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**CLOSE-KIN MARK RECAPTURE AS A TOOL FOR ESTIMATION OF SPAWNING BIOMASS IN  
PACIFIC BLUEFIN TUNA: SAMPLING DESIGN AND SAMPLING PLAN**

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# Close-kin Mark Recapture as a Tool for Estimation of Spawning Biomass in Pacific Bluefin Tuna: Sampling Design and Sampling Plan

Outcomes from a Workshop<sup>1</sup> on Developing CKMR techniques for Pacific Bluefin  
NOAA National Marine Fisheries Service  
Southwest Fisheries Science Center  
May 27-29, 2015

## 1.0 Introduction

Pacific bluefin tuna, *Thunnus orientalis* (PBF) is an iconic species that is highly sought after for the quality of its flesh and its attributes as a fighting fish for sport. Exceptionally high prices and a trans-Pacific migratory pattern make PBF a highly targeted species at almost all life stages and regions of the Pacific. Almost all types of fishing gear are used to harvest PBF resulting in a rich tapestry of multi-national, fishery-dependent data that demands an international approach to management. Changes in targeting as a result of depletion as well as changes in catchability due to range shifts in response to climate variation may limit the effectiveness of relative abundance indices derived from fishery-dependent data (CPUE) as the only source of stock abundance information. Better estimates of absolute spawning stock biomass (SSB) are needed. Measures based on spawner output are difficult as are aerial or acoustic surveys. Most analysts rely on mark-recapture approaches, but well designed conventional tagging studies are problematic due to: high costs; inadequate sample designs and uncertainties associated with post-tagging survival, tag shedding and tag reporting rates. Faced with a similar situation for Southern Bluefin Tuna (SBT), scientists at CSIRO exploited recent advances in genetic parentage markers, high-throughput analytical methods, and life-history specific population modeling, to develop a quasi fishery-independent tagging approach that estimates spawning stock biomass based on the likelihood of detecting parent offspring pairs (POPs) in a sample of fisheries landings. While this approach requires the same attention to sampling design as any abundance estimation technique, it solves many of the problems associated with conventional tagging and can be accomplished using only fish that are taken during the course of normal fishing operations. A workshop was held at the Southwest Fisheries Science Center on May 27-29, 2015 to accomplish three goals: 1. Evaluate the theory and promise of Close Kin Mark Recapture (CKMR) population estimation; 2. Review the known and unknown aspects of PBF life history that could influence sampling design; and 3. Develop a sampling design and sampling program that would build on: currently monitored fisheries; existing fisheries sampling programs; current modeling approaches and the existing management structure of national and international fisheries organizations.

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This proposal details a sampling design and a sampling plan for PBF-CKMR research designed to produce a preliminary estimate in three years and a more precise estimate in five years of SSB. The overall plan has three components: biological sampling; high throughput genetic screening; and population modeling. This document focuses on the biological sampling required based on expectations of stock size derived from the 2014 PBF stock assessment and known aspects of PBF life history. The contents of the report and the proposed next steps should be considered as a possible way forward. We encourage continued discussion and refinement of ideas. The workshop agenda and the list of attendees are appended at the end of this document.

## **1.1 Background and Need for Study**

PBF consists of a single, Pacific-wide stock that is managed jointly by the Western and Central Pacific Fisheries Commission (WCPFC) and the Inter-American Tropical Tuna Commission (IATTC). The scientific basis for management is provided by the International Scientific Committee for Tuna and Tuna-like Species (ISC). Pacific Bluefin tuna have been harvested as a commodity for a least the last century and landings have been recorded as early as 1804 in Japan and in the early 1900's in the United States. While reported landings have fluctuated greatly since records were kept in earnest (1952; peak of 40,383 t in 1956, trough 8,643 tons in 1991), PBF represents a lucrative and important resource across the North Pacific Ocean. Total landings, size compositions, and relative indices of abundance were used to inform total removal from the corresponding size/age of fish caught and trend of the abundance in the assessment (ISC stock assessment reference). Management reference points have not been formally adopted but recent stock assessment (2014) suggests that PBF is overfished and has experienced overfishing based on a suite of reference points. Additionally, recruitment in 2012 was the 8<sup>th</sup> lowest recruitment estimated in 61 years and the standing stock (2012) was estimated to 3-4% of pre-exploitation levels. Given the low stock level and limitation of relative abundance indices, it is critical for us to be able to monitor what spawning stock biomass remains in order to properly manage this impacted resource.

## **1.2 PBF Life History as a Determinant of CKMR Sampling Design**

An ideal CKMR sampling design would have random samples of uniformly mixed and known aged individuals of both reproductively mature and juvenile groups. Alternatively, sources of bias must be understood and accounted for in the sampling design. Workshop participants developed a list of life history properties that were deemed critical to fully implementing a CKMR study of PBF. It was felt that some of these topics could be determined by consulting experts and published literature, while other topics such as determining birth location by otolith microchemistry signatures may require new research done in conjunction with the CKMR study. Age at maturity, size-dependent reproductive success, spawning duration, population structure and juvenile migration rates between the western and eastern Pacific feeding grounds were all important life-history considerations for developing a CKMR sampling design. While a complete life-history model is as yet unavailable, there are key pieces of information that are known which allow many assumptions of CKMK to be met.

### **1.2.1 Age and Growth**

Studies of otolith aging and other aging techniques have been reviewed extensively (e.g., Shimose, et al., 2008; 2009; Shimose and Takeuchi, 2012). These studies indicate that individuals may live in excess of 20 years and reach a maximum size of ~250cm or larger TL. Reproductive maturity begins around age 3 or about 100 cm TL. Growth rates in PBF are variable and it remains unclear if size or age determines the time of first spawning. For purposes of assessments, all fish over 5 years old are considered mature (S. Teo, pers. comm.).

