Otolith mass as a predictor of age in kokanee salmon (*Oncorhynchus nerka*) from four Colorado reservoirs

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Abstract: Estimating ages of individuals in fish populations is crucial for determining characteristics necessary to effectively manage sport fisheries. Currently, the most accepted approach for fish age determination is using thin sectioned otoliths for interpretation. This method is labor-intensive, requires extensive training, and subjectively determines age. Several studies have shown that otolith mass increases with age, yet use of otolith mass to determine fish age is relatively underutilized. However, determining fish age using otolith mass requires relatively little training, is relatively nonsubjective, and is faster compared with other aging techniques. We collected kokanee salmon (i.e., landlocked sockeye salmon, *Oncorhynchus nerka*) in 2004 from four reservoirs and from 2000 to 2009 in one reservoir to evaluate the efficacy of using otolith mass to determine fish ages. We used a machine learning technique to predict kokanee salmon ages using otolith mass and various other covariates. Our findings suggest this method has potential to substantially reduce time and financial resources required to age fish. We conclude that using otolith mass to determine fish age may represent an efficient and accurate approach for some species.

Résumé : L'estimation de l'âge des individus au sein de populations de poissons est essentielle à la détermination de caractéristiques nécessaires à une gestion efficace de la pêche sportive. La méthode de détermination de l'âge des poissons la plus largement acceptée à l'heure actuelle repose sur l'interprétation de lames minces d'otolithes. Il s'agit d'une méthode laborieuse qui nécessite une formation poussée et qui établit l'âge de manière subjective. Si plusieurs études ont démontré que la masse des otolithes augmente avec l'âge, l'utilisation du poids des otolithes pour déterminer l'âge des poissons demeure une approche relativement sous-utilisée. Pourtant, cette méthode nécessite une formation relativement peu poussée et est moins subjective et plus rapide que d'autres méthodes de détermination de l'âge. Nous avons prélevé des saumons rouges (*Oncorhynchus nerka*) de quatre réservoirs en 2004 et d'un réservoir de 2000 à 2009 dans le but d'évaluer l'efficacité d'utiliser le poids des otolithes pour déterminer l'âge des poissons. Nous avons fait appel à une technique d'apprentissage automatique pour prédire l'âge de saumons rouges à partir du poids des otolithes et de diverses autres covariables. Nous résultats suggèrent que cette méthode pourrait permettre des réductions significatives du temps et des ressources financières nécessaires pour déterminer l'âge de poissons. Nous concluons que cette méthode constitue une approche efficiente et exacte de détermination de l'âge des poissons pour certaines espèces.

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Introduction

Fish age interpretation is often an essential component of stock assessments and subsequent management of valuable fisheries globally (Francis and Campana 2004). Currently, the most commonly used methods to interpret fish ages rely on estimates derived from length–frequency relationships or hard structures from individual fish, including otoliths, spines, scales, fin rays, and cleithra. Fish age interpretation from hard structures requires a trained age interpreter ("reader" from here forward) to accurately estimate the age by distinguishing annuli formed during periods of varying growth (seasons). However, age interpretations of fish hard structures are subjective, and their accuracy depends largely on reader experience, as well as the experience of those preparing the hard structures for age interpretation. Preparation of hard structures (otoliths and spines) for fish age interpretation generally include the time- and labor-intensive procedures of embedding, sectioning, polishing, and imaging under magnification. Researchers have avoided these procedures in the past by using relatively easy to prepare scale samples for fish age interpretation, but this method has

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largely been abandoned, as it is considered inaccurate when compared with otolith and spine aging methods (Erickson 1983; Welch et al. 1993; Maceina and Sammons 2006). Thus, widely accepted fish aging methods generally require time-consuming and costly preparation methods and personnel training.

