The performance of larval *Seriola lalandi* (Valenciennes, 1833) is affected by the taurine content of the *Artemia* on which they are fed.

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Abstract

This study describes the effects of feeding taurine-supplemented *Artemia* on the growth, survival, whole-body taurine content and jaw malformation rate of larval yellowtail kingfish *Seriola lalandi*. Larvae were fed rotifers containing no supplemental taurine from 3 to 15 dph and *Artemia* co-enriched with taurine from 12 to 22 dph. *Artemia* were supplemented at concentrations of either 0, 0.8, 1.6, 2.4, 3.2 or 4.0 grams of taurine per litre during the 18 hour HUFA enrichment process. Taurine content in the *Artemia* increased from 0.76 ± 0.04% DW in those without supplementation to 3.95 ± 0.17% DW in those supplemented at 4.0 g L^{-1}. Survival rates of larval yellowtail kingfish were significantly lower in all taurine supplemented treatments compared to the unsupplemented control. Growth was significantly improved in those larvae fed taurine supplemented *Artemia*, however we cannot attribute this improvement solely to taurine, as improved growth may have been a function of the reduced survival, and therefore increased prey availability, in these treatments. The whole-body taurine content of larvae fed unsupplemented *Artemia* was significantly lower (1.85 ± 0.03% DW) than those fed supplemented *Artemia*, which did not differ from each other (pooled average 2.48 ± 0.03% DW), suggesting either a functional excretion mechanism is in place or that this represents the saturation value for larvae of this age. Jaw malformation rates were not affected by *Artemia* taurine content. The results of this research suggest yellowtail kingfish larvae may have a lower requirement and/or a reduced tolerance to excess dietary taurine than juveniles.

1. Introduction

Yellowtail kingfish *Seriola lalandi* is an established aquaculture species in Japan and Australia (Nakada 2002; Fielder 2013) and is being investigated in many other countries and regions including New Zealand (Poortenaar, Hooker & Sharp 2001), the Americas (Benetti, Nakada, Shotton, Poortenaar, Tracy & Hutchinson 2005) and Europe (Abbink, Blanco Garcia, Roques, Partridge, Kloet & Schneider 2012). Unlike Japan, growout production in Australia is reliant upon hatchery produced juveniles and production is somewhat constrained by relatively low larval survival rates and juvenile quality, particularly jaw malformations (Cobcroft, Pankhurst, Poortenaar, Hickman & Tait 2004). The rotifers and *Artemia* used to feed yellowtail kingfish larvae contain lower concentrations of many nutrients and trace elements than the wild zooplankton on which marine fish larvae naturally feed, including taurine, and such deficiencies may be at least partially responsible for such malformations (Cobcroft *et al.* 2004).

Taurine is a neutral β-amino acid. It differs from most amino acids in that it lacks a carboxyl group and does not form peptide bonds, but it is the most abundant free amino acid in animal tissues, including in marine fish larvae, accounting for up to 50% of the free amino acid pool (Conceicao, van der Meeren, Verreth, Evjen, Houlihan & Fyhn 1997). It is found throughout the body in muscle, brain, ocular tissues, intestines, plasma and blood cells (Ripps & Shen 2012; El-Sayed 2013). It is involved in many different physiological processes such as digestion via bile acid metabolism and the regulation of blood cholesterol levels, neuromodulatory actions, cardiac Ca^{2+} modulation, osmoregulation, vision, renal function, brain development and reproduction

(Ripps & Shen 2012). It acts as a broad-spectrum cytoprotective agent and antioxidant (Ripps & Shen 2012) and has been found to improve gut development (Li, Mai, Trushenski & Wu 2009). It is considered a growth promoter in many fish species possibly due to its role in enhancing the absorption of lipids and lipid-soluble vitamins. Furthermore, and of potential importance to *Seriola* larviculture, is taurine's role in bone growth via the stimulation of bone-forming osteoblasts and preventing formation of bone-degrading osteoclasts (Salze, Craig, Smith, Smith & McLean 2011).

