DATE OF MARINE ANNULUS FORMATION IN ATLANTIC SALMON (*Salmo salar*) AND IMPLICATIONS FOR RETROSPECTIVE GROWTH ANALYSES USING SCALES

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Abstract

Scales have increasingly been used to quantify annual and seasonal growth trends and in efforts to link growth to environmental conditions. Understanding the timing of formation of an annulus (a group of narrowly spaced circuli) is critical when assessing the influence of marine ecosystem conditions on seasonal growth patterns of Atlantic salmon, yet the literature does not provide consistent answers regarding the timing or drivers of marine annulus formation. We demonstrate a novel method for estimating stock-specific annulus formation timing based on marked individuals with known emigration and return/recovery dates. We applied an equation to estimate the date of annulus completion for Atlantic salmon (*Salmo salar*) using known dates, the number of circuli after the most recent annulus, and marine circulus deposition rate. We tested five marine circulus deposition rate scenarios, some of which accounted for individual, seasonal, and age-related variability, and others which use previously published marine circulus deposition rates. Based on these results, we present an argument to reconsider the practice of assigning annulus formation dates to winter solstice in favor of dates estimated by a scenario that accounts for individual, seasonal, and age-related variation in circulus deposition. This scenario suggests that annulus formation occurs between mid-February and late March. In this case, the annulus would be formed during the coldest part of the year in the primary overwintering area for North American Atlantic salmon.

Key Words: Atlantic salmon, annulus completion date, Carlin tag, circulus deposition rate

1 Introduction

Scales can provide a comprehensive record of growth over the life of individual fish. For fish with leptoid scales, such as Atlantic salmon (*Salmo salar*), the scale radius increases approximately linearly as the fish grows in length (Fisher & Pearcy, 1990; Fukuwaka & Kaeriyama, 1997; Panfili *et al.*, 2002). As the radius increases, circuli (mineralized ridges that appear as concentric rings) are deposited, and the interaction between the scale's growth rate and circulus deposition rate determines the spacing between circuli (Thomas *et al.*, 2019). Bands of widely spaced circuli are thought to indicate periods of fast somatic growth when circulus deposition is relatively slow compared to scale radius growth (Fisher & Pearcy, 1990; Friedland *et al.*, 2000). In contrast, bands of narrowly spaced circuli (also known as annuli) are thought to indicate periods of slow somatic growth when circulus deposition is relatively fast compared to scale radius growth (Fisher & Pearcy, 1990; Shearer, 1992).

The record of a fish's growth experience documented on the scale is used for a variety of scientific and management purposes. For Atlantic salmon, intercirculi spacing patterns are interpreted to determine the age of a fish and can be used to distinguish growth experiences in freshwater and marine habitats (ICES, 2011). The scale growth record is also used for investigating relationships between growth and ecosystem conditions (Jensen *et al.*, 2012; Peyronnet *et al.*, 2007). However, linkage of growth and variable conditions such as temperature is often constrained because circuli are not deposited at a constant rate and periodicity is not well understood (Thomas *et al.*, 2019), making it difficult to assign a date to the deposition of circuli (Todd *et al.*, 2014). The annulus is one of a few highly distinguishable growth markers that could potentially be tied to a specific time in the life cycle of an Atlantic salmon. However, a limited understanding of when and why annuli form complicates the direct linkage of growth trends with spatially and temporally specific ecosystem conditions experienced by an individual salmon. Understanding the relationship between annulus formation and time is critical for assessing the influence of changing oceanographic and ecosystem conditions on growth patterns of Atlantic salmon during their marine life stage, yet at present, the literature does not provide consistent answers to questions regarding the timing or drivers of annulus formation.

A few studies on Pacific salmon have addressed annulus formation timing using observational data from salmon caught at sea. Chinook salmon (*Oncorhynchus tshawytscha*) caught throughout the year in coastal waters of Washington had mostly completed annulus formation by March or April, but very few had completed annulus formation in January or February (Hyun *et al.*, 1998). Pink salmon (*O. gorbuscha*) in Russia formed an annulus in early spring after a period of arrested growth in winter (Vedensky, 1954 as referenced by Fukuwaka, 1998). Based on scales collected by research vessels throughout the Gulf of Alaska in winter, chum salmon (*O. keta*) completed their annulus in February or March (Bilton & Ludwig, 1966). The same study found that sockeye (*O. nerka*) and pink salmon likely completed their annulus in December or January (Bilton & Ludwig, 1966). Barber and Walker (1988) cited Bilton and Ludwig's findings for December annulus formation of sockeye salmon and concluded that annulus formation must be initiated by photoperiod because regional sea surface temperatures do not reach a minimum until April. The assumption that photoperiod drives annulus formation resulted in assignment of winter solstice as an approximate annulus completion date (Barber & Walker, 1988). Despite a lack of similar observational datasets for Atlantic salmon, the assertion that annulus formation is associated with a period of decreasing photoperiod has been widely perpetuated in the Atlantic salmon literature as well (Friedland *et al.*, 2009b; Hubley *et al.*, 2008; Jensen *et al.*, 2011; Todd *et al.*, 2014). As a result, annulus formation for Atlantic salmon is commonly assigned to winter solstice (Friedland *et al.*, 2009a; Hogan & Friedland, 2010; Izzo & Zydlewski, 2017; Todd *et al.*, 2014) without species-specific evidence for this conclusion.

Some studies have attempted to partition growth into seasonal or monthly increments to link growth to finer-scale environmental or population trends (Friedland *et al.*, 2009a; Hogan & Friedland, 2010; Izzo & Zydlewski, 2017; McCarthy *et al.*, 2008). When attempting to align sub-annual growth patterns with environmental trends such as sea surface temperature, it becomes important to understand the timing of scale growth transitions, specifically the shift from faster to slower growth. Using the assumed annulus formation date, winter solstice, to anchor temporal alignment of sub-annual growth patterns may lead to mismatch between scale increments and environmental metrics, further obscuring these potentially important relationships.

Our objective was to use data from marked individuals to produce region- and species-specific estimates of marine annulus formation timing for Atlantic salmon based on ecological principles and growth markers of individual scales and establish a methodology that could be applied to other marked stocks across the species' range. Whereas previous studies have relied on population-level data to make inferences about marine circulus deposition rate and associated time periods (Hubley *et al.*, 2008), uniquely marked individuals with known emigration and return/recovery dates provide a novel opportunity to gain direct insight into marine circulus deposition rate (Todd *et al.*, 2014) and timing of annulus formation. Maine's historical Carlin tag release/recapture dataset (described in Miller et al., 2012) consists of release records of Carlin-tagged smolts since the early 1960s and subsequent marine and freshwater recapture throughout their migratory range. We use this dataset, one of few for which marine emigration dates are known, to provide new insights into marine circulus deposition rate and annulus formation timing. We believe this information is critical for future work linking seasonal growth dynamics and environmental trends, and we discuss how differential assignment of annulus formation date may shape inferences about environmental conditions experienced by Atlantic salmon.