Fisheries scientists have explored the utility of using otolith mass to estimate fish ages in the past. Otoliths provide a useful indicator of fish age because slow-growing, long-lived fish tend to have relatively heavy otoliths, while fast-growing, younger fish tend to have relatively light otoliths (Radtke et al. 1985). This method has potential benefits because it limits the amount of subjectivity in fish age interpretation and personnel training generally required when designating fish ages using hard structures. In addition, this method has been found to be more accurate for assigning fish ages when compared with the most commonly used nonsubjective fish aging technique (i.e., length–frequency relationships) in several species evaluated (Boehlert 1985; Fletcher 1991; Francis and Campana 2004). Though assigning fish ages using otolith mass has been applied in several studies over the past three decades, these efforts have been primarily focused on saltwater fish species, with a few examples from brackish fish species, and only one example from a freshwater species (conducted in a laboratory) of which we are aware (Mosegaard et al. 1988).

Although aging fish using otolith mass shows promise, other factors can be incorporated into modeling efforts to more accurately predict fish ages (Francis and Campana 2004). For example, fish length is often highly correlated with fish age (as well as otolith mass) and could provide additional useful information for predicting fish age. Further, in several studies, fish sex was found to influence variation in otolith mass (Wilson et al. 1991; Fletcher 1995; Vallisneri et al. 2008). It has also been shown that the location of collection of fish of the same species can influence otolith mass (Worthington et al. 1995). Finally, annual variation has been shown to occur in otolith masses of fish of the same species collected over time (Pilling et al. 2003; Lou et al. 2007). In many systems, these variables are relatively easy to measure and should be incorporated when they have potential to increase fish age prediction accuracy and precision.

In this study, we evaluated the utility of using otolith mass (coupled with fish length, sex, and collection information) to designate fish ages. We selected to study landlocked populations of sockeye salmon (i.e., kokanee, *Oncorhynchus nerka*) in four Colorado (USA) reservoirs because of their relatively short lifespan (generally dying after 2–4 years of growth), relatively fast growth, and important role (i.e., providing fish for harvest by anglers, eggs for future stocking efforts, and forage for other sport fish) in fisheries in the western United States and Canada (Wydoski and Bennett 1981; Rieman and Myers 1992; Johnson et al. 2002). Thus, understanding the population age structure of kokanee salmon and developing appropriate management strategies and objectives for this species are crucial throughout their range. We used ages interpreted from sectioned kokanee salmon otoliths as training data to predict individual fish ages. Further, we included fish otolith mass, length, sex, day of capture, system of origin (in an analysis evaluating reservoir as a covariate), and year of capture (in a separate analysis evaluating year as a sequence of indicator covariates) in the model. Fish age prediction was performed using a random forests (RF) approach: a nonlinear machine learning statistical tool rarely used in fisheries science, but highly useful for prediction (Breiman 2001; Cutler et al. 2007). Specifically, this method allows for the inclusion of correlated variables with predictive power (e.g., fish length and otolith mass) into a single model, while this multicollinearity can be problematic in many other approaches. Further, the RF approach simultaneously accounts for interactions and nonlinear effects of all variables included in predictive models. This approach is the first to use otolith mass, of which we are aware, to assign ages to freshwater fish outside of the laboratory.

Materials and methods

Kokanee salmon were collected in 2004 from Blue Mesa (Gunnison County, Colorado, USA), Granby (Grand County, Colorado, USA), Shadow Mountain (Grand County, Colorado, USA), and Williams Fork (Grand County, Colorado, USA) reservoirs. To evaluate the relative importance of temporal variability in our analyses, kokanee salmon were also collected from 2000 to 2009 in Williams Fork Reservoir. Kokanee salmon were collected during spawning runs (September to November) at annual egg-take operations conducted by Colorado Parks and Wildlife. Sex and length of the fish were recorded, and both sagittal otoliths were extracted, removed from the sacculus, cleaned (all tissue removed), and archived in coin envelopes. Sagittal otoliths were weighed only when intact (i.e., no missing chips or pieces) to the nearest microgram, and mean masses of the left and right otolith were calculated. If one sagittal otolith was missing or damaged, the mass of the remaining otolith was used for analyses to increase sample size. Left and right otolith masses were highly correlated (linear regression; $F = 2657$, $N = 276$, $p < 0.01$) with a slope ~1. A paired two-sample t test showed that left and right otolith masses were not significantly different (t statistic = 1.65, $N = 276$, $p = 0.10$).

