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# Evaluation of dietary taurine concentrations in microparticulate diets provided to larval California yellowtail (*Seriola dorsalis*) post larvae.

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## 1. Abstract

Taurine is an important amino acid derivative for marine and freshwater fish, especially during early development. We investigated the range of taurine concentrations that influence the growth and survival rates of California yellowtail (CYT; *Seriola dorsalis*) during transition from live feeds to microparticulate diets, as well as the extent to which nutrient leaching from the microparticulate diets affect these ranges. We tested particle assisted rotationally agglomerated (PARA) particles with four levels of taurine: 0.4 (Low Taurine; LT), 4.5 (Medium Taurine; MT), 9.3 (High Taurine; HT), and 12.2% (Very High Taurine; VHT). Our results showed that CYT post larvae had no significant differences in growth, survival and feed consumption rates between the MT, HT and VHT treatments. However, it should be noted that the PARA particles containing 122 g $kg^{-1}$ (VHT) taurine were especially prone to leaching and may have had taurine concentrations as low as 34.9 g $kg^{-1}$ before they settled on the bottom of the tank. Therefore, the actual dietary taurine concentrations experienced by the larvae were likely lower than the initial dietary concentrations. More research is needed to evaluate the potential nutrient toxicity of elevated dietary taurine concentrations for marine fish larvae and juveniles.

## 2. Introduction

Taurine (2-aminoethanesufonic acid) is an important amino acid derivative for vertebrates, with beneficial effects on mammals being extensively studied for several decades (Hayes et al. 1975, Knopf et al. 1978, Hayes & Trautwein 1989, Huxtable 1992, Stapleton & Bloomfield 1993, Di Leo et al. 2002, Lourenco & Camilo 2002, Kuzmina et al. 2010). However, only recently have the effects of taurine on fish physiology (osmoregulation, antioxidation, feeding stimulation, and vision) and metabolism been extensively researched (Huxtable 1992, Schaffer et al. 2000, Militante & Lombardini 2002, Lima 2004, Kim et al. 2007, 2008, El-Sayed 2013). Nutritional requirement studies have shown that taurine is an essential or conditionally

essential nutrient for various life stages of marine fish and some freshwater fish (Jacobsen & Smith 1968, Takeuchi 2001, Yokoyama et al. 2001, Kim et al. 2005, Gaylord et al. 2006, Takagi et al. 2008, Salze et al. 2012, Jirsa et al. 2014a, Jirsa et al. 2014b, Hawkyard et al. 2015a, 2015b). In mammals, the major pathway for taurine biosynthesis from cysteine involves the oxidation of cysteine to cysteine sulphinate, following decarboxylation of this product to hypotaurine and then to taurine (Worden & Stipanuk 1985). Cysteine sulphinate decarboxylase (CSD) plays an important role in this pathway, because it catalyzes the reaction that produces hypotaurine. Several species are deficient in this enzyme, including many species of marine fish (Salze & Davis 2015). In fish, the capacity to biosynthesize taurine varies among species and throughout ontogenesis (Goto et al. 2001; Goto et al. 2003; Kim et al. 2008). For example, CSD activity in red seabream (*Pagrus major*) and Japanese flounder (*Paralichthys olivaceus*) is approximately half the levels observed in rainbow trout (*Oncorhynchus mykiss*) (Yokoyama et al. 2001). The wide-range of biosynthesis of taurine by fish necessitate that taurine requirements need to be evaluated on a species and stage specific basis.

Rearing marine fish typically begins with offering live prey (rotifers or *Artemia*) until the fish can be weaned to consume formulated microparticulate diets. The taurine concentrations measured in rotifers and *Artemia* have been reported to be much lower than the concentrations measured in copepods, the primary natural food source for marine fish larvae (van der Meeren et al. 2009, Maehre et al. 2013, El-Sayed 2013, Takeuchi 2014, Hawkyard et al. 2015a). Studies involving species such as cobia (*R. canadum*), Japanese flounder (*P. olivaceus*), red sea bream (*P. major*), amberjack (*Seriola dumerili*), yellowtail (*S. quinqueradiata*), northern rock sole (*Lepidopsetta polyxystra*) and California yellowtail (*S. lalandi*), have shown taurine-enriched live prey improved not only growth and survival rates, but also helped with morphological development and impacted the activities of digestive enzymes (Chen et al. 2004, Chen et al. 2005, Matsunari et al. 2005, Salze et al. 2012, Matsunari et al. 2013, Hawkyard et al. 2014; 2015a, 2015b). Juvenile taurine requirements for marine finfish have also been described in the literature for species such as, white seabass (*Atractoscion nobilis*), Atlantic Salmon (*Salmo salar*), *S. lalandi*, *S. quinqueradiata*, *P. olivaceus*, *P. major*, and *R. canadum* (Kim et al. 2003, Takagi et al. 2006a, 2006b, Lunger et al. 2007, Kousoulaki et al. 2009, Jirsa et al. 2014a, 2014b). In these studies, juvenile growth, survival and additional metrics of health status were impacted

by dietary taurine concentrations, with effective concentrations ranging from 0.2 to 3.9% of the feed.

Transitioning marine fish larvae from live feeds to microparticulate diets is a challenging and yet critical part of marine fish culture. This transition often utilizes a co-feeding strategy where fish are simultaneously offered both live feeds and formulated diets for a period of time. Generally, the proportion of live feeds with respect to formulated diets is decreased over time until only the formulated diet is being fed, a process referred to as weaning. Co-feeding has been shown to improve diet acceptability and improve larval growth and survival in a number of species (Rosenlund et al. 1997). While many nutritional studies have focused on either the larval (live feed) or juvenile (after weaning onto formulated diets) stages, very little attention has been focused on the transition between these two phases.

During and immediately after weaning, marine fish larvae are fed microparticulate diets that require special considerations when compared to juvenile and adult feeds. Most commercially available microparticulate diets are microbound whereby nutrients are held together in a matrix using a binding substance. These particles can be formed directly into microparticles (250 - 700 µm) through processes such as agglomeration or they may be made by producing large particles (> 1mm), generally by extrusion or pelletization, and then crushed and sieved to the desired size range (crumbled diets). Microparticulate diets are particularly prone to losses of low molecular weight water-soluble nutrients, hereafter “nutrient leaching”, due to high surface area to volume ratios (Langdon & Barrows 2011). Likewise, microparticulate diets may undergo dramatic declines in water-soluble nutrients before they are consumed by the fish. Unlike proteinogenic amino acids, which are predominantly protein-bound, taurine is generally present in animal tissues in the free form (Huxtable, 1992). In addition, taurine is a relatively low molecular weight nutrient (125.1 g $mol^{-1}$) and has a high water-solubility (~100 g $L^{-1}$ at standard conditions) both of which make it susceptible to nutrient leaching. Accounting for nutrient leaching may influence our perception of nutrient intake rates, which are often based on the initial (pre-leached) taurine concentrations in the diet. In the present study, we evaluate the leaching of taurine from particle assisted rotationally agglomerated (PARA) particles, a commercial processing method for microbound diets. Our overall objectives were to: 1) identify the range of taurine concentrations that influence the growth and survival rates of California yellowtail (CYT; *S. dorsalis*) during transition from live feeds to microparticulate diets and 2)

determine the extent to which nutrient leaching from microparticulate diets affect these ranges. Based on results from Martinez-Takeshita et al. (2015), CYT species name has changed from *S. lalandi* to *S. dorsalis*, we will now refer to CYT as *S. dorsalis*.

