**Abstract**—In this study we describe the courtship and spawning behaviors of captive yellowfin tuna (*Thunnus albacares*), their spawning periodicity, the influence of physical and biological factors on spawning and hatching, and egg and early-larval development of this species at the Achotines Laboratory, Republic of Panama, during October 1996 through March 2000. Spawning occurred almost daily over extended periods and at water temperatures from 23.3° to 29.7°C. Water temperature appeared to be the main exogenous factor controlling the occurrence and timing of spawning. Courtship and spawning behaviors were ritualized and consistent among three groups of broodstock over 3.5 years. For any date, the time of day of spawning (range: 1330 to 2130 h) was predictable from mean daily water temperature, and 95% of hatching occurred the next day between 1500 and 1900 h. We estimated that females at first spawning averaged 1.6–2.0 years of age. Over short time periods (<1 month), spawning females increased their egg production from 30% to 234% in response to short-term increases in daily food ration of 9% to 33%. Egg diameter, notochord length (NL) at hatching, NL at first feeding, and dry weights of these stages were estimated. Water temperature was significantly, inversely related to egg size, egg-stage duration, larval size at hatching, and yolksac larval duration.

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# Spawning and early development of captive yellowfin tuna (*Thunnus albacares*)

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The yellowfin tuna (*Thunnus albacares*) is found worldwide in tropical and subtropical oceans. Yellowfin tuna reproduction is characterized by serial batch spawning and asynchronous oocyte development that is typical of tunas (Schaefer, 2001a). Yellowfin tuna are broadcast spawners and exhibit extremely high batch and annual fecundities over protracted spawning seasons (McPherson, 1991; Schaefer 1996, 1998).

Tuna reproduction has been studied predominantly through histological analyses of the gonads of fish sampled at sea. Estimates have been made of either spawning seasons, spawning intervals, fecundities, or energetic costs of spawning for yellowfin tuna (Joseph, 1963; McPherson, 1991; Schaefer, 1996), skipjack tuna, *Katsuwonus pelamis* (Goldberg and Au, 1986; Hunter et al., 1986; Schaefer, 2001b), bigeye tuna, *Thunnus obesus* (Nikaido et al., 1991), albacore, *Thunnus alalunga* (Ramon and Bailey, 1996), and southern bluefin tuna, *Thunnus maccoyii* (Farley and Davis, 1998). Egg and early larval development of tunas has been described in a number of identification guides or descriptive reviews (Nishikawa and Rimmer, 1987; Ambrose, 1996; Richards, 2006). Most descriptions of early life stages of tunas are based on examinations of specimens collected at sea.

Our knowledge of the spawning dynamics and early development of tunas remains incomplete, and most of our understanding comes from studies of cultured tunas. Before 1980, there were several small-scale efforts in Japan to artificially spawn tunas and to rear the larvae and juveniles (Harada[1]). In the past decade several programs have been developed worldwide to induce spawning and to rear tunas in captivity. Maturation and spawning of tuna broodstock in sea pens in Japan has been described

[1] Harada, T. 1980. Progress and future prospects in tuna culturing studies. *In* Proceedings of the 1979 Japan Tuna Research Conference, Shimizu, Japan, p. 50–58. [Engl. Transl. no. 50 by T. Otsu, 1980, 8 p., avail. Pacific Islands Fisheries Science Center, National Marine Fisheries Service, 2570 Dole Street, Honolulu, HI 96822.]

for yellowfin (Masuma et al.[2]) and bluefin tuna, *Thunnus thynnus* (Miyashita et al., 2000a). Spawning of captive yellowfin tuna in landbased tanks in Bali occurred in late 2004 (Nakazawa[3]). Descriptions of larval and juvenile development stemming from these culturing programs have been presented for yellowfin tuna (Kaji et al., 1999; Margulies et al., 2001; Wexler et al., 2001) and bluefin (Kaji et al., 1996; Miyashita et al., 2001).

Little is known about the spawning behavior of tunas or the influence of physical factors on spawning or egg and larval development. Almost nothing is known about the manner in which tunas aggregate for spawning, their courtship and spawning behaviors, the duration of spawning events, or the effects of physical variables on spawning dynamics or early life stage development. Since 1996, the Inter-American Tropical Tuna Commission (IATTC) has maintained a spawning population of yellowfin tuna in large landbased tanks at the Achotines Laboratory in Panama (Scholey et al., 2001; Wexler et al., 2003). Our broodstock yellowfin tuna have spawned over protracted time periods (nearly year-round on a daily basis) since October of 1996. This spawning pattern has provided a unique opportunity to study the daily spawning dynamics of this species over multiple years. In this article we describe the courtship and spawning behaviors of captive yellowfin tuna, their spawning periodicity, the influence of physical and biological factors on spawning and hatching, and the egg and early-larval development of this species.

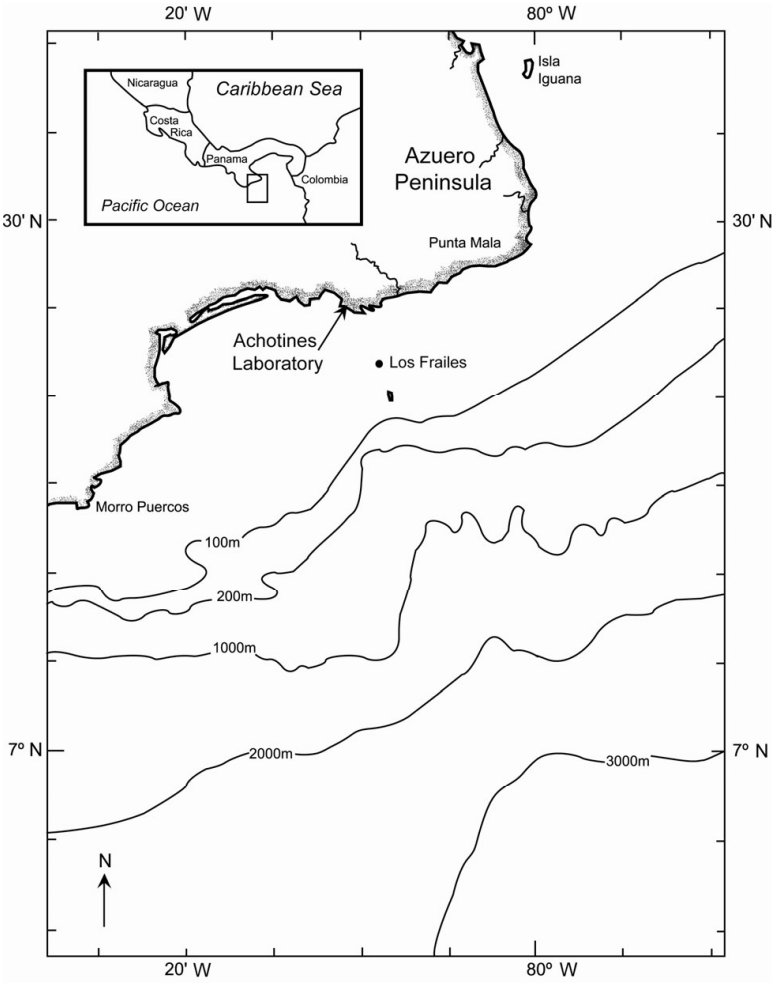


**Figure 1**

Location of the Inter-American Tropical Tuna Commission's Achotines Laboratory, Republic of Panama.

## Materials and methods

### Development of the broodstock

The yellowfin tuna broodstock was developed at the IATTC's Achotines Laboratory, located at the southern tip of the Azuero Peninsula of Panama in the northwestern portion of the Panama Bight in the Pacific Ocean (Fig. 1). The broodstock was developed in collaboration with the Overseas Fishery Cooperation Foundation (OFCF) of Japan. The design of the seawater system, plus specific details of the capture, handling, and feeding procedures for the broodstock yellowfin tuna are described by Wexler et al. (2003). We began collecting this species in coastal waters in the vicinity of the Laboratory in early 1996, and initially placed 55 individuals in the main broodstock tank (concrete, in-ground, 17 m diameter, 6 m deep) in June and September of 1996. We captured an additional 24 fish in September and October 1996 and maintained them in a smaller (8.5 m diameter, 6 m deep) in-ground tank as a reserve group. The tanks received filtered seawater by intake lines that extended outside Achotines Bay, and both tanks received biological filtration and partial recirculation. The water delivery for the main broodstock tank flowed through an aeration tower (400 m3/h) designed to aerate and degas the makeup and recirculated water entering the tank. We installed several translucent panels in the roof above the main tank to allow exposure to the natural photoperiod. Water temperature, salinity, dissolved oxygen (DO), and oxygen saturation in the tanks were recorded on a daily basis. We measured ammonia, nitrite, nitrate, and carbon dioxide on a weekly or semi-weekly basis.

[2] Masuma, S., N. Tezuka, K. Teruya, M. Oka, M. Kanematsu, and H. Nikaido. 1993. Unpubl. data. Yaeyama Experimental Station, Japan Sea Farming Association, 148 Ohta Ishigaki, Okinawa 907 Japan.