Kokanee salmon ages were interpreted to inform the RF model by assigning ages to randomly selected fish (≥15 males and ≥15 females) from each reservoir and each year (2000–2009 in Williams Fork Reservoir). These sectioned otoliths ($N = 429$) represented a subset of fish that were to be aged outside of the scope of this study ($N = 2210$ fish). Intact left sagittal otoliths (based on precedence set historically in Colorado) were embedded in Epofix embedding resin and sectioned using a Buehler IsoMet 1000 low speed precision saw with a 15.24 cm diamond wafering blade rotating at 350 $r \cdot min^{-1}$ (1 r = 2π rad). Transverse thin sections were polished using 600 grit, followed by 1000 grit sandpaper. Finally, the otolith thin sections were immersed in mineral oil, placed on a microscope slide, and photographed with an InfinityX-21C camera (Lumenera Corporation) under 25× magnification. Ages were interpreted from these images by two independent readers. When the two readers disagreed (only two instances in our case), a third reader was used to settle the disagreement. All kokanee salmon ages are presented as whole numbers representing complete annuli, though a large amount of growth had taken place after the last complete annuli, considering the fish were sampled in the fall of each year.

Statistical analyses

We used a nonlinear machine learning technique (RF) to predict kokanee salmon ages (Cutler et al. 2007) with two approaches. In the first approach, we used data from kokanee salmon collected in 2004 from Blue Mesa, Granby, Shadow Mountain, and Williams Fork reservoirs to evaluate the relative importance and effectiveness of using kokanee salmon length, otolith mass, sex, location of collection, and collection day of year as predictors of kokanee salmon age. In the second approach, we used data from kokanee salmon collected from 2000 to 2009 from Williams Fork Reservoir to evaluate the relative importance and effectiveness of using kokanee salmon length, otolith mass, sex, year of collection, and collection day of year as predictors of kokanee salmon age. The first approach was used to evaluate the relative importance of reservoir as a predictor of kokanee salmon age, and the second approach was used to evaluate the relative importance of year as a predictor of kokanee salmon age.

The RF approach represents an ideal tool for prediction because it is based on a form of cross-validation, is entirely nonlinear, accounts for obscure interactions, and simultaneously evaluates multicollinear variables (Breiman 2001; Cutler et al. 2007). Where other approaches (e.g., linear models) preclude the inclusion of multicollinear variables and thus the exclusion of potentially valuable predictive data, the RF approach can incorporate all readily available data for prediction. In our case, kokanee salmon length and otolith mass are often highly correlated, and with most approaches, one variable or the other would need to be excluded from any single predictive model. Using the RF approach allowed us to incorporate all variables of interest that were easily obtained during kokanee salmon spawning operations (kokanee salmon length, otolith mass, sex, collection day of year, year of collection, and location of collection).

The RF approach falls under a much larger class of machine learning methods, commonly used in applications where prediction is of primary interest (e.g., banking and marketing). The concept of “bagging” is the key to their success in that it essentially refers to the method of fitting regression trees to numerous bootstrapped versions of the training data (i.e., observations sampled with replacement from the larger data set). The resulting trees are then model-averaged to yield a predictor with low bias and variance. The RF method itself is an improvement over the standard bagged regression tree approach because it lowers the variance further by reducing correlation among the trees through random selection of the covariates. As a more detailed description of random forests is beyond the scope of this article, we refer the interested reader to chapter 15 of Hastie et al. (2009) for further information. From a practical perspective, RF methods require very little algorithmic tuning and are therefore much more user-friendly than alternative machine learning predictive approaches. To perform our RF analyses, we used the “randomForest” package (Liaw and Wiener 2002; package 4.6–6) in R (R Development Core Team 2011). A regression approach was used, and all predicted kokanee salmon ages were rounded to the nearest whole number to classify individuals by age-class. This was done because kokanee salmon had discrete year classes in the study systems, being raised from eggs in hatcheries, stocked in the spring, and sampled in the fall while collecting eggs during their spawning runs to begin the cycle again. This approach would also be useful if fish ages are desired at a subannual scale, but the method that satisfies the research need and provides the least amount of misclassification based on cross-validation should be used.