Because taurine is not involved in protein synthesis, it is often considered to be 'non-essential', however based on its many physiological roles and broad distribution throughout the body, taurine is clearly a critical nutrient (Ripps & Shen 2012). Taurine is certainly essential for those species which lack the ability to produce it from its precursors, L-cysteine and methionine, due to a lack of cysteinesulfinic acid decarboxylase (CSD) activity (Ripps & Shen 2012). Many pelagic marine species fall into this category and whilst the CSD activity of yellowtail kingfish has not been studied, Japanese yellowtail *Seriola quinqueradiata* (Temminck & Schlegel) has been demonstrated to be completely lacking in this enzyme (Yokoyama, Takeuchi, Park & Nakazoe 2001). The rotifers and *Artemia* used to culture *Seriola* sp. contain lower concentrations of taurine than the zooplankton on which they would naturally feed. The taurine content of wild zooplankton, for example, ranges from 0.58% to 1.77% DW, whereas *Artemia* have been reported to contain only 0.63% to 0.83% DW (van der Meeren, Olsen, Hamre & Fyhn 2008 ; Yamamoto, Teruya, Hara, Hokazono, Hashimoto, Suzuki, Iwashita, Matsunari, Furuita & Mushiake 2008) and rotifers even less at 0.04% to 0.19% DW. Furthermore, it has been hypothesized that larval fish may

have a higher requirement for taurine than older fish due to the development of their organ systems and based on the fact that egg and yolk-sac larvae contain high levels of taurine (Pinto, Figueira, Ribeiro, Yúfera, Dinis & Aragão 2010 ; Pinto, Figueira, Santos, Barr, Helland, Dinis & Aragão 2013). These factors suggest that taurine supplementation may be necessary for the larviculture of yellowtail kingfish. Indeed, preliminary studies with this species have demonstrated a benefit of feeding taurine-enriched rotifers (Rotman, Stuart & Drawbridge 2012), however studies have not been conducted during the *Artemia* feeding stage. In a preliminary, unpublished study we found reduced survival of yellowtail kingfish larvae fed *Artemia* enriched with taurine at the concentration of 4 g L^{-1} as per the methods of Salze *et al.* (2011). The aim of this study was therefore to confirm these findings and investigate the effect of lower taurine enrichment concentrations on the taurine content of *Artemia* and the subsequent effect these *Artemia* have on the growth, survival, jaw malformation rate and whole-body taurine content of yellowtail kingfish larvae.

2. Materials and methods

2.1 Larval fish rearing system

Fertilized yellowtail kingfish eggs were sourced from captive broodstock held at Clean Seas Tuna Ltd., Arno Bay (South Australia) and transported to the Australian Centre for Applied Aquaculture Research (Western Australia). The eggs were hatched in a 1000 L incubator at 22 °C. After hatching, 1 day post hatch (dph) larvae were randomly stocked into twelve 300 L tanks at 60 larvae L^{-1}. All 12 tanks were treated equally during the

first 12 days. The rearing tanks were part of a flow-through system supplied with filtered seawater (34 g L^{-1} with an exchange rate of 54 L h^{-1} (400% daily water exchange) in each tank. Each of four rearing tanks were floated in three 5000 L tanks to maintain the water temperature at 24 °C throughout the experiments. All tanks were completely independent of each other and no mixing of water between them was possible. Two airstones were placed in each tank to maintain the dissolved oxygen levels close to saturation. During the first 15 days a diffused metal halide light (400 W) above each 5000 L tank provided a surface light intensity of 4600 ± 1250 lux at the center of each rearing tank for a photoperiod of 12 h light (0800 to 2000 h) and 12 h dark. Microalgal paste (*Nannochloropsis* sp., Reed Mariculture Inc., USA) was automatically dosed into the rearing tanks during daylight hours to maintain turbidity within the range of 1.7 to 1.9 NTU (equivalent to a secchi disk depth of 55 to 60 cm) from initial stocking until the end of the rotifer feeding stage. On 15 dph microalgal additions ceased and the light intensity was reduced to 800 ± 150 lux at the centre of each rearing tank.