2 Materials and methods

2.1 Carlin tag mark-recapture dataset

Approximately 1.5 million tagged Atlantic salmon smolts were released in Maine rivers between 1966 and 1996. Smolts were reared at federal hatchery facilities in Maine USA, and broodstock were sea-run adults captured in Maine rivers. Smolts were tagged with Carlin tags and released into rivers during late March or early April. Telemetry studies show that hatchery-reared smolts begin emigration almost immediately after release into the river and reach marine waters within 200 hours (Renkawitz *et al.*, 2012), so release date is approximately equivalent to marine emigration date. Tagged individuals were recaptured at various points during their migration, and when possible, length, weight, and scale samples were collected and archived. Recaptures can be described using two categories: at-sea recaptures in distant waters and homewater recaptures in natal rivers. At-sea recaptures occurred primarily in coastal, nearshore fisheries in Canada and Greenland. We limited our sample to individuals for which a scale sample was available, that were recaptured during their second summer at sea, and had formed a first marine annulus (n=1,310). Homewater recaptures are adult returns captured in fishway traps or by recreational fishers in natal rivers. We limited our sample to 2SW individuals for which a scale sample was available (n=193) and coincidentally, all eligible samples were collected in Penobscot River fishway traps.

2.2 Scale analysis

Scales were cleaned and mounted between glass microscope slides using protocols described by ICES (2011). Scale images were taken using a computer image analysis system with 0.78 total lens magnification resulting in 4800 x 3600 pixel images, which were then measured at 1.9 µm/px using Image Pro Plus 7.0.1 (Media Cybernetics, www.mediacy.com). The reader placed a calibrated line transect from the center of the focus to the edge of the scale, along the longest axis. The software then marked the middle of each circulus based on luminescence values and extracted the distance from the focus to each circulus along the transect line. The reader manually corrected any misplaced marks and also marked and extracted the distance from the focus to the first marine circulus and each marine annulus using the definitions of these reference points as described by ICES (2011).

2.3 Annulus date estimation

Our initial objective was to develop a method to estimate annulus completion timing based on data from marked individuals. We applied an equation (1) to estimate the date of annulus completion (T_a):

$$T_a = T_1 - (RC_a) \tag{1}$$

where T_1 is the known recapture date, C_a is the number of circuli after the most recent annulus, and R is the marine circulus deposition rate in units of days per circulus, such that the product of RC_a is an estimate of the number of days since the annulus was formed.

Release date, recapture date, and the number of circuli after the annulus are known for each individual, but there are a variety of marine circulus deposition rates that could be applied to this equation. We calculated annulus completion timing under five marine circulus deposition scenarios: 1) ‘individual constant’ which allows for variation in marine circulus deposition rate between individuals but assumes constant deposition for an individual throughout its life; 2) 'seasonally variable' which allows an individual to experience seasonal variation in their marine circulus deposition rate; 3) 'seasonal and lifetime variable' which allows an individual to experience seasonal variation in their marine circulus deposition rate and also adjusts for asymptotic growth; 4) 'constant winter' which assumes all individuals have a post-annulus circulus deposition rate of 14 days/circulus, and 5) 'constant summer' which assumes that all individuals have a post-annulus circulus deposition rate of 7 days/circulus.

The 'individual constant' scenario accounts for individual variation in circulus deposition rate but holds that rate constant across the entire growth period (Figure 1a). The 'individual constant' scenario uses a lifetime average marine circulus deposition rate (R) calculated using equation (2) for each fish:

$$R = (T_1 - T_0){C_m}^{-1} \tag{2}$$

where T_0 is the known release date, $T_1 - T_0$ is the number of days between the release date and recapture date, and C_m is the total number of marine circuli. For clarity, this means that the product of the total number of marine circuli and the lifetime average marine circulus deposition rate (days/circulus) is equal to the number of days at sea.

The 'seasonally variable' scenario accounts for both individual and seasonal variation in marine circulus deposition rate, given that the actual deposition rate for each circulus is likely faster (fewer days/circulus) than average during periods of rapid growth and slower (more days/circulus) during periods of reduced growth. Under the assumption that scale growth rate and circulus deposition rate are positively correlated (widely spaced circuli indicate periods of faster growth; Fisher & Pearcy, 1990; Shearer, 1992), we can use the intercirculi spacings (distance between consecutive circuli) to estimate a unique deposition rate for each circulus (described visually in Figure 1b), thus allowing deposition rate to vary seasonally based on patterns of intercirculi spacings (Jensen *et al.*, 2012). We began with the assumption that if a salmon experienced a constant growth rate over its lifetime, it would deposit evenly spaced circuli and it would take the same amount of time to deposit each circulus. Therefore, if a fish experienced its average marine circulus deposition rate constantly, it should deposit all circuli at rate R with an average marine spacing value (S) according to equation (3):

$$S = I_m {C_m}^{-1} \tag{3}$$

where I_m is the total marine increment (distance between first marine circulus and radius) and C_m is the total number of marine circuli. We centered each intercirculus spacing (S_c) on the average marine spacing (S) for that individual fish, thus creating a metric of the deviation from average marine spacing. Where deviation was positive ($S_c > S$), fish were growing faster than average, so we assumed that the seasonal circulus deposition rate (R_c) was faster than R at a value proportional to the value of the deviation. Where deviation was negative ($S_c < S$), fish were growing slower than average, we assumed that R_c was slower than R at a value proportional to the value of the deviation (further explanation of methods in Supplementary Information). The purpose of this adjustment was to allow for seasonal, growth-rate dependent variation in circulus deposition rate (R_c; Figure 1b), without changing sampled values: number of marine circuli (C_m) and number of days at sea ($T_1 - T_0$).

The 'seasonal and lifetime variable' scenario accounts for asymptotic growth considering that declining somatic growth rate over the life of an individual may translate to slower circulus deposition rates later in life (Figure 1c). Atlantic salmon grow rapidly upon marine entry and the somatic growth rate slows as fish approach asymptotic length (Miller *et al.*, 2014), as often described by the von Bertalanffy growth function. To adjust for age-related variation in marine circulus deposition rate over an individual's lifespan, we described the relationship between the marine increment (I_m) and the number of days at sea ($T_1 - T_0$) with a quadratic regression fitted as a generalized linear model (Figure 2) using R statistical software (R Development Core Team, 2019). As to include a range of marine ages, we used the entire Carlin dataset (for which days at sea and marine increment was available) for this analysis (n=1,599). We used the relationship between fitted values for fish with two marine annuli and fish with one marine annulus to calculate an adjustment factor that, in comparison to the 'individual constant' average circulus deposition rate, would hasten the average marine circulus deposition rate before completion of the first marine annulus, slow the average marine circulus deposition rate after completion of the first marine annulus, and further slow the average marine circulus deposition rate after the second marine annulus (if present). The sum of the age-adjusted average deposition rates is still equal to the number of days at sea. The age-adjusted average deposition rates were then further modified using the previously described process to account for seasonal variation in marine circulus deposition rate based on intercirculi spacings (further explanation of methods in Supplementary Information).

