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BRIEF COMMUNICATION

Key transitions in morphological development improve age estimates in white sturgeon *Acipenser transmontanus*

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We reared white sturgeon *Acipenser transmontanus* under laboratory conditions and found that a random-forest model containing scute counts and total length predicted age significantly better than total length alone. Scute counts are rapid, inexpensive and non-lethal meristics to gather in the field. This technique could improve age estimates of imperilled sturgeon populations.

KEYWORDS
age-prediction, fisheries management, larval fish, ontogeny, random-forest model

Larval growth estimates are a critical tool in fish population management, as small fluctuations in growth can affect survival and recruitment to older age classes (Houde & Hoyt, 1987; Post & Prankevicius, 1987). Larval growth determination requires an assessment of age in days, which can be done morphometrically using structures that form daily rings, such as otoliths (Campana & Neilson, 1985). However, otoliths can only be extracted from dead fish, which is problematic for live-caught larval fishes, including those that are legally protected due to conservation concerns. Thus, techniques to age larval fish without otolith extraction are needed. Length–age curves have been used to address this problem, but they carry several assumptions including no size-selective mortality, no multiple recruitment events and no migration (Campana, 2001). Estimating age and growth for population management may therefore benefit from using structure development, as has been demonstrated with tooth analysis, zygomatic arch breadth and other bone measurements (Gipson *et al*., 2000; Karels *et al*., 2004; Zimmerman, 1972).

Sturgeon (Acipenseridae) populations around the world are declining and are the focus of intensive management efforts (Billard & Lecointre, 2001; Birstein, 1993; Pikitch *et al*., 2005). The primary threats to sturgeon populations are barriers to migration and overfishing (De Meulenaer & Raymakers, 1996; Jager *et al*., 2001; Rochard *et al*., 1990). Even when these threats are mitigated, an exceptionally long generation time slows population recovery (Boreman, 1997). Population mortality is highest at the larval life stage, yet few studies focus on the early life stages of sturgeons (Le Cren, 1962; Sifa & Mathias, 1987). Larvae from all North American sturgeon species have been captured in screw traps or bottom trawls and aging of these specimens could allow estimates of growth. However, larval age and growth are difficult to estimate for wild sturgeon populations. Otoliths are not reliable for estimating age in sturgeons (Brennan & Cailliet, 1991) and the first pectoral-fin ray does not develop until fish are larger. Larval sturgeons have high variance in length–age curves (Figure 1a) so age in the wild cannot currently be estimated. Previous studies of growth in larval sturgeons have been possible only by releasing hatchery-raised larvae (Braaten *et al*., 2008; Braaten *et al*., 2012). These studies are sizable efforts and may be biased by genetic or plastic responses to hatchery practices. A reliable, non-lethal aging technique would benefit management of larval sturgeons by allowing estimates of growth, but no technique currently exists to obtain

