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Spawning and Larval Rearing of California Yellowtail (*Seriola lalandi*) in Southern California

Kevin STUART* and Mark DRAWBRIDGE*

Abstract：Hubbs-SeaWorld Research Institute (HSWRI) has been rearing California yellowtail (*Seriola lalandi*) in San Diego, California, since 2003. The broodstock spawn naturally between 16.5-22.0°C in a 140 m^3 maturation pool. Egg quality measurements have shown inconsistent spawn quality, with highest quality seen early in the spawning season. Hatch rates ranged from 10-90% and survival to first feeding at two days post-hatch (dph) ranged from 5 -80%. Recent research results on yellowtail larvae have shown no significant differences in growth or survival at 10 dph among egg stocking densities of 50, 100, and 200 eggs/L. There were no significant differences in growth or survival when larvae were fed at densities of 15, 30, and 45 rotifers/ml. Larvae were able to consume 1st instar brine shrimp (*Artemia franciscana*) as early as 6 dph. Finally, we determined that greenwater rearing conditions created using either INVE SanoLife® ALG, algae paste, or live algae all produced similar results. Current culture protocols for production are based on these results and include 1) stocking eggs at a density of 100/L; 2) maintaining water temperature at 21.0-22.0°C; 3) providing rotifers (*Brachionus rotundiformis*) from 2 to 9 dph at 20/ml; 4) moving larvae from tall, narrow 1600 L egg incubators to shallow, wide 8,000 L tanks at 10 dph; 5) providing 1st instar *A. franciscana* from 6-10 dph and 2nd instar *A. franciscana* from 10-35 dph; 6) 24 hr overhead light at 5,000-13,000 lux; and 7) using Green water from 2-17 dph. These procedures yielded consistent survival rates from egg to juvenile of up to 5%. High larval mortality, from unidentified causes at around 17-20 dph and deformity rates as high as 40% are focal areas for future improvement.

Keywords：*Seriola lalandi*, aquaculture, green water culture, live feed management

Introduction

The California yellowtail, (*Seriola lalandi; CYT*), is a member of the family Carangidae, found in temperate waters of the Pacific and Indian oceans (Baxter, 1960; MacCall *et al.,* 1976; Sumida *et al.,* 1985; Kolkovski and Sakakura, 2007). In the Eastern Pacific, adult and juvenile CYT range from southern Washington to Chile, including the Gulf of California, and are seasonally abundant in southern California. Larval distribution is limited to warmer waters from as far north as Point Conception and south to Baja California (Suminda *et al.,* 1985). In the Southern California Bight, CYT larvae have been found from April through October (Suminda *et al.,* 1985). Because of its status as a popular sport and commercially-caught fish, the CYT, along with several other species in the genus *Seriola*, are a focus of the growing worldwide aquaculture industry.

Multiple *Seriola* spp. are cultured commercially in several countries around the Pacific (Benetti *et al.,* 2001; Nakada, 2002; Fowler *et al.,* 2003; Verner-Jeffreys *et al.,* 2006). These species include yellowtail, *S. quinqueradiata* (Japan and Taiwan);

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* Hubbs-SeaWorld Research Institute, 2595 Ingraham St., San Diego, CA 92109, USA
Kstuart@hswri.org

amberjack, *S. dumerili* (Japan and Hawaii); and almaco jack, *S. rivoliana* (Hawaii). CYT is being commercially cultured in Japan, South Australia, and New Zealand, with Japanese farmers relying on wild caught juveniles (Nakada, 2002), and farmers in Australia and New Zealand producing juveniles from domestic broodstock (Fowler *et al.,* 2003). Currently, commercial culture of *Seriola* spp., in the U.S. occurs only in Hawaii (Verner-Jeffreys *et al.,* 2006). However, CYT is considered a top candidate for culture in southern California because of its local availability and high market value. The Hubbs-SeaWorld Research Institute (HSWRI) began an experimental CYT breeding program in 2003 with wild locally caught adults. The research program is conducted from HSWRI's research laboratory in San Diego, California.