In the 'constant winter' scenario, we assume that circuli after the annulus are deposited at the winter rate of 14 days/circulus. In the 'constant summer' scenario, we assume that circuli after the annulus are deposited at the summer rate of 7 days/circulus. These rates were selected based on assumptions provided by Friedland and Reddin (2000) which asserted that circuli are deposited at a rate of 14 days per circulus in winter and 7 days per circulus in spring and summer. These and similar circulus deposition rates have been widely cited as standard for Atlantic salmon growth (Friedland *et al.*, 2005; Hubley *et al.*, 2008; Thomas *et al.*, 2019; Todd *et al.*, 2014).

2.4 First annulus completion date determination for homewater recaptures

Our method was designed to estimate the date of annulus completion for the most recent marine annulus. For homewater recaptures, two annuli had been completed, so the second marine annulus completion date was calculated. To calculate the first marine annulus completion date (T_f) for homewater recaptures using equation (4), we used the previously described equation (1), but replaced the number of circuli after the most recent annulus (C_a) with the number of circuli after the first marine annulus (C_f).

$$T_f = T_1 - \left(RC_f\right) \tag{4}$$

The estimate of first marine annulus completion date for homewater recaptures is useful for describing the validity of our methods: we would expect the estimated first annulus completion date for homewater recaptures to fall within the range of first annulus completion dates estimated for at-sea recaptures. Most North American Atlantic salmon are thought to winter in the south Labrador Sea, so individuals in our sample likely experienced similar environmental conditions when they were forming their first and second marine annuli. If annulus formation is related to environmental conditions, be it photoperiod or temperature, we would expect the first and second marine annuli to form around the same time.

We cautiously applied this method for the ‘individual constant,’ ‘seasonally variable,’ and ‘lifetime and seasonally variable’ scenarios. However, the ‘constant winter’ and ‘constant summer’ deposition rates we used are published as seasonal and the length of each “season” is not defined in the literature that describes them. Because calculating the first marine annulus for homewater recaptures requires defining a circulus deposition rate over multiple “seasons,” when applying the ‘constant winter’ and ‘constant summer’ scenarios, we opted to estimate only the most recent annulus completion date for both at-sea and homewater recaptures (the first marine annulus for at-sea recaptures and the second marine annulus for homewater recaptures).

2.5 Relationships to monthly sea surface temperature

To relate estimated annulus completion dates to monthly temperature trends in presumed wintering areas, we obtained mean monthly sea surface temperatures from the Extended Reconstructed Sea Surface Temperature (ERSST) v5 dataset for the period of 1961 to 1991. We included data that falls within the presumed wintering area of the south Labrador Sea (Reddin, 1985). To quantify how differential assignment of annulus formation date may shape inferences about environmental conditions experienced by Atlantic salmon, we calculated the difference in accumulated thermal units (number of days in the month × mean monthly temperature; Izzo & Zydlewski, 2017) between the previously assumed date of annulus formation (winter solstice) and estimated annulus completion dates under each marine circulus deposition rate scenario, for each individual. Because accumulated thermal units (ATU) are infrequently used in studies of marine growth, it is difficult to link ATU with realized physical growth differences. However, it provides a metric for the difference in experienced environmental conditions between winter solstice and the estimated annulus formation date, the magnitude of which is not fully achieved by reporting difference in time or difference in mean temperature alone.

3 Results

3.1 Estimated marine annulus completion dates

Under the 'individual constant' scenario, the median first marine annulus completion date for at-sea recaptures was 10 March (Table 1, Figure 3a). The median first marine annulus completion date for homewater recaptures was 30 May, and the median second marine annulus completion date for homewater recaptures was 30 April. Circulus deposition rates used in the individual constant scenario were based on calculated average marine circulus deposition rates for each individual. For at-sea recaptures, 95% of average marine deposition rates were between 9 and 13.5 days/circulus ($\bar{x}$ = 11.1 days/circulus, SD = 1.4, Figure 4a). For homewater recaptures, 95% of average marine deposition rates were between 11.6 and 15.6 days/circulus ($\bar{x}$ = 13.6 days/circulus, SD = 1.3, Figure 4b).

For the 'seasonally variable' scenario, the median first marine annulus completion date for at-sea recaptures was 15 March. The median first marine annulus completion date for homewater recaptures was 18 May, and the median second marine annulus completion date was 11 April (Table 1, Figure 3b). Under the 'seasonal and lifetime variable' scenario, the median first marine annulus completion date for at-sea recaptures was 20 February. The median first marine annulus completion date for homewater recaptures was 22 March and the median second marine annulus completion date for homewater recaptures was 25 March (Table 1, Figure 3c).

The 'constant winter' scenario resulted in a median annulus completion date of 26 January for the first marine annulus of at-sea recaptures and 29 April for the second marine annulus of homewater recaptures (Table 1, Figure 3d). The 'constant summer' scenario resulted in a median annulus completion date of 7 May for the first marine annulus of at-sea recaptures and 28 May for the second marine annulus of homewater recaptures (Table 1, Figure 3e).

3.2 Monthly sea surface temperature trends

Sea surface temperatures in the south Labrador Sea began decreasing in September and typically declined until March (Figure 5). Median annulus completion dates for the 'individual constant', 'seasonally variable,' and 'seasonal and lifetime variable' scenarios fall close to the coldest part of the year, or just as temperatures began increasing. The 'winter constant' and 'seasonal and lifetime variable' scenario had the least differences in ATU between winter solstice and estimated annulus completion dates, and the 'constant summer scenario' had the greatest differences. In general, the ATU differences varied widely, between 100 and 900 ATUs dependent on the scenario and individual (Figure 6).

4 Discussion

We used scale data from uniquely marked individuals with a known release and recapture date to estimate annulus completion dates from Atlantic salmon based on the current understanding of biological mechanisms driving scale growth. Within the extensive Atlantic salmon literature that uses scales as a means of assessing growth trends, the idea that annulus formation occurs sometime around winter solstice is commonly cited (Friedland *et al.*, 2009a; Todd *et al.*, 2014). We argue that annulus formation date estimates produced by our 'seasonal and lifetime variable' scenario should be used rather than the common assumption of winter solstice as the date of annulus completion.