[3] Nakazawa, A. 2004. Personal commun. OFCF (Overseas Fishery Cooperation Foundation), Sankaido Bldg. 9-13, Akasaka 1, Minato-ku, Tokyo 107-0052, Japan.

We fed the broodstock a controlled diet of market squid (*Loligo opalescens*), Argentine shortfin squid (*Illex argentinus*), Pacific thread herring (*Opisthonema* spp.), Pacific anchoveta (*Cetengraulis mysticetus*), and bigscale anchovy (*Anchovia macrolepidota*). The daily ration was usually divided into 50% squid and 50% fish.

Nonlinear least-squares procedures were used to obtain growth parameters for the yellowfin broodstock (Tomlinson[4]) in order to estimate growth rates and sizes at age and length (Wexler et al., 2003).

### Spawning

During October 1996, the fish in the main broodstock tank began to exhibit courtship behavior in the late afternoons (see subsection "Spawning behavior"). We began monitoring the broodstock tank for egg production during this time. On 8 October 1996, we collected fertilized eggs in the tank for the first time. The fish spawned sporadically throughout October and November 1996, and by December 1996 were spawning daily. Using the estimated ages of wild yellowfin tuna in the eastern Pacific Ocean (Wild, 1986) and applying them to the lengths and weights of our broodstock fish (Wexler et al., 2003), we estimated the average age of the broodstock fish at the time of first spawning. During 1999–2000, we estimated the spawning periodicity and age at first-spawning of individual females. We compared the mitochondrial DNA genotypes of the female broodstock with those of their offspring (eggs and yolksac larvae) on a weekly basis from August 1999 through August 2000. Mitochondrial DNA analysis was conducted by using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) methods. The genetic analysis is described in greater detail in Niwa et al. (2003).

To determine the time of spawning each day, we checked approximately every 15 minutes for fertilized eggs at the surface of the tank with a hand-held dipnet, usually beginning in mid- afternoon because the fish most often spawned during the late afternoon or evening. Yellowfin tuna eggs, when fertilized, are positively buoyant and rise to the surface in a tank. The time at which eggs were first caught in the dipnet was noted as the "time of spawning." This is a conservative estimate because the fish spawned at different depths and locations in the tank, and we always sampled for eggs at the same location in the tank. We recorded the temperature of the broodstock tank at the time of spawning.

Although we did not anticipate spawning to occur in the reserve tank, the yellowfin tuna began spawning after 7 to 8 months in captivity in mid-April 1997. After the initial spawning in the reserve tank, we monitored the tank daily for fertilized eggs. In October 1997, we collected eggs spawned by the one remaining pair of fish to study Mendelian inheritance of nuclear DNA variants (Chow et al., 2001). We sacrificed the breeding pair after six spawning events and took samples of their muscle tissue for genetic analysis.

### Spawning behavior

When the fish spawned before sunset, we made visual observations to describe the courtship and spawning behavior. In addition, we used an underwater video camera connected to a surface video recorder to tape the spawning behavior. On several occasions, we positioned the camera at a depth of 1 to 4 m in different locations and at different angles in the main broodstock tank. The camera recorded continuously for approximately 1 h. We viewed the video tapes and chose footage that showed both courtship and spawning behaviors for behavioral analysis.

### Egg collection

In early 1997, we constructed an egg-collection system with three PVC pipes, placed at different depths between the water surface and 70 cm below the surface, so that eggs were siphoned into a square egg-collection basket (1 m × 1 m) made of porous fabric (mesh size 200 $\mu$m). We also collected eggs with dipnets and with an egg seine that sampled the entire surface of the tank. In early 1998, we attached a stationary drift net (trapezoidal opening 70 cm height × 20 cm [top width] and 40 cm [bottom width] × 1.6 m long) to one of the siphons to further standardize the egg collections. We considered the siphon system (1997) and the drift net+siphon system (1998–2000) as equivalent sampling systems because the same area in the water column (70 cm × 20 cm) was sampled, siphoned into the same egg collection basket (1 m × 1 m), and sampled daily for the same period.

We collected and counted eggs from each spawning event approximately two hours after the estimated time of spawning. We washed the eggs from the collection basket into a 20-L container. We then set the egg collection basket back into place until the next morning, when we made a second, supplementary collection. We included the second collections in the daily estimates of egg numbers, but these eggs were not used in developmental or experimental analyses.

To determine the number of eggs collected, we brought the egg collection container to 10 L of water volume and lightly agitated the mixture until the eggs were well distributed. We took three 5-mL samples with a wide-mouthed pipette and placed them in three separate, glass counting dishes. We counted the individual eggs in each of the three dishes under a dissecting microscope, calculated the mean, and estimated by extrapolation the total number of eggs in the container. Standardized egg production in the main tank was calculated daily as the number of eggs collected divided by the total biomass of females assumed to be spawning (all those ≥20 kg) in the tank.

[4] Tomlinson, P. 2001. Personal commun. Inter-American Tropical Tuna Commission, 8604 La Jolla Shores Drive, La Jolla, CA 92037.

### Determination of egg stage and average egg size

We determined the stage of development of the eggs (egg stage) and measured the egg diameter and oil globule diameter of 30 fresh, randomly selected eggs from each standard collection made at 2 h after fertilization. We measured the eggs to the nearest 0.1 mm with a dissecting microscope fitted with an ocular micrometer. Eggs were classified developmentally according to the terms used by Fritzsche (1978). Egg-stage duration was estimated as the time period from fertilization to 50% hatching (defined below).

### Incubation of eggs

We incubated the eggs in conical fiberglass tanks containing 300 L of 1-$\mu$m-filtered, UV-sterilized seawater. Through mid-1997, the incubation tanks were exposed to ambient air temperatures and indirect ambient light. Beginning in mid-1997, the incubation tanks were housed in a room with indirect fluorescent lighting and at a constant temperature. The incubation tanks were in total darkness during the night, and were never exposed to direct sunlight or overhead fluorescent light at any time. We used tanks with both flow-through and closed systems for incubation, but we detected no notable differences in hatching success between the two systems. We rinsed the eggs in a 500-$\mu$m sieve before placing them in the incubation tanks to eliminate any potentially harmful organisms such as *Benedenia* trematodes or parasitic copepods that might have transferred with the egg sample from the broodstock tank. Beginning in January 1997, we recorded the daily temperatures of the incubation tanks approximately four times between initial stocking and the time of hatching.

### Determination of time at hatching

To determine the time at 50% hatching, we collected a sample of eggs from the incubation tank at 15-min intervals, beginning about 12 to 15 h (depending on water temperature) after the estimated time of spawning. When 50% of the eggs in the sample were hatched, we recorded the time. Approximately 2 hours after 50% hatching, we made a final estimate of the mean hatching rate. We took three 300-mL samples from the incubation tank and counted the total number of dead and live eggs and yolksac larvae. Live unhatched eggs, which made up a small portion (<5%) of the samples, were considered to be almost ready to hatch because our experience has shown that the majority of these eggs eventually hatch. The estimated final percent hatching was calculated as

$$\textit{Final \% hatching} = (\textit{no. larvae} + \textit{no. live eggs}) / (\textit{no. larvae} + \textit{no. live eggs} + \textit{no. dead eggs} + \textit{no. dead larvae}) \times 100.$$

### Collection of yolksac larvae

After all the larvae were hatched (final hatching), we made morphometric measurements on 20 randomly selected yolksac-stage larvae from each daily cohort. For each larva we measured total length (TL), notochord length (NL), yolk length, yolk height, and oil globule diameter to the nearest 0.1 mm.

### Developmental series

Periodically we followed the development of a daily cohort of eggs and larvae from fertilization to first-feeding (normally a duration of 3.5 days). We initiated a developmental series whenever the daily mean temperature of the broodstock tank changed by at least 1°C. Each developmental series entailed sampling eggs at 15-min intervals for the first 4 hours after fertilization, at 1-h intervals for the next 6 h, and then at 2-h intervals until the time at 50% hatching. After final hatching, we sampled yolksac larvae at 6-h intervals until the larvae were ready to feed. We considered a larva to be at first-feeding stage when its retina was pigmented, the alimentary tract was formed, and the mouth was fully developed. We took morphometric measurements on 20–30 fresh eggs and yolksac larvae, as described previously. At first feeding, we measured 20 live larvae for TL, NL, the height and length of any remaining yolk, and the diameter of the oil globule. We measured mouth width on freshly fixed (5% formalin) first-feeding larvae because of the difficulty in obtaining accurate mouth measurements on live specimens.

### Dry weights of eggs, yolksac larvae, and first-feeding larvae

For each developmental series examined, we obtained fresh dry weights of 20–30 eggs, yolksac larvae, and first-feeding larvae. We used 8-mm diameter aluminum pans that were dried in an oven at 60°C for 24 h, desiccated for 24 h, and then individually weighed to the nearest 0.1 µg on a microbalance. After measuring, we rinsed the larvae and eggs multiple times with distilled water to remove salts and particulate matter. We placed an individual egg or larva in a preweighed aluminum pan, dried it at 60°C for 48 h, desiccated it for 48 h, and then weighed the specimen to the nearest 0.1 $\mu$g.

