REVIEW AND INSIGHTS INTO THE DIFFERENCES IN REPRODUCTIVE PARAMETER ESTIMATES BETWEEN EASTERN AND WESTERN ATLANTIC BLUEFIN TUNAS STOCKS

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SUMMARY

There are differences in estimates of reproductive parameters for eastern and western stocks of Atlantic bluefin tuna, with much younger age at maturity/spawning for the eastern stock relative to the western stock. This report reviews the methods used to estimate ABFT reproductive parameters, and provides insights into the potential causes of the observed differences. It is clear from this review that the estimate of "spawning fraction" for both stocks requires validation. Estimates of maturity-at-age for the eastern stock are based on ovaries sampled in the Mediterranean Sea and are likely to be biased towards mature fish (overestimating proportion mature at age). Estimates of spawning fraction-at-age for the western stock are based on catch data from the Gulf of Mexico and may be biased if selectivity is not proportional to residency time, or if the data analysed was not representative of the whole spawning stock Research recommendations are provided. It may be possible to obtain an independent maturity ogive for each stock through a well-designed, length stratified, sampling program on winter feeding grounds, when both immature and mature females are present.

RÉSUMÉ

Il existe des différences dans les estimations des paramètres liés à la reproduction pour les stocks de thon rouge de l'Atlantique Est et Ouest, les âges d'arrivée à maturité et le frai étant beaucoup plus précoces pour le stock de l'Est que pour le stock de l'Ouest. Le présent rapport passe en revue les méthodes utilisées pour estimer les paramètres de la reproduction du thon rouge de l'Atlantique et donne un aperçu des causes potentielles des différences observées. Il ressort clairement de cet examen que l'estimation de la fraction reproductive pour les deux stocks doit être validée. Les estimations de la maturité par âge pour le stock de l'Est sont basées sur des ovaires échantillonnés de la mer Méditerranée et sont probablement biaisées en faveur des poissons matures (proportion surestimée de la maturité par âge). Les estimations de la fraction reproductive par âge pour le stock occidental sont basées sur les données de capture du golfe du Mexique et peuvent être biaisées si la sélectivité n'est pas proportionnelle au temps de résidence ou si les données analysées ne sont pas représentatives de l'ensemble du stock reproducteur ; des recommandations de recherche sont fournies. Il peut être possible d'obtenir une ogive de maturité indépendante pour chaque stock grâce à un programme d'échantillonnage bien conçu et stratifié par taille dans des zones trophiques hivernales, lorsque des femelles tant matures qu'immatures sont présentes.

RESUMEN

Existen diferencias en las estimaciones de los parámetros reproductivos para los stocks oriental y occidental de atún rojo del Atlántico (ABFT), con una edad de madurez/desove mucho menor para el stock oriental que para el stock occidental. Este informe revisa los métodos utilizados para estimar los parámetros reproductivos del ABFT, y aporta ideas sobre las posibles causas de las diferencias observadas. Queda claro a partir de esta revisión que la estimación de la "fracción reproductora" para ambos stocks requiere una validación. Las estimaciones de madurez por edad para el stock oriental se basan en ovarios muestreados en el Mediterráneo y es probable que estén sesgadas hacia peces maduros (sobrestimando la proporción de madurez por edad). Las estimaciones de la fracción reproductora por edad para el stock occidental se basan en datos de captura del golfo de México y podrían estar sesgados si la selectividad no es proporcional al tiempo de residencia o si los datos analizados no eran representativos de todo

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el stock reproductor. Se presentan recomendaciones sobre investigación. Podría ser posible obtener una ojiva de madurez independiente para cada stock mediante un programa de muestreo bien diseñado y estratificado por talla en las zonas tróficas invernales, cuando están presentes tanto las hembras maduras como las inmaduras.

1. Introduction

Atlantic bluefin tuna (ABFT) are widely distributed in the Atlantic Ocean and undertake long migrations between spawning and feeding grounds. The fisheries that harvest them are managed by the ICCAT as two stocks separated at 45°W (e.g., Rooker *et al.* 2007). The eastern stock spawns in the Mediterranean Sea while the western stock spawns in the Gulf of Mexico and nearby waters (e.g., Baglin 1982, Corriero 2003, Karakulak *et al.* 2004, Nemerson *et al.* 2000, Goldstein *et al.* 2007). Tagging, otolith chemistry and genetic studies have shown that the feeding grounds are mixed in the Atlantic Ocean but the stocks appear to show high spawning grounds fidelity (Block *et al.* 2005, Carlsson *et al.* 2007, Rooker *et al.* 2007).

Accurately estimating reproductive parameters (e.g., maturity-at-age) for ABFT is complex given their broad distribution, migration patterns and spawning strategy. For stock assessment purposes, the ICCAT uses different maturity ogives for the eastern and western stocks based on work undertaken in the Mediterranean Sea and the Gulf of Mexico spawning grounds respectively. For eastern ABFT, maturity is assumed to occur at younger ages (50% mature at age 4) than for western ABFT (50% spawning at ages ~8-10 or older). Large differences in reproduction and maturity parameters of ABFT stocks are not expected given their similarity in biological traits such as maximum size and growth rates (Cort 1991, Anon 2017b, Ailloud 2017). Given this, it is important to determine whether the differences in reproductive parameters are real (i.e., due to variation in life-history parameters between the populations) or due to methodological differences and/or challenges obtaining representative samples and data.

At the 2017 ICCAT bluefin tuna data preparatory meeting, it was recommended that a workshop of experts in tuna reproduction, life history and ecology be arranged to clarify the terminology and methods used in ABFT reproductive studies and to make recommendations on further research needs (Anon 2017b). A planning meeting was held in early 2018 where it was recommended that an independent review of available information on east/west reproductive differences be undertaken to provide insights about the causes of the current discrepancies in the assumptions about age at maturity (first maturity, 50% and 100%) with the aim to facilitate agreement within ICCAT on this issue.

This review provides insights into the causes of the differences in reproductive parameter estimated obtained for Atlantic bluefin tuna in western and eastern areas based on relevant published papers and reports. In particular, we focused on the differences of methodologies used for estimating age at maturity/spawning fraction. The report is structured into 7 main sections:

1. Maturity parameters used in recent ICCAT stock assessments
2. Brief overview of maturity, spawning fraction and reproductive potential
3. Spawning and feeding areas for Atlantic bluefin tuna
4. A review of relevant eastern ABFT reproductive/maturity studies
5. A review of relevant western ABFT reproductive/maturity studies
6. An overview of reproductive parameters estimated for Pacific and southern bluefin tunas
7. Summary and recommendations

2. Maturity parameters used in recent stock assessments

For eastern ABFT, the same maturity ogive has been used in stock assessments since 1997, where age at 50% maturity is assumed to be at age 4 years (115 cm/30 kg) and 100% maturity at age 5 (**Table 1, Figure 1**). The assessment model then assumes all females contribute equally (per unit of spawning stock biomass) to egg production. The ogive used in the assessment is similar to the ogive estimated by Corriero *et al.* (2005) based on the analysis of ovaries from the Mediterranean Sea.

There is uncertainty about the age at maturity ogive for western ABFT. In 2008, a knife edge maturity ogive for females was used, based on work by Baglin (1982), which specifies that age classes 1-7 are immature and make no contribution to the spawning biomass (reproductive output) and all age classes 8+ (190 + cm) fish are mature and contribute in proportion to their weight (Anon 2008).

In 2010, the knife edge was updated to age 9 using new growth data from Restrepo *et al.* (2011) (Anon 2011). This ogive was used in the base case model for the 2012 and 2014 stock assessments. Since 2010, routine sensitivity analyses have also been performed to assess the effect of different maturity ogives on stock assessments. These have included 'early maturity ogives' where 50% maturity is assumed to be age 4 (from the eastern ABFT stock) and age 5 (from gonad work by Mathers *et al.* 1995) (Anon 2011; 2012). 'Late maturity ogives' have also been included based on analysis of various size data from longline catches in the Gulf of Mexico with 50% maturity estimated at between age ~11-15(Anon 2011, Diaz and Turner 2007, Diaz 2011). However, as described in section 3.2 and below, the 'late maturity ogive' is analogous to a 'spawning fraction ogive'.

In 2017, two alternate vectors for the "proportion of fish contributing to the spawning output" were used to assess the western stock (**Table 1, Figure 1**). Vector 1 was based on the maturity ogive used in the eastern assessment where 50% mature was at age 4. For this vector, it was assumed that maturity alone determined the contribution to the spawning stock (i.e. annual egg production does not vary with fish size/age). Vector 2 was from Porch and Hanke (2017) where the fraction of fish contribution to spawning output (spawning fraction) was estimated by age class using catch data from the Gulf of Mexico (see section 6.2). Age at 50% spawning was estimated at age ~10.