Between 3 and 12 dph larvae were fed only rotifers enriched with SPRESSO (INVE Aquaculture, Belgium) and without taurine enrichment under the hybrid feeding protocol described by (Woolley & Partridge 2015). From 12 dph, feeding on taurine-enriched *Artemia* metanauplii began. *Artemia* were enriched with SPRESSO (INVE Aquaculture, Belgium) according to manufacturer's directions. *Artemia* were coenriched with taurine (Henan Aowei International, China) during the 18 hour enrichment period at one of six concentrations 0, 0.8, 1.6, 2.4, 3.2 and 4.0 g TAU L^{-1} following the method of Salze *et al.* (2011). During 12 to 15 dph larvae were co-fed

with rotifers and *Artemia*. Treatments were randomly allocated across the 12 experimental tanks, with duplicate larval rearing tanks per treatment. *Artemia* were fed to larvae according to the adaptive feeding method described by Woolley, Partridge & Qin (2012).

2.3 Sampling Protocol

Sub-samples (ca. 100 g) of *Artemia* from each enrichment tank were taken on two different days, rinsed in freshwater, frozen and then freeze-dried. Total taurine was analyzed as part of total amino acid profile on each sample via HPLC following homogenization then hydrolysis in 6 M HCl with 0.5% phenol for 24 hours at 110°C according to Rayner (1985) and Barkholt & Jensen (1989). Purity of the taurine was also determined via this method by comparing against a pure taurine standard (Sigma Alrich, T-0625). Heavy metal analysis of the taurine was conducted by preparing an acidified 1% solution in deionised water following analysis on an Agilent 730 Axial Simultaneous CCD ICP-OES.

Larval dry weight were assessed on 23 dph on 20 randomly selected larvae per tank. Individual *Artemia* were quantified in the larval guts (5 per tank) by counting undigested *Artemia* eyes under a stereomicroscope one hour after the first feed on 13, 15, 17 and 19 dph in squash-mounted fresh larvae. The trial was terminated on 23 dph and larvae from each tank were hand counted to determine the survival. One hundred larvae from each tank were anesthetized without recovery, and fixed in 10% formaldehyde then transferred to 70% ethanol for jaw deformity assessment according

to methods described by Cobcroft *et al.* (2004). Jaws classified as a commercial cull by the industry are represented as a percentage of the total number of larvae assessed. The remaining larvae from each tank were pooled, rinsed to remove seawater and frozen for analyses of taurine as described above. Following the freeze-drying and grinding of these pooled larvae a 2 gram subsample was analysed for taurine as described above.

2.4 Statistics

One-way ANOVA was used to determine differences between treatments in final growth of larvae, jaw deformity rates and larval taurine uptake. A repeated measures ANOVA was used to determine the effect of larval age and *Artemia* enrichment treatment on the number of *Artemia* consumed. Regression analysis was used to determine the effect of dosage concentration on the taurine uptake in *Artemia* and the relationship between taurine content in *Artemia* and larval taurine whole body content. Data were arcsine transformed where necessary to ensure homogeneity of variance. Significance was set at $P < 0.05$ and values are presented as mean ± SE. All statistical analyses were performed using IBM Statistics 20 (Release 20.0, Chicago, IL, USA).

3. Results

The purity of the taurine was measured at 99.55% and all heavy metals were below their respective detectable limits.

Artemia taurine content was significantly affected by enrichment concentration ($P < 0.001$; Figure 1). Those *Artemia* not receiving taurine supplementation had a total taurine content of 0.76 ± 0.04% DW, significantly lower than all treatments receiving supplementation. Total *Artemia* taurine content increased from 1.79 ± 0.27% at the lowest concentration of 0.8 g L^{-1} to 3.77 at 3.2 g L^{-1}. *Artemia* taurine plateaued at an enrichment concentration of 3.2 g L^{-1}, with no significant difference in *Artemia* taurine content between a concentration of 3.2 and 4.0 g L^{-1} (3.77 ± 0.13 % and 3.95 ± 0.16% DW, respectively). The relationship between the taurine content of the *Artemia* and the taurine enrichment concentration was described by the following equation with an R^2 of 0.99; Equation 1: *Artemia* taurine content (% DW) = $0.76 + 4.35 \times (1 - \exp^{(-0.34 \times \text{taurine concentration})})$.

