



## Tank culture of yellowfin tuna, *Thunnus albacares*: developing a spawning population for research purposes

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### Abstract

A land-based culture facility for research on yellowfin tuna, *Thunnus albacares*, was developed at the Achotines Laboratory in the Republic of Panama. Six concrete tanks, and seawater and life support systems were built to maintain a yellowfin broodstock. On average, 50% of the yellowfin caught survived capture and handling, and approximately 30% became broodstock in Tank 1 (17 m diameter, 6 m depth) or Tank 2 (8.5 m diameter, 3 m depth). Each fish was tagged with a microchip implant tag, then weighed, measured, and injected with oxytetracycline (OTC) prior to stocking. Daily rations of primarily market squid, *Loligo opalescens*, and Pacific thread herring, *Opisthonema* spp., were regulated based on the feeding activity and energy requirements of the fish. Feeding activity of the broodstock decreased when the water temperatures decreased, and the fish ate decreasing daily rations and increasing calories with increasing size. Spawning occurred in both tanks within 6–8 months of capture. Spawning first occurred in Tank 1 when 24 females ranged in size from 6 to 16 kg and 65 to 93 cm fork length (FL). Spawning was intermittent during the first 2 months and occurred near daily thereafter. Tank size appeared to affect survival rates, the types of mortalities that occurred, and the growth of the fish. Survival rates after 1 year in captivity were higher, and the fish were larger, on average, in Tank 1 than in Tank 2. Most of the mortalities in Tank 1 were the result of wall strikes, which occurred more frequently after the fish reached their highest density of 0.64 kg m<sup>-3</sup> and sizes greater than 96 cm FL and 19 kg. Non-linear growth models were fitted to the initial stocking sizes and final sizes of fish that died

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or were removed from Tank 1 during 1996–1999. Estimated growth rates in length (11–48 cm year<sup>-1</sup>) for fish between 51 and 150 cm FL decreased with increasing length. Estimated growth rates in weight ranged from 9 to 19 kg year<sup>-1</sup> for fish less than 19 kg and 20–23 kg year<sup>-1</sup> for fish greater than 19 kg. The results of this work demonstrate that the stable environment of a land-based culture facility may be the preferred system for long-term maintenance of a yellowfin broodstock.

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## 1. Introduction

The yellowfin tuna, *Thunnus albacares*, is an important species in tuna fisheries worldwide. In 1998, the global catch of yellowfin was over 1.1 million metric tons (FAO, 2000). Although many aspects of the biology and population structure of yellowfin in wild populations have been studied (summarized in Suzuki, 1994; Wild, 1994), few attempts have been made to culture yellowfin for experimental or aquaculture purposes. From 1970 to 1980 in Japan, yellowfin were reared in small-scale studies, with some limited success at artificial spawning and rearing of larvae and early juveniles (Harada et al., 1971; Mori et al., 1971; Harada et al., 1980). Beginning in 1986, scientists at the Japan Sea Farming Association (JASFA) held yellowfin in anchored sea pens at Ishigaki Island, Okinawa Prefecture, and these fish spawned naturally for the first time in 1992 (Masuma et al., 1993). The spawning of this broodstock resulted in several studies of their early life history, including a description of the morphological development of larvae and early juveniles (Kaji et al., 1999), food selectivity of larvae (Margulies et al., 2001), and age validation and growth of larvae (Wexler et al., 2001). Since 1993, scientists at the Tuna Research and Conservation Center (TRCC), a collaborative program of the Monterey Bay Aquarium and Stanford University, have held yellowfin in tanks for research and husbandry purposes (Farwell et al., 1997; Farwell, 2000, 2001). Prior to these studies, no other attempts to rear and spawn yellowfin in captivity have been made.

Previous spawning and rearing of captive yellowfin have depended upon artificial fertilization of eggs from wild fish or natural spawning of broodstock held in sea pens. These methods are dependent upon either the well-timed collection of wild, mature fish or the uncontrolled variability of physical conditions in sea pens. Another approach to develop a yellowfin broodstock is to rear the fish in large, concrete tanks. The use of a land-based culture facility allows for more control of physical conditions and rearing methods.

Since 1985, the Inter-American Tropical Tuna Commission (IATTC) has maintained the Achotines Laboratory in Los Santos Province, Republic of Panama for the purpose of studying the early life histories of tunas (Olson and Scholey, 1990; Margulies, 1993; Wexler, 1993; Lauth and Olson, 1996). In December 1993, the IATTC, the Overseas Fishery Cooperation Foundation (OFCF) of Japan, and the government of the Republic of Panama reached an agreement to investigate the feasibility of maintaining yellowfin

broodstock in large, in-ground tanks to produce eggs, larvae, and juveniles for research purposes at the Achotines Laboratory.

This paper reports the first successful development of a spawning population of yellowfin tuna in a land-based culture facility. The characteristics of the rearing system, the collection and handling of the broodstock, husbandry techniques, and food consumption, growth, spawning, and survival of the captive fish are described.

## 2. Methods

### 2.1. Physical systems

The seawater and life support systems were designed to maintain a healthy broodstock and reserve broodstock of yellowfin tuna for several years in captivity.

#### 2.1.1. Seawater system

Seawater was drawn through dual 30-cm diameter PVC intake lines that extended 550 m from the intakes, at 16 m depth outside Achotines Bay, to a pump house on the beach (Fig. 1). The pump house contained two 10-hp fiberglass pumps that lifted  $45 \text{ m}^3 \text{ h}^{-1}$  each to a slow sand filter (described by Huguenin and Colt, 1989) located 16 m above the pump house. At the filter, the water percolated through layers of sand and gravel that retained particles larger than about  $50 \mu\text{m}$ . The filter was divided into two  $3.5 \times 3.5 \times 2.5$  m sections, so that one section could be used while the other was undergoing backwash, rinse, or maintenance. From the filter, the water flowed by gravity into a  $60\text{-m}^3$  header tank, through  $16\text{-}\mu\text{m}$  pleated-paper cartridge filters, through ultra-violet sterilizers, and into the tanks.

#### 2.1.2. Broodstock tanks

Six concrete tanks (Table 1) were constructed and housed in a  $1300 \text{ m}^2$  roofed, open-walled building. The main broodstock tank (Tank 1) was designed to be large enough to reduce the stress of captivity and enhance the chances of spawning. Vertical black stripes, 2.5 cm wide at 65-cm intervals, were painted on the entire interior tank wall to improve visibility of the wall. Tuna purse-seine netting was strung around the perimeter of the tank on fixed 1.5-m high PVC poles to prevent fish from jumping out of the tank. A translucent panel in the roof above Tank 1 was installed to allow exposure to the natural photoperiod, and a pair of red, 25-W light bulbs illuminated the egg-collecting box at night.

The metabolic demands of yellowfin tuna are very high (Olson and Boggs, 1986; Boggs and Kitchell, 1991; Dewar and Graham, 1994), and bioenergetics calculations suggested that substantial aeration would be needed to meet the oxygen requirements of a broodstock. An aeration tower (3 m diameter, 4.3 m height) was constructed to aerate and degas the water entering Tank 1 (Fig. 1) based on a design by the Monterey Bay Aquarium (C. Farwell and D. Thomasberg, Monterey Bay Aquarium, Monterey, CA 93940, personal communication). Water ( $400 \text{ m}^3 \text{ h}^{-1}$ ) was pumped from the surface of the tank sump (to reduce the uptake of solids to the aeration tower) to the top of the tower (Fig. 1) by two