Materials and Methods

Egg Collection: Broodstock were collected in 2003 and 2004 off San Diego and Catalina Island, California. The broodstock were housed in a 140 m^3 fiberglass pool (9.1 m diameter x 2.4 m deep). The fish were exposed to shaded natural light and ambient seawater temperatures of 13-23℃. The seawater for the broodstock pool was recirculated at a rate of 1,135 L/min using an airlift-driven bead filter (0.7 m^3 PolyGeyser Bead Filter, Aquaculture System Technologies, New Orleans, LA). The bead filter performed the critical processes of solids capture and biofiltration. Water supplied to the pool by the airlift flowed by gravity from the top of the pool and a central bottom drain into an egg collector, so all eggs were collected during the study period spanning 2009 through 2010. The egg collector measured 1.27 x 1.14 x 0.64 m and contained a 500 μm mesh bag to collect the eggs before the water returned to the filter. Makeup water drawn from Mission Bay was sand-filtered and sterilized with ultraviolet light before being supplied to the pool at a rate of 5-20 L/min. A supply of pure oxygen was available to maintain dissolved oxygen (DO) levels above 7 mg/L (90-100% saturation) during the summer months.

The egg trap was checked daily at 0800, and any eggs found were collected with a fine mesh aquarium net. Only the floating eggs were considered viable. Eggs were disinfected with 100 ppm of formalin for one hour prior to stocking. The following egg and larval parameters were recorded: egg diameter, oil diameter, hatch rate (%) and survival to first feeding (%). A picture was taken of a subsample of eggs from the floating portion of the spawning event (Image Pro Plus; Leica MZ16 Macro). Once the picture was taken, the egg and oil diameters were measured from 20 qualifying eggs. A qualifying egg was one that was in full view and could be measured. Hatch rates were determined by setting up ten floating eggs on the day of collection (minus 2 days post hatch [dph]) in each of 10, 1.0 L beakers filled with 800 ml of sterilized seawater. The number of hatched larvae was counted in each beaker, and percent hatch was calculated as the average for the 10 replicates (totaling 100 eggs) for that spawning event. Survival to first feeding tests were conducted using the same methodology as the hatching test, except 0 dph larvae were used instead of eggs. Each day the number of surviving larvae in each beaker was counted until first feeding (2 dph).

Larval Rearing Trials: All larval rearing trials were conducted in the same experimental system, which maintained consistently good water quality. The experimental system consisted of 24,320 L black conical bottom tanks. DO, pH, and temperature were monitored daily using a portable meter (Hatch model HQ40d, Hach Company, Loveland, Colorado). Total ammonia nitrogen, nitrite, and nitrate were measured weekly with Hach test kits (Hach Company, Loveland, Colorado). Separate rearing trials were completed to investigate stocking density (50, 100, 200 eggs/L), rotifer feed density (15, 30, 45 rotifers/ml); brine shrimp weaning (6, 8, 10 dph), and green water replacement (live algae, algae paste, dry algae, SanoLife® ALG (INVE Aquaculture, Inc., Salt Lake City, Utah), clay, tracer dye).

The stocking density was set at 50 eggs/L for all but the stocking density trial. The tanks were supplied with temperature-controlled (22 ℃) recirculating seawater at 1.5-2.0 L/min. A containment screen was placed in the center of each tank along with an aeration ring to maintain good water circulation. Three hundred μm screens were used from -2-10 dph and 500 μm screens were used

from 10-14 dph. Fluorescent lights were placed 0.7 m above the tanks for illumination (Lithonia, Conyers, Georgia). The light intensity (1,500 to 3,000 lux) was set at the surface using a light meter (EXTECH Instruments, Waltham, Massachusetts), and light duration was set at 24 hr. The tanks were cleaned once a week for the duration of the trial, and screens were changed as needed.

Live prey was added four times daily at 0700, 1000, 1300, and 1600. Live feed densities were maintained at 15-45 rotifers/mL or 5 *Artemia*/mL throughout each trial. Prey densities were measured twice daily in each tank to maintain the target levels. This was accomplished by taking a 10-20 ml sample of seawater from each tank after which prey counts were made from three, 1 ml aliquots of the total sample. Green water was used for each trial and was developed by adding algae paste (Reed Mariculture, Campbell, California) at a cell level of 300,000-500,000 cells/mL with each feeding. The green water replacement trial was standardized by using similar Secchi disc readings (28-35.6 cm) for the clay (Bentonite Clay, New Mexico Clay Company, Albuquerque, New Mexico) and tracer dye (Bright Dyes, Kingscote Chemicals, Miamisburg, Ohio) treatments.