### **1.2.2 Reproductive output**

Although the basic spawner-recruit relationship is often weak in high fecundity species such as tuna species, understanding reproductive output is important for the success of a CKMR study since the likelihood of identifying a parent-offspring pair depends on knowing the reproductive contributions of smaller newly mature animals as well as larger older adults. Spawning fraction, spawning frequency and spawning seasonal duration are also important and have been studied (Ashida et al. 2015). Batch fecundity for PBF has been estimated at  $F = 3.2393 \times 10^5 \times L - 5.2057 \times 10^7$  (where F = fecundity and L = fork length; Chen, et al., 2006). Since spawning output and the potential for POPs is also a function of the number of years a fish remains in the spawning population prior to capture by the fishery, the age of the fish at time of capture is important as is the need to take otoliths for aging as well as a tissue sample for CKMR genotyping.

### **1.2.3 Spawning sites and stock structure**

Adult PBF are iteroparous spawners, and spawning grounds for PBF are currently understood to occur in the western North Pacific Ocean in two discreet areas. In what is considered the main, southern spawning grounds, spawning commences in April near the Ryukyu Islands and off eastern Chinese Taipei largely in the Pacific Ocean (i.e., outside of the East China Sea) (Nishikawa et al. 1985; Kitagawa et al. 2010). Spawning generally progresses from southwest to northeast along the archipelago linking Taiwan and southern Japan. A secondary, northern, spawning area is used from July to August in the Sea of Japan (Yonemori, 1989; Abe et al. 2014). Most individuals (80%) are reproductively capable at age 3 and ~30kg in the northern spawning grounds (Sea of Japan; Tanaka, 2006). In contrast PBF sampled in the southern spawning grounds are larger (60kg and >150cm fork length corresponding to 5 years old; Tanaka, 2006).

### **1.2.4 Distribution and Movements**

PBF are largely concentrated in sub-tropical and temperate latitudes from 20°N to 40°N, however they are occasionally encountered in tropical waters and in the southern hemisphere. Patterns in movements of age 0-1 fish are variable inter-annually, however they tend to move northward along the coasts of Japan and Korea during summer months, and southward in the winter (Inagake, et al., 2001; Itoh, et al., 2003; Kitagawa, et al., 2004; Yoon et al., 2012).

An unknown proportion of juveniles spawned in the western Pacific migrate to the eastern Pacific (the “trans-Pacific migration of Bayliff, et al., 1991) where they reside for ~3 years

before returning to the spawning grounds (Inagake et al., 2001). This migration has been suggested to be driven by inter-annual fluctuations in the abundance of PBF preferred food sources in the western Pacific (Polovina, 1996), however this has yet to be quantified. While in the eastern Pacific, movements of PBF are somewhat predictable. In the spring, PBF are resident off the southern coast of Baja California. As the water warms, PBF in the eastern Pacific move northwest into the southern California bight in summer, and by fall are off of central California (Domeier, et al., 2005; Kitagawa, et al., 2007; Boustany, et al., 2010)

Following a period of ~3-4 years, PBF move westward presumably for purposes of spawning as no spawning grounds have been observed outside of the western Pacific. This westward migration has been observed from December to March as PBF begin their southward migration along the coast of California (Boustany et al., 2010). Of the tagged fish that have been observed on the westward migration, many have shown temporary residency at mid-Pacific ocean ridges until eventually completing their crossing to Japanese waters (Block et al., 2003).

Mature adults in the western Pacific generally disperse north and east to feeding grounds after spawning, although a small proportion of fish move to a relatively small area in the western South Pacific, although these movements are not well understood (Itoh, 2006; Shimose and Farley, 2015).

## **2.0 Objectives**

1. To implement phase one (sampling design and sampling plan) of a fishery independent estimation of PBF-SSB using a Close-Kin Mark Recapture approach.
2. To develop an outline of an overall research plan and organizational structure and identify points of contact for the three parts of a successful CKMR project: sampling, genetic analysis and population modeling.

## **3.0 Methods**

The following sections describe CKMR and a potentially viable sampling strategy to acquire sufficient data for analysis. This strategy was largely developed at the CKMR workshop with representatives from Korea, Japan, Taiwan, Mexico, Australia, and U.S.A.

### **3.1 Overview of Close-kin Genetic Tagging**

Close-kin genetic tagging is a new method which makes use of the rapidly advancing field of genetic research. The overarching goal of the technique is to take advantage of heritable genetic information that can be collected from each and every individual sampled and use this to obtain an estimate of the spawning stock biomass for use in assessment models. The “ultimate” data that are used in the estimation process are parent-offspring-pairs, or POPS. The basic idea is that each juvenile “tags” its two parents, so the number of tags found (via pairwise comparisons) and their pattern in time can be used similarly to conventional mark-recapture.

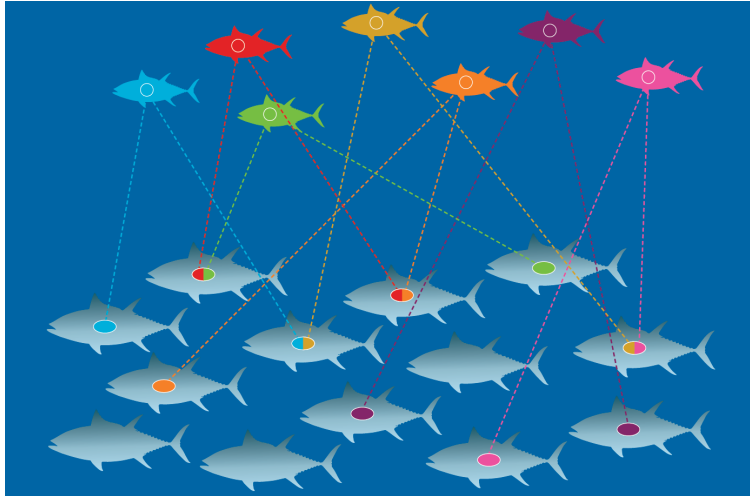


Figure 1. Schematic of parent-offspring pair (POP) relationships. Juveniles are shown in various colors at the top, and lines to larger fish represent parent-offspring connections.