In both RF implementations described here, 2000 regression trees were used to calculate accuracies and error rates for each observation using out-of-bag predictions (i.e., predicting data that were withheld from each tree). The prediction of data that were not used to fit the model can be considered as a form of cross-validation. Variable importance can then be assessed by comparing the increase in (*i*) mean squared prediction error and (*ii*) node purity associated with each individual covariate. We refer the interested reader to Gini (1912), Liaw and Wiener (2002), and Cutler et al. (2007) for more information.

Effort determination

Approximate effort, in the form of personnel hours, was documented during sample preparation and analysis to allow for a qualitative comparison of the time expended aging fish by otolith sectioning alone versus weighing otoliths to inform the RF model. This comparison was qualitative because we were unable to account for the amount of training and experience required for readers (i.e., cost of education and salary) to become proficient at interpreting ages from otoliths, which varies by the individual. However, the two approaches involve different contributions of otolith reader effort: a relatively large amount of effort when sectioning and aging otoliths and a relatively small amount of effort when weighing otoliths to inform the RF model.

Results

The RF approach predicting kokanee salmon ages from fish collected from Blue Mesa, Granby, Shadow Mountain, and Williams Fork reservoirs in 2004 had an overall error (an error defined from here forward as age misclassification relative to ages determined from sectioned otoliths) rate of 9.2% (Table 1). In this analysis, kokanee salmon length, otolith mass, sex, location of collection, and collection day of year were included as covariates for predicting kokanee salmon ages. Based on the variable importance indices of increase in mean square prediction error and node purity, kokanee salmon otolith mass, length, collection day of year, location of collection, and sex had the most predictive value, in that order (Fig. 1). Of the 11 kokanee salmon predicted ages (out of 120) that did not agree with ages determined from sectioned otoliths, seven (64% of those misclassified) were age-4 fish (Table 1) and constituted the largest source of error with respect to age-class.

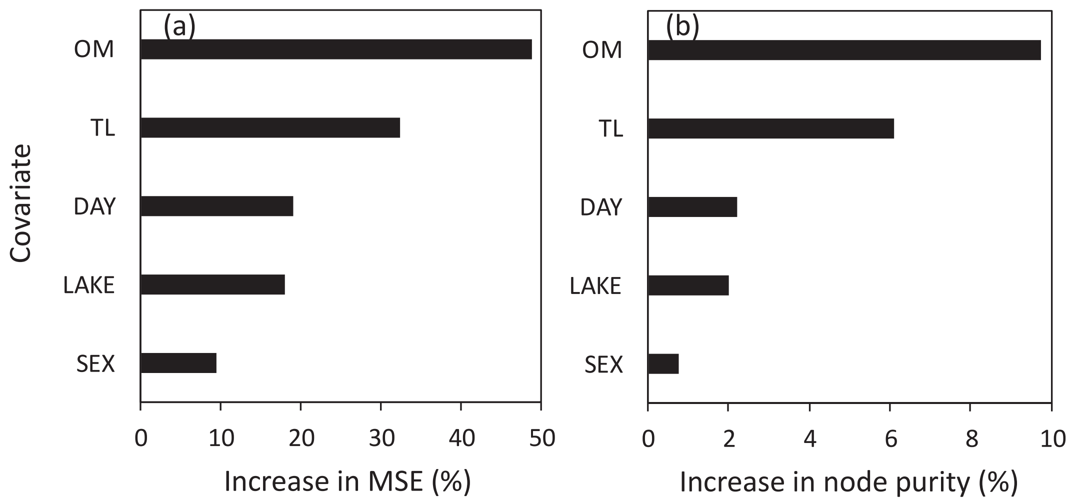
The RF approach predicting kokanee salmon ages from fish collected from Williams Fork Reservoir from 2000 to 2009 had an overall error rate of 7.4% (Table 2). In this analysis, kokanee salmon length, otolith mass, sex, year of collection, and collection day of year were included as covariates for predicting kokanee salmon ages. Based on the variable importance index of increase in mean square prediction error, kokanee salmon otolith mass, length, year of collection, collection day of year, and sex had the most predictive value, in that order (Fig. 2). Based on the variable

