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## DOCUMENT SAC-06-10c REVIEW OF RESEARCH AT THE ACHOTINES LABORATORY

by

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#### 1. INTRODUCTION

The egg, larval, and juvenile stages of marine fishes are characterized by high rates of mortality and growth. Most marine fishes, particularly pelagic species, are highly fecund, produce small eggs and larvae, and feed and grow in complex aquatic ecosystems. The identification of environmental or biological factors that are most important in controlling survival during the early life stages of marine fishes is a potentially powerful tool in stock assessment.

Because vital rates (mortality and growth) during the early life stages of marine fishes are high and variable, small changes in those rates can have profound effects on the properties of survivors and recruitment potential (Houde 1989). Understanding and predicting the factors that most strongly influence pre-recruit survival are key goals of fisheries research programs.

#### 2. RESEARCH ON THE EARLY LIFE HISTORY OF TUNAS

The Antigua Convention states that the Commission shall perform the following functions, giving priority to tunas and tuna-like species:

(a) Promote, carry out and coordinate scientific research concerning the abundance, biology and biometry in the Convention Area of fish stocks covered by this Convention and, as necessary, of associated or dependent species, and the effects of natural factors and human activities on the populations of these stocks and species.

Although decades of research have provided considerable information on the populations of adult tunas, relatively little is known about the early life history stages and the factors that affect pre-recruit survival. Tunas are among the most commercially-valuable marine fish stocks in the world (FAO 2014), and recruitment variability is one of the most important factors affecting their population fluctuations (IATTC-CIAT 2004).

Tuna stocks are characterized by order-of-magnitude recruitment fluctuations, but the underlying mechanisms controlling the variability in recruitment remain poorly understood. Yellowfin tuna (*Thunnus albacares*) are recruited to the surface fishery in the eastern Pacific Ocean (EPO) at approximately 30 cm

in length and 6 months of age (Aires-da-Silva and Maunder 2012). Yellowfin recruitment in the EPO has fluctuated by a factor of 3.2x over the past 30 years (Minte-Vera *et al.* 2014). Yellowfin are highly fecund (batch fecundities > 1,000,000 oocytes per female) and spawn almost daily during their reproductively-active periods (Schaefer 2001). Yellowfin early life stages are characterized by high mortality rates, high metabolic rates, and exponential growth (Margulies *et al* 2007a, Wexler *et al*. 2007). This pattern of reproduction and early life history has strong potential for regulation of recruitment during larval or early-juvenile stages, when initial numbers in a cohort are large and vital rates (mortality and growth) are high (Houde 1987, Margulies *et al*. 2001). Most tunas exhibit similar patterns of high reproductive potential and pre-recruit life stages that are characterized by fast growth and high mortality (Davis *et al*. 1991, Tanaka *et al*. 1996, Margulies *et al*. 2007a).

Prior to the 1980s, few studies had been undertaken to examine the mechanisms that control pre-recruit survival of tunas or to estimate their vital rates during their early life stages. These considerations motivated the IATTC to establish a research facility at Achotines Bay in the Republic of Panama for the purpose of studying the early life histories of tropical tunas and tuna-like fishes (scombrids).

#### 3. THE ACHOTINES LABORATORY AND THE IATTC EARLY LIFE HISTORY PROGRAM

The Achotines Laboratory is located on the southern coast of the Azuero Peninsula in the Los Santos province of the Republic of Panama (Figure 1). This region is in the northwestern portion of the Panama Bight. The continental shelf is quite narrow at this location; the 200-m depth contour occurs only 6 to 10 km (3 to 5 nm) from shore. This provides the scientists working at the Achotines Laboratory with ready access to oceanic waters where spawning of tunas occurs during every month of the year. The annual range of sea-surface temperature in these waters is approximately 21° to 29° C.

The early life history research program involves laboratory and field studies of tropical scombrids aimed at gaining insight into the recruitment process and the factors that affect it. Previous research on recruitment of non-scombrid fishes suggests that abiotic factors, such as temperature, light, current patterns, and wind conditions, and biological factors, such as feeding, growth, and predation, can affect recruitment (Houde 1997). As the survival of pre-recruit fishes is probably controlled by a combination of these factors, the IATTC research program addresses the interaction between the biological system and the physical environment (Lauth and Olson 1996, Owen 1997).

Research on tropical scombrids at the Achotines Laboratory has involved two distinct phases. The first phase of research was directed predominantly at coastal, tropical scombrids, mainly black skipjack (*Euthynnus lineatus*), bullet and/or frigate tunas (*Auxis* spp.), sierra (*Scomberomorus sierra*), and striped bonito (*Sarda orientalis*), during the period from 1984 to 1995. From 1996 to present, the focus of research shifted to the reproductive biology and early life history of yellowfin tuna, utilizing eggs spawned by captive yellowfin broodstock.

In this report, we review the research conducted on reproductive biology and the early life history of tropical tunas at the Achotines Laboratory. We also summarize the key research findings from the studies and present brief summaries of 4 areas of research that hold great promise for linkage with stock assessment research.

#### 4. RESEARCH ON COASTAL TROPICAL SCOMBRIDS

#### 4.1. Early experimental studies and surveys at sea

During 1986-1994, we began our studies of coastal scombrids in the Panama Bight. We developed methods for the collection of large numbers of live early-juvenile scombrids at sea. The fish were collected by dipnet after attraction to an underwater light (24 V DC, 300 W) and transported alive to laboratory tanks. They were fed in the laboratory with wild zooplankton size-graded between 333 and  $1000~\mu m$  in body width, predominantly cladocerans and copepodite and adult stages of copepods. The successful collection and maintenance of scombrids in the laboratory supported numerous experiments

examining the growth, nutrition, and development of larvae and early juveniles. Since small changes in growth rates and mortality can have a significant impact on recruitment variability (Houde 1989), we directed a large portion of our research toward characterizing growth during the early life stages of several tuna species. We examined a number of physical and biological factors that could potentially affect survival and growth, using both laboratory and field applications.

