced by reduced temporal resolutions (i.e., low FFF). Low FFF implies a blurring of fast-moving objects or the inability to detect them at all [49–51,54–56]. We specifically investigated the effects of dietary taurine level on FFF (Fig 6), but we could not demonstrate any effects. We therefore conclude that there was no effect of dietary taurine level on retinal anatomy in terms of the degree of the convergence of receptor cells onto bipolar cells.

In terms of spectral sensitivity, our results imply that the European seabass visual system has three visual pigments and, more importantly, that spectral sensitivity can be impacted by dietary levels of taurine (Fig 7). To the best of our knowledge, we are the first to demonstrate this phenomenon in fishes. Our results could be due to: (1) changes in the relative abundance of the three visual pigments [57–59], or (2) changes in the amino acid composition of opsin proteins (which influence peak wavelength absorbancies of visual pigments) [60–61]. Changes in amino acid composition of opsin proteins (due to changes in gene expression) have been shown to occur over ontogeny, through adaptation of individuals to different light spectra, or changes diurnal lighting patterns [62–66]. Our data do not allow us to differentiate between changes in the relative abundance of visual pigments or changes in amino acid composition of opsin proteins. Our results do, however, demonstrate that there is a threshold effect of dietary taurine in European seabass, in that there were no discernable alterations in spectral sensitivity of fish fed 0% and 1.5% taurine diets, but a clear difference in spectral sensitivity of fish fed a 5% taurine diet. The net effect of reduced taurine diets was a broader overall spectral sensitivity, with the peak in spectral sensitivity being leftward-shifted (a greater sensitivity to shorter wave lengths). Our results are in contrast with the effects a taurine deficiency (and light-induced photoreceptor degeneration) on spectral sensitivity of rats where cone cells containing short wavelength pigments are most negatively affected [67].

We note, however, that one of reasons for undertaking this study was to determine if fishes being cultured for stock enhancement programs fed a plant-based diet lacking enough taurine could show decreased survival and fitness following release. If so, programs rearing fish in this manner for restocking would be less able to meet their ultimate objective of enhancing population abundance. (We do acknowledge, however, that this will not be an issue for programs releasing fish at the fry or fingerling stage because they rely on natural feeds such as copepods.) Our demonstration that reduced dietary taurine levels influence spectral sensitivity (Fig 7) implies that this is the case. This conclusion is congruent with both the visual pigment sensitivity hypothesis and the contrast sensitivity hypothesis. The former posits that for optimal visual function the absorbance of visual pigments must correspond to the spectral distribution of the light environment [68–70]; whereas the latter contends that aquatic animals maximize contrast sensitivity to acquire information effectively in a turbid (i.e., low contrast) environment [71–73]. Under both situations, however, changes in the relative abundance of visual pigment are likely to reduce fitness in cultured fish released for restocking. It unknown, however, if these deficits would remain following release of cultured fish, or if the visual properties of fish fed 0% and 1.5% taurine diets we observed would converge onto those of fish fed a 5% taurine diet after the switch to a natural prey diet. These questions are worth investigating.

Conclusions

We could not demonstrate any effects of dietary taurine level on retinal anatomy, nor the functional properties of luminous sensitivity or temporal resolution (i.e., FFF) in European sea

bass. We did, however, find an effect on spectral sensitivity. The peak of spectral sensitivity of individuals fed a 5% taurine diet was rightward shifted (i.e., towards longer wavelengths) relative to that of fish fed 0% or 1.5% taurine diets. This difference in spectral sensitivity was caused by a relatively lower level of a middle wavelength pigment (maximum absorbance .500 nm) in fish fed a 5% taurine diet. Changes in spectral sensitivity resulting from diets containing different taurine levels are unlikely to be detrimental to fish destined for market but could be in fishes fed plant-based diets that are being reared for stock enhancement through the release of large fish.

Supporting information

S1 Data. Excel spreadsheet containing data values plotted in all main figures.
(XLSX)

Acknowledgments

Author Contributions

Conceptualization: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place, Mary E. M. Larkin, Renate Reimschuessel.

Data curation: Richard W. Brill, Allen R. Place, Mary E. M. Larkin.