Table 1. Age misclassification by the random forests (RF) approach using kokanee salmon (*Oncorhynchus nerka*) from four Colorado reservoirs in 2004.

Reservoir	*N*	% misclassified	Ages misclassified
Blue Mesa	30	13.3	2,4,4,4
Granby	30	3.3	2
Shadow Mountain	30	3.3	2
Williams Fork	30	16.7	2,4,4,4,4

Note: Reservoir names are provided (Reservoir), the number of fish aged (*N*), and the percentage and ages of fish misclassified.

Fig. 1. Variable importance indices for classifying kokanee salmon (*Oncorhynchus nerka*) ages using the random forests (RF) approach with reservoir as a variable. Fish were collected from four different reservoirs in Colorado from 2004. OM is otolith mass, TL is total length, DAY is collection day of year, LAKE is the system from which an individual was collected, and SEX is the sex of an individual. MSE is mean square error. Panel (*a*) represents increase in MSE, and panel (*b*) represents increase in node purity.



importance index of increase in node purity, kokanee salmon otolith mass, length, collection day of year, year of collection, and sex had the most predictive value, in that order (Fig. 2). Of the 25 kokanee salmon predicted ages (out of 339) that did not agree with ages determined from sectioned otoliths, 14 (56% of those misclassified) were age-4 fish (Table 2) and constituted the largest source of error with respect to age-class.

Across both analyses, the RF approach error rate never exceeded 20% in any reservoir or year. Further, the RF approach only exceeded an error rate of 10% in reservoirs and years that had relatively high numbers of age-4 kokanee salmon. Every kokanee salmon determined to be age-4 with sectioned otoliths ($N = 21$ out of a total of 429 sectioned otoliths) was categorized as age-3 using the RF approach. Across both RF analyses, when age-1 and age-2 kokanee salmon (determined using sectioned otoliths) were misclassified, they were overestimated by 1 year. Across both RF analyses, when age-3 and age-4 kokanee salmon (determined using sectioned otoliths) were misclassified, they were underestimated by 1 year.

Based on our evaluation of effort expended during otolith weighing and otolith preparation, sectioning, and aging, we determined that using otolith mass coupled with a subset of sectioned otoliths for aging to inform the RF modeling approach was approximately five times faster than preparing, sectioning, and aging an otolith from each individual in this study. We were able to rapidly predict the ages of 429 kokanee salmon using the approach described here. If we were to use the informed RF models to estimate the ages of more fish from the cohorts analyzed here, otolith mass would be the only additional data required. Thus, the more fish ages that are predicted with this approach, the more cost effective it would become relative to sectioning an otolith from every individual.

Table 2. Age misclassification by the RF approach using kokanee salmon (*Oncorhynchus nerka*) from Williams Fork Reservoir from 2000 to 2009.