## 2. Materials and Methods

### *2.1 Production of PARA particles*

Particle assisted rotationally agglomerated (PARA) particles were prepared using the methods described in Barrows & Lellis (2006). Briefly, this process uses the rotational forces of a spheronizing system where a grooved plate on the bottom of a cylinder spins at a high rate of speed. The addition of 3 mm plastic beads helps agglomerate the ingredients into complete particles. This low pressure method results in a particle that has a slower sinking rate than a micro-extruded particle. The binding characteristics of the protein ingredients themselves holds the particle together without the need to add indigestible binders.

After particle formation and drying, PARA particles were size selected using a series of metal sieves. Particles from the 250-425 and 425-700 μm fractions were retained for leaching trials, determination of sink rates and feeding trials. Particles containing increasing concentrations of taurine were prepared using the formulas shown in Table 2. Feed samples were analyzed according to standard methods (AOAC 1995) on a Leco thermogravi- metric analyzer (TGA701, LECO Corporation, St. Joseph, MI, USA). Protein (N X 6.25) was determined by the Dumas method (AOAC 1995) on a Leco nitrogen determinator (TruSpec N, LECO Corporation). Total energy was determined by isoperibol bomb calorimetry (Parr 6300, Parr Instrument Company Inc., Moline, IL, USA). Lipid was determined by petroleum ether extraction using an Ankom XT10 (Ankom Technologies, Macedon, New York, USA). Yttrium oxide ($Y_2O_3$), used as an inert feed marker, was added to taurine-containing PARA particles at a concentration of 10 g $kg^{-1}$ of the total mash (w/w; wet weight). Particles containing sodium fluorescein (28803; Fluka, St. Louise, MO) were produced for use in leaching studies. Sodium fluorescein was added to PARA particles at a concentration of 10 g $kg^{-1}$ of the mash.

### *2.2 Sinking rate of microparticulate diets*

Sinking rates were measured by placing 1 g of each diet in a 10.2 cm diameter glass cylinder with a water depth of 50.6 cm and timing the decent of the particles with a stopwatch. This procedure was replicated twenty times for each diet. Sink rate (cm $sec^{-1}$) was calculated by dividing the cylinder depth (cm) by the average decent time (seconds) of the particles in each replicate.

*2.3 Retention of water-soluble compounds by microdiets*

Particles suspended in seawater are prone to nutrient leaching prior to consumption by target organisms, which can impact water-soluble nutrients concentrations in the feed. For this reason it was necessary to evaluate the retention efficiencies (RE) of taurine in PARA particles following suspension in seawater. We approached this in two steps: First, we conducted leaching trials to evaluate the retention of sodium fluorescein in PARA particles. This allowed us to develop a comprehensive data set of retention efficiencies from which we were able to perform model selection. Sodium fluorescein was used as a representative compound because it is chemically stable in seawater and is easy and inexpensive to quantify. We assumed that sodium fluorescein could be used as a proxy for other water-soluble compounds, notably taurine, because it is highly water-soluble (~500 g $L^{-1}$ in water at standard conditions) and has a relatively low molecular weight (376.3 g $mol^{-1}$) comparable to that of taurine (125.2 g $mol^{-1}$). We then conducted leaching trials with PARA particles containing taurine and developed specific model parameters for the retention efficiencies of taurine. Model parameters were used to interpolate taurine retention following a 2 minute suspension in seawater.

Leaching trials were conducted as follows: before the trial period began, 100 mg of PARA particles was added to a 50 ml centrifuge tube. One test tube was used for each treatment and sampling time, in triplicate, ensuring sample independence. At the beginning of the experimental period (0 min), 40 ml of autoclaved seawater was added to each tube. After the allotted time, the contents of each tube were poured onto a Whatman GF/C filter under vacuum. Leached particles were scraped from the surface of the filter and transferred to a clean centrifuge tube. Particles containing taurine were collected after 15 and 30 minutes whereas PARA particles containing sodium fluorescein were collected at 10, 30, 60, 120 and 240 minutes. In addition, triplicate samples of untreated PARA particles were collected to determine the initial (0 min)

taurine and sodium fluorescein concentrations. Leaching rates were measured from PARA particles in the 250-425 μm size range because small particles have a higher surface area to volume ration and should leach at a higher rate than large particles (Langdon 2003). Therefore, leaching from the small diameter particles should represent a "worst case" condition. Particles were freeze dried in a Freezone freeze dryer (Labconco, Kansas City, MO) for 72 h and were then ground to a powder with a mortar and pestle. Water-soluble compounds were extracted from powdered, freeze dried particles as described in sections 2.9 and 2.10. Retention efficiencies (RE) were calculated as follows:

*RE (%) = Conc. measured in particles at time of sampling / Initial conc. measured in particles X 100*

Where: "Conc. measured in particles at time "Y" was the concentration of taurine or sodium fluorescein (g $kg^{-1}$) measured in the PARA particles at the time of sampling and "Initial conc. measured in particles" was the concentration of taurine or sodium fluorescein (g $kg^{-1}$) measured in the PARA particles at prior to leaching, i.e. T0.

*2.4 Larval Rearing*

Larvae were produced from resident broodstock populations of CYT that are maintained at the Hubbs-SeaWorld Research Institute (HSWRI). Eggs were stocked at 100 eggs $L^{-1}$ and larvae were reared from -2 through 15 days post hatch (dph) in 1600L black conical bottom tanks. CYT were fed rotifers (*Brachionus plicatilis*) enriched with Ori-Green (Skretting, Tooele, UT) from 2 through 7 dph. At 6 dph larvae were co-fed rotifers and 2$^{nd}$ instar *Artemia* (*Artemia franciscana)* enriched with S.Presso (Inve Aquaculture, Salt Lake City, UT). Enriched second instar *Artemia* were offered to the larvae in the experimental tanks from 15 to 35 dph at a density of 1 – 3 *Artemia* $mL^{-1}$. Samples of S.Presso-enriched *Artemia* were collected periodically throughout the trial (n = 3) on a 140 um sieve and rinsed with distilled water. *Artemia* samples were frozen (-80° C), freeze-dried (Labconco Freezone freeze-drier; Kansas City, MO) and analyzed for taurine as described in section 2.9.

*2.5 Experimental Design*

The trial was stocked with 15 dph larvae and each treatment had four corresponding replicate tanks. Larvae were stocked at a density of 6.6 larvae $L^{-1}$ (2,000 larvae) into an

experimental system consisting of twenty, 320 L black conical-bottom tanks. The experimental tanks were supplied with temperature controlled (21°C) recirculating seawater at a rate of 2.0 – 3.0 L $min^{-1}$. A containment screen (500 µm) was placed in the center of each tank along with an aeration ring to maintain good water circulation. Fluorescent lights were placed 0.7 m above the tanks for illumination (800 – 1,500 lux lights, Lithonia, Conyers, GA), the lights were set at a continuous photoperiod until 30 dph and then were placed on a 18 light: 6 dark photoperiod. The duration of the trials was 26 days (15 to 41 dph).