In most animal species on the planet, an individual has two parents, each which contribute to that individual's genetic makeup. Typically, an individual will have two copies (alleles) for each gene in its genome, one from its father, and one from its mother. If enough alleles for enough genes are examined, it is possible to ascertain with a high degree of confidence if two individuals represent a parent-offspring-pair (POP).

If population numbers are high, the probability of finding a POP is low; chance in sampling from a large population does not favor finding POPs. If population numbers are low, the reverse is true: one is more likely to sample a POP when there are not many individuals from which to choose. Thus, the expected number of POPs in a sample is inversely proportional to population size.

### **3.2 Sample Collection Method**

Genetic information for CKMR is obtained through the extraction of DNA from tissues sampled from individual fish. These tissues can be in any form, and fin clips have proven to be an effective and efficient tissue type. Due to the nature of the PBF fishery, large numbers of individuals are routinely sampled for other programs including dockside and shipboard monitoring of size and landings data. Our proposal would add a simple, inexpensive, and time efficient addition to most sampling protocols already in place.



Figure 2. Dockside sampling for CKMR. 1. Removal of small piece of fin, 2. Place in ethanol, 3. Place in numbered vial, 4. Record total length, date, location and all other relevant biological information if available (e.g. sex, reproductive condition, if otoliths were obtained).

A major benefit of the active fisheries for PBF is that sampling for CKMR can not only be accomplished over a broad geographic area, but also among a broad range of age classes with minimal cost added to already existing monitoring programs. Fourteen major PBF fleets have been identified by ISC based on location, fishing gear type, and age composition of landings. With modest contributions from a subset of these fisheries, phase one sampling can be accomplished in a reasonable time frame with sufficient samples to ensure the identification of POPs (see Appendix I for detailed rationale for sampling discussed below).

### 3.3 Sample Collections by Fishery

There are three target groups of juveniles that should be sampled: 1. East Pacific 1-3 years old, 2. West Pacific 1-3 years old, and 3. West Pacific age-0. Based on the conditions described in Appendix I and the current ISC PBF assessment, the following number of samples per area/fleet should be as follows (and shown in Figure 2):



1. East Pacific (Mexico-F12, USA-F13) – 1300 individuals from 1-3 years old.
2. West Pacific (Japan-F5, Japan and Korea-F2) – 1300 individuals from 1-3 years old.
3. West Pacific (Japan-F5) – 1300 individuals from age class zero.
4. West Pacific (Taiwan-F11) – 740 individuals  $\geq 4$  years old.
5. West Pacific (Japan-F1) – 1480 individuals  $\geq 4$  years old.
6. Sea of Japan (Japan-F3) – 1680 individuals  $\geq 4$  years old.

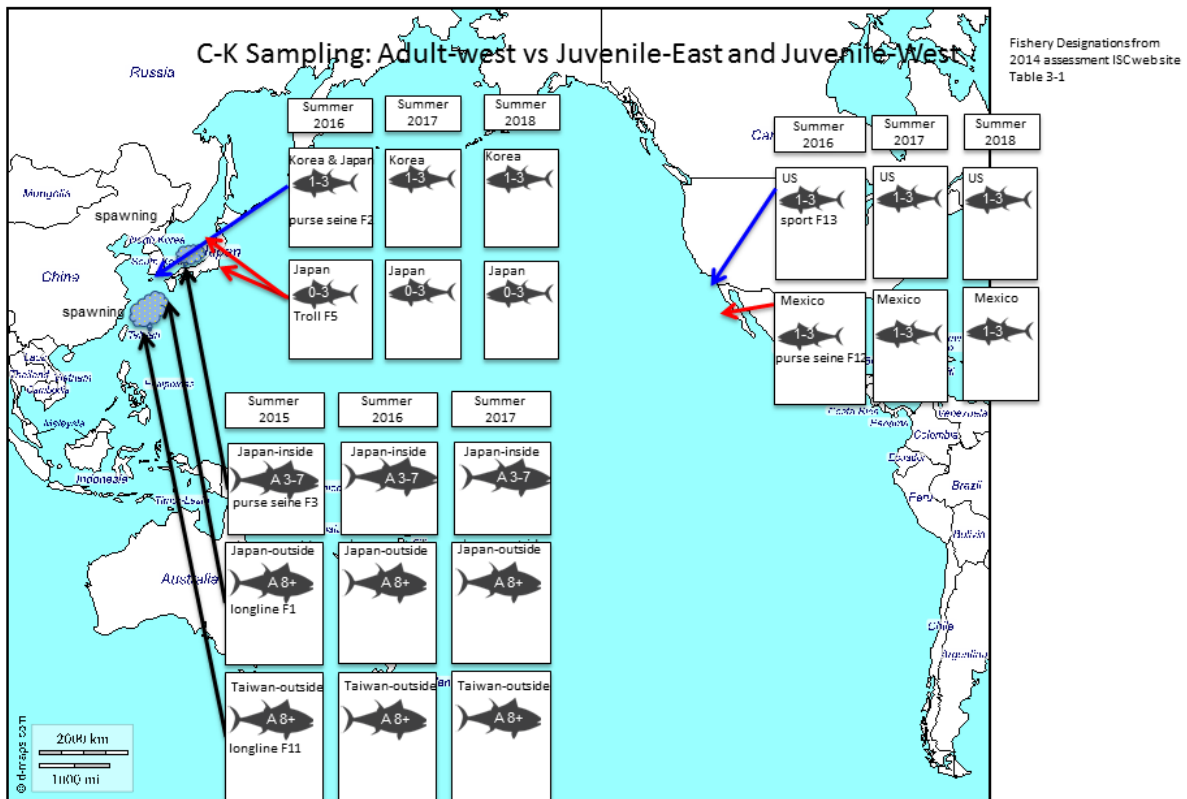


Figure 3. Sampling of Pacific Bluefin tuna by fishery for collaborative Close Kin Mark Recapture study.