Formal analysis: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place, Mary E. M. Larkin, Renate Reimschuessel.

Funding acquisition: Andrij Z. Horodysky, Allen R. Place.

Investigation: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place, Mary E. M. Larkin, Renate Reimschuessel.

Methodology: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place, Mary E. M. Larkin, Renate Reimschuessel.

Project administration: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place, Renate Reimschuessel.

Resources: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place, Mary E. M. Larkin, Renate Reimschuessel.

Software: Richard W. Brill.

Supervision: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place.

Validation: Richard W. Brill, Renate Reimschuessel.

Writing – original draft: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place, Mary E. M. Larkin.

Writing – review & editing: Richard W. Brill, Andrij Z. Horodysky, Allen R. Place, Mary E. M. Larkin, Renate Reimschuessel.

References

1. Silva SS, Anderson TA. Fish nutrition in aquaculture. London: Chapman & Hall; 1995.

2. Lunger AN, Craig SR, McLean E. Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. Aquaculture 2006; 257:393–399

3. Tacon A, Metian M. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 2008; 285:146–158.

4. Pauly D, Christensen V, Dalsgaard J, Froese R, Torres F J r. Fishing down marine food webs. Science 1998; 279:860–863. PMID: 9452385

5. Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MC, Clay J, et al. Effect of aquaculture on world fish supplies. Nature 2000; 405:1017–1024. https://doi.org/10.1038/35016500 PMID: 10890435

6. Gatlin DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW et al. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac Res. 2007; 738:551–579.

7. Barrows FT, Gaylord TG, Sealey WM, Smith CE, Porter L. Supplementation of plant-based diets for rainbow trout (*Oncorhynchus mykiss*) with macro-minerals and inositol. Aquac Nutr. 2010; 16:654–661.

8. Gaylord TG, Barrows FT, Teague AM, Johansen KA., Overturf KE, Shepherd B. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 2007; 269:514–524.

9. Zhou QC, Wu Z, Chi S, Yang Q. Dietary lysine requirement of juvenile cobia (*Rachycentron canadum*). Aquaculture 2007; 273:634–640.

10. Turchini GM, Trushenski JT, Glencross BD. Thoughts for the future of aquaculture nutrition: realigning perspectives to reflect contemporary issues related to judicious use of marine resources in aquafeeds. N Am J Aquacult. 2018; https://doi.org/10.1002/naaq.10067

11. Fernandez-Jover D, Arechavala-Lopez P, Martinez-Rubio L, Tocher DR, Bayle-Sempere JT, Lopez-Jimenez JA, et al. Monitoring the influence of marine aquaculture on wild fish communities: benefits and limitations of fatty acid profiles. Aquacult Environ Interact. 2001; 2:39–47.

12. Lorenzen K, Agnalt A-LA, Blankenship HL, Hines AH, Leber KM, Loneragan NR, Taylor MD. Evolving context and maturing science: aquaculture-based enhancement and restoration enter the marine fisheries management toolbox. Rev Fish Sci. 2013; 21:213–221.

13. Agnisola C, McKenzie DJ, Taylor EW, Bolis CL, Tota B. Cardiac performance in relation to oxygen supply varies with dietary lipid composition in sturgeon. Am J Physiol. 1996; 271:R417–425. https://doi.org/10.1152/ajpregu.1996.271.2.R417 PMID: 8770143

14. McKenzie DJ. Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. Comp. Biochem Physiol A Mol Integr Physiol. 2001; 128:607–621. PMID: 11246048

15. McKenzie DJ, Piraccini G, Steffensen JF, Bolis CL, Bronzi P, Taylor EW. Effects of diet on spontaneous locomotor activity and oxygen consumption in Adriatic sturgeon (*Acipenser naccarii*). Fish Physiol Biochem. 1995; 14;341–55. https://doi.org/10.1007/BF00003373 PMID: 24197526

16. Randall DJ, Mckenzie DJ, Abrami G, Bondiolotti GP, Natiello F, Bronzi P, Bolis L, Agradi E. Effects of Diet on Response to Hypoxia in Sturgeon (*Acipenser naccarii*). J Exp Biol. 1992: 125;113–125.