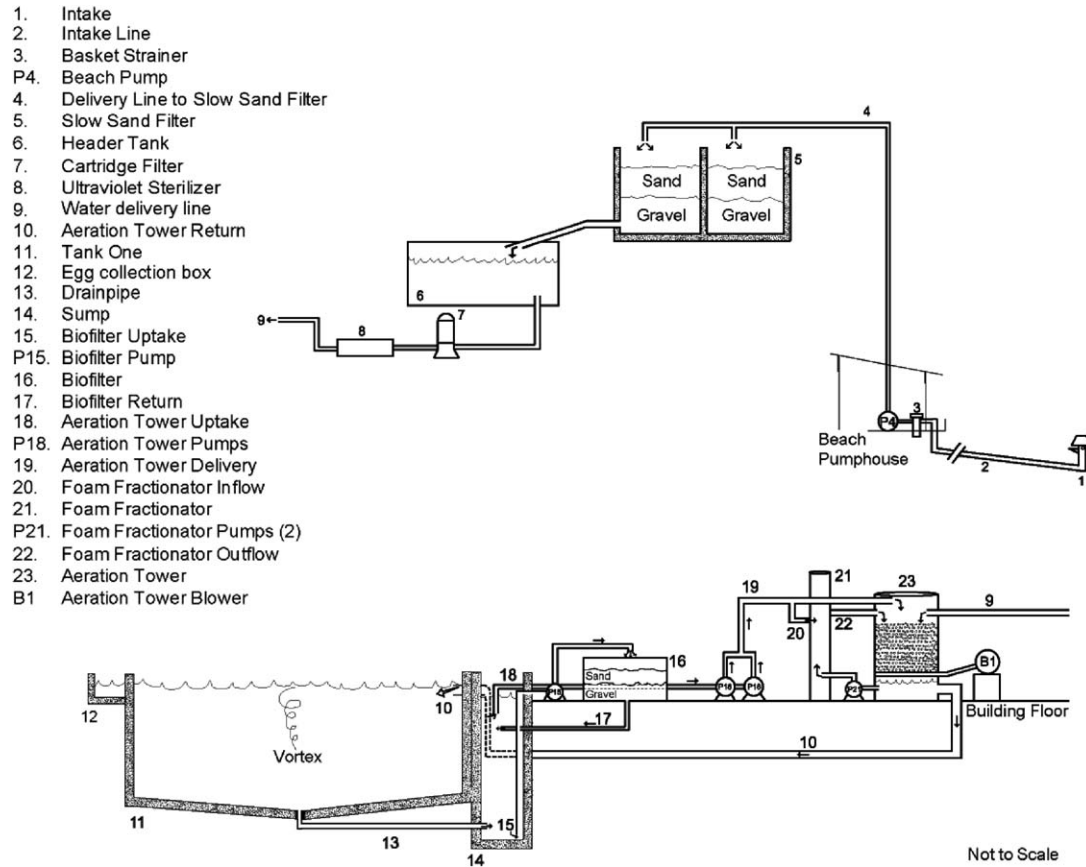


Fig. 1. Seawater delivery and filtration systems (upper diagram) and broodstock tank (Tank 1) systems (lower diagram) at the Achotines Laboratory. The reserve holding Tanks 2 and 6 also have biofiltration (lower diagram) but water is not circulated through an aeration tower or foam fractionator.

Table 1  
 Characteristics of experimental, reserve, and broodstock tanks for yellowfin tuna at the Achotines Laboratory

Tank number	Dimensions (diameter × depth) (m)	Capacity (m <sup>3</sup> )	Seawater system	Water exchange rate (tank volumes/day)	Biofilter dimensions (m)	Purpose/supporting fish density and duration
1	17 × 6	1362	Semi-closed	7	4 × 4 × 1.9	Main broodstock tank/0.5–0.75 kg m <sup>-3</sup> for >3 years
2	8.5 × 3	170	Semi-closed	2.5	1.5 × 1.5 × 1.6	Holding reserve fish/ <0.4 kg m <sup>-3</sup> for up to 1 year
3	8.5 × 1.5	85	Open	1–3	None	Short-term experiments/ <0.5 kg m <sup>-3</sup> for up to 3 months
4	8.5 × 1.5	85	Open	1–3	None	Short-term experiments/ <0.5 kg m <sup>-3</sup> for up to 3 months
5	8.5 × 1.5	85	Open	1–3	None	Short-term experiments/ <0.5 kg m <sup>-3</sup> for up to 3 months
6	8.5 × 3	170	Semi-closed	2.5	1.5 × 1.5 × 1.6	Holding reserve fish (not used in this study)

7.5-hp fiberglass pumps. The water cascaded through the packed column and returned by gravity to the tank via four 25-cm diameter pipes that exited the tank wall near the surface at an angle, to induce a clockwise flow in the tank. The water exited the tank through a screened, 30-cm diameter drain in the center of the tank floor. Supplemental aeration for Tanks 2–6 was supplied by airstones.

Water was drawn from the bottom of the sump with a 5-hp fiberglass pump that delivered 114 m<sup>3</sup> h<sup>-1</sup> to a slow sand filter, and returned by gravity to the sump (Fig. 1). Uneaten food and feces were retained by the sand filter and flushed away daily by backwashing. Makeup water was added to Tank 1 daily to replace 10–35% of the water. About 10% of the water pumped to the aeration tower was diverted through a foam fractionator (0.76 m diameter, 5 m height) to remove small particles and dissolved organics, and then returned to the aeration tower.

The design of Tanks 2 and 6 was similar to that of Tank 1, but on a reduced scale (Table 1). These tanks were used to maintain smaller tuna as reserve broodstock for up to 1 year.

Water parameters, including temperature, dissolved oxygen, salinity, and pH, were measured daily in all tanks. Ammonia, nitrite, nitrate, and carbon dioxide levels in Tank 1 were measured weekly or semi-weekly.

## 2.2. Capture and handling procedures

Yellowfin were collected in coastal waters in the northwest Panama Bight from 1996 through 1999. Fish captured in February through August 1996 were used as the principal

spawning stock for Tank 1. The fish were captured by either rod and reel or hand line, using barbless hooks, from either a 7.6-m or an 8.2-m boat. Each fish was lifted by the hook and line and placed, without touching the body, into either a rectangular tank of the 7.6-m boat (1.5 × 1.9 × 0.6 m, length × width × depth) or an oval tank of the 8.2-m boat (2 × 1.4 × 0.5 m). One to three fish were transported to the Laboratory within 1 h after capture. Upon arrival at the Laboratory, each fish was weighed, measured, and tagged with a microchip implant tag (AVID brand) in the dorsal musculature for individual identification. After a preliminary experiment to test for the effect of oxytetracycline (OTC) on survival (Sections 2.4 and 3.2), all fish were injected with 1.5 ml of 100 mg ml<sup>-1</sup> OTC. Each fish was then moved to either a 4.6-m diameter treatment tank, or to Tank 2, 3, or 4 (Table 1). After a preliminary experiment to examine the effect of sodium nifurstyrenate (NFS) on survival, all fish were treated with NFS following procedures detailed in Section 2.4. Beginning in July 1996, the yellowfin were treated once or twice for 1 h each in a solution of 200 ppm formalin to eliminate external parasites before stocking.

### 2.3. Capture mortalities

The yellowfin that died at sea, during transfer or handling, or shortly after placement in a tank were classified as capture mortalities (CMs), and the fish that survived capture and transport were classified as capture survivors (CSs). Shortly after death, the CMs were measured and weighed, and their sex was determined. Analysis of variance ( $\alpha = 0.05$ ) was used to compare mean lengths and weights of the CMs and CSs within and between 1996 and 1998, to examine if fish size explained the variability in their survival after capture and handling.

### 2.4. Preliminary experiments

During April–June 1996, two experiments were conducted to: (1) examine the effects of the antimicrobial agent NFS on yellowfin survival after capture and handling; and (2) examine the short-term (2–3 weeks) effect of OTC injection on survival.

The first experiment was designed to determine if immersion of yellowfin in NFS resulted in a measurable reduction in mortality compared to non-treatment. NFS has been used by the National Research Institute of Aquaculture in Japan for the treatment of bacterial skin infections on young bluefin tuna (*Thunnus orientalis*) caused by handling (Sako et al., 1991). Thirty yellowfin were captured (see Section 2.2 for methods) over a 2-month period, and placed into either a 4.6-m diameter, 20-m<sup>3</sup> treatment tank or directly into Tank 3 or 4 (Table 1) without treatment. Most of the fish were placed at random, and the last few fish were non-randomly selected so that equal numbers of fish ended up in each treatment. In the treatment tank, the fish were immersed in a solution of approximately 7 ppm NFS for two periods of 1 h each separated by 12 h. The tank was allowed to flush after each treatment. The 15 treated fish were then randomly transferred into either Tank 3 or 4 with the non-treated fish. The experiment was terminated 62 days after the first fish was captured. A daily survival rate (DSR) was calculated for each group and 95% confidence intervals (95% CI) were calculated for the DSRs directly from a binomial distribution (DeMaster and Drevanak, 1988). The differ-

ence between the two groups was tested with the variance test for homogeneity of the binomial distribution (Snedecor and Cochran, 1967).

The second experiment was designed to determine if OTC injection has an adverse effect on survival of yellowfin in the laboratory. OTC injection was shown to be detrimental to the health of captive summer flounder (*Paralichthys dentatus*) (Monaghan, 1993). OTC injected into the musculature of tunas establishes a temporal mark in the otoliths and vertebrae that can be detected by microscopic examination with ultraviolet light for studies of growth (Wild and Foreman, 1980; Foreman, 1996). It was, therefore, desirable to inject all captive yellowfin, if doing so did not reduce survival, to examine the influence of environmental variables and the onset of spawning on otolith and vertebral growth. Every second fish captured (Section 2.2) was injected with 1.5 ml of 100 mg ml<sup>-1</sup> OTC (approximately 0.3–0.7 ml kg<sup>-1</sup> body weight). All fish were placed into Tank 2. Of the 29 yellowfin captured for the experiment over a period of 1 week, 14 were injected with OTC. The experiment was terminated 2 weeks after the last fish was placed in the tank.

### 2.5. Stocking densities

A maximum stocking density of 0.5 kg m<sup>-3</sup> was targeted for a spawning population of yellowfin in Tank 1 (Table 1), based on the experience of JASFA scientists (S. Masuma, JASFA, Amami Station, 955-5 Hyo Setouti, Ooshima, Kagosima 894–24, Japan, personal communication).

Yellowfin (55 fish total) were transferred from Tanks 2, 3, and 4 (Table 1) to Tank 1 in June and September 1996. Additional fish were caught in 1998 and 1999 and added to Tank 1 in August 1999 to supplement the spawning population. When the fish were transferred, they were re-measured, re-weighed, and re-injected with OTC (to provide another temporal mark in their otoliths). Twenty-four fish that were captured in September and October 1996 were maintained in Tank 2 for 1 year as a reserve group, and they were never moved to the broodstock tank.