Prior to the 1980s, little was known about the distribution and abundance of scombrid larvae in relation to seasonal or annual variability in the physical environment. During 1989-1993, we conducted a series of field surveys in the northwestern Panama Bight designed to investigate the spatial and temporal distribution patterns of scombrid larvae in relation to the local oceanographic and secondary production conditions of the region. Our approach was to sample repetitively over a small spatial scale for several years. Sampling was conducted from a 25-ft (7.6-m) Boston Whaler outfitted with a mast, boom, and hydraulic winch. Ichthyoplankton tows and CTDO (conductivity, temperature, depth, and oxygen) casts were made at each sampling station. Both bongo net (integrated-depth tows) and Tucker trawl (discrete-depth) surveys were conducted.

#### 4.2. Key research findings from early life history studies of coastal scombrids

Studies of the early life history and reproductive biology of coastal, tropical scombrids during the first decade of operation of the Achotines Laboratory produced new and important findings on the biology of tropical scombrids. The most important findings were as follows:

- 1. We described for the first time the growth dynamics of larval and juvenile tropical scombrids in the Pacific Ocean, and learned that late-larval black skipjack are proficient feeders and capable of fast growth even during the wet season, when secondary production is lower. We learned that juvenile black skipjack, bullet/frigate tunas, and sierra are capable of extremely fast growth in the laboratory (1-5 mm day<sup>-1</sup>) and possess a large scope for growth.
- 2. We developed methods for the collection and husbandry of late-larval and early-juvenile scombrids. These proved invaluable in conducting laboratory experiments to examine the growth dynamics, nutrition, and physiology of tropical scombrids.
- 3. During 1991, we completed the life cycle for black skipjack in captivity by rearing field-caught larvae to reproductive maturity over a period of 1 year. This was the first time that a species of tuna was reared from the larval stage to reproductive size. In 1993 we developed, also for the first time, a captive spawning population of black skipjack.
- 4. We provided the first estimates for tropical waters of the incidence of starvation in larval and juvenile scombrids. First-feeding larvae of black skipjack, bullet/frigate tunas, and sierra exhibited a high potential for starvation during the wet season in the Panama Bight. We concluded that starvation mortality alone for first-feeding tropical tuna larvae approached 45% day<sup>-1</sup>. Early juveniles, however, exhibited a very low incidence of malnourishment.
- 5. We gained important insights into the temporal and spatial distribution of larval scombrids in the Panama Bight. Black skipjack and bullet/frigate tunas appear to spawn year-round in the study area, and the Gulf of Panama appears to be a major spawning area for these species. Yellowfin and/or bigeye (*Thunnus* spp.) larvae were rare in collections, and appear to spawn in waters further offshore (south and/or west of the study area). Descriptions of the diel, vertical distribution, growth, starvation rates, and diets of larvae, based on Tucker trawl surveys during 1990-1993, await further analyses of archived samples.
- 6. We validated daily increments in the otoliths of larval and early-juvenile black skipjack and bullet/frigate tuna to determine ages and estimate *in situ* growth rates based on length-at-age data. Daily increments had not been validated in the otoliths of most scombrid larvae previous to our studies.

7. We described, for the first time, the development of the visual system of larval and early-juvenile scombrids. The visual system is advanced, and undoubtedly contributes to rapid improvements in foraging abilities and the early onset of piscivory. We also supported the first studies to describe the minimum size for endothermy in tunas (Dickson 1994).

#### 5. RESEARCH ON YELLOWFIN TUNA

#### 5.1. Background on the development of yellowfin research at the Achotines Laboratory

During 1992 and 1993, the IATTC's Early Life History group and the Japan Sea Farming Association (JASFA) conducted joint studies in Japan of captive spawning and early life history of yellowfin. Based on the success of the joint studies conducted in Japan, and positive results of larval and adult black skipjack research at the Achotines Laboratory, in December 1993 the IATTC, the Overseas Fishery Cooperation Foundation (OFCF) of Japan, and the government of the Republic of Panama agreed to undertake a larger joint study, funded mostly by the OFCF and IATTC, of yellowfin tuna at the Achotines Laboratory. In general, the yellowfin project was designed to gain new insights into the reproductive biology and early life history of the species by maintaining a spawning population of adults and studying the egg, larval, and juvenile stages in the laboratory. The joint project extended from 1993 through March 2001. Since March 2001, the yellowfin research program has been continued by the IATTC research group.

Expansion of and improvements to the infrastructure of the Achotines Laboratory were necessary to carry out the objectives of the joint yellowfin project. We initiated a major expansion of the Laboratory in 1994 with funding provided primarily by the OFCF. To rear broodstock yellowfin, we carried out the construction of new broodstock and rearing tanks and a seawater system from late 1994 through early 1996, with additional minor construction continuing through 1999 (Wexler *et al.* 2003). The broodstock and rearing tanks were operational by early 1996. We designed the main broodstock tank (Tank 1) to be large enough to minimize the stress of captivity and enhance the chances of spawning (Margulies *et al.* 2007b).

During this period we upgraded the "wet" laboratory facilities with additional experimental tanks fitted with small heaters and chillers to control water temperatures, lighting to regulate photoperiod, and aeration equipment. We also constructed a building dedicated to the storage and preparation of broodstock food and the culturing of food organisms for the larvae resulting from spawning by the broodstock fishes. A new office and "dry" laboratory building with an expanded array of analytical equipment and microscopes was also built. We purchased two new panga-style boats for collection and transfer of fish; the boats carry transport tanks and are 7.3 m (24 ft) and 8.2 m (27 ft) in length, respectively. We added a concrete pier and boat ramp in 1999 to facilitate boat operations. All of the infrastructure upgrades to the Laboratory were done with OFCF funding.

