BRIEF DESCRIPTION OF THE PROJECT (including objectives, materials, methods and an explanation of how the research results will contribute to the improvement of the conservation, welfare or management of the animal populations in zoos):

We propose to electronic tag up to 10 Atlantic bluefin tuna in the waters off Spain. Electronic tagging of Atlantic bluefin tuna has emerged as a powerful tool to reduce uncertainty in scientific knowledge about their population structure and seasonal distributions. Data obtained from electronic tags has the potential to improve modeling of key parameters of life history traits related to maturation, spawning, and natural mortality as well as movements, and habitat utilization. Population models rely on biological assumptions, and reducing the uncertainty in these assumptions with electronic tag data is vital to improving stock assessment models. Despite the rapid advances in knowledge on Atlantic bluefin tuna, key questions remain about the level of population mixing from eastern Mediterranean spawning grounds to the western Atlantic, spawning ground dynamics, spawning habitat environmental preferences, and the number of independent spawning populations in the Mediterranean Sea. Furthermore spatial movements and mixing vary with ontogeny of each Atlantic bluefin tuna population and need to be accounted for in models. We propose to use electronic tagging techniques to study the eastern bluefin tuna population that spawns in the western Mediterranean Sea off the Spanish coast near Barcelona. This mature group of fish may provide the opportunity to obtain one year tracks that provide key information on spawning ground locations that would lead to maximal protection of the remaining spawning biomass. The project will enable Zoo personnel to increase their handling knowledge of Atlantic bluefin tuna and help protect this critical species in waters off the coast. In addition the use of camera tags will enable spectacular underwater video to be obtained that will improve knowledge of spawning ground behaviors in the Mediterranean. Its possible the first ever spawning video will be obtained in the wild. The major objective is to deploy the tags with the funding and matching support from Stanford and the Monterey Bay Aquarium and TAG A Giant fund, will provide additional tag deployment support and salary funds for personnel conducting tagging and data analyses. Our goal is to work together to increase capacity, improve the opportunity for recovery of bluefin populations and to identify critical spawning habitats. The results will enable improving models of how mature bluefin use the Mediterranean foraging an spawning grounds. The Camera tags will provide high resolution information on behaviors.

GIVE A DETAILED EXPLANATION OF WHETHER THE METHODOLOGY APPLIED MAY HAVE ANY NEGATIVE IMPACT ON THE ANIMALS OR VISITORS AT THE ZOO:

Satellite tagging has been shown previously to be highly successful at tracking big fish in the sea- Camera tagging video may be used by Barcelona Zoo in exhibits.

DURATION OF THE PROJECT: One Year

OTHER INSTITUTIONS INVOLVED: Stanford University, Monterey Bay Aquarium and TAG A Giant Fund of the The Ocean Foundation

BUDGETARY FRAMEWORK

SUMMARY OF EXPENDITURE

We request 13,500 Euros to be spent on purchasing Pop up satellite archival tags for deployment on bluefin tuna in waters off the coast of Spain.

SUMMARY OF INCOME:

The TAG A Giant Fund will match any funding the Barcelona Zoo puts forth for the project enabling purchase of additional tags.

PROPOSED GRANT OR FINANCIAL AID REQUESTED OF BARCELONA ZOO

TOGETHER WITH THIS FORM AND THE COVERING LETTER OF THE PROJECT (Appendices 1 and 2 of these Rules)**, YOU SHOULD SUBMIT THE PROJECT WITH THE DETAILED REPORT ON THE PROGRAMME SUBMITTED FOR THE APPLICATION FOR THE GRANT OR AID:**

- **PLAN AND PROGRAMME**
- **SCIENTIFIC JUSTIFICATION OF THE PROJECT**
- **PRESENTATION OF THE PARTICIPATING TECHNICIANS, RESEARCHERS AND INSTITUTIONS** (if appropriate)
- **FORECAST COSTS**
- **DETAILED PLANNING SCHEDULE**
- **LIST OF MATERIALS**
- **POSSIBLE PUBLICATIONS**
- **REQUIREMENTS REQUESTED OF THE ZOO**
- **ETC.**

PROJECT PLAN and PROGRAM

INTRODUCTION

The migrations of Atlantic bluefin tuna, *Thunnus Thynnus*, have captured the interest of scientists and fishers for thousands of years. These fish are an iconic species, highly prized as catch by commercial and recreational fishers throughout the north Atlantic and exploited by fisheries throughout their range. These fish are large and long lived, reaching a mass of 679 kg and a lifespan of over 35 years (Neilson & Campana, 2008). The populations of bluefin in the Atlantic declined severely in the 1970s and 1990s but have remained stable through implementation and enforcement of stringent quotas. The eastern population is hypothesized to be recovering. Bluefin tuna are among the largest and longest lived fish in the sea. The unique endothermic physiology of the bluefin coupled with their large size, is associated with the capacity of the fish to migrate from polar seas to warm temperate waters in short durations. The size, power and speed of the giant bluefin has made it a challenge to study their biology. Atlantic bluefin tuna are distributed from the Mediterranean Sea to Iceland, Greenland, and Canary Islands in the East Atlantic to the Gulf of Mexico and Newfoundland in the west Atlantic.

Giant bluefin tuna have co-existed for centuries with humans, who are their major predator. Prior to the 1960s, fishing was limited to subsistence fisheries or small-scale commercial ventures involving nets, traps or harpoons since Roman times. Although exploitation has been on-going for centuries bluefin tuna fishing was sustainable. Currently, all three species of bluefin tunas (Atlantic, Pacific and Southern) are considered overexploited and the western Atlantic population of Atlantic bluefin as well as the Pacific bluefin tuna species have been severely depleted where less then 10% of virgin spawning biomass remains. Importantly, the demise of the bluefin populations has occurred during a period when management and conservation has been coordinated internationally among major fishing nations. Fishing efforts have and during the past five years, the Atlantic-wide landings of bluefin have reached a historical high in human history. The unbridled commerce surrounding the Atlantic bluefin tuna is a major factor associated with the decline of the stocks. However, recently there has been evidence of a recovery in the Mediterranean Sea. In this grant we will examine the capacity to do long term 1 year tags- from Spanish waters that might improve our knowledge of spawning and foraging areas for these recovering populations.