### 2.6. Food organisms

Five species of food were fed to the broodstock yellowfin: market squid (*Loligo opalescens*); Argentine shortfin squid (*Illex argentinus*); Pacific thread herring (*Opisthonema* spp.); Pacific anchoveta (*Cetengraulis mysticetus*); and bigscale anchovy (*Anchovia macrolepidota*). All food organisms were stored frozen at –19 °C.

A sample of 5–10 individuals from every batch of food was measured and weighed. They were then coarsely ground, dried to a constant weight at 60 ± 2 °C, and homogenized for proximate analysis. The dried samples were sent to a commercial laboratory where the ash, protein, and lipid contents were measured using standard methods (Association of Official Analytical Chemists, 1990). The carbohydrate content was considered to be negligible, and was not measured. The caloric equivalents of the food were calculated based on metabolizable energy values (heats of combustion adjusted for digestibility and non-fecal excretory losses) of protein (4.23 kcal g<sup>-1</sup>) and lipid (8.0 kcal g<sup>-1</sup>), according to Brett and Groves (1979).

### 2.7. Feeding

The yellowfin in Tank 1 were fed once per day at 10:30 h or twice per day during brief acclimation periods or to increase egg production for laboratory experiments. The reserve yellowfin in Tank 2 were fed twice per day during the first 8 months and once per day thereafter.

The desired target ration (% body weight day<sup>-1</sup>) of food was based on the best estimate of the biomass in Tank 1 on that day minus the weight of any individuals that were not feeding. The fish and squid were thawed, drained, and weighed before being offered (usually in equal amounts). Food items were usually offered whole; some of the food was cut into pieces when the yellowfin were still small or when smaller fish were added to the tank with the larger fish. A vitamin formulation (Y. Takahashi, National Fisheries University, Shimonoseki, Japan) at 0.5–1.5% of the weight of the food, and vitamin C at 500 mg fish<sup>-1</sup> day<sup>-1</sup> were packaged in gel capsules and placed in the mantle cavities of the squid or the gill cavities of the fish.

Feeding procedures for Tank 1 were developed to ensure that (1) the food was eaten before reaching the tank bottom, (2) the yellowfin ate the food that, judging from their behavior, was least desirable (usually the squid) along with the preferred food (usually the fishes), (3) food was offered in several locations in the tank so that the less aggressive yellowfin were not kept away from the food by the more aggressive individuals, and (4) collisions between yellowfin were avoided during active feeding behavior. Feeding behavior was monitored carefully and ultimately determined the amount of food eaten. The amount of food of each type offered, the estimated amount that reached the tank bottom uneaten, and the number of yellowfin that did not feed were recorded.

A food conversion ratio was calculated as the wet weight of food consumed divided by the biomass gained during 33 time intervals of 12–103 days, over which the numbers of yellowfin in Tank 1 were constant. Time intervals of less than 10 days were omitted from the analysis. The biomass of the yellowfin was estimated using procedures outlined in Section 2.10.

### 2.8. Bioenergetics

Predictions from a bioenergetics model developed for captive yellowfin (R. Olson, IATTC, 8604 La Jolla Shores Drive, La Jolla, CA 92037-1508, personal communication) were used to estimate the food requirements of the broodstock in Tank 1. The goal was to provide the yellowfin with sufficient energy to, initially, fuel high growth rates and, subsequently, to fuel egg production and spawning, and to avoid overfeeding the fish, which could result in excessive fat accumulation and poor condition. The model incorporated parameters from [Olson and Boggs \(1986\)](#), [Olson \(1990\)](#), [Boggs and Kitchell \(1991\)](#), [Dewar and Graham \(1994\)](#), and [Schaefer \(1998\)](#). The model predicted oxygen and caloric requirements of the yellowfin as a function of their size, whether they were spawning or expected to spawn, and water temperature. The energy requirements were converted to biomass of food based on the proximate composition of the food and the proportion of the diet comprised by each food taxon. The proximate composition of the captive yellowfin, determined from sacrificed individuals and those that had recently died

from wall strikes, for example, was used to compute the caloric equivalent of respired oxygen (the oxy-calorific equivalent) (Beamish, 1974).

### 2.9. Survival estimates

Expected survival estimates (Ricker, 1975; Margulies, 1989) of fish that were stocked in Tanks 1 and 2 during 1996 were calculated at 3-month intervals. The expected survival estimates the numbers of fish that would have survived to the end of each time interval if the sampling mortalities had not occurred. The calculations are based on the relationship:  $N_t = N_0 e^{-Zt}$ , where  $N_t$  = number of survivors at  $t$  days after stocking,  $N_0$  = initial number of fish stocked, and  $Z$  = instantaneous total mortality rate. Also,  $Z = F + M$ , where  $F$  = sampling mortality rate and  $M$  = “natural” mortality rate in the tank. The planned removals of fish for nutritional analysis were considered sampling mortalities, and all other mortalities were considered “natural.” For any particular  $t$ , when  $N_0$ ,  $N_t$ ,  $Z$ , and  $F$  were known,  $M$  was solved, from which the expected number of survivors ( $EN_t$ ) as  $EN_t = N_0 e^{-Mt}$  was calculated (Ricker, 1975; Margulies, 1989).

### 2.10. Growth curve fitting procedures and analyses

Non-linear least-squares estimation procedures (P. Tomlinson, IATTC, personal communication) were used to obtain the best estimates of growth parameters for the yellowfin stocked in Tank 1 in 1996 and maintained through 1999.

The following Richards growth model (Richards, 1959) for the expected size in length or weight ( $\hat{Y}_2$ ) of a fish after an elapsed time ( $\Delta t$ ), given the initial size in length or weight is  $Y_1$  and the parameters of the model are  $K$ ,  $Y_\infty$ , and  $m$ , is:

$$\hat{Y}_2 = [Y_\infty^{(1-m)} - (Y_\infty^{(1-m)} - Y_1^{(1-m)})e^{(-K)(1-m)(\Delta t)}]^{1/(1-m)}, \quad (1)$$

where  $Y_\infty$ ,  $m$ , and the product  $K(1-m)$  are all constrained to numbers greater than or equal to zero (Pella and Tomlinson, 1969). For 39 of the 55 yellowfin in Tank 1, data were available for their size when transferred to Tank 1 ( $Y_1$ ), their size at death ( $Y_2$ ), and  $\Delta t$  in years. The model parameters,  $K$ ,  $Y_\infty$ , and  $m$ , from Eq. (1) were estimated for the 39 fish. For the length measurements, the best estimate of  $m$  was equal to 0 (von Bertalanffy, 1938), and the variance of the parameters  $K$  and  $Y_\infty$  were estimated using 1000 bootstraps (Efron, 1982; Efron and Tibshirani, 1993). Each bootstrap was a resample, with replacement, from the 39 fish. For the weight measurements, the best estimate of  $m$  was greater than 0 (Ricker, 1979), and estimating the parameter variances using bootstrapping was computationally too difficult.

Growth rates ( $\Delta y/\Delta t$ ) in  $\text{cm year}^{-1}$  and  $\text{kg year}^{-1}$  for fish at any given length or weight ( $Y$ ) were estimated using the following equation:

$$\Delta y/\Delta t = K(Y^m)(Y_\infty^{(1-m)} - Y^{(1-m)}). \quad (2)$$

By assuming that each fish has the same  $K$ , the growth curve (for either length or weight) of each fish was forced through the observed  $Y_1$  and  $Y_2$  by estimating  $\hat{Y}_\infty$  for each fish, using the equation:

$$\hat{Y}_\infty = \{[Y_2^{(1-m)} - (Y_1^{(1-m)} e^{(-K)(\Delta t_1)(1-m)})] / [1 - e^{(-K)(\Delta t_1)(1-m)}]\}^{1/(1-m)}. \quad (3)$$

This procedure allows for the estimation of the size of each fish on any given day by using Eq. (1) with a common  $K$  and  $m$ , and by using the individual  $\hat{Y}_\infty$  values (Eq. (3)) where  $\Delta t_1$  corresponds to the elapsed time in years for each day. The sum of the estimated weights provided total biomass and density (biomass/tank volume) estimates for each day. Of the 55 fish stocked in Tank 1 in 1996, 9 were not included in the daily biomass estimates because they died shortly after introduction to the tank.

Growth functions were not fitted to the length and weight data of fish in Tank 2. However, the mean lengths and weights of the fish that survived 1 year in captivity in Tank 1 (includes only yellowfin stocked in September 1996) and Tank 2 were compared.

### 2.11. Post-mortem analyses

Tissues from nine yellowfin removed from Tank 1 during 1996 and 1997 and from some of the fish that had recently died in the tank, but were not deteriorated, were analyzed to check the condition and fat accumulation of the fish (Section 3.9), and to calibrate the bioenergetics model (Section 2.8). Each fish was identified by its tag number, weighed, measured, and examined for external parasites and the apparent cause of death. Each fish was dissected to determine its sex, to examine the condition of its internal organs, and to check for internal parasites. The gonad and liver were weighed, and a section of the gonad was fixed in 10% formalin for histological classification of reproductive status (Schaefer, 1998). The otoliths were extracted, cleaned, and stored dry, and the 34th and 35th vertebrae were frozen for age and growth analyses. A blood sample (5 ml, usually from the heart) was taken from some of the freshly dead fish for hematocrit and hemoglobin content. A 500-g sample of white muscle was removed from the dorsal loin area just posterior to the second dorsal fin, coarsely ground, dried to a constant weight at  $60 \pm 2$  °C, homogenized, and analyzed for proximate composition using standard methods (Association of Official Analytical Chemists, 1990). The caloric equivalents of the muscle sample were calculated based on the heats of combustion for protein ( $4.8 \text{ kcal g}^{-1}$ ) and lipid ( $9.45 \text{ kcal g}^{-1}$ ) (Phillips, 1969).