*2.6 Food and feeding regime*

Larvae were transitioned from *Artemia* starting at 16 dph by co-feeding with the assigned microdiet treatment. The live prey was added four times daily at 07:00, 10:00, 13:00, and 16:00. The microparticulate diets were fed by hand four times daily as well as with an automatic feeder (FISH MATE; Pentair AES, Apopka, FL) that provided food for 18 hrs. Beginning at 16 dph, 5 g of each microdiet was offered to each experimental tank. This amount was increased to 10 g at 27 dph and again at 35 dph to 15 g. Tanks were cleaned once daily and screens were changed as needed.

CYT larvae were fed one of four PARA formulated diets as shown in Table 2. The treatments included four levels of taurine: 0.4 (Low Taurine; LT), 4.5 (Medium Taurine; MT), 9.3 (High Taurine; HT), and 12.2% (Very High Taurine; VHT) (Table 2). The diets were offered within two size ranges: 250 – 425 µm and 425 – 710 µm. The smaller size range was fed from 15 to 23 dph, and the larger size range from 18 – 41 dph.

*2.7 Larval sampling and measurements*

Larval growth was measured on subsamples of 20 larvae per replicate at four times during the trial (16, 23, 30, and 40 dph). Larvae were euthanized with MS-222 prior to taking the measurements. Twenty larvae were placed under a MZ16 Macro microscope (Leica Microsystems, Inc., Bannockburn, IL), a digital photograph was taken, and standard length (SL) was measured to the nearest 0.1 mm using Image-Pro Plus software (Media Cybernetics, Bethesda, MD). All 20 larvae were then weighed as a batch to the nearest 1 g wet weight. Dividing by 20 provided an average individual larval wet weight.

Gut contents were examined every three days from 20 – 40 dph. Gut content analyses was accomplished by sub sampling ten larvae from each tank one hour after the first feeding of the day at 8:00 am. On these sample days the only food offered to the larvae was the microparticulate diet. Larvae were placed on a microscope slide and the gut contents were dissected so that food items could be identified. Feeding incidence was calculated by dividing the number of larvae observed with food in the gut by the total number of larvae sampled (N = 10). Each diet was marked with the rare earth metal yttrium oxide ($Y_2O_3$). Larvae that were found with microparticulate diets in the gut were saved for yttrium analysis.

*2.8 Analysis of yttrium oxide*

Yttrium oxide was included into the PARA particles in order to determine feeding rates. Both PARA particle samples and fish samples were analyzed for yttrium oxide using inductively coupled plasma-optical emission spectroscopy (ICP-OES) with a Perkin Elmer Optima 3000 Radial ICP-OES (Perkin Elmer Instruments, Norwalk, CT). Larval fish and diet samples were prepared as in Johnson et al (2009) with a modification whereby sample ash was digested overnight at room temperature. The wavelength employed was 371.029 nm and standards were obtained from Ultra Scientific (North Kingstown, RI). Feed consumption was calculated from yttrium concentrations as follows:

*Feed consumption = Yttrium conc. in fish /Yttrium conc. in food*

Where: "Feed consumption" was the weight of PARA particles estimated in the gut of a fish (μg $larvae^{-1}$), "yttrium conc. in fish" was the total quantity of yttrium measured in the fish (μ g $larvae^{-1}$) and "yttrium concentration in food" was the concentration of yttrium oxide measured in PARA diets (μg $g^{-1}$).

*2.9 Analysis of taurine*

Taurine concentrations were determined in unleached microparticulate diets, leached microparticulate diets and whole body fish samples (sampled on 41 dph). Samples were packed in dry ice and sent to Midwest Laboratories (Omaha, NE) where the samples were analyzed for taurine using method HPLC PROC 23 derived from AOAC 999.12. Briefly, taurine was extracted using a weak acid and no hydrolysis was carried out. The extract was analyzed using

HPLC with post column derivatization where ninhydrin was used to produce a Ruhemann's Purple derivative that was measured using an ultra-violet (UV) detector.

*2.10 Analysis of sodium fluorescein*

Powdered, freeze dried samples of sodium fluorescein containing microdiets were placed in 15 mL centrifuge tubes and sonicated (B. Braun Labsonic sonicator, Bethlehem, PA) at medium power for 1 min. Samples were then soaked overnight in a refrigerator to allow full dissolution of sodium fluorescein. A 1 mL aliquot was removed from each tube, filtered with a 0.22 μm syringe filter into a 1.5 mL microcentrifuge tube. Fifty microliters was removed and transferred to a 96 well plate and measured on a Wallac Victor 2 flourometer (Perkin-Elmer, Waltham, MA) plate reader with a 485 nm narrow band excitation and a 535 nm sharp cut emission filter. Samples were diluted as necessary to fall within the range of the standard curve (R-square = 0.987).

*2.11 Water-quality*

Nitrogen compounds (nitrate, nitrite, ammonia; ± 0.001 mg $L^{-1}$) and pH (± 0.01) were monitored weekly, while dissolved oxygen (± 0.01 mg $L^{-1}$ and ± 0.1 percent saturation) and temperature (± 0.1°C) monitored daily. Temperature, DO, and pH were measured with a model HQ40d meter (Hach Company, Loveland, CO). Total ammonia, nitrite, and nitrate were measured with a model DI/890 colorimeter (Hach Company, Loveland, CO).

*2.12 Statistics*

Statistical analyses were performed using Statistica version 6.1 (StatSoft, Inc., Tulsa, OK) and JMP 11.0.0 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was used to compare sink rates of PARA particles among particle sizes and treatments. Retention efficiencies were compared among treatments (particle types) and among sampling times using a generalized linear model (GLM). Model selection for retention efficiencies of sodium fluorescein was accomplished using Akaike information criteria (AIC) and Bayesian information criteria (BIC). The resultant model was fitted to taurine leaching data and was used to estimate the retention of taurine at 2 minutes. Standard lengths, wet weights, total amino acids, taurine and yttrium concentrations in experimental diets and fish tissues, feeding incidences, feed consumption rates

and survival data were analyzed using ANOVA. As necessary data were normalized using *ln*- or arcsin-square root transformations, raw data are displayed in figures and tables. Post-hoc comparisons were performed with Tukey's HSD with a significance level of $p < 0.05$.

## 3. Results

### *3.1 Microparticulate diets*

Proximate composition of the experimental diets showed that protein (%), lipid (%), and energy density (kcal $g^{-1}$) were similar between the treatments (Table 2). Total amino acid composition of the experimental microparticulate diets is shown in Table 4. Amino acid levels tended to decrease with increasing taurine addition (Table 4).

The sink rate of PARA particles was not significantly different among microdiet treatments (ANOVA, $p = 0.733$). However, large particles (425-700 μm in diameter) sank significantly faster ($0.9 \pm 0.4$ cm $sec^{-1}$) than small particles (250-425 micron; $0.6 \pm 0.2$ cm $sec^{-1}$). The 320 L tanks used in the feeding trial had a depth of 63.5 cm and therefore, we estimate that large particles remained in suspension for $1.2 \pm 0.5$ minutes and small particles for $1.8 \pm 0.3$ min. These estimates assume that tank characteristics, such as turbulence and aeration, did not impact the sink rate of particles. CYT larvae feed only on suspended particles, meaning that after ~2 minutes (maximum) the PARA particles were no longer available for consumption. Therefore, we estimated the quantities of taurine that would have remained in PARA particles after 2 minutes of suspension in seawater; nutrient leaching/retention trials are described in section 3.2.