Larval survival to 23 dph was significantly affected by treatment (Figure 2). Survival in the control (0 g L^{-1}) treatment (10.4 ± 1.1%), was significantly higher than all taurine enriched treatments, which did not differ from each other (pooled average 4.7 ± 1%; $P = 0.001$). As a result of the significant differences in survival, final larval density was also significantly affected by treatment ($P = 0.001$). Larval density at the end of the trial in the control treatment (5.5 ± 0.4 larvae L^{-1}) was more than double that in all taurine enriched treatments, which did not differ from each other (pooled average 2.5 ± 0.18 larvae L^{-1}).

Final larval dry weights were significantly affected by enrichment concentration ($P = 0.01$ and $P = 0.04$, respectively). Those larvae fed *Artemia* without taurine enrichment were significantly smaller (3.8 ± 0.3 mg DW; Figure 3) than those receiving *Artemia* at

all enrichment concentrations, except those enriched at 2.4 g L^{-1}. The average final dry weight of the larvae was strongly and negatively correlated with their survival (R^2 = 0.81; Figure 4). *Artemia* ingestion by larvae increased significantly with age ($P > 0.001$) but was equal across all treatments ($P = 0.05$; Figure 5).

Larval whole-body taurine content was significantly affected by *Artemia* taurine content ($P < 0.001$). Those larvae receiving unsupplemented *Artemia* had a significantly lower whole-body taurine content (1.85 ± 0.03% DW) than all taurine supplemented treatments, which did not differ from each other (pooled average 2.48 ± 0.03% DW). The relationship between the taurine content of the *Artemia* and the whole body taurine content of the larvae was described by the following equation with an R^2 of 0.98 (Figure 6).

Equation 2: $\textit{Larval whole body taurine} = 0.79 + 1.75 \times (1 - \exp^{(-1.2 \times \textit{Artemia taurine content})})$.

There was no effect of taurine enrichment concentration on jaw deformity levels (P= 0.77; Figure 7). Deformities considered a 'commercial cull' by the industry were 17.3 ± 1.5% (pooled mean ± SE).

4. Discussion

This appears to be the first study investigating the response of live foods to a range of different taurine enrichment concentrations. Our results demonstrate that *Artemia* effectively take up taurine during the enrichment process in a relationship that is linear

236 at low concentrations then plateaus at the highest concentrations tested. The *Artemia*
237 taurine levels we achieved in all but the lowest enrichment concentration were higher
238 than typically seen in wild zooplankton (range 0.58 to 1.70% DW; (van der Meeren *et*
239 *al.* 2008 ; Yamamoto *et al.* 2008). The rates of supplementation we selected were based
240 on the study by Salze *et al.* (2011) in which larval cobia *Rachycentron canadum*
241 (Linnaeus) were fed rotifers and *Artemia* which had both been enriched with taurine at a
242 concentration of 4.0 g L^{-1}. Whilst the taurine content of the live foods were not
243 presented by these authors, they were reported in a later publication as being ca. 0.08%
244 and 0.23%, on a wet weight basis for unsupplemented and supplemented *Artemia*,
245 respectively (Salze, McLean & Craig 2012). To convert these values to % DW, we
246 measured the water content of *Artemia* at 90% and subsequently calculated these values
247 to equate to ca. 0.8% DW and 2.3% DW, respectively. Whilst this level in the
248 unsupplemented *Artemia* is equivalent to that reported here, the level we achieved in
249 *Artemia* supplemented at 4.0 g L^{-1} was much higher than achieved by Salze *et al.* (2012)
250 (3.95% DW cf. 2.3% DW). These differences may have been due to differences in
251 enrichment time, as it has been demonstrated that taurine uptake by live feeds is time-
252 dependent (Chen, Takeuchi, Takahashi, Tomoda, Koiso & Kuwada 2004 ; Chen,
253 Takeuchi, Takahashi, Tomoda, Koiso & Kuwada 2005). Further studies are therefore
254 required to fully elucidate the interactive effects of enrichment time and concentration
255 on the taurine content of live foods.
256
257 All larvae receiving taurine enriched *Artemia* in the current trial experienced
258 significantly lower survival than those receiving unsupplemented *Artemia* and this
259 could not be attributed to impurities in the taurine used. This finding is consistent with