The last four fish in Tank 2 were also sacrificed after 1 year in captivity to provide baseline data on genetic variation in mitochondrial and nuclear DNA (Chow et al., 2001).

## 3. Results

### 3.1. Yellowfin collections

During 1996, a total of 248 yellowfin were caught, and 55.2% survived capture and handling (Table 2). A total of 79 healthy yellowfin, 32% of those captured in 1996, were

Table 2

Capture, survival, and mortality data for yellowfin tuna caught for captive broodstock in the northwestern Panama Bight during 1996 and 1998. The yellowfin that died at sea, during transfer or handling, or shortly after placement in a tank were classified as capture mortalities

Year	Number caught	Fishing trips	Average number caught per trip	Percent survival	Capture mortalities		
					Males	Females	Unknown sex
1996	248	103	2.41	55.2	49	53	9
1998	151	111	1.36	43.7	40	43	2

stocked in Tank 1 (55 fish—53% females, 46% males) and Tank 2 (24 fish—58% females, 42% males). In 1998, 151 yellowfin were captured, and 43.7% of these survived as a reserve broodstock (Table 2). However, less than 2% were healthy enough to stock in Tank 1 during 1999.

When caught, the yellowfin ranged from 32.5 to 81.2 cm FL and from 1.8 to 10.6 kg (Fig. 2). In general, the smaller fish survived capture and handling better than the larger fish (Fig. 2). The CMs were, on average (mean  $\pm$  1 S.E.), significantly larger ( $58.7 \pm 0.54$  cm and  $3.9 \pm 0.13$  kg) than the CSs for 1996 ( $56.4 \pm 0.42$  cm and  $3.4 \pm 0.07$  kg) ( $P \leq 0.001$ ) and larger in weight during 1998 (CM =  $3.6 \pm 0.13$  kg; CS =  $3.0 \pm 0.10$  kg) ( $P < 0.0001$ ).

### 3.2. Preliminary experiments

The average daily survival rates for fish treated and not treated with NFS over the 62-day experimental period were 0.9849 (95% CI, 0.9748–0.9947) and 0.9881 (95% CI, 0.9799–0.9973), respectively, and were not significantly different ( $P > 0.25$ ). Individuals survived for 4–62 days after capture during the experiment. A total of 8 of the 15 treated fish and 6 of the 15 untreated fish died due to eye and/or body infections during the experiment.

OTC injection did not affect the short-term survival of the captive yellowfin. All fish in the control and treatment groups survived throughout the experimental period.

### 3.3. Tank seawater parameters

Seasonal fluctuations in ocean upwelling and rainfall influenced seawater temperatures and salinities in the tanks. Daily mean water temperatures in Tank 1 during the study period ranged from 20.1 to 29.7 °C, averaging 27.5 (S.D. = 1.4) °C (Fig. 3). The salinity ranged from 26.0 ‰ to 36.1 ‰, with the highest values occurring during periods of low rainfall. The dissolved oxygen (DO) and oxygen saturation ranged from 4.23 to 7.70 mg l<sup>-1</sup> and 65.27% to 107.33%, respectively. The lowest oxygen values occurred during July through November 1997, when water temperatures and fish densities were high. Pure oxygen was added to the tank using airstones when the DO fell below 5.0 mg l<sup>-1</sup>, which raised the oxygen saturation above 100%. The pH ranged from 7.6 to 8.3, within normal values for the ocean and for marine aquaculture systems (Raymont, 1963;

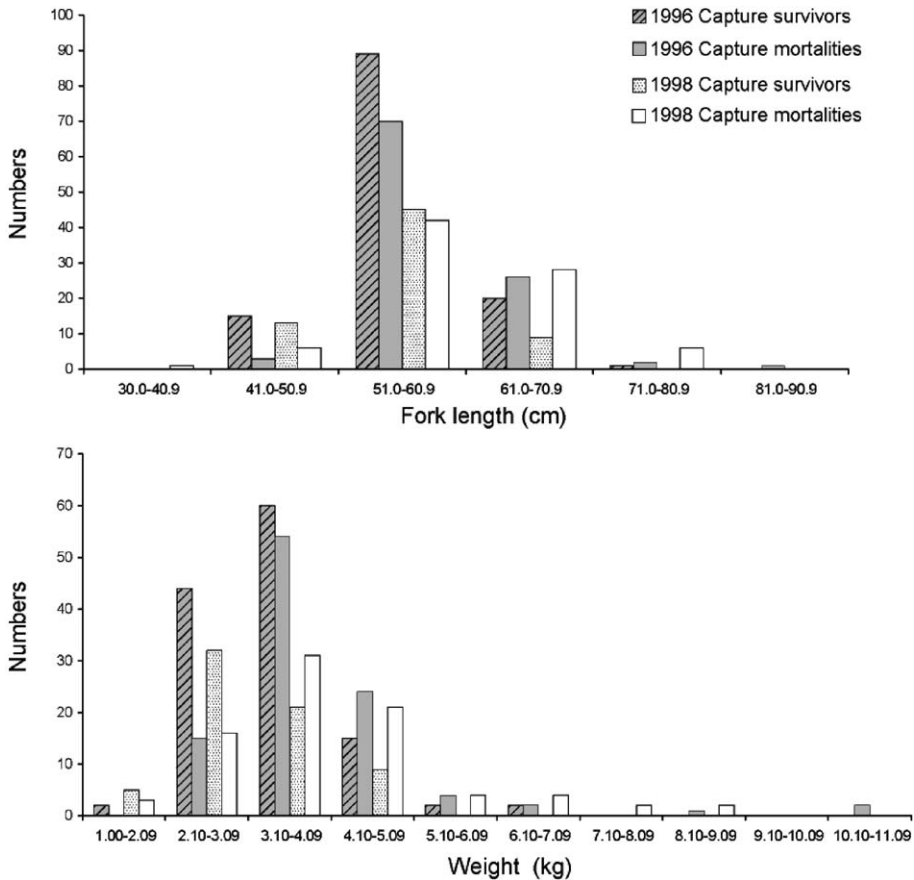


Fig. 2. Length- and weight-frequency distributions of capture survivors (yellowfin that survived capture and handling) and capture mortalities (yellowfin that died at sea after capture or shortly after placement in a tank) during 1996 and 1998.

Huguenin and Colt, 1989). Ammonia levels were always below the detection limit, nitrites ranged from 0.01 to 0.03 mg l<sup>-1</sup>, and nitrates ranged from 0 to 2 mg l<sup>-1</sup>. All were within acceptable levels for marine fishes (Spotte, 1979). Carbon dioxide ranged from 15.2 to 56.8 mg l<sup>-1</sup>. It exceeded 50 mg l<sup>-1</sup> (maximum guidelines of Van Gorder, 1994) four times during 1999, but this was not of great concern because the oxygen saturation was higher than 85% and the pH was higher than 7.7 (Wedemeyer, 1996).

The daily mean water temperature in Tank 2 ranged from 20.1 to 29.0 °C, averaging 27.1 (S.D. = 1.9) °C. The lowest daily mean temperatures occurred in March 1997, when seasonal upwelling conditions were prevalent. The DO and oxygen saturation ranged from 4.67 to 8.10 mg l<sup>-1</sup> and from 72.85% to 109.30%, respectively. Salinities ranged from 26.0‰ to 34.1‰, and the pH ranged from 7.6 to 8.3.

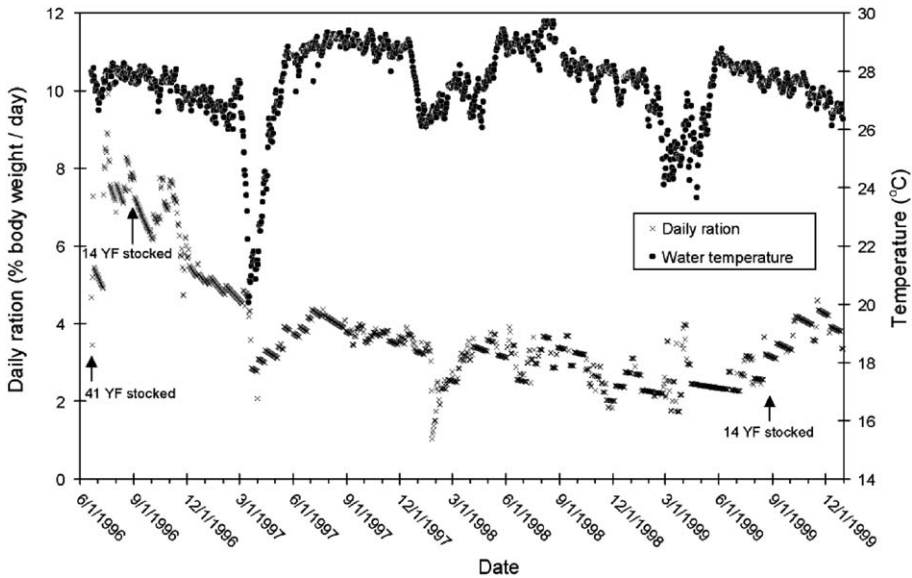


Fig. 3. Daily rations fed to yellowfin tuna (YF) broodstock and water temperatures in Tank 1 during the study.