The 'individual constant,' 'seasonally variable,' and 'seasonal and lifetime variable' scenarios have comparable spreads and similar distribution shapes. However, the 'seasonal and lifetime variable' scenario results in median annulus completion dates that are much more internally consistent than the other two scenarios. Because we expect that first and second marine annuli are formed at the same time of year for Atlantic salmon in this population, the similarity between median annulus completion dates is likely indicative of better estimation ability by this scenario. In addition, the 'seasonal and lifetime variable' scenario is our best attempt to account for biological variability as manifested in seasonal changes in growth rate and asymptotic growth over a salmon's lifetime. Finally, the early spring annulus completion date (mid-February to late March) that this scenario produces is most logical given our current understanding of salmon growth biology. If annuli are completed in early spring as this scenario suggests, that means that narrowly spaced circuli comprising the annulus would be deposited during a period of decreasing temperature in the south Labrador Sea, and wider spaced circuli would be deposited around the time when sea surface temperatures increase in spring. Some experimental studies on salmonids have found a relationship between temperature and scale growth rates (Skurdal & Andersen, 1985) or intercirculi spacings (Beakes *et al.*, 2013). In contrast, other studies have found a weak or nonlinear relationship between temperature and intercirculi spacings (Bigelow & White, 1996; Thomas *et al.*, 2019) or no relationship at all (Fukuwaka, 1998). Though a relationship between scale growth and temperature is often assumed, additional experimental studies are needed to elucidate the relationship between scale growth and temperature. This relationship would inform our understanding of the annulus formation mechanism, and presumably, provide context to the timing of marine annulus formation.

The 'constant winter' scenario produced annulus completion estimates closest to winter solstice using the constant deposition rate of 14 days/circuli; however, only the 90% interval for the first marine annulus of at-sea recaptures overlapped winter solstice (Table 1). Although the 'constant winter' deposition rate produces estimates closer to winter solstice, using a deposition rate of 14 days/circulus is problematic conceptually. The circuli formed after the annulus are more widely spaced than those that form the annulus. Under the assumption that circulus deposition rates are inversely related to intercirculi spacings, the relatively wider-spaced circuli formed after the annulus would be deposited at a more rapid rate than those circuli deposited within the annulus. If the circuli after the annulus form at a rate of 14 days/circulus, then the narrower circuli within the annulus must have formed at a slower rate. This would result in average marine circulus deposition rates (R) much higher than the calculated averages (Figure 4a; 97% of average marine circulus deposition rates are less than 14 days/circulus for at-sea recaptures). For our sample, a post-annulus deposition rate as suggested by the 'constant winter' scenario is ecologically possible only if intercirculi spacings and deposition rate are unrelated.

We found substantial individual variation in annulus completion date in all scenarios; long tails of density plots are at least partly due to individual and annual variability in annulus completion dates (these dates are estimated based on 1,503 individuals during 29 years; Figure 3). Individual variation may have many sources such as thermal conditions, food availability, predatory ability, latitude, body size and condition, smolt age, genetically innate growth rate, or other smolt-year related annual factors, but it is difficult to partition the origin of that variation using observational data. Regardless of the source of the differences, we find it important to acknowledge that individual variation likely manifests itself in the timing of annulus completion. Experimental studies to understand individual variation in circulus deposition rate and development of methods to account for this variation could further improve the utility of future studies seeking to relate growth and environmental conditions in space and time.

Despite the variability in estimates of annulus completion dates, our analyses point towards a temperature influence on annulus formation. This finding is in contrast to the widely accepted idea that photoperiod is the primary driver of marine annulus formation. Aligning the timing of annulus formation with the winter solstice may confound ecological analyses that would be affected by seasonal conditions. For example, using the winter solstice versus the 'seasonal and lifetime variable' scenario for first annulus completion of at-sea recaptures results in a difference in ATU between 160 °C to 487 °C (5^{th} to 95^{th} percentiles of the estimated date), which corresponds to a difference from solstice of 36-132 days, and a mean sea surface temperature difference in the south Labrador Sea SST of 0.57-2.59 °C. Making the same comparison between the 'seasonal and lifetime variable scenario' for first annulus completion of homewater recaptures results in a difference of accumulated thermal units between 100 °C to 473 °C, where difference from solstice is 31-139 days, and monthly sea surface temperature difference is 0.57-2.59 °C, with similar differences for the second annulus of homewater recaptures. Mis-aligning dates of biological events or underestimating thermal exposure can substantially affect results of analyses that attempt to link ecosystem conditions to population dynamics, bioenergetics, or other biological responses. The annulus formation date estimates presented herein offer an alternative to the widely used winter solstice date and an opportunity to represent uncertainty in the alignment of environmental variables with this growth marker.

4.1 Limitations

A few recent studies have cited the importance of partitioning growth into sub-annual units or assigning a date to circulus deposition (Izzo & Zydlewski, 2017; McCarthy *et al.*, 2008; Todd *et al.*, 2014). Though we used our equation to produce estimates of annulus completion timing, the approach in its current form is not appropriate for assigning dates to the deposition of individual circuli, given that our deposition rates are estimated based on a set of assumptions.

With regards to assumptions, we note the limitations of our method for applying a seasonally variable deposition rate. Our method assumes that there is an inverse, linear relationship between intercirculi spacings and deposition rate (Fisher & Pearcy, 1990), but recent experimental work only weakly supports the linearity of this relationship (Thomas *et al.*, 2019), and further work is needed to test these assumptions. Also, age presents an additional source of non-linearity. Though we attempted to correct for asymptotic growth and incorporate it in the 'seasonal and lifetime variable' scenario, the relationship between scale growth and somatic growth is poorly understood as Atlantic salmon reach asymptotic length. The relationship is especially ambiguous when salmon begin allocating resources to reproduction, transitioning back to freshwater habitat, or increasing mass rather than skeletal growth (Fisher & Pearcy, 2005). Finally, there is no evidence that hatchery-raised fish would have different annulus formation timing than wild populations of the same origin, but we note that our sample is comprised entirely of hatchery-raised fish.

Additionally, we acknowledge the uncertainty around calculating the annuli other than the most recent annulus (such as for the first marine annulus of homewater recaptures). The certainty of our estimates can be thought of as a bimodal distribution; we have higher certainty of date assignments close to the release and recapture dates, but we have lower relative certainty of date assignments furthest from those known dates (Figure 7). Because this certainty cannot be described quantitatively or assigned more than a relative, subjective value, we feel it is important to highlight that these estimates have both unknown and varying levels of certainty. For this reason, we urge cautious and reasonable application of this method for estimating dates other than that of the most recent annulus.