Note that the growth curves estimated for the two stocks are very similar (i.e., from Cort 1991 and Restrepo *et al.* 2011). The 2017 assessment for western ABFT used a new growth curve developed by Ailloud *et al.* (2017) based on tag-recapture data and direct age from otoliths (Anon 2017b) (**Figure 2a**). A Richards growth model was used as it fitted the data better than a von Bertalanffy model, especially for ages >16. The 2017 assessment of eastern Atlantic bluefin used the growth curve from Cort (1991) (**Figure 2a**), although it was noted that the curve didn't fit the length-at-age data for older fish (>20 years) (**Figure 3**) (Anon 2017a, b). The body weight to age relationships for the two stocks are show in **Figure 2b**.

3. Maturity, spawning fraction and reproductive potential

Knowledge of key reproductive parameters, and how they are estimated, is important for this review. Below is a brief summary of the key reproductive parameters relevant to ABFT.

3.1 Proportion mature at length/age (maturity ogive)

As noted above, the reproductive parameter input for the 2017 assessment of the eastern stock of ABFT was a maturity ogive. A maturity ogive is the estimated proportion of the population mature at length/age. Length or age at 50% maturity (L_{50}, A_{50}) is the standard maturity parameter for assessing fish stocks. It is assumed that females determine the dynamics (males are not limiting) so the reproductive potential of a stock depends on the number and fecundity of mature females present. There are three main requirements for estimating a maturity ogive (Schaefer 2001, Shimose and Farley 2013):

1. Precise criteria to identify mature and immature fish.
2. Representative sampling of ovaries from fish in the appropriate size range, which includes both immature and mature females, and at the time of year when it is possible to distinguish between the two reproductive states.
3. Fitting an appropriate statistical model to the maturity at length (or age) data to estimate the maturity ogive (maturity schedule).

Histological analysis of ovaries is the most informative method to determine the maturity status, but it involves complex methodology. For species where immature and mature fish are mixed year-round (i.e., a spawning migration does not occur), sampling can be conducted just prior to and/or during the spawning season as this is the time of year when it is assumed that all mature females will display histological evidence of maturity in their ovaries. Mature females are identified by the presence of advanced yolked (vitellogenic) oocytes/eggs or atresia of yolked oocytes. Females with early yolked oocytes are not considered sexually mature. As discussed below, estimating a maturity ogive is complicated for Atlantic bluefin tuna as mature fish migrate to particular areas to spawn where immature fish are very likely to be underrepresented.

3.2 Spawning fraction at size/age

The reproductive parameter inputs for the 2017 assessment of the western stock of ABFT were *spawning fraction* ogives, i.e., the proportion of fish at age contributing to the spawning output of the population (rather than simply the proportion mature at age). The first ogive was the maturity ogive from the eastern stock, with the assumption that annual egg production does not vary with fish size/age. The second ogive was calculated using catch data from the Gulf of Mexico spawning ground (see section 6.2) and, in principle, it accounts for the possibility that there may be size/age related differences in spawning duration (residency time). If estimated accurately, spawning fraction will equal proportion mature x residence time on the spawning ground for a given age class. However, duration/residency on the spawning ground does not necessarily equal spawning duration as individuals may not spawn every day for the entire period they are present. Electronic tagging data indicated that western ABFT may only spawn for a proportion of time spent on the spawning ground (Teo *et al.* 2007).

Note that *spawning fraction* used here should not be confused with *daily spawning fraction* used in reproductive studies, which is the fraction of the mature population that are spawning each day. Daily spawning fraction is used to estimate mean spawning frequency (see below).

3.3 Spawning potential at length/age

A maturity ogive or a spawning fraction ogive can be used in the estimation of spawning stock biomass (SSB). That is, numbers-at-age x weight-at-age x proportion mature (or proportion spawning) at age. Ideally, sex ratio should also be included. Remember that proportion spawning (spawning fraction) accounts for spawning duration to some extent, while proportion mature does not. However, the estimate of SSB using either ogive does not account for variability in egg production (fecundity) among fish of different sizes or ages. Recent work has shown that large fish produce disproportionately more (and larger) eggs than small fish (Barneche *et al.* 2018) and contribute disproportionately to successful reproduction, i.e. surviving progeny (Bravington *et al.* 2016). It is not known if this is the case for ABFT. Therefore, it is important that accurate estimates of the relationship between size/age and annual fecundity are obtained to estimate reproductive potential (the number of offspring produced by a spawning stock).

Tunas are batch spawners with indeterminate fecundity and asynchronous oocyte development (Schaefer 2001). This means that potential annual fecundity is not fixed prior to spawning as unyolked oocytes are continually matured and spawned during the spawning season. To determine annual fecundity, estimates of reproductive parameters such as spawning frequency and batch fecundity via histological analysis of ovaries, are required. Since large females are generally more fecund than smaller fish, length/age dependent estimates of reproductive parameters are essential.

Females with late stage migratory nucleus or hydrated stage oocytes are used to estimate batch fecundity (the number of eggs spawned per batch). The most common approach to estimate batch fecundity is by the gravimetric method of Hunter *et al.* (1985) where the number of migratory nucleus or hydrated oocytes in small (weighed) ovary subsamples are multiplied up to the total weight of the ovary. Alternate methods use stereological methods to count post ovulatory follicles (POFs) or migratory nucleus oocytes in histological sections (e.g., Medina *et al.* 2007).

The presence/absence of POFs in ovaries is used to calculate daily spawning fraction and spawning frequency. The mean spawning fraction is calculated as proportion of all mature females with POFs <24 hours old (Hunter and Macewicz 1985). The mean spawning frequency is the reciprocal of the spawning fraction. For example, if the ovaries of 30% of mature females contain POFs, and POFs remain visible in the ovary for only 24 hours, then the mean spawning fraction is 0.3. The spawning frequency is 1/0.3, which is 3.33 days. Therefore, females spawn once every 3.33 days on average. It is generally found that POFs remain visible in tuna ovaries for 24 hours before they are completely resorbed.

4. Spawning and feeding areas for Atlantic bluefin tuna

Knowledge of spawning and feeding areas for ABFT is important for assessing methods that have been used to estimate size/age at maturity/spawning fraction. Of particular importance is the spatial distribution of adults and sub-adults (i.e., mature and immature fish) for each stock. Here we provide a brief overview of spawning and feeding areas, which is useful when considering how maturity/spawning fraction ogives have been estimated for ABFT. However, a full review of the distribution and migration of ABFT was not part of this review.

As previously mentioned, the two main spawning grounds for ABFT are in the Mediterranean Sea (eastern stock) and the Gulf of Mexico (western stock). Piccinetti *et al.* (2013) reviewed much of the literature on eastern ABFT reproduction and concluded that spawning could occur in most of the Mediterranean Sea but that some areas are better studied than others (**Figure 4**). It is generally agreed that spawning is concentrated in the Balearic Sea, the south Tyrrhenian Sea, around Malta and in the Levantine Sea. Spawning generally occurs between May and July although spawning as late as September has been recorded. The intensity of spawning can vary spatially and temporally depending on local environmental conditions, and the timing of spawning appears to be related to sea surface temperature (Heinisch *et al.* 2008, Piccinetti *et al.* 2013). Spawning occurs earlier in the eastern Mediterranean (May and early June) than in the central (June) and western Mediterranean (late June and July) (Heinisch *et al.* 2008, Rooker *et al.* 2007).

The Mediterranean Sea is also an important feeding area for ABFT as a proportion of the adult and juvenile population may remain within the Mediterranean Sea year-round (Mather *et al.* 1995, Heinisch *et al.* 2008, Fromentin and Lopuszanski 2013, Cermeño *et al.* 2015). Piccinetti *et al.* (2013) highlighted areas of non-spawning (feeding) in the Mediterranean Sea (**Figure 4**).

Electronic and conventional tagging data, however, has shown that juveniles also migrate out of the Mediterranean Sea (Block *et al.* 2005, Rooker *et al.* 2007). Juveniles tagged in the eastern Atlantic can undertake trans-Atlantic migrations and it has been hypothesised that they may remain in the western Atlantic for several years before returning to the Mediterranean Sea (Block *et al.* 2005). Conventional tag data indicated that of the 14668 young fish (aged <4) tagged in the eastern Atlantic between 1954 and 2005, only 13.8% at liberty >365 days were recaptured in the Mediterranean Sea (Rooker *et al.* 2007). The remaining were recaptured west of Gibraltar (4.3%), in the Bay of Biscay (66.4%), the western Atlantic (9.5%) and other areas (6.0%). Using otolith stable isotope analysis, Siskey *et al.* (2016) found that in the 1990s 73% of small fish <100 cm fork length (FL) sampled in the western Atlantic were of eastern origin, providing further evidence that immature fish migrate out of the Mediterranean Sea and are capable of undertaking long trans-Atlantic migrations.

The presence of a substantial proportion of the population of eastern ABFT sub-adults (immature) outside the Mediterranean Sea needs to be accounted for in estimates of maturity-at-age. Maturity estimates based on ovaries collected from the Mediterranean Sea will be negatively biased if immature fish are underrepresented in the sampling program.