### Data analysis

We analyzed spawning parameters and the characteristics of eggs and early-stage larvae in relation to biological and physical data, including water temperature, time of day, and lunar cycle, from October 1996 through March 2000. We analyzed the relationships of daily ration and female size with egg size and egg production. We considered 20 kg as the minimum size for actively spawning females, based on the size at first-spawning from the genetic analysis (Niwa et al., 2003) and our observations of courtship and spawning behaviors. We

analyzed egg parameters possibly affected by size of females (standardized egg production, egg size) only during the period from June 1997 through July 1999. During this period only the original broodstock group was spawning, and we estimated that the majority of females in the tank were actively spawning (i.e., ≥20 kg each). This eliminated any confounding effects due to newly introduced immature females. Statistical analyses of the data included linear regression, correlation, and multiple regression, and followed the methods of Zar (1984). Statistical programs were run in Microsoft Excel and S-Plus 6.0 (Mathsoft, Inc., Seattle, WA).

## Results

The spawning patterns and subsequent egg and larval development are described only for the fish in the main broodstock tank in the first 11 subsections. A short synopsis of the spawning patterns of the fish in the reserve tank is presented in the last subsection.

### Broodstock fish

A total of 55 yellowfin tuna were initially stocked in the main broodstock tank in June and September of 1996. At stocking, the fish ranged in length from 51 to 78 cm fork length (FL) (mean of 62 cm FL) and weighed between 3 and 8 kg (mean of 5 kg). The sex composition of the original 55 fish (determined later at death) was 54% female and 46% male. Spawning first occurred in the main broodstock tank on 8 October 1996. At that time, there were 24 females (ranging from 6 to 16 kg and 65 to 93 cm FL) and 20 males (ranging from 6 to 14 kg and 66 to 86 cm FL) in the tank. From observations of courtship behavior during the first 2 to 4 months of spawning, it appeared that only a small group of larger individuals (generally >80 cm FL) was spawning. This pattern changed over time, as more of the broodstock fish attained reproductive size, and by mid-1997 it appeared from the courtship behavior that most of the broodstock fish were participating in the spawning. By mid-1997, there were 19 females (ranging from 13 to 30 kg and 87 to 108 cm FL, averaging 20 kg and 98 cm FL) and 16 males (ranging from 15 to 30 kg and 89 to 112 cm FL, averaging 22 kg and 99 cm FL) in the main tank. Using the estimated ages of wild yellowfin tuna in the eastern Pacific Ocean (Wild, 1986) and applying them to the lengths and weights of our broodstock fish (Wexler et al., 2003), we estimated that the average age of the broodstock fish in mid-1997 was approximately 2 years.

The broodstock population ranged in number from 55 (in September 1996) to five (in July 1999). In mid-August of 1999, we added 14 fish (ranging from 8 to 15 kg and 74 to 89 cm FL) to the main tank to supplement the spawning group. In February of 2000, we added another group of six fish (ranging from 4 to 14 kg and 58 to 71 cm FL) to the tank. The longest time that any individual fish from these broodstock groups survived in captivity was 4.7 years, although average survival in captivity was 1.9 years. Estimated ages of broodstock fish at death averaged 3.1 years and ranged from 1.4 to 6.1 years. Mortalities occurred gradually over time—most often caused by strikes against the tank wall—during the early morning hours before feeding. The average size of the broodstock fish increased over time, and reached a maximum in mid-August 1999. At that time, there were four females (ranging from 46 to 77 kg and 141 to 150 cm FL, averaging 67 kg and 147 cm FL) and one male (50 kg and 133 cm FL) in the main tank. Several of these fish attained sizes >85 kg and 155 cm FL at the time of their deaths in March and October 2000. Estimated growth rates in length for the broodstock fish ranged from 0.9 to 4.0 cm/month and decreased with increasing lengths of the fish. Estimated growth rates in weight ranged from 0.8 to 1.6 kg/ month for fish <19 kg and 1.7 to 1.9 kg/month for sizes >19 kg (Wexler et al., 2003).

### Water temperature, spawning, and egg incubation

The mean daily water temperature in the main broodstock tank fluctuated with the ocean temperatures and ranged from 20.1° to 29.7°C, averaging 27.3°C (SD=1.5) (Fig. 2A). Periods of reduced temperatures occurred each year during the coastal upwelling season from January through March. Spawning occurred when the daily mean tank temperature was 23.3° to 29.7°C, and averaged 27.7°C (SD=1.1) at the time of spawning (Fig. 2A). The minimum spawning temperature during most years and seasons was about 24.0°C. The daily mean water temperature decreased to 24.0°C or below during three of the four years of the study; however, only two spawnings occurred at mean temperatures below 24.0°C. During most years, spawning occurred daily and continuously for several months, but spawning ceased when tank temperatures decreased below 24°C (March 1997, March 1999, and February 2000) or decreased by at least 0.5°C for at least one week (December 1997, October 1998, and August 1999). The cessation of spawning in October 1998 extended for 5.5 months. During this period, water temperatures were sufficient for spawning, although they were steadily decreasing (from 28°C to <24°C) during most of the period.

The mean water temperatures in the egg incubation tanks ranged from 21.9° to 29.8°C, averaging 27.6°C (SD=1.0) (Fig. 2B). The pattern of incubation temperatures closely paralleled the pattern of mean daily water temperatures for the main broodstock tank, although the incubation temperatures did not decrease as much as those of the main broodstock tank during the upwelling period in early 1997 (because of the influence of higher air temperatures on the incubation tanks). From January 1997 through March 2000, incubation temperatures and the broodstock tank temperatures were highly correlated ($r=0.74$, df=886, $P<0.001$), and we considered the two data sets as equally representative of water temperatures observed in the laboratory.

### Seawater characteristics and spawning

Spawning in the main broodstock tank occurred at salinities of 26 to 36 ppt, but during most months salinities ranged from 30 to 34 ppt. Dissolved oxygen ranged from 65% to 107% of saturation but usually exceeded 80% of saturation. The seawater pH ranged from 7.6 to 8.3, and ammonia was always below detection levels. During periods of cessation of spawning, salinities were generally stable between 30 and 34 ppt, and dissolved oxygen, pH, and ammonia were also within normal ranges.

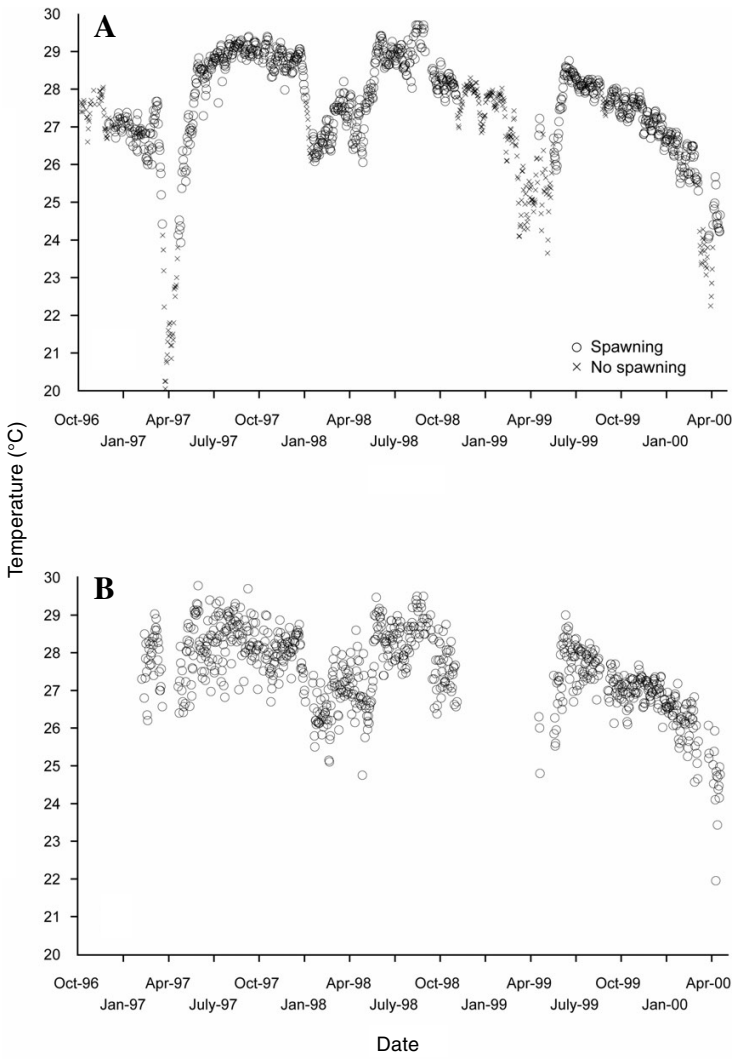


**Figure 2**

Mean daily water temperature in (**A**) the main broodstock tank, and (**B**) the incubation tanks from October 1996 through March 2000. In (A), plotted symbols are individual dates on which there was either spawning (indicated by o) or no spawning (indicated by x). In (B), plotted symbols are individual dates on which egg incubation occurred.