#### 5.2. Research on reproductive biology and early life history of yellowfin

From 1996 to present, the IATTC has conducted research on the reproductive biology in captivity and early life history of yellowfin. The objective of the research is to develop a more complete understanding of daily mortality processes occurring during pre-recruit life stages (larval and early-juvenile stages) and how mortality is influenced by key environmental and biological factors. The ultimate goal of our experimental program on yellowfin early life history is the contribution of new insights into recruitment variability. The ability to forecast yellowfin recruitment, prior to the age at entry to the fishery (6 months), would be a powerful stock assessment tool.

Yellowfin research at the Achotines Laboratory has focused on important aspects of adult growth, spawning dynamics, genetics of spawning fish, early life stage development, growth dynamics of larvae and early-juveniles (in the laboratory and *in situ*), and the effects of important physical factors on pre-recruit survival and growth. The results of this research are summarized in a series of publications listed on the <u>IATTC website</u>.

#### 5.3. Key research findings from studies of yellowfin tuna

The studies of the reproductive biology and early life history of yellowfin tuna conducted since 1996 at the Achotines Laboratory have contributed significantly to our understanding of yellowfin biology and the factors that influence pre-recruit survival. The key findings to date of the yellowfin research program are as follows.

- 1. A spawning population of yellowfin was established, which represents the first occurrence worldwide of sustained spawning by yellowfin in land-based facilities. The spawning dynamics, growth, genetics, physiology, and early life history of yellowfin were studied over multiple years.
- 2. Methods for the successful capture, transfer, and husbandry of yellowfin were developed. A diet of 50% squid and 50% fish, such as thread herring or anchoveta, seems to provide adequate nutrition for broodstock yellowfin and fuels almost continuous spawning. Estimates of growth in length of captive fish decreased with increasing lengths of the fish, ranging from 18 to 37 cm year<sup>-1</sup> during `1996-2001 and from 11 to 62 cm year<sup>-1</sup> during 1999-2014 (Figure 2). Growth in weight was estimated at 11 to 26 kg year<sup>-1</sup> during 1996-2001 and 4 to 36 kg year<sup>-1</sup> during 1999-2014, and these estimates also decreased with increasing weights of the fish. The stable environment of onshore tanks seems to promote good health and sustained spawning of yellowfin.
- 3. The spawning patterns of yellowfin in relation to physical and biological factors have been described. The broodstock fish spawned as long as they received adequate daily food rations and water temperature was >23.3° C. Water temperature appears to be the main exogenous factor controlling the occurrence and timing of spawning for yellowfin (Figure 3). Courtship and spawning behaviors are ritualized, and yellowfin appear to have the ability to adjust the timing and final maturation processes of spawning based on minute changes in water temperature. For example, yellowfin adjust the time of day of spawning in relation to water temperature, resulting in a narrow range for the time of day at hatching. This pattern appears to be adaptive as a means to maximize the survival of yolk-sac larvae by maintaining the timing of hatching during periods of dimming light or darkness (late afternoon and early evening).
- 4. The age at first spawning for female yellowfin in captivity was estimated at 1.3 to 2.8 years, averaging slightly less than 2.0 years. Over short periods (<1 month), spawning females increased their egg production by 30 to 234% in response to short-term increases in daily ration of 9 to 33%. The ability to increase egg production in response to greater food abundance has adaptive significance, and would allow yellowfin to exploit patchy food resources and periodic increased production in the ocean.
- 5. Genetic monitoring of the spawning yellowfin was conducted by comparing mitochondrial DNA variation of spawning females with those of their eggs and larvae. The analysis provided the identification of individual spawning females and estimates of their spawning periodicity. Individual females are capable of spawning daily for extended periods of time as long as they remain in the appropriate range of water temperatures (>23.3° C) and have sufficient food. The genetic variation of the mtDNA *D-loop* region of yellowfin appears to be so high that it is probably useful not only for identification of individual fish but also for investigations of population structure in the wild.
- 6. Water temperature is significantly, inversely related to egg size, egg stage duration, larval size at hatch, and yolk-sac larval duration of yellowfin. Fertilized yellowfin eggs average 1.0 mm in diameter and 43 μg in dry weight. Hatched larvae average 2.5 mm SL and 30 μg in weight, while larvae at first feeding average 3.3 mm SL and 22 μg in weight. The growth potential from early-stage larva to size at recruitment (30 cm, 6 months of age) is very high, approaching 10<sup>6</sup> to 10<sup>7</sup> times.
- 7. The development of visual sensitivity in larval, juvenile, and adult yellowfin has been described. Adult yellowfin have at least three visual pigments in the retina, and may be able to distinguish among colors (*i.e.* they may not be color blind). Larvae have not only mixtures of the adult cone

pigments (violet- and blue-sensitive) but also a third green-sensitive pigment. Juvenile sensitivity tends to converge to the adult condition. The spectral sensitivity of adult yellowfin is probably adaptive to the ambient bluish light characteristic of the open sea, while larval sensitivity over an extended spectral range is probably adaptive for planktivory in mixed layer habitats.

- 8. The lethal limits of water temperature and dissolved oxygen on yellowfin eggs and larvae were estimated in laboratory trials. The results have indicated that the vertical distribution, and to some extent the horizontal distribution, of yolk-sac and first-feeding larvae in the ocean is determined by the physical limitations of water temperature and dissolved oxygen. In the Panama Bight, critical depths for survival of yellowfin larvae would occur above 30 m during the upwelling season (when mixed layer depth is shallower), and above 50 m during the reduced upwelling season, based on water temperature alone. Oxygen deficits would probably not occur at depths above 50 m.
- 9. Trials have been conducted at the Achotines Laboratory to investigate the feasibility of developing bycatch-reduction devices, such as sorting grids and bubble curtains, which will allow smaller fish to escape from tuna purse seines while retaining larger fish. Results from the trials have indicated that yellowfin swim through sorting grids and are reluctant to pass through bubble curtains. Both of these techniques are worthy of further trials under field conditions to test their effectiveness as sorting methods to reduce the mortalities of unmarketable fish.
- 10. Tests have been conducted at the Achotines Laboratory to examine the use of implanted archival tags for detection of feeding and spawning events of yellowfin. Preliminary results indicate that the archival tags can detect changes in peritoneal temperature that correspond to both feeding and spawning signals. This technology holds great promise for the detection of feeding and spawning signals in wild fish.