### 3.4 Outline of Research Plan, Organizational Structure and Time Line

The CKMR approach can be cost effective if existing port sampling infrastructure of national fisheries agencies is leveraged. However, the required samples and the required mix of juveniles and adults from different spawning grounds and juvenile habitats must be sampled within the proper year. Therefore coordination is key. The ISC and the member nations provide an excellent



structure to make this possible. If sampling objectives and sampling opportunities are clearly defined the program is likely to succeed.

A potential organizational structure is shown in Figure 4. The three components of the proposed research organization: sampling; genetics and modeling are not temporally linear and it was a consensus of the Workshop that the Modeling Group should be highly involved from the beginning in developing the sampling design and in monitoring preliminary outcomes. The Genetics Group need not be exclusive to PBF and there is a strong argument to share costs for marker development across all countries and RFMO's considering genetic tagging approaches for the three species of bluefin tuna. There will be significant data management needs for archiving tissues and monitoring DNA extractions, marker detection and trouble-shooting, and searching for potential POPs. A cost per sample of \$30 was used for SBT, but the rapid progression of high throughput genotyping techniques suggests that costs will continue to drop precipitously after marker development is completed and routine screening begins.

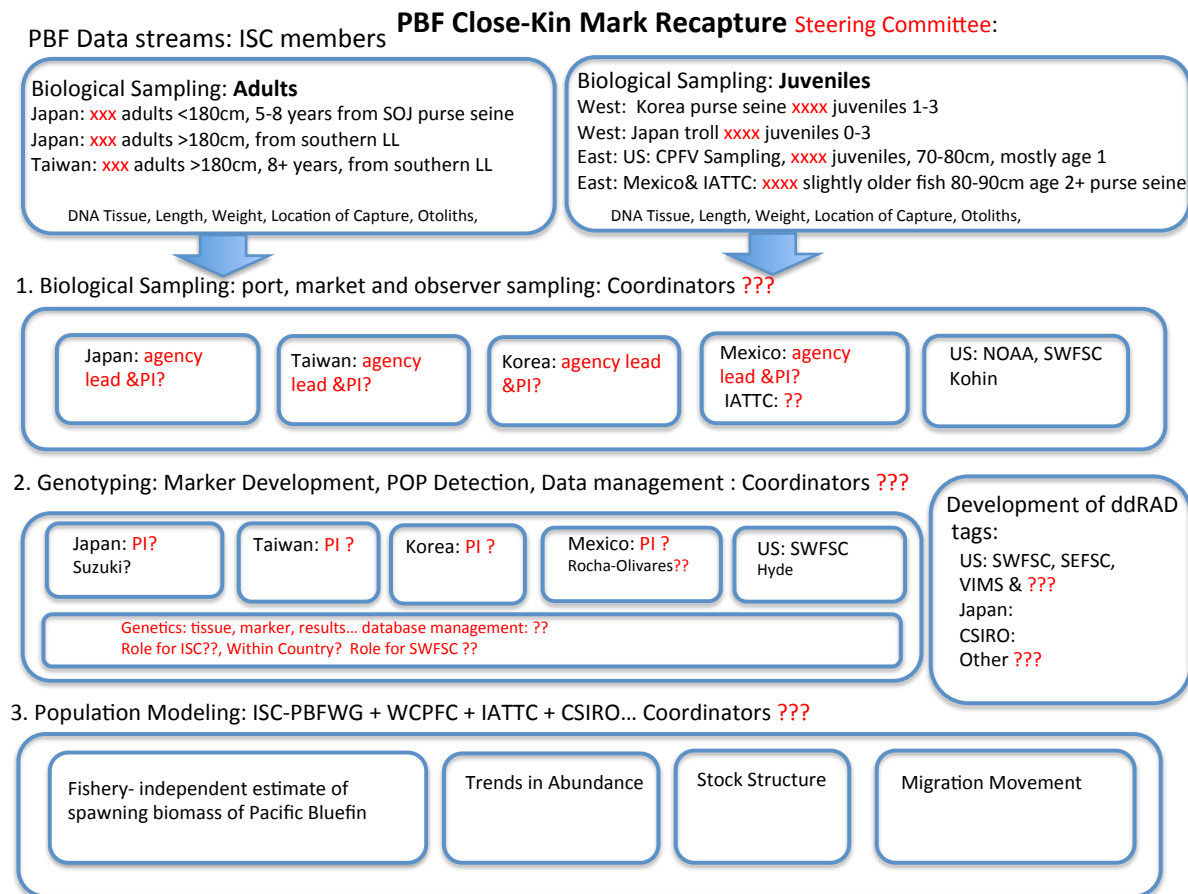


Figure 4. Proposed Organizational Structure

### **3.5 Supporting Research**

#### **3.5.1 Otolith Aging**

Otolith aging techniques are reasonably well studied (see 1.2.1). It is considered very useful if not imperative to know the age of adults as well as size as a proxy for age. This might be relaxed in later sampling years if size is shown to be a reasonable substitute for age. In the beginning it should be a goal to collect otoliths from every sample for CKMR. A logical way to reduce subsequent work load would be to age only those individuals that appear in a POP comparison. Damage to the specimen pre-sale is a concern for the best quality adult fishes. The aging manual of Shimose and Ishihara (2015) illustrates three protocols for obtaining otoliths either at the point of sale or after the fish is sold.