Electronic tagging of Atlantic bluefin tuna, *Thunnus thynnus,* has emerged as a powerful tool to reduce the uncertainty in scientific knowledge of this species and to inform the spatial and temporal patterns necessary for proper fisheries management (Block *et al.,* 1998, 2001, 2005; Teo *et al.,* 2007a,b, Walli *et al.,* 2009, Wilson *et al.* 2010, Lawson *et al.,* 2010, Lutcavage *et al.,* 1999, 2000, Royer *et al.,* 2005, Taylor *et al.,* 2011, Galuardi and Lutcavage 2012). Tagging, genetics and microconstituent analyses provide the capacity to demonstrate that discrete eastern and western Atlantic bluefin tuna populations exist, move to separate spawning grounds, and mix on foraging grounds (Rooker *et al.,* 2008, Carlsson *et al.,* 2007, Boustany *et al.,* 2008, Secor 2015). The International Commission for the Conservation of Atlantic Tunas (ICCAT) presently manages northwest Atlantic and northeast Atlantic-Mediterranean Sea bluefin tuna resources as two distinct management units. Under this management regime one stock is recognized in the eastern Atlantic with a breeding area within the Mediterranean Sea and a second stock is thought to exist in the western Atlantic ocean with a breeding ground in the Gulf of Mexico. Each stock is subjected to different management regimes with the most prominent difference being the strict quotas for the western fishery and the rising quotas for the eastern population. We know from the tagging that the management is more complex then current models recognize and the objective of this proposal is to help generate 1 year tagging records that enable a better understanding of the western Mediterranean population structure. As the bluefin of this region recover it will be important to improve our knowledge of their stock structure in and around the Mediterranean Sea where Spanish fleets take large tonnage for ranching operations.

The eastern population uses the Mediterranean Sea and spawning ages are thought to vary between populations. Conventional tagging (Fromentin 2002), electronic tagging (Block *et al.,* 1998, 2001, 2005, Lutcavage *et al.,* 2000, Walli *et al.,* 2009), population genetics (Boustany *et al.,* 2008, Riccioni *et al.,* 2010), organochlorine tracer analyses (Dickhut *et al.,* 2009) and otolith microchemistry studies (Rooker *et al.,* 2008, 2014) all indicate that as many as three or more (Riccioni *et al.,* 2010) populations of Atlantic bluefin tuna exist in the North Atlantic. Two populations (Riccioni *et al.,* 2010) or more, spawn in the eastern and western Mediterranean Sea in summer months, and a smaller population spawns in the GOM in the spring months. Site directed fidelity to these spawning grounds has been observed using electronic tags (Block *et al.,* 2005, Teo *et al.,* 2007a) and this is hypothesized to maintain the observed genetic structure (Riccioni *et al.,* 2010). This proposal will focus on tagging off Spain in what we hope will be the western Med population. By combining Barcelona Zoo funds, and Stanford and TAG funds we hope to get out 10 tags in the region.

Recent biological data indicate a high degree of spawning site fidelity and foraging ground homing in bluefin populations in the Mediterranean Sea and Gulf of Mexico (Block *et al.* 2005, Rooker et al. 2008, 2014, Cermeno et al. 2015, Secor 2015). These migration behaviors also influence fisheries stock assessments and population estimates as well as management decisions. We propose herein to use well established electronic tagging techniques to study the eastern Atlantic bluefin tuna population that spawns in the Mediterranean Sea off the coast of Spain. Our proposal will focus specifically on priority areas #3.3 (To contribute to research into natural habitats), priority area #4 ('To produce and use quality science that helps in decision-taking in managing the fauna, collections of animals and related programmes and projects. Priority area #8, (To internationalise research and conservation programmes.) and #9 (To stimulate top-quality research concerning wildlife and captive species, whereby through publications in high-profile and widely published scientific journals the name of Barcelona Zoo becomes associated with top-quality research and conservation work.). The research will also improve our understanding of preferred habitats for spawning and enable dynamic management of the closed areas for bluefin tuna longlining in the Gulf of Mexico, which in theory would also reduce mortality on spawning sized bluefin.

SCIENTIFIC JUSTIFICATION

Despite the rapid advances in our understanding of bluefin tuna biology, key questions remain about spawning, population mixing, recruitment dynamics, maturity schedules, abundance trends. Electronic tagging datasets can improve management models by providing accurate information on key parameters of life history traits related to movements, maturation, fisheries and natural mortality (Kurota *et al.,* 2009, Taylor *et al*., 2011, Kerr *et al*., 2012), as well as providing information on critical habitat selection. Additionally, using mark recapture frameworks, fisheries mortality and abundance estimates on individual year classes are possible (Whitlock *et al.*, 2012). This information reduces the biological uncertainty in assessment models, thus improving their overall accuracy. The Tag-A-Giant (TAG) team has developed a spatially explicit model called MAST (Taylor *et. al.,* 2011) that demonstrates the importance of incorporating the spatial and temporal movements, as well as population mixing, observed with electronic tags. MAST models the seasonal movements of the eastern and western Atlantic bluefin tuna spawning stocks through five discrete fishing grounds in the north Atlantic Ocean (i.e., the Mediterranean Sea, northeast Atlantic, northwest Atlantic, Gulf of St. Lawrence and the Gulf of Mexico) and accounts for inter-annual and seasonal trends in the mixing rates of the two stocks on these fishing grounds. The model uses quarterly time steps (seasonal) rather then a single, annual time step. Newer models also are emphasizing the need for spatially explicit data (Kerr *et al.,* 2012). By continuing the tagging on mature eastern bluefin tuna, we're increasing the accuracy of all spatially explicit models.

The rebuilding of the Mediterranean spawning population also requires fisheries managers to have knowledge about how the population is influenced by environmental variation, and how susceptible mature bluefin will be to environmental changes. Ultimately this influences where the fish are, what sectors will have access and where protected areas will have the greatest impact. Management of Atlantic bluefin tuna at the population level requires knowledge of survival, growth and reproduction, movements and habitat utilization as well as changes that occur with ontogeny and gender. Electronic tags provide information on these parameters, as well as informing locations of critical spawning and foraging hot spots which can then be target areas for protection as these regions represent areas where catchability increases due to aggregation behavior. Electronic tags also provide information on geographic and physiological ranges, responses to oceanographic forcing, and human impacts (oil spills). Understanding the risk of marine species to anthropogenic stressors requires information on the patterns of habitat use and movements of marine animals as well as the spatial and temporal patterns of their stressors.

We propose to electronic tag Atlantic bluefin tuna that spawn in the Mediterranean. Our objectives are to: a) deploy 8 pop up satellite archival tags to Atlantic bluefin tuna and b) two camera tags to obtain high resolution behavioral data of how bluefin tuna use the waters off Spain. We will analyze the satellite archival tag data to assess critical Mediterranean foraging and spawning habitats for these mature bluefin tuna, d) investigate bluefin habitat selection in relationship to environmental variables. The overall objective is to improve our knowledge of spawning fish migrations and habitat use in the western Mediterranean Sea

We will bring to the Barcelona zoo our collaborative electronic tagging program called Tag-A-Giant (TAG). To date, we have deployed from 1996-2016 over 1300 electronic tags on adolescent and mature fish in the Atlantic, the Mediterranean and the Gulf of Mexico. Over the past decade about 100 tags have been released by TAG researchers in Mediterranean fisheries but very few of these have gone in off the coast of Spain. This grant seeks to test long term deployments, in collaboration with Spanish recreational fishers and the Barcelona Zoo. Electronic tagging is expensive, long-term deployments are difficult to achieve and our program has been built on small deployments annually such as the one requested here. We will focus these deployments in Mediterranean waters close to Barecelona, to learn more about Mediterranean spawning populations. This grant will focus on placing tags on fish captured in recreational fisheries. To date no other bluefin population on the globe has the collection of spawning area tracks and behavioral results that the TAG data set is generating. Many of the obstacles of tagging large spawning sized individuals have been overcome in the past five years.