### 6. PROMISING LINKS BETWEEN YELLOWFIN EARLY LIFE RESEARCH AND STOCK ASSESSMENT

#### 6.1. Laboratory and in situ growth of larval and juvenile yellowfin

For pre-recruit life stages, it is not the mortality rate (M) alone that determines stage-specific survival, but also the ratio of mortality/growth (M/G), the stage-specific or 'physiological' mortality rate (i.e. mortality per unit of growth) (Houde 1997). Small variability in either the instantaneous mortality or specific growth rates can generate major changes in stock level at defined stages. Growth variability alone has the potential to influence stage durations and cumulative mortality during the larval and juvenile life stages (Houde 1989). We have focused much of our experimental efforts with yellowfin on investigations of growth dynamics during the larval and early-juvenile stages.

Since 1997, we have studied growth in the laboratory of yellowfin larvae and juveniles reared from eggs from our yellowfin broodstock. We have investigated the effects of food availability, water temperature, and other physical factors on the survival and growth of yellowfin larvae and juveniles up to 100 days after hatching. Early-larval growth (the first 2 weeks) is exponential in length and weight (<0.35 mm day in length and 20 to 35% body weight day-1), but growth increases significantly during the late-larval and early-juvenile stages (>0.6 mm day-1 and ca. 30-50% body weight day-1) (Figures 4 and 5). Yellowfin larvae become piscivorous at around 6.5 mm SL, and the timing of the onset of piscivory probably determines, in part, an individual's growth potential. Laboratory cohorts that are early piscivores (ca. 6.0-7.0 mm SL) grow more rapidly, and individuals that remain zooplanktivorous lag in growth and/or are cannibalized. The fastest-growing laboratory cohorts appear to be influenced more by high food levels, high water temperatures and/or lower stocking densities (see following discussions of density-dependent growth), and the slowest-growing cohorts appear to be affected more by lower food levels, higher stocking densities and/or low water temperatures. In comparison with growth rates of field-collected yellowfin, our most rapid laboratory growth rates to date have been 17% less than those of the slowest-growing group in the field; however, the sizes at age of the laboratory fish approach those of the field-

collected fish shortly after a piscivorous diet is introduced (Figure 6).

Growth rate variability in the larval and juvenile stages of marine fishes is substantial, and has strong potential to influence the M/G ratio during pre-recruit life stages (Houde 1997). Density-dependent regulation of growth has been identified as a significant potential factor in the control of pre-recruit survival (Shepherd and Cushing 1980, Rothschild 1986). For yellowfin, density-dependent mortality may weaken any relationship between egg production and recruitment consistent with the IATTC stock assessment of yellowfin (Minte-Vera et al. 2014). It is possible that relative growth rate or densitydependence in feeding success and growth during the larval stage could contribute to variations in prerecruit survival of yellowfin. Faster growth shortens the period of greatest vulnerability to daily mortality by predation. A juvenile growth index, perhaps estimated quarterly in the Panama Bight, may prove useful as an index of recruitment strength (Margulies et al. 2007a). This type of sampling program to estimate in situ juvenile growth could be developed at the Achotines Laboratory via quarterly or seasonal sampling and aging of juveniles collected by nightlighting. We have conducted similar analyses of in situ growth during selected years in the Panama Bight, and we found some localized correspondence between high growth rates of larvae and recruitment estimates (Wexler et al. 2007). Our experimental results have indicated an early onset of substantial density-dependent growth of yellowfin during the first 2.5 weeks after hatching. Increases of 2-4 times in larval density have resulted in growth deficits up to 56% during larval stages. We have also noted strong indirect evidence of density-dependent growth in larval cohorts during certain years in the Panama Bight (Wexler et al. 2007). Our experimental evidence suggests that density-dependence in growth persists into the early-juvenile stages of yellowfin. Even subtle density effects on growth during the relatively long pre-recruit juvenile stage (5 months) could have a "finetuning" effect on recruitment and the mean biomass of a cohort. This association requires further study.

#### 6.2. Effects of wind-induced turbulence on yellowfin larval survival

Feeding success of marine fish larvae can be influenced by the levels of wind-induced microscale turbulence in the feeding environment (Rothschild and Osborn 1988, Cury and Roy 1989). The probability of prey encounters and feeding success of larvae may increase with increases in wind-induced microscale turbulence up to an asymptotic wind and turbulence level and then decrease at higher levels of turbulence (MacKenzie *et al.* 1994). Our studies of feeding of yellowfin larvae in Japan in 1992 indicated a strong potential for the influence of microscale turbulence on the feeding success of yellowfin larvae. We expanded these investigations during 1997-2000 in a series of laboratory experiments at the Achotines Laboratory which examined the survival of yellowfin larvae during the first week of feeding under conditions of variable microturbulence. Turbulence in the experimental tanks was measured as the mean horizontal velocity of a neutrally-buoyant surface drogue; in 1999 and 2000 these velocities were calibrated against velocities measured at depth with a microacoustic Doppler current meter. The analysis of the data is summarized in a recently-completed draft manuscript, but preliminary results are reported by Kimura *et al.* (2004).

Our preliminary analysis of the 1997-2000 data indicates that survival during the first week of feeding is up to 2.7 times higher at intermediate levels of microturbulence (ca. 7.4 x 10<sup>-9</sup>m<sup>2</sup>s<sup>-3</sup> to 2.25 x 10<sup>-8</sup>m<sup>2</sup>s<sup>-3</sup> as an energy dissipation rate) than at lower or higher levels of turbulence (Figure 7). Using a boundary layer model that equates microturbulence levels in the mixed layer of the ocean with wind speed, we have made preliminary estimates of optimal wind speeds for larval yellowfin survival, based on depths of 5-20 m for the maximum concentration of the larvae (estimated from larval field survey data in the literature). The optimal wind speed estimates range from 2.0 to 4.5 m sec<sup>-1</sup>. These are the first such estimates reported for yellowfin tuna early life stages, and among the first estimates of microturbulence effects on survival of marine larvae based on extended experimental trials.