The situation is slightly different for the western stock of ABFT. Sexually mature adults spawn in the Gulf of Mexico primarily between April and June, a shift of one month earlier than in the Mediterranean Sea (Teo *et al.* 2007). However, catch data shows they are presence early as February (Diaz 2007). Spawning has also been recorded in the Straits of Florida and the Caribbean (Rivas 1954, Richards 1976, Mathers *et al.* 1995) and more recently in the Slope Sea (Richardson *et al.* 2016). After spawning, adults migrate to feeding grounds in the North Atlantic. Juveniles are also found on North Atlantic feeding grounds with seasonal north-south movements, but they do not enter the Gulf of Mexico until mature.

5. Review of eastern Atlantic bluefin tuna reproductive studies

5.1 Minimum size/age at maturity

There have been many studies examining the ovaries of eastern ABFT in the Mediterranean Sea using modern histological techniques. Most provide estimates of length or age at first spawning (i.e., the smallest/youngest mature fish sampled) (**Table 2**) rather than average length/age at 50% mature. In all studies, sampling was during the spawning season ort just prior to.

Tawil *et al.* (2002) examined the gonads of ABFT in the coastal waters off Libya in the central Mediterranean Sea, during 2000 and 2001 (May and June) (115-172 cm FL) using histological technique. They found that all females were sexually mature independently of their size or age, and that the smallest sampled (115 cm, age 4) was actively spawning.

Medina *et al.* (2002) examined the ovaries of 60 bluefin tuna caught by trap off Barbate in 1999 and 2000 (30-217 kg) as they entered the Mediterranean Sea in April and June. The reproductive development stage of fish was identified using appropriate histological methods. The majority of females were classed as mature but non spawning (91.7%) and the rest were classed as immature (sizes not provided). In addition, the ovaries of 24 females caught by purse seine around the Balearic Islands in June and July were also examined. All were mature and actively spawning. The two smallest fish examined were estimated to be ~116 and 126 cm FL based on converting weight to length.

Corriero *et al.* (2003) collected and analysed ovaries from 131 ABFT caught in the western Mediterranean Sea between February 1999 and September 2000 (63-236 cm FL). The fish were caught in 7 locations from Barbate in the west to the Gulf of Taranto and the south Adriatic Sea in the east. All sampling locations were suggested feeding areas apart from the Balearic Islands (spawning area) and the Bocche di Bonifacio (migration route into the Mediterranean Sea). The reproductive development stage of the fish examined was identified using appropriate histological methods including late stages atresia ('brownish granules and yellow-pigmented globules') as maturity markers. The maturity state of fish caught in February to April were not classified as all atretic follicles were resorbed, making it difficult to distinguish immature from mature fish prior to the spawning season. Size at maturity was not estimated in the study. Based on the data provided, the smallest mature female was 110 cm FL and the largest immature female was 107 cm FL (assuming that the 130 cm FL fish was misclassified as immature since late stages atresia was identified indicating it was mature).

Karakulak *et al.* (2004) was the first to report Atlantic bluefin tuna spawning in the eastern Mediterranean Sea. Gonads from 50 fish (27 females and 23 males) were collected from May and June 2003 from purse seiners in the Levantine Sea. Fork length ranged from 107 to 226 cm. All females were mature; 20 were actively spawning, four were mature but non-spawning, and three were post spawning. Again, immature females were not sampled to examine length/age at maturity.

Zupa *et al.* (2009) reported on the histological analysis of ovaries of 326 adult ABFT (>135 cm FL/age 4-5 years) sampled in the Mediterranean Sea in May to September between 1998 and 2008. Some of the data were possibly obtained from Corriero *et al.* (2005). Three fish were classed as non-reproductive, 90 as actively spawning, 12 as post-spawning and the remaining as reproductive but not spawning. Since there were only three non-reproductive fish, the authors suggest it is "unlikely that non-reproductive individuals aggregate with reproductive ones during their migration towards spawning grounds" in the Mediterranean Sea. However, these fish may have been skipped spawners as one was in resting condition and the other two has early yolked oocytes with 100% atresia of yolked oocytes.

Knapp *et al.* (2014) also examined 40 gonads from ABFT (120-240 cm FL) collected in June-July 2008 in the western Mediterranean Sea. All were classed as mature using histological techniques.

5.2 *Proportion mature at length/age*

Corriero *et al.* (2005) undertook one of the largest studies of reproduction and maturity of eastern ABFT in the Mediterranean Sea. They collected the ovaries from 501 females across several locations (east and west Mediterranean) between May and September of 1998 to 2004 (>60 cm FL). The fish were caught by longline, drift net, purse seine and trap. The ovaries were histologically analysed using the classification scheme of Corriero *et al.* (2003) and the reproductive state was assessed using criteria from Schaefer *et al.* (1998). Length at 50% maturity (L_{50}) was estimated at 103.6 cm FL and length at 100% maturity was 135 cm FL. Based on the analysis of fin spines, the age of females in the 100-104 cm length class were predominantly age 3 and females in the 135-139 cm length class were predominantly age 5. The estimate of L_{50} is less than the minimum size that mature females were sampled in other studies (see section 5.1) and slightly less than used in the stock assessment for the eastern stock (A_{50} = 4 years, L_{50} = 115 cm).

The maturity ogive of Corriero *et al.* (2005) was derived from histological classification of ovaries and included both mature and immature fish, and from the full length of the spawning season. However, it is unclear whether the sampling of mature and immature fish was in proportion to their abundance in the whole population. Only 57 (11.3%) of the 501 females examined were classed as immature, suggesting the sampling from only the Mediterranean Sea and only during the spawning season may have biased in the sampling program towards mature females. The sharp change from 0% mature in the 95 cm length class to ~60% mature in the 100 cm length class also suggests that immature fish were underrepresented. A bias towards mature females could occur if the majority of fish were sampled from spawning areas within the Mediterranean Sea, as immature fish may be underrepresented. Unfortunately, the study does not provide information on the number, size range or proportion mature by area. As noted previously, immature females are also known to occur outside the Mediterranean Sea during the spawning season (Cort 1991, Mathers *et al.* 1995, Rooker *et al.* 2007), which would bias a Mediterranean-based maturity ogive.

5.3 Batch fecundity and spawning frequency

Di Natale *et al.* (2017) presented fecundity data obtained by Rodriguez-Roda (1967) for 10 mature ABFT. Rodriguez-Roda (1967) estimated absolute fecundity by counting mature (vitellogenic) oocytes (i.e., eggs greater than 0.333 mm in diameter) and compared it to fish weight and length. The estimates of absolute fecundity are incorrect (underestimated) as ABFT are batch spawners and will continue to develop vitellogenic oocytes during the spawning season (see section 3.3).

Medina *et al.* (2002) estimated mean batch fecundity of females caught near the Balearic Islands at 93 oocytes per gram of body weight from stereological counts of migratory nucleus oocytes in the ovaries. Mean spawning frequency was estimated at 1.2 days. Medina *et al.* (2007) suggested that batch fecundity estimated by Medina *et al.* (2002) was overestimated as shrinkage of tissue was not considered, and was likely to be 82 oocytes per gram. Note that estimates of relative batch fecundity by Medina *et al.* (2002) from fish caught in the Strait of Gibraltar (Barbate) are not accurate as they used counts of late vitellogenic (advanced) oocytes.

Medina *et al.* (2007) found differences in reproductive parameters between female bluefin tuna sampled in purse seine (PS) (surface) gear compared to females sampled from longline (LL) (deeper set, 50-125 m) gear around the Balearic Islands in May to July. Fish from PS had higher gonadosomatic index values and a greater proportion of females were actively spawning compared to LL caught fish, although a higher proportion of LL fish were actively spawning at the end of the spawning season. Mean batch fecundity was 59 versus 1.2 oocytes per gram of body weight from PS and LL respectively. Mean spawning fraction (proportion of fish with POFs) was 0.84 vs 0.33 (PS vs LL) and spawning frequency was 1.2 days vs 3.1 days. Medina *et al.* (2007) calculated the average annual fecundity for purse sein caught fish at approximately 77 million oocytes /fish, assuming individuals spawn for 14 days for the season.

Knapp *et al.* (2014) examined ovaries from ABFT collected in the western Mediterranean Sea (n = 40, June-July) and the Gulf of Mexico (n = 147, April-June) using histological methods. For the Mediterranean Sea fish, they estimated the spawning fraction at 0.6, giving a mean spawning frequency of 1.67 days, and relative batch fecundity of 45 oocytes /gram of body weight. For the Gulf of Mexico fish, mean spawning frequency was 2.22 days and relative batch fecundity was 28 oocytes /gram of body weight (also see section 6.3 below).

Size/age-based trends in reproductive parameters (fecundity, spawning frequency, spawning duration) are not documented for eastern ABFT.