BACKGROUND

The successful rebuilding of Atlantic bluefin populations requires an understanding of their spatial structure, population mixing and seasonal movements (Taylor *et al.,* 2011). Atlantic bluefin have been historically challenging to study due to their size, speed and range. Integrated population assessment models depend upon data that incorporates spatial and temporal mixing of the western (GOM) and eastern (Mediterranean) breeding populations. New information on genetics, maturity and microconstituent analyses are also considered essential. Our tagging studies and those of our colleagues (Lutcavage *et al.,* 2000, Galiuardi and Lutcavage, 2012) have demonstrated that all age classes of Atlantic bluefin are capable of transits across ocean basins from the Mediterranean to the western Atlantic and back. The enormous scale of these migrations has made it difficult to follow these fish over long durations. Understanding the connectivity between eastern and western populations of bluefin tuna is critical to management efforts. Uncertainty about fidelity to spawning grounds, the timing and age of maturity, the size at which bluefin tuna move into spawning grounds (breeding and maturity schedules), temporal timing of spawning, duration of occupation on the spawning ground and exit dates from the spawning grounds, has resulted in a lack of clarity in fisheries stock assessment models (population assignments). We propose to use Barcelona Zoo funds in concert with Stanford and TAG a giant funds to electronically tag bluefin of Mediterranean origin We propose to use the electronic tag data to capture the adult spawning habitat in Mediterranean for a year (Teo et al. 2007a, Teo et al. 2010, Wilson et al. 2015), and integrate the behavioral diving data with oceanographic satellite data, and compare outputs to the Gulf of Mexico spawning data sets.

Satellite Archival Tagging Breeding Adults

In recent years, TAG has focused on electronic tagging of large bluefin tuna in the Canadian commercial fishery off Port Hood, Nova Scotia, Canada and in Morroco. The purpose has been to examine behaviors of adult fish and mixing from east to west Atlantic and back. The Canadian foraging aggregation has been the main location for electronic tagging by US scientists since 2007, and to date TAG scientists have placed 140 satellite tags on these fish (measured mean size 266.1 cm ± 22.2 CFL) over a decade. By taking small steps, putting out 10-12 tags a year- over time the data sets became formidable. 74% of the bluefin tuna whose tags remained attached until the peak spawning month (May, n=51) went to the Gulf of Mexico spawning ground from the Canadian foraging grounds, and two individuals carrying satellite tags went to the western and central Mediterranean Sea and two were at Gibraltar when their tags jettisoned. Population assignments of the individuals that move into spawning grounds have been verified using genetic microsatellite DNA assignments from fin clip analyses which were obtained during tagging. These results confirm GOM fish go to GOM spawning grounds, and have a GOM genotype; and fish that return to the Mediterranean spawning grounds have a distinctly different genotype, consistent with the Mediterranean population identification (Boustany *et al.,* 2008, Reeb *et al*., unpublished data).

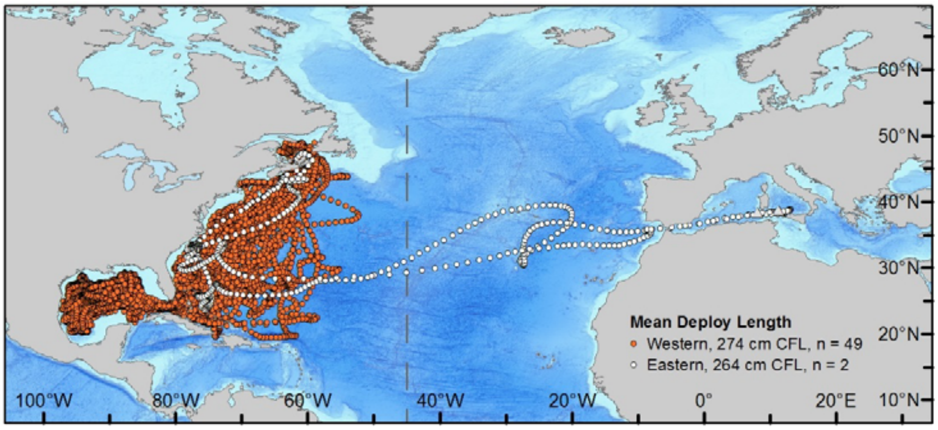

Figure 1. Geolocations from PAT satellite archival tags (n=51) deployed in Canadian waters that remained on fish. Circles are geolocations from light and SST based estimations and colors are tracks of satellite tagged bluefin tuna that are coded by their movement on to known spawning grounds (orange, Gulf of Mexico, white Mediterranean).

In addition to geolocation tracks, the satellite archival tags provide detailed time series on behaviors including potential foraging activity, putative spawning behaviors, and oceanographic preferences. Tags record temporal data on the journey from the west Atlantic foraging ground to the Mediterranean and Gulf of Mexico spawning grounds (Teo *et al.,* 2007a, 2007b, Wilson *et al.,* 2010, Block et al. 2013, Wilson et al. 2015).

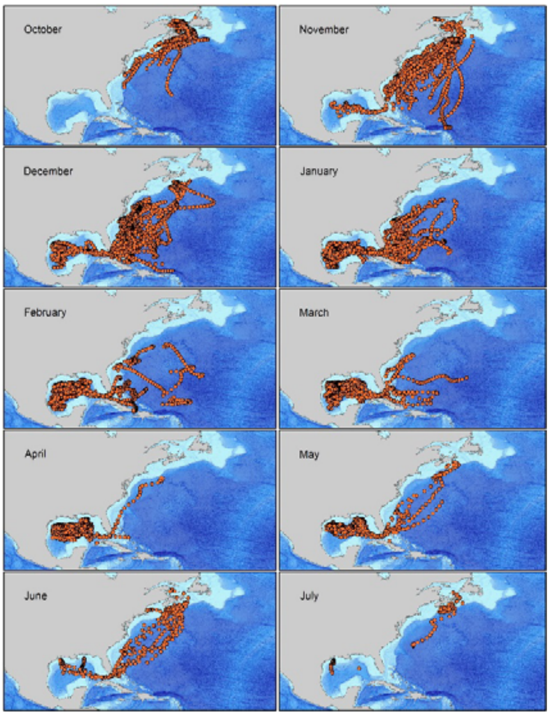

Figure 2. Pooled monthly geolocations from all satellite archival bluefin tuna tags that showed visitation to the GOM spawning site. Movement into the GOM begins as early as November by some individuals. Exit from the GOM is by early July. These tracks provide informative information on how small tag deployments can add up to show large movement trajectories.