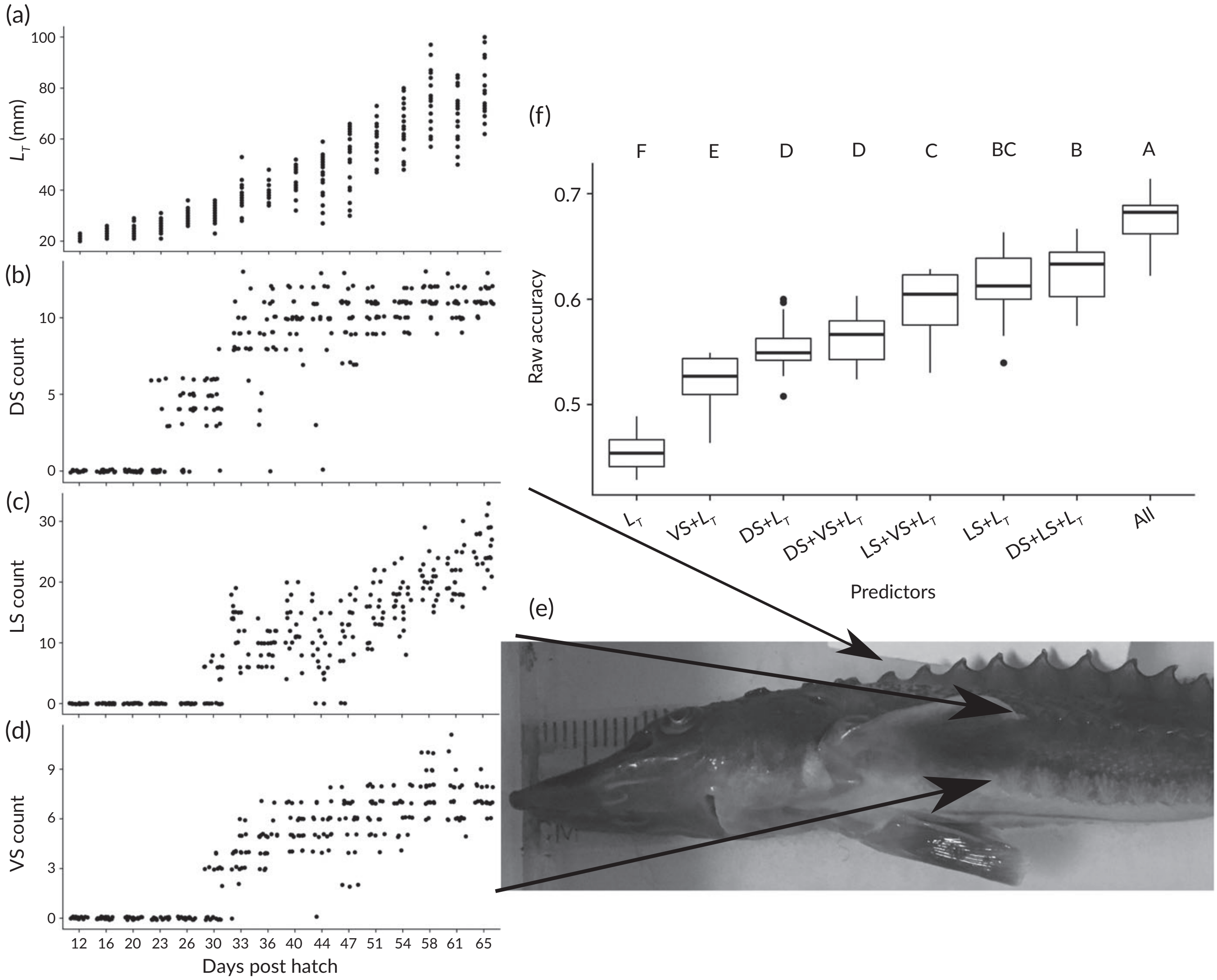

FIGURE 1 Accuracy of *Acipenser transmontanus* age-prediction models based on (a) total length (L_T) and (b) dorsal scute (DS), (c) lateral scute (LS) and (d) ventral scute (*VS*) counts *v.* days post hatch. (e) Illustration of dorsal, lateral, and ventral scutes. (f) Accuracy of age estimation using L_T and different morphological predictors. Raw accuracy boxplots (–, median; □, 25th–75th percentiles; |, 95% range; O, outliers) generated with 20 bootstrap iterations. Letters, above, are results of grouping with Tukey HSD *post hoc* test

accurate age estimates during the larval life stage. In this study, we tested the potential for using developmental stage of morphological structures as a non-destructive method to improve age estimates for larval sturgeons.

To better understand how key developmental transitions are related to age and size, we reared white sturgeon *Acipenser transmontanus* Richardson 1836 in the laboratory and collected a developmental series from the beginning of exogenous feeding through to the completion of the larval life stage. *Acipenser transmontanus* is an anadromous species and the largest freshwater fish in North America. It is a popular sport fish and is farmed for caviar, but its long generation time of 10–20 years makes this species vulnerable to overharvesting (Conte, 1988; Doroshov, 1985). Spawning and rearing habitats of *A. transmontanus* have been altered by dams throughout the species' range, including California's Central Valley (Kynard & Parker, 2005). This species was selected because it is readily available from hatcheries and represents a valuable recreational fishery. It is also a closely related species to threatened green sturgeon *Acipenser medirostris* Ayres 1854, which would also benefit from improved aging techniques.

We reared *A. transmontanus* in a single 1.8 m diameter tank (455 l) at the Center for Aquatic Biology & Aquaculture at the University of California Davis. Water was from a designated well and aerated before being pumped into the tank. Temperature of the water coming from the well into the tank was 18.6°C (daily σ = 0.3°C) throughout rearing. Approximately 6500 larvae were acquired from Sterling Caviar LLC (www.sterlingcaviar.com) and were progeny from 4–6 males and 5–6 females (Van Eenennaam *et al.*, 2004, Van Eenennaam, J. *pers. comm.*). Adult females were induced to ovulate on the same day and eggs were removed by caesarean section. Adult males were induced to produce milt and fertilised eggs were placed in hatching jars. Larvae were transitioned into Rangen RSM Starter Feed (www.rangen.com; 55% protein, 17% fat, <2% fibre, <10% ash) *ad libitum*, then fed Rangen semi-moist feed (45% protein, 19% fat, <2% fibre, <9% ash) *ad libitum* using a 24 h continuous feeder. Average mass of fish in the tank was calculated weekly to adjust feed rates for optimum feeding as fish grew (Deng *et al*., 2003; Lee *et al*., 2014).

Twenty fish were randomly collected biweekly from 12 to 65 days post hatch (dph) for a total of 320 individuals. Fish were euthanized with MS-222 (0.5 g l^{-1} MS-222 buffered with 0.42 g l^{-1} sodium bicarbonate and 6 g l^{-1} salt; UC Davis Institutional Animal Care and Use Protocol #18767) then total length (to the nearest 1 mm) and wet mass (to the nearest 0.0001 g) was recorded before fish were fixed in 10% formalin. In the laboratory, larval *A. transmontanus* were transferred to distilled water for 2 days to remove traces of formalin before development stage was assessed. Calcified dorsal scutes, lateral scutes and ventral scutes were then counted on each individual using a laboratory probe.