### Courtship and spawning behavior

The daily courtship and spawning behavior of the broodstock fish followed a consistent pattern. Courtship behavior usually began in the afternoon, and spawning occurred most often in the late afternoon or evening. The courtship behavior was usually initiated with a loose aggregation by most of the fish in the central, bottom area of the tank, although at times some pairing behavior was observed before formation of the aggregation. Smaller groups of fish (two to five individuals per group) would break off from the main aggregation and exhibit courtship behavior that included paired swimming, chasing, and bursts of speed throughout the tank. The paired swimming and chasing was often in the pattern of loops throughout the water column. A courtship group consisted of a single fish, presumed to be a female, followed closely by one to three fish, assumed to be males. During the courtship process males would often flash vertical bars (also termed feeding bars by tuna biologists) along the sides of their bodies. Fish presumed to be females would often release concentrated discharge trails from their vents during the late stages of courtship. Courtship behaviors would continue unabated for 1 to 4 h prior to actual spawning.

Two to eight spawning groups would eventually break off from the courtship aggregation and spawning would take place nearly simultaneously in the tank. When the fish spawned, they would typically swim in an ever-tightening circle—one female in front and one to five males following closely behind in single file. During actual spawning we always observed the trailing individuals releasing milt. As the females in each group began to release their eggs, they would tighten their swimming circle and the males would do the same while they released milt. This action resulted in the entire spawning group swimming in a very tight circle, which appeared to facilitate the mixing of eggs and milt. During spawning, the swimming speeds of the fish usually decreased, compared to the speeds during courtship, but at times swimming would remain quite rapid while eggs and milt were released. The fish within spawning groups moved horizontally as they spawned, but the fish often added some vortex-like movement upward through the water column as spawning occurred. Within each group, spawning was usually completed within 30 to 45 sec. The entire spawning event was often finished within 60 to 90 sec, although at times spawning events would continue for up to 5 to 10 min.

### Spawning periodicity and size and age at first-spawning

The yellowfin tuna broodstock spawned 963 times, at almost daily frequencies, over the study period, although not all the fish were sexually mature during the entire period. Genetic analysis of female broodstock and their eggs and larvae (Niwa et al., 2003) corroborated our observations and videotape analysis of courtship and spawning behaviors and spawning frequencies. The mitochondrial DNA analysis confirmed that multiple females were contributing to individual spawns over protracted time periods (weeks to months). From August 1999 through August 2000, there were ten identified females in the tank. We identified up to six female genotypes in egg or larval samples on individual spawning dates, and we observed the same genotypes for at least five females on successive sampling dates over 1 to 3 months. The spawning frequencies estimated by this analysis were undoubtedly conservative because the sampling was not conducted every day, even though spawning was occurring daily.

When spawning first occurred (8 October 1996), we estimated that the average age of the broodstock females was 1.6 years (range 1.3–2.0 years, $n$=24). For the 1999–2000 period, we estimated that the weight of a female at first spawning was 12 to 28 kg and its size was 75 to 112 cm FL. These measurements correspond to an average age of 2.0 years (range 1.6–2.8 years, $n$=7), where the majority of females would be slightly younger than 2.0 years.

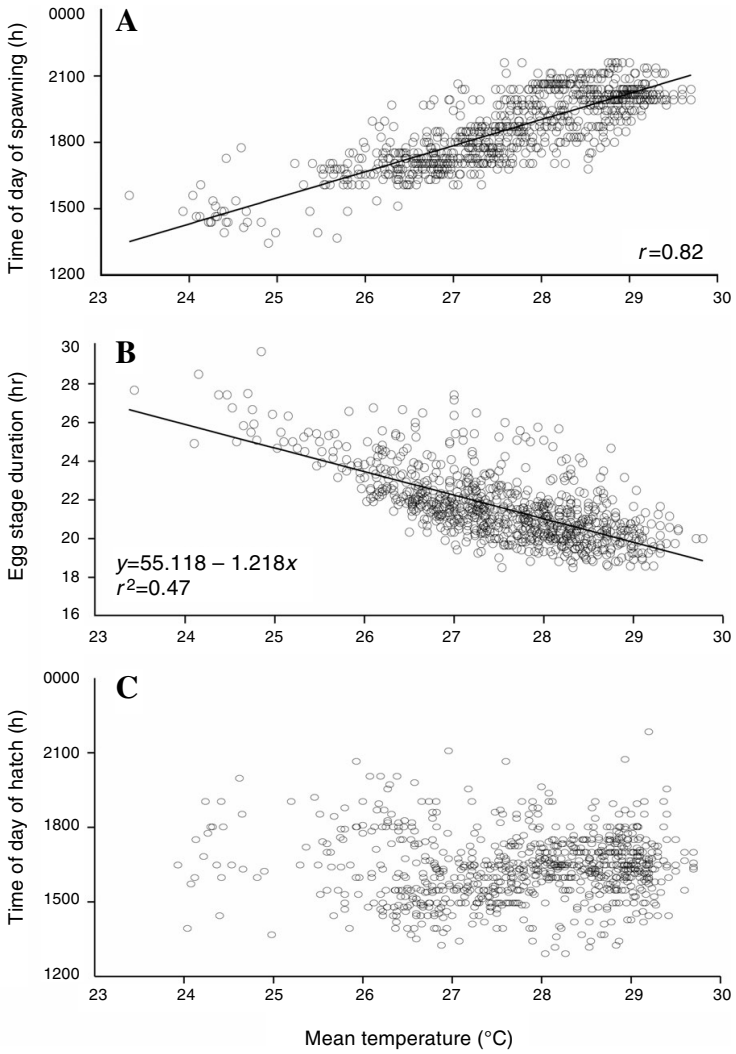


**Figure 3**

The relationship between mean daily water temperature and (**A**) time of day of spawning, (**B**) egg-stage duration, and (**C**) time of day of hatching for yellowfin tuna (*Thunnus albacares*) in captivity. Plotted values are daily estimates.

### Timing of spawning and hatching

The time of day that spawning occurred ranged from 1330 to 2130 h, and was most frequent between 1600 and 2100 h (Fig. 3A). The time of day of spawning was strongly and positively correlated ($r$=0.82, df=937, $P$<0.001) with mean daily water temperature. For example, at mean daily water temperatures of 25°C or below, spawning occurred in the afternoon between 1330 h and 1745 h. At temperatures near 27°C, spawning took place near 1800 h, and at water temperatures of 27.5°C or above, spawning occurred at night between 1845 h and 2130 h.

Egg-stage duration was inversely related to mean incubation temperature (Fig. 3B). Mean egg-stage duration ranged from a maximum of 28 h at 24°C to a minimum of 18 h at 29°C or above. The relationship was best described by a highly significant linear regression ($r^2$=0.47, df=827, $P$<0.001).

Water temperature imposed opposite effects on the time of day of spawning (direct relationship) and egg-stage duration (inverse relationship), and the net effect of these relationships was a narrow range for the time of day at hatching (Fig. 3C). Over the 3.5-yr study period, the eggs hatched between 1300 h and 2145 h. However, over 95% of the hatch times occurred in a narrow time frame between 1500 h and 1900 h, regardless of water temperature.

### Egg production and daily ration

For the original broodstock group, there was no definitive relationship between standardized egg production and mean size of spawning females, nor was there a significant overall correlation between standardized egg production and daily ration over the entire study

period ($r$=0.15, df=564, $P$>0.10). However, over short time periods (<1 month), the spawning females increased their egg production in response to short-term increases in daily ration. Over the period of June 1997 through July 1999, we varied the daily ration of food for the broodstock fish from approximately 1.0% to 4.5% body wt/day. During eight periods, we purposely increased daily food ration from 9% to 33% over time durations of 3 to 14 days. The rations were usually increased in response to greater food requirements, signaled by increasing feeding activity, or in attempts to effect increased egg production. During all eight of these periods, the standardized egg production in the tank increased from 30% to 234% (Fig. 4). There was a time lag until peak egg production occurred (indicated by numbers in parentheses in Fig. 4) after the initial increase in ration. Egg production increased, and peaked from 4 to 21 (average of 12) days after the introduction of increased rations. The percentage increase in egg production tended to be greater with greater increases in daily ration, but the relationship was not significant ($r$=0.36, df=7, $P$>0.10). The increases in egg production occurred over a range of water temperatures from 27.3° to 29.4°C; however, there was no clear association between water temperature and standardized egg production.

