JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

doi: 10.1111/jwas.12478

A Comparison of the Effects of Two Prey Enrichment Media on Growth and Survival of Pacific Bluefin Tuna, *Thunnus orientalis*, Larvae

Maria S. Stein[1], Daniel Margulies, and Jeanne B. Wexler

Early Life History, Inter-American Tropical Tuna Commission, 8901 La Jolla Shores Drive, La Jolla, California, 92037, USA

Vernon P. Scholey

Achotines Laboratory, Inter-American Tropical Tuna Commission, Las Tablas, Los Santos, Republic of Panama

Katagiri Ryo, Tomoki Honryo, and Tsukasa Sasaki

Oshima Branch, Aquaculture Research Institute, Kinkdai University, Oshima 1790-4, Kushimoto, Wakayama, 649-3633, Japan

Angel Guillen

Aquatic Resources Authority of Panama, Panama, 0819-05850, Republic of Panama

Yasuo Agawa and Yoshifumi Sawada

Oshima Branch, Aquaculture Research Institute, Kinkdai University, Oshima 1790-4, Kushimoto, Wakayama, 649-3633, Japan

Abstract

Three experiments were conducted to investigate the growth, survival, and standardized cohort biomass of Pacific bluefin tuna, *Thunnus orientalis*, larvae fed nutritionally enhanced prey during the first week of feeding using two commonly used, commercially available enrichment media, AlgaMac Enrich and Marine Glos. *T. orientalis* larvae exhibited exponential growth in standard length and dry weight. The daily specific growth rates in length and weight are the first reported for *T. orientalis* larvae and the averages ranged from 3.8 to 4.1% and 27.5%, respectively, for larvae in the AlgaMac treatment and from 4.1 to 6.1% and 31.5%, respectively, in the Marine Glos treatment. Average daily growth rates in length ranged from 0.16 to 0.23 mm/d for larvae in the AlgaMac treatment and from 0.17 to 0.27 mm/d for those in the Marine Glos treatment. Daily growth rates in length were similar to those reported for other tuna larvae reared in the laboratory but slower than most published estimates for larval tunas *in situ* at similar water temperatures. Mean prey number per gut was positively associated with mean prey level in the tank. Both enrichment media appear to be good sources of nutritional improvement of planktonic prey for *T. orientalis* larvae.

KEYWORDS

growth rate, prey enrichment, survival, *Thunnus orientalis*, tuna larvae

The Pacific bluefin tuna, *Thunnus orientalis*, one of the largest species of tunas, inhabits temperate waters (Bell 1963), but spawns in the tropics, and the larvae and juveniles also inhabit tropical and subtropical waters of the Pacific Ocean. In the eastern Pacific Ocean, *T. orientalis* occur from Guadeloupe Island, Baja California, and Southern California with occasional forays

[1] Correspondence to: mstein.ocean@gmail.com

as far north as Shelikoff Strait, Alaska, in the central Pacific Ocean from the equator to 40°N latitude, and in the western Pacific Ocean from Sakhalin Island south to southwestern Australia and New Zealand (Collette and Nauen 1983; Bayliff 1994). Spawning of *T. orientalis* occurs in the warm seas of the northwestern Pacific Ocean between the Philippines and the Ryukyu Islands of Japan and in the Sea of Japan; spawning generally occurs at sea-surface temperatures >22.0 C (Tanaka et al. 2006; Masuma et al. 2008). *T. orientalis* is a high-value species in global fresh-fish markets, particularly in the sashimi and sushi markets of Japan, and it is being intensively studied in aquaculture programs throughout Japan (Masuma et al. 2008). Since the early 1990s, the catch of *T. orientalis* in the Pacific Ocean has been dominated by young-of-the-year and juveniles of 0–3 yr (ISSF 2013). Bayliff (2001, 2002) reported that increases in the catch at this age would likely decrease productivity of the adult fishery, based on yield-per-recruit and cohort analyses. Recent stock assessments (ISC 2012, 2014) have indicated that spawning biomass is near historically low levels, and the most recent stock assessment conducted in 2016 is equally pessimistic (ISC 2016).

Identifying the environmental and biological processes that influence vital rates (e.g., survival and growth) of early life stages is crucial to an understanding of prerecruit survival of marine fish species. Recruitment and cohort strength are often strongly linked to prejuvenile survival (Bailey and Houde 1989). Several bottlenecks to successful production of *T. orientalis* in full-life-cycle aquaculture systems occur during the prejuvenile stages. These bottlenecks can include low feeding success, reduced growth, and poor nutrition during the first 2 wk of life (Masuma et al. 2008). Tunas such as *T. orientalis* exhibit reproductive modes characterized by very high batch and annual fecundities and early life histories characterized by fast growth potential, high metabolic rates, and high natural mortality rates (Tanaka et al. 1996; Margulies et al. 2007a). This type of early life history has strong potential for recruitment control during prejuvenile stages, although the relatively long juvenile stage of *T. orientalis* and other long-lived tuna species cannot be ruled out as a recruitment-control life stage (Houde 1989).

Studies of the early life history of *T. orientalis* have been conducted in recent decades in Japan, aided by captive spawning and rearing programs (Miyashita et al. 2001; Miyashita 2002; Sawada et al. 2005; Masuma et al. 2009). Studies of *in situ* distribution, growth, and survival of *T. orientalis* larvae have also been carried out in the western Pacific Ocean (Tanaka et al. 2006; Satoh et al. 2013). There is strong interest worldwide in refining understanding of early life history processes of *T. orientalis* and other bluefin tuna species, not only for resource management purposes but also to develop improved aquaculture practices leading to successful full-life-cycle production (Masuma et al. 2008).

Growth during the larval stage can be an important determinant of prerecruit survival in marine fishes (Miller 2007). Laboratory growth of *T. orientalis* larvae during the first 30 d after hatching has been described by Kaji et al. (1996), Miyashita et al. (2001), and Miyashita (2002) in terms of either growth in length or growth in wet weight in order to estimate a length–weight relationship, but there are no published studies of growth rates or survival of *T. orientalis* larvae focused specifically on the first 1–2 wk of feeding, a crucial period for feeding success and cohort survival during the early life history of a species that spawns in warm seas (Houde 1989). The first 2 wk of feeding and growth are also crucial in the success of full-life-cycle aquaculture programs for *T. orientalis* (Masuma et al. 2008).

As part of a multiyear comparative study of the early life histories of *T. orientalis* and yellowfin tuna, *Thunnus albacares*, the effects of two enrichment media used to produce nutritionally enhanced prey for *T. orientalis* larvae were experimentally compared, and the effects of those preys on the growth and survival of the larvae were investigated. Nutritional enrichment of planktonic prey is routinely used in rearing of high-growth, high-metabolism marine fish larvae such as tunas, in order to boost the highly unsaturated fatty acid (HUFA) content of the prey to near-equivalent levels found in

wild planktonic prey (Tocher et al. 2008). AlgaMac (Aquafauna Bio-Marine, Hawthorne, CA, USA) and Marine Glos (Nisshin Marinetech Co., Yokohama, Japan) are both popular commercial products commonly used to increase HUFA content in rotifers for rearing of *Thunnus* spp. larvae (Nakagawa et al. 2011; Kurata et al. 2015; Margulies et al. 2016). The specific objective of these laboratory experiments was to compare the efficacies of two common enrichment media used successfully in the past to enhance the nutritional value of planktonic prey of tuna larvae by (1) estimating feeding incidence and the daily instantaneous growth and specific growth rates in length and dry weight of *T. orientalis* larvae at variable food levels and (2) estimating survival and standardized cohort biomass (i.e., a larval cohort weight index) of *T. orientalis* larvae during the first week of their feeding.

Materials and Methods

Collection of Eggs

The experiments were carried out at the Aquaculture Research Institute of Kindai University's (KU) Oshima Branch, in the Wakayama Prefecture of Japan (Fig. 1). Oshima is a small island in the Pacific Ocean 1.8 km offshore from the southern-most point of Honshu. Scientists at KU completed the life cycle of *T. orientalis* in 2002 (Sawada et al. 2005) and maintain multiple broodstock populations in sea pens in the waters between Oshima and Kushimoto, the nearest coastal town on the mainland. Broodstock are also held in an enclosed bay of Amami Island, northeast of Okinawa and 380 km south of Kyushu. Amami Island is bordered by the East China Sea to the west and the Pacific Ocean to the east.