Notwithstanding these qualifications, we feel confident that our methods provide a more robust estimation of annulus completion timing for Atlantic salmon than current reliance on observational studies based on Pacific salmon and the assumption that annulus formation aligns with winter solstice. Based on evidence of wide variation in timing of annulus completion among species of Pacific salmon (Bilton & Ludwig, 1966; Hyun *et al.*, 1998), major geographical differences, and life history differences, there is little reason to assume that annulus completion timing for *O. nerka*, or any other Pacific salmon, can be directly applied to Atlantic salmon.

4.2 Conclusions

We recommend further experimental investigation of the biological and physiological mechanisms driving marine circulus deposition (i.e., the effect of temperature, photoperiod, food availability, smolt age) to fully elucidate drivers of annulus formation. In the interim, we provide a method for estimating stock-specific annulus formation timing, and we recommend that this method be applied to similar datasets across the species' range. For the North American Atlantic salmon, annulus formation likely occurs at a time associated with the coldest sea surface temperatures in their wintering area rather than on winter solstice. Based on the 'seasonal and lifetime variable' circulus deposition rate scenario, annuli are likely completed between mid-February and late March. This improved understanding of annulus formation timing may inform work attempting to partition growth into seasonal or other sub-annual increments, especially those hoping to align growth with ecosystem conditions such as monthly mean sea surface temperatures.

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Contributions

L.G.C., K.E.M. and T.F.S. conceptualized the paper; L.G.C. and M.D.T. planned and conducted the analyses; L.G.C., K.E.M. and T.F.S. wrote the paper; L.G.C., M.D.T., K.E.M., and T.F.S. reviewed, edited, referenced the paper; T.F.S. and K.E.M. contributed funding for analysis and write-up.

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Supplementary Information

A repository including data, methods, and reproducible code for this analysis can be found at: https://github.com/LGCarlson/10.1111-jfb.14763

Table 1

Deposition rate scenario	Sources of variability			First marine annulus (at-sea recaptures)			First marine annulus (homewater recaptures)			Second marine annulus (homewater recaptures)		
	Individual	Seasonal	Lifetime	Median completion	SD (days)	90% between	Median completion	SD (days)	90% between	Median completion	SD (days)	90% between
Individual constant	X			Mar 10 (DOY=69)	30	Jan 26-May 2 (26-122)	May 30 (DOY=150)	40	Mar 26-Jul 28 (85-209)	April 30 (DOY=120)	36	Mar 11-Jul 3 (70-184)
Seasonally variable	X	X		Mar 15 (DOY=74)	31	Jan 25-May 8 (25-128)	May 18 (DOY=138)	41	Mar 7-Jul 13 (66-194)	Apr 11 (DOY=101)	40	Feb 20-Jun 21 (51-172)
Seasonal and lifetime variable	X	X	X	Feb 20 (DOY=51)	33	Dec 31-Apr 17 (365-107)	Mar 22 (DOY=81)	35	Jan 21-May 9 (21-129)	March 25 (DOY=84)	60	Jan 26-Jun 30 (26-181)
Winter constant (14 days/circulus)				Jan 26 (DOY=26)	53	Nov 27-Apr 17 (331-107)	-	-	-	Apr 29 (DOY=119)	39	Mar 5-Jul 9 (64-190)
Summer constant (7 days/circulus)				May 7 (DOY=127)	33	Mar 25-Jul 16 (84-197)	-	-	-	May 28 (DOY=148)	30	Apr 28-Aug 14 (118-226)

Figure 1. Theoretical representation of marine circulus deposition rates in days per circulus (solid black lines) implemented under the individual constant scenario, seasonally variable scenario, and seasonal and lifetime variable scenario. Because intercirculus spacings (mm) are related to somatic growth, intercirculus spacings (dashed grey lines) are used here as a visual proxy for somatic "growth rate" in order to show the relationship between growth rate and deposition rate in each scenario. In the individual constant scenario (a), the deposition rate does not vary regardless of season or in relation to growth rate. In the seasonally variable scenario (b), the deposition rate is inversely related to growth rate to represent seasonal fluctuations in circulus deposition rate, but this relationship is constant over the lifetime. In the seasonal and lifetime variable scenario (c), the deposition rate is inversely related to growth rate to represent seasonal fluctuations in circulus deposition rate, but this rate is more rapid early in life, then slows after each subsequent annulus. Solid gray lines show the "averaged" rate to illustrate the concept that averaged 'seasonally variable' rates are equivalent to the 'individual constant' rate and that the 'seasonal and lifetime variable' scenario results in faster rates before the annulus and slower rates after annulus completion.

Figure 2. The relationship between marine increment (mm) and the number of days at sea shows declining growth rate with increasing age. The solid line represents the fitted quadratic generalized linear model (pseudo $R^2 = 0.7$) used in the 'seasonal and lifetime variable' scenario to account for asymptotic growth, and dashed lines represent the 95% confidence interval of the regression.

Figure 3. Frequency distributions for estimated annulus completion dates based on the five considered scenarios. The median, 5%, and 95% percentiles are denoted by solid vertical lines and winter solstice indicated by dashed line. Distributions are truncated to dates with > 0.5% probability. †Completion dates of the first marine annulus for homewater recaptures were not estimated for the constant winter or constant summer scenarios because the season length associated with these seasonal rates is not described in the literature which calculated them initially.

Figure 4. Histograms showing circulus deposition rates for a) individuals captured at sea with one marine annulus (n=1,310) and b) homewater captures with two marine annuli (n=193). Dashed lines show group means a) 11.1 days/circulus b) 13.6 days/circulus.

Figure 5. Between 1966 and 1991, sea surface temperatures in the south Labrador Sea were typically decreasing until March. The dashed vertical line represents the previously proposed annulus completion date (winter solstice) and the solid vertical lines represent the median annulus completion dates (first marine annulus of at-sea recaptures) estimated by each scenario.

Figure 5. Between 1966 and 1991, sea surface temperatures in the south Labrador Sea were typically decreasing until March. The dashed vertical line represents the previously proposed annulus completion date (winter solstice) and the solid vertical lines represent the median annulus completion dates (for the first marine annulus of at-sea recaptures) estimated by each scenario.

Figure 6. Median and interquartile range of the difference in accumulated thermal units in °C between the commonly cited date of annulus formation, winter solstice (December 21) and the annulus formation date estimated under each scenario. †Completion dates of the first marine annulus for homewater recaptures were not estimated for the constant winter or constant summer scenarios, so differences in accumulated thermal units were not calculated.

Figure 7. Conceptual representation describing our cautious interpretation of date estimates other than that of the most recent annulus. The y-axis represents our theoretical "relative certainty of" annulus completion date. The same principle could be applied to other markers of interest for individuals of any age: we are more certain of date assignments for markers that formed closer to the known release and recapture dates.