our preliminary unpublished study where *Artemia* were enriched at 4.0 g L^{-1}. The majority of published studies dealing with the taurine enrichment of live foods for marine fish larvae report no benefit or negative impact on survival. For example, survival of Japanese flounder *Paralichthys olivaceus* (Temminck & Schlegel), red sea bream *Pagrus major* L., Pacific cod *Gadus macrocephalus* L. and northern rock sole *Lepidopsetta polyxystra* (Orr & Matarese) larvae were not improved by increasing the taurine content of rotifers to between ca. 0.3 and 0.45% DW (Hawkyard, Laurel, Barr, Stuart, Drawbridge & Langdon 2014a; Chen *et al*., 2005; Chen *et al*., 2004; Matsunari, Arai, Koiso, Kuwada, Takashi & Takeuchi 2005a). Likewise there was no benefit to the survival of gilthead sea bream *Sparus aurata* L. larvae when fed rotifers whose taurine content had been increased from 0.88% of total amino acids to 1.41% (Pinto *et al*., 2013). On the other hand, Salze *et al.* (2011) reported that cobia larvae fed both taurine enriched rotifers (estimated taurine content 0.5% DW) and *Artemia* (estimated taurine content 2.3% DW) experienced ca. four times greater survival than those fed unsupplemented live feeds. These authors did not separate the performance of the cobia larvae between the rotifer and *Artemia* feeding phases and the reasons for the very different response of cobia larvae to supplemental taurine compared with both the yellowtail kingfish in the current trial and the other aforementioned studies on marine fish larvae is unclear.

Despite a lack of published studies showing a negative impact of taurine on the survival of marine fish larvae, there does appear to be an emerging body of evidence that live feed taurine contents higher than those reported above in the studies on Japanese flounder, red sea bream, Pacific cod and gilthead sea bream can be detrimental to

284 marine fish larvae. In two independent trials (performed in successive years), Koven,
285 Nixon, Azouli, Allon, Gaon, El Sadin, Falcon, Besseau, Escande & Tandler (2014) fed
286 rotifers with taurine contents of 0.11% DW (unenriched), 0.44% DW or 0.64% DW to
287 Atlantic bluefin tuna *Thunnus thynnus* (Temminck & Schlegel) larvae. In both studies
288 those larvae fed the highest level of taurine exhibited lower survival than those fed the
289 intermediate concentration of taurine. Similarly, Hawkyard, *et al.* (2014a) presented
290 data showing a reduction in the survival of yellowtail kingfish larvae when fed rotifers
291 enriched to contain 1.5% DW of taurine relative to the control of unsupplemented
292 rotifers. The lowest enrichment concentration tested in the current study (0.8 g L^{-1})
293 yielded *Artemia* with a taurine content of 1.8% DW; higher than those which caused a
294 negative impact on Atlantic Bluefin and yellowtail kingfish larvae by the former two
295 authors.
296
297 In terms of larval growth, most published studies report a positive benefit of enriching
298 live feeds with taurine. For example in the aforementioned trials on red sea bream,
299 Japanese flounder, Pacific cod and northern rock sole, all species grew significantly
300 faster when fed rotifers enriched to contain between 0.3% and 0.45% DW of taurine
301 than those larvae fed unsupplemented rotifers (Matsunari *et al.* 2005a ; Chen *et al.*
302 2004 ; Chen *et al.* 2005 ; Hawkyard, Laurel & Langdon 2014b). Senagalese sole *Solea*
303 *senegalensis* (Kaup) larvae fed microcapsules containing taurine grew faster than those
304 fed microcapsules without taurine (Pinto *et al.* 2010) and cobia larvae grown on taurine
305 enriched live foods grew significantly faster than those receiving unsupplemented live
306 foods (Salze *et al.* 2011).
307

