REVISED FINAL REPORT

Deliverable #3

ICCAT GBYP 08/2017 - Biological Studies Atlantic-wide Research Programme on Bluefin Tuna (ICCAT GBYP- Phase 7)

Submitted to:

Executive Secretary of ICCAT

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To: ICCAT GBYP 08/2017 - Biological studies Atlantic-wide Research Programme on Bluefin Tuna (ICCAT GBYP- Phase 7

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NOAA Collaborators: Robert Allman, and Gary Fitzhugh, Ph.D. Panama City Laboratory, Southeast Fisheries Science Center, Panama City, FL, USA.

Closing Data Gaps in the Reproductive Biology of Atlantic Bluefin Tuna in the Northwestern Atlantic

Task Deliverables Summary

A) Completed:

Reproductive Sampling:

We submitted the LPRC gonad sample collection (~720 samples) to Mass Histology, Inc. for completion of sample preparation (cutting, mounting, and H&E staining) and slide production. In January, 2018, the laboratory service director noted that processing would be delayed beyond the usual processing period (4-6 weeks) because of the large size of the submitted archive. Once samples were processed, the service provider completed processing of the gonads and shipped the final portion of LPRC's archive slides to Drs. Gary Fitzhugh and Robert Allman of the Southeast Fisheries Science Center (SEFSC) Panama City Laboratory, FL.

LPRC processed payment to Riverside Technology, Inc., the contractor used by the Panama City SEFSC laboratory for evaluation of H&E slides, assignment of reproductive stage, proofing, and data entry.

LPRC submitted draft data tables (Tables 1-5) of descriptive information for the gonad sample archive submitted for inclusion into the NOAA archive, where the data fields will be reformatted to match that for biological samples taken by federal fisheries observers and contractors. The Panama City Laboratory will finalize LPRC's archive data records matched to processed slides, and will also be matched to ages assigned and supplied by Dr. Walter Golet. In the future, LPRC may also submit additional stained and evaluated slides to the SEFSC if Robert Allman determines that fish LPRC and federal observers jointly sampled in the Gulf of Mexico are not included in their existing archive.

It is important to note that the LPRC gonad archive for the years before 2012 were collected mainly for studies of bluefin reproduction and energetics conducted for PhD projects, and were not collected specifically for NOAA or ICCAT. Data fields, at minimum, include size by length or mass, and location of catch, if known, in the Gulf of Maine. Fish sampled in Canada and the US are included in our draft data files. All samples were obtained from only two areas in the NW Atlantic, sampling strata 35 and 34e. There are no other sources of representative biological samples for these regions for Atlantic bluefin tuna. Historical gonad and other biological samples taken by Chief Scientist Peter C. Wilson during the US Bureau of Commercial Fisheries exploratory longline cruises for tunas in the NW Atlantic, originally stored at the SEFSC in Miami, have been lost for decades.

Submission of all of LPRC's gonad archive and associated information will complete the deliverables for the gonad archive portion of our contract. Final formatted data tables will be submitted by NMFS and the files included here are meant to represent

the nature of the archive, not its final representation and format. Evaluation of results from the assignment of reproductive stage by Riverside Technology, Inc. will be completed in the future, hopefully in 2018, as a collaboration between LPRC and the Panama City SEFSC investigators, Drs. Robert Allman and Gary Fitzhugh. The objective will be to examine reproductive stage in relation to month, year, fish size, and oceanographic region, and to compare results with previous work conducted by our lab for large, commercially landed ABFT sampled in 2000-2002 in the Gulf of Maine[1] .

B. Endocrine Sampling:
Our objectives for endocrine sampling were to measure pituitary gonadotropins in ABFT of two size classes, 40-55 inches (CFL), and > 73 inches CFL, by obtaining brain-liver-gonad tissue of ABFT (20 of each size class) aboard commercial and recreational fishing vessels, in Canada and the US, respectively. These size classes correspond to strata code 34, 34d, 34e & 35 listed in *ICCAT_GBYP_Sampling_Strata_2017.xlsx*. We could not meet these objectives. As described in Progress Report #2, and our earlier final Draft Report, this sampling could not be accomplished mainly because of the lack of sufficient lead time and personnel to organize the US recreational sampling of smaller fish. For the adult fish, we did not have access to Canadian scientific sampling quota sufficient to enable brain/endocrine sampling in early September off SW Nova Scotia. We attempted sampling but only had access to a single female ABFT landed during our fishing trip. The head cut for a second fish made available to us by an adjacent fishing vessel was incorrectly made and the brain was not present. Consequently, no endocrine samples were shipped for analysis by Dr. Hanna Rosenfeld in 2017.