Three experiments were conducted in total; two experiments during July–August 2011 and one experiment in July 2012. Fertilized *T. orientalis* eggs were collected from sea cages off the coast of Amami Island by KU staff and air shipped to Oshima Station as per KU's standard egg collection and shipping protocols (Sawada et al. 2005). The broodstock was made up of 64–74 fish between 4 and 5 yr old over the time period of these collections. The broodstock diet consisted of approximately 90% finfish (*Scomber* spp.) and 10% squid during the spawning season and was consistent between years. Eggs were received at Oshima and stocked into an incubation tank (1600 L) equipped with a 500-μm mesh egg net ($85 \times 85 \times 69$ cm) suspended in the water. Water temperature during shipment ranged from 23.9 to 25.8 C (mean = 24.7 C, $s = 0.98$, $N = 3$) and after arrival, eggs were incubated at 24.0–25.0 C.

Physical Systems

Hatching occurred between 27 and 32 h after the observed spawning time, depending on the water temperatures throughout spawning, transportation, and incubation. Six circular experimental tanks, three replicate tanks for each treatment, of approximately 1016 L volume were stocked with yolk-sac (YS) larvae shortly after hatching. Larval stocking densities ranged from 13.1 to 15.1 YS/L. Experimental tanks were plumbed with a flow-through system, and intake water flow rates were set to allow 2.5 daily water exchanges. The seawater entering the experimental tanks first received sand filtration and ultraviolet treatment. Temperature was controlled in each tank via a sensor feedback system connected to both hot and cold water inflow systems. Water temperature was gradually increased from the time of hatching to the time of first feeding (FF) and the target tank temperature was maintained at a mean of 27.5 C (range of daily mean 27.1–28.0 C) during all three experiments. A mean experimental temperature of 27.5 C was chosen to represent a common developmental temperature for *T. orientalis* larvae in the western Pacific Ocean (Miyashita et al. 2001). A mean salinity of 31.4 g/kg (range of 30.0–32.4), mean percent oxygen saturation of 109.9% (range of 91.0–139.4%), mean dissolved oxygen of 7.3 mg/L (range of 6.0–9.1), and a mean pH of 8.11 (range of 7.79–8.24) were recorded in the experimental tanks over the course of the trials. FF occurs in *T. orientalis* larvae when their eyes are fully pigmented and the mouth is open and fully functional (Margulies et al. 2001; Miyashita et al. 2001). The

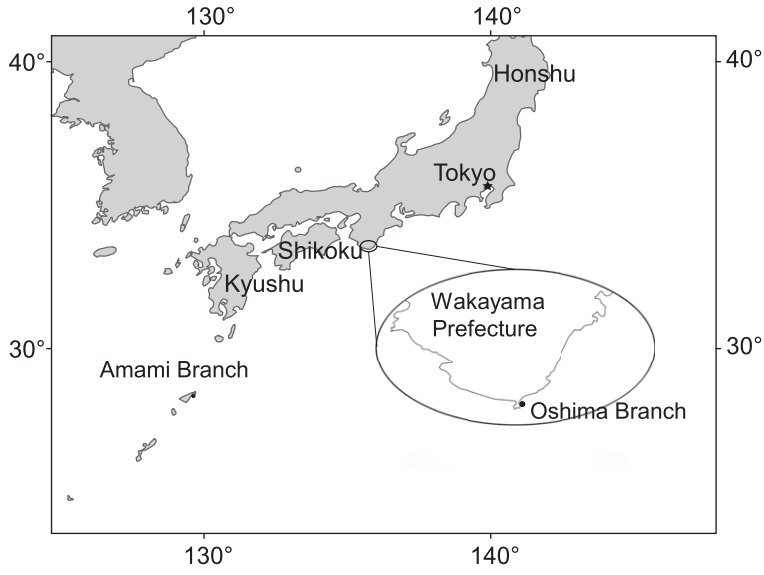

FIGURE 1. *Map of Japan indicating Oshima and Amami Branches of Kinkdai University's Aquaculture Research Institute (created using ArcMap™ of Esri).*

average standard length (SL) of FF *T. orientalis* larvae of this cohort was 3.38 mm ($s = 0.22$, $N = 269$) and the mean dry weight at FF sampled from the 2012 experimental season was 25.5 µg ($s = 0.0038$, $N = 60$). The larvae in these experiments reached FF between 51 and 59 h after hatching, or 2.1–2.4 d after hatch (d.a.h.), which we will refer to as 2 d.a.h. from this point forward.

Three single, florescent light bulbs were hung above each tank (range of 22.26–27.82 µmol/sec/m^2 light intensity at 30 cm depth from the water's surface) and the photoperiod was maintained on a 12 h light; 12 h dark cycle beginning at the time of FF and through the duration of the experiments.

The two trials that took place in 2011 were conducted for 8.2 and 8.4 d of feeding (10.2 and 10.4 d.a.h.), respectively, while the single trial in 2012 was conducted for 7 d of feeding (9 d.a.h.). The first week of feeding was selected for study because this stage is a time of very high mortality and rapidly changing vital rates for *Thunnus* larvae (Margulies et al. 2007b). All three trials began at FF (2 d.a.h.). Larvae in all trials were fed enriched rotifers for the first 7 d of feeding. Enriched *Artemia* (San Francisco Bay strain, second instar stage) was introduced as an additional prey item beginning on the eighth day of feeding in the 2011 experiments, when rotifer concentrations were successively decreased on each of the final days. Food levels were measured by taking the mean of two 100-mL water samples from each tank three times per day with food levels adjusted accordingly to maintain nominal levels (Table 1). Within each trial, food was maintained at consistent levels for each enrichment treatment. The food level was adjusted between trials in order to examine the effects of prey enrichment on vital rates under relatively lower and higher food conditions. The overall range of food levels (1900–3500 rotifers/L) was chosen based on the Inter-American Tropical Tuna Commission's (IATTC) previous work with *T. albacares* (Margulies et al. 2016) and was considered to represent mid-level food concentrations (i.e., neither food-limiting nor an excessive concentration that would likely never be encountered in the wild).

Microturbulence was created in each experimental tank via aeration by six air diffusers on the floor of each tank. Turbulence conditions were maintained at appropriate levels for each stage of larval development, according

TABLE 1. *Mean number of prey items in the gut of Pacific bluefin tuna larvae,* Thunnus orientalis*, sampled during the experiments from each enrichment treatment (mean ± 2 SEM).*

		Mean number of prey items in the gut for each experiment by treatment					
		2011 Expt. 1[a]		2011 Expt. 2[b]		2012 Expt.[c]	
Day of experiment	Day after hatch	ALG-E	MG-M	ALG-E	MG-M	ALG-E	MG-M
1	2	3.2 (±1.6)	4.2 (±1.5)	3.0 (±1.1)	4.0 (±1.4)	10.2 (±2.9)	7.9 (±2.5)
3	4	7.6 (±1.2)	7.0 (±1.4)	3.8 (±2.1)	5.9 (±1.8)	14.5 (±2.6)	12.5 (±2.9)
5	6	6.4 (±2.3)	7.8 (±1.8)	7.1 (±1.6)	7.1 (±1.7)	15.1 (±3.0)	14.5 (±3.0)
7	8	7.7 (±1.7)	6.9 (±1.9)	9.6 (±2.2)	6.9 (±2.1)	17.4 (±6.0)	17.0 (±6.0)
9	10	5.9 (±1.8)	6.6 (±3.5)	8.2 (±1.6)	7.0 (±2.0)	–	–

ALG-E = AlgaMac Enrich treatment; MG-M, Marine Glos-Modified treatment.
[a]Mean food concentration: 2600 (±317) rotifers/L.
[b]Mean food concentration: 1900 (±118) rotifers/L.
[c]Mean food concentration: 3500 (±474) rotifers/L.

to protocols developed by the IATTC research group with *T. albacares* (Margulies et al. 2007b). Turbulence levels were quantified based on surface measurements of a neutrally buoyant drogue. The lowest level possible (<0.5 cm/sec) was used during the YS stage and an increased level of approximately 5.9 cm/sec was used once larvae reached the FF stage (2 d.a.h.).

Algal concentrations of *Chlorella* sp. (Super Chlorella V-12; Chlorella Industry Co., LTD, Tokyo, Japan) were also maintained in each experimental tank to aid in visual contrast of prey and added at concentrations in order to create similar conditions to those of rearing protocols previously developed by the IATTC (Loew et al. 2002; Margulies et al. 2007b). Cell counts in the same tanks under similar conditions ranged between 310,000 and 420,000 cells/mL. The algal density was maintained by visually judging the clarity at a set point at depth in each tank. The algae were added to each tank via a calibrated drip line above the tanks and the cell density of the algae added was the same for all tanks. The volume and clarity of algae added to each tank was based on the IATTC's previous experience with green water additions for *T. albacares* larval rearing.