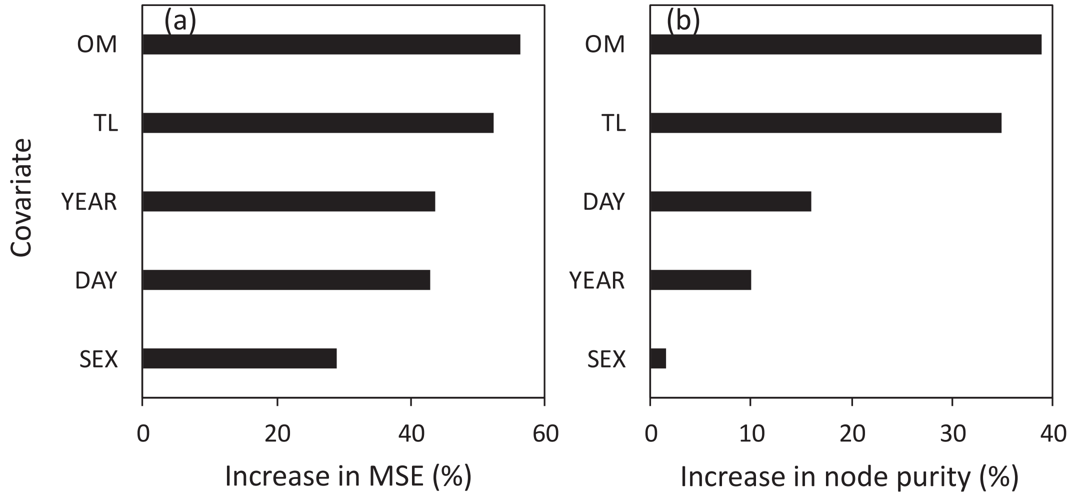
Year	*N*	% misclassified	Ages misclassified
2000	30	6.7	3,4
2001	30	0	None
2002	30	10	2,4,4
2003	37	18.9	2,2,3,4,4,4,4
2004	30	13.3	4,4,4,4
2005	35	8.6	1,3,3
2006	33	3	2
2007	36	0	None
2008	37	2.7	3
2009	41	9.8	3,4,4,4

Note: Years of collection are provided, along with the number of fish aged (*N*) and the percentage and ages of fish misclassified.

Discussion

The results of our RF approach using otolith mass coupled with a variety of other covariates to predict kokanee salmon

Fig. 2. Variable importance indices for classifying kokanee salmon (*Oncorhynchus nerka*) ages using the RF approach with year as a variable. Fish were collected from Williams Fork Reservoir from 2000 to 2009 (YEAR). OM is otolith mass, TL is total length, DAY is collection day of year, and SEX is the sex of an individual. MSE is mean square error. Panel (*a*) represents increase in MSE, and panel (*b*) represents increase in node purity.



age suggested that this method has promise as an alternative to other methods. Error rates between the RF approach and kokanee salmon ages determined from sectioned otoliths were relatively low, with few predictable exceptions, and were always within what is considered acceptable for many standard fisheries assessments (Maceina et al. 2007; 20% error). Further, this approach is more efficient and less subjective when considering the amount of time and level of training required to ensure personnel are properly embedding, sectioning, polishing, and imaging otoliths, and subsequently interpreting ages from these sections. We suggest that these activities be restricted to highly trained individuals and that these individuals' time be used as efficiently as possible, making the RF approach informed with a subset of sectioned otoliths, coupled with otolith mass data, an attractive option for maximizing the efforts of highly trained personnel.

The RF approach described here relies on aging a subset of otoliths to inform the model; thus, subjectivity is not completely eliminated. However, once trained personnel have put in relatively little effort sectioning and interpreting fish ages from a subset of otoliths, there is very little limitation on how many fish can be aged using the RF approach by weighing otoliths. Relatively untrained personnel can weigh hundreds of otoliths in a single day, essentially eliminating any subjectivity that they may impart on further analyses. Additionally, other available covariates that are easily obtained (e.g., fish length, sex, sampling date, year and location, etc.) can be incorporated in the RF modeling approach to increase estimate precision without adding further subjectivity. Importantly, if otoliths in the subset to be aged after sectioning are difficult to age because of poor sectioning, opacity, or other challenges, they can simply be replaced by other better examples that readers have more confidence in, and the masses of unsuitable otoliths can still be used to predict fish age.