The estimated optimal wind speeds for larval survival were examined for correlations with historical yellowfin recruitment estimates in the EPO for select 2°x2° areas. Wind speed data for the 1987-2007 period were obtained from the Blended Sea Winds Database, National Oceanic and Atmospheric

Administration (NOAA), National Environmental Satellite, Data, and Information Service (NESDIS), National Climatic Data Center (NCDC) (Zhang et al 2006). The percentage of days with optimal wind speeds within a given 2°x2° area was estimated and correlations were calculated with IATTC quarterly estimates of yellowfin recruitment (time-lagged 6 months to account for pre-recruit development). A spatial pattern was observed both latitudinally and longitudinally for the areas selected (Figure 8). The areas closer to shore, east of 100°W, showed positive correlation values, while the correlation coefficients became negative further offshore and west of 100°W. All areas south of the equator exhibited positive correlations. The correlation analysis was also conducted for quarter-year combinations (e.g. quarters 1 and 2, quarters 1 and 3, etc.). For the six positively correlated areas in the southeast region of the study area, quarters 1 and 2 contributed most strongly to the positive correlation between optimal wind speed and recruitment. In nearly all of these regions, the correlations became significantly positive when only the first two quarters of each year were considered (Area 11 off Peru was marginally non-significant). The areas west of 100°W showed negative correlations regardless of quarter-year combination.

The wind speed-recruitment analysis can be refined and expanded, but this analysis is promising for assessing yellowfin recruitment patterns. The correlation analysis reported here involves different spatial scales of variables (EPO-wide recruitment estimates versus 2°x2° estimates of wind speed). More geographical coverage would improve the analysis and ongoing development of spatial components to the IATTC's recruitment estimates would allow the examination of wind speed data and recruitment on the same spatial scale.

#### 6.3. Comparative studies of the early life histories of yellowfin and Pacific bluefin

In 2011, the IATTC, Kinki University (KU) of Japan, and the Autoridad de los Recursos Acuáticos de Panama (ARAP) began a 5-year comparative study of the reproductive biology and early life history of yellowfin and Pacific bluefin tuna (Science and Technology Research Partnership for Sustainable Development, SATREPS). The joint research project is funded by the Japan International Cooperation Agency (JICA) and Japan Science and Technology Agency (JST), and is being conducted mostly at the Achotines Laboratory and the Fisheries Laboratories of Kinki University in Wakayama Prefecture, Japan. The studies are the first in the world to investigate important comparative aspects of the reproductive biology, genetics, and early life histories of Pacific bluefin tuna and yellowfin tuna. Although Pacific bluefin are temperate to subtropical and yellowfin are tropical to subtropical in their adult life histories, the early life stages of both species require warm-water (> 24°C) ecosystems as nursery grounds, thus providing a common background for comparative studies. Experimental results will also be used to comparatively model mortality processess occurring during the pre-recruit life stages of both species. An additional objective of the project is to develop technologies for the aquaculture of juvenile yellowfin, including sea-cage culture.

Comparative experiments are ongoing, but preliminary results indicate that Pacific bluefin larvae hatch and initiate feeding at slightly larger sizes than yellowfin. Bluefin larvae, given their larger size and greater endogenous energy reserves, exhibit greater resistance to starvation at first-feeding (15-25 hrs longer, depending on temperature) compared to yellowfin. However, larger size confers no apparent advantage to Pacific bluefin larvae in growth or survival when small microzooplankton prey are the prevalent forage (Figure 9). Yellowfin larvae exhibit greater growth potential and higher survival when foraging on small microzooplankton prey. However, greater size of Pacific bluefin larvae may confer feeding and growth advantages when foraging on large zooplankton prey, and this hypothesis is being experimentally investigated in 2015.

#### 6.4. The effects of ocean acidification on yellowfin eggs and larvae

The 5<sup>th</sup> Intergovernmental Panel on Climate Change (IPCC) assessment (Stocker *et al.* 2013) estimates a global average decline in ocean surface pH of 0.30-0.32 by 2100 due to increasing concentrations of dissolved carbon dioxide (pCO<sub>2</sub>) from anthropogenic activities. Across regions of the Pacific Ocean where yellowfin tuna spawn and develop, mean surface water pH is predicted to decrease by 0.26-0.49

pH units by 2100 (Ilyina *et al.* 2013). Ocean acidification is a concern for its potential effects on the growth, development, and survival of early life stages of tunas in oceanic habitats and on the spatial extent of suitable nursery habitat for tunas.

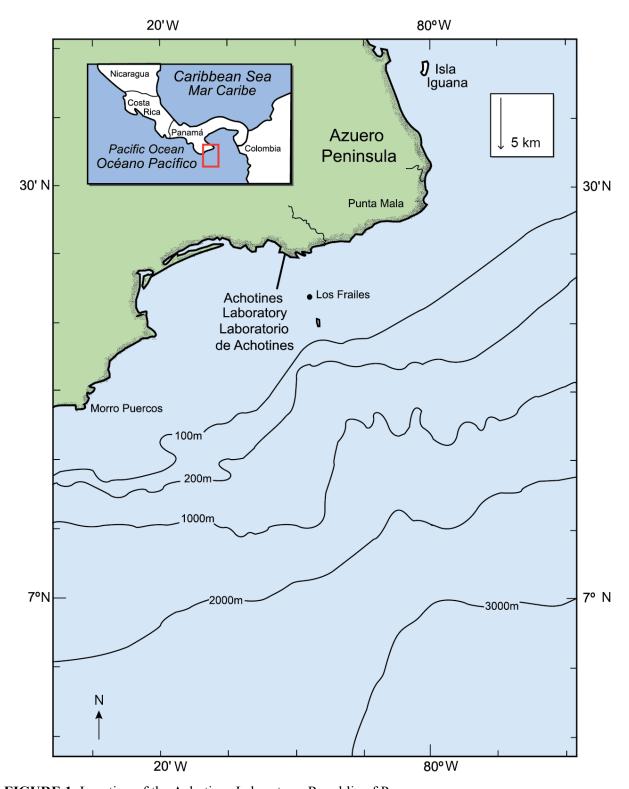
To investigate the potential effects of ocean acidification on yellowfin early life stages, a laboratory study was conducted by multiple collaborating organizations at the Achotines Laboratory in 2011. Two separate trials were conducted to test the impact of increased pCO<sub>2</sub> on eggs, yolksac larvae, and first-feeding larvae. Acidification levels tested ranged from present day to levels predicted to occur in some areas of the Pacific within the next 100 years (near future) to 300 years (long term). The study results were variable between trials, but did indicate the potential for significantly reduced survival (Figure 10) and size of larvae and prolonged egg hatch times at acidification levels that are similar to near future predicted levels (Bromhead *et al.*, 2015).

