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Dietary taurine improves vision in different age gilthead sea bream (*Sparus aurata*) larvae potentially contributing to increased prey hunting success and growth

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ABSTRACT

The effect of different levels of dietary taurine on vision, prey hunting success and growth was tested in developing larval gilthead sea bream (GSB; *Sparus aurata*). Fifteen 17 L aquaria were stocked with 3000 fertilized eggs aquarium^{-1} from a local GSB broodstock, which allowed the testing of 5 prey taurine treatments in triplicate aquaria treatment^{-1}. Nine aquaria tested the effect of the non-taurine enriched rotifer (*Brachionus rotundiformis*) control (1.7 mg g^{-1} DW) and two taurine enriched rotifer treatments (6.6 and 11.0 mg g^{-1} DW) in 2 to 13 dph larvae followed by feeding non-taurine enriched *Artemia* nauplii (36.6 mg g^{-1} DW) to all treatments from 14 to 31 dph. Larvae in another 6 aquaria were fed control rotifers (1.7 mg g^{-1} DW) from 2 to 13 dph followed by testing the effect of taurine enriched *Artemia* nauplii (51.5 and 61.9 mg g^{-1} DW) on 16 to 31 dph larvae. In parallel, GSB fertilized eggs from the same broodstock were stocked (100 eggs L^{-1}) in fifteen 400 L conical, polypropylene tanks, which tested the 5 rotifer and *Artemia* taurine levels in triplicate tanks treatment^{-1}. Only rotifer consumption (mastax number) in 7 and 13 dph larvae and dry weight of 31 dph larvae were determined in the aquaria, while total length, essential fatty acid profile, taurine level, opsin abundance and gene expression of selected opsin genes in different age larvae were determined from the much larger biomass of the 400 L tanks.

The growth of 3 and 13 dph larvae were significantly ($P < 0.05$) correlated with rotifer and larval body taurine levels in a dose-dependent manner. Moreover, rotifer ingestion (mastax number) significantly ($P < 0.05$) improved in 13 dph larvae with rotifer taurine level. The growth promoting effect of rotifer taurine continued to significantly ($P < 0.05$) influence larval length in older 21and 26 dph larvae, although this effect diminished ($P > 0.05$) in 31dph larvae. The contribution of taurine to improved vision was supported in 31 dph larvae, which exhibited significantly ($P < 0.05$) increasing retinal opsin protein abundance with rotifer taurine level in a dose dependent manner. In fact, dietary taurine markedly ($P < 0.05$) improved the gene expression of 5 different opsins in 8 and 31 dph larvae. Nevertheless, the correlation between rotifer taurine and larval fatty acid content suggests that the taurine-modulated production of bile salts facilitated lipid digestion and absorption, which also might have promoted growth.

1. Introduction

The β-amino sulfonic acid taurine is one of the most abundant, low molecular weight, organic constituents of animal tissues. This amino acid-like compound contains a negatively charged sulfonic acid group (Nusetti et al., 2005) but lacks a carboxyl group, as in other amino acids, and therefore cannot be incorporated into proteins (Li et al., 2009). Taurine plays an array of critical roles that promote fish growth, through bile salt synthesis, anti-oxidative defense, cellular osmoregulation, modulation of neural transmitters as well as contributing to muscular, neural and visual function (Huxtable, 1992; Fang et al., 2002; Omura and Inagaki, 2000). Taurine is known to promote differentiation of retinal cells in vertebrates during development (Altshuler et al., 1993) as well as accumulating in the outer segments of retinal photoreceptors

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where they play a shielding role against prolonged light exposure (Pasantes-Morales and Cruz, 1984). An extended period of light exposure can cause oxidative stress and significant peroxidation damage to the DHA rich photoreceptor membranes (Organisciak and Vaughan, 2010).

The eyes of fish larvae are conspicuously large (Koven et al., 2012), which broadly hints at the importance of vision during prey identification and hunting success. However, most fish larvae can only detect prey over a visual distance of approximately one to two body lengths (Wahl et al., 1993), underscoring the need for an effectively functioning visual system to ensure good growth and survival (Job and Shand, 2001). The accumulating evidence of the importance of taurine in photoreceptor function in vertebrates (Militante and Lombardini, 2002; Ripps and Shen, 2012; L'Amoreaux, 2014; Tao et al., 2019) suggests that enhanced visual prey detection may have been a contributing factor to improved larval growth in species such as amberjack (*Seriola dumerili*) (Matsunari et al., 2013) and Senegalese sole (*Solea senegalensis*) (Pinto et al., 2010).

In nature, taurine is abundantly found in copepods, the larvae's natural zooplankton prey (van der Meeren et al., 2008). However, rotifers, the initial live food organism ubiquitously fed to early developing larvae in marine aquaculture farms world-wide, is deficient in adequate levels of taurine (Maehre et al., 2013). In contrast, *Artemia* nauplii, that are fed at a later stage of larval development in most teleosts grown in captivity, have considerable endogenous levels of taurine (Rotman et al., 2016; Partridge and Woolley, 2016), which is also the dominant free amino acid of this zooplankter (Aragão et al., 2004).

The fact that taurine particularly concentrates in retinal tissue of insects and vertebrates has been well documented (Bicker, 1991; Marc and Cameron, 2001; Fletcher and Kalloniatis 1996; Lake et al., 1978) and a number of studies have verified this is true with fish. Omura et al. (1997) reported intense and moderate immunostaining for taurine in the retinal cone-like cells and retinal outer membranes of rod-like cells of the ayu (*Plecoglossus altivelis*) while Omura and Yoshimura (1999) found that the retinal photoreceptor layer showed the highest taurine concentration, particularly in the outer and inner segments as well as in the synaptic terminals in the left-eye flounder (*Paralichthys olivaceus*). Interestingly, during light stimulation taurine generally moves out of the outer membranes of vertebrate photoreceptors (Pasantes-Morales et al., 1973; Pasantes-Morales and Cruz, 1985; Schmidt and Berson, 1978) in order to shield them from prolonged light-induced peroxidation (Omura and Inagaki, 2000; Pasantes-Morales and Cruz, 1985 Studies have reported on the effect of taurine on photoreceptor development and repair in vertebrates (Tao et al., 2019; Omura and Yoshimura, 1999; Altshuler et al., 1993) as well as photoreceptor degeneration in the absence of this nutrient (Hadj-Saïd et al., 2016; Pasantes-Morales and Cruz, 1984; Hayes et al., 1975). Nevertheless, the current literature is lacking in studies on the effect of taurine on vision, particularly the photopigments of cones and rods, and its impact on prey acquisition and growth during fish larval development. Photopigments in photoreceptors are comprised of retinal and one of a variety of opsin proteins, which differ in the wavelengths they absorb, and are responsible for converting photons of light into neural signals to the brain which would affect vision quality and ultimately prey hunting success and growth.

The aims of the current study were (1) to test the effect of different levels of rotifer and *Artemia* nauplii taurine content on prey hunting success and growth on different age larval gilthead sea bream (GSB; *Sparus aurata*) and (2) to investigate the effect of taurine on larval vision, in terms of retinal opsin abundance and the pattern of the gene expression of various opsins during larval development.

2. Methods and materials

2.1. Larval rearing

The effect of prey taurine content during early rotifer (*Brachionus rotundiformis*) and later *Artemia* nauplii feeding was examined in order to evaluate the efficacy of dietary taurine as a function of GSB larval development, in terms of prey consumption, growth and eye development.

The aim of this study was investigated using two experimental systems: 17 L aquaria and 400 L conical V-tanks. This was because sampling large numbers of larvae can be a confounding factor causing stress and affecting growth results. In order to minimize this effect, the 15 aquaria system was only sampled for mastax number, which requires a relatively small sample size (*ca* 10 larvae tank^{-1}) as well as dry weight at the end of the experiment at 31 dph. On the other hand, all sampling for larval total length, fatty acid profiles, taurine level, immunohistochemistry as well as RNA extraction for gene expression requires larger sample sizes and was carried out in the 400 L conical V-tanks.