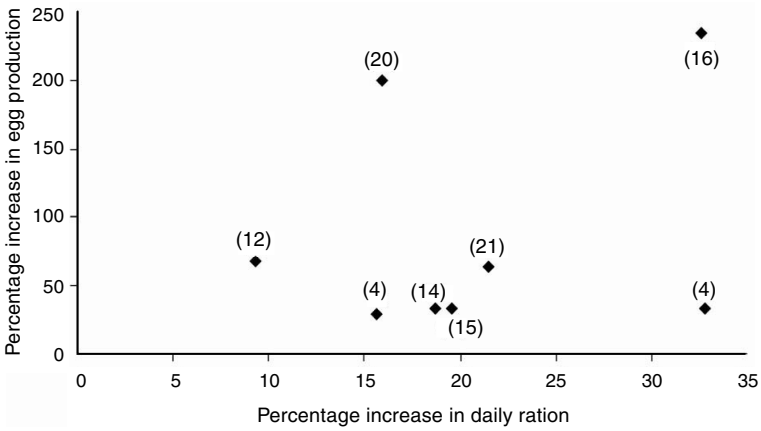


**Figure 4**

Increases in egg production by female yellowfin tuna (*Thunnus albacares*) broodstock during eight periods of planned increases in daily ration. Each period is represented by a plotted symbol. For each period, the value in parenthesis is the elapsed number of days from the initial increase in ration until the peak egg production was observed.

### Spawning and photoperiod

Given the tropical latitude of the Achotines Laboratory (7°25′N), photoperiod was relatively constant during the study. From October 1996 through March 2000, day length varied by only 53 min, and changed by only 3 to 12 min/month (U.S. Naval Observatory Astronomical Applications Database[5]). No strong relationships between either frequency of spawning or standardized egg production and photoperiod were apparent. Cessations in spawning associated with short-term decreases in tank water temperature (in December 1997, October 1998, and August 1999) occurred during periods of decreasing photoperiod, while cessations in spawning due to water temperature decreases below the apparent-minimum for spawning (<24°C) (in March 1997, March 1999, and February 2000) occurred during periods of increasing photoperiod.

### Spawning and lunar phase

Spawning occurred almost daily and showed no definitive relationship with lunar phase on a daily or monthly basis. Also, there was no significant correlation between standardized egg production and lunar phase ($r$=0.10, df=564, $P$>0.20). In each of the 26 months for which standardized egg production was calculated (June 1997 through July 1999), the monthly maxima egg production occurred equally during the first two quarters (new moon to half-moon) and last two quarters (half-moon to full moon) of each lunar cycle. However, over the 2-year period, the maximum egg production from individual spawns occurred most often at greater illumination phases of the moon. Of the 20 spawns with the greatest standardized egg production, 90% occurred during the third or fourth quarters (i.e., half-moon to full-moon), although only one of these 20 spawns occurred directly on a full moon.

### Egg-stage duration and egg size

Egg-stage duration increased with larger egg size, although the predictive power of the linear regression was low ($r^2$=0.09, df=837, $P$<0.001). Water temperature, as presented previously (Fig. 3B), exhibited a strong inverse relationship with egg stage duration. We used stepwise regression to build a model to predict egg-stage duration as a function of egg size and temperature:

$$Y = 27.96\ (\pm 1.69\ \mathrm{SE}) + 29.05\ (\pm 1.55)\ D - 1.28\ (\pm 0.04)\ DT \quad (r^2=0.58,\ \mathrm{df}=826,\ P<0.001),$$

[5] U.S. Naval Observatory Astronomical Applications Database. 2002. Table of sunrise/sunset, Astronomical Applications Dept., Data Services. Website: http://aa.usno.navy.mil/data (accessed August 2002).

**Table 1**

Morphometric measurements and dry weights of eggs, yolk-sac larvae, and first-feeding larvae of yellowfin tuna (*Thunnus albacares*).

| Eggs | Egg diameter (mm) | Oil globule (mm) | Weight (μg) | |
|---|---|---|---|---|
| Mean | 0.97 | 0.22 | 42.8 | |
| Standard error | <0.01 | <0.01 | 0.4 | |
| Minimum | 0.85 | 0.15 | 33.6 | |
| Maximum | 1.13 | 0.28 | 59.7 | |
| *n* | 27,343 | 27,359 | 197 | |
| Yolksac larvae | Total length (mm) | Notochord length (mm) | Weight (μg) | |
| Mean | 2.61 | 2.51 | 30.1 | |
| Standard error | <0.01 | <0.01 | 0.4 | |
| Minimum | 2.10 | 2.00 | 25.0 | |
| Maximum | 3.6 | 2.90 | 40.8 | |
| *n* | 15,832 | 15,849 | 132 | |
| First-feeding larvae | Total length (mm) | Notochord length (mm) | Mouth width (mm) | Weight (μg) |
| Mean | 3.49 | 3.32 | 0.262 | 21.7 |
| Standard error | <0.01 | <0.01 | <0.01 | 0.4 |
| Minimum | 2.90 | 2.70 | 0.225 | 13.8 |
| Maximum | 4.10 | 3.90 | 0.350 | 29.6 |
| *n* | 272 | 272 | 126 | 105 |

where $Y$ = egg stage duration (h),
$D$ = mean egg diameter, and
$T$ = mean incubation temperature (°C).

The diameter of individual fertilized eggs ranged from 0.85 to 1.13 mm and averaged 0.97 mm (Table 1). Each egg contained a single oil globule that averaged 0.22 mm in diameter. The dry weight of individual eggs ranged from 34 to 60 μg and averaged 43 μg (Table 1). Mean daily egg diameter was inversely related to water temperature during the period when the original broodstock (stocked in 1996) were spawning (Fig. 5A; df=892, $P<0.001$), although there was considerable scatter about the regression ($r^2=0.23$). Mean egg diameter was significantly correlated ($r=0.45$, df=553, $P<0.001$) with the mean weight of spawning females (all females ≥20 kg) in the original broodstock group (Fig. 5B).

### Larval size at hatching and hatching success

At hatching, the larvae averaged 2.5 mm NL (range 2.0–2.9 mm NL; SD=0.16) (Table 1). Larvae at hatching had unpigmented eyes, no alimentary tract nor mouth, and exhibited a large, elliptical yolk mass containing a single posterior oil globule. The dry weight of individual larvae at hatching ranged from 25 to 41 μg, averaging 30 μg (SD=4) (Table 1). Mean larval length at hatching was positively correlated ($r=0.46$, df=498, $P<0.001$) with mean egg diameter (Fig. 6A), and negatively correlated ($r=-0.27$, df=774, $P<0.05$) with mean incubation temperature (Fig. 6B).

The percentage hatching of eggs spawned in captivity ranged from 9.5% to 99.0% and averaged 83% (SD=14.7%). In general, the hatching success was high, with over 84% of the hatchings occurring at rates >70%. The percentage hatching showed no relationship with either water temperature or larval length.

### Egg and early larval development

Yellowfin tuna egg development was rapid. The developmental duration from fertilization to hatching followed a curvilinear pattern (Fig. 7), and the egg-stage duration was temperature dependent (Fig. 3B). At a modal incubation temperature of 27.0°C, egg-stage duration was 21.65 h (Fig. 7). At 27.0°C, cell cleavage occurred <1 h after fertilization, early embryo gastrulation occurred about 6 h after fertilization, the embryo first formed after 8 h of development, and the embryo was in the tail-free stage approximately 17 h after fertilization. The specific gravity of fertilized eggs changed with development. Upon fertilization, eggs were positively buoyant and would rise to the surface neuston layer of the broodstock tank. Eggs remained positively buoyant into the tail-free stage, but 2 to 4 h (dependent on temperature) before hatching the specific gravity changed and the eggs became negatively buoyant until they hatched. The nega-

tive buoyancy in the late egg stage was observed at all salinities at which incubation occurred (26 to 36 ppt).

The duration of the yolksac larval stage was inversely (but weakly) related to temperature and ranged from 56 h at 29.0°C to 65 h at 23.5°C. Eye pigmentation and mouth formation in larvae occurred almost simultaneously, and at the stage of mouth formation there was usually only a trace of yolk remaining. First-feeding larvae averaged 3.3 (SD =0.18) mm NL (range 2.7–3.9 mm) and weighed between 14 and 30 $\mu$g (average 22 $\mu$g) (Table 1). The mouth width of first-feeding larvae ranged from 225 to 350 $\mu$m, averaging 262 $\mu$m (Table 1).