The potential impacts of ocean acidification on early life stages are an important consideration in future assessments of tunas in the EPO. If acidification does progress to predicted levels for the Pacific Ocean, it is unclear whether tunas possess the capacity to adapt to acidification through selection for more resistant individuals (Bromhead *et al.*, 2015). It is also unclear whether resistant individual traits are heritable (Munday *et al.*, 2012). To date, there is evidence that near future levels of ocean acidification can have significant negative effects on development, survival and growth of yellowfin eggs and larvae. These results can allow models such as SEAPODYM (Lehodey *et al.* 2008) to be parameterised to include acidification effects and to incorporate the effects of acidification in the development of spawning-habitat indices.

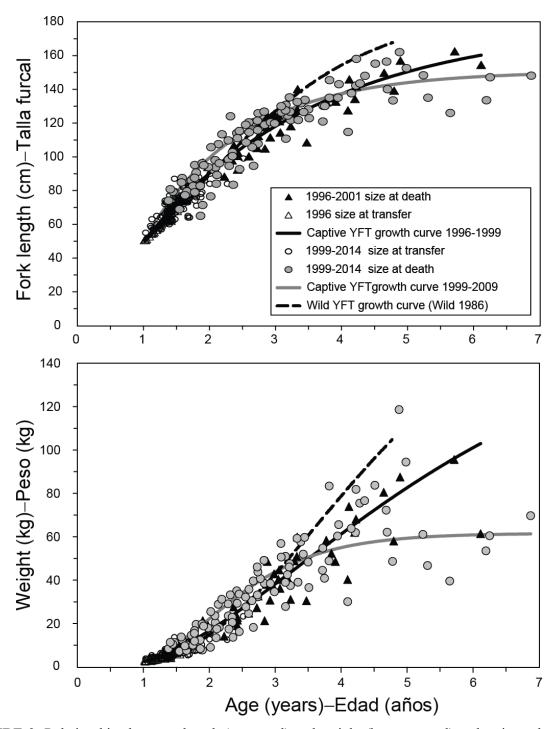
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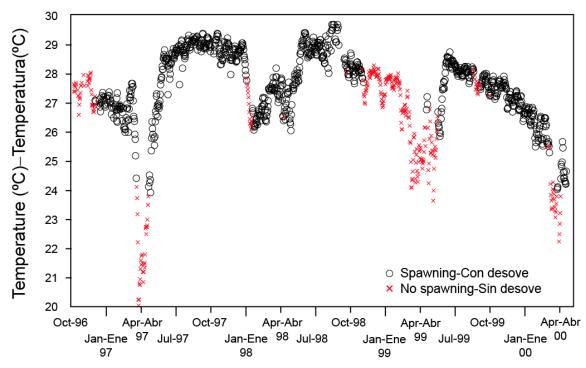


**FIGURE 1**. Location of the Achotines Laboratory, Republic of Panama. **FIGURA 1**. Ubicación del Laboratorio de Achotines, República de Panamá.

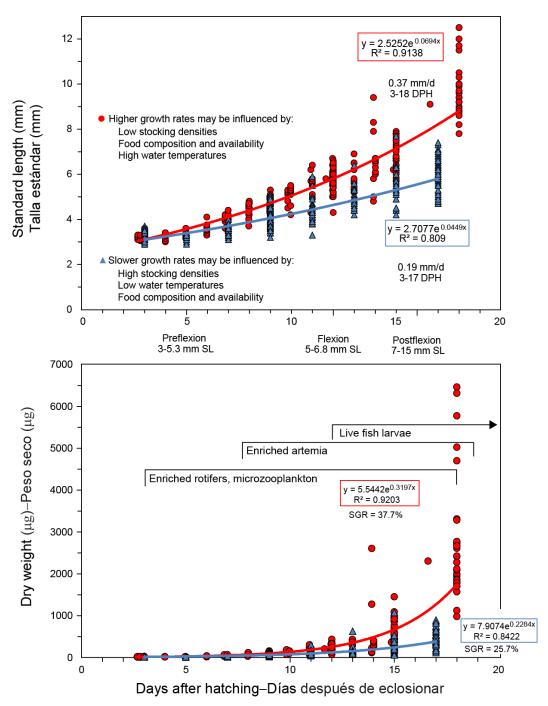


**FIGURE 2**. Relationships between length (top panel) and weight (bottom panel) and estimated ages of wild yellowfin in the eastern Pacific Ocean (Wild, 1986) and captive yellowfin in Tank 1. (After Wexler *et al.* 2003)

**FIGURA 2**. Relación entre talla (panel superior) y peso (panel inferior) y edad estimada de aletas amarillas en el Océano Pacífico oriental (Wild, 1986) y aletas amarillas cautivos en el Tanque 1. (De Wexler *et al.* 2003)