#### **3.5.2 Otolith Microchemistry**

Otolith microchemistry is an emerging field of study that seeks to determine origins and residence based on the capacity for calcified tissue to incorporate the unique signatures of the source waters surrounding the growing fish (Campana 1999). The annual ring deposition of otoliths allows the elemental signatures in an annulus to be tied to a given year of life and the core can indicate birth location. Fish born in the more oceanic southern spawning grounds surrounding the Ryuku Islands should be able to be separated from those spawned in the more coastal waters of the Sea of Japan spawning grounds that are subject to terrestrial riverine inputs. Rooker et al (2001) demonstrated the potential of the technique but more needs to be done.

### **4.0 Expected Results**

The sampling plan outlined in this proposal is intentionally robust to enable background research and allow a deeper analytical approach if results reveal that the basic assumptions of the sampling design are not as anticipated. Because of the rich history of research surrounding PBF it is unlikely that basic assumptions are greatly in error but more can always be learned. It is highly likely that future sampling efforts can be reduced once more certainty is gained on the presence or absence of stock structure and differential migration patterns. As noted in the appendix the addition of age 0 fish is not necessary but is anticipated to shed light on recruitment patterns.

Careful attention to the collection of otoliths in conjunction with CKMR can provide valuable information on the contributions of different spawning seasons and spawning locations as well as the relative contributions of younger and older spawners.

It is expected that within three years there will be sufficient POPs to provide a preliminary estimate of SSB. By the end of five years there should be sufficient information on estimates of precision and accuracy to allow full incorporation into the assessment process.

At the end of five years costs per sample and the number of samples needed should be greatly reduced since there will be a large pool of genotyped juveniles. The decision will need to be made to continue the study as a time series to chart the recovery of the stock.

## 5.0 References

- Ashida, H., Suzuki, N., Tanabe, T., Suzuki, N., Aonuma, Y. 2015. Reproductive condition, batch fecundity, and spawning fraction of large Pacific bluefin tuna *Thunnus orientalis* landed at Ishigaki Island, Okinawa, Japan. *Environ. Biol. Fish.* 98: 1173-1183.
- Abe, O. and others. 2014. 4. Current status of spawning grounds and periods of Pacific Bluefin Tuna. Working paper submitted to ISC-PBFWG available on ISC website.
- Bayliff, W.H., Y. Ishizuka and R. Deriso. 1991. Growth, movement, and attrition of northern bluefin tuna, *Thunnus Thynnus*, in the Pacific Ocean, as determined by tagging. *IATTC Bull.* 20: 3-94.
- Block, B.A., D.P. Costa, G.W. Boehlert and R.E. Kochevar. 2003. Revealing pelagic habitat use: the tagging of Pacific pelagics program. *Oceanologica Acta* 25: 255-266.
- Boustany, A.M., R. Matteson, M. Castleton, C. Farwell and B.A. Block. 2010. Movements of Pacific bluefin tuna (*Thunnus orientalis*) in the Eastern North Pacific revealed with archival tags. *Prog. Oceanogr.* 86: 94-104.
- Bravington MV, Grewe PG, Davies CR (2014). Fishery-independent estimate of spawning biomass of Southern Bluefin Tuna through identification of close-kin using genetic markers. *FRDC Report 2007/034*. CSIRO, Australia.
- Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar Ecol Prog Ser* 188:263-297.
- Chen, K-S., Crone, P., Hsu, C-C. 2006. Reproductive biology of female Pacific Bluefin Tuna *Thunnus orientalis* from south-western North Pacific Ocean. *Fisheries Science*, 72: 985-994.
- Domeier, M.L., D. Kiefer, N. Nasby-Lucas, A. Wagschal and F. O'Brien. 2005. Tracking Pacific bluefin tuna (*Thunnus thynnus orientalis*) in the northeastern Pacific with an automated algorithm that estimates latitude by matching sea-surface-temperature data from satellites with temperature data from tags on fish. *Fish. Bull.* 103: 292-306.
- Inagake, D., Yamada, H., Segawa, K., Okazaki, M., Nitta, A., and Itoh, T. 2001. Migration of young bluefin tuna, *Thunnus orientalis* Temminck et Schlegel, through archival tagging experiments and its relation with oceanographic condition in the western North Pacific. *Bull. Natl. Res. Inst. Far Seas Fish.* 38: 53-81.
- Itoh, T. 2006. Sizes of adult bluefin tuna *Thunnus orientalis* in different areas of the western Pacific Ocean. *Fish. Sci.* 72: 53-62.
- Itoh, T., Tsuji, S., and Nitta, A. 2003. Migration patterns of young PBF (*Thunnus orientalis*) determined with archival tags. *Fish. Bull.* 101: 514-534.
- Kitagawa, T., Kimura, H.N., Yamada, H. 2004. Diving behavior of immature, feeding Pacific Bluefin tuna (*Thunnus thynnus orientalis*) in relation to season and area: the East China Sea and the Kuroshio-Oyashio transition region. *Fish. Oceanogr.* 13:3(161-180).