### *3.2 Retention and inclusion rates of taurine and sodium fluorescein*

PARA particles produced with sodium fluorescein were $7.7 \pm 0.5$ g $kg^{-1}$ sodium fluorescein (w/w; wet weight). Retention of sodium fluorescein by PARA particles was best modeled using an exponential decay model ($RE = 97.25 \times e^{-0.0427} \times Time$; Adjusted R-squared = 0.93; Figure 1) and yielded the lowest AIC and BIC values of all models evaluated (Table 1). Based on these results, an exponential decay model was used to fit taurine retention data in subsequent analyses.

The taurine concentrations measured in the four experimental microparticulate diets, prior to nutrient leaching, were significantly different among diets (ANOVA, $p < 0.001$; Table

3). Taurine retention efficiencies (RE; %) varied significantly based on time of suspension (time), taurine concentration in PARA diets (treatment), and showed a significant interaction between taurine concentration and time of suspension (GLM, $p = 0.001$, $p < 0.001$ and $p = 0.001$, respectively). The retention efficiencies of taurine, modeled as exponential decay functions are shown in Figure 2 and model parameters were used to derive estimates of RE at 2 minutes (Table 3). Yttrium oxide concentrations varied among experimental microparticulate diets (ANOVA, $p = 0.016$; data not shown), consequently, diet-specific yttrium concentrations were used to calculate feed consumption values for their respective treatments. *Artemia* nauplii enriched with S-Presso had a mean taurine concentration of $8.1 \pm 0.6$ g $kg^{-1}$.

*3.3 Growth, survival and whole body taurine concentrations of CYT*

Natural-log transformed standard lengths (SL) and *ln*-transformed wet weights (WW) of CYT post larvae were significantly different among treatments (ANOVA, $p = 0.025$ and 0.012, respectively); untransformed values are shown in Table 5 along with the results of Tukey's HSD. Arcsin-square root transformed survival rates, determined at the end of the feeding trial, were not significantly difference among treatments (ANOVA, $p = 0.221$). Whole body taurine concentrations (Figure 3) measured in the fish at the end of the trial varied with experimental treatment (ANOVA, $p < 0.001$; Figure 3). Specifically, treatments MT, HT, and VHT were greater than the LT treatment.

*3.4 Feeding incidence and feed consumption*

Arcsin-square root transformed feeding incidences (proportion of fish with feed in their guts) varied significantly among sampling times but not among treatments (Repeated-Measures ANOVA, $p < 0.001$ and $p = 0.698$, respectively). At the end of the trial, feed consumption rates were significantly different (Figure 4) among treatments in terms of the amount of feed per larvae (µg $larvae^{-1} \pm$ SD) but not by the amount of feed per dry weight of the larvae (g $kg^{-1}$ DW; ANOVA, $p = 0.005$ and $p = 0.11$, respectively). Specific pairwise comparisons can be seen in Figure 4.

*3.5 Water-quality*

Water quality measurements for both trials were similar and within an acceptable range for marine fish larvae. Water temperature was maintained at 21.7 ± 0.3°C and salinity was 34.6 ± 0.5 ppt. Dissolved oxygen ranged from 8.0 to 8.7 mg $L^{-1}$ (92 – 97% saturation), and mean total ammonia nitrogen and unionized ammonia levels were < 0.01 and 0.0025 mg $L^{-1}$, respectively.

**4. Discussion**

Taurine is lowly concentrated in most terrestrial plants, such as soy, and therefore, it is largely believed that taurine may be particularly limiting for fish when feeds are produced using plant-based protein (Salze & Davis 2015). However, in the present study we found that CYT post larvae benefitted from dietary taurine concentrations above those provided by taurine-unsupplemented PARA particles (0.4% TAU treatment) that had been prepared with marine animal protein sources (krill and squid meal). These results show the importance of taurine supplementation in a wide range of fish diets, beyond those simply produced with alternative protein sources. Our results are consistent with other studies that evaluated the effects of taurine supplemented diets on the growth of early stage marine fish. For instance, Matsunari et al. (2005) showed that *S. quinqueradiata* juveniles grew at higher rates when fed increasing concentrations of dietary taurine. In the 2005 study, diets included fish, krill and squid meal for the primary protein sources. Pinto et al. (2010) found that Senegalese sole (*Solea senegalensis*) had enhanced rates of growth and metamorphosis when fed microcapsules supplemented with taurine when compared to those fed microcapsules without taurine supplementation. In the 2010 study, microcapsules were prepared with a mixture casein as well as fish and cuttlefish meals for the primary protein sources. Kim et al. (2007) found that Japanese flounder juveniles (*Paralichthys olivaceus*) showed increasing growth rates and whole body taurine concentrations with increasing dietary taurine concentrations. While, diets in the 2007 study were also prepared from marine fish protein (jack mackerel meal) it should be noted that the fish meal had been pre-washed to partially purify the diets of taurine.

In this study, CYT were co-fed with *Artemia* from the beginning of the trial until 35 dph and the experiment was terminated on 42 dph. The average taurine concentration of *Artemia* used in this study was 8.1 ± 0.6 g $kg^{-1}$ DW. While feed intake of the microparticulate diets was measured throughout the study, the intake of *Artemia* nauplii was not. Therefore the relative consumption of *Artemia* when compared to the microparticulate diets by CYT cannot be

determined in this study and is subject to speculation. Presumably, *Artemia* were the major source of food (and therefore taurine) during the first phases of weaning and microparticulate diets were the major source of food towards the later phases. However, given that fish grew faster and stored higher quantities of taurine in body tissues in treatments with diets that were supplemented with taurine suggests that *Artemia* were not a sufficient source of taurine during the weaning phase. There are two factors that may explain this: 1) *Artemia* have relatively low concentrations of taurine and have been a poor source of taurine for the fish and/or 2) *Artemia* may have represented a small fraction of the total diet. In either case, our data suggest that CYT benefit from taurine-supplemented microparticulate diets during the weaning phase.

We found that sodium fluorescein and taurine were rapidly leached from PARA particles, as shown by the low retention efficiencies, when suspended in seawater. The high leaching observed in this study is likely due to the small particle sizes and therefore high surface area to volume ratios of the microparticulate diets (Langdon 2003). Retention efficiencies of sodium fluorescein were best modeled with an exponential decay model. Furthermore, exponential decay models provided good fit for the taurine leaching data (all adjusted R-squared values > 0.96). We also found that retention efficiencies decreased, i.e. increased leaching, with increasing taurine inclusion rates in microparticulate diets (GLM, $p < 0.001$). The trends we observed were similar to those reported by Watson et al. (2013), whereby taurine leaching rates increased with increasing taurine concentrations. However, leaching rates of taurine (overall mean of 59.13% ± 17.54 after 40 min suspension) in the 2013 study were lower than those in the present study (>80% after 15 min in all treatments) which is likely due to differences in particle size, i.e. 4 mm particles in 2013 study vs. ~0.25 mm in the present study.