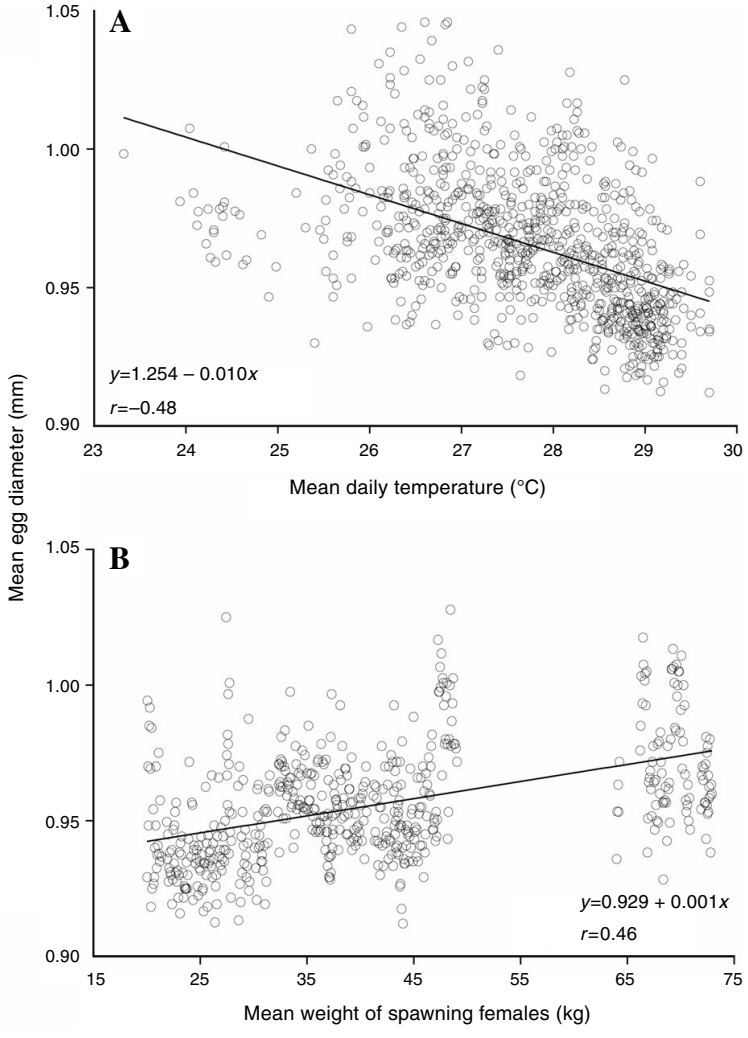


**Figure 5**

(**A**) The relationship between mean egg diameter and mean daily water temperature, and (**B**) the relationship between mean egg diameter and mean weight of spawning females of yellowfin tuna (*Thunnus albacares*) in captivity.

### Spawning in the reserve tank

The fish in the smaller reserve tank began spawning in mid-April 1997. At that time there were four females and four males in the tank. When spawning first commenced, the females ranged from approximately 7.0 to 10.5 kg (average 9 kg) and 70 to 74 cm FL (average 72 cm), while the males ranged from 5.5 to 9.0 kg (average 7.5 kg) and 62 to 74 cm FL (average 66 cm). We did not determine how many fish were involved in the initial spawning, but it appeared from courtship behavior that only a few of the larger individuals were participating in spawning during the first 1 to 2 months. Spawning continued into October 1997, when we sacrificed the one remaining pair of fish. At the time of death, the female was 16 kg and 94 cm FL and the male was 12 kg and 79 cm FL. We did not monitor the numbers or characteristics of eggs or larvae in the reserve tank. Spawning in the reserve tank occurred over a water temperature range of 24.4° to 29.2°C.

## Discussion

The spawning by the yellowfin tuna broodstock at the Achotines Laboratory, beginning in 1996, represents the first occurrence worldwide of sustained spawning by yellowfin tuna in landbased facilities. Over 3.5 years, the broodstock fish spawned in our large broodstock tank (1362 $m^3$) at near-daily frequencies over extended time periods. In general, the fish spawned as long as they received adequate daily rations of food and the water temperature was 24°C or higher. The reserve group also spawned for a 6-month period in a tank of reduced volume (270 $m^3$).

### Courtship and spawning behaviors

No courtship behavior or spawning aggregation has ever been observed in tunas in nature. The sustained spawning by the yellowfin tuna broodstock in this study allowed us to observe and analyze reproductive behavior that has not been described previously. The courtship and spawning behavior of the captive yellowfin tuna was ritualized and consistent among three groups of broodstock fish over almost four years. The courtship behavior (chasing by males, paired swimming) always occurred after the initial formation of a central aggregation of participating fish. During the actual spawning events, males were not monogamous to single spawning groups and would often move from

one spawning female to another. Spawning behavior (swimming in tightening circles with females in the lead position) ensured that eggs and milt would be well mixed. Most spawning groups moved over a range of depths in the broodstock tank, so it is likely in nature that yellowfin tuna in spawning groups move vertically to some degree during release of gametes.

The aggregations and courtship behavior usually occurred unabated for several hours before spawning and represented a significant energetic investment by the broodstock fish. The daily energetic cost of maturing a batch of eggs for a single spawning by yellowfin tuna sampled at sea was estimated at 1.06% of body weight by Schaefer (1996), but that estimate did not include the energetic costs of courtship or spawning behavior. If the behavior of our captive spawning group is representative of yellowfin tuna behavior in nature, then we believe that yellowfin tuna in the wild probably form large spawning aggregations and individual fish may invest considerable time (1 to 4 h) and energy (costs unknown) on daily courtship and spawning activities.

The behavior of the yellowfin tuna broodstock is predictable on a daily basis, which would indicate that a synchronization mechanism is inherent to the behavior. Most of the courtship and spawning behavior of the fish appeared to be driven by female behavior (i.e., females led, males followed). In teleost fishes, the release of eggs and milt during spawning is synchronized by the release of pheromones, predominantly by females (Liley et al., 1991). Sex hormones and hormone metabolites are water soluble and indicate the sender's reproductive status. Pheromones studied to date appear to be of two types, steroids and prostaglandins, and they are released by females in concentrated urine trails (Stacey, 1984, 1991). Female yellowfin tuna in our spawning group often released discharge trails during the courtship process just before spawning, and at times we misinterpreted these trails as egg emissions. Given the ritualized nature of the courtship and spawning behavior of both sexes in our broodstock, most of the discharge trails during late-courtship may have been pheromone releases by the females, although future confirmation with biochemical or immunoassay analyses would be required.

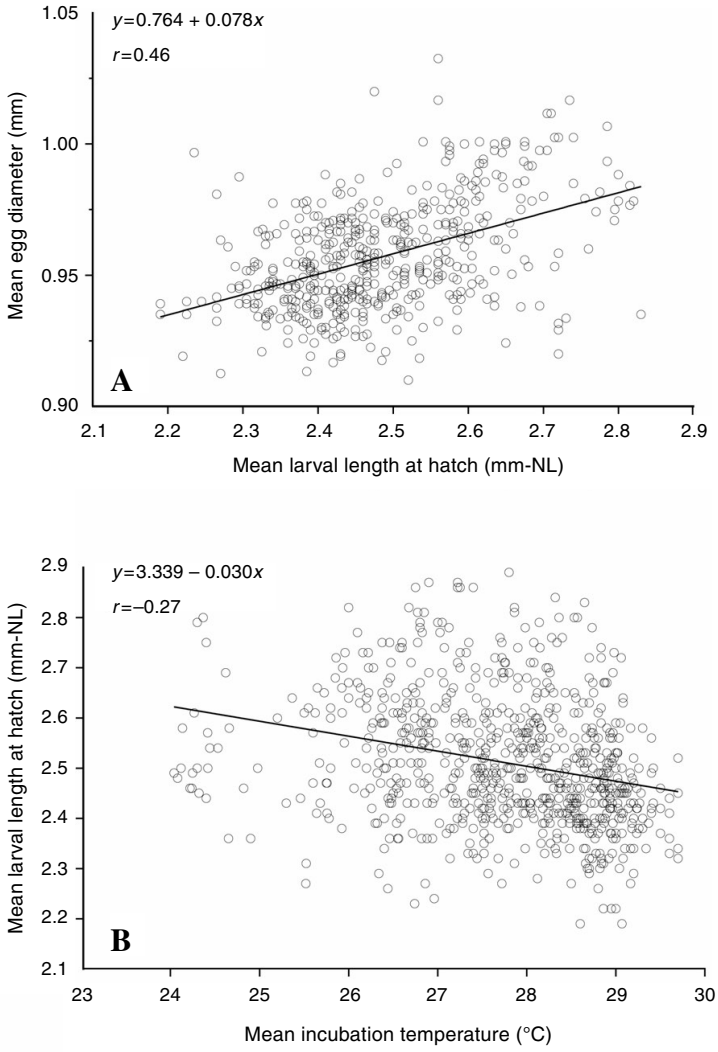


**Figure 6**

(**A**) The relationship between mean larval length at hatching and mean egg diameter, and (**B**) the relationship between mean larval length and mean incubation temperature for yellowfin tuna (*Thunnus albacares*) in captivity.

### Diel patterns of spawning and egg hatching

The diel timing of spawning by the broodstock yellowfin tuna was finely tuned to water temperature and, combined with the strong inverse relationship between water temperature and egg duration, resulted in a predictable and narrow range in the time of hatching. The majority of spawning occurred after sunset and at higher (generally >27°C) water temperatures. However, when water temperatures decreased, the broodstock fish spawned earlier in the day. This temporal shift resulted in nearly all hatching occurring in the late afternoon or early evening. We believe that this pattern for the diel timing of spawning and hatching is important and has adaptive significance for the early life history of yellowfin tuna. In nature, the maintenance of a conservative time for egg hatching would ensure that newly hatched yellowfin larvae spend the first 12 to 15 h after hatching in dimming light or darkness. Because yolksac larvae of marine

fishes are vulnerable to predation by planktivorous fishes and invertebrates (Bailey and Houde, 1989), minimizing the amount of time spent in daylight appears to be a mechanism for maximizing survival for yolksac larvae.