17. Wagner GN, Balfry SK, Higgs DA, Lall SP, Farrell AP. Dietary fatty acid composition affects the repeat swimming performance of Atlantic salmon in seawater. Comp Biochem Physiol A Mol Integr Physiol. 2004; 137;567–76. https://doi.org/10.1016/j.cbpb.2003.11.005 PMID: 15123193

18. Ripps H, Shen WK. Review: Taurine: A "very essential" amino acid. Mol Vis 2012; 18: 2673–2686. PMID: 23170060

19. Schaffer SW, Jong CJ, Ramila KC, Azuma J. Physiological roles of taurine in heart and muscle. J Biomed Sci. 2010: S2;1–2.

20. L'Amoreaux W. The roles of taurine in the retina. In: Taurine in Health and Disease, El Idrissi A, L'Amoreaux W, editors. Kerala, India: Transworld Research Network; 2015. p. 215–254. ISBN: 978-81-7895-520-9.

21. Schuller-Levis GB, Park E. Taurine: new implications for an old amino acid. FEMS Microbiol Lett. 2003: 226;195–202. https://doi.org/10.1016/S0378-1097(03)00611-6 PMID: 14553911

22. Hillenkamp J, Hussain AA, Jackson TL, Constable PA, Cunningham JR, Marshall J. Compartmental analysis of taurine transport to the outer retina in the bovine eye. Invest Ophth Vis Sci. 2004: 45;4099–4105.

23. Lake N. Electroretinographic deficits in rats treated with Guanidinoethyl sulfonate, a depletory of taurine. Exp Eye Res. 1986: 42;87–91. PMID: 3956606

24. Lake N, Malik N. Retinal morphology in rates treated with a taurine transport antagonist. Exp Eye Res. 1987: 44;331–346. PMID: 3595755

25. Gisselmann G, Galler A, Friedrich F, Hatt H, Bormann J. Cloning and functional characterization of two glycine receptor alpha-subunits from the perch retina. Eur J Neurosci. 2002: 16;69–80. PMID: 12153532

26. Marc RE, Cameron D. A molecular phenotype atlas of the zebrafish retina. J Neurocytol. 2001: 30;593–654. PMID: 12118163

27. Negishi K, Drujan BD. Effects of some amino acids on horizontal cells in the fish retina. J Neurosci Res. 1979: 4;351–263. https://doi.org/10.1002/jnr.490040504 PMID: 118265

28. Nusetti S, Obregón F, Quintal M, Benzo Z, Lima L. Taurine and zinc modulate outgrowth from goldfish retinal explants. Neurochem Res. 2005: 30:1483–1492. https://doi.org/10.1007/s11064-005-8825-9 PMID: 16362767

29. Omura Y, Yoshimura R. Immunocytochemical localization of taurine in the developing retina of the lefteye flounder *Paralichthys olivaceus*. Arch Histol Cytol. 1999: 62;441–446. PMID: 10678573

30. Omura Y, Hach A, Furukawa E, Ueck M, Lake N. Immunocytochemical localization of taurine in the pineal organ and retina of an anadromous fish, *Plecoglossus altivelis*. Arch Histol Cytol. 1997: 60;153–162. PMID: 9232179

31. Lombardini JB. Taurine: retinal function. Brain Res Rev. 1991: 16;151–169. PMID: 1760655

32. Brill R. Magel C, Davis M, Hannah R, Rankin P. Effects of rapid decompression and exposure to bright light (simulated capture) on the visual function of black rockfish (*Sebastes melanops*) and Pacific halibut (*Hippoglossus stenolepsis*). Fish Bulletin. 2008: 106;427–437.