Enrichment

Prey items were enriched with one of two different products to enhance their nutritional value. The IATTC research group routinely uses AlgaMac enrichment products produced by Aquafauna Bio-Marine, Inc. for rearing *T. albacares* larvae in the laboratory (Margulies et al. 2016). Aquafauna Bio-Marine developed a custom blend, AlgaMac Enrich (ALG-E), designed to increase uptake efficiency of the available essential HUFA, specifically docosahexaenoic acid (DHA), during the rotifer enrichment process. Since these trials, the company now offers ALG-E as one of its standard commercially available products, with the addition of taurine and ascorbyl palmitate, which were not included in the formula at the time of these trials. The ALG-E treatment will be referred to as ALG-E for the purposes of this paper. Marine Glos (Nisshin Marinetech) is frequently used to boost the HUFA content of prey for rearing of *T. orientalis* larvae in the laboratory (Nakagawa et al. 2011; Kurata et al. 2015). Salmon egg oil (Seoka et al. 2008) and taurine (Matsunari et al. 2013) have also been used as diet enrichments to enhance growth and survival in larval fish. Because of this history, Marine Glos supplemented with salmon egg oil and taurine was chosen as the second enrichment medium used in these trials. This treatment will be referred to in this paper as MG-M (short for Marine Glos-Modified).

The rotifers for these trials were enriched in conical tanks at a concentration of 50 million rotifers per 100 L of water volume (or 500 rotifers/mL). The tank water for the MG-M enrichment was maintained at 25.0 C. The ALG-E enrichment tank water temperature was

maintained at 27.5 C. Salmon egg oil (1.5 g $100\,L^{-1}$ water), taurine (40 g $100\,L^{-1}$ water), and Super V-12 chlorella (50 mL $100\,L^{-1}$ water) were added to the MG-M enrichment tank 18 h prior to harvest, followed by Marine Glos (150 mL $100\,L^{-1}$ water) 9 h prior to harvest. ALG-E (15 g $100\,L^{-1}$ water) was added to the ALG-E enrichment 9 h prior to harvest.

Artemia (second instar) was introduced as a prey item after the seventh day of feeding in addition to rotifers. The *Artemia* was enriched with ALG-E in the amount of 1 g per 330,000 cycsts, 12 h prior to harvest in all treatments.

Sample Analyses

Samples of 15 larvae were removed and fixed in 5% formalin from each tank on the first day of feeding (2 d.a.h.) and every 2 d thereafter for growth analysis during both of the 2011 trials. Preserved specimens were measured to the nearest 0.1 mm total length (TL) and SL using a calibrated Nikon Eclipse 80i (Tokyo, Japan) stereo microscope.

Samples of 15 larvae were removed on the first, fourth, and eighth days of the experiment (after 7 full days of feeding, or 2, 5, and 9 d.a.h.) during the 2012 trial and were measured fresh to the nearest 0.1 mm TL and SL using image capture analysis and measuring software (MeasurePRO Version 1.0; Shodensha, Inc., Osaka, Japan) with a stereo microscope (Olympus SZX12) fitted with a 5 MP digital camera (model number HDCE-50B; Shodensha, Inc.). Dry weights were obtained on the same fresh specimens following measurements on the final day of sampling. Larvae were rinsed in a series of distilled water baths to remove salts and particulate matter. Larvae were placed in preweighed aluminum pans, dried in a drying oven (DS44; Yamato-HiTEC, Inc., Northbrook, IL, USA) at 60 C for 48 h, then removed from the oven and desiccated for 48 h, and then weighed to the nearest 0.1 µg on a Mettler Toledo ultra microbalance (model XP2UV) (Margulies 1989; Margulies et al. 2007a). Weight on the first day of feeding (2 d.a.h.) was given an assigned value of 25.5 µg, based on a mean of 60 dry weights from a different cohort of the same age due to lack of equipment on the first day of this experiment. The microbalance was only available for the 2012 trial, and subsequently only growth in length was analyzed in each of the 2011 trials.

Samples of 5–10 larvae were collected from each tank at 2-d intervals and fixed in 5% formalin for later gut analysis. During processing, the alimentary tract of each fixed larva was teased apart in glycerine and the number and identity (rotifer or *Artemia*) of prey were recorded (Margulies et al. 2001).

Proximate Analysis of Enriched Rotifers

Rotifers from each treatment were collected after a full enrichment cycle with a fine mesh collection net and vigorously washed with tap water. The collection was then strained and compressed to release the excess water before being packaged and stored in an ultra-freezer at −80 C for later nutritional analysis. Each analysis was conducted in triplicate. Proximate composition of rotifers enriched with each formula was analyzed by standard methods (AOAC 1995). The carbohydrate content was measured by the phenol-sulfuric acid method (Hodge and Hofreiter 1962).

Lipid for fatty acid analysis was extracted according to Folch et al. (1957). The fatty acid methyl esters were formed by using methanolic 2 M NaOH solution and methanolic 2 M HCL solution according to Yoshinaka and Satoh (1989) and analyzed with a gas chromatograph (GC-4000; GL Science, Inc., Tokyo, Japan) equipped with an Ultra Alloy® capillary column (30 m × 0.25 mm interior diameter; Frontier Laboratories, Fukushima, Japan) and a flame ionization detector. Tricosanoic acid methyl ester (Sigma-Aldrich, St. Louis, MO, USA) was used as an internal standard. The column oven temperature was increased from 180 to 240 C at a rate of 4 C/min and then the temperature was maintained at 240 C for 20 min. The carrier gas was nitrogen, and source and column head pressure were 5 and 1 kerosene-gas oil fractions per cm^2, respectively. The final temperatures for the injector and detector were 260 and 290 C, respectively. Peak quantification

was performed using EZChrom Elite compact software Ver. 1.65 (Agilent Technologies, Santa Clara, CA, USA).

Data Analyses

The mean final SL for each treatment of all three trials and the mean final dry weights between treatments in the 2012 trial were compared, using the $F_{\max}$ test for homogeneity of variance followed by either Student's t test or Mann–Whitney U test, when appropriate (Zar 1996). Regression analysis was performed on length and weight data versus age expressed as d.a.h. and the residuals were examined to determine the best description of the growth relationship. All statistical programs were run in Microsoft Excel and XLSTAT 2012 (Microsoft Office 2010; Addinsoft, Paris, France).

An exponential model best described the relationships in both length and weight at age; therefore, the instantaneous daily growth in length of all experiments and weight of the 2012 experiment was calculated based on lengths and weights measured on the first and final days of the experiment (2 and 9–10 d.a.h.), using the following expressions: $L_t = L_0 e^{Gt}$, where L_t = SL at t days, G = instantaneous daily growth coefficient, and L_0 = SL at time zero, or 2 d.a.h., and $W_t = W_0 e^{Gt}$, where W_t = dry weight at t days, G = instantaneous daily growth coefficient, and W_0 = dry weight at time zero, or 2 d.a.h. (Ricker 1975; Margulies 1989). Specific growth rates (%/d) were calculated in both dry weight and SL as $100(e^G - 1)$ (Ricker 1975; Margulies 1989). Instantaneous daily growth and specific growth rates in length were calculated based on lengths measured at 2 and 10 d.a.h. for the 2011 experiments and at 2 and 9 d.a.h. for the 2012 experiment. In most cases, specific growth rates (percentages) were arcsine transformed for Student's t test comparison between treatments. Mann–Whitney U comparisons were made instead of Student's t test when more appropriate ($\alpha = 0.05$) (Zar 1996). Average daily growth rates in terms of mm/d were also calculated for all of the length-at-age data for each treatment of each experiment through differentiation of the exponential equation fitted to the data.

Prey number per larva was compiled by tank and day. Data were then analyzed by ANOVA ($\alpha = 0.05$) and pooled by treatment for each day followed by the Student's t test to determine differences in mean prey number between treatments (Zar 1996). A Pearson's correlation test was used after a log10-transformation of the data to analyze the relationship between prey number larvae per gut and mean food level for each treatment.

Expected mean survival in laboratory trials was calculated based on the following relationships (Ricker 1975): $Nt = N_0 e^{-Zt}$ and $Z = F + M$, where N_t = number of survivors at t days after the first day of a specific trial, $N_{0\_}$ = initial number of larvae for that trial, t = number of days of the trial, and Z = instantaneous total mortality rate. Z is equal to the sum of F, the sampling mortality, and M, the natural mortality rate. The number of larvae processed for either growth or gut analysis was considered the sample mortality (F), and all other mortality was considered natural mortality (M) (Margulies 1989). In the first experiment in 2011, one of the replicate tanks in the MG-M treatment was terminated early due to low survival and a final survival estimate of 0.01 was estimated for this tank in order to calculate expected survival, which requires a positive value.