The fifteen 17 L aquaria were each stocked with 3000 fertilized, gastrula stage eggs aquarium^{-1} from GSB broodstock maintained at a local fish farm (Ardag Ltd., Eilat, Israel). This allowed the testing of 5 prey taurine treatments in triplicate aquaria treatment^{-1}. Nine aquaria tested the effect of non-taurine enriched rotifers (*B. rotundiformis*) as the control, which contained 1.7 mg taurine g^{-1} dry weight (DW), against two taurine enriched rotifer treatments (6.6 and 11.0 mg taurine g^{-1} DW) during early larval development (2 to 13 dph) followed by feeding all treatments non-taurine enriched *Artemia* nauplii, which contain substantial endogenous taurine (36.6 mg taurine g^{-1} DW), from 14 to 31 dph. In the second group of 6 aquaria, larvae were fed the taurine unenriched rotifer control from 2 to 13 dph followed by testing the effect of two taurine enriched *Artemia* nauplii treatments (51.5 and 61.9 mg taurine g^{-1} DW) on later developing larvae from 14 to 31 dph. The control for both groups of aquaria were larvae fed the taurine unenriched rotifers and *Artemia* nauplii. In parallel, GSB fertilized eggs at the gastrula stage were stocked (100 eggs L^{-1}) in fifteen 400 L conical, polypropylene tanks, which tested the 5 rotifer and *Artemia* taurine treatments in triplicate tanks treatment^{-1}. Fatty acid, protein, vitamin and mineral content were identical in all rotifer and *Artemia* enrichment treatments as only the commercial preparation "Red Pepper" (Bernaqua, Belgium) was used according to manufacturer's instructions. In order to minimize disturbing the larvae, only rotifer consumption (mastax number), length from 2 to 13 dph larvae and dry weight in 31 dph larvae at the end of the study were determined in the two groups of aquaria. On the other hand, larvae were sampled for total length, fatty acid profile, taurine level, opsin abundance and gene expression of selected opsin genes in different age larvae were determined from the much larger biomass of the 400 L tanks. Table 1 summarizes the experimental set up in the 17 L aquaria and 400 L tanks as well as the analyses carried out.

The aquaria and tanks were supplied with filtered (10 μm), UV treated (Atlantium, Israel) ambient seawater (40‰) initially at 19 ± 0.5 °C during hatching and then was raised incrementally to 22 °C through a computer control system (Gavish, Israel) while seawater salinity was gradually decreased from 40 to 25‰ (Gavish, Israel) according to Tandler et al. (1995). Seawater entered at the bottom of the tanks and aquaria and exited near the top through 150 μm filters at a flow rate of 2 tank exchanges day^{-1} during hatching, which was increased to 4 tank exchanges day^{-1} for the remainder of the experiment, while the daily exchange rate for the aquariums ranged from 5 to 10 exchanges day^{-1} by 31 dph. Protein skimmers were placed on the water surface to collect organic material remaining after hatching. Exit filters were changed to 250 μm during the *Artemia* naupli feeding. At 2 dph, the photoperiod was adjusted to 12 h (light): 12 h (dark) when the larvae have begun to feed exogenously, following eye pigmentation and the opening of the mouth and anus. Microalgae (*Nannochloropsis oculata.*) were added to the tanks and aquaria twice a day to reach a concentration of 5 × 10^5 cells mL^{-1}. From 2 to 13 dph the larvae were fed twice a day (morning and afternoon) with the rotifer treatments (10 rotifers mL^{-1}). When the larvae reached the age of 14 dph they were offered unenriched *Artemia* nauplii for 2 days and from 16 dph the larvae were fed the Artemia nauplii treatments twice a day at a concentration ranging from 0.2 nauplii mL^{-1} to 4 nauplii mL^{-1} according to larval demand. Water

Table 1
Description of taurine treatments in the fifteen 400 L V-tanks and 17 L aquaria, zooplankton feeding, environmental parameters as well as larval analyses from each experimental system.

	Experimental systems	
	Fifteen 400 L V-tanks	Fifteen 17 L aquaria
	• 5 treatments (3 replicates treatment^{-1})	• 5 treatments (3 replicates treatment^{-1})
Stocking	100 eggs L^{-1}	175 eggs L^{-1}
Treatments for tanks and aquaria	Rotifer taurine treatments + Artemia control	
	1. 1.7 mg taurine g^{-1} DW rotifers +36.6 mg taurine g^{-1} DW *Artemia**	
	2. 6.6 mg taurine g^{-1} DW rotifers +36.6 mg taurine g^{-1} DW *Artemia*	
	3. 11.0 mg taurine g^{-1} DW rotifers +36.6 mg taurine g^{-1} DW *Artemia*	
	Rotifer control + Artemia taurine treatments	
	4. 1.7 mg taurine g^{-1} DW rotifers +51.5 mg taurine g^{-1} DW *Artemia*	
	5. 1.7 mg taurine g^{-1} DWrotifers +61.9. mg taurine g^{-1} DW *Artemia*	
	*Rotifer and *Artemia* Control	
Zooplankton Feeding	1. Rotifers (10 rotifers mL^{-1}) from 2 to 13 dph 2. Non-enriched *Artemia* nauplii (0.2 mL^{-1}) from 14 to 15 dph 3. Enriched *Artemia* nauplii (0.2 to 4 nauplii mL^{-1} according to demand) from 16 to 30 dph.	
Water exchange	2–4 tank exchanges day^{-1}	5–10 aquarium exchanges day^{-1}
Temperature increase	19 °C - 22 °C	19 °C - 22 °C
Salinity decrease	40–25‰	40–25‰
Analyses of larvae	• Larval total length • Fatty acid (6 and 13 dph) • Taurine level • Opsin abundance • Gene expression of opsin genes	• Rotifer consumption (mastax number) • Dry weight 31 dph

salinity (±1 ppt) (Atago, master S100, Japan), temperature (±0.5 °C) and oxygen saturation (±1%) (Oxyguard, Handy Polaris 2, Denmark) were performed daily in the morning in the larval and live feed rearing tanks. Oxygen was maintained above 95% saturation through air stones placed in the tanks.

2.2. Rotifer feeding

Rotifers were stocked (1000 rotifers mL^{-1}) for taurine enrichment in 65 L plastic bins and supplied with (*N. oculata*; 4.5 × 10^6 cells mL^{-1}) followed by adding one of three levels of taurine (0, 400 and 600 mg taurine L^{-1} enrichment media) for 21 h. After 13 h of taurine enrichment, the rotifers were additionally fed (according to manufacturer's instructions) a commercial fatty acid and nutrient preparation (Red Pepper, Bernaqua, Belgium) for a period of 8 h. The enriched rotifers of each of the different treatments were washed, counted and fed to the larvae (10 rotifers mL^{-1}) twice daily.

2.3. Artemia feeding

Iron coated *Artemia* cysts (INVE, Ghent, Belgium) were hatched according to Sorgeloos et al. (1977) in 200 L V-tanks filled with filtered (10 μm) and UV treated (Atlantium, Israel), diluted (30‰) seawater at 28–30 °C for 24 h. After the incubation, approximately 50–60 × 10^6 *Artemia* nauplii were harvested using the "Sep art" (INVE, Ghent, Belgium) magnetic pipe that separates the iron coated cyst shells from the hatched *Artemia* nauplii. The 24 h old nauplii were counted under a stereoscopic microscope (Optika, Italy) and then stocked (0.2 × 10^6 nauplii L^{-1}) and enriched with the "Red Pepper" preparation for 16 h in three 15 L conical tanks and one 65 L plastic bin filled with 28 °C, filtered (10 μm) and UV treated (Atlantium, Israel) seawater (40‰). The bin and a 15 L conical tank were necessary to supply the non-taurine enriched Artemia control treatment to the larvae in the 9 aquaria and 400 L tanks. The taurine enriched nauplii (400 and 600 mg L^{-1}) in the two 15 L conical tanks were sufficient to feed the larvae in the six aquaria and 400 L tanks. The treatment nauplii were prepared daily, washed, counted and fed to the larvae (0.2 to 4 nauplii mL^{-1}) twice daily according to fish demand.

2.4. Rotifer and Artemia sampling for fatty acid and taurine analysis

Rotifers and *Artemia* from the taurine enrichment treatments were sampled on 5 separate days during the course of the experiment for analysis of their fatty acid profile and taurine content. The rotifers or *Artemia* nauplii were concentrated on a circular mesh (70 μm and 150 μm, respectively) then washed with fresh water followed by double distilled water (DDW). The washed rotifer and *Artemia* samples were collected into Eppendorf (~1 mL) tubes and stored at −20 °C under nitrogen until analysis.

2.5. Larval sampling from aquaria

All GSB larvae sampled were first immobilized in ice water and then quickly euthanized in concentrated tricaine methanesulfonate (MS222; Sigma Aldrich, Israel). From each aquarium, 10 larvae were sampled at 7 and 13 dph, 90 min after feeding to determine gut mastax number. These larvae were placed in Eppendorf tubes filled with 10% buffered formalin and stored at 4 °C until mastax analysis. Following storage, the larvae were rinsed well with fresh water and DDW and then transferred to a glass slide where under a microscope (Optika, Italy) the number of mastaxes (indigestible part of the rotifer's mouth) were counted after gently compressing the larval gut under a glass coverslip. At the end of the study at 31 dph, about 30 larvae from each aquaria were first rinsed with fresh water and then DDW on a circular mesh filter and then transferred to 3 Eppendorf tubes (10 larvae tube^{-1}), which were stored at −20 °C under nitrogen until further analysis.