Based on sink rates, we estimated that PARA particles were available to the larvae for less than 2 minutes after adding them to the tanks. For this reason, we estimated the minimum quantities of taurine, i.e. lower 95% CI that would have remained in the PARA particles after 2 minutes of suspension in seawater. We found that they ranged from 29 to 65% (based on the lower 95% C.I.) of the initial taurine provided in the diets. These values represent worst-case losses and do not likely represent the actual dietary taurine levels experienced by the fish since much of the feeding occurred before 2 minutes. The potential for high leaching from microparticulate diets suggests that the results of the feeding trial must be interpreted with

caution. These results highlight the importance of considering nutrient leaching when conducting larval feeding trials utilizing microparticulate diets for nutrient delivery.

We used yttrium oxide ($Y_2O_3$) as a feed marker of PARA diets and were able to compare feed consumption by the CYT as a consequence of dietary treatment. We found that the quantity of feed per larvae ($ug^{-1}$ larvae) was significantly lower in the LT treatment when compared to the HT and VHT treatments and that the quantity of feed per animal body weight was lower in the LT treatment when compared to all others ($\mu g\ g^{-1}$; Figure 4). The most likely explanation is that fish may have eaten more because they were larger. That is, fish in the MT, HT and VHT treatments were significantly larger than those in the LT treatment and may have consumed more food as a result. This is strongly supported by the finding that feed consumption rates were not significantly different when standardized by larval body weights. There are two alternative, though be it less likely, explanations: First, it is possible that taurine may have improved the taste or smell of the feed and thereby increased feeding by CYT, serving as a feed stimulant or attractant. Few studies have investigated the role of taurine as a feed stimulant and those few studies have provided little evidence for such an effect. Although it was shown that the common carp (*Cyprinus carpio*) had an attraction effect when offered a formulated diet with an inclusion rate of 0.4% (Xiaocong et al. 2006). Second, increased dietary taurine may have resulted in larvae that were not only larger but had more acute senses, when compared to those that received low levels of dietary taurine. Specifically, elevated dietary taurine may have resulted in more improved development of visual and olfactory senses of CYT, which may have enhanced the abilities of larvae to locate and ingest food in the MT, HT and VHT treatments. This explanation is plausible given that taurine is known to play a role in neurological function and in eye development (Huxtable 1992, Bouckenooghe et al. 2006). More research is needed to address these hypotheses.

Based on growth (SL and WW) differences and whole body taurine concentrations measured at 41 dph, it appears that optimal taurine concentration for newly weaned CYT is > 4 but ≤ 45 g $kg^{-1}$ based on initial (pre-leached) diet concentrations. However, corrected for nutrient leaching, this range may be expanded to > 2.6 and but ≤ 45 g $kg^{-1}$ (Table 3). Our results agree with previous studies that have investigated the effects of dietary taurine on *Seriola* larvae and juveniles. For instance, Hawkyard et al. (2015b) showed that the naturally occurring taurine concentrations in *Artemia* meta nauplii (~7 g $kg^{-1}$) were sufficient to maximize larval growth (SL

and DW) of CYT larvae. In the same study they found that larval whole body taurine concentrations where higher when larvae were fed *Artemia* enriched with taurine to a concentration of 16 g $kg^{-1}$ when compared to those fed taurine-unenriched *Artemia*. Matsunari et al. (2005) showed that *S. quinqueradiata* juveniles (starting weight 0.5 g $fish^{-1}$) showed increased growth and body taurine concentrations over a range of dietary taurine concentrations between 3.9 to 13.0 g $kg^{-1}$, however, dietary taurine concentrations above 13 g $kg^{-1}$ had little effect. Jirsa et al. (2014b) estimated that CYT juveniles (starting weight 5.0 g $fish^{-1}$) had a taurine requirement of 3.2 g $kg^{-1}$. Future research is needed to develop a requirement for newly weaned CYT post larvae. This work should include dietary taurine treatments in the range of 4 and 45 g $kg^{-1}$.

Our results provide evidence that CYT post larvae were not adversely affected by the highest taurine inclusion rates tested in this study (122 g $kg^{-1}$ of the diet), i.e. nutrient toxicity was not observed. Specifically, growth, survival and feed consumption rates were not significantly different in the MT, HT and VHT treatments. However, PARA particles containing 122 g $kg^{-1}$ (VHT) taurine were especially prone to leaching and may have had taurine concentrations as low as 34.9 g $kg^{-1}$ before they settled on the bottom of the tank (2 min). Therefore, the actual dietary taurine concentrations experienced by the larvae were likely lower than the initial (pre-leached) dietary concentrations. It should be noted that, growth (wet weight and standard length) and survival were not significantly higher in the VHT treatment suggesting a minor negative effect of high dietary taurine concentrations. Of course, at some inclusion level, taurine will have a negative effect on the fish due to nutrient dilution; presumably fish cannot survive on taurine alone. More research is needed to evaluate the potential nutrient toxicity of elevated dietary taurine concentrations for marine fish larvae and juveniles.

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Table 1. Model selection criteria (AIC and BIC) for candidate models used for retention efficiencies of sodium fluorescein from PARA particles.

| **Model** | **AIC** | **BIC** |
|---|---|---|
| Linear | 175.4 | 176.4 |
| Quadratic | 160.5 | 161.0 |
| Cubic | 144.0 | 143.5 |
| Logistic | 132.7 | 133.1 |
| Exponential | 129.3 | 130.3 |

Table 2. Ingredients used to produce experimental microparticulate diets. Values represent the quantities (g $kg^{-1}$) of ingredients added to the mash unless otherwise specified.

| | **Experimental microparticulate diets used in feeding trial** | | | |
|---|---|---|---|---|
| **Ingredient** | LT | MT | HT | VHT |
| Squid Meal [a] | 392 | 381 | 370 | 356 |
| Krill Meal [b] | 256 | 249 | 240 | 232 |
| Fish Oil [c] | 105 | 102 | 99 | 95 |
| Lecithin [d] | 100 | 97 | 94 | 91 |
| Wheat Gluten Meal [e] | 90 | 87 | 85 | 82 |
| Vitamin premix [f] | 20 | 19 | 19 | 18 |
| Di-calcium Phosphate | 20 | 19 | 19 | 18 |
| Taurine [g] | 0 | 29 | 57 | 91 |
| Astaxanthin [h] | 5 | 5 | 5 | 5 |
| Vitamin-C [i] | 2 | 2 | 2 | 2 |
| $Y_2O_3$ | 10 | 10 | 10 | 10 |
| Protein (%) | 49 | 49 | 50 | 49 |
| Lipid (%) | 26 | 25 | 25 | 25 |
| Energy density (kcal $g^{-1}$) | 5.9 | 5.7 | 5.7 | 5.6 |

[a] Wilbur-Ellis, Adams, Oregon, USA, 723 g $kg^{-1}$ crude protein

[b] International Proteins Corp., New York, New York,USA.

[c] Mixed Pacific Fish Oil, Bio-Oregon Proteins, Warrenton, Oregon, USA

[d] Soy, refined, American Lecithin, Oxford, Connecticut, USA

[e] MGP Ingredients, Inc., Atchison, KS, USA, 820 g $kg^{-1}$ crude protein.

[f] ARS 702; contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 gm: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5; inostitol 600.