C: Participation in the GBYP Planning Workshop on Reproduction. Drs. Molly Lutcavage and Tim Lam were invited to participate in the 13-14 Feb, 2018 meeting in Madrid, and prepared a presentation and background materials. Dr. Tim Lam attended the meeting and represented the LPRC's research on reproductive biology of ABFT in the NW Atlantic. Lam and Lutcavage reviewed the resulting report and made comments and recommendations to the GBYP committee.

D. Map sampling area shown below (Figure 1).

E. Data Tables 1-5 attached as Addenda

[1] Goldstein, J., Heppell, S., Cooper, A., Brault, S., and M. Lutcavage. 2007. Variability in the reproductive status and body condition of Atlantic bluefin tuna in the Gulf of Maine, 2000-2002. Marine Biology 151:2063-2075.

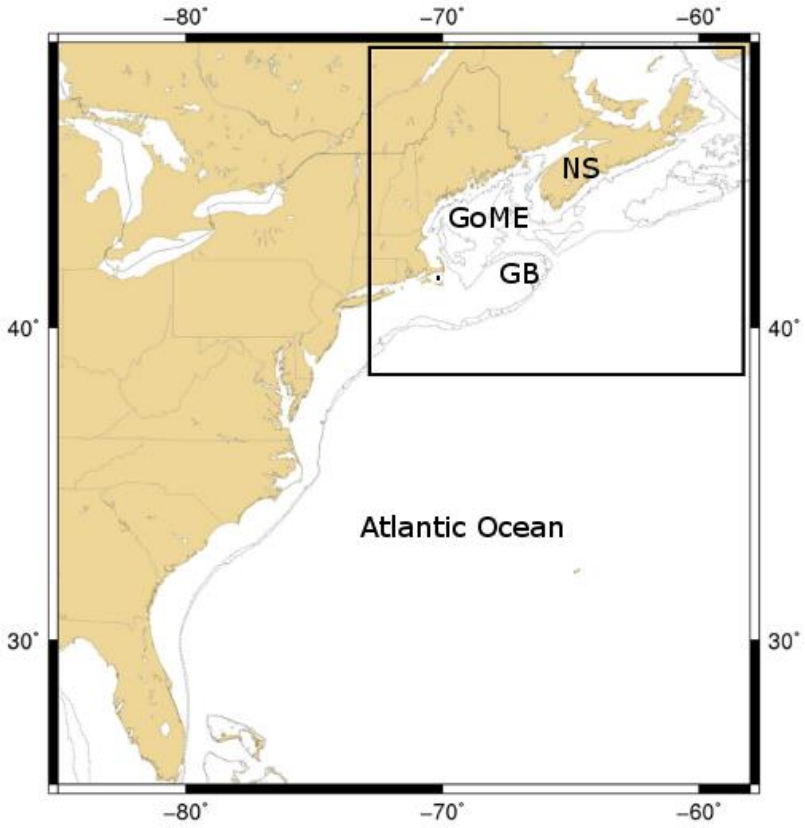

Figure 1. ICCAT strata areas 34 and 35 where Atlantic bluefin tuna reproductive samples were obtained for the LPRC gonad archive from the US commercial and recreational fisheries.

Further Considerations

Sexual maturity and reproduction is a key life history parameter that needs to be accurately portrayed in biological assumptions used in stock assessments, and is an important aspect of the resiliency of the population. Surprisingly, a complete understanding of reproduction for Atlantic bluefin tuna (ABFT) in the NW Atlantic, as well as its spawning habits, have not been achieved (Heinisch et al., 2016). The SCRS of ICCAT has identified this top research priority due to its substantial effect on estimates of stock productivity, calculation of spawning stock biomass (Richardson et al., 2016), steepness (Mangel et al., 2010; Simon et al., 2012) and assessment and management actions that determine sustainable catches for the commercial and recreational tuna fisheries (Pons et al., 2017).

