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Age validation of Pacific cod (*Gadus macrocephalus*) using high-resolution stable oxygen isotope ($\delta^{18}O$) chronologies in otoliths

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ABSTRACT

The Pacific cod (*Gadus macrocephalus*) fishery in Alaska is large and economically important; in 2014 its ex-vessel value was $203.8 million. Management relies on integrated assessments that employ survey abundance trends and fishery catches as well as age compositions from assessment surveys. However, Pacific cod age determination based on otolith growth zone counts has historically been difficult, adding to the uncertainty in biological reference points. Further, a mismatch exists where modes in fish length frequencies are larger than mean lengths at otolith-based age. To address ageing inaccuracy, we conducted an age validation study using stable oxygen isotopes ($\delta^{18}O$). This approach is based upon the principle that variability in marine carbonate $\delta^{18}O$ is inversely related to water temperature, which we independently verified in Pacific cod otoliths ($r^2 = 0.74$). We sequentially microsampled 40 Pacific cod otoliths from the core to the margin and measured the aragonite $\delta^{18}O$ by isotope ratio mass spectrometry. This provided a detailed $\delta^{18}O$ life history chronology for each specimen. First, we identified seasonal variation (the cyclical pattern of otolith $\delta^{18}O$ values) and determined whether the number of $\delta^{18}O$ maxima, considered to represent "true fish age," was consistent with the age estimated from growth zone counts. Second, we estimated the probability of bias in the ages determined from growth zone counts. Overall, the probability of assigning an age (based on the number of counted growth zones) equal to the true age (number of $\delta^{18}O$ maxima) was approximately 61%. However, the probabilities of over- or underestimating the age by 1 year were 25% and 13%, respectively. The probability of over- or underestimating true age by 2 or more years was very low (<2%). The probability of age misclassification was used to correct bias in mean length at age.

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1. Introduction

Pacific cod (*Gadus macrocephalus*) is the subject of a large and economically important fishery in Alaska. During 2014, the total catch in the Gulf of Alaska (GOA), Aleutian Islands, and eastern Bering Sea (EBS) was 334,200 metric tons, with an ex-vessel value of $203.8 million (http://www.afsc.noaa.gov/REFM/Docs/2015/economic.pdf). Management of this important resource is conducted through stock assessments using age-structured models in which fish length-at-age data are key to estimating mortality, recruitment, and recommended harvest levels. Hence, Pacific cod age determination accuracy is critical. The reliability of Pacific cod age determination, however, has been called into question. A problem often encountered during Pacific cod age determination is that the patterns of otolith growth zones deposited during the first 2–3 years of life can be difficult to interpret (Roberson et al., 2005; Lai et al., 1987), and may lead to an age determination error of 1–2 years in some fish (Fig. 1). Specifically, a mismatch exists where modes in fish length frequencies, presumed to represent 2-and 3-year-old fish, are larger than mean length at age based on otolith growth zone counts (http://www.afsc.noaa.gov/REFM/docs/2010/BSAIpcod.pdf). This mismatch suggests the presence of age determination bias, and brings into question the accuracy of the estimated ages.

To improve age determination in Pacific cod, virtually every possible hard structure and preparation method has been exam-

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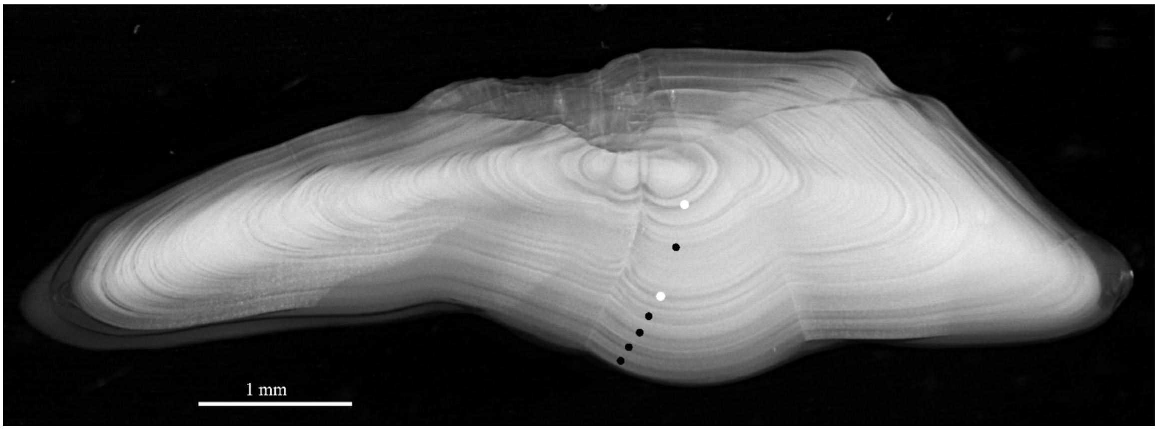

Fig. 1. Pacific cod (*Gadus macrocephalus*) otolith transverse thin section which demonstrates difficulty in growth zone interpretation. Presumed annual growth zones are indicated with black dots and questionable (possibly not annual) translucent growth zones are indicated with white dots.