6. Review of western Atlantic bluefin tuna reproductive studies

6.1 Minimum size/age at maturity

There have also been many studies examining the ovaries of western ABFT using modern histological techniques (**Table 3**). Baglin (1982) assessed the ovaries of 119 small and medium sized (<180 cm FL) Atlantic bluefin tuna caught in the Mid-Atlantic Bight (1974-1977) using histological analysis. The histological staging was based on the dominant oocyte stage present. Baglin (1982) found that all females aged 1 to 3 were immature. The majority of females aged 4 to 7 were also immature, but a few showed signed of initial oocyte development (early yolked oocytes undergoing resorption) in June. Two individuals aged 5 and 6 also sampled in June had fully yolked oocytes undergoing resorption, suggesting they were mature post-spawning. However, the relative number of atretic oocytes present was not given so it is unclear if these fish were truly post-spawning with significant atresia of yolked oocytes, or had initiated minor yolk development but had not spawned (i.e., 'simulation of gonad maturation"; Baglin 1982). It is also not clear whether Baglin (1982) specifically looked for late stage atretic bodies (brown bodies) in the ovaries of the small and middle-sized females, which if present would indicate they were mature. Baglin (1982) found large masses of brown bodies in the ovaries of giant bluefin sampled in March to October in the Gulf of Mexico, confirming they are present in ABFT ovaries.

Baglin (1982) concluded that the mid-Atlantic Bight was not a significant spawning area for ABFT in summer, although ovaries were only collected in June from fish aged ≤ 7. He also concluded that the North Atlantic was not an important spawning area in March-April based on the examination of ovaries from five giant ABFT; only one had yolked oocytes. Finally, Baglin (1982) concluded that age 6 would be the "earliest age at which a majority of females could possibly reach maturity". It is not clear how this conclusion was reached as only one of 27 females aged 6 or 7 had (atretic) yolked oocytes. As noted in section 2, the maturity ogive used in the stock assessment for western ABFT up until 2014 was based on the Baglin (1982) study. In the assessment, length at 50% maturity is assumed to be 190 cm FL, which was estimated at age 9 using the growth curve of Restrepo *et al.* (2011), but Baglin (1982) only examined fish <180 cm.

Goldstein *et al.* (2007) assessed the reproductive status of 195 large (≥185 cm curved fork length, CFL) ABFT caught in the Gulf of Maine in June to October 2000-2002. All were assumed to be aged ≥7 and all females were assessed as being mature based on histological analysis of ovaries. A fish 185 cm CFL would now be considered age ~8 using the growth curve of Restrepo *et al.* (2011) after converting curved FL (CFL) to straight FL (SFL). Briefly, the study found that females were either stage 1 (immature or resting adults) or stage 6 (early post-spawning). The authors note the difficulty in differentiating immature from resting females because the ovaries are similar, although identifying 'maturity markers' (see section 8.4) may have helped. They found that stage 1 females were caught in all months, and large fish (>235 cm CFL) were more likely to be stage 1. Given their size, these fish were probably resting females. The authors propose that these large females had migrated from the Gulf of Mexico spawning ground and had resorbed all yolked oocytes during the journey. Conversely they found that stage 6 early post-spawning females were only caught in June and July, and that small fish (185-235 cm CFL) were more like to be early post-spawning. The authors proposed that these fish were mature and must have migrated a shorter distance from their spawning ground, relative to the larger fish, because they had not fully resorbed all the yolked oocytes. This is a logical assumption and if it is the case, then it suggests there are spawning areas outside the Gulf of Mexico. However, as noted by Goldstein *et al.* (2007), the small fish may have developed vitellogenic oocytes but it is not known whether they spawned. Again, the presence of maturity markers in the ovary (such as muscle bundles) may help determine whether these fish were likely to have spawned or not. Another scenario to consider is that the large resting females may be of eastern origin and had time to resorb all yolked oocytes if they had migrated from the Mediterranean Sea. Genetic analysis of tissue samples would be useful to accurately identify eastern and western fish in the sample (see Puncher *et al.* 2018, and ongoing work).

Knapp *et al.* (2013) histologically examined 529 gonads collected in the NW Atlantic in 2004-2011 as part of a broad study also examining hormone levels in fish to assess maturity (see Heinisch *et al.* 2014, below). The gonads were collected from fish 107-292 cm curved fork length (CFL) and they were staged histologically following Goldstein *et al.* (2007). Forty percent of the gonads were from females and the smallest classed as mature was 157 cm CFL as it had extensive atresia of vitellogenic oocytes, suggesting it had been capable of spawning if conditions were favourable. It is not clear if other females were also mature as most of the ovaries examined contained early yolked oocytes (lipid stage), which may indicate early development but are not diagnostic for previous active spawning or that they would spawn in the coming season. A 157 cm CFL fish would be estimated to be age ~6 based on length (converted to SFL) and the growth curve Restrepo *et al.* (2011).

Knapp *et al.* (2014) examined 147 gonads from ABFT (172-326 cm CFL) collected in February to July 2007-2009 in the Gulf of Mexico. All were classed as mature using histological techniques. The smallest female analysed (172 cm CFL) was estimated age 7–8 and was actively spawning. The authors noted that young fish such as these would have been classified as immature based on the maturity ogive used at the time (knife edge 100% mature at age 8), and that the minimum age at maturity is younger than the age at 50% maturity estimated by Diaz (2007) and Diaz and Turner (2011). The study concluded that broader sampling was required to better determine the presence of smaller mature females spawning in the Gulf of Mexico, but did not recommend the need for representative sampling off the spawning grounds. Note that fish < 180 cm CFL were also classes as mature by Diaz (2007) and Diaz and Turner (2011) (see section 6.2 below).

Heinisch *et al.* (2014) reported on a new endocrine (hormone) approach developed to help better understand maturity schedules in western ABFT. The pituitary was removed from 10 fish caught in the Gulf of Mexico in May (>213 cm CFL), 119 fish caught in several locations in the NW Atlantic from June to September (134-292 cm CFL), and 17 Young of Year (YOY) fish caught off Virginia (26-37 cm CFL). The authors note that most fish sampled were likely to be from western origin based on previous work by Rooker *et al.* (2007, 2008). The study found that all fish ≥134 cm CFL had hormone levels < 0.4 (mean ratio of pituitary follicle stimulating hormone/leutinizing hormone, FSH/LH), which is similar to the spawning ABFT in the Mediterranean Sea. FSH/LH was significantly lower in fish ≥134 cm CFL than in the YOY, but were not significantly different between fish 134-184 cm CFL and ≥185 cm CFL. The authors conclude that all fish >134 cm CFL were mature and that these results do not support the age at maturity used in the stock assessment (knife edge at age 9). Based on the growth curve of Restrepo *et al.* (2011), a fish 134 cm CFL (~ 128 cm SFL) would be aged 4-5. Unfortunately, fish between 37 and 134 cm CFL were not available for hormone analysis to determine the minimum size that fish could be classed as mature based on hormone levels.

Histological analysis of gonads from the sampled fish was also undertaken by Heinisch *et al.* (2014). The authors found that most females were in a perinucleolar stage and they detected no histological differences between females of different sizes. However, using recent histological classification schemes of Brown-Peterson (2011) or Farley *et al.* (2014), it may be possible to confirm whether all females ≥134 cm CFL were mature (i.e., had spawned) based on the presence of maturity markers in the ovaries (see section 8.4), thus confirming (or otherwise)

the results of the endocrine approach. **Figure 2** (bottom left) in Heinisch *et al.* (2014) indicates that maturity markers are present in western ABFT ovaries (muscle bundles and brown bodies; 205 cm CFL). By comparison, maturity markers do not seem to be apparent in the ovary of the smaller fish depicted in **Figure 2** (top right), although the image resolution is not sufficient to be certain. If validated, the endocrine approach could be used to estimate a maturity ogive for western ABFT if a well-designed, length stratified, sampling program can be developed outside spawning areas (the Gulf of Mexico or elsewhere). However, the two approaches may provide different results. The endocrine approach may indicate that a fish is mature but does it have the resolution to determine whether the fish is mature and had spawned? Histological analysis of ovaries can provide evidence of previous spawning activity (see section 8.4) and are easier to collect than the pituitary.

6.2   *Spawning fraction at age*

Diaz and Turner (2007) developed an indirect method to estimate a maturity ogive for western ABFT using catch data from the Gulf of Mexico. The approach was based on estimating the proportion of the stock that had recruited onto the spawning ground by comparing the age distribution of the commercial catch on the spawning ground catch with the age distribution of the population estimated using "catch curve" analysis. Diaz and Turner (2007) used catch data from the Japanese (1974-1981) and the US (1981-2005) longline fleets in the analysis. Fork length of fish was estimated from round weight data using agreed conversion factors. The lengths were then converted to ages using cohort slicing and the growth curve of Turner and Restrepo (1994). The method assumes that all fish caught in the Gulf of Mexico are mature and that the age distribution of the longline catch represents the age distribution of the spawning population. Although rare, fish <180 cm were classes as mature based on this approach as they were caught on the spawning ground. Age at 50% maturity was estimated at 11.8 years.