Standardized cohort biomass (i.e., a "larval cohort weight index") takes into account both the expected survival and absolute growth in weight (mean final weight) estimated for each tank, standardized by the number of larvae originally stocked in each tank. Standardized cohort biomass is an estimate of the weight of a cohort at the end of the study period, standardized by the initial number of larvae in the cohort. Cohort biomass is a component of cohort production estimates and provides a comparison of the relative contributions of survival and growth to the final weight of the cohort. The standardized cohort biomass for each tank on the final day of the 2012 trial was estimated as: $B_s = \frac{(\overline{w_t})(N_t)}{N_0}$, where B_S = standardized cohort biomass (µg/larva), $\overline{W}_t$ = mean dry weight of larvae at t (final) days, N_t = number of larvae at t

(final) days, and N_0 = initial number of larvae at time 0 (Ricker 1975).

Proximate composition (% dry weight) and fatty acid composition (% total fatty acids, dry basis) are presented as mean ± 2 SEM, $N = 3$, for each enrichment formula. A Student's t test was performed between the two treatments for each fatty acid to test for differences between the means.

Results

Survival

Survival of *T. orientalis* larvae after approximately 1 wk of feeding ranged from 0.1 to 11.5% in individual tanks during all experiments (Fig. 2). The mean survival of each treatment ranged from 1.4 to 7.5%. There were no significant differences ($P > 0.05$) in mean survival between the ALG-E and MG-M treatments for each experiment (Fig. 2).

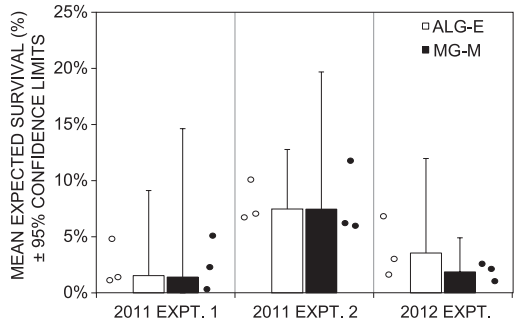

FIGURE 2. *Expected survival of* Thunnus orientalis *larvae for 2011 experiment 1 (10.2 days after hatch [d.a.h.]), 2011 experiment 2 (10.4 d.a.h.), and the 2012 experiment (9 d.a.h.). Bars indicate mean expected survival by treatment while circles represent the expected survival of individual tanks. Open bars and circles represent AlgaMac Enrich treatment while solid bars and circles represent Modified Marine Glos treatment. Error bars are ± 95% confidence limits.*

Growth

Mean lengths at 2 d.a.h. were similar between treatments in each of the experiments (ANOVA, $P > 0.05$). In the first experiment in 2011, larvae in the ALG-E treatment exhibited a specific growth rate in SL of 3.8 ± 0.4%/d (mean ± 95% confidence interval [CI]) after 8 d of feeding and growth (2–10.2 d.a.h.) (Fig. 3). The specific growth rate in the MG-M treatment was similar at 4.1%/d ± 0.3% (mean ± 95% CI). The growth rates were not significantly different ($U = 620$, df = 72, $P > 0.05$). The average daily growth rates were 0.1611 mm/d (SEM = 0.0094, $N = 5$) and 0.1676 mm/d (SEM = 0.0099, $N = 5$) for the ALG-E and MG-M treatment groups, respectively. The final mean SL in the ALG-E treatment (4.6 ± 0.1 mm, mean ± 2 SEM) was also not significantly different ($U = 575.5$, df = 73, $P > 0.05$) from that in the MG-M treatment (4.7 ± 0.1 mm, mean ± 2 SEM) (Fig. 4).

Greater growth rates in SL were attained in the second 2011 experiment after 8.4 d of feeding and growth (2–10.4 d.a.h.) when the larvae were fed at a lower mean daily food level than that of the previous experiment (Table 1). Specific growth rate in the ALG-E treatment was 4.9 ± 0.3 and 5.6 ± 0.3%/d (mean ± 95% CI) in the MG-M treatment (Fig. 3). A significant difference between treatments was indicated ($t = 3.59$, df = 178, $P < 0.001$). The average daily growth rates were 0.2155 mm/d (SEM = 0.0167, $N = 5$) and 0.2449 mm/d (SEM = 0.0212, $N = 5$) for the ALG-E and MG-M treatment groups, respectively. The final mean SL in the ALG-E treatment (4.9 ± 0.1 mm, mean ± 2 SEM) was also significantly less than that in the MG-M treatment (5.2 ± 0.1, mean ± 2 SEM) ($t = 3.17$, df = 178, $P < 0.015$) (Fig. 4).

Growth in SL was greater in the 2012 experiment than in either of the 2011 experiments (Fig. 3) when the mean daily food level was higher (Table 1). Mean specific growth rates after 7 d of feeding and growth (2–9 d.a.h.) were 5.5 ± 0.4%/d (mean ± 95% CI) for the ALG-E treatment and 6.1 ± 0.4%/d (mean ± 95% CI) for the MG-M treatment. A significant difference in growth rates was indicated ($t = 2.29$ df = 88, $P < 0.05$). The average daily growth rates were 0.2302 mm/d (SEM = 0.0253, $N = 3$) and 0.2678 mm/d (SEM = 0.0335, $N = 3$) for the ALG-E and MG-M treatment groups, respectively. The final mean SL for the ALG-E treatment was 5.1 mm ± 0.1 (mean ± 2 SEM), while the mean SL for the MG-M treatment was greater at 5.5 mm ± 0.2 (mean ± 2 SEM), and

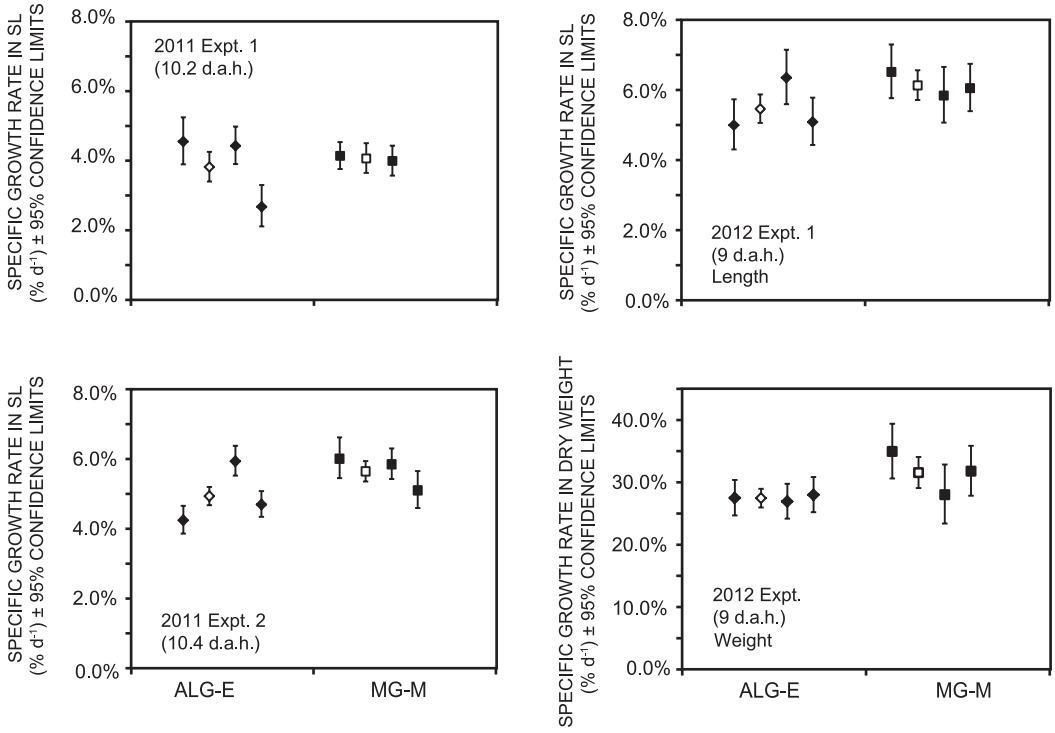

FIGURE 3. *Specific growth rate in standard length (SL) and dry weight for each experiment with days after hatch (d.a.h.) indicated. Specific growth rates were calculated based on lengths and weights measured on the first day of feeding (2 d.a.h.) and at 9–10.4 d.a.h. for AlgaMac Enrich (ALG-E) treatment and Modified Marine Glos (MG-M) treatment. Solid symbols are individual tank means while open symbols are overall treatment means. Error bars represent ± 95% confidence limits.*

the means were significantly different ($t = 3.70$, df = 88, $P < 0.001$) (Fig. 4).