ined. This includes scales (Kennedy, 1970; Kimura and Lyons, 1990), whole otoliths (Moser, 1954; Ketchen, 1970), broken-and-burnt otoliths (Lai, 1985; Kimura and Lyons, 1990), thin-sectioned otoliths (Roberson, 2001), vertebrae (Ketchen, 1970), opercula (Lai, 1985), and thin-sectioned dorsal and pectoral fin rays (Lai et al., 1987; Kimura and Lyons, 1990). Among all these methods, growth zones are most visible in thin-sectioned otoliths, dorsal fin rays, and broken-and-burnt otoliths (Johnston and Anderl, 2012). Importantly, radiometric age validation has indicated that ages estimated by the broken-and-burnt method are generally accurate up to an age of about 7 years (Andrews, 2015). However, consistent problems with interpreting growth zones deposited in the first several years of life remain, so ages determined from these methods may not be free of error or bias and therefore require validation (Fig. 1). Currently, at the Alaska Fisheries Science Center (AFSC) a version of the otolith break-and-burn method, the break-and-bake method (Johnston and Anderl, 2012), is used to efficiently determine fish age in up to 5000 specimens each year. Using this method, the maximum estimated age is 17 years, but fish over 12 years of age are rarely seen (http://www.afsc.noaa.gov/REFM/Docs/2015/EBSpcod.pdf). The accuracy of the break-and-bake method was evaluated in this study.

Despite intensive efforts to identify an appropriate ageing structure and method of preparation, a direct validation of estimated ages or age determination methods has yet to be conducted for Pacific cod. The current "gold standard" method of age validation is based on assaying bomb-produced radiocarbon (^{14}C) in otoliths (Kalish, 1995). This method requires specimens extant during the 1950s–1970s, when an abrupt increase in atmospheric and oceanic ^{14}C occurred due to nuclear testing, providing a known time marker within fish otoliths. Unfortunately, for short-lived species such as a Pacific cod, archived otoliths from that era are lacking, so the bomb-produced ^{14}C validation approach is not possible. Alternative chemical markers in fish otoliths have been identified to measure the accuracy of age estimation, and include both micro-chemical constituents (Clarke et al., 2007) and stable isotopes (Weidman and Millner, 2000), which are the focus of this study.

Otoliths are composed of calcium carbonate in the form of aragonite that is usually precipitated in isotopic equilibrium with ambient seawater (Campana, 1999; Thorrold et al., 1997). In marine calcium carbonate structures, the fractionation of oxygen isotopes ($^{18}O/^{16}O$, measured as $\delta^{18}O$) is inversely related to temperature (Hoie et al., 2004a). To some degree, oxygen fractionation is also a function of salinity (Grossman and Ku, 1986; Jones and Campana, 2009; Lécuyer et al., 2004; Campana, 1999), the ramifications of which are discussed later in this paper (Section 4). Because otoliths are a metabolically stable recording mechanism operating over the life of a fish, they can provide a way to reconstruct its temperature history (Hoie and Folkvord, 2006; Darnaude et al., 2014; Thorrold et al., 1997).

This general relationship between temperature and $\delta^{18}O$ has been used in a variety of studies. High-resolution $\delta^{18}O$ measurements of marine coral and shells (Grossman and Ku, 1986; Culleton et al., 2009; Nielsen and Nielsen, 2009; Lopez Correa et al., 2010), and fish otoliths (Weidman and Millner, 2000; Hoie et al., 2004a,b) have proven useful to derive environmental temperature and salinity profiles over an organism's lifetime (Kalish, 1991; Thorrold et al., 1997). Grossman and Ku (1986) documented the relationship between temperature and $\delta^{18}O$ in aragonite foraminifera. Hoie and Folkvord (2006) provided extensive evidence to show that $\delta^{18}O$ cycles in North Atlantic cod (*G. morhua*) otoliths represent annual signals. In a seminal study, Weidman and Millner (2000) made use of this $\delta^{18}O$-temperature relationship, with $\delta^{18}O$ cyclic signals as a proxy for seasonal temperature cycles, and successfully validated ages from North Atlantic cod otolith growth zone counts.

Our study was based on the concept that numerated seasonal cycles within a high-resolution lifetime $\delta^{18}O$ chronology (i.e., the number of $\delta^{18}O$ maxima, representing winter low temperatures) are an independent measure of fish age. The EBS has seasonal bottom temperature changes of at least 5 °C (Stabeno et al., 2012), which provides the basis for the $\delta^{18}O$ chronology. Therefore, if the number of $\delta^{18}O$ maxima are considered a proxy for "true age," validity of fish ages estimated by growth zone counts can be determined through comparison with this proxy. With this application of $\delta^{18}O$ in mind, our first goal was to use $\delta^{18}O$ to validate age estimates from Pacific cod otolith growth zone counts. A critical step in achieving this goal was to confirm the relationship between EBS Pacific cod otolith $\delta^{18}O$ and measured seawater temperature (i.e., to develop a fractionation curve) using tagged adult fish and juvenile fish. Specifically, our objective was to use high-resolution microsampling of Pacific cod otoliths and subsequent isotope ratio mass spectrometry to develop detailed lifetime $\delta^{18}O$ chronologies for age validation for untagged adult Pacific cod. Age estimates from growth zone counts were considered accurate if they were equal to our proxy for true age. Our second goal was to estimate ageing bias, or the probability of age misclassification for a given age.

2. Materials and methods

2.1. Selection and preparation of otoliths

To develop the fractionation curve of otolith $\delta^{18}O$ and seawater temperature we used two collections of otoliths with paired water temperature measurements. The first collection was EBS and GOA