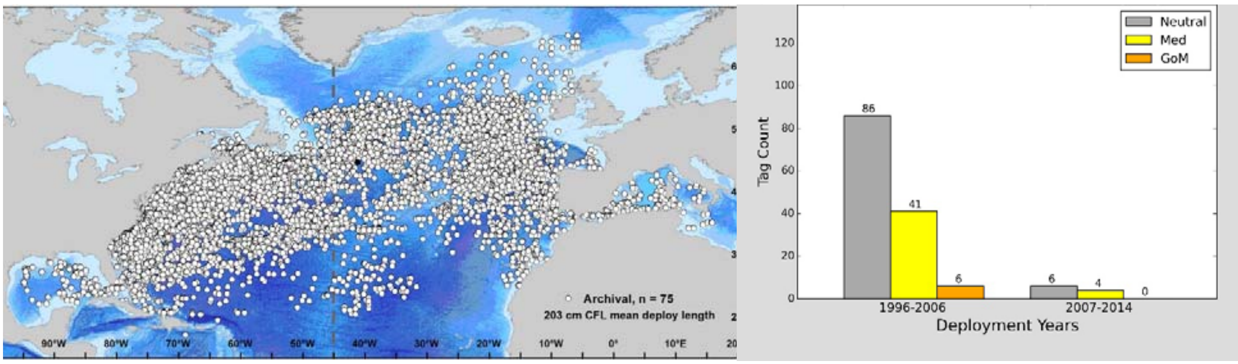

Figure 3. A) Positions (geolocations) of Atlantic bluefin tagged and released in the west Atlantic winter fishery with archival tags that recorded 30 days of tracking data or more (recovered from 1997-2012). The tags were deployed on fish with a mean measured length of 203 cm (CFL) from waters off Morehead City, and Hatteras, NC. Right panel -The tracks visiting a spawning area go primarily to the Mediterranean Sea (45) and fewer tracks recorded visitation to the GOM (6). Neutral track are most likely adolescent fish that had one year or less of data.

The Canadian foraging ground fisheries are highly concentrated foraging areas where bluefin tuna aggregate primarily to feed on herring and mackerel. We learned here how to electronically tag large fish that are breeders on cool foraging grounds, we have obtained 25 full satellite archival tracks to the spawning ground (complete 1 year round trips) and our request for Mediterranean funding should result in 8-10 tag deployments off Spain. We anticipate this will yield similar full archival records from Mediterranean spawners who will either remain in the Med or exit and move off into the North Atlantic prior to coming back. The tag we will be using is called a mini-pat satellite archival tag and is well tested. The advancement in satellite tagging techniques in the Canada waters now provides the latest information on the annual cycle for bluefin tuna breeding in the Gulf of Mexico as shown in

Figures 1-3. To date, the new Canadian derived bluefin tuna tracks on mature tuna provide the highest level of information on how individual adult bluefin tuna use the spawning areas (figure 4). Tracks show mean size and age of entry of bluefin into the Gulf of Mexico. The mean size of the bluefin tuna that recorded visitation to the GOM was 275 $\pm$ 14 cm (CFL $\pm$ SD, n = 41) and tagged bluefin entering the GOM ranged from 243 to 313 cm (Figure 7). The mean travel duration from the GSL to the GOM was 94 $\pm$ 41 days (SD) (n=41). Atlantic bluefin entered the GOM over an extended period from 10 November to 4 April (mean entry date = 17 January $\pm$ 42 days (SD), (n=41) and exited from 5 April to 10 June (mean exit date = 20 May $\pm$ 16 days (SD), n = 17). Both entry and exit dates were available for 17 individuals (Figure 4). The mean residency period within the GOM was 118 $\pm$ 50 days (SD) and ranged from 46 to 197 days. The peak GOM residency, measured by the number of tagged bluefin in the GOM each month, occurred during the months of April and May. All of this information is needed in the Mediterranean fisheries for bluefin tuna.

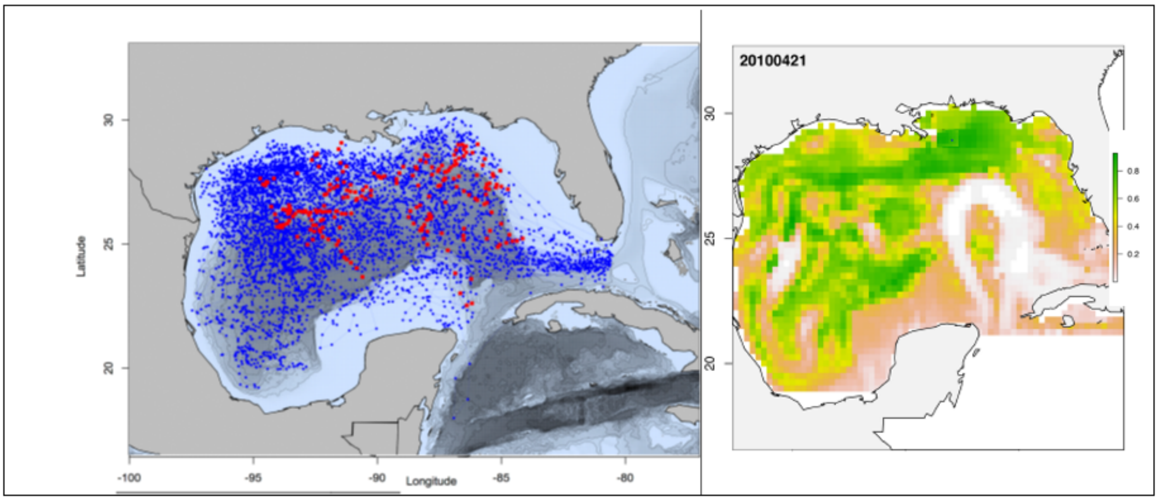

Figure 4. Tagging provides a unique data set of information on how bluefin tuna use habitat in the waters of the Gulf of Mexico. All geolocations (blue) of Atlantic bluefin tuna that visit the Gulf of Mexico from satellite archival and implantable archival tags, and red squares (areas where spawning is predicted to occur based on robust analyses of time series from archival records and coding of unique diving and thermal behaviors that only show up in consecutive days in the Gulf). Shown on the right is environmental correlates predicting spawning habitat with high suitable spawning probability habitat (in green) present in the month of April 2010, based on mapping positions, and associated error, with environmental correlates of these regions (from Hazen *et al.*, in preparation and discussed in objective 3 below).

Identifying Spawning Habitat for Bluefin in the Gulf of Mexico

Electronic tags provide detailed movements of bluefin tuna into the western Atlantic spawning grounds and evidence for reproductive philopatry of GOM fish to specific areas (Block *et al.,* 2001, Block *et al.,* 2005, Teo *et al.,* 2007a). We have recently placed implantable archival tags in the Mediterranean area (Spain, Corsica and Italy) but to date, we have few satellite tags deployed (Orange dots in Figure 6), and few tag tracks showing breeding or spawning behaviors. By deploying near Spain we hope to assure long term deployments- that help us discern where bluefin tuna are spawning and what habitats they are selecting.