This temporal pattern of hatching would also minimize the exposure of yellowfin tuna yolksac larvae to the ultraviolet effects of sunlight. Although the vertical distribution of yellowfin tuna yolksac larvae in the ocean has not been described, feeding-stage larvae of yellowfin tuna are found predominantly in the upper mixed layer (Leis et al., 1991; Boehlert and Mundy, 1994). The deleterious effects (mostly genetic damage) of ultraviolet radiation are strongest in the upper mixed layer and may affect yolksac larvae more than other early life stages of marine fishes (Vetter et al., 1999). Thus, any adaptation that reduces the amount of time yellowfin tuna yolksac larvae spend in daylight increases their chances of survival.

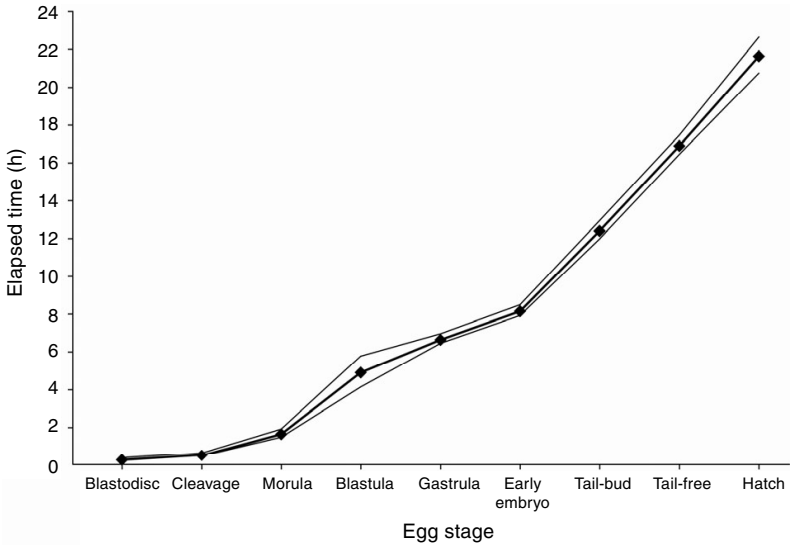


**Figure 7**

The development of yellowfin tuna (*Thunnus albacares*) eggs from fertilization to hatching at 27°C. Plotted values are mean hours elapsed to reach specific developmental stages. Error envelopes are ±2 SEs.

Most tunas are reported to be nocturnal spawners, according to histological analyses of the ovaries of adult fishes caught at sea (McPherson, 1991; Nikaido et al., 1991; Schaefer, 1998, 2001a). Our experimental results indicate that the view that yellowfin tuna spawn exclusively at night should be reconsidered. The assumption of nocturnal spawning by tunas in past studies has been based on histological analyses of oocyte development and new postovulatory follicles in the ovaries of adult fishes in the wild (collected predominantly at sea surface temperatures >27°C), but the actual water temperatures encountered by the fish before and during spawning were not known. Our results indicate that yellowfin tuna respond to variations in water temperature by altering their time of spawning and that the diel changes in spawning time are precise and predictable. The ability to control the timing of spawning by adults may be mediated by the timing of the release of sex hormones and maturation processes in response to water temperature. It is possible that our results are simply an artifact of captivity because our broodstock fish were confined to a tank and not able to thermoregulate by changing water depth or location as wild fish can. However, we believe that our experimental evidence for the control of the timing of spawning by yellowfin is compelling. The adaptive significance of this spawning pattern is most likely related to the ultimate effects of temperature on development rates of eggs and yolksac larvae (i.e., maintenance of a consistent time of hatching to maximize early larval survival). In the only other similar study on tunas, Miyashita et al. (2000a) reported that cultured bluefin tuna also spawned predominantly before sunset. Daily mean water temperatures in their 4-yr study ranged from 21.6° to 29.2°C, and the mean temperatures during the spawning season ranged from 23.6° to 27.0°C.

### Spawning cues

Spawning substrate, food availability, water temperature, photoperiod, and lunar cycle can strongly influence many functions of the teleost reproductive system (Bye, 1984; Stacey, 1984). We consider each of these potential cues for reproduction in yellowfin tuna

Because yellowfin tuna are pelagic spawners, they have no specific substrate requirements. The yellowfin tuna in our study received controlled daily rations to fuel daily spawning costs (Wexler et al., 2003). Under the nonlimiting food conditions that we provided, no relationship was apparent between spawning occurrence and food abundance. However, this pattern of spawning in captivity does not rule out the possibility that the timing of yellowfin tuna reproduction in tropical or subtropical waters could be influenced by seasonal fluctuations in food abundance, such as those associated with seasonal upwelling. Some species of tropical and subtropical marine fishes exhibit reproductive cycles that coincide with periods of upwelling or increased productivity and food availability (Bye, 1984). Tunas in tropical regions usually spawn year-round, but those occurring in the subtropics exhibit seasonal spawning patterns (Fritzsche, 1978; Schaefer, 1998), whereas bluefin tuna and albacore migrate to warm waters to spawn in distinct areas during restricted periods (Collette and Nauen, 1983). Our yellowfin tuna broodstock

did not exhibit any patterns of spawning occurrence that were related to food abundance in captivity (but see the following subsection on "Egg production and daily rations").

Our focus on the processes controlling maturation and spawning of captive yellowfin tuna has centered on the influences of water temperature, photoperiod, and lunar cycle. Water temperature appeared to provide the main exogenous control over the occurrence and timing of spawning of captive yellowfin tuna. The fish spawned over a range of daily mean temperatures from 23.3° to 29.7°C. Spawning occurred at daily mean temperatures <24°C on only two out of 963 dates (single spawning events at 23.3° and 23.9°C), and spawning became intermittent or ceased within 24 hours after those two dates. This thermal range for spawning of yellowfin tuna is similar to that reported from collections at sea of reproductively active adults (Schaefer, 1998) and early-stage larvae (Nishikawa et al., 1985; Boehlert and Mundy, 1994; Lauth and Olson, 1996). In general, tunas spawn at water temperatures ≥24°C (Collette and Nauen, 1983), although larvae of bullet or frigate tunas (or larvae of both) (*Auxis* spp.) and skipjack tuna have been collected at sea at temperatures near 22°C (Richards and Simmons, 1971; Boehlert and Mundy, 1994). In their study of spawning of captive bluefin tuna in Japan, Miyashita et al. (2000a) reported spawning temperatures from 21.6° to 29.2°C.

This study is the first to investigate the relationship on a daily basis between water temperature and spawning by yellowfin tuna over a protracted period. Our results indicate that the sensory ability of yellowfin tuna to detect ambient water temperature and the associated feedback mechanisms involved in neuroendocrine control of ovulation and spawning behavior are rapid and characterized by subdaily response times. Spawning usually ceased within one day after water temperatures decreased by only 0.1 to 0.2°C, and usually recommenced in less than one day after similar minute changes in water temperature. The broodstock altered the time of day of spawning predictably in relation to water temperature, but this behavioral change in the time of day of spawning occurred only after several days of exposure to changes in water temperature. Maturation and spawning in female fishes, in particular the initiation of vitellogenesis, oogenesis, and ovulation, is under hormonal control (Goetz, 1983). Sensory inputs to neuroendocrine control of reproduction include information on external water temperature transmitted via temperature-sensitive afferent nerve fibers (Van der Kraak et al., 1998). The spawning periodicity of the broodstock and their ability to adjust the time of day of spawning in response to water temperature indicate that yellowfin tuna have the ability to rapidly integrate sensory information on water temperature and, often within hours, adjust the hormonal control of final maturation processes and spawning.

Our analysis of spawning by the yellowfin tuna broodstock did not indicate a strong influence of lunar cycles on the timing of spawning or egg production within monthly periods. We originally anticipated some lunar periodicity to spawning because many tropical marine species exhibit lunar spawning rhythms that usually peak around the new moon or full moon (spring tides) (Johannes, 1978; Bye, 1984). Peak spawning on new or full moons in the tropics may be an adaptation to maximize offshore transport of eggs and larvae by spring tides away from increased predation pressure in coastal habitats (Johannes, 1978). Our captive yellowfin tuna, however, were exposed only to lunar cycles in captivity, but not to tidal influences. The mean daily egg production from individual spawnings in our study was highest during phases of greater lunar illumination. This trend in egg production could be viewed as evidence that yellowfin tuna increase their spawning efforts just prior to full moons, but the trend from our data is not definitive. Although adult yellowfin tuna in nature sometimes aggregate around islands and seamounts (Boehlert and Mundy, 1994), spawning by yellowfin tuna is widespread throughout the tropical and subtropical oceans (Nishikawa et al., 1985). Because yellowfin tuna often spawn in pelagic open-ocean habitats, they may not require the same level of synchronization with lunar cycles to aid in egg and larval dispersal as that required by coastal tropical species.