Whilst we also achieved significantly greater larval growth in all taurine supplemented treatments, we are unable to attribute this difference solely to taurine, as such differences may also be at least partially attributable to differences in prey availability and/or larval density as a result of the significantly different survival rates between treatments. On the basis of previous data we suggest that larvae should not have been food limited. Based on the survival rates achieved in each treatment and with the feeding regime employed, we have calculated that larvae in the taurine enriched treatments would have had access to 416 ± 30 *Artemia* larvae^{-1} meal^{-1} at the end of the trial, whilst those in the control treatment had access to 182 ± 13 *Artemia* larvae^{-1} meal^{-1}. Whilst this difference is significant, those larvae in the control treatment had access to a similar number of *Artemia* to those in Woolley *et al*. (2012), which received approximately 140 *Artemia* larvae^{-1} meal^{-1} at the same age. That there was no correlation between survival and larval size in this aforementioned trial, even with a lower feed rate, suggests that larvae in the current trial should not have been food limited. Furthermore, the lack of difference in the measured *Artemia* ingestion rates in the current trial also supports our hypothesis that larvae were not food limited. We therefore consider that differences in larval density may have played a more important role than food availability in any differences in fish size not attributable to taurine. Larval density at the end of the trial in the control treatment (5.5 ± 0.4 larvae L^{-1}) was more than double that in all taurine enriched treatments (pooled average 2.5 ± 0.18 larvae L^{-1}) and the average final dry weight of the larvae was strongly correlated to their final larval density (R^2 = 0.81). Density dependent growth has been described in other marine fish larvae. Increasing the density of yellowfin tuna *Thunnus albacares* (Temminck & Schlegel) larvae from 2 to 18 L^{-1}, for example, resulted in growth

reductions of up to 35% (Margulies, Scholey, Wexler, Olson, Suter & Hunt 2007). Density dependent larval growth was also observed in unpublished trials investigating the effects of larval density in yellowtail kingfish (Chen, B. pers. com. 2014). Whilst supplementation with taurine may have been responsible for the improved growth of larvae in those treatments, further studies in which reduced survival does not lead to significant differences in prey availability and final larval density will be required to prove this hypothesis.

Given that taurine plays a role in bone growth and development (Salze *et al.* 2011), we hypothesised that taurine supplementation may reduce the incidence of jaw malformations in yellowtail kingfish larvae. That no significant differences in jaw malformations were found demonstrates there to be no beneficial effects of the taurine supplementation regimes employed in this trial. Further studies investigating the effects of lower *Artemia* taurine supplementation concentrations may yield different results.

Despite significant differences in the taurine content of *Artemia* at all but the two highest taurine supplementation rates, there were no significant differences in the whole-body taurine contents of the larvae that consumed these different supplemented *Artemia*. This suggests that either larvae of this age already possess the functional mechanisms required for excreting taurine in excess of their requirements, or that their bodies are fully or super-saturated with taurine. The former hypothesis would imply that the bodies of these larvae have reached a state of homeostasis and that any taurine beyond the larvae's requirements is being excreted; a process that may have been demanding on these larvae and reduced their survival. The negative impact on survival