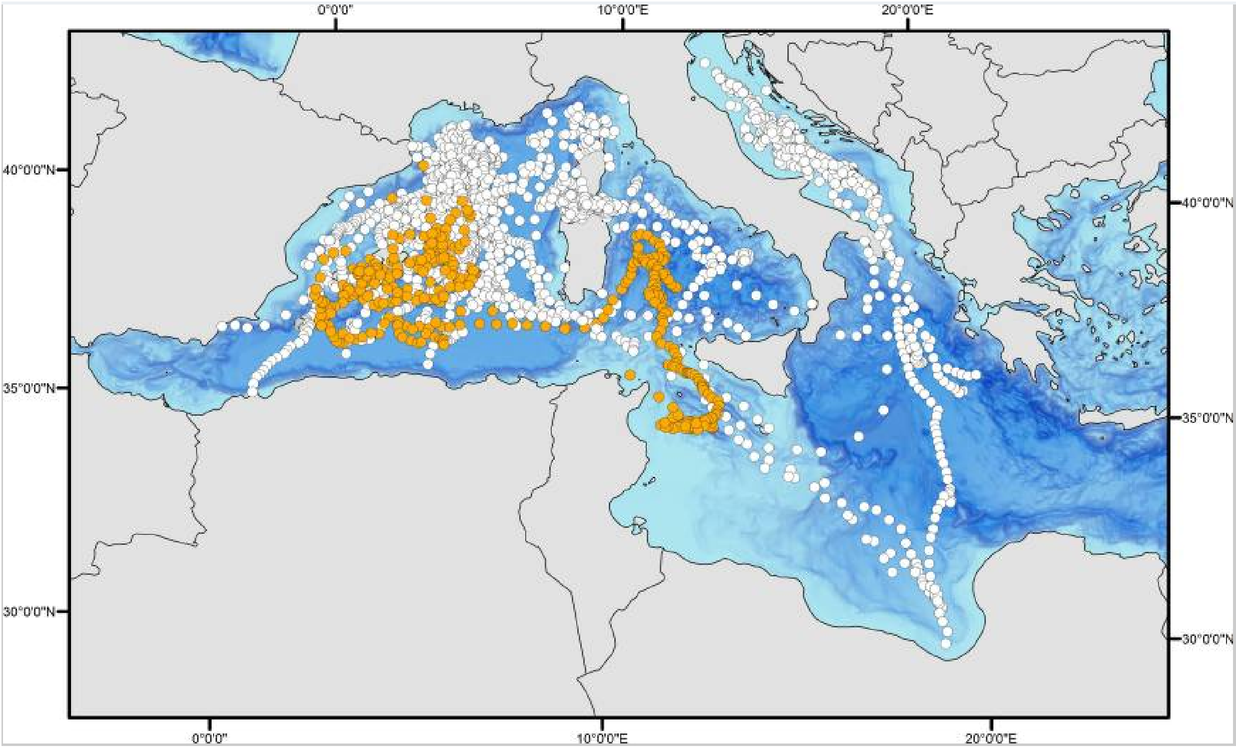

Figure 5. Electronic tag tracks (dots) of 38 bluefin tuna tagged in the Mediterranean Sea. Orange dots are tracks from pop up satellite tags, and white dots from archival tags. (From Cermeno et al. 2015)

We have developed dynamic habitat utilization models that provide information on environmental preferences of bluefin during the spawning season (Teo *et al.,* 2007a, b, Teo *et al,* 2010, Hazen, Carlisle et al. *In preparation*). These data have the potential to allow us to identify areas in the GOM areas being used by mature Atlantic bluefin tuna in relation to oceanographic conditions and newly developed closed areas (Figure 4). Mature bluefin tuna have been shown to use the western and eastern GOM slope waters for breeding along frontal zones of the Loop Current and within cyclonic features (Teo *et al.,* 2007a, b, 2010). Their habitat preferences appear to be slightly wider in environmental tolerances then the models generated for larvae (Muhling *et al.*, 2010, 2012). Bathymetry, sea surface temperature, eddy kinetic energy, sea surface height anomaly and surface chlorophyll concentration all have had significant effects on pelagic longline "Catch Per Unit Effort" and habitat use patterns of tagged breeding bluefin tuna (Teo *et al.*, 2010). We hypothesize that the optimal environmental conditions at bluefin tuna spawning sites represent a balance between the physiological requirements of tuna larvae (Muhling *et al.,* 2010, 2012) and habitat preferences of adult bluefin tuna.

We propose to analyze all Mediterranean data with the same tools developed for the Gulf of Mexico. All spawning tracks acquired previously (Cemeno et al. 2015), plus the new tracks generated by future 2016 tagging (~8-10 can be expected from these deployments), with the objectives of building a state space "switching" models (Jonsen et al., 2005) that helps to define when behaviors in the time series record change significantly along the track. We have already identify "spawning proxies" (Hazen et al., In prep), these mathematical codes highlight the unique behaviors we have identified that are characteristic in the time series records in the GOM. To date, we have identified a cluster of these "spawning proxies" that include shallow oscillating diving above the thermocline (Figure 7), presence in warmer SSTs, and when body temperature is available, higher then median internal body temperatures. In addition, tags provide more information on environmental data sets (increased opacity, decreased light extintion of the water masses coincident with presence in cyclonic eddies). In our new proposed research, we will continue to identify from location data, oceanographic conditions (extracted from environmental grids) that are correlated with these time series changes in behaviors to discern what environmental habitat shifts correlate with the behavioral shifts in archival data (see also Teo *et al.,* 2007a). To incorporate environmental conditions from satellite oceanographic inputs, we propose to use general additive mixed models (GAMMs; Block *et al.,* 2011, Hazen *et al.*, 2012) to examine the track and behavioral switching in the context of the oceanographic environment (SST, SSH, chlorophyll, geostrophic flow, thermocline depth, eddy kinetic energy, and gradients). Together this will provide predictictions for putative spawning site areas based on real track data, and actual behaviors from high resolution time series, that can be observed in relationship to the currently proposed April and May closed areas.