Another major challenge to bluefin reproduction studies is that most research has been conducted on known spawning grounds during the spawning season. As noted in a recent review of ABFT reproduction (Heinisch et al., 2016) a root cause of the lack of scientific resolution of maturity and spawning grounds of western ABFT, since Ray Baglin's work (e.g., 1982), for example, is the challenge of observing and sampling ABFT across its entire range. Consequently, reproductive sampling in the NW Atlantic has been limited. Its physiology dictates that spawning fish must be sampled within 24-48 h of a spawning event, because post ovulatory follicles (POVs), the hallmark of spawning, are reabsorbed in warm-bodied ABFT within that time, and remnants of old POVs are not found (e.g., Hunter, 1985; Goldstein et al., 2007). Similarly, very few ABFT have been analyzed for reproductive status in the pelagic areas where larvae have been found since the US Bureau of Commercial Fisheries exploratory longline cruises in the 1950's- 1960's. Logistics and management measures that restrict retention of ABFT by the US pelagic longline fleet (PLL) have also limited opportunities for reproductive sampling.

Since 2009, the SEFSC Panama City Lab has archived several hundred ABFT reproductive samples obtained by the Observer Program and by an outside contractor, and LPRC holds over 720 gonad samples, but until 2017, they were not processed and analyzed, nor integrated with existing information from our earlier studies. Both the SEFSC and LPRC archives have gaps in offshore samples and smaller size classes of ABFT. A major goal of this research was to complete processing and H&E staining for LPRC's gonad archive, and to combine it with Panama City's gonad archive for a comprehensive record of fish sampled in the NW Atlantic. Because of the short contract period and the large size of the archive, we knew there would not be sufficient time for the slides to be evaluated and assigned reproductive stage by Panama City's research technician. However, the scientific goal of the research project is to eventually have a comprehensive "atlas" of (histological) reproductive status by size, assigned age, year and sampling location.

Recent hormonal analyses and endocrine research have resolved sexual maturity in ABFT sampled across NW Atlantic foraging grounds, confirming similarity to Eastern ABFT (3-5 y, Corriero et al., 2005;Heinisch et al., 2014). This is consistent with similar growth rates, trophic relationships, and higher than expected rates of mixing (Heinisch et al.2014). In addition, fecundity and spawning characteristics were found to be mostly similar between the known spawning areas in the Mediterranean Sea and northern Gulf of Mexico (Knapp et al., 2014).

In contrast, no experimental, physiological or biological evidence has been presented to date that confirms delayed or late maturity for western ABFT, even though it was the prevailing US management paradigm (e.g., Diaz and Turner, 2007), with some recommending that sexual maturity may be delayed to even older size classes, above 10-12 y based on size of ABFT bycatch catch sizes in the Gulf of Mexico (Diaz, 2011). Although some bluefin experts questioned the long standing assumption and proposed broader spawning areas (e.g., Takeuchi et al., 1999; 2008), the challenged maturity ogive and exclusive GOMEX spawning stood for western stock assessments. What has not been seriously addressed by the SCRS is the assumptions of vastly different ages of maturity and lack of convergence in bluefin biology and life styles between East and West Atlantic, while recognizing some phenotypic variability and selective fishing, violated life history and selection principles operating on most vertebrates and fish species (Roff, 1992; Law, 2000; Reznick et al., 2002; Kawabata et al., 2015). There has been little focus on how such differences could be explained, in terms of evolutionary or ecological principles of life history (Fonteneau, 2009; Heinisch et al., 2016).