Larval growth was measured at 0, 3, 10, and 16 dph on subsamples of 50 larvae per treatment. Larvae were euthanized with MS-222 prior to measurement. Twenty larvae were placed under a microscope (Leica MZ16 Macro, Bannockburn, Illinois), and a photo was taken with a digital camera (Image Pro Plus, Media Cybernetics, Bethesa, Maryland). Notochord length (NL) was then measured to the nearest 0.1 mm. All 50 larvae were rinsed with de-ionized water on a 200-600 μm filter, poured through glass filter paper, and dried on glass filter paper dishes in an 80°C oven for 48 hrs. Dishes were weighed before adding larvae and then after the drying period to the nearest μg. To calculate the individual dry weight (DW), the difference between the weights was divided by the total number of larvae sampled (n = 50). Statistical analyses were performed using Statistica (Statistica, Tulsa, Oklahoma). ANOVA was used to determine differences between treatments, and arcsine transformation of the data was completed when appropriate.

Larval Production: Eggs were stocked into 1,600 L (1.2 m diameter and 1.6 m deep) cone-bottom larval rearing incubator tanks for culture. Flow rates were maintained at 3-6 turnovers per day, depending on the developmental stage of the larvae. Mild aeration was provided with airstones and bubble ring diffusers. Controlled lighting was installed above the incubator tanks providing an illumination of 7,000-11,000 lux (four 54-watt Metalux® bulbs, Cooper Lighting, Peachtree City, Georgia) at the surface. Larvae were reared in the incubators until 10 dph, at which time they were gently moved to a large diameter (3.6 m), shallow (0.9 m), flat-bottom 6,000 L pool. The seawater supplied to the pools was maintained at 21-22°C. Larvae were reared on recirculating water through metamorphosis (35 dph). The recirculating system was equipped with a fluidized bed, bead filter, and 50 μm bags to filter any particulates and/or uneaten rotifers or *Artemia*. At approximately 2-3 dph, larvae were fed rotifers (*Brachionus plicatilus*) enriched with Arti-Kol (Nutra-Kol Nutrition Solutions, Western Australia). In order to provide the larvae with enriched rotifers throughout the day, rotifers were cold-stored the day before at 8-10°C in an insulated cooler. The larvae from 2-10 dph were fed rotifers four times per day, maintaining a tank density of 20 rotifers/ml with algae paste (Reed Mariculture, Campbell, California) added to the larval tank with each feeding to achieve a concentration of approximately 300,000-500,000 cells/ml until the larvae were 17 dph. Unenriched 1st instar *Artemia* were fed from 6-14 dph and 2nd instar *Artemia* enriched with Arti-Kol were fed from 14-35 dph. A dry micro diet (Otohime, Reed Mariculture, Campbell, California) was fed starting at 15 dph and overlapped with 2nd instar *Artemia* through to 35 dph.

Results

Egg Production: Each year, spawning occurred when water temperatures were 16-22°C, with egg production decreasing at the end of the season when water temperature was above 21°C. Eggs were found as early as 1600 and as late as 0100, and spawning occurred earlier in the evening as the

Table 1. Egg production and quality numbers for CYT broodstock

Parameter	2009	2010
Number of spawn events	37	43
Egg diameter (mm)	1.36 ± 0.03^{b}	1.40 ± 0.05^{a}
Oil diamter (mm)	0.29 ± 0.02	0.31 ± 0.05
Hatch Rate (%)	75 ± 11	70 ± 19
Survival to First Feeding (%)	64 ± 18	63 ± 22

*Values sharing superscripts are not significantly different.

spawning season progressed. Courtship behavior was usually observed early in the afternoon, with pairs of fish swimming faster than normal and a male nudging at the abdomen of the female. The mean egg diameter in 2010 was significantly larger than in 2009; oil diameters also increased but not significantly. Hatch rates and survival to first feeding remained constant from 2009 through 2010 (Table 1).

Larval Rearing Trials: Water quality was similar for all experiments and within acceptable ranges for fish larvae. Water temperature was maintained at 21.4±0.3°C and salinity was 34.5±0.5 ppt. Dissolved oxygen ranged from 8.0-8.6 mg/L (94-96% saturation), and mean total ammonia nitrogen and unionized ammonia levels were <0.01and 0.0025 mg/L.

Egg stocking density trials showed no significant differences in growth or survival at 50, 100, and 200 egg/L up to 10 dph. The highest survival (15.3±8.1%) and the largest larvae (215 ± 41 μg) were produced in the 100 eggs/L treatment, although they were not significantly different from the other treatments.

Larvae were fed 15, 30, and 45 rotifers/ml all showed similar in growth and survival rates, although the largest larvae came from the 45 rotifer/ml treatment. Larvae fed 1st instar *Artemia* at 6 dph had significantly higher growth than larvae fed 1st instar *Artemia* at 8 and 10 dph and were able to ingest a 1st instar *Artemia* at 6 dph.