356 in all supplemented treatments also supports our alternative hypothesis that the bodies
357 of larvae in all supplemented treatments are fully or super-saturated and subsequently
358 that these whole-body levels are in excess of the larvae's requirements. A comparison of
359 the whole-body taurine contents of wild juvenile conspecifics supports this theory.
360 Larvae fed taurine supplemented *Artemia* in this study had a higher whole-body taurine
361 content (2.48% DW) than for both 30 mm wild juvenile Japanese yellowtail (2.3% DW)
362 (Matsunari, Takeuchi, Takahashi & Mushiake 2005b) and for wild juvenile amberjack
363 *Seriola dumerili* (Risso)(2.1% DW) of 28 to 44 mm in length (Yamamoto *et al.* 2008).
364
365 Given that taurine is involved in many different physiological mechanisms, it is
366 conceivable that one or more of these mechanisms are being overwhelmed by excessive
367 taurine in underdeveloped larvae. Larvae such as those used in the current trial may
368 therefore have a lower requirement and/or a reduced tolerance to excess taurine than
369 juvenile fish. For example, juvenile Japanese flounder fed zooplankton (mysids) have a
370 similar whole-body taurine content (2.4% DW) to the aforementioned wild Japanese
371 yellowtail and amberjack (Matsunari *et al.* 2005b ; Yamamoto *et al.* 2008), yet larvae
372 of this same species fed taurine enriched rotifers have a whole-body taurine content of
373 only 0.45% DW (Takeuchi, Park, Seikai & Yokoyama 2001). Furthermore, data
374 showing that juvenile marine fish tolerate compound diets containing taurine levels far
375 in excess of their requirements without negative consequence also supports the
376 hypothesis that juveniles have the ability to effectively excrete excess taurine. Salze,
377 Rhodes, Davis, Jirsa & Drawbridge (2014), for example, fed diets containing taurine at
378 concentrations up to ca. 10% DW to Florida pompano *Trachinotus carolinus* L., white

seabass *Atractoscion nobilis* (Ayres) and yellowtail kingfish with no adverse effects on growth or survival.

Despite the above argument, if we were to assume that the whole-body taurine content of wild juvenile conspecifics were a useful guide as to an appropriate whole-body taurine content of larvae, then the function derived for the relationship between *Artemia* taurine content and larval taurine content (Equation 1), demonstrates that the *Artemia* taurine content required to obtain a whole-body larval taurine content equal to that of wild Japanese yellowtail (2.3% DW) or amberjack (2.1% DW) are 1.6% DW and 1.2% DW, respectively. Then using the function describing enrichment concentration vs *Artemia* taurine content (Equation 2), shows that the taurine enrichment concentration required to achieve these *Artemia* taurine concentrations are 0.6 g L^{-1} and 0.3 g L^{-1}, respectively.

Based on the evidence reported here we recommend that further studies be undertaken supplementing *Artemia* at lower concentrations to determine if there is any benefit to growth and/or survival or whether the naturally occurring levels of taurine within *Artemia* are sufficient to meet the taurine requirements of this species at this life stage without further supplementation.

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Figure Legends

Figure 1. Relationship between taurine enrichment concentration (g L^{-1}) and *Artemia* taurine content (% dry weight) following an 18 hour enrichment period (R^2 = 0.99, *Artemia* taurine content (% DW) = 0.76 + 4.35 × (1-exp $^{(-0.34 \times \text{taurine concentration rate})}$).

Figure 2. Final survival (% mean ± S.E., *n* = 2) of yellowtail kingfish larvae at 23 days post hatch fed *Artemia* co-enriched with increasing concentrations of taurine. Symbol *asterisk* indicates significant differences between treatments.

Figure 3. The final dry weight (mean ± S.E., *n* = 2) of yellowtail kingfish larvae at 23 days post hatch fed at varying levels of taurine co-enrichment during the *Artemia* feeding phase. Different superscripts indicate significant difference between treatments.

Figure 4. Relationship between the final dry weight (mg larvae^{-1}) and survival of yellowtail kingfish larvae ($R^2 = 0.81$).

Figure 5. *Artemia* ingestion, measured as the number of undigested *Artemia* eyes per larval gut one hour after the first feed, of yellowtail kingfish larvae from 13 to 19 days post hatch. Five larvae per sample time per replicate tank, values are mean ± S.E. (n = 2).

Figure 6. Correlation between the taurine content (% dry weight) in *Artemia* and yellowtail kingfish larvae fed *Artemia* co-enriched at increasing concentrations of taurine ($R = 0.98$, $\textit{Larval whole body taurine} = 0.79 + 1.75 \times (1 - \exp^{(-1.2 \times \textit{Artemia taurine content})})$.

Figure 7. Jaw deformity levels in yellowtail kingfish fed varying amounts of taurine enriched *Artemia* from 12 to 23 days post hatch. Levels represent deformities that would represent a commercial cull by industry. Values are mean ± S.E. (n = 2).