We were able to obtain acceptable age interpretation precision (Maceina et al. 2007) with a subset of approximately 30–40 sectioned otoliths from each year or reservoir. These subsets then allowed us to predict the ages of thousands (2210) of kokanee salmon outside of the scope of this study. Throughout the RF analyses we predicted all ages of kokanee salmon well, except those that were age-4 (likely because of low representation in the sample). Thus, in the future, we will focus our sampling on particular groups of fish that are underrepresented in the data set (e.g., age-1 and age-4 fish) to systematically improve the error associated with the RF approach. Currently, we are able to classify kokanee salmon of all ages with confidence because we are sectioning the otoliths from the smallest and largest individuals. It is important to note that we started the RF analyses with a relatively small subset of sectioned otolith samples to inform the RF approach, and it still performed well. Therefore, this method showed promise with relatively little effort from highly trained personnel and might be improved with additional minimal effort.

Based on the RF results, otolith mass was the most important covariate for classifying kokanee salmon by age, followed by length. These two predictors were more useful relative to the collection day of year, the year and location of collection, and sex. Many fishery assessments have relied on length–frequency histograms to indicate the age structure of fish populations (Pauly and Morgan 1987; Gulland and Rosenberg 1992). Our results, and others, suggest that using otolith mass may be a more accurate approach (Boehlert 1985; Fletcher 1991; Francis and Campana 2004). Additionally, using the RF method allowed us to include correlated covariates (otolith mass and fish length) to classify kokanee salmon ages, which may be useful in other studies relying on fish aging to determine the characteristics of a population for management or research purposes. For example, Francis and Campana (2004) suggested that although otolith mass appeared to be a potentially useful predictor of fish age, age predictions could benefit from multiple predictive variables included in a single model. The flexibility of the RF approach allowed us to include several easy to collect covariates increasing prediction precision, whether correlations between the covariates existed or not.

Our evaluation of the efficiency of the RF model informed with a subset of kokanee salmon ages interpreted from sectioned otoliths showed that the method was approximately fivefold faster than interpreting ages by preparing, sectioning, and aging an otolith from each individual. This discrepancy would increase as more otoliths were weighed for age estimation. For example, we estimated the ages of 429 individuals and used these data to inform the RF model. If we were to use the informed RF model to estimate ages from an additional 1000 kokanee salmon by weighing otoliths (achievable by relatively untrained personnel), the RF approach would be fivefold more efficient than relying solely on otolith sectioning (requiring highly trained personnel). These increases in efficiency translate to monetary savings, especially when considering the personnel training required to conduct each approach. These findings corroborate those of others in which they observed significant increases in efficiency by aging fish with an approach using otolith mass (McDougall 2004; Cardinale and Arrhenius 2004; Steward et al. 2009). Additionally, our evaluation is conservative and does not account for the time spent training personnel to properly handle and interpret ages from otoliths. Thus, this method is very attractive for rapidly and cost-effectively predicting fish ages as long as the population is representatively sampled (an assumption made in most statistical models).

We selected a short-lived, fast-growing fish species for the analyses described here. Based on the observations of others, this species was an ideal candidate for this research (Radtke et al. 1985). The maximum age of kokanee salmon in our study was four, limiting the amount of possible categories to which a fish could be assigned. Although we selected this species because of its favorable characteristics, our findings suggest that this approach might prove useful for assigning ages to other freshwater fishes. The method was more efficient and less subjective, and the RF approach allows one to include a multitude of multicollinear covariates in analyses to classify fish age. The method described here has been adopted to manage the kokanee salmon fishery in Blue Mesa Reservoir, Colorado, which provides the majority of kokanee salmon eggs to sustain fisheries throughout Colorado. Using otolith mass to assign ages to freshwater fishes is essentially absent from the literature, and we suggest coupling this approach with RF analysis could greatly increase our capabilities of conducting age- and growth-based research, assessing sport fish stocks, and management of valuable freshwater fisheries.

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