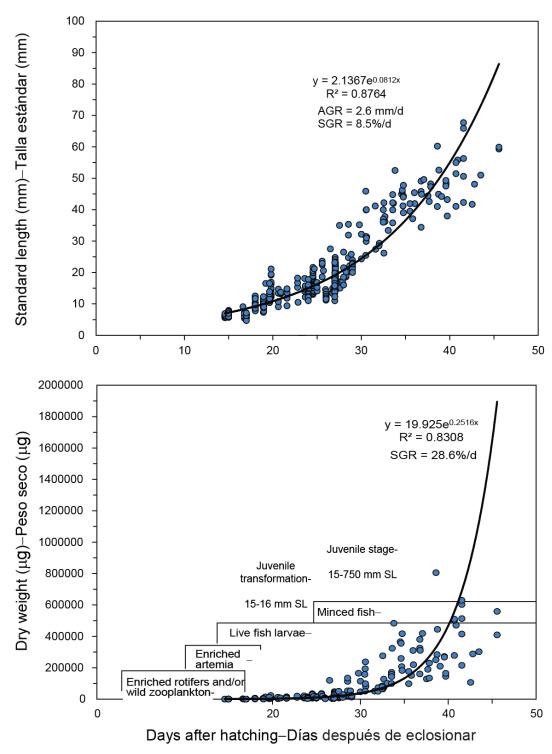
**FIGURE 3**. Mean daily water temperature in Tank 1. Plotted symbols are individual dates on which there was either spawning (indicated by 'o') or no spawning (indicated by 'x'). (After Margulies *et al.* 2007b) **FIGURA 3**. Temperatura media diaria del agua en el Tanque 1. Los símbolos corresponden a fechas individuales en las que ocurrió desove (indicadas con 'o') a sin desove (indicadas con 'x'). (De Margulies *et al.* 2007b)



(Stage terminology follows Kendall, et al. (1984))

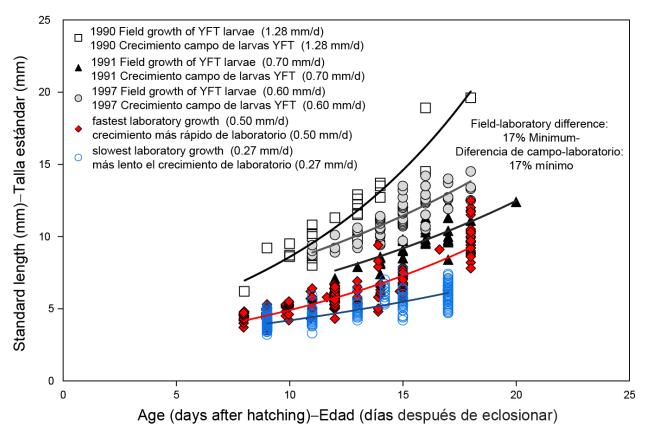
**FIGURE 4**. Relationships between standard length (top panel) and dry weight (bottom panel) and age in days after hatching of yellowfin reared in the laboratory for the fastest- (red line) and slowest-growing (blue line) cohorts. Prey type at age routinely offered in the laboratory and stage terminology at standard length are also shown.

**FIGURA 4**. Relaciones entre talla estándar (panel superior) y peso seco (panel inferior) y edad en días desde eclosión de aletas amarillas criados en el laboratorio correspondientes a las cohortes de crecimiento más rápido (línea roja) y más lento (línea azul). Se indican también el tipo de presa por edad ofrecido rutinariamente en el laboratorio y la terminología estándar de etapas por talla estándar.



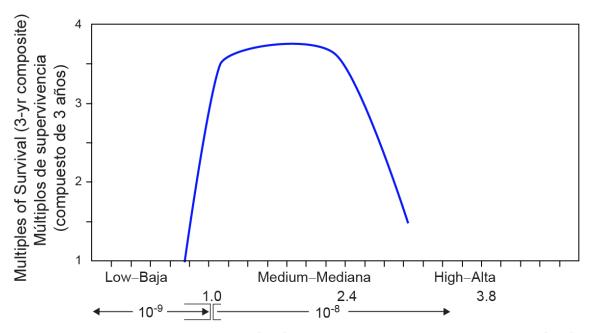
**FIGURE 5**. Growth in standard length (top panel) and dry weight (bottom panel) versus days after hatching of yellowfin larvae and early-juveniles reared in the laboratory. The bottom panel also shows the feeding regime.

**FIGURA 5**. Crecimiento en talla estándar (panel superior) y peso seco (panel inferior) como función de días desde eclosión de aletas amarillas larvales y juveniles tempranos criados en el laboratorio. En el panel inferior se indica también el régimen de alimentación.

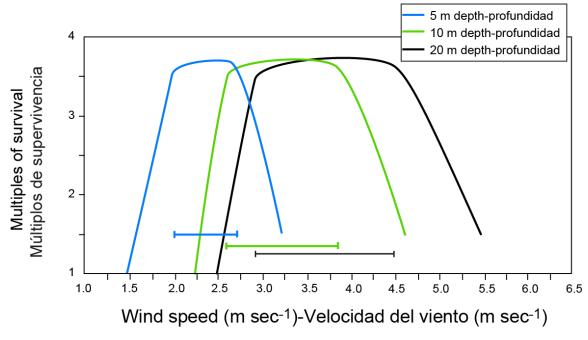


**FIGURE 6**. Relationship between standard length and age in days estimated from otolith increment counts of larval yellowfin collected during June 1990, September 1991, and August 1997 in the northwestern Panama Bight (after Wexler *et al.* 2007) and for the slowest- and fastest-growing cohorts of yellowfin larvae reared in the laboratory.

**FIGURA 6**. Relación entre la talla estándar y la edad en días estimada a partir de conteos de incrementos en los otolitos de aletas amarillas larvales capturados durante junio de 1990, septiembre de 1991, y agosto de 1997 en el noroeste de la Bahía de Panamá (de Wexler *et al.* 2007) y para las cohortes de crecimiento más rápido y más lento.