- Kitagawa, T., A.M. Boustany, C.J. Farwell, T.D. Williams, M.R. Castleton and B.A. Block. 2007. Horizontal and vertical movements of juvenile bluefin tuna (*Thunnus orientalis*) in relation to seasons and oceanographic conditions in the eastern Pacific Ocean. *Fish. Oceanogr.* 16: 409–421.
- Macdonald, J. I., Farley, J. H., Clear, N. P., Williams, A. J., Carter, T. I., Davies, C. R., Nicol, S. J. 2013. Insights into mixing and movement of Southern Pacific albacore *Thunnus alalunga* derived from trace elements in otoliths. *Fish. Res.* 148: 56-63.
- Nishikawa, Y, Honma, M., Ueyanagi, S., Kikawa, S. 1985. Average distribution of larvae of scombrid fishes, 1956-1981. *Bull. Far. Seas. Fish. Res. Lab.* 12, 1-99.
- Polovina, J.J. 1996. Decadal variation in the Trans-Pacific migration of northern Bluefin tuna (*Thunnus thynnus*) coherent with climate-induced change in prey abundance. *Fish. Oceanogr.* 5:114-119.
- Rooker, J. R., Secor, D. H., Zdanowicz, V. S., Itoh, T. 2001. Discrimination of northern Bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry. *Mar. Ecol. Prog. Ser.* 218: 275-282.
- Shimose, T., Kai, M., Tanabe, T., Chen, K.-S., Hsu, C.-C., Muto, F., and Yamasaki, I. 2008. Age and growth of PBF, *Thunnus orientalis*, validated by the sectioned otolith ring counts. Working paper submitted to the ISC PBF Working Group Meeting, 28 May-6 June 2008, Shimizu, Japan. ISC/08/PBFWG-1/08.
- Shimose, T., Tanabe, T., Chen, K.-S., and Hsu, C.-C. 2009. Age determination and growth of PBF, *Thunnus orientalis*, off Japan and Taiwan. *Fish. Res.* 100: 134-139.
- Shimose, T. and Takeuchi, Y. 2012. Updated sex-specific growth parameters for PBF *Thunnus orientalis*. Working paper submitted to the ISC PBF Working Group Meeting, 31 January-7 February 2012, La Jolla, California, USA. ISC/12/PBFWG-1/12.
- Tanaka, S. 2006. Maturation of bluefin tuna in the Sea of Japan. Working paper submitted to the ISC PBF Working Group Meeting, 16-20 January 2006, Shimizu, Shizuoka, Japan. ISC/06/PBFWG/09.
- Yonemori, T. 1989. To increase the stock level of the highly migrated pelagic fish. In *Marine ranching (Agriculture, Forestry and Fisheries Research Council secretariat, eds.)*, 8–59. Koseisha-Koseikaku, Tokyo, Japan.
- Yoon, S., Kim, Z., Lee, S., Lee, M., and Lee, D. 2012. Catch characteristics and resources management of PBF caught by offshore large purse seine in Korean waters. Working paper submitted to the ISC PBF Working Group Meeting, 10-17 November 2012, Honolulu, Hawaii, USA. ISC/12-3/PBFWG/09

## Appendix I. PBF CKMR Study Design Rationale

The general strategy proposed here is to sample adults on (all) spawning grounds, and juveniles at various ages, comparing adults to juveniles to look for POPs, as with SBT. It is important to use up-to-date genetic methods because these will also reveal many HSPs among juveniles and some POPs amongst adults alone, which provide considerable extra information on abundance and demography.

The design is based on the assumption that there is just one population (i.e. complete interbreeding), but that individual PBT will preferentially use one of the two spawning grounds depending on age/size. If this hypothesis is wrong, it will become obvious during the study, because unexpected patterns will appear in the POPs [footnote: For example, if SoJ and Echina Sea are entirely separate populations and old fish in SoJ are just unavailable to the fishing gear, then POPs among adults alone (i.e. rather than between adults and juveniles) will not be crossed between SoJ and ECS.].

There are currently too many unknowns about PBT biology/dynamics (e.g., about growth in adults; fecundity; movement; juvenile mixing) to try designing a detailed “optimal” sampling scheme yet (Another way to express this, is that there are many parameters of PBT life-history which are relevant to formulating a CKMR model but which still need to be estimated. The relative efficiency of different possible designs would vary depending on the true values of those parameters, which we currently do not know. Therefore we need a design which will allow estimation of those unknown parameters, rather than focusing too narrowly on getting an immediate abundance estimate). Instead, we propose below a broad and robust strategy which should quickly reveal enough POPs to (i) understand juvenile mixing, (ii) design a more sophisticated and efficient sampling strategy for the longer-term, and (iii) estimate abundance without having to rely on untestable assumptions. Because of the need to quickly understand juvenile mixing for PBT before an absolute estimate of adult abundance can be made, the number of POPs required is considerably higher--- for this initial phase of CKMR--- than for SBT. Assuming the approach is successful, long-term sampling levels to keep the abundance estimate up-to-date could be considerably lower.

General points:

1. For SBT CKMR, juveniles could safely be sampled anywhere because there is no risk of “correlation” between offspring sampling location and parent sampling location--- all adults use the same spawning ground, and that is where they are sampled. (The only known SBT summertime juvenile aggregation is in the Great Australian Bight, but the SBT CKMR strategy would not be compromised even if another aggregation did exist somewhere else.) However, PBT has clearly-separated spawning grounds and clearly-separated groups of 1-3yo on both sides of the Pacific, so there is the possibility that spawning site might be correlated with juvenile destination--- in other words, that juvenile mixing might be incomplete. For example, fish spawned in SoJ might be more likely to go the E Pacific as 1-3yo, while fish spawned in ECS might be more likely to stay in W Pacific. Also, the total mortality rate experienced by juveniles

(between birth and CKMR sampling) may depend on where they were spawned. Since there is no way to sample adults “randomly” across spawning grounds, there is a risk that--- for example--- sampling juveniles only in E Pacific (with no other information on where they were spawned) might “over-compare” with SoJ-sampled adults and “under-compare” with ECS-sampled adults. A naive CKMR analysis of such data assuming full juvenile mixing would lead to some bias in estimates of adult abundance and reproductive-output-at-age, and there would not be enough data to develop a more sophisticated CKMR analysis that allows for and is robust to incomplete juvenile mixing. There are three options for addressing this, not mutually exclusive:

(a) It may be possible to analyze juvenile otoliths to distinguish spawning site (SoJ or ECS). This would be very useful, allowing more precise estimates with lower required sample sizes. Otoliths would not need to be collected or read from all juveniles sampled, only: (i) enough read to estimate the proportion of each type of juvenile in each set of juvenile samples (set = place and year); and (ii) more collected but not initially analyzed, but which can be analyzed later if a parent of that juvenile is eventually found. However, although there are promising initial results from microchemistry and isotope studies, the origin-by-otolith approach cannot currently be guaranteed to work.