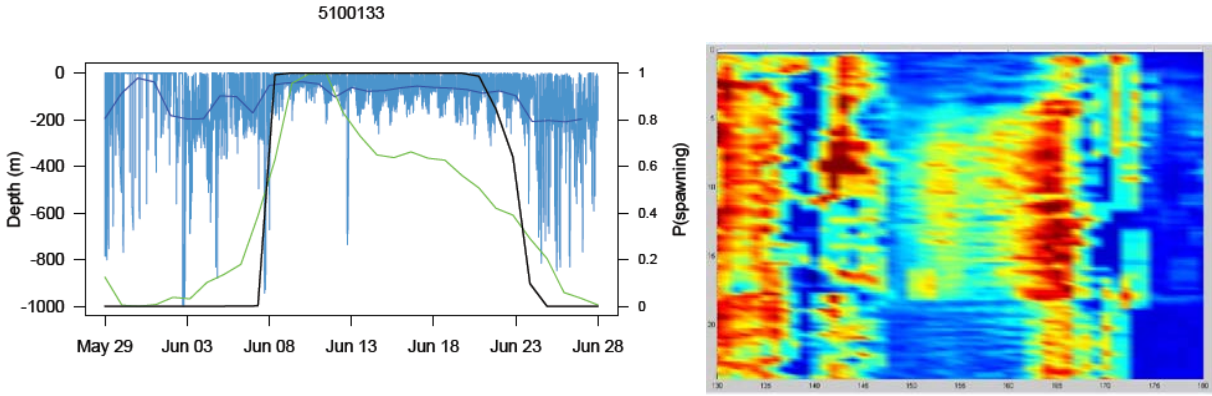

Figure 6. (**Left**) Designing algorithms that can identify specific behaviors for spawning bluefin tuna. A month of bluefin tuna diving behaviors from a recovered satellite archival PAT tag during the period of an individual bluefin tuna in the GOM. The extensive time series on the archival tags enables examining the diving behaviors (blue). Mean depth (dark blue line). From these data we can identify "switching" from one state (deep diving on May 29-June 7) to another state (shallower diel diving, June 08-June 23). Similar work can be done with temperature and other parameters recorded by the tags that provide proxies for behavioral changes. Black line provides a probability of spawning index that can be used to integrate environmental data and test hypotheses. (**Right**). Wavelet code has been developed that enables recognition of high frequency versus low frequency diving in an archival record from a bluefin tuna while retaining the time period (Julian date on x axis). So in this figure the wavelet code was developed that can recognize the particular low frequency diving that is characteristic of the spawning period in the GOM as exemplified by the archival tag record on the left- the example shows the switch to the lower frequency diving.

In the past two years, we developed a state-space framework for a statistical approach to analyze location data from archival double-tagging experiments (Figure 1-2; Jonsen *et al.*, 2007, Block *et al.*, 2011, Winship *et al.*, 2011, Wilson *et al.* 2015). This state-space framework has advantages over simple data comparisons because it acknowledges errors in all geolocation data, not just the less precise data around solstices, and it can accommodate a range of data types with different temporal resolutions without the need for data averaging, filtering or interpolation. A state-space models can be fitted to as many location data types as are available. Furthermore, because the state-space model incorporates an underlying model of animal movement, one can make appropriate inferences about true animal locations and movement while simultaneously estimating measurement errors. Thus taken together the geolocation validation and the new model output for tracks provides a measurement equation that accounts for the errors in the observed locations.

Camera Tags

To date 4 camera tags have been deployed on bluefin tuna in the North Atlantic. The tags provide spectacular new data on how bluefin tuna move in the ocean over very detailed periods- 1-7 days. The tags provide information on actual behavior while simultaneously providing underwater film of the animal moving beneath the sea. We will donate two camera tags to the project – and simultaneously attached them to pop up satellite tagged fish. This has been done in the western Atlantic.

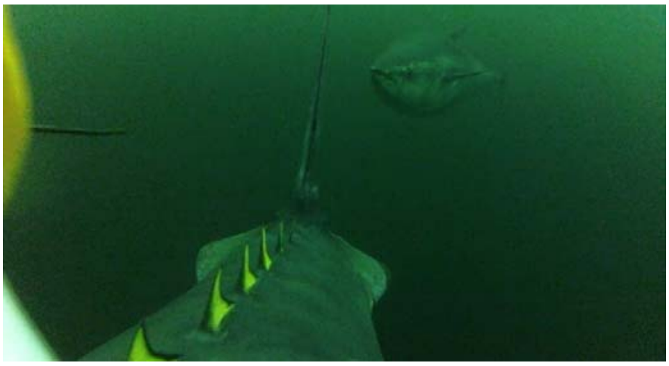

Figure 7. Camera tags pointing toward tail provide a new view of bluefin in the wild. The tags provide information on movements, tailbeat and compelling video that can be used in exhibits at the zoo.

Project Detailed Plan

Experimental Objectives:

1) We propose to deploy 8-10 mini-PAT satellite archival tags (Wildlife Computers) on mature bluefin tuna in the Mediterranean Sea waters. The electronic tags will provide vital information about the habitat utilization of mature fish in the western Mediterranean spawning ground, and improve our capacity to model habitat utilization used by these mature western fish in the GOM and North Atlantic. Tags will be set for 12 month deployments. Potentially the fish will return to the deployment location and tags can be recovered when they jettison. The tagging portion of the project occur in the first quarter of the project in the summer of 2016.

2) We propose to double-tag two bluefin tuna with 1-4 day camera tags, that we already own and have purchased. Tags will be put on the same bluefin we tag in experimental objective 1. Double tagging has been done like this before and great video has been obtained.

3) We will analyze the satellite archival tag data for location (state space model tracks) and time series data (behavioral data) to identify core bluefin tuna habitats and behaviors (e.g. transit, spawning) in the GOM. State-space modeled tracks combined with remotely sensed oceanographic data will be inputs into our habitat preference models for adult tuna in the GOM and North Atlantic. A state space switching model will be coded and used to identify the periods of the tracks where putative spawning behaviors occur (location of aggregations, shallow diving, diel behavior, oscillatory diving, higher SSTs). By integrating these behavioral and location data in the context of oceanographic variables (SST, SSH, chlorophyll, geostrophic flow, thermocline depth, eddy kinetic energy, and gradients), general additive mixed models (GAMMs) can be used to predict influences of the variable Mediterranean environment on bluefin tuna. To examine presence or absence in the new closed areas, state space location data will be used to assess the probability of individuals remaining in the protection zones during their GOM periods. This portion of the project will begin with prior data sets, involve a collaborative team and will be on going during the entire funding period. We will use new data as a validation of the models developed in the first three quarters in the last 4th quarter of the project.

Objective 1. Satellite Archival Tagging Bluefin tuna to obtain Tracks and Behavior on the Mediterranean Spawning Grounds

We propose to use Barcelona Zoo funds to purchase and deploy satellite archival tags Atlantic bluefin in the Mediterranean Sea. The goal is to obtain western Mediterranean spawning data sets on spawning bluefin tuna. To conduct this tagging we will deploy the tags in the Mediterranean recreational fishery in the months of August and September off the Spanish coast (potentially the coastal city of Roses). The procedures will be similar to how we've conducted the prior tagging for recreational fishers. Two scientists will come to Spain and work with Barcelona Zoo scientists (Pablo Cermeno) and due to weather considerations, stay two weeks to work with recreational charters, and private anglers we've come to know in Spain. The Wildlife Computers miniPAT satellite tags will be deployed with specially designed tethers and titianium darts, and programmed to remain attached for periods of 9-12 months (pop up satellite tag dates will be set for one year durations. Tags will transmit time series (recording data on depth, light and ambient temperature), and when recovered provide full archival records. Thus, miniPATs function as externally-attached archival tags, capable of collecting high-resolution time series data for the full duration of the track. Using this generation of smaller tags, it is possible to obtain oceanographic-quality profiles, diving behaviors, thermocline depth information, light extinction, chlorophyll maximum identified (Teo *et al*., 2007a, Teo *et al.*, 2009, Walli *et al.*, 2009).