[g] Alliance Nutrition, Twin Falls, Idaho, USA.

[h] Carophyl Pink 10, DSM Nutritional Products, Boulder, Colorado, USA

[i] Stay C, DSM Nutritional Products, Boulder, Colorado, USA

Table 3. Taurine concentrations measured in experimental microparticulate diets post-production as well as the estimated TAU concentrations remaining after 2 minutes of suspension in seawater. Estimates were based on the exponential decay model derived from taurine retention data. Treatments sharing superscript letters are not significantly different.

| **Treatment** | **Initial TAU conc.** | **Est. TAU conc. after 2 min suspension (g $kg^{-1}$)** | |
|---|---|---|---|
| | **(g $kg^{-1}$ ± SD)** | Lower 95% | Upper 95% |
| LT | 4.0 ± 0.2[a] | 2.6 | 3.3 |
| MT | 45.0 ± 4[b] | 27.5 | 37.5 |
| HT | 93.0 ± 6[c] | 41.0 | 70.8 |
| VHT | 122 ± 9[d] | 34.9 | 115.3 |

Table 4. Total amino acid (TAA mg AA $g^{-1}$ DW ± SD) composition of the experimental microdiets. Treatments sharing superscript letters within rows are not significantly different.

| | **Experimental microparticulate diets used in feeding trial** | | | |
|---|---|---|---|---|
| | LT | MT | HT | VHT |
| **AA** | | | | |
| Ala | 2.37 ± 0.02$^{a}$ | 2.24 ± 0.06$^{ab}$ | 2.19 ± 0.01$^{bc}$ | 2.10 ± 0.04$^{c}$ |
| Arg | 2.93 ± 0.01$^{a}$ | 2.73 ± 0.04$^{ab}$ | 2.70 ± 0.01$^{bc}$ | 2.58 ± 0.01$^{c}$ |
| Asp | 4.53 ± 0.04$^{a}$ | 4.24 ± 0.08$^{ab}$ | 4.19 ± 0.01$^{b}$ | 4.02 ± 0.04$^{b}$ |
| Glu | 8.71 ± 0.07$^{a}$ | 8.38 ± 0.24$^{ab}$ | 8.12 ± 0.13$^{bc}$ | 7.83 ± 0.18$^{c}$ |
| Gly | 2.20 ± 0.04$^{a}$ | 2.06 ± 0.01$^{b}$ | 2.02 ± 0.03$^{bc}$ | 1.93 ± 0.02$^{c}$ |
| His | 1.18 ± 0.01$^{a}$ | 1.09 ± 0.02$^{ab}$ | 1.08 ± 0.01$^{b}$ | 1.04 ± 0.01$^{b}$ |
| Ile | 2.19 ± 0.01$^{a}$ | 2.05 ± 0.05$^{ab}$ | 2.00 ± 0.02$^{bc}$ | 1.95 ± 0.04$^{c}$ |
| Leu | 3.81 ± 0.01$^{a}$ | 3.62 ± 0.12$^{ab}$ | 3.51 ± 0.02$^{b}$ | 3.40 ± 0.08$^{b}$ |
| Lys | 3.05 ± 0.01$^{a}$ | 2.84 ± 0.05$^{ab}$ | 2.81 ± 0.02$^{b}$ | 2.70 ± 0.01$^{b}$ |
| Met | 1.24 ± 0.01$^{a}$ | 1.15 ± 0.03$^{ab}$ | 1.13 ± 0.01$^{b}$ | 1.09 ± 0.01$^{b}$ |
| Phe | 1.99 ± 0.01$^{a}$ | 1.87 ± 0.07$^{ab}$ | 1.81 ± 0.01$^{ab}$ | 1.79 ± 0.05$^{b}$ |
| Pro | 2.69 ± 0.16 | 2.31 ± 0.43 | 2.23 ± 0.30 | 2.37 ± 0.01 |
| Set | 2.22 ± 0.02$^{a}$ | 2.12 ± 0.05$^{ab}$ | 2.07 ± 0.02$^{bc}$ | 1.97 ± 0.03$^{c}$ |

| | | | | |
|---|---|---|---|---|
| Thr | 2.06 ± 0.02[a] | 1.94 ± 0.04[ab] | 1.91 ± 0.01[bc] | 1.82 ± 0.02[c] |
| Tyr | 2.07 ± 0.04[a] | 1.99 ± 0.02[ab] | 1.97 ± 0.01[ab] | 1.80 ± 0.01[b] |
| Val | 2.18 ± 0.01[a] | 2.03 ± 0.06[ab] | 1.98 ± 0.02[b] | 1.94 ± 0.04[b] |

Ala-alanine; Arg-arginine; Asp-aspartate; Glu-glutamine; Gly-glycine; His-histidine; Ile-isoleucine; Leu-leucine; Lys-lysine; Met-methionine; Phe-phenylalanine; Pro-proline; Ser-serine; Thr-threonine; Tyr-tyrosine; Val-valine

Table 5. Growth and survival for California yellowtail (S. dorsalis) at 41 dph. Values sharing lowercase superscripts are not significantly different.

| Treatment | Wet Weight (g ± SD) | Standard Length (mm ± SD) | Survival (Percent ± SD) |
|---|---|---|---|
| LT | 0.24 ± 0.12[a] | 21.3 ± 3.7[a] | 33.9 ± 4.3[a] |
| MT | 0.29 ± 0.12[b] | 24.5 ± 3.7[b] | 31.3 ± 2.4[a] |
| HT | 0.28 ± 0.11[b] | 23.8 ± 3.5[ab] | 34.5 ± 3.1[a] |
| VHT | 0.26 ± 0.11[ab] | 23.4 ± 3.5[ab] | 30.1 ± 3.2[a] |