(b) A robust alternative is to (tissue-)sample juveniles from several fisheries in E and in W Pacific, then (initially at least) construct separate CKMR estimates based on each set of juveniles separately, but using the same full-mixing model each time. By comparing the separate estimates, it will be possible to ascertain the extent of any bias, and to develop a more sophisticated integrated CKMR model allowing for incomplete juvenile mixing. Even in this case, though, it would still be beneficial (in terms of more precise estimates and lower sample size requirements) to have origin established from otoliths for at least some juveniles.

(c) For some 0yo juveniles at some times of year, the spawning site may be obvious from the location of capture.

2. Bias in estimated abundance is only of importance if it reaches, say, 10%. To reliably detect a difference of that size by comparing two abundance estimates from different sets of juveniles, about 300 POPs would be needed from each [footnote: Straightforward calculations from the Poisson distribution;  $\sqrt{300} \approx 15$  so 300 vs 330 is about 2 standard deviations.]. Sampling needed to find that many POPs should be spread across at least 3 years, because of (i) the possibility of skip-spawning in young adults (demonstrated for SBT), (ii) possible variations in juvenile mixing from year to year, and (iii) the need to avoid comparing juveniles to adults caught in the same spawning season (Note that adults caught during a spawning season have not had the full opportunity to contribute reproductively in that year, so that 0yo juveniles (see below) caught in year 2 can only be compared to adults caught in year 3 or later, and 3 years are needed to cover 2 juvenile cohorts of 0yo. Suppose sample sizes are chosen so that each juvenile fishery sampled is expected to record 50 POPs per year against adults caught in the same year. The number of cross-year POPs (e.g. between juveniles caught in year 1 and adults caught in year 2) will be similar (not exactly the same because of parental mortality and growth,

and avoidance of point (iii) above). After 3 years we might therefore expect to comfortably reach that target of 300 POPs (150 same-year POPs and at least the same number of cross-year POPs) for each juvenile fishery. In addition, the demography guarantees that there will also be about as many HSPs as POPs.

3. Overall, roughly equal numbers of adults & juveniles are close to optimal for POP-finding (i.e. highest precision for a given total of samples). For adults, it is important to have samples across the mature age range and spawning grounds. We propose sampling from each of three fisheries in proportion to their estimated current removal of spawning potential [footnote: Assumed proportional to percent-mature times average-bodyweight at age.], to obtain roughly equal numbers of parents from each spawning-ground fishery and as far as possible across the mature age range. The latter helps for estimating reproductive-output-at-age and consequently abundance, as well as for refining the sampling design in future. No length-stratified subsampling is required. It may be wise to collect and archive more than the sample sizes proposed here (cheap) but only genotype a subset (since genotyping is the most expensive step); the extra samples are a reserve which could be genotyped later if initial analysis reveals any need to do so.

(a) Samples can be taken randomly with respect to catch within each spawning-ground fishery, except that genotyping of 3yo spawners should be avoided for now since they will be excluded from POP comparisons (and the sample size for SoJ JPS fishery excludes 3yo, so any 3yo collected are additional; need to set a length-based criterion for this). Note that we still get direct information about the relative reproductive contribution of 3yo adults even without genotyping any 3yo, because of retrospective comparisons (e.g. comparing a 4yo adult caught in 2017 to a 1yo juvenile caught in 2017 which would have been born in 2016 when the adult was 3yo).

4. For juveniles, we propose splitting the sampling equally between the following three areas, to provide the best basis for comparing estimates. The actual breakdown by fishery/country within area is not important; however, it is desirable to sample from all ages 1-3yo in (a) and (b) below, because that maximizes the timespan of cohorts covered in the initial study. Strictly, either (c) on its own, or (a) and (b) together, should be enough (in other words, the project is not doomed if (c) turns out to be impossible). But the best would be (a), (b), and (c).

(a) E Pacific 1-3yo (Mexico, USA);

(b) W Pacific 1-3 (Japan, Korea);

(c) W Pacific 0-group (Japan, from two distinct fisheries either side of Honshu; samples collected at times-of-year where spawning ground should be obvious; roughly equal sample sizes from both; note that only a total of 50 POPs per year combined across both of these 0-group fisheries is required)



5. To achieve 50 same-year POPs per juvenile-fishery-group (4a, 4b, and 4c) under the conditions above, and based on numbers from the current assessment, the annual sampling levels would be:

- (a) E Pacific 1-3yo: 1300
- (b) W Pacific 1-3yo: 1300
- (c) W Pacific 0-group: 1300
- (d) Taiwan ECS long-line: 740
- (e) Japan ECS long-line: 1480
- (f) Japan SoJ purse-seine for younger adults: 1680

6. Milestones and background work might be as follows:

(a) Development of genetic techniques can begin straightaway--- this needs to be coordinated internationally, and should make use of the latest and best techniques so that HSPs as well as POPs can be found.

(b) So can work on otolith-origin. This is of very scientific high priority, since it will improve abundance estimates and the ability to infer differential juvenile mixing whatever the long-term sampling strategy turns out to be--- it gives more flexibility in future sampling design.

(c) So can statistical model development.

(d) After 1 or 2 years, if reality (e.g. adult abundance) is vastly different from current estimates, then it will be clearly obvious in the data (since there will be a lot of POPs).