Daily growth in weight was analyzed for the first time in the 2012 experiment. Daily specific growth rate in weight averaged 27.5 and 31.5% in the ALG-E and MG-M treatments, respectively (95%CI: ±1.5%, ±2.5%) (Fig. 3). The difference in growth rate was statistically significant ($U = 1275.5$, df = 85, $P < 0.01$). Final mean dry weight in the ALG-E treatment (144.3 ± 11.1 µg) was significantly lower than in the MG-M treatment (189.8 ± 22.0 µg, mean ± 2 SEM) ($U = 1275.5$, df = 85, $P < 0.01$) (Fig. 4).

Growth analyses indicated that exponential models best described the length and weight data at age in all three experiments (Fig. 5). Regression results in weight are not presented here due to the lack of sampling points in dry weight.

Standardized Cohort Biomass

The standardized cohort biomass was analyzed for the 2012 experiment, and the means were not significantly different between enrichment treatments ($t = 0.754$, df = 4, $P > 0.05$) (Fig. 6), although the absolute mean of 5.5 ± 2.1 µg/larva of the ALG-E treatment was 1.5 times that of 3.7 ± 1.1 µg/larva in the MG-M treatment (mean ± 2 SEM).

Gut Contents Analysis

A positive correlation between mean food level and mean number of prey per gut was found in each treatment (MG-M, $r_s(6) = 0.8214$, ALG-E $r_s(8) = 0.4000$) (Table 1). Mean number of prey per gut increased with larval age in the majority of cases. No significant differences were found in the mean number of prey per gut between the treatments on any of the sampling days (Student's t test, $\alpha = 0.05$). All prey items consumed

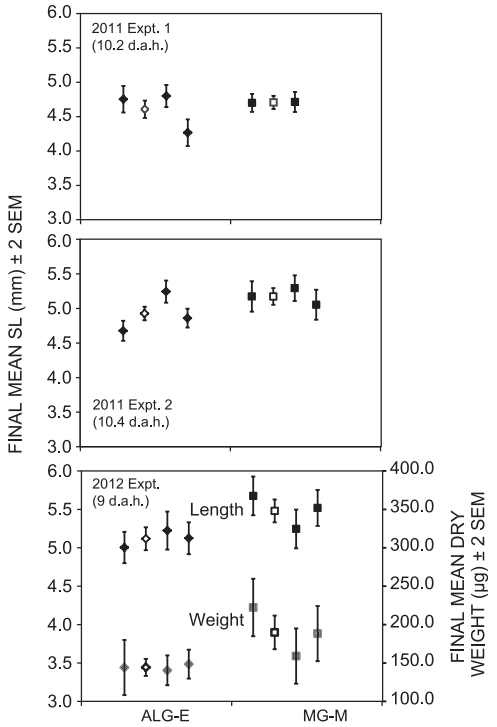

FIGURE 4. *Mean standard length (SL) on the final day of each experiment and mean dry weight for 2012 experiment only. Days after hatch (d.a.h.) indicated for each experiment. Solid symbols are individual tank means while open symbols indicate overall treatment means. ALG-E represents the AlgaMac Enrich treatment and MG-M the Modified Marine Glos treatment. Error bars are ± 2 SEM.* L_S *taken on samples fixed in 5% formalin during both 2011 trials and on fresh specimens in 2012. No correction for fixative shrinkage applied to data.*

during the experiments were rotifers. Because of the absence of *Artemia* in the stomach samples and for ease of presentation, the calculations of daily mean food levels were based on the rotifer concentrations only.

Proximate Composition and Fatty Acid Analyses

The proximate composition analysis revealed similar composition of rotifers enriched with each of the two formulas (Table 2). No significant differences in the mean values of eicosapentaenoic acid (EPA) or DHA levels were found (Table 3).

TABLE 2. *Proximate composition of rotifers cultured with two enrichment products (% dry weight,* N = *3).*

Constituent	ALG-E Mean (±2 SEM)	MG-M Mean (±2 SEM)
Crude protein[a]	71.8 (±5.2)	69.5 (±3.8)
Crude lipid	12.2 (±3.4)	14.5 (±1.0)
Crude carbohydrate	6.2 (±0.5)	6.7 (±0.4)
Ash	6.1 (±0.2)	5.8 (±0.4)

ALG-E = AlgaMac Enrich treatment; MG-M = Marine Glos-Modified treatment.

[a]*N* factor = 6.25.

TABLE 3. *Fatty acid composition of total lipids extracted from the enriched rotifers (dry basis, % total fatty acids,* N = *3).*

Fatty acid	ALG-E Mean (±2 SEM)	MG-M Mean (±2 SEM)
C14:0	2.36 (±1.2)	1.91 (±0.4)
C14:1	0.34 (±0.2)	0.20 (±0.1)
C15:0	0.73 (±0.1)	0.66 (±0.4)
C15:1	0.17 (±0.0)	0.29 (±0.2)
C16:0	30.45 (±9.5)	38.75 (±8.6)
C16:1	1.30 (±0.5)*	2.82 (±0.1)*
C17:0	0.94 (±0.3)	1.04 (±0.2)
C17:1	0.70 (±0.5)	1.02 (±0.8)
C18:0	9.19 (±2.9)	10.38 (±2.1)
C18:1n9	2.14 (±0.1)*	4.43 (±0.4)*
C18:2n6	9.41 (±9.7)	8.53 (±16.6)
C18:3n6	0.35 (±0.1)	0.40 (±0.1)
C18:3n3	4.20 (±3.7)	6.91 (±5.0)
C20:0	0.69 (±0.3)	0.69 (±0.1)
C20:1n9	0.70 (±0.0)	1.22 (±0.5)
C20:2	4.84 (±1.1)	5.35 (±2.3)
C20:3n6	2.98 (±2.9)	2.38 (±1.3)
C20:3n3	1.23 (±0.4)	1.26 (±0.3)
C20:4n6	1.94 (±0.6)	1.75 (±0.8)
C20:5n3(EPA)	2.35 (±1.0)	2.27 (±1.8)
C22:0	3.33 (±2.0)	0.77 (±0.2)
C22:1n9	5.66 (±3.6)*	0.53 (±0.2)*
C22:2	3.37 (±3.8)	0.63 (±0.7)
C23:0	2.19 (±0.6)*	0.59 (±0.1)*
C22:5n6	3.34 (±3.0)	1.50 (±0.9)
C24:0	0.59 (±0.5)	1.58 (±1.6)
C22:6n3(DHA)	1.46 (±0.6)	1.57 (±0.1)
C24:1n9	3.06 (±3.6)	0.57 (±0.3)
ΣSFA	56.38	50.46
ΣMUFA	11.08	14.06
ΣPUFA	32.54	35.47
ΣPUFA n-3	12.00	9.24
ΣPUFA n-6	14.56	18.01
ΣHUFA	10.72	13.30

ALG-E = AlgaMac Enrich; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; HUFA = highly unsaturated fatty acid; MG-M = Modified Marine Glos; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; SFA = Saturated fatty acid.

*Mean values are significantly different at $P < 0.05$.