33. Horodysky AZ, Brill RW, Warrant EJ, Musick JA, Latour RJ. Comparative visual function in five sciaenid fishes inhabiting Chesapeake Bay. J Exp Biol. 2008: 211;3601–3612. https://doi.org/10.1242/jeb.023358 PMID: 18978225

34. Horodysky AZ, Brill RW, Warrant EJ, Musick JA, Latour RJ. Comparative visual function in four piscivorous fishes inhabiting Chesapeake Bay. J Exp Biol. 2010: 213; 1751–1761. https://doi.org/10.1242/jeb.038117 PMID: 20435826

35. Horodysky AZ, Brill RW, Crawford KC, Seagroves ES, Johnson AK. Comparative visual ecophysiology of mid-Atlantic temperate reef fishes. Biol. Open. 2013: 2;1371–1381. https://doi.org/10.1242/bio.20136825 PMID: 24285711

36. Stavenga DG, Smiths RP, Hoenders BJ. Simple exponential functions describing the absorbance bands of visual pigment spectra. Vis Res. 1993: 33;1011–1017. PMID: 8506642

37. Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K. In search of the visual pigment template. Vis Neurosci. 2000: 17;509–528. PMID: 11016572

38. Burnham KP, Anderson DR. Model selection and multimodel inference: a practical information-theoretic approach. New York: Springer; 2002.

39. Martins N, Estevão-Rodrigues T, Diógenes AF, Diaz-Rosales P, Oliva-Teles A, Peres H. Taurine requirement for growth and nitrogen accretion of European sea bass (*Dicentrarchus labrax*, L.) juveniles. Aquaculture 2018: 494;19–25.

40. Schmidt SY, Berson EL, Watson G, Huang C. Retinal degeneration in cats fed casein III. Taurine deficiency and ERG amplitudes. Invest Ophthalmol Vis Sci. 1977: 16;673–678. PMID: 873729

41. Rust MB, Barrows FT, Hardy RW, Lazur AL, Naughten K, Silverstein J. Alternative feeds initiative. The future of aquafeeds. National Oceanic and Atmospheric Administration / United State Department of Agriculture 2011:NOAA Tech. Memo. NMFS–F/SPO–124.

42. Hardy RW. New developments in aquatic feed ingredients, and potential of enzyme supplements. In: Cruz-Saurez LE, Ricque-Marie D, Tapia-Salazar M, Olvera-Novoa MA, Civera-Cerecedo R, editors. Avances En Nutrition Acuicola V. Memorias Del V Simposium Internacional de Nutricion Acuicola. 2000 Merida, Yucatan, Mexico, p. 216–226.

43. Barrows FT, Gaylord TG, Sealey WM, Porter L, Smith CE. The effect of vitamin premix in extruded plant-based and fish meal based diets on growth efficiency and health of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 2008: 283;148–155.

44. Palti Y, Silverstein JT, Wieman H, Phillips JG, Barrows FT, Parsons JE. Evaluation of family growth response to fishmeal and gluten-based diets in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2006: 255;548–556.

45. Watson AM, Kissil GW, Barrows FT, Place AR. Developing a plant-based diet for cobia *Rachycentron canadum*. Int. Aquafeed. 2012: 15;34–38.

46. Espe M, Holen E. Taurine attenuates apoptosis in primary liver cells isolated from Atlantic salmon (*Salmo salar*). British J Nutr. 2013: 110;20–28.

47. Hayes KC, Rabin AR, Berson EL. An ultrastructural study of nutritionally induced and reversed retinal degeneration in cats. Am J Pathol. 1975a: 78;504–524.

48. Hayes KC, Carey RC, Schmidt SY. Retinal degeneration associated with taurine deficiency in the cat. Science 1975b: 188;949–951.

49. Warrant EJ. Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. Vis Res. 1999: 39;1611–1630. PMID: 10343855

50. Warrant E. Vision in the dimmest habitats on earth. J Comp Physiol A. 2004: 190;765–789.

51. Fritsches KA, Marshall NJ, Warrant EJ. Retinal specializations in the blue marlin: Eyes designed for sensitivity to low light levels. Mar Freshwater Res. 2003: 54;333–341.