Diaz (2011) revised the analysis of Diaz and Turner (2007) by using just the Japanese catch data and the growth curve of Restrepo *et al.* (2011). The authors note that the Japanese fishery catch larger fish than the US possibly due to gear differences or age truncation due to fishing pressure since the US was catch fish at a later period than the Japanese (Diaz 2011). Age at 50% maturity was estimated higher at 15.8 years.

Porch and Hanke (2017) updated the work of Diaz (2011) for use in the 2017 stock assessment. The catch data used in the analysis was obtained from the US and Mexican fisheries operating in the Gulf of Mexico and nearby waters (2009-2014). To avoid the cohort slicing approach to convert length to age, otolith-based age estimates were also obtained from fish caught by the US fleet in the same years (n=198). The size range of aged fish was smaller than for the whole catch. To account for this, the age data was weighted so that the two length compositions were comparable. The length classes were aggregated into 11 bins, which included a bin for all lengths <190 cm FL. Given there were very few age estimates for these sized fish, the age distribution estimates for small fish in the catch will be more uncertain. The youngest fish aged was 8, but fish as small as ~140-160 cm were caught and could have been aged 6-7. Despite this, the age distribution of the catch (assumed to represent the age distribution of the spawning population in the Gulf of Mexico) was compared to the age distribution for the stock derived from the 2014 base case stock assessment (see the paper for full details involving plus-groups). A "spawning fraction ogive" was estimated with 0% at age ~7, 50% at age ~10, and 100% at age ~13. Given that the 2014 stock assessment may use data from both eastern and western stocks of ABFT, the analysis was repeated using data from a "western only" assessment of Cadrin *et al.* (2017) which had a higher relative abundance of juveniles. This gave a flatter ogive with 50% spawning fraction at age ~11 years.

Porch and Hanke (2017) noted several assumptions:

1)   selectivity by the fishery is directly proportional to residency time of fish-at-age on the spawning ground,
2)   lengths and ages are estimated accurately,
3)   the age distribution of the population is well estimated by the assessment model, and
4)   the Gulf of Mexico (and surrounding waters) is the main spawning ground for western ABFT, and/or the age and size distribution of fish analysed is representative of fish spawning in spawning locations.

The authors specifically highlight the possibility of spawning activity off Cuba, and that there is some evidence that fish younger than those present in the Gulf of Mexico may spawn elsewhere (Lutcavage *et al.* 1999, Richardson *et al.* 2016). The potential of additional spawning sites needs to be investigated.

It is also important to recognise that the "spawning fraction ogive" is not a maturity ogive *per se*, and cannot be directly compared to the maturity ogive of Corriero *et al.* (2005) based on histological data from the Mediterranean Sea. As the spawning fraction ogive uses catch data from the spawning ground, in principle it accounts for the possibility that there may be size/age related differences in spawning duration (residency on the spawning ground)

in addition to size/age differences in proportion mature. For example, small fish may spend less time on the spawning ground and are less likely to be caught compared to larger fish. It also accounts for the possibility that some fish may skip spawning and are not present on the spawning ground at all in a given year.

However, as noted in section 3.2, duration on the spawning ground does not necessarily equal spawning duration. The method does not account for the possibility that spawning frequency (the number of time a fish spawns while on the spawning ground) or batch fecundity varying with size and/or age. The method also assumes that all fish caught on the Gulf of Mexico are mature and spawning. This needs to be validated using histological analysis of the gonads. Other factors that need to be considered are whether there is depth partitioning by size on the spawning ground, as identified for southern bluefin tuna (Davis and Farley 2001). Finally, the spawning fraction ogive is combined for both sexes as males and females are not separated in the catch data. Since males may mature at younger ages than females, the ogive will be biased if the stock assessment requires a female only maturity ogive.

6.3   *Batch fecundity and spawning frequency*

The fecundity estimates of Baglin (1982) for eastern ABFT are incorrect as they were based on the number of yolked oocytes (diameter >0.32 mm and >0.44 mm) present in the ovary.

As discussed in section 5.3, Knapp *et al.* (2014) examined ovaries from ABFT collected in the western Mediterranean Sea and the Gulf of Mexico using histological methods. They found that daily spawning fraction and relative batch fecundity were both smaller in the Gulf of Mexico suggesting fecundity (spawning output) may be lower for western Atlantic ABFT. Although Knapp *et al.* (2014) noted the difficulty in obtaining representative samples of gonads in the Gulf of Mexico to accurately estimate reproductive parameters.

7.   **Maturity ogives for other bluefin species**

7.1   *Pacific bluefin tuna*

The spawning grounds of Pacific bluefin tuna are thought to be two main areas, Nansei area and Sea of Japan (Ashida *et al.* 2015, Okochi *et al.* 2016, Ohshimo *et al.* 2018). Recently, a new spawning ground in the Sanriku area was detected (Ohshimo *et al.* in press). The predominant age classes in the Sea of Japan, Sanriku and Nansei are 3-6, 7-9 and over 10, respectively (**Figure 5**). This indicates the spawning habitats of Pacific bluefin tuna are segregated by age/size.

The reproductive characteristics of Pacific bluefin tuna are reported in the Sea of Japan by Okochi *et al.* (2016) and Ohshimo *et al.* (2018), in the Sanriku area by Ohshimo *et al.* (in press) and in the Nansei area by Ashida *et al.* (2015) and Shimose *et al.* (2018). Ohshimo *et al.* (2018) calculated the proportion mature by length based on the analysis of number of 6767 ovaries collected in the Sea of Japan. The annual proportion mature was calculated by 5 cm FL bins based on classification of the development of the oocyte. The maturity ogive estimated using a logistic function is shown in **Figure 6**. Ohshimo *et al.* (2018) reported that the annual proportion mature at length varied annually from 100-130 cm FL (age 2-4). This indicates that accurate estimation of the proportion mature requires substantial numbers of samples, in particular from small sized fish.

The maturity ogives estimated for Pacific bluefin in the Sea of Japan and the Pacific are quite different (**Figure 7**). The length at 50% maturity was smaller in the Sea of Japan compared to the Pacific, indicating spatial differences in the proportion mature by length/age for Pacific bluefin.

The relative batch fecundities among the three spawning grounds, the Sea of Japan, Sanriku and Nansei area were reported by Ohshimo *et al.* (in press). The mean relative fecundity in the Sea of Japan was 52.6 ± 20.2 (mean ± SD) eggs/gram of body weight. In comparison, previous studies have found mean fecundity values of 56.4 ± 37.9 in the Nansei area (Ashida *et al.* 2015) and 109.8 ± 34.0 in the Sea of Japan (Okochi *et al.* 2016). The results from the Sea of Japan were significantly different, but did not differ significantly from those reported in the Nansei area (p = 0.956) (Ohshimo *et al.* in press). This indicates that both percent mature and relative batch fecundity of Pacific bluefin tuna different among size/age classes and areas. The differences could be caused by physiological or environmental differences.

7.2 Southern bluefin tuna

Southern bluefin tuna (SBT) is the smaller cousin of Atlantic and Pacific bluefin tuna, reaching a maximum length of only ~220 cm FL. Reproductively mature SBT migrate to a single spawning ground between the Indonesian archipelago and the north-west coast of Australia between September and April, (Farley and Davis 1998), which is substantially longer than the spawning seasons for eastern and western ABFT. Only adults ~150-190 cm FL (~8-30 years) are caught on the spawning ground and individuals quickly migrate from the area after spawning, some as early as October (Farley and Davis 1998). The waters of the Southern Ocean are important winter feeding grounds for sub-adult and adult SBT.

Monitoring the catch of SBT on the spawning ground began in the early 1990s and size data and otoliths have been collected annually since that time. In addition, two large biological sampling programs have been completed with over 1,115 ovaries collected and histologically analysed. Using these data, knowledge of the size/age structure of the spawning stock as well as reproductive dynamics and maturity of SBT have increased greatly.

Davis and Farley (2001) found that SBT segregate by size and depth on the spawning ground. They discovered this using a novel approach to estimate fishing depth for Indonesian longline vessels, that catch SBT as a bycatch, based on the theory that bigeye are generally caught deeper than yellowfin. A bigeye (BE) index was calculated for each landing as: weight of bigeye / (weight of bigeye + yellowfin). If the BE index was high, the fishing depth was assumed to be deep (and vice versa); the index was used as a proxy for fishing depth. By determining the BE index of 15,882 Indonesian landings, and examining the size distribution of SBT in those landings, Davis and Farley (2001) were able to show that small SBT were more abundant in deep-set catches and larger fish were more abundant in shallow-set catches. Davis and Farley (2001) used data from Farley and Davis (1998) (below) to show that the size partitioning by depth was related to spawning activity patterns. It appears that large fish spend proportionally more time spawning, or are more likely to spawn, while on the spawning ground, than small fish. Since spawning occurs at the surface, the larger fish are more likely to be caught in the shallow longline sets.