### 3.4. Food organisms

Squid and thread herring comprised the majority of the food of the captive yellowfin. The lipid content, and hence the caloric density, of the squid was lower than those of the thread herring, anchoveta, and bigscale anchovy (Table 3). These differences were less pronounced for the Argentine shortfin squid than for the market squid, although the sample size for proximate analysis of the former was low. The means of the proximate composition parameters of each food species were weighted by the biomass of the different batches fed to the yellowfin (Table 3). Overall, the weighted mean caloric content of the food during the study was  $1055 \text{ cal g}^{-1}$ .

The bioenergetics model was used to illustrate the tradeoffs between a diet of lipid-rich thread herring versus a diet of market squid. The model was set to simulate an average (by sex) 100-cm yellowfin tuna at  $27^\circ\text{C}$  that is not spawning. The proximate composition of the food was assumed to equal the weighted means for thread herring and market squid from Table 3. For various proportions of thread herring and market squid in the diet, the model predicts a nearly constant energy requirement of about  $38 \text{ cal g}^{-1} \text{ day}^{-1}$  (Fig. 4A). However, in terms of biomass, about two-thirds more squid ( $5.0\% \text{ body weight day}^{-1}$  or  $1.0 \text{ kg}$ ) as thread herring ( $3\% \text{ body weight day}^{-1}$  or  $0.6 \text{ kg}$ ) is required to fulfill the predicted energy requirements (Fig. 4A and B).

### 3.5. Feeding and food conversion

The broodstock yellowfin in Tank 1 ate decreasing daily rations ( $\% \text{ body weight day}^{-1}$ ) (Fig. 3) and increasing calories with increasing size. Daily rations ranged from

Table 3

Sizes, proximate composition (% of wet weight), and caloric equivalents of the food organisms fed to captive yellowfin tuna broodstock at the Achotines Laboratory. “No. of indiv.” is the number of individuals that were measured and weighed. “No. of batches” is the number of food batches that were analyzed for proximate composition. The mean values for ash, water, protein, lipid, and calories are weighted by the biomass of each food batch fed to the yellowfin. The carbohydrate content was considered to be negligible, and was not measured

Food organism	No. of indiv.	Mean (S.D.)	Mean		No. of batches	Weighted mean				
			Length (cm)	Weight (g)		Ash (%)	Water (%)	Protein (%)	Lipid (%)	Cal/g <sup>a</sup>
Market squid <i>Loligo opalescens</i>	43	12.8 (1.2) <sup>b</sup>	41.2 (13.3)	7	2.6	79.0	16.4	0.2	712	
Shortfin squid <i>Illex argentinus</i>	6	41.5 (10.9) <sup>b</sup>	173.8 (50.3)	1	1.8	77.2	15.5	3.3	922	
Thread herring <i>Opisthonema</i> spp.	31	18.5 (1.6) <sup>c</sup>	70.7 (20.0)	4	5.1	69.8	17.7	6.1	1237	
Anchoveta <i>Cetengraulis mysticetus</i>	8	15.7 (0.5) <sup>c</sup>	41.1 (3.9)	2	7.1	64.7	19.6	7.7	1448	
Bigscale anchovy <i>Anchovia macrolepidota</i>	15	16.7 (0.7) <sup>c</sup>	55.5 (5.1)	2	4.9	66.8	16.0	10.9	1546	

<sup>a</sup> Assuming metabolizable energy values (heats of combustion adjusted for digestibility and non-fecal excretory losses), 4.23 kcal/g for protein and 8.0 kcal/g for lipid (Brett and Groves, 1979).

<sup>b</sup> Mantle lengths.

<sup>c</sup> Total lengths.

1.0% to 9.9% body weight day<sup>-1</sup>. Caloric consumption ranged from 9.2 to 104.2 kcal kg<sup>-1</sup> day<sup>-1</sup> and from 124.5 to 1439.2 kcal fish<sup>-1</sup> day<sup>-1</sup>.

When the water temperature in Tank 1 decreased, the feeding activity also decreased and the food reached greater depths in the tank before it was eaten. This was presumably due to lower energy requirements of the yellowfin, which was corroborated by predictions from the bioenergetics model. Lower food rations were offered during the periods of colder temperatures (Fig. 3), and the rations were increased again when the fish's activity and the bioenergetics model indicated that more energy was needed. Feeding duration and food depth ranged from 6 to 17 min and 0.2 to 6.0 m, respectively.

Food conversion ratios (FCRs) ranged from 10.9 to 34.6 (Fig. 5). The smaller yellowfin, early in the study, had the lowest FCRs. The weighted mean FCR, weighted by the number of days in each time interval, was 18.2.

The fish in Tank 2 were initially fed daily rations of approximately 10% body weight day<sup>-1</sup>, and rations were gradually reduced to approximately 4% per day over the 1-year period. The lowest food rations were consumed in March 1997, when water temperatures were low.

### 3.6. Spawning

Spawning first occurred in Tank 1 in October 1996, 4 months after the first fish were transferred to the tank. At the time of first spawning, the estimated sizes of 24 females

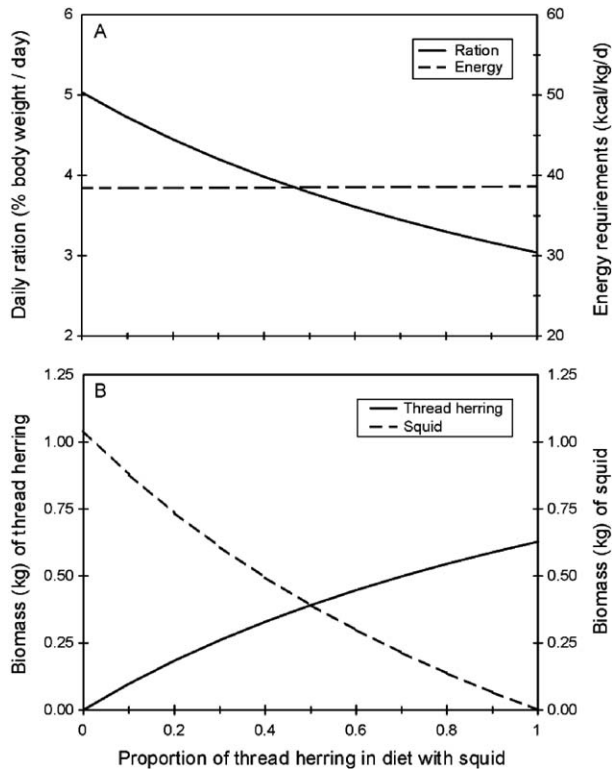


Fig. 4. Bioenergetics model predictions of food daily ration and energy requirements (panel A) and food biomass of thread herring and market squid (panel B) when fed in various proportions (by weight) of the diet, for a non-spawning 100-cm yellowfin tuna at 27 °C (see Section 2.8). In the model, the proximate composition of the food was set to the weighted means for thread herring and market squid from Table 3.

ranged from 6 to 16 kg and 65 to 93 cm FL. Spawning was intermittent during the first 2 months and occurred near daily thereafter (IATTC, unpublished data<sup>1</sup>).

It was not anticipated that the yellowfin in Tank 2 would spawn because it was a much smaller tank, but after 7–8 months in captivity the yellowfin also began spawning daily. Spawning first occurred in mid-April 1997 when four females and four males inhabited the tank. Spawning continued daily until the fish were sacrificed in September and October 1997. The four females ranged from 9 to 17 kg and 79 to 94 cm FL at the time of death.

### 3.7. Survival

The expected survival of the yellowfin stocked in Tank 1 in 1996 was high during the first year in captivity, and the density increased as the fish grew (Fig. 6A). During the

<sup>1</sup> A manuscript on the spawning and early development of the yellowfin in captivity is currently in preparation and will provide a more comprehensive account of the spawning characteristics of the broodstock.