2.6. Larval sampling from 400 L tanks

Total lengths from 10 larvae sampled from each 400 L tank were measured under a microscope (Optika, Italy) during rotifer (3 and 13 dph) and *Artemia* (21, 26 and 31 dph) feeding. At 6, 13 and 27 dph, 30 larvae from each 400 L tank were collected, and transferred to 3 Eppendorf tubes (10 larvae tube^{-1}) and freeze dried (BYK FD80, China) for taurine analyses. In addition, 30 larvae at 8 and 31 dph from each 400 L tank were collected into 3 Eppendorf tubes (10 larvae tube^{-1}) for RNA extraction (gene expression of different opsin proteins) and stored at −80 °C. A further 10 larvae at 31 dph were sampled from each 400 L tank into Eppendorf tubes filled with 3% glutaraldehyde in 0.15 M cacodylate buffer and stored at 4 °C for immunohistochemistry assay (ICC).

2.7. Fatty acid analysis

Fatty acid analysis was carried out by chloroform- methanol (2,1) extraction of total lipid from freeze dried tissue samples (Folch et al., 1957). The lipid-containing chloroform upper phase was removed and evaporated to dryness under a stream of nitrogen and total lipid weighed (GH-120 analytical balance, A&D, San Jose, CA, USA). This was followed by the addition of the internal standard 17:0 (heptadecanoic acid; Sigma, St. Louis, MO, USA) to each of the samples. The samples were then transmethylated to their fatty acid methyl esters (FAME) by adding 1 mL per 1 mg lipid of a 14% solution of boron trifluoride methanol (BF3) and sonicated for 1 h at 50 °C. To the samples were added 0.5 mL DDW and 1 mL per mg lipid of analytical grade hexane (99%), which was vortexed and then centrifuged (2300 rpm) for 2 min. The FAME was

analyzed in a Varian 450–220 GC/MS/MS (Agilent technologies, California, USA). Injected FAME samples (1 μL) were separated on a Varian WCOT fused silica column (50 M × 0.32 mm) at a flow rate of 1.5 mL min^{-1} and identified by known purified standards and quantified using a response factor to the internal standard. The 30 min oven temperature program began at 70 °C for 4 min following injection and then increased to 300 °C at 10 °C min^{-1} for 3 min.

2.8. Taurine analyses

A standard taurine linear curve was first generated ($r^2 = 0.999$) from a taurine concentration series (0, 20, 40, 60, 80, 100 μg taurine mL^{-1}DDW) after similarly treating the standards and sampled rotifers, *Artemia* and larvae, which is briefly described here. Freeze dried diet samples of 2–5 mg for Varian 325–410 HPLC (Agilent Technologies, California, USA) taurine analysis were prepared by adding 3 mL of 6 M HCL and 0.5% phenol and homogenizing them under a fume hood. The samples were flushed with nitrogen and placed in a heating block for 24 h at 108–110 °C. After cooling samples to room temperature and filtering (0.45 μm; cellulose nitrate), 0.5 mL carbonate buffer (pH 9), 0.5 mL DMSO (dimethyl sulfoxide) and 0.1 mL DNFB (1-fluoro-2,4 dinitrobenzene) were added and the samples mixed well followed by heating for 15 min at 40 °C then cooled for 10 min. To the samples were added 6.5 mL of 0.01 M of buffered phosphate, vortexed for 30 s and then left to stand for 5 min. The samples were then transferred to HPLC vials and injected (10 μL) into an Acclaim ™120 C18 (5 μm, 4.6 × 150 mm) HPLC column (Thermo Scientific, USA). Column flow rate was 1.5 mL min^{-1} where specific ratios of buffer phosphate 0.01 M (pH 6) and acetonitrile (90,10, 10:90, 10:90, 90:10, 90:10) were introduced into the column at different times (0, 10, 11, 11.01, 18 min), respectively.

2.9. Immunohistochemistry (ICC) for opsin proteins in the retina

The larvae fed the different taurine enrichment rotifer treatments were embedded in resin and sections of their retina were prepared for immunohistochemistry (ICC). In brief, larval samples were fixed in 3% glutaraldehyde in 0.15 M cacodylate buffer, washed in buffer (3 × 10 min), dehydrated through an ethanol series and processed for embedding in Lowicryl resin. Semi-thin sections (10 μm thick) and ultrathin sections (collected on nickel grids) were obtained using a Leica Ultracut R microtome (Wetzlar, Germany). For the ICC procedure, the sections were incubated 20 min in a phosphate buffer saline (PBS) containing 0.05 M glycine (PBSG), then incubated in blocking solution (AURION, Biovalley, France) for 30 min. After being washed 2 × 5 min in PBS containing bovine serum albumin (BSA), they were incubated in PBS-BSA containing a 1/250 dilution of the primary antibody (anti-opsin IgG1 O 4886, Sigma-Aldrich, France). After an over night incubation at 4 °C, sections were washed (6 × 5 min) in buffer and incubated (2 hRT) in a buffer solution containing a 1/20 solution of the second antibody (goat anti-Mouse labelled with 20 nm gold particles) for 2 h. After this, the grids were washed successively in buffer PBSG-BSA (6 × 5 min) and then in PBS (2 × 5 min). The grids were then fixed in 2% glutaraldehyde in PBS buffer for 10 min, washed (2 × 5 min) in DDW and then blotted dry. Before observation they were stained in 2% uranyl acetate. The immuno-labelled grids were then observed under a Hitachi 7500 electron microscope (Fukuoka, Japan) and processed using ImageJ software (Rasband, W.S., ImageJ, US) for opsin quantiication.

2.10. Primer design

Six opsin genes were identified, five of which were chosen for quantitative gene expression in order to examine the taurine effect on eye ontogeny. The identified opsin sequences in other species were used to build a phylogenetic tree based on the maximum parsimony algorithm. In order to select the closest group of fish to *S. aurata*, the rhodopsin gene that was already sequenced in *S. aurata* (Levitan, 2013) was used, as well as the sequenced opsin genes in members of the Sparidae family; *Acanthopagrus berda*, *Pagrus major* and *Acanthopagrus schlegelii* (Wang et al., 2009). Following this, the "clustalw" program for multiple sequence alignment was used to find conserved sequences to design primers that will be used to amplify each opsin gene in *Sparus aurata.* Primers were designed and sent to Hy-Laboratories Ltd. (Rehovot, Israel) for primer synthesis. The opsin genes were then amplified using PCR with these primers. The amplified genes were sent to AGENTEC (Tel Aviv, Israel) for sequencing and real time PCR primers design with the Primer Express® Software v. 3.0 (Applied Biosystems).

2.11. RNA extraction

Total RNA was extracted from larvae samples with the Bio-Tri RNA reagent (Bio lab, Israel) according to manufacturer protocol based on the method of Chomczynski and Sacchi (1987). RNA concentration was determined by measuring absorbance at 260 nm in a spectrophotometer plate reader BioTek (Synergy HT, USA) and by using the program Gen5 2.0. RNA cleanliness from contaminations was measured based on the absorbance ratio between 260/230 nm (salts) and 260/280 nm (proteins). RNA samples were cleaned from residual DNA by using perfeCta® DNase 1 kit (Quanta Biosciences USA). Samples were treated with DNase 1 enzyme according to the manufacturer's protocol. Following incubation for 30 min at 37 °C the enzyme was then inactivated by adding 1 μl of "stop" buffer and incubated for 10 min at 65 °C. RNA samples were transformed into cDNA by using qScript cDNA synthesis kit (Quanta biosciences USA) according to the manufacturer's protocol. Reaction was performed in a PCR device (Tpersonal-48 Thermocycler, Biometra, Germany). Reaction conditions were 22 °C for 5 min, 42 °C for 30 min and then 85 °C for 5 min.

PCR reaction was performed with Verso 1 step RT-PCR Reddy Mix kit (Thermo scientific, Israel). The primers, that were designed for each of the opsin genes and 18S as a reference gene, were used for amplification according to the kit's protocol (AB-1454/LD/A, Thermo Scientific, Israel), in a 50 μl volume. The reaction was performed in a PCR thermocycler (Tpersonal-48 Thermocycler, Biometra, Germany). Reaction conditions were as follows: 50 °C for 15 min (cDNA synthesis), 95 °C for 2 min (enzyme deactivation), 35 cycles of 95 °C for 20 s (denaturation), 55 °C for 30 s (annealing), 72 °C for 1 min (extension), 72 °C for 5 min (final extension). The opsin DNA products were later quality tested on agarose gel and sent for sequencing at the AGENTEC laboratory (Tel Aviv, Israel).