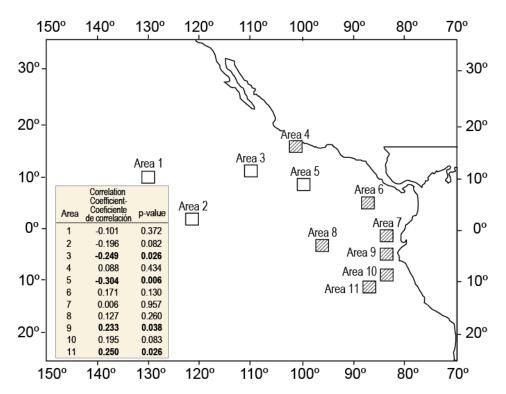


Turbulent dissipation rate (m<sup>2</sup> s<sup>-3</sup>)-Tasa de disipación turbulenta (m<sup>2</sup> s<sup>-3</sup>)



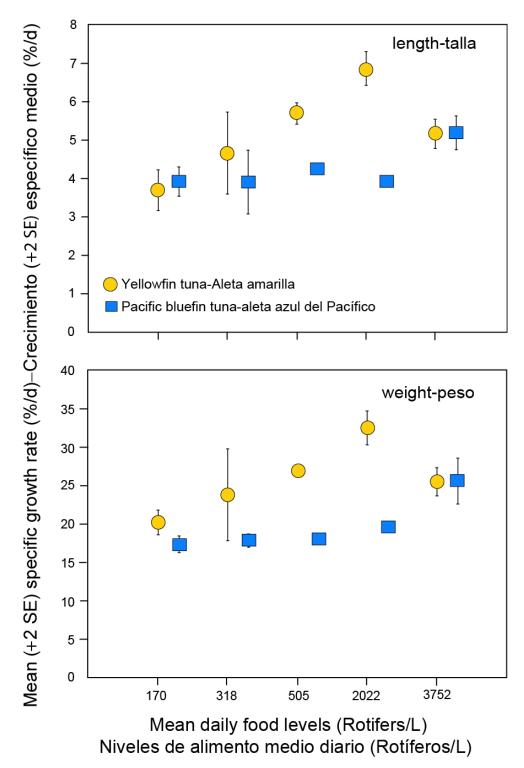
**FIGURE 7**. Relationship between microturbulence (estimated in the top panel as the turbulent dissipation rate and converted in the bottom panel to wind speed) and survival of yellowfin larvae during the first week of feeding. The survival curve is a smoothed, composite curve representing the mean survival estimated during 4 trials over 3 years.

**FIGURA 7.** Relación entre microturbulencia (estimada como tasa de disipación turbulenta y convertida en velocidad del viento en el panel inferior) y supervivencia de larvas de aleta amarilla durante la primera semana de alimentación. La curva de supervivencia es una curva compuesta suavizada que representa la supervivencia media estimada durante 4 pruebas en 3 años.



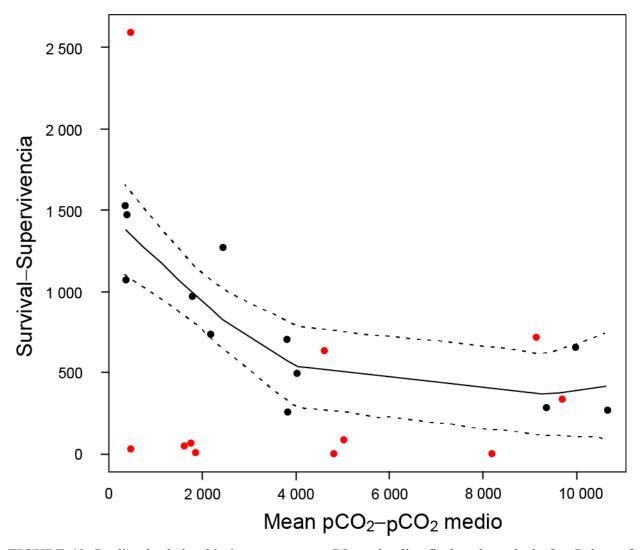
**FIGURE 8**. Correlation analysis results of recruitment (R) and the percentage of days with optimal wind speeds for selected  $2^{\circ}x2^{\circ}$  areas of the eastern Pacific Ocean. Shaded boxes signify areas of positive correlation and open boxes negative correlation (bold values are statistically significant at an alpha level of 0.05).

**FIGURA 8**. Resultados del análisis de correlación del reclutamiento (R) y el porcentaje de días con vientos de velocidad óptima en áreas seleccionadas de 2°x2° en el Océano Pacífico oriental. Los cuadros sombreados señalan áreas de correlación positiva, y los blancos una correlación negativa (los valores en negritas son estadísticamente significativos en un nivel alfa de 0.05).



**FIGURE 9**. Mean specific growth rates in standard length (top panel) and dry weight (bottom panel) for yellowfin and Pacific bluefin larvae over a range of mean daily food levels during the first 10 days of feeding.

**FIGURA 9**. Tasas de crecimiento específico medio en talla estándar (panel superior) y peso seco (panel inferior) de larvas de aleta amarilla y aleta azul del Pacífico correspondientes a una gama de niveles de alimento medio diario durante los 10 primeros días de alimentación.



**FIGURE 10.** Predicted relationship between mean pCO<sub>2</sub> and yellowfin larval survival after 7 days of growth (Trial 1, black line) (Trial 2, red dots). Dashed lines for Trial 1 represent 95% confidence intervals; points indicate the data used to fit the models (Bromhead *et al.* 2015).

**FIGURA 10.** Relación entre pCO<sub>2</sub> medio y supervivencia de aletas amarillas larvales al cabo de 7 días de crecimiento (Prueba 1, línea negra) (Prueba 2, puntos rojos). Las líneas de trazos en la Prueba 1 representan los intervalos de confianza de 95%; los puntos indican los datos usados para ajustar los modelos (Bromhead *et al.* 2015).