The bluefin tags will pop-up automatically and provide data via satellite. In addition, the remarkable homing behavior to the bluefin means that a year post-deployment potentially post-pop up the bluefin tags can be recovered. When retention is high, and reporting rates are high, recovery is relatively easy.

Objective 2. Camera tagging bluefin to obtain high resolution behaviors on the Mediterranean Spawning Grounds

In addition to the satellite tags, we propose to double-tag the fish with a camera tag set for 2-4 days. This tag will provide the capacity for additional tracking of an individual Mediterranean bluefin tuna for a week, where high resolution video, accelerometry and behavioral data can be obtained. This technique has been regularly used in prior years in the west Atlantic, with high success. Tags are fin mounted – for short periods, then pop- up and are recovered. A satellite tag on the tags- enables recovery.Both types of electronic tags (satellite and acoustic) will be externally attached to bluefin tuna near the second dorsal fin (Wilson *et al*. 2015). We have conducted similar double-tagging experiments in prior years and with the high detection rates and reporting of both types of tags, retention.

Personnel

The tagging research requires skilled personnel in tuna handling and tagging. We will send Dr. Barbara Block and Research scientists Mr. Robbie Schallert and Aaron Carlisle. In addition, the Monterey Bay Aquarium will support a technician, Mr. Ethan Estess who will help with the tag preparations on site in Monterey, California, and potentially with deployments. We will partner with Dr. Pablo Cermeno of the Barcelona Zoo and his personnel and pass on knowledge of tuna handling and tagging. Dr. Cermeno, a former Post-doc with the TAG team. He has previously deployed tags on several expeditions with our team. Data analyses will be done by members of the Block laboratory in Pacific, Grove, Ca.

Tagging Protocol

Bluefin tuna in will be tagged with techniques that are approved by Stanford and Monterey Bay Aquariun animal care (Figure 8). The TAG team acquires all IACUC APLAC animal care approvals from Stanford University. Recreational vessels that catch bluefin tuna are used to tag and catch bluefin tuna with heavy tackle and circle hooks. We also have developed use "transfer" techniques that permit a fish from one vessel to be transferred to a tagging vessel. The likelihood of encountering bluefin tuna to tag during the short period we are permitted to tag in the region is maximized with these techniques. By working with a team of recreational fishers who have used our techniques, using several vessels simultaneously, we can maximize tagging effort.


Figure 8. TAG team members Dr. Wilson and R. Schallert tag a mature bluefin in Canada's GSl (B. Skerry, NGS photo).

All captured fish are pulled aboard the tagging vessel by slipping a specially designed titanium lip-hook through the lower jaw. The designated tagging boat has a large transom door and we use a large vinyl mat (Figure 8), that protects the animal and allows the fish to slip easily once wet onto the deck. A saltwater hose is inserted in the fish's mouth to oxygenate the gills and a wet cloth is placed over the eyes helps to keep the fish calm. The cloth is removed (above picture the cloth had just been removed as they were about to swing the mat around), and the fish is returned with the mat to the sea. TAG developed these techniques in 1996-1997 off North Carolina winter fishery, and we have had no problems employing these techniques in 2007-2013 deployments in Canada on larger fish. The titanium lip hooks are stronger, and the number of scientists and fishers required to pull a fish on board has increased, mats are larger, but overall the same methods have worked well, as evidenced by the retention and recovery of acoustic tagged fish and PAT tag reporting success. Fish are measured, sampled for genetics (*i.e.*, fin clip), tagged and released. The fish is typically out of the water for less then two minutes.

The miniPAT tags (Figure 9) are secured externally using a two-point attachment (front end titanium and back end looped with a second titanium dart). Tethers are constructed of three layers including monofilament, a Kevlar-like material covering the mono, and two layers of shrink wrap on top. The miniPAT tags log ambient temperature, pressure and light intensity. At a pre-programmed time, the tags release from the fish, float to the surface and transmit the recorded data to orbiting Argos satellites. Time series data are transmitted from the miniPATs in 5' to 15' intervals. Light curves are used to calculate sunrise and sunset, and an accurate internal clock allows mathematical estimation of longitude and latitude based on time of local noon and day length using a custom program called SST lats (Block *et al.,*2001, 2005, 2011; Wilson *et al.,*2010, Teo *et al.,*2004, Stokesbury *et al.,*2004). A state-space switching model (SSM) developed in the past two years by Dr. Ian Jonsen of Macquarie University and James Ganong in the Block laboratory (Figures 1-3; Wilson et al., 2015), based on prior SSM model work (Block *et al.,*2011, Winship *et al.,* 2012), is used to generate a probablistic track. This SSM model algorithm incorporates a bathymetry filter that actively uses the deepest daily diving behaviors of the fish, to improve the fit of latitude position for these tracks.

Recovery

To obtain full time series records archived in the tags' memory, we have been retrieving the tags once they pop up and begin communicating with the Argos satellite system. This approach has worked so well that we're now programming this into the budget, including both shiptime and personnel. Retrieval is accomplished with a prepared team ready to move as much as 50 nm offshore with a hand-held Argos satellite tag finder. Mr. Schallert would guide the vessel to the current location of the transmitting tag, which is readily available and updated by Argos, and the team reliably locates the tag with the ARGIS PTT receiver on board the boat. By programming the tag to stay on until the pop-off date (June-August 2014), and then planning for small vessel recovery program upon return to the GSL foraging grounds in the summer post tagging, success has been high in the past three years (22 recovered mini-pat tags as of 2015, 19 analyzed and 3 recent recoveries).

A B

Figure 9. A) The small size of a Wildlife Computers mini-PAT satellite archival tag (in hand) has increased retention on giant bluefin to 11-12 month deployments. B) Double tagging (satellite archival and camera tags) permits getting both instruments out on a single fish- both come off the fish leaving no marks on the fish or injury.

Objective 2. Double tagging Mediterranean Bluefin Tuna Camera Tags

We have demonstrated in the past five years that camera tags can be important tools for discerning behavior and biology of bluefin tuna. We will deploy two of these tags with rear and front facing cameras- on bluefin tuna during operations to satellite tag. These tags are applied with a pop-off technique that will enable the tag to be retrieved locally with special ARGOS locator receivers already owned by the Stanford/TAG team. Video from the tags is combined with movement and accelerometer data to better understand how bluefin move.