52. Lisney TJ, Collin SP. Retinal ganglion cell distribution and spatial resolving power in elasmobranchs. Brain Behav Evol. 2008: 72;59–77. https://doi.org/10.1159/000146082 PMID: 18679025

53. Litherland L, Collin SP. Comparative visual function in elasmobranchs: spatial arrangement and ecological correlates of photoreceptor and ganglion cell distributions. Vis Neurosci. 2008: 25;549–561. https://doi.org/10.1017/S0952523808080693 PMID: 18606042

54. Frank TM. Comparative study of temporal resolution in the visual systems of mesopelagic crustaceans. Biol Bull. 1999: 196:137–144. https://doi.org/10.2307/1542559 PMID: 28296477

55. Frank TM. Temporal resolution in mesopelagic crustaceans. Philos Trans R Soc. 2000: 355;1195–1198.

56. Frank TM. Effects of light adaptation on the temporal resolution of deep-sea crustaceans. Integr Comp Biol. 2003: 43;559–570. https://doi.org/10.1093/icb/43.4.559 PMID: 21680464

57. Wald G. The significance of vertebrate metamorphosis. Science 1958: 128;1481–1490. PMID: 13615297

58. Dartnall HJA, Lythgoe JN. The spectral clustering of visual pigments. Vision Res. 1965:5;81–100.

59. McFarland WN, Allen DM. The effect of extrinsic factors on two distinctive rhodopsin-porphyropsin systems. Can J Zool. 1977: 55;1000–1009. PMID: 884629

60. Cheng CL, Flamarique IN. Opsin expression: new mechanism for modulating colour vision. Nature 2004: 428;279.

61. Bowmaker JK. Evolution of vertebrate visual pigments. Vis Res. 2008: 48;2022–2041. https://doi.org/10.1016/j.visres.2008.03.025 PMID: 18590925

62. Schweikert LE, Grace MS. Altered environmental light drives retinal change in the Atlantic Tarpon (*Megalops atlanticus*) over timescales relevant to marine environmental disturbance. BMC Ecol. 2018: 18;1, https://doi.org/10.1186/s12898-018-0157-0 PMID: 29347979

63. Schweikert LE, Grace MS. Spectral sensitivity change may precede habitat shift in the developing retina of the Atlantic tarpon (*Megalops atlanticus*) Physiol Biochem Zool. 2017: 90;553–563. https://doi.org/10.1086/692993 PMID: 28665184

64. Johnson AM, Stanis S, Fuller RC. Diurnal lighting patterns and habitat alter opsin expression and colour preferences in a killifish. Proc R Soc B 2013: 280;20130796. http://dx.doi.org/10.1098/rspb.2013.0796.

65. Shand J, Davies WL, Thomas N, Balmer L, Cowing JA, Pointer M. The influence of ontogeny and light environment on the expression of visual pigment opsins in the retina of the black bream, *Acanthopagrus butcheri*. J Exp Biol. 2008: 211;1495–1503. https://doi.org/10.1242/jeb.012047 PMID: 18424684

66. Taylor SM, Loew ER, Grace MR. Ontogenic retinal changes in three ecologically distinct elopomorph fishes (Elopomorpha: Teleostei) correlate with light environment and behavior. Vis Neurosci. 2015: 32;1–15.

67. GarcPa-Ayuso D, Di Pierdomenico J, Hadj-Said W, Marie Agudo-Barriuso M, Vidal-Sanz M, et al. Taurine depletion causes ipRGC loss and increases light-induced photoreceptor degeneration. Invest Ophth Vis Sci. 2018: 59;1396–1409.

68. Munz FW. Vision: visual pigments. In: Hoar WS, Randall DJ, editors. Fish Physiology vol 5. New York: Academic Press; 1971: p 1–32.

69. Munz FW, McFarland WN. The significance of spectral position in the rhodopsins of tropical marine fishes. Vision Res. 1973: 13;1829–1874. PMID: 4746986

70. Crescitelli F, McFall-Ngai M, Horwitz J. The visual pigment sensitivity hypothesis: further evidence from fishes of varying habitats J Comp Physiol A. 1985: 157;323–333. PMID: 3837092

71. Lythgoe JN Vision in fishes: ecological adaptations. In: Ali MA, editor. Environmental physiology of fishes, NATO Advanced Study Institutes Series (Series A: Life Sciences) vol 35. Boston: Springer; 1980:p 431–445.

72. Lythgoe JN. Light and vision in the aquatic environment. In: Atema J, Fay RR, Popper AN, Tavolga WN, editors. Sensory biology of aquatic animals. New York: Springer; 1988:p 57–82.

73. McFarland W Light in the sea: the optical world of elasmobranchs. J Exp Zool. 1990: 256;3–12.