Finally the green water replacement trial showed that SanoLife ALG produced the highest survival (28.6±9.2 %), and there were no significant differences in growth among the algal products. The highest growth was seen in the clay treatments, although the lowest survival also came from the clay and tracer dye treatments (Table 2).

Larval Production: Survival was quantified for all 50 dph larvae and ranged from 2.5-5.8% from egg. Transfer of the larvae at 10 dph was based on the behavior of the larvae. From the time of eye pigmentation (2-3 dph) through 10 dph, the majority (70-80%) of the larvae were at the surface of the water allowing for easy transfer. Prior to transfer, swim bladder inflation was assessed and ranged from 50-100% among cohorts. There was no noticeable mortality seen from transfer of larvae at 10 dph; the majority of the larval mortality began to occur close to the completion of flexion (17-21 dph) and most of the mortality was associated with spinning larvae at the surface. The cause of the spinning was investigated but not determined. Losses after flexion, occurring between 35 and 50 dph, were the result of aggressive behavior that included cannibalism. No disease outbreaks were observed during the culture period, however the deformity level seen during 2009 and 2010 ranged from 25-40%.

Discussion

Egg production and quality: CYT broodstock in this study spawned between April and July when water temperatures were 16.5-22°C. It has been reported that CYT spawn off southern California between April and August when water temperatures are 17-21°C (Baxter, 1960; Sumida *et al.*, 1985). A similar temperature range for *S. lalandi* spawning has been reported by researchers in New Zealand (Moran *et al.*, 2007). Eggs were seen in the trap as early as 1600 and as late as 0100, and timing of spawning events got earlier as the season progressed. This has also been described in other CYT, with fish

Table 2. CYT larval rearing trials completed in 2009 and 2010.

Trial	Treatments	End of Trial (dph)	DW (µg ± SD)	NL (mm ± SD)	Survival (% ± SD)
Egg Stocking	50 eggs/L	10	205 ± 19	5.51 ± 0.38	6.8 ± 3.5
	100 eggs/L	10	215 ± 41	5.40 ± 0.35	15.3 ± 8.1
	200 eggs/L	10	195 ± 44	5.41 ± 0.36	7.0 ± 4.9
Rotifer Density	15 rotifers/ml	10	146 ± 20	5.06 ± 0.38	0.2 ± 0.2
	30 rotifers/ml	10	133 ± 12	5.05 ± 0.37	4.3 ± 6.2
	45 rotifers/ml	10	160 ± 20	5.18 ± 0.33	1.1 ± 1.3
Artemia Weaning	6 dph	14	685 ± 181	7.15 ± 0.65^{A}	6.0 ± 1.8
	8 dph	14	590 ± 131	6.60 ± 0.86^{B}	5.3 ± 3.4
	10 dph	14	530 ± 157	6.83 ± 0.73^{B}	5.3 ± 4.4
Green water Replacement	Live algae	10	340 ± 121^{AB}	5.81 ± 0.55^{B}	16.3 ± 0.9^{B}
	Algae paste	10	292 ± 64^{B}	5.75 ± 0.46^{B}	17.2 ± 9.9^{AB}
	Dry algae	10	270 ± 48^{B}	5.59 ± 0.44^{BC}	11.4 ± 6.1^{B}
	SanoLife (ALG)	10	350 ± 48^{AB}	5.79 ± 0.46^{B}	28.6 ± 9.2^{A}
	Clay	10	485 ± 87^{A}	6.23 ± 0.56^{A}	1.4 ± 0.6^{C}
	Tracer dye	10	284 ± 104^{B}	5.43 ± 0.45^{C}	1.1 ± 1.4^{C}

*Values within each experimental column sharing superscripts are not significantly different.

spawning just prior to dawn in the first half of the season and closer to dusk thereafter (Moran *et al.,* 2007). The reproductive behavior described here is also similar to what Moran *et al.* (2007) described for captive CYT in New Zealand, where a single male positioned himself beneath the female, his snout directly beneath the female's gonoduct.