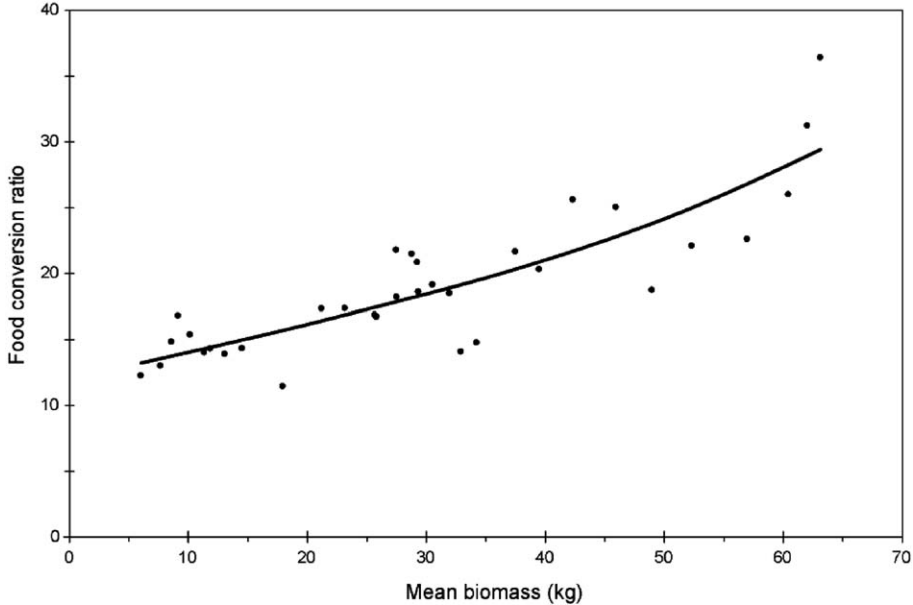


Fig. 5. Food conversion ratio (food consumed/biomass gained) versus mean biomass of individual yellowfin tuna during several time intervals over which the numbers of fish in Tank 1 were constant. The line represents the data smoothed with a smoothing spline.

second year, the fish continued to grow well (Section 3.8), and survival decreased considerably when the stocking density reached a maximum of  $0.64 \text{ kg m}^{-3}$  (Fig. 6A). The wall strikes continued throughout 1998 and 1999, and after 3.5 years in captivity the expected survival was 7%.

The expected survival in Tank 2 decreased sharply to about 46% during the first 2 months of captivity (Fig. 6A) due to high mortality associated with bacterial and parasitic infections. After 1 year in captivity, the survival in Tank 2 was substantially lower than that in Tank 1 (Fig. 6A).

### 3.8. Growth

The parameters of the von Bertalanffy model that provided the best fit to the length data of yellowfin in Tank 1 were  $Y_{\infty} = 179.2$  (S.D. = 15.0) and  $K = 0.3761$  (S.D. = 0.0704). Estimated growth rates (Section 2.10, Eq. (2)) ranged from 11 to  $48 \text{ cm year}^{-1}$ , and decreased with increasing length of the fish. In comparison to wild yellowfin in the EPO (Wild, 1986), the growth in length of the broodstock appeared to be slightly higher initially until they reached 96 cm, after which the growth was slower (Fig. 7A). The slowing in growth coincided with the time when the yellowfin reached their highest biomass (Fig. 6B) and density (Fig. 6A) after 1.4 years in captivity.

The parameters of the Richards model fitted to the weight data were  $Y_{\infty} = 169.3$ ,  $K = 0.6671$ , and  $m = 0.5682$ . Estimated growth rates (Section 2.10, Eq. (2)) ranged from 9 to  $19 \text{ kg year}^{-1}$  for fish less than 19 kg, and 20 to  $23 \text{ kg year}^{-1}$  for fish greater than 19 kg.

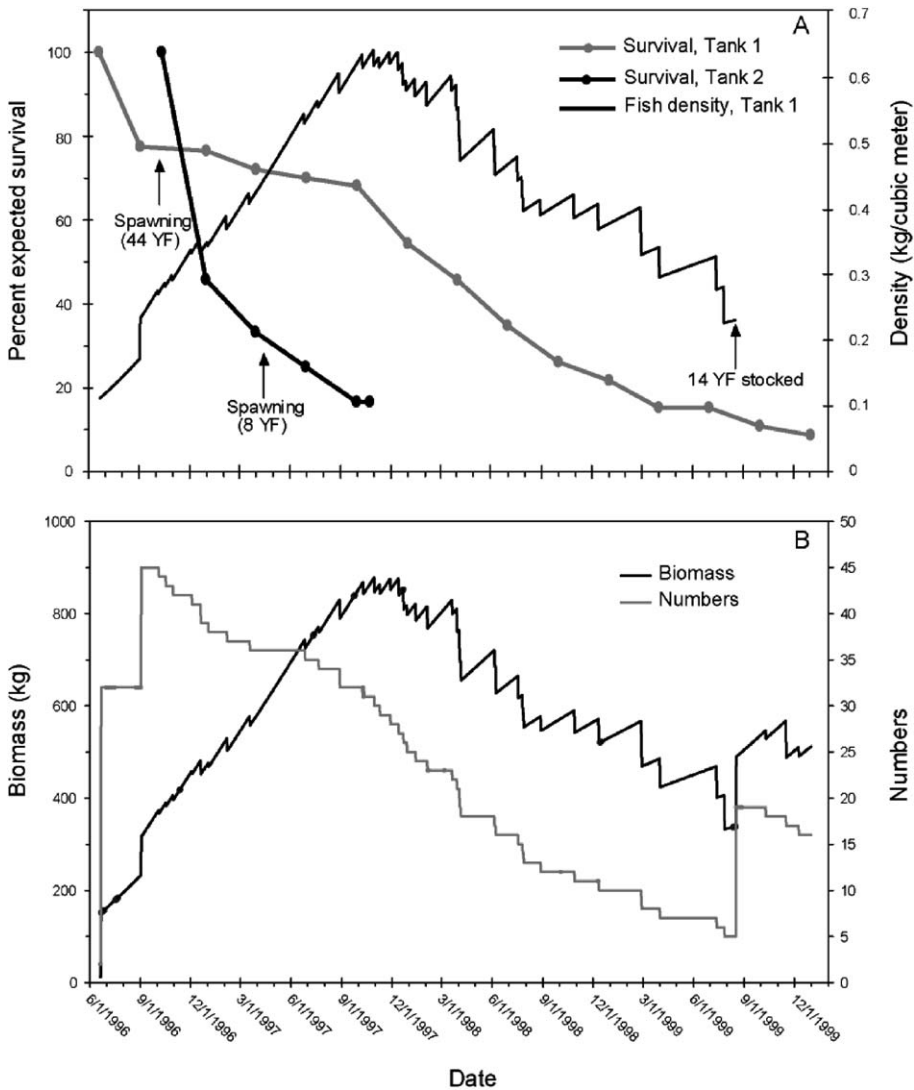


Fig. 6. (A) Percent expected survival estimates of yellowfin stocked in Tank 1 in June (44 fish) and September (14 fish) 1996 and of yellowfin stocked in Tank 2 in October 1996 (24 fish). The points represent the percent expected survival calculated over 1- to 3-month intervals from the first stocking date. The dates and numbers of yellowfin (YF) when spawning first occurred in both tanks are indicated. Daily density estimates for fish that were stocked in Tank 1 in 1996 are also shown. (B) Daily estimates of the biomass and numbers of yellowfin in Tank 1 from June 1996 through December 1999.

In comparison with wild yellowfin in the EPO, growth in weight of our broodstock was slightly higher for fish less than 10 kg, but became much slower after they reached 20–30 kg in weight and 2.5 years of age (Fig. 7B). The maximum rate of growth of our