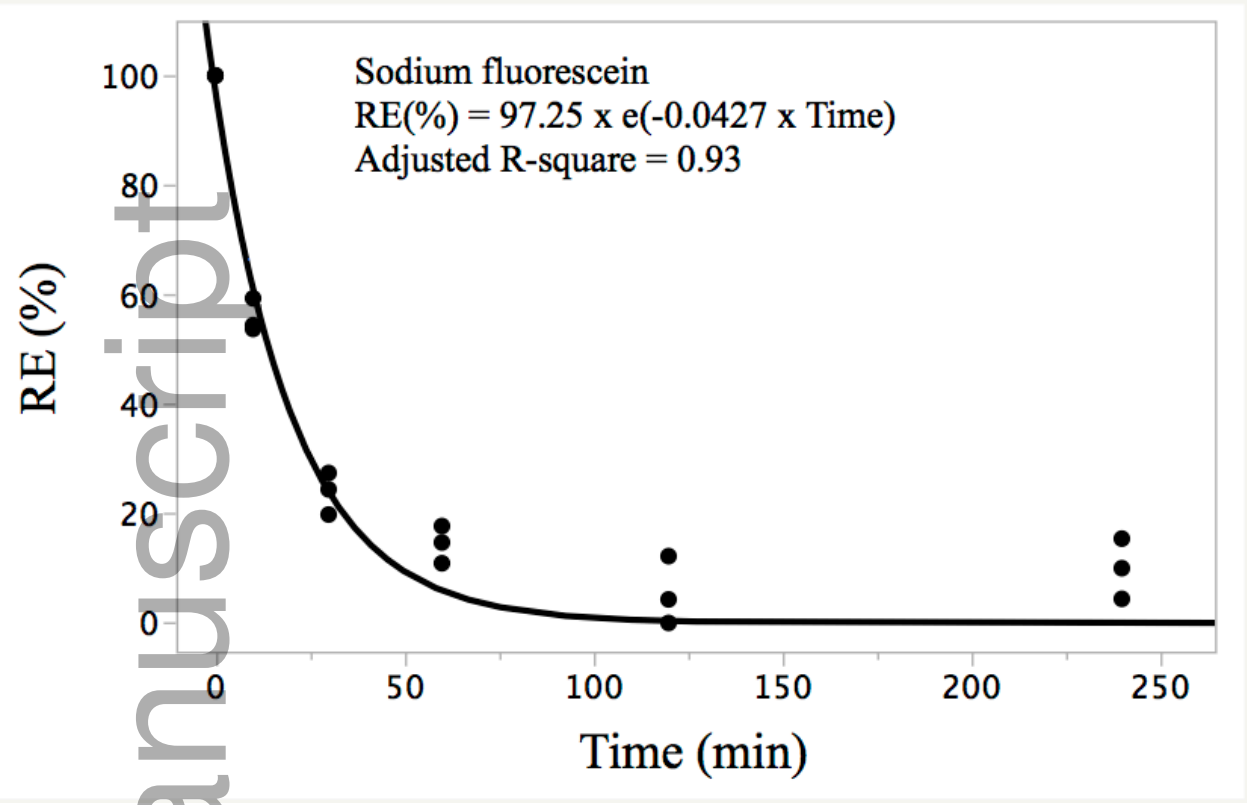


Figure 1. Retention efficiencies (RE; %) of sodium fluorescein measured in PARA particles following suspension in seawater. Sodium fluorescein was used as a proxy for water-soluble nutrients and allowed for model selection using AIC and BIC (Table 1). An exponential function (shown) was selected as the best model and was used for all subsequent analyses (i.e. with taurine). Adjusted R-square is displayed as a summary measure and was not used for model selection.

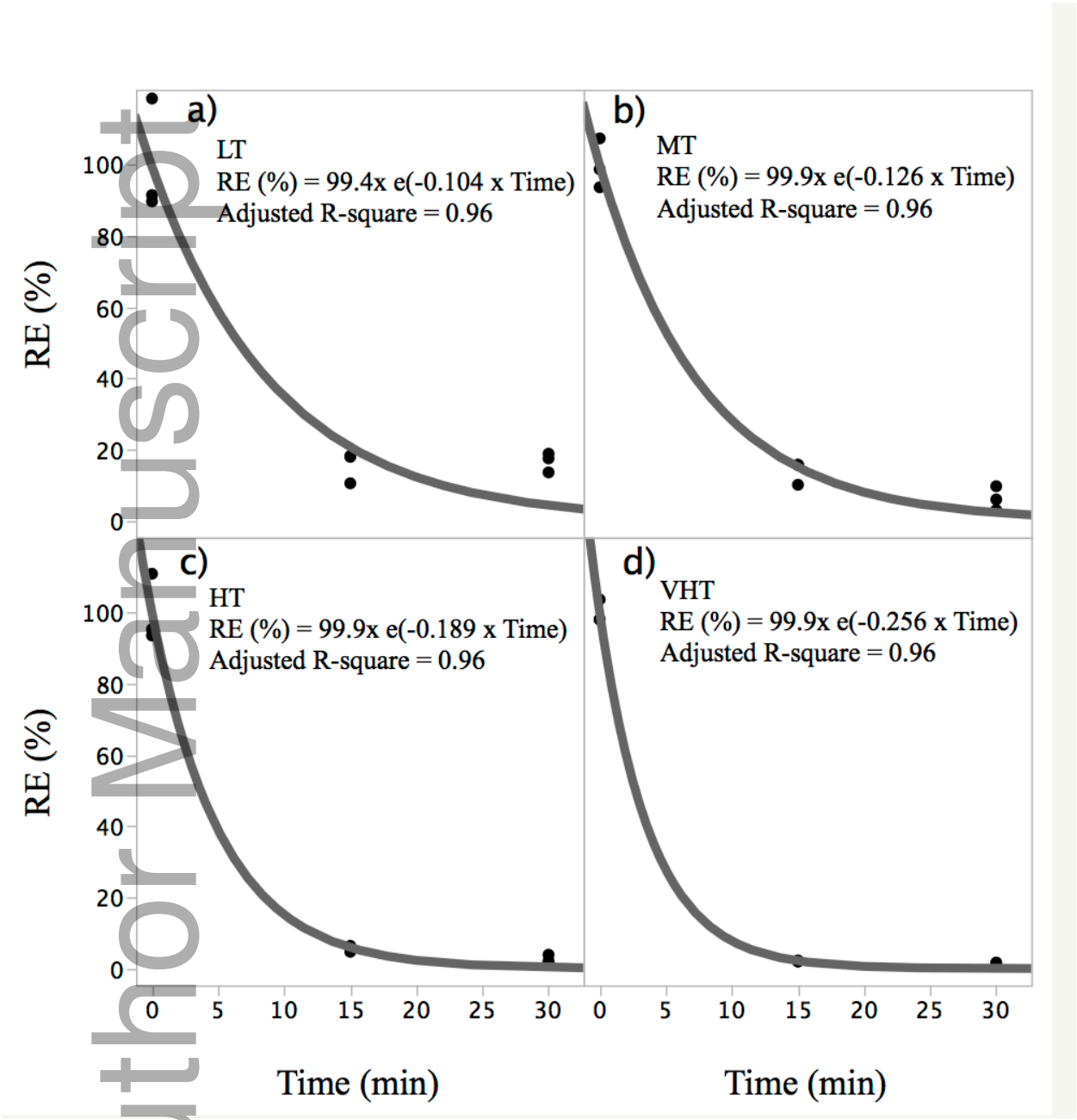


Figure 2. Retention efficiencies (RE; %) of taurine measured in PARA particles following suspensions in seawater. The exponential decay model shown and was used to estimate taurine retention at 2 minutes and T75 values (Table 3). Adjusted R-square values are displayed as summary measures and were not used for model selection.