Table 1 Median annulus completion date estimates for Atlantic salmon (*Salmo salar*) as calendar and ordinal date (DOY), standard deviation (SD), and date ranges between which 90% of annulus completion estimates fall (as calendar and ordinal dates) for the five considered circulus deposition rate scenarios. Individual variability is based on days at sea/number of marine circuli, seasonal variability is based on an inverse relationship with intercirculi spacing patterns, and lifetime variability is based on the relationship between postsmolt, 1SW, and 2SW scale growth.

Figure 1

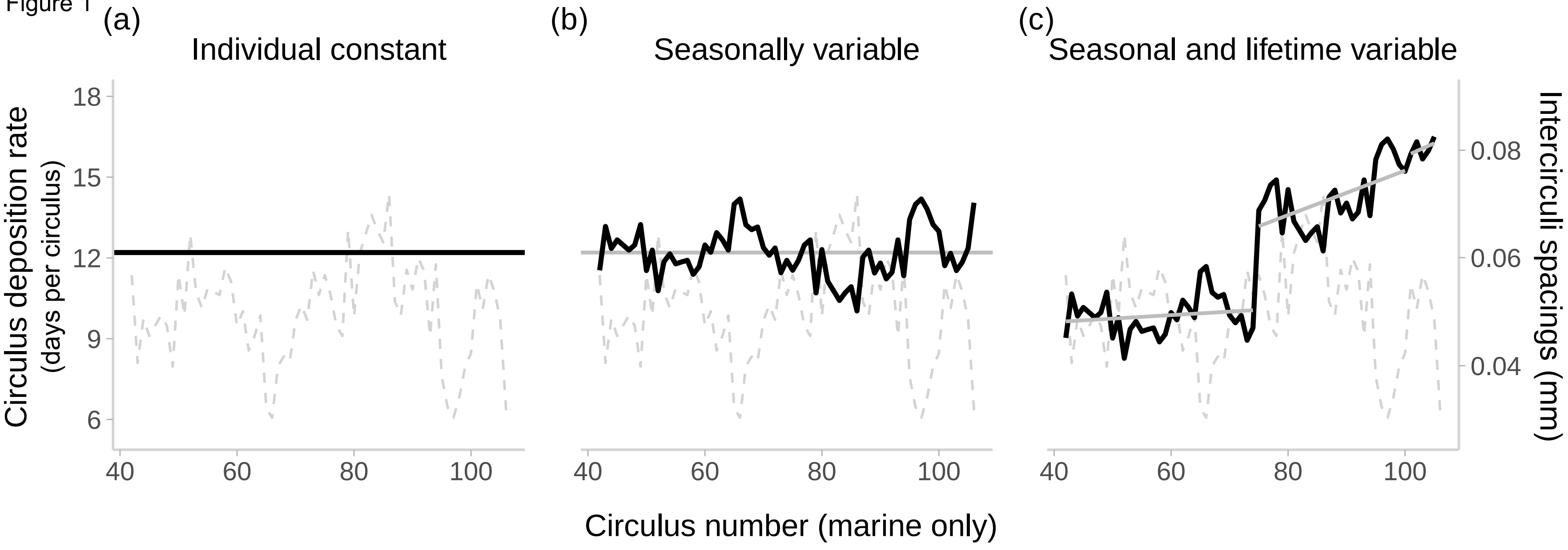

(a)
Individual constant
(b)
Seasonally variable
(c)
Seasonal and lifetime variable
Circulus deposition rate
(days per circulus)
18
15
12
9
6
40
60
80
100
Intercirculi spacings (mm)
0.08
0.06
0.04
Circulus number (marine only)