(e) After 3 years, there should be enough data to perform an initial CKMR analysis and start investigating issues around juvenile mixing. Depending on the results, it may be possible to make an adult abundance estimate at that point, but it will probably be necessary to wait for another year or two, so that an appropriately sophisticated and robust analysis can be developed. This is also the moment to review sampling levels and broad design issues; there may well be logistical and statistical reasons to change the design (i.e. the annual sample size by fishery) substantially to focus on some fisheries (and/or size-ranges) rather than others.

(f) After 5 years, a final adult abundance estimate (actually, a short time series of abundance estimates) should be ready. So should a long-term sampling strategy for monitoring abundance through CKMR, without relying on fishery-derived CPUE that may well change its relationship to abundance as management and economic factors are modified. It is pointless to speculate at

this stage about the shape of any long-term design, because so much will depend on what is discovered about juvenile mixing and on the feasibility of origin-by-otolith, but it is safe to say that long-term sample sizes could be lower than in this initial study (unless the abundance turns out to be much higher than currently thought).

## Agenda for Pacific Bluefin (PBF) Workshop

### Close-Kin:

### A Fishery Independent Estimate of Spawning Stock Biomass

**Date:** Wednesday, 27-29 May 2015

**Location:** Southwest Fisheries Science Center  
8901 La Jolla Shores Drive  
La Jolla, CA 92037

**Contacts:** Russ Vetter ([russ.vetter@noaa.gov](mailto:russ.vetter@noaa.gov))  
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### Objectives:

- Review recent advancements in genetic identification of individuals and parent-offspring relationships and their use in fisheries conservation.
- Evaluate the theory and lessons learned from the application of parent-offspring measures of spawning stock biomass of Southern Bluefin Tuna
- Discuss the merits and challenges of applying a similar method to Pacific Bluefin Tuna
- Review existing multi-national PBF fisheries sampling programs, the remaining uncertainties in the life history of Pacific Bluefin, and next steps needed to implement a close-kin genetic analysis.
- Develop a research plan that will:
  - Identify existing sampling programs that are likely to continue.
  - Identify the needs for additional sampling protocols.
  - Discuss the alternatives and costs of genetic analyses and data management
  - Identify potential biases and the research needed to resolve uncertainties in PBF life history.
  - Identify the intellectual resources needed to incorporate C-K results into the PBF assessment
  - Discuss options for coordination and oversight.

**Schedule:**

Wednesday, May 27, 2015

- 10:00 Welcome, opening remarks and introductions (R. Vetter)
- 10:15 Relatedness Measures in Conservation and Management  
Overview of Genetic Methods (J. Hyde)  
Overview of Relatedness and Conservation Questions (J. Hyde)
- 10:30 Why Close-Kin for Southern Bluefin Tuna?  
Context and Background (C. Davies)
- 11:00 Close-Kin Theory  
Theoretical Estimation of Absolute Abundance (M. Bravington)  
Additional Complexities of Real World Sampling (M. Bravington)
- 11:30 Incorporation of C-K Results in CCSBT Operating Model  
Process and Assumptions (R. Hillary)
- 12:00 Lunch
- 1:00 Initial Thoughts on Applying C-K to Pacific Bluefin Tuna  
(M. Bravington)
- 1:30 Atlantic Bluefin Tuna  
SEFSC C-K pilot study (M. Laretta)
- 2:00 Pacific Bluefin Tuna

Japan C-K pilot study (T. Irie)

- 2:30 PBF Assessment and Stock Status  
Overview of model and assessment results (K. Piner)
- 3:00 Break
- 3:30 PBR Assessment and Stock Status continued  
Data gaps and uncertainties (S. Teo, M. Maunder, A. De Silva)  
Potential for changes in fishery-dependent data sources (H. Lee)
- 4:00 Follow-up Questions and Discussion for Tomorrow
- 5:00 Adjourn

Thursday May 28, 2015

- 9:00 Summary of Pacific Bluefin Life History (H. Dewar)
- 9:30 Possible PBT C-K Sampling Designs (R. Vetter)
- 10:00 Summary of Western Pacific Fisheries and Sampling Opportunities  
Taiwan Adult Fishery Sampling (W. Chen)  
Japan Adult Fishery Sampling (T. Irie)  
Juvenile Sampling Opportunities, Larval, YOY and Juvenile (Z. Kim)

10:30 Summary of Eastern Pacific Fisheries and Sampling Opportunities  
US Recreational Fishery Sampling (H. Dewar)  
Mexican Purse Seine Fishery Sampling (M. Dreyfus and A. De Silva)

11:00 PBT Preliminary Sampling Design

12:00 Lunch

1:00 Sampling Design Continued:  
Point Estimate or Time Series (M. Bravington & C. Davies)  
Other Required Life-History Data?  
Other Desirable Life-History Data?

2:00 Project Management SBT and Lessons Learned (C. Davies, M. Bravington, R. Hillary)

2:30 Pacific Bluefin Project Management (R. Vetter)  
Steering Committee, Organizational Umbrella  
Fisheries Agency Sampling: DNA and Life History Information  
Tissue and DNA archiving: Central or Distributed  
Marker Development:  
Inter-lab Marker QA/QC  
Data Management  
Data Analysis and Incorporation in Assessments

5:00 Adjourn

Friday, May 29

9:00

Continued Discussion and Wrap-up

Value of an Absolute Estimate of SSB for PBT

Role of C-K in Evaluating Management Alternatives

Role of C-K in Evaluating Results of Management Actions

Impacts of Management Decisions on C-K Design

Additional Information from Genetic Relatedness Measures

12:00

Adjourn



Close-kin Mark Recapture as a Tool for Estimation of Spawning Biomass in Pacific Bluefin  
Tuna: Sampling Design and Sampling Plan

Workshop on Developing CKMR techniques for Pacific Bluefin

NOAA National Marine Fisheries Service

Southwest Fisheries Science Center

May 27-29, 2015

List of Attendees

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