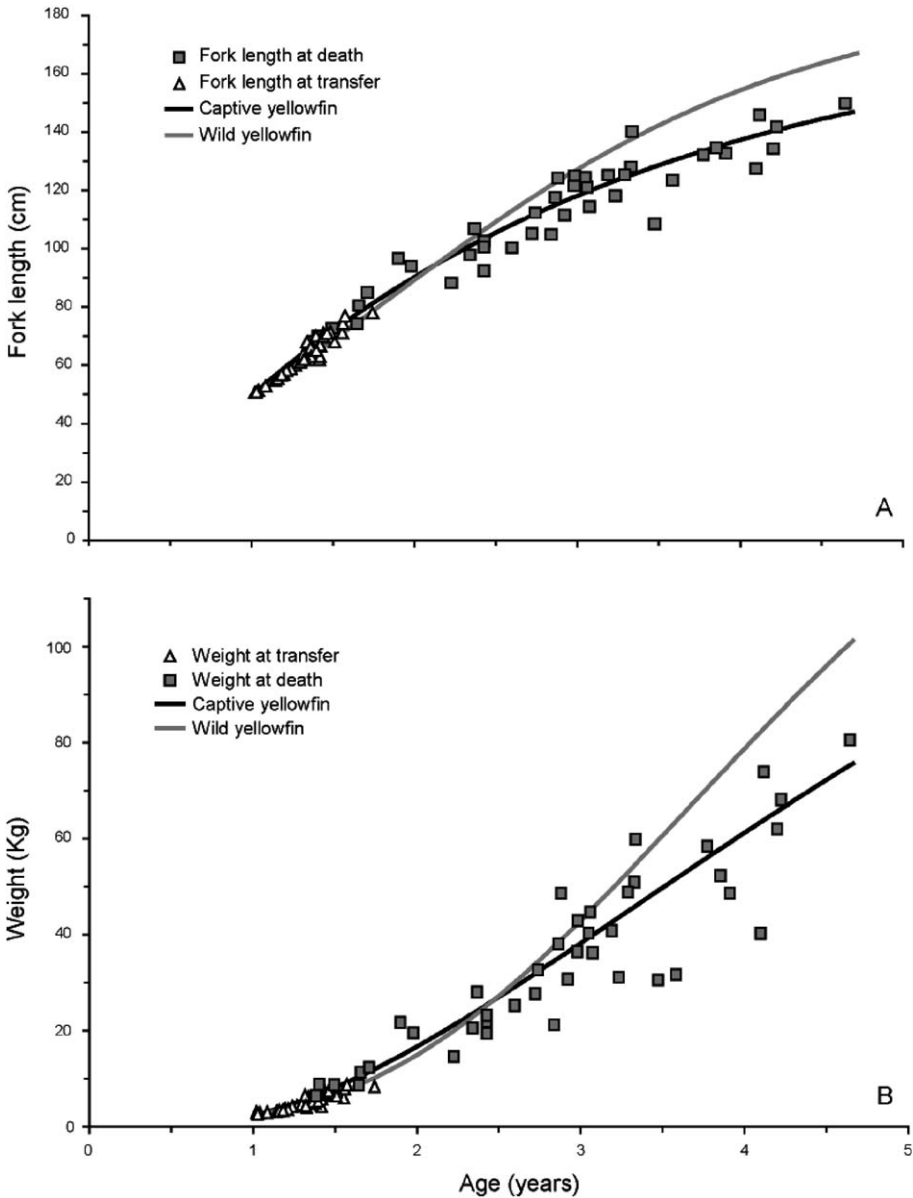


Fig. 7. Relationships between length (panel A) and weight (panel B) and estimated ages of wild yellowfin in the eastern Pacific Ocean (Wild, 1986) and captive yellowfin in Tank 1. Ages were assigned to each of the captive fish by applying Eq. (6) of Wild (1986) to their lengths and the corresponding weights at capture. The applicable  $t$  range in years (the amount of time in Tank 1) was 0.0877–3.4055. The Richards model (Wild, 1986, Eq. (1), Table 5) for wild yellowfin and the von Bertalanffy model for the captive yellowfin were superimposed on the length data (panel A). The Gompertz model (Wild, 1986, Eq. (2), Table 5) for wild yellowfin and the Richards model for the captive yellowfin were superimposed on the weight data (panel B). See Section 3.8 for parameter estimates of the growth models.

broodstock ( $23 \text{ kg year}^{-1}$ ) was much lower than that of yellowfin sampled in the EPO ( $36 \text{ kg year}^{-1}$ ).

The weight–length relationship of the broodstock was compared to that of the wild yellowfin in the EPO (Wild, 1986). The captive yellowfin were heavier relative to their lengths than the wild fish when their sizes were greater than 96 cm and 19 kg (Fig. 8). The weight–length data of the captive and wild fish were log transformed to linearize the two data sets and the slopes were compared. The variances were homogeneous ( $P>0.50$ ) and the slopes of the two groups were significantly different ( $t_{0.05(2),235} = 4.309$ ,  $P < 0.001$ ).

Despite a similar size range at capture and a higher daily food ration for fish in Tank 2, the fish in Tank 1 were generally larger (mean  $\pm 1 \text{ S.E.} = 100.5 \pm 2.09 \text{ cm}$ ,  $22.3 \pm 1.59 \text{ kg}$ ) than those in Tank 2 (mean  $\pm 1 \text{ S.E.} = 85.3 \pm 3.17 \text{ cm}$ ,  $13.6 \pm 1.33 \text{ kg}$ ) after 1 year in captivity.

### 3.9. Condition and mortalities

Fat deposition in the captive broodstock was not excessive. Individuals that were removed from the tank and sacrificed and those that died by sudden trauma averaged about 10.3% lipid and  $2047 \text{ cal g}^{-1}$  wet weight (Table 4). These values are higher than those of freshly captured yellowfin in the Panama Bight (Table 4), but are not excessive. In contrast, the yellowfin that were starving had reduced intra-muscular lipids (about 1.0%) and caloric densities ( $1032 \text{ cal g}^{-1}$ ).

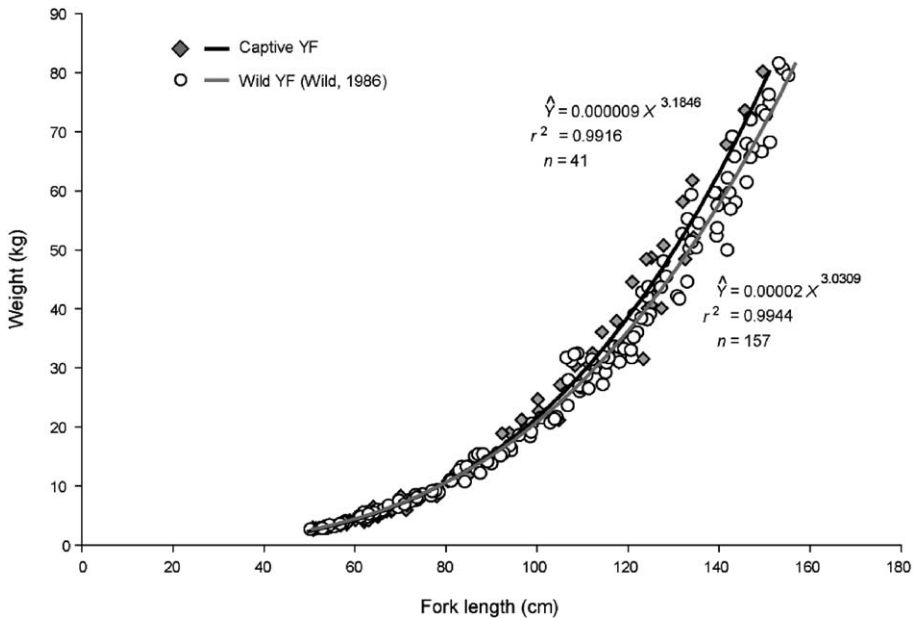


Fig. 8. Relationships between weights and lengths of captive and wild yellowfin tuna of a similar size range. The data for wild yellowfin are from Wild (1986).

Table 4

Sizes, proximate composition (% wet weight), and caloric equivalents of yellowfin tuna broodstock that died in Tank 1 and during capture. Accidental deaths were caused by wall strikes and jumping out of the tank

Cause of mortality	n	Mean (S.D.)						
		Length (cm)	Weight (kg)	Ash (%)	Water (%)	Protein (%)	Lipid (%)	Cal/g <sup>a</sup>
Sacrificed	9	91.2 (12.38)	17.68 (7.39)	2.1 (0.72)	62.7 (4.44)	24.5 (1.81)	10.0 (4.12)	2125 (416)
Starved	3	92.3 (33.96)	18.22 (14.59)	1.4 (0.67)	77.2 (3.58)	19.5 (3.94)	1.0 (1.70)	1032 (216)
Accidental	10	109.2 (17.85)	32.45 (14.30)	1.5 (0.61)	67.8 (9.14)	20.5 (6.81)	10.5 (5.06)	1977 (646)
Wild caught	5	56.4 (4.66)	3.49 (0.82)	1.4 (0.01)	71.1 (0.04)	28.7 (0.04)	0.05 (<0.01)	1384 (213)

<sup>a</sup> Assuming 4.8 kcal/g for protein and 9.45 kcal/g for lipid (Brett and Groves, 1979).

The general condition of the body and internal organs of the fish in Tank 1 at the time of death indicated that most were in good health. Only 20 of the 51 fish (39%) that died from 1996 through 1999 showed evidence of physical damage or disease. Most of the physical damage involved blindness in at least one eye, resulting from secondary tissue growth around the eyes that was probably caused by the initial capture and handling. Some of the fish with eye damage were otherwise in good physical condition. Only nine (18% of the total) of the fish showed signs of poor health (i.e., diseased internal organs or infection). The hematocrit of freshly dead individuals averaged 39.7% (range, 17–55%), and the hemoglobin content averaged 14.2 g 100 ml<sup>-1</sup> (range, 11.5–16.5 g 100 ml<sup>-1</sup>).

Most of the mortalities (64%) that occurred in Tank 1 were due to wall strikes that began when the fish were greater than 96 cm FL and the biomass in the tank reached its highest levels (Fig. 6B). The wall strikes took place mostly during early morning hours prior to feeding.

A much greater percentage (54.2%) of fish in Tank 2 than in Tank 1 (2%) exhibited secondary bacterial or parasitic (*Benedenia* trematode, parasitic copepod) infections at the time of death, and most died of starvation from their weakened condition.

The histological classification by K. Schaefer (IATTC, personal communication) of the gonads of five males and five females that died in Tank 1 during 1996 and 1997 indicated that all were sexually mature. At the time of death, three of the females were classified as reproductively active, and spawning was imminent in one of these, based on the presence of hydrated oocytes (Schaefer, 1998).

#### 4. Discussion

This study represents the first successful development of a spawning population of yellowfin tuna in onshore tanks. The physical systems developed at the Achotines

Laboratory provided good water quality and a tank habitat of suitable size and shape to promote long-term survival, growth, and spawning.

The results of this study and that of the TRCC in Monterey, CA (Farwell et al., 1997; Farwell, 2000, 2001) demonstrate that land-based culture facilities may be preferable to sea pens for maintaining yellowfin tuna in a stable environment over long periods of time. Raising tunas for commercial grow-out and harvest in sea pens has been successful over short time periods (Smart, 1996; Carter et al., 1998; Lee, 1998), but storm events have caused major mortalities in several of the offshore operations (Lee, 1998). The JASFA was also successful in raising broodstock yellowfin (Masuma et al., 1993) and bluefin (Lee, 1998) tunas in anchored sea pens, but onshore tanks provide more protection and control of physical conditions, which is important for continuous spawning. If the objective of a culture facility is to maintain a healthy, spawning population of yellowfin for long periods of time, large, in-ground tanks with adequate life support systems may be preferable to sea pens.