Photoperiod is an important environmental cue for spawning in temperate fish species (Lam, 1983). However, in the tropics, photoperiod hardly varies throughout the year and is usually not a major factor in the control of maturation and spawning of tropical fishes (Lam, 1983). Photoperiod changed very little during our study, and the yellowfin tuna broodstock showed no detectable responses to slight changes in day length.

### Egg production and daily rations

Yellowfin tuna in this study exhibited the ability to boost egg production in response to periodic increases in daily food rations. Egg production peaked over periods of 1 to 3 weeks (average 12 days) after food rations were increased. These increases in daily egg production sometimes exceeded 200%, providing strong evidence that yellowfin can convert peaks in exogenous energy consumption into higher egg production in a matter of days or weeks. The adaptive significance of this reproductive pattern is obvious. The ability to increase egg production in response to greater food abundance in oceanic habitats would provide yellowfin tuna with the opportunity to exploit patchy food resources and periodic increased production that can occur in the vicinity of islands, seamounts, and in coastal or equatorial upwelling zones. Results from several field studies, where the abundance of tuna larvae was examined, support our laboratory results. Boehlert and Mundy (1994) suggested that increased abundance of *Thunnus* spp. larvae on the leeward side of Oahu, Hawaii, may be related to greater forage for adult tunas in that region. Lauth and Olson (1996) reported peak abundance of *Euthynnus lineatus* and *Auxis* spp. larvae during periods of peak seasonal upwelling and secondary production in the Panama

Bight, and they suggested that spawning adult tunas may increase batch fecundities in response to greater forage.

Yellowfin tuna are multiple-spawning fish, and their fecundity is not fixed at the beginning of any spawning period (Schaefer, 2001a). Batch fecundity of yellowfin tuna can vary annually or geographically (Schaefer, 1998), although information on daily patterns in batch fecundity of tunas is lacking. Wootton (1979) reported one of the few studies to demonstrate a positive relationship between batch fecundity and daily food ration in nonscombrid species. Our results with captive yellowfin tuna agree with Wootton's data and indicate that over short time periods yellowfin tuna can boost egg production in response to increased food abundance.

### Size and age at first spawning and sex ratios

Our estimates of the range of sizes at first spawning for our female yellowfin tuna in 1999–2000 (12 to 28 kg, 75 to 112 cm FL) (Niwa et al., 2003) are not directly comparable to estimates from some studies of wild yellowfin tuna because of slight differences in the reproductive conditions measured. We estimated size at first spawning, whereas estimates from studies of wild fish have included estimates of size at maturity and size of reproductively active fish. Size at maturity, although related to size at first-spawning, is a more conservative estimate than size at first-spawning and does not indicate true reproductive activity. Schaefer (1998) reported that the minimum length of reproductively-active wild fish observed in the eastern Pacific Ocean was 60 cm FL. Fifty percent of the fish in that study were mature at 92 cm FL, and the portion spawning per day at that size was 0.61. McPherson (1991) estimated that the mean length at maturity of yellowfin tuna in the western Pacific Ocean was 108 cm FL. In general, the sizes of reproductively active fish reported for wild yellowfin tuna are comparable to our estimates of size at first spawning. Also, Schaefer's (1998) estimate of 92 cm FL as the length at 50% maturity for wild fish is similar to the average length of our broodstock fish in mid-1997, when we estimated that most of our fish appeared to be spawning.

It appears that the majority of our broodstock began spawning at slightly earlier ages than did the wild yellowfin tuna. Schaefer (1998) estimated the age at first maturity of yellowfin tuna in the eastern Pacific Ocean to be approximately 2 years. Our estimates of age at first spawning for the original broodstock fish ranged from 1.3 to 2.0 years, and averaged 1.6 years, and the estimated age at first spawning for the 1999–2000 females ranged from 1.6 to 2.8 years and averaged 2.0 years. However, the majority of these females were estimated to be slightly younger than 2 years. The more precocious spawning by most of the captive fish was most likely due to greater food rations and higher growth rates compared to those of wild fish during the first 1–2 years in captivity. Our results indicate that fish size, rather than age, is the best predictor of reproductive status of yellowfin tuna.

The sex ratio of the original group of yellowfin tuna in this study was initially 1.2:1.0 (female:male), and females remained slightly more abundant in the spawning group over 3.5 years. A dominance by males in larger length classes has been reported for wild yellowfin tuna, and has been attributed to potential differences in natural mortality rates between the sexes (Suzuki, 1994; Wild, 1994). We saw no evidence of greater mortality in larger females in our broodstock group. However, mortality rates in captivity are expected to be less than in nature because food is not limiting and predators are absent.

### Egg and larval development

Yellowfin tuna eggs and newly hatched larvae are morphologically typical of marine pelagic fishes. Fertilized yellowfin tuna eggs average about 1 mm in diameter, and the larvae hatch at a relatively small size (ca. 2.5 mm SL). The weight data that we present in this study are either the first (yolksac larvae, first-feeding larvae) or second (eggs) published estimates for tuna. Yellowfin eggs weigh about 43 $\mu$g dry, and weight loss occurs during the embryonic phase, and larvae lose about 33% of the original total weight at hatching and almost 50% of the original weight at first-feeding. The weight loss is due to utilization of yolk and oil during the egg and yolksac stages.

Scombrid early life history is characterized by high mortality rates, high metabolic rates, and exponential growth (Davis et al., 1991; Margulies, 1993; Wexler et al., 2001). Yellowfin tuna larvae at first feeding are intermediate in size (3.3 mm SL, 22 $\mu$g in this study) compared to other scombrid larvae (Tanaka et al., 1996), and they exhibit a large scope for growth as juveniles (Kaji et al., 1999; Wexler et al., 2007). Given the low initial weights of yellowfin eggs and yolksac larvae and the high growth rates of larvae and early-juveniles, the potential for weight gain from the egg stage to the stage at first-recruitment for yellowfin (30 cm FL, 6 months of age, IATTC[6]) is very high and approaches a gain in weight from $\times 10^6$ to $\times 10^7$.

Water temperature influences almost every aspect of the egg and yolksac larval stages of yellowfin tuna. Water temperature is inversely related (significantly) to egg size, egg-stage duration, larval size at hatching, and yolksac larval duration. Inverse developmental relationships between water temperature and the sizes and durations of egg and yolksac stages are common in marine fishes (Blaxter, 1969) and have also been reported for cultured bluefin tuna (Miyashita et al., 2000a, 2000b). These inverse relationships are most likely the result of slower responses of ontogenetic pro-

[6] IATTC (Inter-American Tropical Tuna Commission). 2000. Annual report of the Inter-American Tropical Tuna Commission, 1998, 357 p. 8604 La Jolla Shores Drive, La Jolla, CA 92037.

cesses to lower water temperature during oogenesis and embryonic stages (Chambers, 1997). At lower water temperatures, the durations of developmental stages are longer, and eggs and hatched larvae are larger.

An interesting aspect of egg development in yellowfin tuna is the change in buoyancy as the eggs develop. Fertilized eggs are positively buoyant throughout development until a few hours before hatching, when they become negatively buoyant. We assume that the onset of negative buoyancy occurs as the chorion of the egg begins to break down and more water diffuses into the egg. Just before hatching, the chorion of fish eggs is slowly liquefied by proteolytic enzymes (Blaxter, 1969), and it is likely that this process occurs also in late-stage yellowfin tuna eggs . The change in buoyancy of eggs has not been studied extensively in tunas. Mayo (1973) reported a similar change in buoyancy in the eggs of seven taxa of scombrids from Florida waters, and we have observed a similar pattern in fertilized eggs of black skipjack (*Euthynnus lineatus*, first author, personal commun.). In nature, the adaptive significance of negative buoyancy in late-stage eggs is not clear, but presumably the process of sinking just before hatching would remove the late-stage eggs and newly hatched yolksac larvae from the neuston layer and reduce mortality caused by wave action, wind, and damage from ultraviolet radiation.

Egg size increased with female yellowfin tuna size. This is a common trait in many fish species, although a direct relationship is apparent in some stocks but not in others (Hempel and Blaxter, 1967; Marteinsdottir and Able, 1988; Chambers and Leggett, 1996). The relationship between egg size and female size is relatively unstudied in tunas and is probably less important, in terms of reproductive potential, than the relationship between fecundity and female age and size (Chambers, 1997).

For aquaculture purposes, the relationship of egg size to female size in our study could be used to determine the optimum size of broodstock females required to produce maximum egg sizes. For our original broodstock group, the largest females produced the largest eggs and larvae. However, larger egg size or hatching size had no relationship with hatching success in our study. Whether larger sizes at hatching confer some greater fitness for yellowfin tuna during prerecruit stages in nature is unclear, and may depend on processes of growth, feeding, and development that occur later during development. The ecological implications of the effects of size during early life stages of yellowfin tuna or other tuna species remain unknown and would require detailed investigations of spawning and early life history traits in wild tuna populations.

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