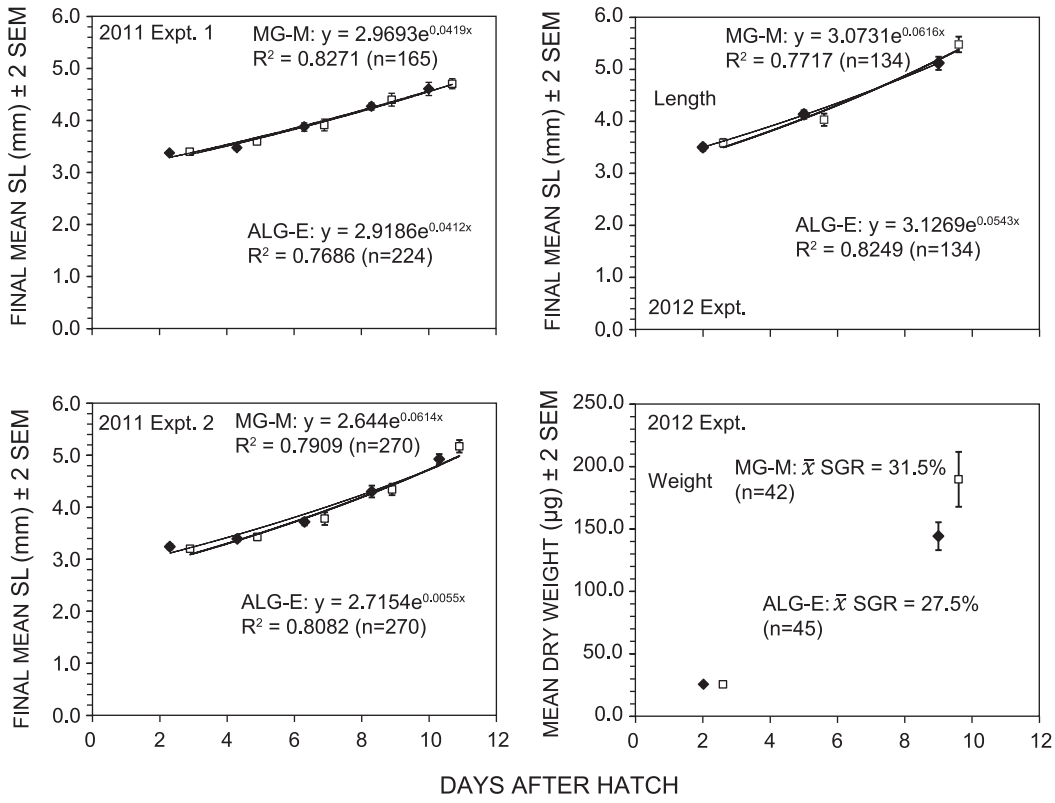

FIGURE 5. *Mean standard length (SL) versus days after hatch for all three experiments and mean dry weight versus days after hatch for the 2012 experiment. ALG-E, AlgaMac treatment, indicated by solid symbols and MG-M, Modified Marine Glos treatment, indicated by open symbols. Error bars are ± 2 SEM.*

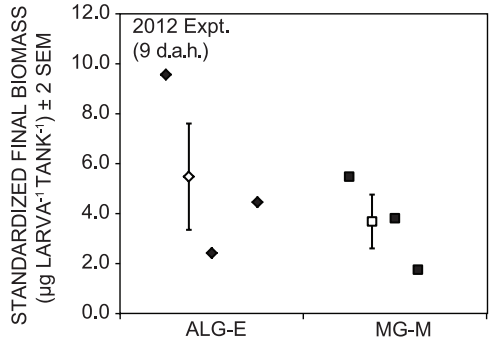

FIGURE 6. *Final standardized biomass (µg larva^{-1} tank^{-1}) for each treatment in the 2012 experiment. Days after hatch (d.a.h.) of experiment indicated. Open symbols indicate treatment means while solid symbols are individual tank values. ALG-E, AlgaMac Enrich treatment; MG-M, Modified Marine Glos treatment. Error bars are ± 2 SEM.*

Discussion

Because ALG-E and MG-M are the most commonly used enrichment products for prey in rearing and experimental investigations of tuna larvae, it is instructive to compare the performance of these products. Overall performance of the ALG-E and MG-M enrichment types was similar, and both enrichment media appear to be good sources of nutritional improvement of planktonic prey for *T. orientalis* larvae; the MG-M treatment, however, appears to promote more rapid growth in weight, and in length in two of the three trials, after the first week of feeding. Both enrichment types seem equally good, with subtle differences, in promoting larval survival and cohort biomass. Each enrichment type was applied over slightly different enrichment periods, based on manufacturer's recommendations,

and enrichment period could have subtle effects on larval vital rates as well.

Nutritional Analysis

The rotifers enriched with both the MG-M and the ALG-E formulae were fairly similar in fatty acid composition. The level of essential fatty acids (EFA) as a percentage of total fatty acids attained with both of these enrichment products seems to be sufficient for normal growth and development of *T. orientalis* larvae during the first week of feeding. This is further supported by a history of successful experimental studies at KU that used similar enrichment protocols for larval rearing (Nakagawa et al. 2011; Kurata et al. 2015). AlgaMac products have been used successfully by the IATTC when rearing *T. albacares* larvae at the Achotines Laboratory in Panama (Margulies et al. 2016). The enrichment protocols of both treatments resulted in a substantial increase in DHA as a percentage of the total fatty acid in rotifers when compared to the level in unenriched rotifers, which is near zero (Bell et al. 2003). Natural prey of larval tunas, such as copepod nauplii, copepodites and adults, generally contain much higher DHA content than the levels reported in this study (McEvoy et al. 1998), suggesting that a further increase in the DHA levels through manipulation of the enrichment protocol could support even faster growth and/or higher survival. However, increasing the lipid content of the enrichment product could decrease the amount of available carbohydrates and result in slow-swimming, sluggish rotifers, which are not optimal for the feeding of larval tunas (Margulies et al. 2016). Therefore, the benefits of boosting the EFA should be balanced with the potential adverse effects on prey behavior, or changes in DHA levels should be offset by changes in other fatty acids in order to maintain an optimal lipid:carbohydrate:protein ratio.

Survival

Survival of tuna larvae in artificial rearing settings has been reported to be relatively low compared to other fish species (Miyashita 2002). Instantaneous daily mortality rates of patches of *T. orientalis* larvae tracked *in situ* have been estimated to be higher than those of other marine species (Satoh et al. 2008). The overall mean survival for each treatment in our experiments (1.4–7.5%) was not unreasonably low, especially in the first 10 d.a.h., which is a period of very high mortality for tuna larvae (Margulies et al. 2016). Multiple factors are thought to contribute to larval mortality of tuna in rearing systems during the early larval stages, such as malnutrition, suboptimal physical conditions, including increased bacterial loads, and surface adhesion (Margulies et al. 2016; Honryo et al. 2017).

Standardized Cohort Biomass

Standardized cohort biomass is an estimate of the relative increase in weight of a larval cohort. Standardized cohort biomass for the 2012 experiment mirrored survival. The 2012 results suggest that overall cohort biomass of *T. orientalis* after the first week of feeding is dominated by relative survival, which was 50% higher in the ALG-E treatment, over growth, at least in the laboratory. These results agree with similar patterns of cohort biomass versus survival and growth exhibited by *T. albacares* larvae during the first week of feeding (Margulies et al. 2016). The results also suggest that there may be a trade-off in early larval production between growth and survival. This relationship is likely stage-specific, however, as vital rates fluctuate with larval development (Houde 1997; Margulies et al. 2007b). Changes in growth may dominate the relationship if growth becomes more rapid and the mortality rate decreases at later stages of development. This type of information may be important when attempting to maximize or estimate larval cohort production, which is useful in the development of larval aquaculture or in the assessment of prerecruit survival patterns of larval populations in nature. It should be noted that our laboratory results occurred in the absence of biological factors such as predation. Growth rate may play a much more intrinsic role in the survival of a cohort via greater predation on slower-growing larvae during the early stages in nature, at least prior to

the onset of piscivory and cannibalism, which is the developmental stage at which growth of tuna larvae typically becomes strongly exponential and density effects of growth appear to diminish (Tanaka et al. 1996; Margulies et al. 2016).

Gut Contents

The number of prey per gut was not a consistent indicator of larval growth or survival in *T. orientalis* larvae. Previous work with *T. albacares* larvae at this age has shown similar ambiguity between number of prey per gut and growth and survival (Margulies et al. 2007b; D. Margulies, IATTC, unpublished data). There was, however, a positive correlation between daily mean food level and mean number of prey per gut for both treatments. Average rotifer concentration in the water column during the 2012 experiment was 0.6 to 0.9 times higher than in experiments 1 and 2 of 2011, respectively. This was reflected in the mean number of prey per gut/day in the 2012 experiment, which was 2–3.8 times higher than those of the 2011 trials. The weakness of number of prey per gut as an index of overall growth and survival suggests that a more complicated relationship exists between feeding and these vital rates, possibly involving other physical and biological factors that may influence the behavior, physiology, and metabolic processes of larvae during feeding such as the energetic costs of foraging under various physical or trophic conditions.

Prey Level

The mean daily growth rate for both treatments was influenced by the prey level of each trial; however, the growth rate in length did not always change proportionately with food level. In the 2012 experiment, for which mean prey levels were 84% greater than the lowest prey level of the second 2011 trial, mean specific growth rate increased approximately 11% in length. Compared to the first 2011 trial, specific growth rate in the 2012 trial increased proportionately to food level (35% increase in food level and 46% increase in daily growth in length). These results suggest that the specific growth rate in length (%/d) of *T. orientalis* larvae is not highly variable within the mean food level range of 1900 to 3500 rotifers/L.