Objective 3. Modeling of Spawning Habitat in the Mediterranean

We propose to utilize the tag data, along with environmental habitat modeling procedures,l that will provide information on how bluefin tuna utilize the Mediterranean during the spawning season (Teo et al. 2007a, 2010, Hazen et al. 2015) to better understand how tuna use the waters of the western Mediterranean. Raw archival PAT tag data will be processed in a 3-step process. First, the light-level data will be processed using an algorithm provided by the tag manufacturer to provide longitude estimates based on the time of local noon or midnight. Then latitude estimates will be calculated by matching sea surface temperatures (SSTs) recorded by the tag with remotely sensed SSTs (Teo et al. 2004). A state-space modeling approach will then be used to refine daily position estimates into the most probable track and enabled quantifying the uncertainty associated with each daily position. For this process we will fit a Bayesian state-space model (SSM; e.g., Jonsen et al. 2005, Block et al. 2011, Winship et al. 2012, Wilson et al. 2015) to the geolocation data to regularize the location estimates in time, interpolate through small gaps due to missing observed locations, and account for errors in the light-level derived estimates of longitude and SST derived estimates of latitude (Teo et al. 2004). The model was adapted from Block et al. (2011) to account for bathymetric information that further improved the resulting location estimates. The SSM is comprised of a process model (from Jonsen et al. 2005) that assumes the first differences in locations are a correlated random walk (details of the model are in Block et al. 2011). A bathymetry mask will be applied as a prior on location estimates so that estimated tracks can not go on land or otherwise enter water shallower than that implied by the time series depth data recorded by the tag. To construct the mask, we will use the 30 arc-second resolution (approximately 1 km) Global Predicted Bathymetry dataset (v15.1; Smith & Sandwell 1997). With the archival tag records from recovered tags we can delineate geographical areas of aggregation in the Mediterranean sea and test putative behavioral proxies for linearity in the track versus aggregation behavior, in association with the putative diving records and enviornmental data that suggest putative spawning behaviors. The use of state-space switching models improves our capacity to discern along a track with statistical robustness when the behaviors are initiated (Jonsen *et al.,*2005, Block *et al.* 2011, Bailey *et al.* 2012) and to identify the regions where such behaviors take place by overlaying satellite oceanography. We hope to delineate transit, aggregation and unique diving behavioral patterns and environmental temperatures where spawning might be occurring. Full archival time series records greatly improve the development of these behavioral proxies. A major reason we are continuing the satellite archival tagging of large bluefin is to garner more of the dense time series data sets that reveal in detail how large giant bluefin behave in the GOM. Our SSM models will improve by incorporating: a) new oceanographic products as available from satellite data sets, and b) new spawning proxies. Once the new data come in we'll incorporate the 2015-2016 satellite tag records into the same working model.

Using the tracks derived from these improved SSM algorithms, general additive mixed models (GAMMs; Block *et al.*, 2011, Hazen *et al.*, 2012) will be used to create adult habitat use models as well as spawning models based on presence or absence of adults as well as spawning behaviors in the context of the oceanographic environment (SST, SSH, chlorophyll, geostrophic flow, thermocline depth, eddy kinetic energy, and gradients). The envelope of ocean conditions for habitat and behaviors defined as spawning derived from these analyses will be used to designate areas for spawning bluefin tuna in the GOM. The result of these analyses will be spatially-explicit outputs of animal presence/absence in the presence of oceanographic conditions, and associated strength of statistical comparisons between years. The final analyses outcomes will be presented as a spawning habitat model and we will predict suitable habitat for spawning in future years.

To model spawning habitat, we will use the following proxies to characterize spawning events in each track and archival record: time of entrance to a spawning ground in the Mediterranean (time stamp of moving east of Gibraltar), transit behavior to spawning locations (a linearity index of track straightness), location of potential spawning activity (identified when track linearity is lower, specific diving oscillatory behaviors unique to MED records appear (Teo *et al.*, 2007a and 2007b), SST maximums show up, mean maximum depths shoal, fish spend more time at the surface (increase in depth records of 0 m), distance between consecutive positions gets closer (as fish aggregate and move less during spawning), SST temperatures during spawning (approach constant), exiting (linearity index) and exit timing (east of 80^{o}W longitude). A few new proxies have emerged from recent analyses of the recovered GSL fish data sets that include a wavelet analysis of diving (resulting in a switch in the Mediterranean to “low frequency diving”), particularly when linearity is reduced, a reduction in time spent below the thermocline, and an increase of time spent near the suface at particular time windows. Once spawning events have been identified by using these behavioral proxies (and spawning/non-spawning dates identified), the final animal tracks and spawning locations will be overlaid with ambient ocean condition data (obtained from satellite imagery provided by the Southwest Fisheries Science Center) to help define a set of ocean conditions that are associated with spawning within a GAMM framework.

For this portion of the program, TAG matching funding will permit Dr. Aaron Carlisle and Mike Castleton from Dr. Block’s team to work with Barcelona Zoo scientists to carry out the work. Dr. Carlisle has been a post-doc in the laboratory of who will to work with the Stanford University. His expertise is in analyzing tag and environmental data and he has recently learned how to run the models.

Detailed Planning Schedule

1. Upon Notification of funding: Project tags will be ordered from Wildlife Computers (30 days to 45 days for deliver)
2. Tag will be painted with biofouling paint, and leadered with Titanium Darts upon receipts at the Stanford lab (1 week post delivery)
3. Scientific team from Stanford will fly to Barcelona and meet with Barcelona Zoo team (When project occurs-August or September)
4. Team will stay and attempt to fish, tag and release fish (August and September 2016)
5. Tags will pop off 9-12 months post tagging, and results will be processed within a month, and delivered to Barcelona Zoo and ICCAT
6. A paper will be drafted on the results, within 6 months delivered to a Peer Review Journal

List of Materials to be purchased with 13,000 Euros; $15,500 US

4 Pop Up satellite Archival Tags, Mini-Pats, Purchased from Wildlife Computers: TAG Pricing $3300 (with discount), $13,200
4 Tethers and Loop Construction for the tag Attachments with Titanium Darts (10), $500 Total
Tagging Tips, betadine swabs, and conventional tags for deployments, 10, $200 Total
Two plane tickets to Spain for researchers to join the team: $1600 Total

TAG A Giant will Match the Barcelona Zoo Contribution and these funds will be used to purchase More tags, and provide salary support

Education and Outreach

The TAG team is part of the Monterey Bay Aquarium, and has provided data and tracks to enhance displays of how tagging enables tracking of animals beneath the sea Video tags are captivating and the video can be used by the aquarium.. In addition we've created similar displays at San Jose Science museum, and the Great Ocean Hall at the Smithsonian. Barcelona Zoo can use these data for displays about these important animals in their exhibits areas.

Personnel

Dr. Barbara A. Block, Stanford Professor
Dr. Aaron Carlisle, Stanford Post-doctoral Associate
Mr. Rob Schallert, TAG A Giant, Research Associate
Mr. Ethan Estess, Research Technician, Monterey Bay Aquarium