In order to measure the influence of taurine on opsin gene expression RT-qPCR was performed. Six similar mixes consisting of ultra-pure water, PerfeCTa® SYBR® Green FastMIX™,LowROX™ (Quanta Biosciences, Gaithersburg, MD, USA) and forward and backward RT-qPCR primers, differing only in the gene identity (5 opsins+ reference gene), were distributed (8 μl) into a RT-qPCR 96 wells plates (MicroAmp® applied Biosystems, Waltham, Massachusetts, USA). Each treatment sample cDNA (2 μl) was added into each well (3 technical replicates) giving a total 10 μl reaction volume. The plate was centrifuged for 2 min at 1000 rpm, placed in a 7500 Fast Real time PCR System (Applied Biosystems, Waltham, Massachusetts, USA) and analyzed with the system software. Reaction conditions were 95 °C for 30 s, 40 cycles of 95 °C for 5 s, 65 °C for 15 s, 72 °C for 10 s. To normalize the levels of target genes β-actin was used as reference gene. Negative control without cDNA template was included in every plate to test for possible contamination. Treatment effect on each opsin gene expression was quantified by calculating the relative expression based on the method of ΔΔCt (Livak and Schmittgen, 2001).

3. Statistics

Statistical analysis was performed with the IBM SPSS Statistics 20 (SPSS Science, Chicago, USA) package. All data were tested for normality (Shapiro-Wilk, and Kolmogorov-Smirnov) and for

homogeneity of variance (Levene's test). One-way ANOVA was performed to analyze data.

3.1. Ethics statement

All animal experimental procedures were conducted in compliance with the Guidelines of the European Union Council (86/609/EU) for the use of laboratory animals.

4. Results

In the present study rotifer taurine levels (1.7, 6.6, 11.0 mg g^{-1} DW) corresponded to taurine enrichment content (0, 400 and 600 mg L^{-1} of the enrichment media, respectively) in a dose dependent manner ($P < 0.05$; Table 2). On the other hand, *Artemia* nauplii, which contain considerable endogenous taurine (36.6 mg g^{-1} DW), significantly ($P < 0.05$) increased their body content to 61.9 mg g^{-1} DW when enriched in the 400 mg L^{-1} enrichment medium, but markedly ($P < 0.05$) decreased to 51.5 mg g^{-1} DW in the highest taurine enrichment medium (600 mg L^{-1} of enrichment media). Interestingly, the enrichment of rotifers with 400 mg taurine L^{-1} enrichment media was associated with a significant ($P < 0.05$) increase in docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ArA; 20:4n-6) as well as total monounsaturated fatty acids (MONO). In contrast, the highest taurine enrichment (600 mg L^{-1}) in most cases markedly ($P < 0.05$) decreased the levels of these essential fatty acids to that of the control. *Artemia* enriched with 400 mg taurine L^{-1} enrichment media increased only their DHA content.

Table 3 clearly demonstrated that in both 6 and 13 dph larvae, whole body essential fatty acids (DHA, EPA and ArA), total polyunsaturated fatty acids (PUFA) and total monounsaturated fatty acids (MONO) significantly ($P < 0.05$) increased with increasing rotifer taurine in a dose dependent manner. Larval total saturated fatty acids (SAT) significantly ($P < 0.05$) increased from rotifer 1.7 to 6.6 mg taurine g^{-1} DW, in 13 dph larvae and then significanly ($P < 0.05$) decreased when larvae were fed rotifers containing 11.0 mg taurine g^{-1} DW (Table 3).

Table 4 demonstrated a significant ($P < 0.05$) rotifer taurine dose dependent response in whole body taurine levels of 6 and 13 dph larvae. Having said that, 27 dph larvae that were fed early on the 6.6 and 11.0 mg taurine g^{-1} DW rotifer treatments and then non-taurine enriched control nauplii continued to reflect significantly ($P < 0.05$) higher tissue taurine levels (66.09 and 50.79 mg g^{-1} DW, respectively) compared to the control rotifer and *Artemia* nauplii treatment (35.69). Moreover, 27 dph larval taurine tissue levels were much higher than those of 6 and 13 dph fish due to the considerable content of endogenous taurine in the non-taurine enriched nauplii (Table 4). Nevertheless, the taurine body content of fish fed the taurine enriched Artemia did not exceed body taurine accumulation in fish fed the moderate taurine (6.6 mg g^{-1} DW) rotifers and non-taurine enriched *Artemia* (Table 4).

In Figs. 1, 3 and 13 dph larvae feeding on the high taurine enriched rotifer treatment (11.0 mg g^{-1} DW), grew (total length) significantly ($P < 0.05$) better (3.9 and 4.9 mm, respectively) than the low taurine control larvae (3.7 and 4.0 mm, respectively) in a taurine dose dependent manner. Furthermore, taurine enriched rotifers (11.0 mg g^{-1} DW) fed to 13 dph larvae significantly improved their prey hunting success (Fig. 2), in terms of the number of rotifers consumed, in a taurine dose dependent manner ($P < 0.05$). The growth promoting effect of rotifer taurine continued to be apparent in 21 and particularly 26 dph larvae even though they were offered non-taurine enriched *Artemia* nauplii for 8 and 13 days, respectively (Fig. 3). Having said that, 31 dph larval body lengths and DW were unaffected ($P > 0.05$) by fish consuming high taurine levels in nauplii, later in development (14–31 dph) (Figs. 4a, b).

Nevertheless, a rotifer taurine effect on apparent retinal function in 31 dph larvae was significantly ($P < 0.05$) demonstrated in Fig. 5 where rotifer taurine level (1.7, 6.6 and 11.0 mg g^{-1} DW) on opsin abundance in the photoreceptors (50.3, 102.0 and 174.5 opsin protein section $area^{-1}$) was exhibited in a dose dependent manner (Fig. 5). For technical reasons, the image of a retinal section of larvae feeding on the moderate taurine rotifer treatment (6.6 mg g^{-1} DW) was not developed. However in Fig. 5a, the image of a retinal section of larvae fed the control rotifer-Artemia taurine treatment demonstrated much lower opsin abundance than observed in the retinal section of larvae fed the high taurine rotifers (11.0 mg g^{-1} DW) and non-taurine enriched *Artemia* nauplii in Fig. 5b.

Table 3
The effect of rotifer taurine levels (mg g^{-1} DW) on 6 and 13 dph gilthead sea bream larval essential fatty acids; DHA, EPA and ArA (mg g^{-1} DW) as well as total saturated (SAT), monounsaturated (MONO) polyunsaturated fatty acids (PUFA) and total fatty acids (mg g^{-1} DW). Essential fatty acid and fatty acid group values within a larval age having different letters were significantly ($P < 0.05$) different ($n = 3$, 1-way ANOVA).

Rotifer taurine mg g^{-1} DW	DHA mg g^{-1} DW	EPA mg g^{-1} DW	ArA mg g^{-1} DW	SAT mg g^{-1} DW	MONO mg g^{-1} DW	POLY mg g^{-1} DW	TFA mg g^{-1} DW
6 dph							
1.7	$9.1^{a} \pm 0.5$	$1.4^{a} \pm 0.3$	$1.3^{a} \pm 0.1$	$22.2^{a} \pm 3.7$	$3.7^{a} \pm .7$	$14.9^{a} \pm 1.1$	$35.9^{a} \pm 3.0$
6.6	$16.9^{b} \pm 0.2$	$2.6^{b} \pm 0.1$	$1.6^{a} \pm 0.2$	$52.0^{a} \pm 25.4$	$6.3^{ab} \pm 2.6$	$29.4^{b} \pm 2.4$	$51.3^{ab} \pm 4.2$
11.0	$20.8^{c} \pm 0.2$	$4.2^{c} \pm 0.5$	$3.2^{b} \pm 0.4$	$33.9^{a} \pm 15.7$	$10.6^{b} \pm 0.3$	$42.8^{b} \pm 9.3$	$97.6^{c} \pm 11.9$
13 dph							
1.7	$11.8^{a} \pm 0.2$	$3.5^{a} \pm 0.8$	$3.1^{a} \pm 0.3$	$21.8^{a} \pm 3.8$	$6.9^{a} \pm 0.6$	$26.9^{a} \pm 1.6$	$51.0^{a} \pm 2.6$
6.6	$15.9^{b} \pm 0.2$	$6.4^{b} \pm 0.2$	$4.9^{b} \pm 0.6$	$57.7^{b} \pm 2.2$	$11.5^{ab} \pm 2.9$	$40.3^{b} \pm 2.3$	$95.4^{b} \pm 14.3$
11.0	$21.5^{c} \pm 0.4$	$11.0^{c} \pm 0.3$	$6.9^{c} \pm 0.5$	$38.8^{c} \pm 2.9$	$14.4^{b} \pm 0.7$	$62.5^{c} \pm 2.2$	$115.1^{b} \pm 5.9$

Table 2
The effect of different taurine enrichment treatments (0, 400 and 600 mg taurine L^{-1} of enrichment medium) having constant DHA levels (ca. 5.5% of total fatty acids) on the taurine and the essential fatty acids and fatty acid groups (mg g^{-1} DW) in rotifers (R) and *Artemia* (A). Taurine and fatty acid values, which are shown as means ± SEM, having different letters were significantly ($P < 0.05$) different ($n = 5$, one-way ANOVA).