Growth

This study presents the first estimates of daily instantaneous growth and specific growth rates in length and dry weight of *T. orientalis* larvae. Laboratory growth of *T. orientalis* larvae was moderate to rapid in both enrichment treatments, exhibiting an exponential pattern in length and dry weight that is fairly typical of growth of larval tunas. Laboratory growth in length of larvae of the tuna genera *Thunnus*, *Katsuwonus*, *Auxis*, and *Euthynnus* is generally linear or curvilinear during the first 7–10 d of feeding and is usually estimated at <0.35 mm/d (Tanaka et al. 1996; Wexler et al. 2001; Sawada et al. 2005; Margulies et al. 2007b; Masuma 2013). Growth rate estimates in weight for tuna larvae are rare, but the estimates of specific growth rates in dry weight reported for *T. albacares* larvae during the first week of feeding in the laboratory (range of 20–35%/d) (Margulies et al. 2007b) are consistent with the estimates for *T. orientalis* in this study (treatment means of 27.5–31.5%/d). As previously noted, Alga-Mac enrichment products were used in the studies contributing to the growth estimate for *T. albacares* (Margulies et al. 2016).

Three studies, Kurata et al. (2012, 2015) and Sawada et al. (2000), reported length-at-age data for *T. orientalis* larvae reared using rotifers enriched with Marine Glos products. From the length-at-age data reported in the Kurata et al. (2012, 2015) and Sawada et al. (2000) studies, we calculated specific growth rates in SL of 4.7–7.2%/d for larvae ranging from 7–10 d.a.h.. This estimated range of specific growth in SL is comparable, though slightly higher, than that obtained in this study. The food levels in the Kurata et al. and Sawada et al. studies were much higher (0.4- to 9-fold) than those in our study and there were significant differences in stocking density, both of which may influence growth rates in tuna larvae at this stage of development.

Kaji et al. (1996) reported a weight–length relationship for *T. orientalis* larvae reared from 2 to 30 d.a.h. in the laboratory using a

different enrichment product. We estimated a specific growth rate in weight, using Kaji et al.'s weight–length relationship, $W = 0.000229\ L^{4.77}$ ($r^2 = 0.922$). This analysis yielded an estimate after the first 7 d of growth of approximately 34.2%/d, which is comparable to, although slightly faster than, the estimated specific growth rate in the current study.

Multiple *in situ* studies have been conducted and some published growth information for larvae of *T. orientalis* exists for comparison to our laboratory growth estimates. Tanaka et al. (2006) and Satoh et al. (2008) estimated a growth rate of 0.3–0.5 and 0.4 mm/d, respectively, for 15-day-old *T. orientalis* larvae collected in the northwestern Pacific Ocean compared to our rates of 0.17–0.27 mm/d for 10-d-old larvae in this study. Although dry weight data is scarce in the literature, García et al. (2006) published dry weight-at-age data from previously frozen *T. thynnus* larvae collected in the Mediterranean Sea, from which we estimated specific growth rates of approximately 7.8%/d in length and 36.3%/d in weight after 7 d of growth, compared to our fastest growth rates of 5.5–6.1%/d in length and 27.5–31.5%/d in weight in our 2012 experiment. *In situ* estimates of early larval growth are often higher than laboratory estimates, often attributable to the rapid removal of slower-growing or malnourished individuals from *in situ* larval populations (Margulies 1993), which can influence the size distribution at age of a cohort (Pepin 1988; Wexler et al. 2007). Additionally, it is well documented that wild plankton, specifically copepods that likely comprise the majority of the natural diet of *T. orientalis* larvae (McEvoy et al. 1998; Bell et al. 2003), have relatively high fatty acid profiles compared to the enriched cultured prey of our study. Therefore, comparisons between estimates of *in situ* growth and laboratory growth should be made with caution, but the comparisons do provide some perspective on the scope and variability of larval tuna growth.

T. orientalis larvae, like the larvae of other tuna genera, are fast growing, with exponential growth patterns in SL and dry weight during the first week of feeding. Both enrichment products tested in this study (ALG-E and MG-M) appear to provide high-quality nutritional enhancement of planktonic prey during the first 7–9 d of feeding of larvae (2–10 d.a.h.), which is presumably a period of high metabolic demand required to support exponential growth. Survival had a stronger influence than growth rate on larval standardized cohort biomass during the first week of feeding, although the relationships among growth and survival and feeding incidence and growth are not clear. Further studies should be conducted to elucidate these relationships, including the interactive effects of water temperature, food level, and larval density on growth and survival of *T. orientalis* larvae during the first 7–10 d of feeding.

Acknowledgments

We would like to acknowledge the Global Center of Excellence Program of Kindai University for financial support of this study. We also acknowledge the Science and Technology Research Partnership for Sustainable Development and its funding sources, the Japan International Cooperation Agency and the Japan Science and Technology Agency, We greatly appreciate the current and former students of Kindai University, K. Awa, T. Komiya, K. Saeki, Y. Tsuji, and O. Kay Kay of Sabah University, Malaysia, for their invaluable help carrying out these experiments and D. Dominguez of the IATTC for fixed length and gut analyses. Thanks to P. Boeing and L. Lai of Aquafauna Bio-Marine for their review of the fatty acid data. C. Patnode of the IATTC skillfully performed the final formatting of all of the figures for this paper and M. Román of the IATTC created the map used as "Figure 1." We thank W. Bayliff for his helpful review of the manuscript. Finally, we thank the IATTC's G. Compeán, and R. Deriso, for their support.

Literature Cited

AOAC (Association of Official Analytical Chemists). 1995. Official methods of analysis of AOAC international, vol I. Agricultureal chemicals; contaminants, drugs, 16th edition. AOAC International, Arlington, Virginia, USA.

Bailey, K. M. and E. D. Houde. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Advances in Marine Biology 25:1–83.

Bayliff, W. H. 1994. A review of the biology and fisheries for northern bluefin tuna, *Thunnus thynnus*, in the Pacific Ocean. FAO Fisheries Technical Paper 336(2):244–295.

Bayliff, W. H. 2001. Status of bluefin tuna in the Pacific Ocean. Inter-American Tropical Tuna Commission Stock Assessment Report 1:211–254.

Bayliff, W. H. 2002. Status of bluefin tuna in the Pacific Ocean. Inter-American Tropical Tuna Commission Stock Assessment Report 2:247–264.

Bell, R. R. 1963. Synopsis of biological data on California bluefin tuna *Thunnus saliens* Jordan and Evermann 1926. FAO Fisheries Report 6(2):380–421.

Bell, J. G., L. A. McEvoy, A. Estevez, R. J. Shields, and J. R. Sargent. 2003. Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture 227:211–220.

Collette, B. B. and C. E. Nauen. 1983. FAO species catalogue, Vol 2. Scombrids of the world: an annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. FAO Fisheries Synopsis 125(2):1–137.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. Simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497–507.

García, A., D. Cortés, T. Ramírez, R. Fehri-Bedoui, F. Alemany, J. M. Rodríguez, A. Carpena, and J. P. Álvarez. 2006. First data on growth and nucleic acid and protein content of field-captured Mediterranean bluefin (*Thunnus thynnus*) and albacore (*Thunnus alalunga*) tuna larvae: a comparative study. Scientia Marina 70S2:67–78.

Hodge, J. E. and B. T. Hofreiter. 1962. Determination of reducing sugars and carbohydrates. Pages 380–394 *in* R. L. Whistler and M. L. Wolfrom, editors. Methods in carbohydrate chemistry. Academic Press, New York, New York, USA.

Honryo, T., M. Kurata, A. Guillen, Y. Tamura, A. Cano, M. Stein, D. Margulies, V. Scholey, and Y. Sawada. 2017. Optimal period for the effective promotion of initial swim bladder inflation in yellowfin tuna, *Thunnus albacares* (Temminck and Schlegel), larvae. Aquaculture Research 48:5443–5446.

Houde, E. D. 1989. Comparative growth, mortality and energetics of marine fish larvae: temperature and implied latitudinal effects. Fishery Bulletin 87:471–495.

Houde, E. D. 1997. Patterns and consequences of selective processes in teleost early life histories. Pages 173–196 *in* R. Chambers and E. Trippel, editors. Early life history and recruitment in fish populations. Chapman and Hall, London, UK.

ISC (International Scientific Committee). 2012. Stock assessment of Pacific bluefin Tuna in 2012. ISC for Tuna and Tuna-like Species in the North Pacific Ocean, Shimizu, Shizuoka, Japan.

ISC (International Scientific Committee). 2014. Stock assessment of Pacific bluefin Tuna in 2014. ISC for Tuna and Tuna-like Species in the North Pacific Ocean, La Jolla, California, USA.