Egg and oil diameters reported in this study were smaller than what has been reported for *S. lalandi* from wild populations in waters off southern California (egg diameter 1.44 mm; oil diameter 0.32 mm; Sumida *et al.,* 1985), and larger (1.1 mm) than what was reported by Kolkovski and Sakakura (2007). Measurements from Moran *et al.* (2007) showed egg diameters ranging from 1.33-1.50 mm and oil diameters ranging from 0.30-0.33 mm. Egg sizes can vary both within a species and between populations of the same species (Beacham and Murray, 1985; Brooks *et al.,* 1997). Egg diameter has been shown to decrease over the spawning season for serial spawners (Lavens *et al.,* 1999). This was not seen in 2009 or 2010; egg diameters remained constant throughout each season. However mean egg diameters in the 2010 season were larger than the mean egg diameters in 2009. The larger eggs did not yield better hatch and survival to first feeding rates between 2009 and 2010. The mean hatch rates over the season were slightly higher than what has been reported for *S. dumerili* (65%; Jerez *et al.,* 2006). There was a seasonal variation with consistently poor hatching success occurring closer to the end of spawning season.

Larval Rearing Trials: There was no clear treatment effect when stocking up to 200 eggs/L. Stocking rates for other species can range from 10-500 eggs/L, depending on the species and the purpose of culture (Benetti *et al.,* 2008; Gimenez and Estevez, 2008; Moran, 2007). The current HSWRI stocking density of 100 eggs/L has consistently produced good survival through 10 dph. The results from this trial showed that a stocking density of 200 eggs/L does not influence growth and survival at 10 dph, but it is unknown whether the higher initial stocking density has a negative impact later in development due to the density of surviving larvae.

Live feed management is a critical area in the development of larval culture for any emerging species. The type, amount, and time needed to transition to and from each live feed are main components of high survival and overall production of quality juveniles. It was determined that CYT can be reared in the tank with levels of 15-45 rotifers/ml,

whereas previous HSWRI protocols called for a minimum of 30 rotifers/ml. Based on this research, we have been able to reduce our rotifer needs by over 30%, saving valuable resources. The weaning time onto 1st instar *Artemia* was also determined. Previous weaning schedules began 1st instar introduction at 10 dph, but it was found that not only could CYT larvae ingest *Artemia* at 6 dph, but there was increased growth associated with larvae fed at the earlier age. Interestingly, the weaning times are shorter than what has been previously reported for other *Seriola* spp. (Benetti, 1997; Garcia and Diaz, 1995; Moran, 2007)

The possibility of replacing algae as the turbidity agent may further increase growth and survival through the reduction of organic material added to the culture tank; limitation of microalgae paste has the potential to limit bacteria growth and minimize larval mortality due to disease or infection (Conceicao *et al.,* 2010). Significantly, it was shown that CYT do not need algae to maintain green water or turbid conditions. The use of SanoLife ALG, as well as the appropriate use of clay, has the potential to increase larval survival and quality at an early age.

Larval Production: HSWRI larval production prior to 2009 was highly variable, with overall survival ranging from 0.1-5.0 %. The culture protocol before 2009 called for larvae to remain in the rearing tanks for 30-35 dph (completion of flexion) before transport to a juvenile rearing tank. The culture protocols were shifted to early tank transfers at 10 dph because of larval behavior. Beginning at 2-3 dph the larvae began swarming at the surface of the tanks and staying within the first 20-25 cm of the water column. The larvae remained in large concentrations at the surface until 20-24 dph (8.58-10.71 mm NL), when the larger fish began to exhibit schooling behavior. This change in protocol helped to gain some consistency in survival between cohorts (2.5-5.8%); however more investigation is needed to reach higher overall survival.

Cannibalism and aggressive behavior was noticed from 35 -50 dph. Similar cannibalistic and aggressive behavior was described for both *S. quinqueradiata* and *S. dumerili*, with larger juveniles showing aggression toward smaller individuals (Sakakura and Tsukamoto, 1999; Moran, 2007). Sakakura and Tsukamoto (1999) also showed that biotic factors, such as starvation and high densities, can accelerate the aggression of the dominant fish. The time period described above coincided with weaning off live food onto dry food, and cannibalism was alleviated once the fish were accepting the dry pellets with regularity. Deformity rates seen between 2009 and 2010 ranged from 25 to 40% depending on the cohort. The main deformity seen was cranial malformations. These cranial malformations can be caused by nutritional factors such as a fatty acid or vitamin C deficiency, a high DHA:EPA fatty acid ratio or by wall-nosing or darting into the tank wall (Cobcroft *et al.,* 2001; 2004). Further study is needed to determine the causes and solutions to these malformations.

Conclusion

Since establishing dedicated systems for conducting larval rearing experiments and simulated production runs, we have conducted numerous experiments focused on refining culture methods for larval CYT. These studies have greatly accelerated our understanding of the culture requirements for this high performance species, as well as highlighting important areas for future research. This research is providing the basis for ensuring successful CYT commercial culture in Southern California.

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