Enrichment treatment	Taurine mg g^{-1} DW	DHA mg g^{-1} DW	EPA mg g^{-1} DW	ArA mg g^{-1} DW	SAT mg g^{-1} DW	MONO mg g^{-1} DW	POLY mg g^{-1} DW	TFA mg g^{-1} DW
Rotifers								
R-0	$1.7^{a} \pm 0.4$	$1.2^{a} \pm 0.0$	$0.5^{a} \pm 0.1$	$0.4^{a} \pm 0.06$	$8.6^{a} \pm 1.9$	$8.6^{a} \pm 2.5$	$11.4^{a} \pm 3.2$	$28.6^{a} \pm 1.2$
R-400	$6.6^{b} \pm 1.8$	$5.6^{b} \pm 0.8$	$1.8^{b} \pm 0.15$	$1.8^{b} \pm 0.15$	$12.9^{a} \pm 0.8$	$20.4^{b} \pm 1.2$	$16.8^{a} \pm 2.2$	$50.2^{c} \pm 3.2$
R-600	$11.0^{c} \pm 0.2$	$4.4^{ab} \pm 0.7$	$1.1^{a} \pm 0.25$	$1.1^{a} \pm 0.25$	$7.3^{a} \pm 1.0$	$7.2^{a} \pm 2.0$	$8.8^{a} \pm 1.3$	$23.2^{ab} \pm 4.1$
Artemia								
A-0	$36.6^{a} \pm 1.0$	$1.6^{a} \pm 0.4$	$1.7^{a} \pm 0.3$	$1.3^{a} \pm 0.6$	$28.6^{a} \pm 2.1$	$17.1^{a} \pm 1.4$	$19.2^{a} \pm 3.0$	$64.9^{a} \pm 4.1$
A-400	$61.9^{c} \pm 1.8$	$4.2^{b} \pm 0.7$	$1.3^{a} \pm .3$	$1.4^{a} \pm 0.2$	$30.9^{a} \pm 5.9$	$18.3^{a} \pm 1.4$	$17.9^{a} \pm 1.9$	$66.6^{a} \pm 7.5$
A-600	$51.5^{b} \pm 3.1$	$3.9^{b} \pm 0.3$	$2.9^{a} \pm .9$	$2.0^{a} \pm 0.7$	$36.3^{a} \pm 10.0$	$22.7^{a} \pm 4.1$	$23.0^{a} \pm 5.4$	$65.3^{a} \pm 10.7$

Table 4
The effect of different rotifer and/or Artemia taurine levels (mg g-1 DW) on taurine levels (mg g-1 DW) in 6, 13 and 27 dph gilthead sea bream (*S. aurata*) larvae. Taurine values (means ± SEM) having different letters within a larval age were significantly ($P < 0.05$) different (n = 3, one-way ANOVA).

Taurine treatments (mg g^{-1} DW)	Whole body taurine content of different age larvae (mg g^{-1} DW)		
	6 dph[2]	13 dph[2]	27 dph[3]
Effect of rotifer taurine			
Control rotifer (1.7)[1] Control *Artemia* (36.6)	14.25^{a} ± 1.29	16.42^{a} ± 1.55	35.69^{a} ± 3.06
Rotifer (6.6) Control *Artemia* (36.6)	26.45^{b} ± 0.72	25.28^{b} ± 1.79	66.09^{cd} ± 2.49
Rotifer (11.0) Control Artemia (36.6)	39.71^{c} ± 0.67	43.36^{c} ± 2.20	50.79^{b} ± 2.10
Effect of *Artemia* taurine			
Control rotifer (1.7) *Artemia* (51.5)			62.04^{cd} ± 2.58
Control rotifer (1.7) *Artemia* (61.9)			57.35^{bc} ± 1.21

[1] Control for both the rotifer and *Artemia* taurine treatments.
[2] Larvae sampled before start of *Artemia* feeding at 14 dph.
[3] Larvae sampled after 14 days of *Artemia* feeding.

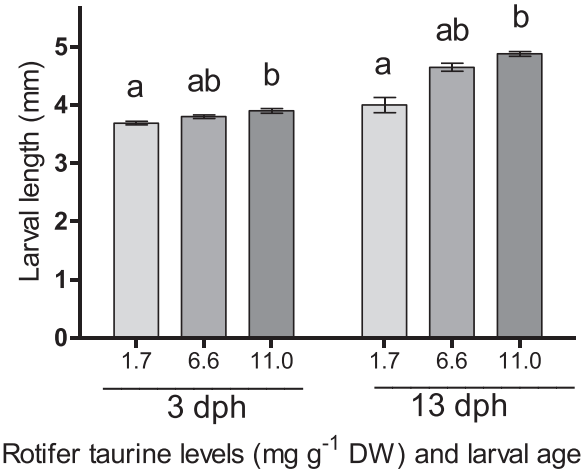

Fig. 1. The effect of different rotifer taurine levels (mg g^{-1} DW) on total length in early developing gilthead sea bream (*S. aurata*) larvae (3 and 13 dph) where the fish are consuming only rotifers. Length values at a specific age having different letters were significantly ($P < 0.05$) different ($n = 30$, one-way ANOVA).

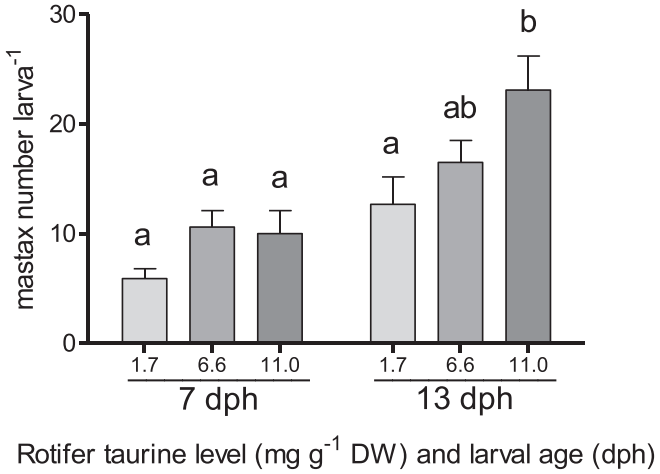

Fig. 2. The effect of rotifer taurine enrichment levels on average mastax number in gut per gilthead seabream (*S. aurata*) larva during rotifer feeding (7 and 13 dph). Values within an age having different letters were significantly ($P < 0.05$) different (n = 30, one-way ANOVA).

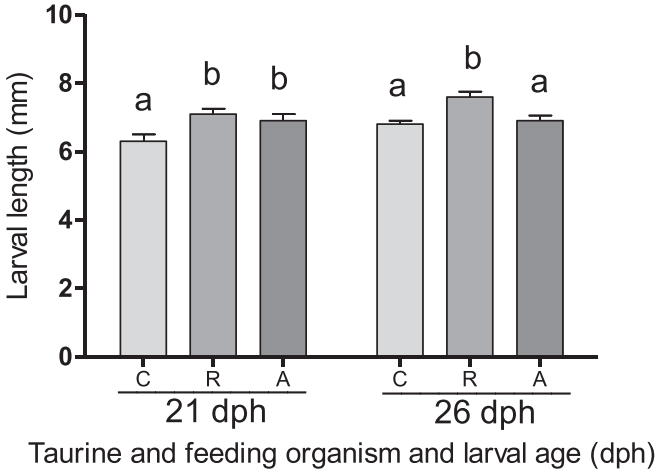

Fig. 3. Comparing the effect of feeding taurine unenriched control rotifers and *Artemia* (C) with the combined treatment effect of taurine enriched rotifers (R) or *Artemia* (A) on total length in later gilthead sea bream (*S. aurata*) larval development (21 and 26 dph). Values within an age having different letters were significantly (P < 0.05) different (n = 30, one-way ANOVA).

In Fig. 6, the partial sequences of the 5 retinal opsin genes; long wavelength sensitive (LWS), short wavelength sensitive1 (SWS1), the green cone opsins rhodopsin-like 2a (RH2a) and rhodopsin-like 2b (RH2b) as well as the rod opsin rhodopsin 1 (RH1) were used to evaluate the effect of prey taurine level on their gene expressions. A significant ($P < 0.05$) effect of rotifer taurine on the gene expression of retinal opsin genes in 8 dph larvae is shown in Fig. 7. Enriching rotifers with taurine (6.6 and 11.0 mg taurine g^{-1} DW) resulted in a marked ($P < 0.05$) increase in RQ expression values of all opsin genes analyzed compared to that of larvae fed the control rotifer treatment (1.7 mg taurine g^{-1} DW). The consumption of rotifer taurine levels of 6.6 mg g^{-1} DW showed the highest expression ($P < 0.05$) in the RH1 and SWS1 genes whereas the 11.0 mg taurine g^{-1} DW rotifer treatment elicited the highest RQ values in RH2a. In the LWS and RH2b genes, the RQ values of both rotifer taurine treatments were alike and significantly ($P < 0.05$) higher than the control (1.7 mg taurine g^{-1} DW).