A life history model completed by Chapman et al. (2011) using empirical information obtained from field and lab studies predicted that smaller, younger ABFT should spawn closer to their foraging grounds and at different times than older, larger fish. This was first proposed by Frank Mather (Mather et al., historical document, 1995), and supported by historic sampling by the late Peter C. Wilson, chief scientist of early US exploratory longline cruises. Historical reproduction findings and biological predictions were eventually confirmed and supported by the presence of larvae (Richardson et al, 2016), and consistent results from year-long migration paths of adult ABFT (e.g., Galuardi et al., 2010) and for younger individuals likely to be spawning in warm water masses of the Bahamas and other areas (Galuardi and Lutcavage, 2012; Knapp et al., in revision). Further confirming their inter-annual occurrence, additional bluefin larvae have been collected each year on the Slope Sea from 2014-2017 (Dr. David Richardson, personal comm.).

In Goldstein et al. (2007), histological analysis confirmed differential reproductive profiles for fish sampled from commercial landings, and suggested the possibility of recent spawning near the Gulf of Maine (GOM) (modification of Fig. 4 in Goldstein et al., 2007, provided). Similarly, fish sampled in Sept. and October off SW Nova Scotia had hormonal profiles indicating spawning in late summer, different from similar sized ABFT sampled in the GOM, but spawning could not be confirmed. There is now a clear research need to more extensively investigate sexually mature ABFT from recreational and commercial fisheries in order to determine their reproductive status and fraction that might spawn in the NW Atlantic. These direct physiological investigations, comparable to the many comprehensive biological studies conducted in the Mediterranean Sea, e.g., see review in Piccinetti et al. (2013) can help guide location of broader larval surveys (and international sampling), and are needed to map potential ABFT spawning grounds in the western Atlantic. They are also needed to establish a realistic understanding of ABFT reproduction and spawning in the face of changing climate and fisheries.

As reviewed in Heinisch et al. (2016) Rosenfeld and colleagues recently developed endocrine and micro-molecular techniques enable measurement of key hormones governing the brain-pituitary-gonad (BPG) axis and sexual maturation, and can now fully elucidate unresolved issues that existed due to inadequate sampling and low-resolution investigative tools. Investigating endocrine factors that govern sexual maturation and reproduction, coupled with conventional

gonad histology, will help define the life history parameters of a mixed ABFT population sampled on foraging and fishing grounds, and not just known spawning grounds.

Richardson et al.'s work at the NOAA Northeast Fisheries Science Center (NEFSC) continues to verify ABFT larvae in the limited areas of tows from non-directed surveys after 2013. Genetic work is also underway. It is important to include cutting edge science in the SCRS discussions and planning, rather than relegating empirical evidence for spawning in the field as a mere hypothesis or anomaly. This was emphasized in the 2016 Independent Performance Review of ICCAT (www.iccat.int/Documents/Other/0-2nd_PERFORMANCE_REVIEW_TRI.pdf):

> *"The SCRS has been slow to incorporating new independent scientific research, e.g. new spawning areas for bluefin tuna in the western Atlantic and the similarity of growth and maturity schedules for the two management units for bluefin tuna. The Panel understands that maintaining consistency from one assessment to the next helps the credibility of the assessments, but there comes a point when the evidence is such that the new scientific information must be adopted. The Panel considers that adopting new scientific findings should be based on the merits of the science, not on the potential effects on the assessment results or credibility.*

Pending additional interest and support, an effort could be made to obtain scientific quota from DFO in 2018 so that multiple fish can be landed and sampled in late August or early September, when the commercial fishery gets underway. ABFT hormonal status at that time is especially important to document the possibility of summer spawning consistent with the presence of bluefin larvae sampled in the northern areas of the Slope Sea by our collaborators (Richardson et al., 2016). Sampling in the US should also be conducted but additional trained personnel would need to accompany fishing vessels in order to accomplish endocrine sampling.

This new effort to establish a comprehensive ABFT reproductive collection, including brain, pituitary and gonad tissues, from eastern and western stocks, remains a priority. Other tuna RMFOs such as the SPC routinely include gonad samples in annual biological sampling and tissue banking programs to support stock assessment (http://www.spc.int/ofp/PacificSpecimenBank/Home/About). The biological profiling of the combined LPRC and NMFS (SEFSC Panama City Laboratory) reproductive archives will establish broad reproductive coverage of ABFT across the NW Atlantic and will help inform planning of future, combined surveys for larvae, biological (repro) sampling, and when possible, endocrine studies.

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