Figure 2

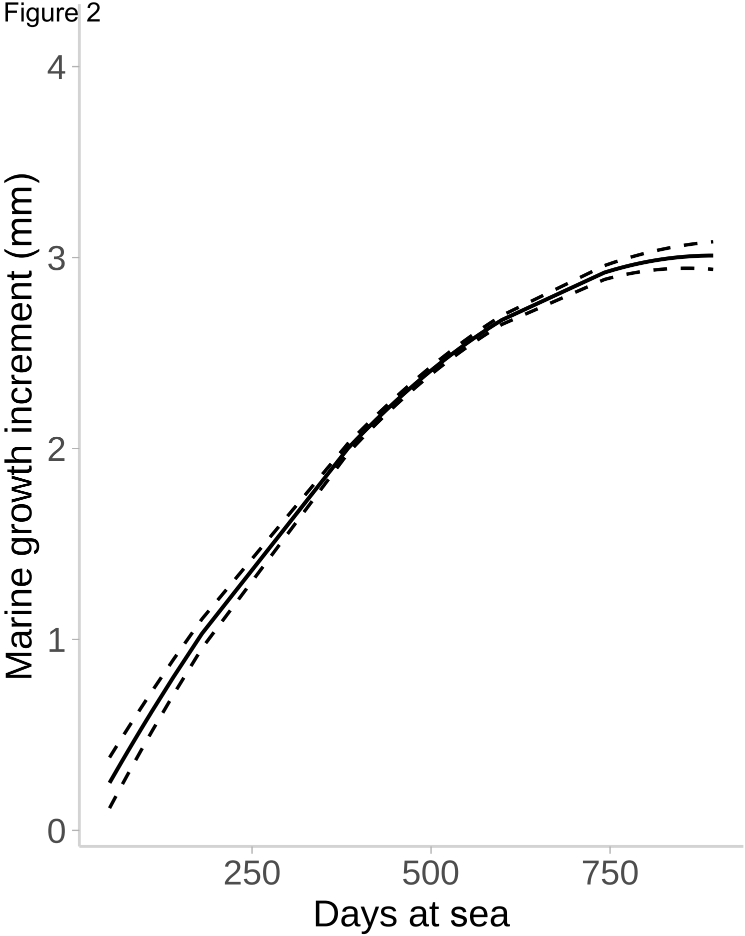


Figure 3

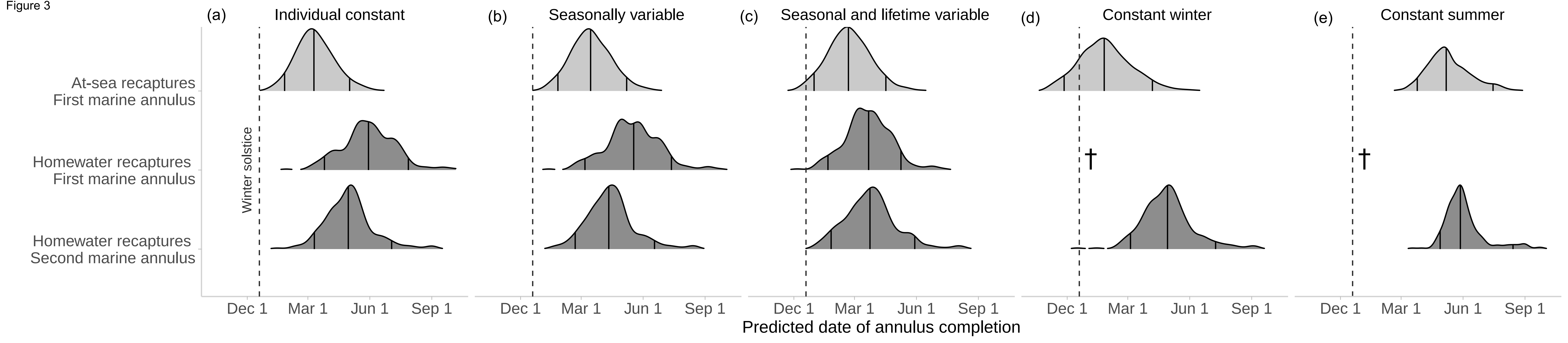

(a)
Individual constant
(b)
Seasonally variable
(c)
Seasonal and lifetime variable
(d)
Constant winter
(e)
Constant summer
At-sea recaptures
First marine annulus
Homewater recaptures
First marine annulus
Homewater recaptures
Second marine annulus
Winter solstice
†
†
Dec 1
Mar 1
Jun 1
Sep 1
Predicted date of annulus completion

Figure 4

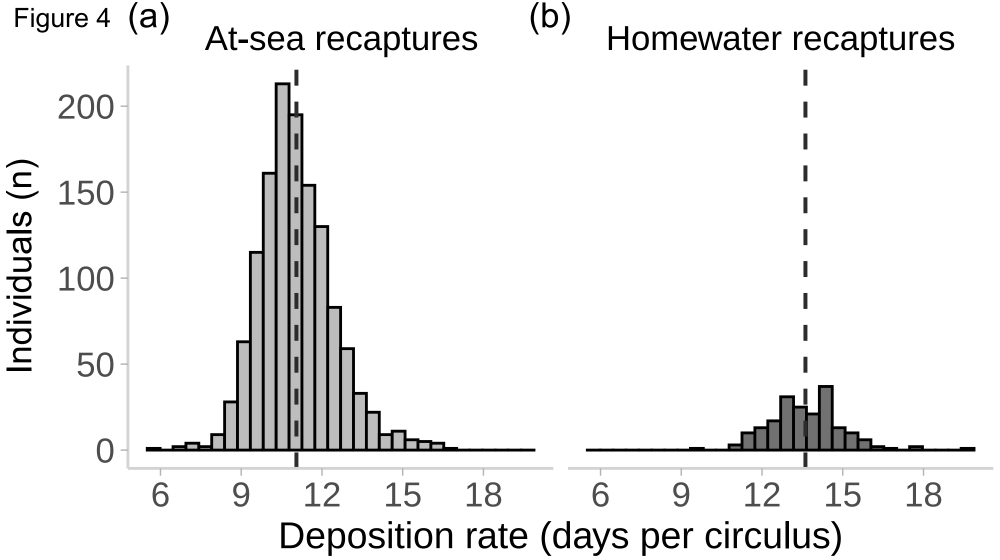
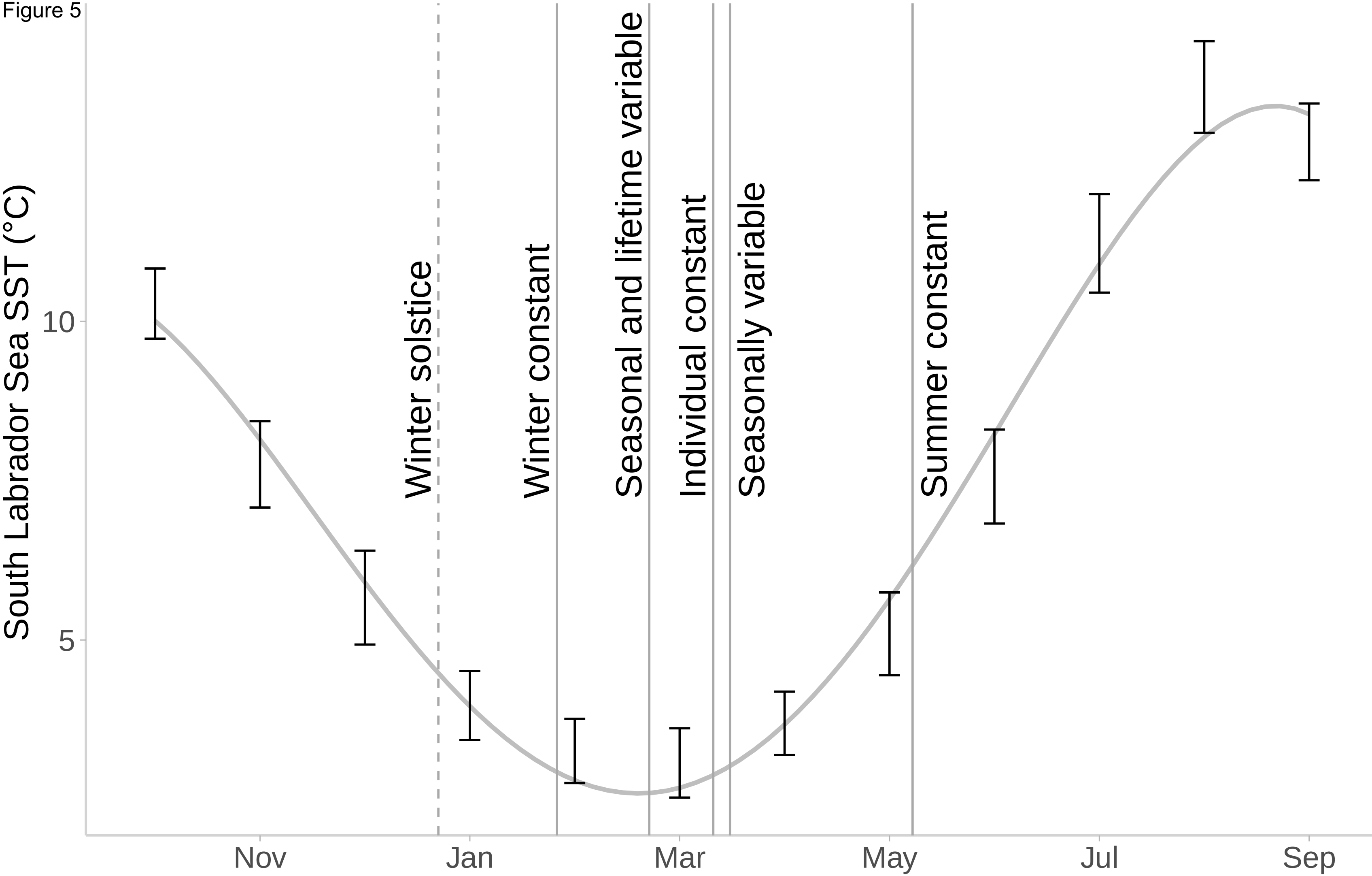
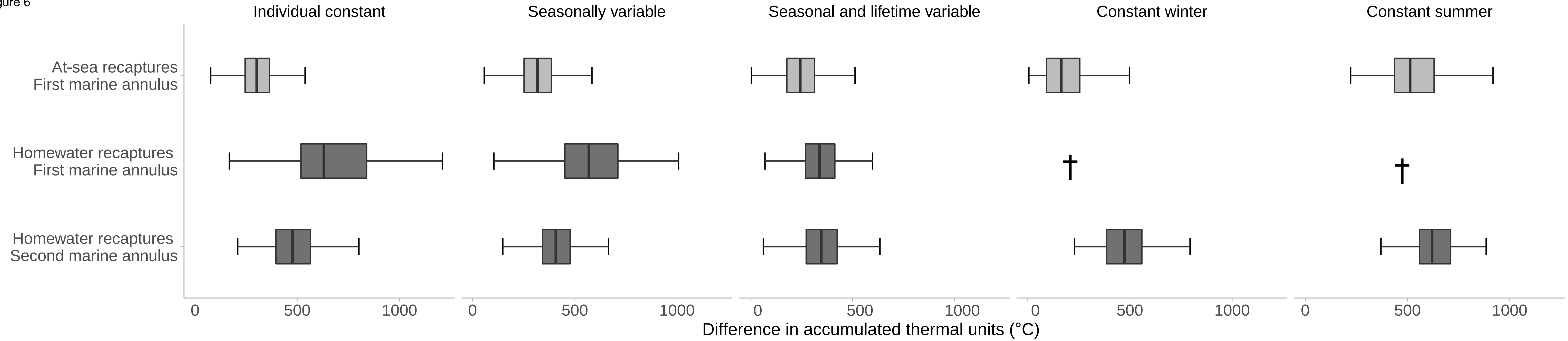