ISC (International Scientific Committee). 2016. Stock assessment of Pacific bluefin Tuna in 2016. ISC for Tuna and Tuna-like Species in the North Pacific Ocean, La Jolla, California, USA.

ISSF (International Seafood Sustainability Foundation). 2013**.** Status of the world fisheries for tuna. ISSF Technical Report 2013-04A**.**

Kaji, T., M. Tanaka, Y. Takahashi, M. Oka, and N. Ishibashi. 1996. Preliminary observations on development of Pacific bluefin tuna *Thunnus thynnus* (Scombridae) larvae reared in the laboratory with special reference to the digestive system. Marine and Freshwater Research 47:261–269.

Kurata, M., M. Seoka, Y. Nakagawa, Y. Ishibashi, H. Kumai, and Y. Sawada. 2012. Promotion of initial swimbladder inflation in Pacific bluefin tuna, *Thunnus orientalis* (Temminck and Schlegel), larvae. Aquaculture Research 43:1296–1305.

Kurata, M., M. Seoka, Y. Ishibashi, T. Honryo, S. Katayama, K. Takii, H. Kumai, S. Miyashita, and Y. Sawada. 2015. Timing to promote initial swimbladder inflation by surface film removal in Pacific bluefin tuna, *Thunnus orientalis* (Temminck and Schlegel), larvae. Aquaculture Research 46:1222–1232.

Loew, E. R., W. N. McFarland, and D. Margulies. 2002. Developmental changes in the visual pigments of the yellowfin tuna, *Thunnus albacares*. Marine and Freshwater Behaviour and Physiology 35(4):235–246.

Margulies, D. 1989. Effects of food concentration and temperature on development, growth, and survival of white perch*, Morone americana*, eggs and larvae. Fishery Bulletin 87:63–72.

Margulies, D. 1993. Assessment of the nutritional condition of larval and early juvenile tuna and Spanish mackerel (Pisces: Scombridae) in the Panama Bight. Marine Biology 115:317–330.

Margulies, D., J. B. Wexler, K. T. Bentler, J. M. Suter, S. Masuma, N. Tezuka, K. Teruya, M. Oka, M. Kanematsu, and H. Nikaido. 2001. Food selection of yellowfin tuna, *Thunnus albacares*, larvae reared in the laboratory. Inter-American Tropical Tuna Commission Bulletin 22(1):9–51.

Margulies, D., V. P. Scholey, J. B. Wexler, R. J. Olson, J. M. Suter, and S. L. Hunt. 2007a. A review of IATTC research on the early life history and reproductive biology of scombrids conducted at the Achotines laboratory from 1985 to 2005. Inter-American Tropical Tuna Commission Special Report 16:63.

Margulies, D., J. M. Suter, S. L. Hunt, R. J. Olson, J. B. Wexler, and A. Nakazawa. 2007b. Spawning and early development of captive yellowfin tuna (*Thunnus albacares*). Fishery Bulletin 105:249–265.

Margulies, D., V. P. Scholey, J. B. Wexler, and M. S. Stein. 2016. Research on the reproductive biology and early life history of yellowfin tuna *Thunnus albacares* in Panama. Pages 77–114 *in* D. D. Benetti, G. J. Partridge, and A. Buentello, editors. Advances in tuna aquaculture from hatchery to market. Academic Press, Waltham, Massachusetts, USA.

Masuma, S. 2013. Studies on broodstock management and spawning ecology of bluefin and yellowfin tuna in captivity. Bulletin of the Fisheries Laboratory Kinki University 13:37–235 (in Japanese, with English summary).

Masuma, S., S. Miyashita, H. Yamamoto, and H. Kumai. 2008. Status of bluefin tuna farming, broodstock management, breeding and fingerling production in Japan. Reviews in Fisheries Science 16:385–390.

Masuma, S., T. Takebe, and Y. Sakakura. 2009. A review of the broodstock management and larviculture of the Pacific northern bluefin tuna in Japan. Aquaculture 315(1&2):2–8.

Matsunari, H., H. Hashimoto, T. Iwasaki, K. Oda, Y. Masuda, H. Imaizumi, K. Teruya, H. Furuita, T. Yamamoto, K. Hamada, and K. Mushiake. 2013. Effect of feeding rotifers enriched with taurine on the growth and survival of larval amberjack *Seriola dumerili*. Fisheries Science 79:815–821.

McEvoy, L. A., T. Naess, J. G. Bell, and Ø. Lie. 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. Aquaculture 163:237–250.

Miller, T. J. 2007. Contribution of individual-based coupled physical-biological models to understanding recruitment in marine fish populations. Marine Ecology Progress Series 347:127–138.

Miyashita, S. 2002. Studies on the seedling production of the Pacific bluefin tuna, *Thunnus thynnus orientalis*. Bulletin of the Fisheries Laboratory of Kinki University 8:1–171 (in Japanese, with English abstract).

Miyashita, S., Y. Sawada, T. Okada, O. Murata, and H. Kumai. 2001. Morphological development and growth of laboratory-reared larval and juvenile *Thunnus thynnus* (Pisces: Scombridae). Fishery Bulletin 99:601–616.

Nakagawa, Y., M. Kurata, Y. Sawada, W. Sakamoto, and S. Miyashita. 2011. Enhancement of survival rate of Pacific bluefin tuna (*Thunnus orientalis*) larvae by aeration control in rearing tank. Aquatic Living Resources 24:403–410.

Pepin, P. 1988. Predation and starvation of larval fish: a numerical experiment of size- and growth-dependent survival. Biological Oceanography 6:23–44.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191:382.

Satoh, K., Y. Tanaka, and M. Iwahashi. 2008. Variations in the instantaneous mortality rate between larval patches of Pacific bluefin tuna *Thunnus orientalis* in the northwestern Pacific Ocean. Fisheries Research 89:248–256.

Satoh, K., Y. Tanaka, M. Masujima, M. Okazaki, Y. Kato, H. Shono, and K. Suzuki. 2013. Relationship between the growth and survival of larval Pacific Bluefin tuna, *Thunnus orientalis*. Marine Biology 160:691–702.

Sawada, Y., S. Miyashita, M. Aoyama, M. Kurata, Y. Mukai, T. Okada, O. Murata, and H. Kumai. 2000. Rotifer-size selectivity and optimal feeding density of Bluefin tuna, *Thunnus thynnus*, larvae. Suisanzoshoku 48:169–177.

Sawada, Y., T. Okada, S. Miyashita, O. Murata, and H. Kumai. 2005. Completion of the Pacific bluefin tuna *Thunnus orientalis* (Temminck et Schlegel) life cycle. Aquaculture Research 36:413–421.

Seoka, M., M. Kurata, R. Tamagawa, A. K. Biswas, B. K. Biswas, A. S. K. Yong, Y. Kim, S. Ji, K. Takii, H. Kumai. 2008. Dietary supplementation of salmon roe phospholipid enhances the growth and survival of Pacific bluefin tuna Thunnus orientalis larvae and juveniles. Aquaculture 275:225–234.

Tanaka, M., T. Kaji, Y. Nakamura, and Y. Takahashi. 1996. Developmental strategy of scombrid larvae: high growth potential related to food habits and precocious digestive system development. Pages 125–139 *in* Y. Watanabe, Y. Yamashita, and Y. Oozeki, editors. Survival strategies in early life stages of marine resources. AA Balkema, Rotterdam, Netherlands.

Tanaka, Y., K. Satoh, M. Iwahashi, and H. Yamada. 2006. Growth-dependent recruitment of Pacific bluefin tuna *Thunnus orientalis* in the northwestern Pacific Ocean. Marine Ecology Progress Series 319:225–235.

Tocher, D. R., E. Å. Bendiksen, P. J. Campbell, and J. G. Bell. 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280:21–34.

Wexler, J. B., D. Margulies, S. Masuma, N. Tezuka, K. Teruya, M. Oka, M. Kanematsu, and H. Nikaido. 2001. Age validation and growth of yellowfin tuna, *Thunnus albacares*, larvae reared in the laboratory. Inter-American Tropical Tuna Commission Bulletin 22:52–91.

Wexler, J. B., S. Chow, T. Wakabayashi, K. Nohara, and D. Margulies. 2007. Temporal variation in growth of yelowfin tuna (*Thunnus albacares*) larvae in the Panama bight, 1990-97. Fishery Bulletin 105:1–18.

Yoshinaka, R. and M. Satoh. 1989. Pages 77–78 *in* Chemistry for fisheries. Kouseisya-kouseikaku, Tokyo, Japan (in Japanese).

Zar, J. H. 1996. Biostatistical analysis. Printice-Hall, Inc., Upper Saddle River, New Jersey, USA.