Histological analysis of ovaries showed that SBT are capable of spawning daily and release an average of 6.5 million oocytes per spawning event (Farley and Davis 1998, Farley et al. 2015). The relationship between estimated batch fecundity and fish length was highly variable. However, analysis of ovary weight data from females caught before and after spawning showed that relative batch fecundity increases with fish length (Farley et al. 2015). It was also shown that SBT cycle in and out of spawning bouts. On average, a 150-cm female spawned sequentially for 3.6 days and then rested for 1.3 days. By comparison, a 190-cm female spawned sequentially for 6.9 days and then rested for 2.2 days (Farley et al. 2015). The length of time that females remain on the spawning ground (cycling through spawning and non-spawning episodes) as a function of fish size has not been directly determined for SBT, although there is evidence to suggest that small fish spend less time on the spawning ground. If residency time was known, it would be possible to determine potential annual fecundity as a function of length.

The size and age that SBT mature is uncertain. Estimates of the mean length/age at 50% maturity have converged at between 152-162 cm and between 11-12 years old (Davis et al. 2001). In 2013, the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) adopted a new maturity ogive to estimate spawning potential by age (Anon 2013). The ogive was estimated in a similar way to the western ABFT spawning fraction ogive using catch data from the spawning ground (i.e., Porch and Hanke 2017; see section 6.2). However for SBT, the length frequency data of fish caught on the spawning ground was compared with that from the Japanese longline catch off the spawning ground (Davis et al. 2001). Length at 50% maturity was estimated for the years 1995 to 2000 to be between 158 and 163 cm FL (~11–12 years).

Over the last few years, a new collaborative sampling program by Commission for the Conservation of Southern Bluefin Tuna (CCSBT) members has begun across the SBT feeding grounds to collected ovaries from the size range of females that include both immature and mature fish. Ovaries have been collected after the spawning season when mature and immature fish will be mixed. Histological analysis of the ovaries will follow the methods of Farley et al. (2014) where 'maturity markers' will be used to differentiate immature from mature-resting female SBT to provide a basis to obtain an estimate of proportion mature. The method has required a broad sampling program to allow for spatial variation in maturity-at-length/age to be accounted for in the maturity estimation models.

8. Summary and recommendations

8.1 Summary

Estimating an accurate maturity ogive is complicated for a species such as ABFT as the mature fish migrate to particular areas to spawn and the immature fish do not (such as the Gulf of Mexico) or are present in lower numbers than their true abundance in the population (such as the Mediterranean Sea). A maturity ogive based on histological analysis of gonads collected only from spawning areas will be biased towards mature females, while a maturity ogive based on analysis of gonads collected off the spawning ground during the spawning season would be biased towards immature females (as mature females will be on the spawning ground).

Different methods are used to estimate maturity parameters for eastern and western ABFT. It is clear from this review that both methods required validation.

8.2 Eastern Atlantic bluefin tuna

The only study to estimate a maturity ogive for the eastern stock of ABFT was by Corriero *et al.* (2005). They used the histological assessment of ovaries to determine the maturity status of females in the Mediterranean Sea. The estimate of L_{50} (103.5 cm FL) is less than the minimum size that mature females were sampled in other studies (~119-116 cm FL) and slightly less than used in the stock assessment for the eastern stock ($A_{50} = 4$, $L_{50} = 115$ cm). The maturity ogive of Corriero *et al.* (2005) may be biased towards mature females if the sampling regime did not cover the full spatial (and depth) range of immature and mature females during the spawning months. It has been shown that sub-adults occur outside the Mediterranean Sea and may not return until mature. Even within the Mediterranean Sea, there is evidence to suggest spatial structuring of sub-adults and adults occurs, which may result in a biased maturity ogive if not accounted for in sampling and, or, the analysis. If there was sufficient data, it would be worthwhile to re-examine the data from Corriero *et al.* (2005) to evaluate if spatial variation in proportion mature by length exists within the Mediterranean Sea. This would provide additional information on the accuracy of the maturity ogive based on the pooled data. This, however, does not account for the proportion of sub-adults that do not return to the Mediterranean Sea until mature. These fish must be included in a maturity ogive for the eastern stock.

The extent of the bias in the maturity ogive currently used in the eastern assessment is unknown and would require direct validation via a well-designed length-stratified sampling program using current histological techniques (see below). The assessment model also assumes all females contribute equally (per unit of spawning stock biomass) to egg production. This assumption is unlikely to be valid given fecundity varies with length and age. Length/age based reproductive parameters (batch fecundity, spawning frequency, and spawning duration) would be required to estimate reproductive potential.

8.3 Western Atlantic bluefin tuna

There are no direct reproductive studies (i.e., based on the analysis of biological samples) that have estimated a maturity ogive for the western stock of ABFT. All studies to date have identified a minimum size/age that a fish may be mature rather than estimates of mean length at maturity (L_{50}). This includes the work by Baglin (1982), which formed the bases of the knife-edge maturity ogive used in assessments up until 2014. The reason for this is due to the migratory nature of Atlantic bluefin and the difficulty in obtaining representative biological samples (e.g., ovaries for histological analysis or tissue for hormone analysis) from mature and immature females in proportion to their abundance in the population.

Heinisch *et al.* (2014) examined hormone levels in ABFT pituitary and concluded all fish ≥ 134 cm were mature. If the methods can be validated/corroborated via histological analysis of ovaries (i.e., confirm that the fish spawn), then the approach may be useful in the future to estimate a maturity ogive, assuming a well-designed length-stratified sampling regime can be established that encompasses the full spatial range of both immature and mature fish. However, further work would be required to collect samples and examine the hormone levels of fish in the size range (37 to 134 cm) that were not available in the Heinisch *et al.* (2014) study. It is also unclear how definitive an endocrine approach would be in determining whether a fish is mature and had spawned. A histological approach would be a more dependable option at this stage.

In 2017, the stock assessment for western ABFT used the spawning fraction ogive of Porch and Hanke (2017), which was based on the analysis of longline catch data in the Gulf of Mexico ($A_{50} = \sim10$). A similar method has been used for SBT (see section 7.2). The accuracy of the ogive is uncertain, given that ogives estimated by Diaz and Turner (2007) and Diaz (2011), using similar approaches but different catch data, gave higher estimates of A_{50} (12-16 years). Direct validation of the ogive is required (see below) given the number of assumptions made including the possibility that young ABFT may spawn outside the Gulf of Mexico. It would also be important to confirm that all fish caught are mature and spawning. Until an independent maturity ogive can be obtained, we suggest conducting further analyses of available catch data in the Gulf of Mexico to evaluate possible effects of area and depth of fishing, and sensitivity to alternative selectivity assumptions. We also suggest additional direct age data is obtained for fish <190 cm FL as very few age estimates for these sized fish were available for analysis by Porch and Hanke (2017). Also note that the spawning fraction ogive is for males and females combined, rather than a female only ogive that is generally used in stock assessment models.

8.4 An independent maturity ogive

It may be possible to obtain a representative maturity ogive for each ABFT stocks through a well-designed, length stratified, gonad (and hard-parts) sampling program on feeding grounds when both immature and mature females are present. Ideally, sampling should occur over the entire spatial range of each stock when mature fish have migrated back to feeding grounds following the spawning season. Although both stocks mix on the feeding grounds in the Atlantic, genetic analysis of tissue from sampled fish could be used to accurately identify eastern and western fish post hoc (see Puncher *et al.* 2018, and ongoing work). Sampling should also occur on feeding grounds in the Mediterranean Sea outside the spawning season.

A difficulty arises when trying to differentiate immature from mature-resting (post-spawning) females during the non-spawning months as the ovaries look similar histologically (Schirripa 2011, Knapp *et al.* 2013, Heinisch *et al.* 2014). Based on recent work on other tunas and swordfish, there are strong arguments that it will be possible to differentiate immature and mature-resting ABFT females in these samples based on the presence of maturity markers, such as late stage atresia (delta or gamma atresia/'brown bodies') and muscle bundles (Farley *et al.* 2013, 2014, 2016, 2017). The method should also account for the presence of sexually mature females that 'skipped' spawning, as the ovaries will appear immature (no histological evidence of recent reproductive activity). The method is currently being applied to SBT (see section 7.2).

Late stage atresia has been identified in ABFT ovaries by Baglin (1982), Corriero *et al.* (2003) and Corriero *et al.* (2005). For western ABFT, preliminary work could be undertaken now using existing histological sections of ovaries collected in the NW Atlantic feeding grounds (e.g., Goldstein *et al.* 2007, Knapp *et al.* 2013, Heinisch *et al.* 2014) to provide the proof-of-concept for the method. If successful, it may be possible to validate the endocrine-based results of Heinisch *et al.* (2014) by direct comparison of results for individual fish if it possible to collect sufficient samples sizes across the appropriate size range.