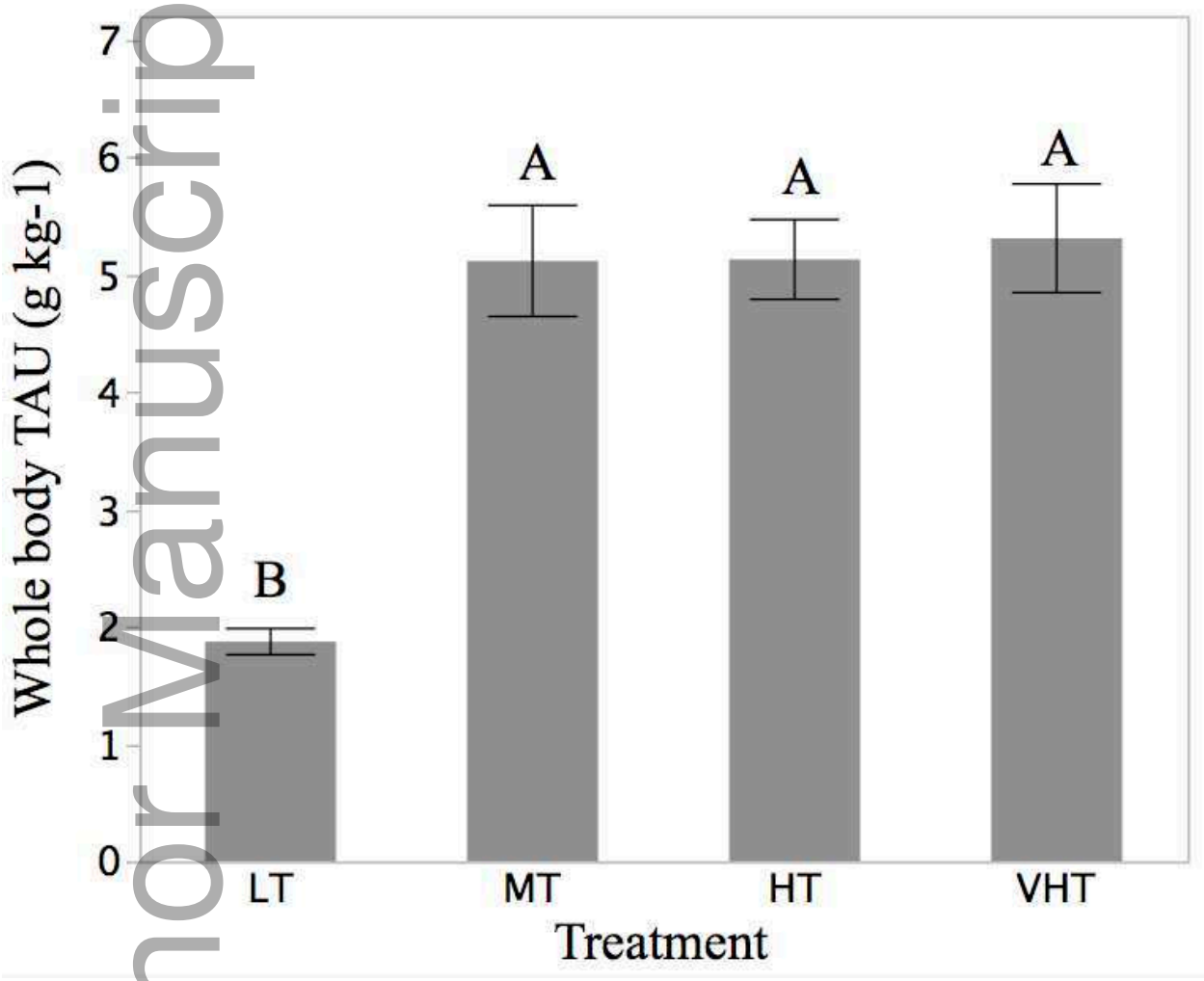


Figure 3. Whole body taurine (TAU) for California yellowtail (*S. dorsalis*) fed four different concentrations of taurine. Treatments sharing capital letters are not significantly different.

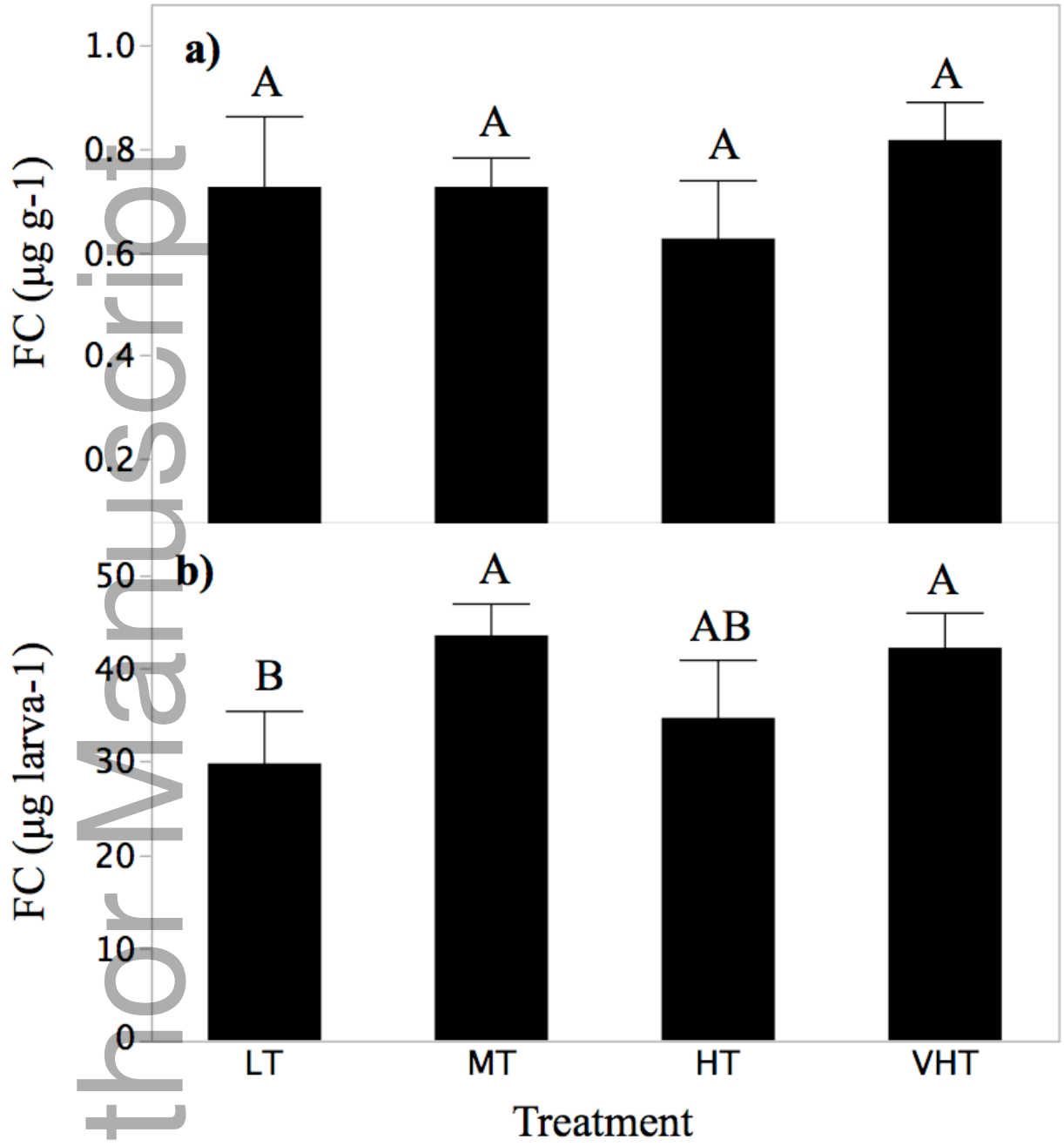


Figure 4. Feed consumption (FC) for California yellowtail (*S. dorsalis*) at 41 dph. Data are expressed as weight specific feed consumption (µg $g^{-1}$; upper) feed consumption per fish (µg $g^{-1}$; lower). Values sharing capital letters are not significantly different.