(a)
At-sea recaptures
(b)
Homewater recaptures
Individuals (n)
0
50
100
150
200
6
9
12
15
18
6
9
12
15
18
Deposition rate (days per circulus)

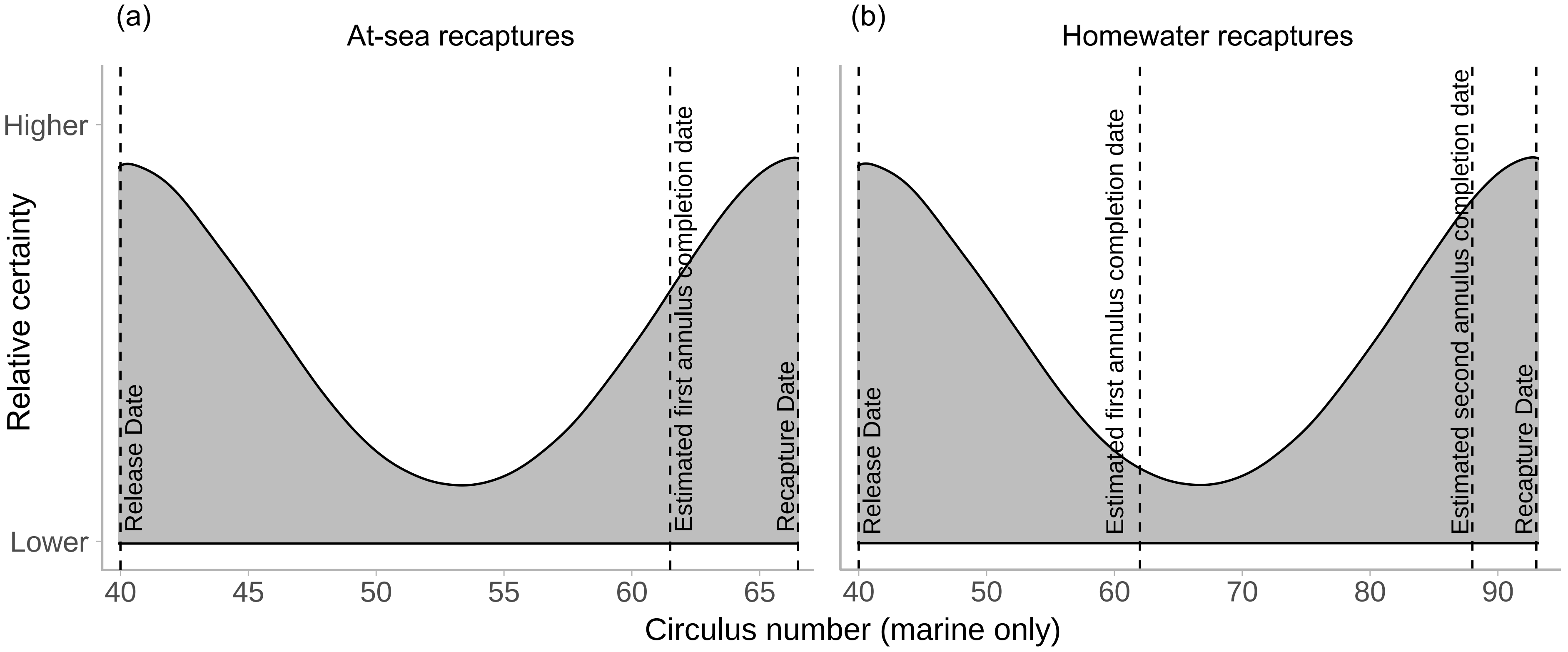
Figure 5



South Labrador Sea SST (°C)
10
5
Nov
Jan
Mar
May
Jul
Sep
Winter solstice
Winter constant
Seasonal and lifetime variable
Individual constant
Seasonally variable
Summer constant

Figure 6


Individual constant
Seasonally variable
Seasonal and lifetime variable
Constant winter
Constant summer
At-sea recaptures
First marine annulus
Homewater recaptures
First marine annulus
Homewater recaptures
Second marine annulus
†
†
0
500
1000
Difference in accumulated thermal units (°C)


(a)
At-sea recaptures
(b)
Homewater recaptures
Relative certainty
Higher
Lower
Release Date
Estimated first annulus completion date
Recapture Date
Release Date
Estimated first annulus completion date
Estimated second annulus completion date
Recapture Date
40
45
50
55
60
65
40
50
60
70
80
90
Circulus number (marine only)