If spatial variation exists in the proportion mature at age for either stock, then methods need to be developed to account for this variation, possibly by weighting the data by the abundance in each region before they are combined into a single maturity ogive for each stock (e.g., following Farley *et al.* 2014)

8.5 Reproductive potential at length/age

As mentioned previously, SSB is calculated as numbers-at-age x weight-at-age x proportion mature (or proportion spawning) at age, and is often used as a proxy for reproductive potential. However, SSB does not account for variability in egg production (fecundity) among fish of different sizes or ages. Accurate size/age-based estimates of batch fecundity, spawning frequency and spawning duration are, therefore, required to estimate reproductive potential.

Size/age based estimates of batch fecundity or spawning frequency-at-age are not available for either stock of ABFT to estimate true reproductive potential-at-age. Mean estimate of batch fecundity and spawning frequency are available (Medina *et al.* 2002, 2007, Knapp *et al.* 2014), however, there were large differences between studies and depth of sampling, indicating further work is needed. Estimates of average duration on the spawning ground (or number of spawning events) could, in principal, be obtained from electronic tagging studies.

9. Acknowledgements

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Table 1. Proportion of fish contributing to spawning output used in the 2017 stock assessment for western and eastern stocks of Atlantic bluefin tuna.

Age	2	3	4	5	6	7	8	9	10	11	12	13	14	15
W	0.000	0.000	0.000	0.000	0.001	0.007	0.039	0.186	0.563	0.879	0.976	0.996	0.999	1.000
E	0.000	0.250	0.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 2. Estimates of length/age at maturity (minimum or L/A_{50}) from recent studies on eastern ABFT. SFL = straight fork length.

Study	Area	Analysis method	N	Size range (cm) SFL	Parameter	Length (cm) FL	Age
Tawil *et al.* (2002)	Libya	Ovary histology	21	115-172	Minimum size/age at maturity	115	4
Medina *et al.* (2002)	Barbate and Balearic Islands	Ovary histology	84	116-?	Minimum size at maturity	116	
Corriero *et al.* (2003)	West Med.	Ovary histology	131	63-236	Minimum size at maturity	110	
Karakulak *et al.* (2004)	East Med.	Ovary histology	50	109-226	Minimum size at maturity	109	
Corriero *et al.* (2005)	Med.	Ovary histology	501	~55-270	Maturity ogive (L_{50})	103.6	
Knapp *et al.* (2014)	West Med.	Ovary histology	40	120-240	Minimum size at maturity	120	

Table 3. Estimates of length/age at maturity (minimum or L_{50}/A_{50}) from recent studies on western ABFT. CFL = curved fork length. *indicates age estimated from length using growth curve of Restrepo *et al.* (2011), where CFL was converted to SFL if required using conversion from ICCAT (2000).

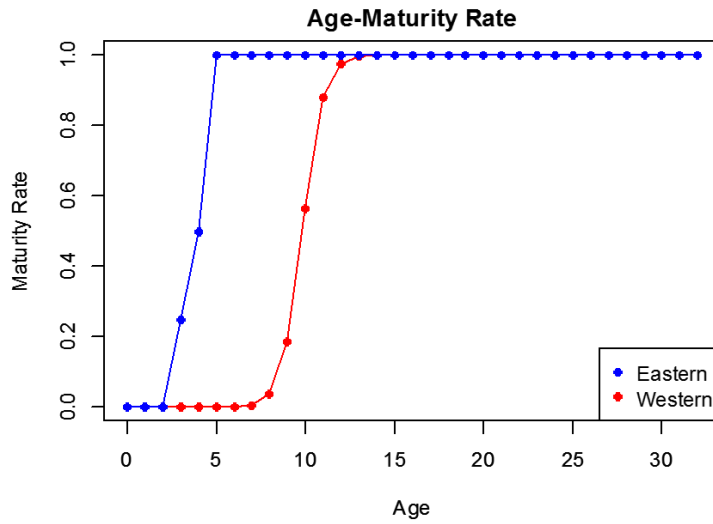| Study	Area	Analysis method	N	Size range (cm) CFL	Parameter	Length (cm) CFL	Age
Baglin (1982)	NW Atlantic	Ovary histology	119	<180	Minimum age at maturity		~6
Goldstein *et al.* (2007)	Gulf of Maine	Ovary histology	195	185-235	Minimum size/age at maturity	185	~8*
Knapp *et al.* (2013)	NW Atlantic	Ovary histology	206	107-292	Minimum size/age at maturity	157	6*
Knapp *et al.* (2014)	Gulf of Mexico	Ovary histology	~59	172-326	Minimum size/age at maturity	172	7*
Heinisch *et al.* (2014)	NW Atlantic and Gulf of Mexico	Hormone levels	110	134-292	Minimum size/age at maturity	134	4-5*
Diaz and Turner 2007	Gulf of Mexico	Catch data			Spawning fraction ogive (A_{50})		11-12
Diaz 2011	Gulf of Mexico	Catch data			Spawning fraction ogive (A_{50})		15.8
Porch and Hanke 2017	Gulf of Mexico	Catch data			Spawning fraction ogive (A_{50})		10

Figure 1. Proportion of fish contributing to spawning output by age vectors used in the 2017 stock assessment for western and eastern stocks of Atlantic bluefin tuna.
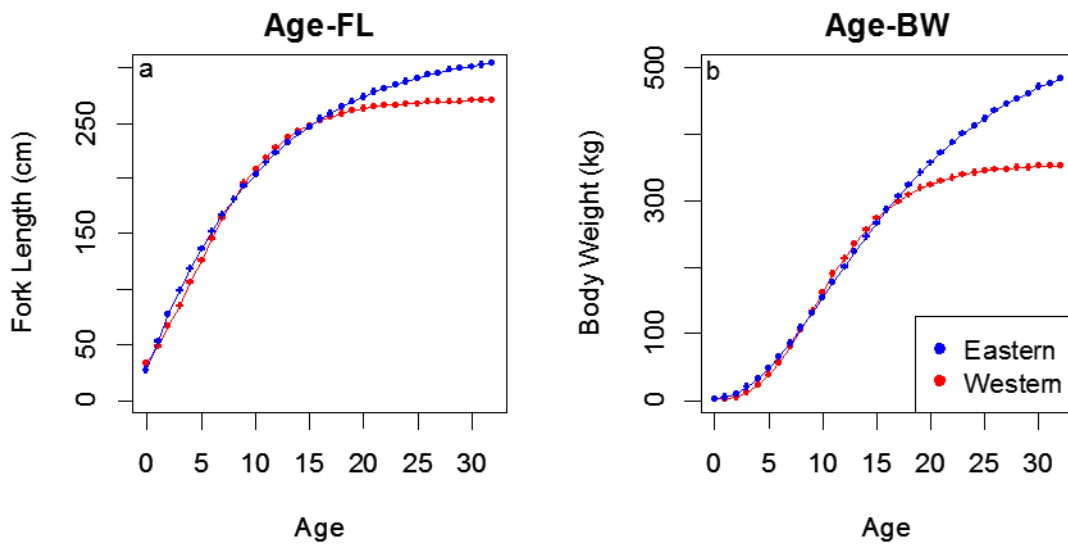