In Fig. 8, the effect of the 5 taurine treatments on the expression of the five retinal opsin genes in 31 dph larvae is shown. There is a non-significant ($P > 0.05$) rotifer taurine dose dependent effect on opsin expression in LWS, RH2a, RH2b, while this effect was significant ($P < 0.05$) in RH1 and SWS1. Nevertheless, larvae fed taurine enriched Artemia nauplii (51.5 and 61.9 mg g^{-1} DW) demonstrated increased opsin gene expression over the rotifer taurine treatments that was not significant ($P > 0.05$) in LWS and RH2a but was significant ($P < 0.05$) in RH1 and RH2b opsin genes in larvae fed the 51.5 mg taurine g^{-1} DW nauplii treatment (Fig. 8).

5. Discussion

Taurine significantly ($P < 0.05$) increased in rotifers in an enrichment dose dependent manner. Interestingly, the 400 mg taurine L^{-1} enrichment treatment also markedly ($P < 0.05$) enhanced levels of rotifer long chain polyunsaturated fatty acids; docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (ArA; 20:4n-6) while this was true to a lesser extent in *Artemia*. The increased presence of these essential fatty acids (EFA) in the prey likely reflects the benefit of taurine to rotifer vitality and their absorption

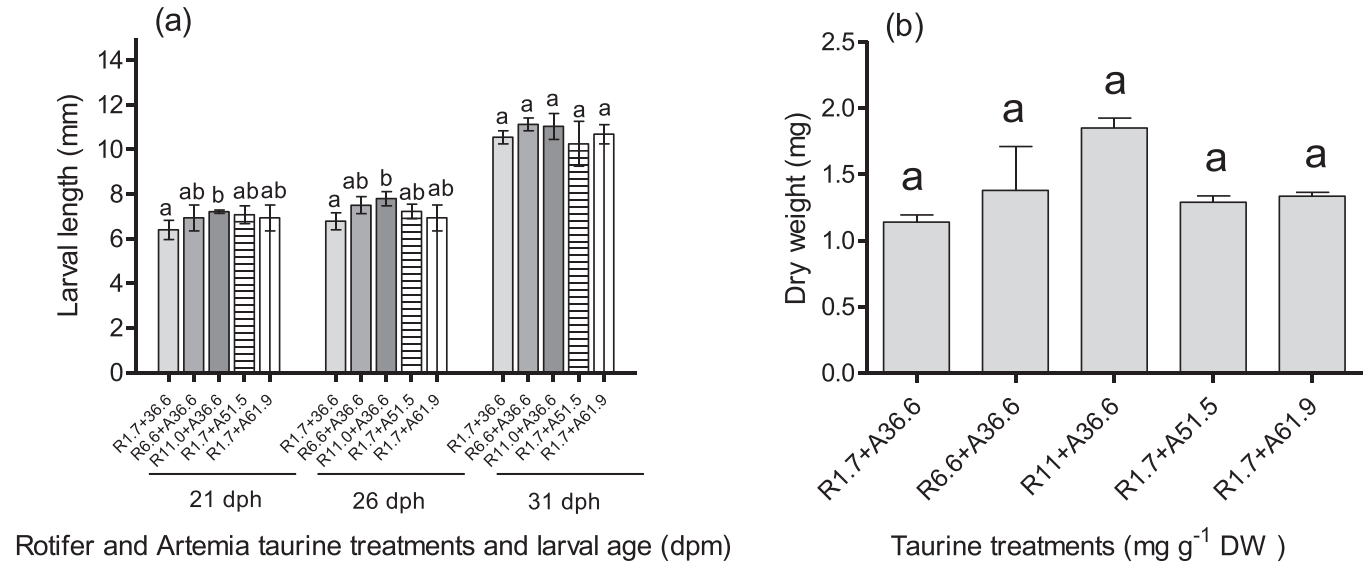

Fig. 4. The effect of feeding rotifer (R) taurine treatments (1.7, 6.6, 11.0 mg g^{-1} DW rotifer) and then the *Artemia* (A) control (36.6 mg g^{-1} DW nauplii) or feeding the rotifer control (1.7 mg g^{-1} DW rotifer) and then different *Artemia* taurine treatments (51.5, 61.9 mg g^{-1} DW) on (a) total length of 21, 26 and 31 dph gilthead seabream (*S. aurata*) larvae and (b) final DW in 31 dph larvae at the end of the study. Larval length values at a specific age having different letters were significantly ($P < 0.05$) different (n = 30, one-way ANOVA). Larval dry weight values at 31 dph having the same letters were not significantly ($P > 0.05$) different (n = 3, one-way ANOVA).

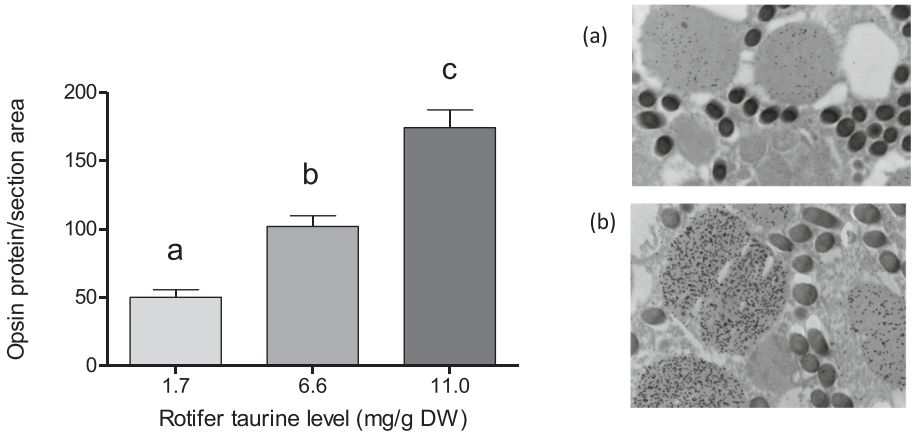

Fig. 5. The effect of rotifer taurine level (mg g^{-1} DW) on opsin abundance/section area in the retinas of 31 dph gilthead seabream (*S. aurata*) larvae that also fed on non-taurine enriched *Artemia* nauplii. Bar values having different letters were significantly ($P < 0.05$) different (n = 3, one-way ANOVA). Retinal sections showing photoreceptors after labeling with opsin antigen marked with gold particles of larvae fed rotifers containing (a) 1.7 and (b) 11.0 mg taurine g^{-1} DW. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

efficiency of this enrichment preparation. Taurine, as a known osmolyte (Huxtable, 1992), may have increased in the free amino acid pool (Aragão et al., 2004) and spared energy designated for osmoregulation in the osmoconformer rotifer (Epp and Winston, 1977) and redirected it to feeding activity. On the other hand, the highest level of taurine enrichment (600 mg L^{-1}) lowered rotifer EFA levels suggesting that taurine is less beneficial to this zooplankter in higher doses. The significant increase in DHA in rotifers fed the 400 mg taurine L^{-1} enrichment treatment may have potentially influenced vision and prey intake as was reported by Koven et al. (2018) in bluefin tuna (*Thunnus thynnus*). However, this effect was considered minor in this species and under the present experimental conditions as the rotifer taurine dose dependent effect on mastax number and larval length in 13 dph fish was significant. Moreover, body taurine levels in 6 and 13 dph larvae showed a direct correlaton with rotifer taurine content, regardless of the variable rotifer essential fatty acid levels. Furthermore, rotifer taurine level was directly correlated with GSB larval growth rate, which indicates a dietary taurine requirement for improved weight gain during the first 13 days of exogenous feeding. Taurine appears to have promoted growth by increasing hunting success, in terms of rotifer ingestion rate, by 13 dph larvae in a dietary taurine dose dependent manner. This increase in rotifer consumption by the larvae may be explained by improved vision as taurine plays an important role in retinal function. Taurine has been associated with improved larval vision and performance in the red sea bream and Japanese flounder (Matsunari et al., 2008; Kim et al., 2005) and is known to promote differentiation of retinal cells in vertebrates (Altshuler et al., 1993). Furthermore, there is evidence for increased uptake of taurine into photoreceptor cells and pigment epithelium in a range of species (Lake et al., 1978). In addition, it has been well documented that taurine can prevent outer segment structural disruption and retinal disfunction caused by prolonged light exposure through shielding the photoreceptor (Pasantes-Morales and Cruz, 1985). Nevertheless, taurine may have also contributed to larval growth through other factors not directly measured in this study. These include its function in cellular osmoregulation, facilitating muscle function as well as modulation of neural transmitters (Huxtable, 1992; Fang et al., 2002; Omura and Inagaki, 2000). Taurine is also the main precursor in bile salt synthesis in fish (Kim et al., 2007). The marked ($P < 0.05$) correlation between rotifer taurine level and the increase in essential fatty acids (DHA, EPA and ArA), well documented as promoters of fish larval growth (Izquierdo