#### 4.1. Capture, handling, and treatments

Although capture of the yellowfin by hook and line and transfer by a small vessel was time consuming and provided small numbers of fish, this technique allowed the fish to be handled carefully and rejected at sea when in poor condition. On average, approximately 50% of the yellowfin caught in a similar manner could be expected to survive capture and handling, and approximately 30% of the fish caught would be healthy enough to use as broodstock. The close proximity of the laboratory to the fishing grounds was advantageous in minimizing the transport time, and resulted in the fairly good survival of the fish. Compared with another tropical tuna, black skipjack (*Euthynnus lineatus*), that has been captured and maintained in Achotines, yellowfin are relatively calm and easy to handle.

Although the experiments did not show that immersion of recently captured yellowfin in the antimicrobial NFS improved survival, NFS treatments may still be useful. In a warm-water environment, fish are more susceptible to bacterial growth and invasion of pathogens than in cold-water environments (Wedemeyer, 1996). Sako et al. (1991) found that using NFS helped reduce the incidence of bacterial infections in young bluefin tuna.

The results from the OTC experiment, and the survival rates of fish that were injected with OTC and maintained in captivity for more than 3 years following treatment indicated that there was no significant adverse effect from OTC at the dosages used. Survival rates of OTC-injected yellowfin and skipjack (*Katsuwonus pelamis*) tunas that were tagged and recaptured in the EPO were also similar to those not injected with OTC (Wild and Foreman, 1980; Wild et al., 1995). The antibiotic properties of tetracycline may, in fact, be beneficial for fish in an aquaculture environment (e.g., Ahmed and Tan, 1992).

#### 4.2. Feeding and food conversion

A diet of 50% squid and 50% fish, such as thread herring or anchoveta, seemed to provide adequate nutrition to the yellowfin over the 3.5-year period. Daily ration

requirements decreased with increasing yellowfin size over time (Fig. 2), as predicted by bioenergetics theory. Food demands versus changes in yellowfin size, water temperature, and proportions of different food types in the diet were consistent with the bioenergetics predictions.

The yellowfin bioenergetics model provided a tool to estimate food requirements when sudden changes in food quality or yellowfin biomass occurred. After some months, food requirements could also be judged by observing the fish's feeding behavior. When feeding activity became high, and the danger of the fish colliding with each other or with the tank wall increased, the bioenergetics model provided a tool to calculate how much food biomass could be increased, while maintaining constant energy levels, by increasing the proportion of the low-calorie squid and decreasing the proportion of the lipid-rich fishes in the diet (Fig. 4). Conversely, when the broodstock were judged to need more dietary lipids to improve spawning success, the model aided in determining a new ration level with a higher proportion of fish in the diet.

Food conversion ratios (FCRs) of the yellowfin broodstock (Fig. 5) were comparable to FCRs (wet weight) of similar-sized southern bluefin tuna (*Thunnus maccoyii*) fed pilchards (*Sardinops neopilchardus*) in floating sea pens in Australia (Smart, 1996). Mean FCRs were lower (4.1 to 11.8), however, for southern bluefin fed manufactured semi-moist diets (Smart, 1996) because manufactured diets have a lower moisture and ash content. Because the FCR is based on food biomass, and not energy, its magnitude depends on the type and composition of the food provided to the fish. The FCRs in this study are higher than would have been achieved if the yellowfin had been fed more thread herring, for example, and less squid (i.e., lower rations, Fig. 4) to fulfill their energy requirements.

#### 4.3. Health and condition

The majority of the yellowfin reared in Tank 1 remained in good health over the entire 3.5 years because the physical systems provided a stable environment with very good water quality. These fish exhibited a low incidence of internal abnormalities and external parasitic infections or damage. The hematocrit in these fish averaged about 40%, within the range of routine hematocrit values (35–40%) reported for skipjack and yellowfin tunas (Brill and Bushnell, 1991). Hematocrit and hemoglobin levels, however, are probably not reliable indicators of fish health because they are strongly influenced by stress, blood loss, and malnutrition (Gallaughan and Farrell, 1998).

Efforts to avoid poor condition due to excessive fat deposition in the captive broodstock were successful. The early efforts at rearing yellowfin in Monterey, CA, at the TRCC at 20–21 °C were met with health problems due to excessive fat accumulation in the muscle (Farwell et al., 1997). Low water temperatures and activity levels exacerbated this situation at TRCC, and the problem was resolved by feeding the fish rations with fewer calories. The challenge at Achotines was different because it was not only necessary to avoid excessive fat accumulation, but also to provide adequate energy for the fish to reach spawning size quickly and to fuel daily production of high-quality eggs. The higher ambient temperatures in the Panama Bight and greater activity levels, especially after courtship began, were beneficial in meeting this challenge.

#### 4.4. *Effects of tank size and fish density*

There appeared to be a strong tank-size effect on overall fish health. Although the fish in Tank 2 eventually spawned over 6 months, they suffered a much higher incidence of external damage and health problems than the fish in Tank 1. The smaller dimensions of Tank 2 apparently caused the increased incidence of pathogen transmission, which is common in crowding or high-density conditions (Wedemeyer, 1996). The smaller tank dimensions also caused increased physical contact with the tank wall and restricted growth of the fish. Based on the health of the yellowfin in Tank 2, the reserve tanks are capable of supporting yellowfin densities of less than  $0.4 \text{ kg m}^{-3}$  for up to 1 year.

Survival in Tank 1 appeared to be influenced more by the increasing fish size and limited tank space than by the increase in the biomass density. With the increasing biomass in Tank 1, density-dependent growth (e.g., Barlow, 1992; Lorenzen, 1996), survival (e.g., Barlow, 1992), and/or some effect on spawning activity were expected. The survival, however, continued to decrease despite a decrease in the biomass density after December 1997 (Fig. 6A). Survival appeared to be associated more with increasing fish size. The maneuverability of the fish became more difficult as they grew, and this may have resulted in the high mortality rates associated with wall strikes. There was, however, no apparent effect of the high mortality rates on spawning activity levels, and the yellowfin continued to spawn almost daily.

It was not possible to determine if fish density affected growth of the broodstock in Tank 1 because we did not control the stocking density at a constant level. The size of the tank, however, appeared to affect the survival and growth of the broodstock once they reached their highest density of  $0.64 \text{ kg m}^{-3}$  and sizes greater than 96 cm and 19 kg. Slower growth was observed during this time, and the fish became heavier relative to their lengths.

Based on the survival and growth of yellowfin in Tank 1, the initial stocking density for a long-term broodstock of yellowfin should not exceed  $0.75 \text{ kg m}^{-3}$ . After the first 12–18 months of captivity, the density should not exceed  $0.5 \text{ kg m}^{-3}$  to maintain a healthy condition. Further, the broodstock should be replenished with younger, smaller fish after no more than 3 years to ensure continuous spawning.

#### 4.5. *Growth*

The growth estimates in this study were compared with two studies of short-term growth of captive yellowfin (Farwell et al., 1997) and southern bluefin tunas (Carter et al., 1998). The growth rates in weight of the 2- to 3-year-old yellowfin in this study ( $0.03\text{--}0.09 \text{ kg day}^{-1}$ ) were up to 7 times higher than those of yellowfin maintained in  $325 \text{ m}^3$  and smaller tanks at the TRCC in Monterey, CA, over a 13-month period (Farwell et al., 1997). Short-term growth of southern bluefin tuna over a size range of 25–34 kg was, on average,  $0.06 \text{ kg day}^{-1}$  during a 131-day period (Carter et al., 1998), which is similar to the average growth rate for yellowfin in this study over the same size range and duration. The southern bluefin, however, grew very slowly in length during this period (Carter et al., 1998) in contrast to the faster growth in length of yellowfin in this study. The southern bluefin and the yellowfin tunas at TRCC were both maintained at cooler water temper-

atures and in smaller enclosures than the yellowfin in Tank 1 at Achotines, and the diets were different, which may explain some of the discrepancies in growth rates.

It is interesting to compare the growth rates in captivity with those of yellowfin over a similar size/age range sampled from the wild population in the EPO (Wild, 1986). The broodstock grew more slowly than the wild fish after they were approximately 2.5 years in age and had reached their highest density in Tank 1. The size of the tank probably contributed to limiting the growth of the captive yellowfin. Although it was not possible to determine the spawning frequency of the individual fish in Tank 1, the broodstock may have expended more energy due to near-daily spawning in captivity than wild yellowfin in the EPO (Schaefer, 1998). This may indicate that energy allocation for spawning may take priority over that for growth in yellowfin tuna that have reached reproductive size.

## **5. Conclusion**

This study demonstrates that a naturally spawning population of yellowfin tuna can be maintained in captivity when reared in a land-based system, such as that at the Achotines Laboratory. The stable environment of the onshore culture facility promoted good health and sustained spawning of the yellowfin broodstock. The results of this study provide the first long-term estimates of food conversion, growth, and survival of tropical tunas in a land-based system, and the husbandry procedures developed can be applied to other aquaculture and research projects of tropical tunas.

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