Figure 2. Relationships between age and (a) fork length and (b) body weight currently used for western and eastern stocks of Atlantic bluefin tuna.
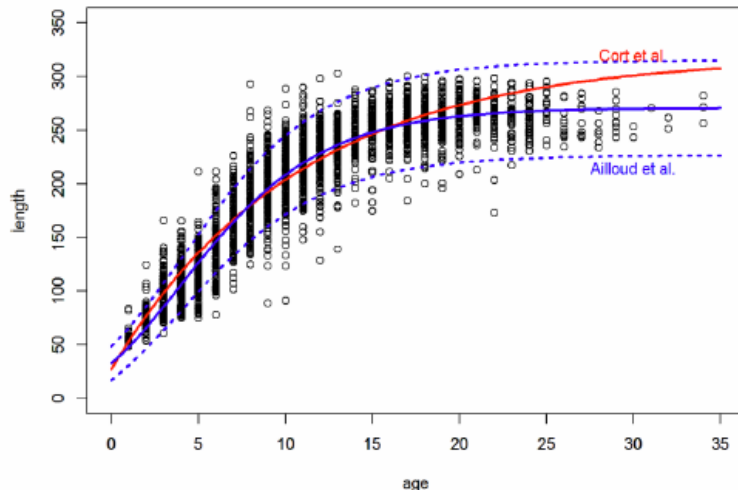
Figure 3. Western otolith data plotted against the growth curves from the Ailloud *et al.* (2017) analysis (solid blue) and the Cort 1991 analysis (solid red). The dashed blue lines represent the 2.5 and 97.5 percentiles of the distribution of the fitted length at age from the Ailloud *et al.* (2017) analysis. Source: Anon 2017b.
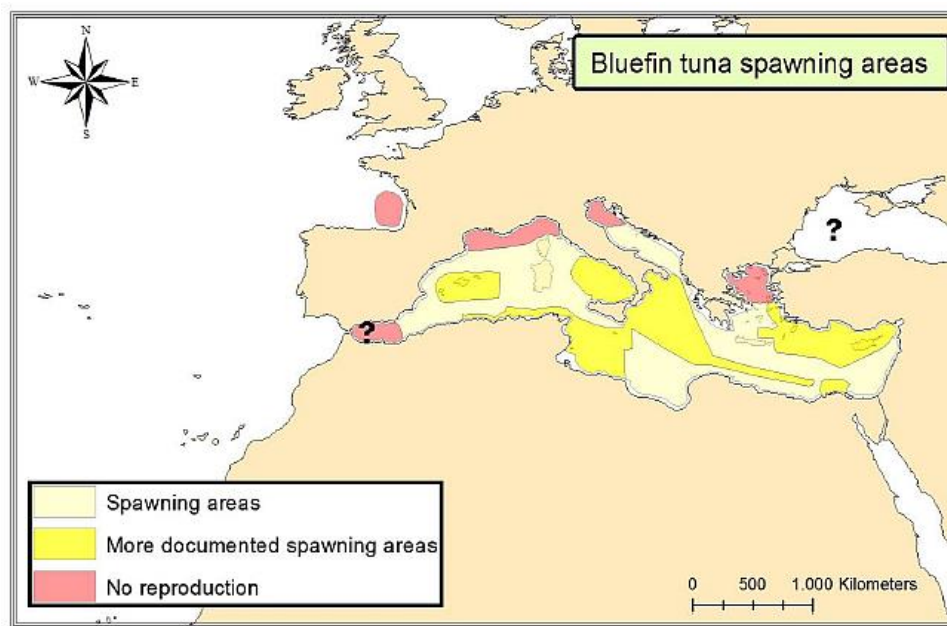

Figure 4. Eastern Aatlantic bluefin tuna spawning and non-spawning areas in the Mediterranean Sea. Source: Piccinetti *et al.* (2013)
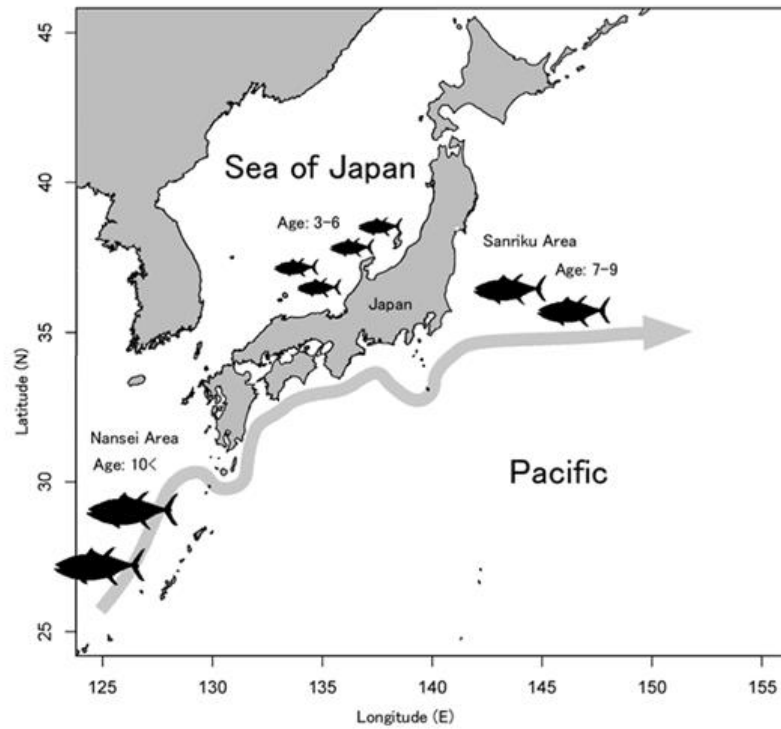
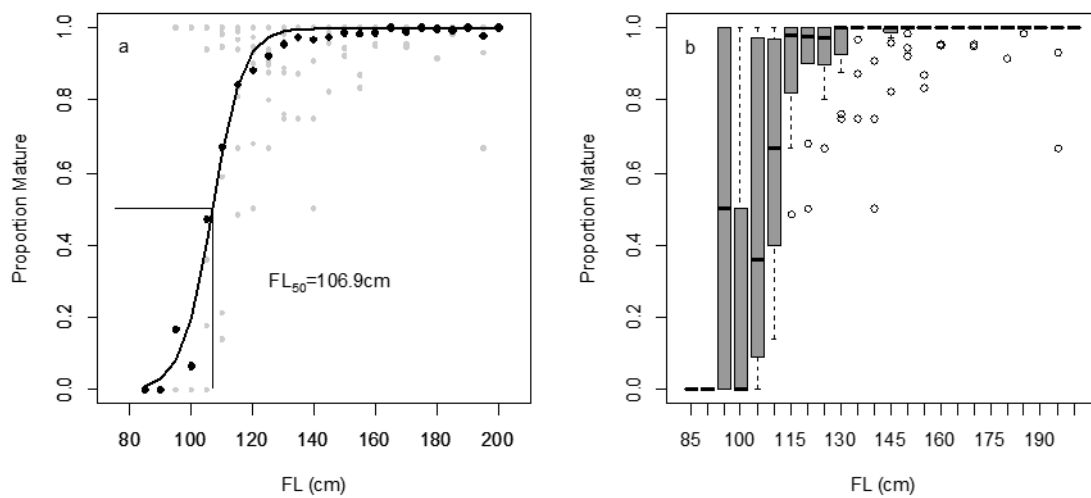**Figure 5.** Spawning habitats of Pacific bluefin tuna.

Figure 6. Maturity schedule for female Pacific bluefin tuna in the Sea of Japan. (a) The solid line and curve indicates the FL at 50% mature and maturity ogive, respectively. Black and grey dots represent mean values (1994–2015) and annual values, respectively. (b) Annual variation of the proportion of mature every 5 cm bins. Box plots show median values (solid horizontal lines), 50th percentile values (box outline), 90th percentile values (whiskers), and outlier values (open circles). Source: Ohshimo *et al.* (2018).
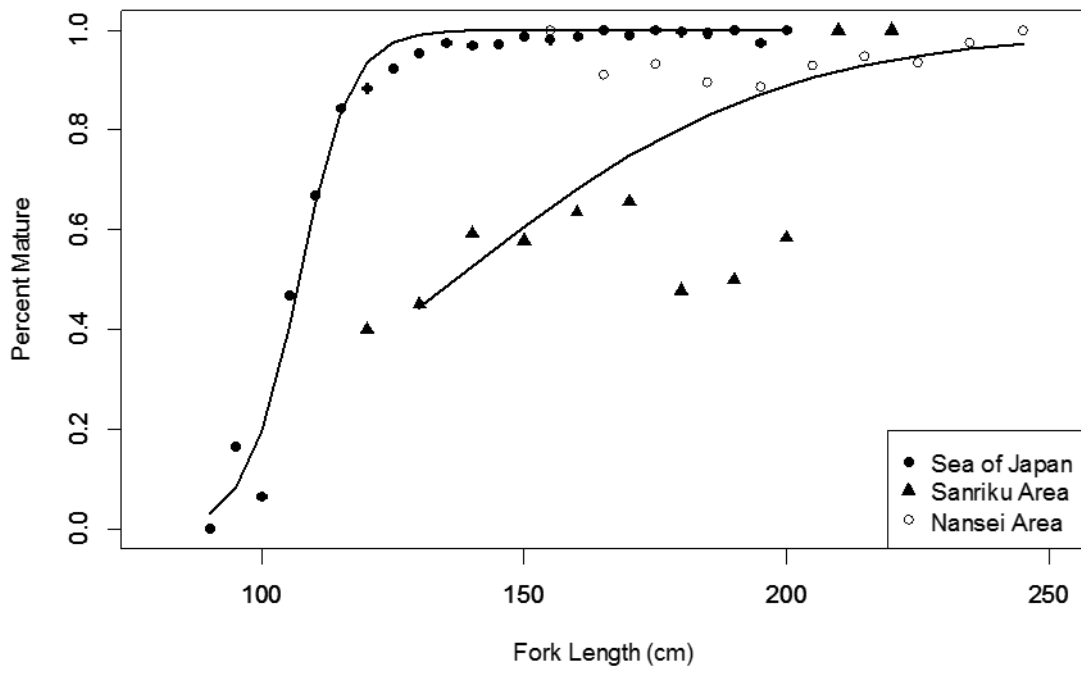
Figure 7. Calculated maturity ogives for Pacific bluefin tuna in the Sea of Japan and the western Pacific (Sanriku and Nansei areas).