LWS

CCAACGGCCTCGTGTTGGTGTCTACAGCAAAGTTCAAGAAACTCAGACACCCACTGAACTGGATCTTAGTCAATCTCGCAATTGCT
GATCTTGGAGAGACAGTTTTTGCCAGCACTATCAGTGTATGCAACCAGTATTTTGGTTACTTTATTCTTGGACACCCGATGTGCGTC
TTTGAGGGCTACACTGTCTCAGTTTGCGGAATTACTGCTCTCTGGTCCCTGACGATCATTTCCTGGGAGAGGTGGATAGTTGTGTG
CAAACCTTTTGGAAACATCAAATTTGATGAAAAATGGGCCATAGGTGGAATAGTATTCTCCTGGGTCTGGTCAGCAGTGTGGTGT
GCTCCCCCCATCTTTGGATGGAGCAGGTACTGGCCTCATGGACTGAAGACTTCCTGTGGACCTGACGTATTCAGTGGAAGCGAAG
ATCCTGGCGTCCAGTCCTACATGATTGTACTTATGATCACATGTTGCTTAATTCCTCTGGCTATCATCATCTTATGCTACCTCGCAG
TCTGGTTGGCTATCCGTGCAGTTGCTATGCAGCAGAAAGAGTCAGAGTCAACCCAGAAAGCCGAGAGGGAAGTATCCAGGA

SWS1

TGTTCCTCGCCTCCGTGAAGGGGTACTACTTCCTCGGTTACACCTTGTGTGCCATGGAGGCCGCCATGGGATCTATAGCAGGTCTC
GTGACGTCCTGGTCTCTGGCCGTCTTGTCTTTCGAGAGATACCTGGTGATCTGTAAACCGTTTGGAGCCTTTAAGTTTGGCAGTAA
CCACGCTTTGGCTGCTGTCGTCTTCACCTGGGTCATGGGCACCAGTTGTGCAATCCCACCTTTCTTCGGCTGGAGCAGGTACATCC
CCGAGGGTTTAGGCTGCTCCTGTGGACC

RH2A

TCGTCTGCAAACCCATGGGAAGCTTCAAGTTCACTGGAACTCACGCTGCAGCGGGAGTCATCTTCACCTGGGTCATGGCTCTGGCT
TGTGCTGCTCCTCCTCTATTCGGCTGGTCCAGGTACCTTCCTGAGGGCATGCAGTGCTCCTGTGGACCCGACTACTACACTCTGGCT
CCAGGCTACAACAATGAATCATTTGTCATTTACATGTTCGTCGTCCACTTCTTCGTCCCtGTCTTCATCATTTTCTTCACCTATGGAA
GCCTTGTGATGACAGTCAAAGCTGCCGCAGCCCAGCAGCAGGAGTCAGAGTCCACCCAGAA

RH2B

GGTGGACCTGACTACTACACTCTGGCCCCAGGCTTCAACAATGAGTCCTATGTCATGTACATGTTCGTCTGCCACTTCTGCGTCCC
TGTCT

RH1

TACACCTCTATGCACGGCTACTTCGTCCTTGGACGCCTTGGCTGCAATATCGAAGGATTCTTTGCTACTCTTGGTGGTGAAATTGCC
CTCTGGTCACTGGTTGTTCTGGCTATTGAAAGGTGGGTGGTTGTCTGCAAGCCCATCAGCAACTTCCGTTTCGGGGAGAATCATGC
CATCATGGGCTTGGCCTTCACCTGGATAATGGCCATGGCTTGTGCTGCCCCCCCCTCtAGTTGGCTGGTCTCGTTACATCCCTGAGGG
AATGCAGTGCTCATGTGGAATTGACTACTACACACGTGCTGAAGGTTTCAACAATGAGTCGTTTGTGATCTACATGTTCATCTGCC
ACTTCTCGATCCCACTGACGATCGTGTTCTTCTGCTATGGCCGTCTGCTCTGTGCTGTCAAGGAGGCTGCTGCTGCCCAGCAGGAG
TCTGAGACCACCCAGAGGGCTGAGAGGGAAGTCACCCGCATGGTTATTATGATGGTCATTGCTTTCCTGGTATGTTGGCTTCCCTA
TGCCGGTGTAGCCTGGTGGATCTTCACACATCAAGGCTCAGAGTTCGGCCCAGTCTTCATGACCATCCCGGCCTTCTTTGCCAAGA
GCTCATCCATCTACAATCCAATGATCTACATCTGCTTGAACAAGCAGTTCCGCCACTGCATGATCACCACCTTG

Fig. 6. Partial sequences of the five opsin genes; long wavelength sensitive (LWS), short wavelength sensitive1 (SWS1), the green cone opsins rhodopsin-like 2a (RH2a) and rhodopsin-like 2b (RH2b) as well as the rod opsin rhodopsin 1 (RH1) in gilthead seabream (*S. aurata*) larvae that were used in Rt-qPCR analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and Koven, 2011), as well as total polyunsaturated and monounsaturated fatty acids suggest that the taurine-modulated enhanced production of bile salts facilitated lipid digestion and absorption. Koven et al. (2016) similarly concluded that dietary taurine increased bile salt production and the incorporation of fatty acids into the eyes of white grouper (*Epinephelus aeneus*) juveniles. In addition, dietary taurine had a strong hypocholesterolemic effect in the rat liver by increasing production of cholesterol 7α hydroxylase (CYP7a1), a key rate-limiting enzyme converting cholesterol to bile salts (Fukuda et al., 2011). Moreover, taurine suppresses acyl CoA cholesterol acyltransferase (ACAT) activity, which binds free cholesterol to a fatty acid, resulting in the β-oxidation of free fatty acids and the channeling of surplus hepatic cholesterol into bile salt synthesis (Fukuda et al., 2011). Bile salt activated lipase (BAL), which has been suggested as the main lipase in fish (Gjellesvik et al., 1992; Koven et al., 1997), requires the presence of bile salts (Lombardo et al., 1980). Chatzifotis et al. (2008) reported that supplemented taurine increased the activity of this non-specific lipase in common dentex (*Dentex dentex*).

Dietary taurine was found to improve larval growth in red sea bream (*Pagrus major*) (Kim et al., 2016), Japanese flounder (*Paralichthys olivaceus*) (Chen et al., 2005), amberjack (*Seriola dumerili*) (Matsunari et al., 2013), California yellowtail (*Seriola lalandi*) (Hawkyard et al., 2015), northern rock sole (*Lepidopsetta polyxystra*) (Hawkyard et al., 2014) and bluefin tuna (*Thunnus thynnus*) (Koven et al., 2018). However, the growth promoting effect of taurine in GSB during rotifer feeding is in stark contrast with Pinto et al. (2013) who argued that taurine enriched rotifers had no effect on larval tissue taurine levels or growth performance in this species. This contradiction may be based on the much lower taurine levels in liposome enriched rotifers and larval body content that was reported in this study.

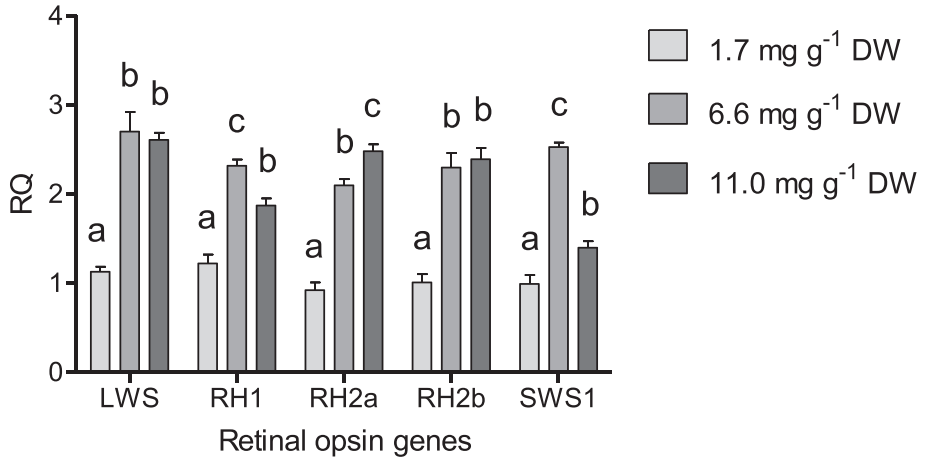

Fig. 7. The effect of rotifer taurine level (mg g^{-1} DW) on the expression of five retinal opsin genes (LWS, RH1, RH2a,RH2b and SWS1 in 8 dph gilthead seabream (*S. aurata*) larvae. Bar values having different letters were significantly ($P < 0.05$) different (n = 3, one-way ANOVA).

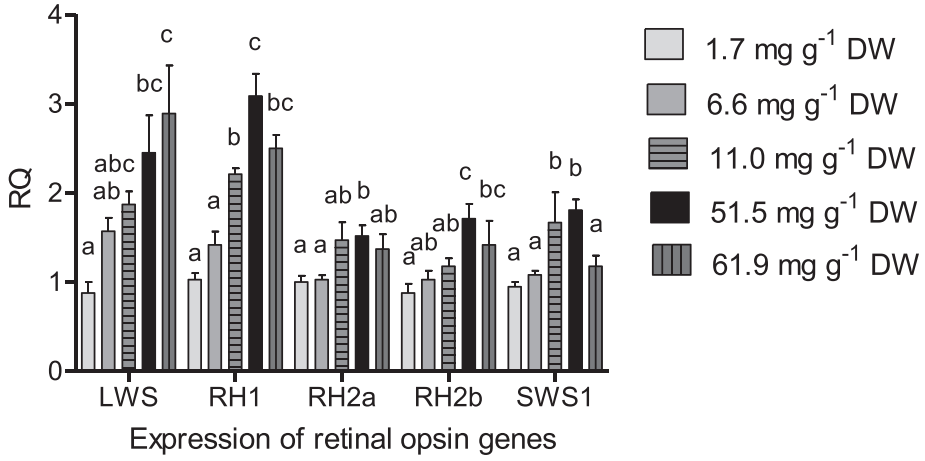

Fig. 8. The effect of rotifer and *Artemia* taurine level (mg g^{-1} DW) on the expression of five retinal opsin genes (LWS, RH1, RH2a, RH2b and SWS1) in 31 dph gilthead seabream (*S. aurata*) larvae. Bar values having different letters were significantly ($P < 0.05$) different. (n = 3, one-way ANOVA).

Interestingly, older 21 dph GSB larvae, that were feeding on control *Artemia* nauplii and no longer consuming the taurine enriched rotifers, demonstrated that the earlier taurine consumption continued to influence growth similarly to the combined effect of rotifer and *Artemia* taurine and markedly ($P < 0.05$) better than the rotifer-*Artemia* control. In fact, in 26 dph larvae, rotifer taurine significantly ($P < 0.05$) impacted on growth more than *Artemia* taurine even though the rotifer taurine treatments had stopped 13 days previously. These results clearly emphasize the importance of the early feeding of taurine on later larval performance. This is surprising as it would be expected that consuming the higher biomass of *Artemia* nauplii would contribute to growth more markedly than the ingestion of smaller rotifers. Indeed, by the time the larvae were 31 dph, the substantial consumption of the larger non-taurine enriched and taurine enriched *Artemia* nauplii became the overriding growth factor, masking the effect of the earlier rotifer feeding. Nevertheless, the pattern of growth in dry weight, which is generally a more sensitive growth parameter than length, suggests that rotifer taurine continues to influence growth in 31 dph larvae, although not quite significantly ($P = 0.07$).

In the present study, there was a significant ($P < 0.05$) effect of dietary taurine on larval GSB retinal opsin abundance, in a dose dependent manner (Fig. 5). Koven et al. (2018) reported in bluefin tuna (*Thunnus thynnus*) larvae, a docosahexaenoic (DHA; 22:6n-3) dietary dose dependent effect on opsin abundance suggesting that this essential fatty acid increases opsin synthesis, which leads to improved prey detection and consumption with an associated promotion of larval growth. This parallel increase in opsin abundance in response to both dietary taurine in the present study and to DHA in bluefin tuna (Koven et al., 2018) broadly hints that the roles of DHA and taurine in the retinal membrane are complementary. However, the physiology underpinning this taurine effect remains unclear. DHA imparts membrane fluidity with its 6 double bonds, which facilitates the enzyme cascade in photo-transduction and the conversion of photons to electrical signals in the photoreceptors that ultimately send a message to the brain. However, DHA enriched membranes of the outer segments of rods and cones are very susceptible to peroxidation damage from exposure to prolonged light intensity. It has been shown that taurine is released from the outer segments of photoreceptors under a light stimulus, suggesting that it plays a role protecting them from photodamage, while during the dark cycle taurine returns to concentrate in the outer segment via membrane taurine zinc-dependent transporters (Nusetti et al., 2005). This would explain the complementary nature of both DHA and taurine preserving the functionality of photoreceptors. In the present study, the rise in opsin abundance with prey taurine level may have been the result of increasing protection of an optimal number of opsin molecules from peroxidation. On the other hand, the results indicated that taurine stimulated increased gene expression, and presumably synthesis, of various types of opsin molecules, and not just merely protecting them.

This added role for taurine in photoreceptor functionality may be only true in early developing animals. Altshuler et al. (1993) demonstrated that taurine promoted rod development in vitro while the addition of a competitive taurine antagonist to the culture (i.e. amino-methyl-sulphonic acid) caused a significant decrease in the proportion of opsin containing cells. Omura and Yoshimura (1999) and L'Amoreaux (2012) suggested that taurine plays a role inhibiting the phosphorylation of proteins involved in the modulating and/or regulating synaptic transmission in the retina. This might, in turn, affect signal transduction (Ramila et al., 2015) and the expression of genes controlling cell growth, proliferation and many other processes. Nevertheless, the link between taurine, protein phosphorylation and opsin synthesis remains obscure and demands further study.

The effect of taurine on the gene expression of various opsin molecules of the cone photoreceptor suggests how this nutrient contributes to improved vision, growth and survival as a function of age. The detection of zooplankton during the larval stages is critical for the survival and growth of the developing fish. Moreover, larval prey is distributed in patches (Britt et al., 2001) in the sea, making it more challenging for the larvae to find and consume adequate levels of food. A failure of the larvae to hunt effectively may lead to reaching the point of no return (PNR), where digestive tract function deteriorates irreversibly leading to almost certain mortality (Yúfera and Darias, 2007). Moreover, the distance at which zooplankton can be detected by the larvae is only 1–2 body lengths (Wahl et al., 1993) further underlining the importance of vision. UV sensitive visual pigments were common in marine fish larvae in 22 species studied from the Pacific Northwest coast of North America (Britt et al., 2001). The early developing 8 dph sea bream larvae in this study also demonstrated opsin SWS1 taurine-stimulated gene expression. This was similar to the other opsins investigated and is indicative of the importance of near surface foraging (UV wavelength is absorbed with depth). Retinal SWS1 opsin would facilitate the detection of tansparent zooplankton, as this prey would reflect UV light. Moreover, prey can also be opaque (Loew et al., 1996) and contrast with their background if they contain UV absorbing compounds such as mycosporine-like amino acids (Johnsen and Widder, 2001), which are found in rotifers and copepods (Hylander, 2020). In older 31 dph larvae, prey taurine continues to significantly up-regulate the expression of the 5 retinal opsin genes. However, the two opsin genes that were upregulated most by dietary taurine was LWS and RH1 suggesting that these older fish are foraging deeper in the water column, which is also indicated by the lower expression levels of SWS1 opsin. The increased expression of the cone LWS opsin gene would allow in the larval retina the absorption of red, yellow and green light that can penetrate to a greater water depth. As gilthead sea bream at this age would inhabit coastal waters, which have higher primary productivity, it would be expected that the capability to detect prey in green light in particular would be advantageous. Britt et al., 2001 found in the larva retina from the 22 species he examined, the presence of green absorbing cones, which would correspond to the dominant greenish light of the inshore waters of the Pacific northwest (Flamarique and Hawryshyn, 1993). As developing larval fish are foraging deeper, all wavelengths of light are attenuated by the water. Prey taurine increased the expression of the rod rhodopsin (RH1) opsin gene, which would facilitate prey detection under the lower light intensity characteristic of deeper water.

In summary, there is a marked rotifer taurine effect on body accumulation of taurine and growth in GSB larvae in a dose dependent manner. This effect in early developing larvae continues to promote growth later in older larvae feeding on *Artemia* nauplii. Dietary taurine demonstrated, as well, a significant effect on retinal opsin abundance and gene expression of 5 main opsins, in largely a dose dependent manner. The taurine effect on the pattern of gene expression of selected opsins in GSB larvae was age dependent and potentially improved vision leading to increased hunting success and weight gain. Moreover, it suggests that the light wavelengths that would optimize the larva's hunting environment would vary with developmental stage and foraging water depth.

CRediT authorship contribution statement

A. Gaon: Conceptualization, Investigation, formal analysis, data curation. **O. Nixon:** Methodology, data curation. **A. Tandler:** supervision, writing-review and editing. **J. Falcon:** Validation, methodology. **L. Besseau:** supervision, methodolgy. **M. Escande:** methodolgy. **G. Allon:** Investigation. **W. Koven:** Conceptualization, funding acquisition, supervision, writing original draft.

Declaration of Competing Interest

None.

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