We built a model to predict larval *A. transmontanus* age using scute counts and total length. We compared raw accuracy of two models: linear discriminant analysis (MASS package; Venables & Ripley, 2002) and Breiman's random-forest classifier (randomForest package; Liaw & Wiener, 2002). Both models are resilient against multicollinearity and can identify the independent variables that are predictive of age. The linear discriminant analysis was trained using leave-one-out cross-validation, while random forest does not require cross-validation. The best model was selected by bootstrapping and then comparing raw model accuracy in predicting categorical age group. Using the best-fit model, we determined which predictors were most important by bootstrapping and sequentially leaving out predictors. Each model was run over a dataset with replacement 20 times and raw accuracy was compared between models using Tukey HSD *post hoc* test from ANOVA comparison of all models.

We used the counts of each calcified dorsal, lateral and ventral scute to build predictive models of age. Fish structures developed in a consistent order: dorsal scutes calcified first and then ventral and lateral scutes simultaneously calcified (Figure 1b–d). A series of models were constructed using all combinations of scute counts in addition to total length. These ranged from models with a single predictor (total length) to a model with all predictors. As there are 16 age groups, the probability of selecting the correct age group if left to random chance is 6.25%. We compared models using raw accuracy, which is scored by the percentage of individual fish that were placed in the correct age group. Every model exceeded the accuracy of this null model (6.25%), indicating that the predictors were effective indicators of age. Bootstrapped model selection showed that the random-forest classifier performed better than the linear discriminant analysis (Welch two-sample t-test: $df = 35$, $t = -43.98$, $P < 0.001$). Mean model accuracy increased from 41% with the linear discriminant analysis to 68% with the random forest classifier. This can probably be explained by the higher resilience of random-forest models to non-parametric variable distributions (Karels *et al*., 2004; Lynn & Brook, 1991; Smith *et al*., 1997); response variables were distributed uniformly while predictors were a zero-inflated negative binomial distribution. Next, we found that prediction accuracy increased as scute counts were included in the model. The model with only total length achieved a mean accuracy of 45%, while the model with total length and all scute counts increased to a mean accuracy of 68% (Figure 1f). Scute count varied in predictive power, with the best being lateral scutes (group BC), next being dorsal scutes (group D) and final being ventral scutes (group E; Figure 1f).

Our results show that scute counts, a simple meristic to measure in the field without euthanizing individuals, significantly increased the accuracy of age estimates over those based on total length alone for fish ranging from 20 to 80 mm. Total length is informative for age-prediction in early larvae, but high variance in age–length relationships for older individuals decreases predictive power (Figure 1a). It is for these later-stage larvae that scute counts become most valuable for age prediction (Figure 1b–d). The current method of aging *A. transmontanus* is to count rings in the pectoral-fin ray and validate estimates with mark–recapture studies (Beamish & McFarlane, 1983; Nguyen *et al*., 2016). However, this method does not work for larval *A. transmontanus* as the pectoral-fin rays have not yet calcified. Larval *A. transmontanus* age predictions could increase accuracy of growth estimates, which is important for management because growth is a predictor of survival and recruitment (Letcher *et al*., 1996; Miller *et al*., 1988).

Temperature affects the rate of physiological processes in sturgeons, including growth, metabolism and development (Cech *et al*., 1984; Geist *et al*., 2005; Hardy & Litvak, 2004). In our experiment, fish were reared at a single temperature, 18.6°C. Therefore, our results at this one temperature should not be extrapolated to age prediction of wild populations without further validation. Furthermore, different sturgeon species (and even populations) may have different relationships between developmental stage, temperature and age. Species and population-specific trials should be conducted. Morphological meristics probably interact with temperature and alter the age-prediction algorithm. Despite this challenge, current age-prediction of larval sturgeons is conducted with total length alone and this method is also susceptible to temperature effects (Kappenman *et al*., 2009). Therefore, developmental stage will probably increase accuracy of age-prediction over total length alone under variable temperatures.

Acipenserid populations around the world are imperilled due to dams and overfishing (Beamesderfer *et al*., 1995; Billard & Lecointre, 2001; Birstein, 1993; Jager *et al*., 2001; Rochard *et al*., 1990). Mitigation for these stressors is hampered by the exceptionally long generation time of sturgeons, which slows population recovery (Boreman, 1997). Larval growth is crucial to understand because growth can drive survival and recruitment (Houde & Hoyt, 1987; Post & Prankevicius, 1987). We developed a non-destructive method that increases age-prediction accuracy by integrating scute meristics into traditional total length measurements, which are simple measurements to take in the field. These findings are important for management of *A. transmontanus* and other sturgeon species as they provide a framework to increase accuracy of larval age-prediction, which can lead to better growth estimates and assist in the recovery of sturgeon species worldwide.

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CONTRIBUTIONS

L.J.Z contributed ideas, data generation, data analysis and manuscript preparation. S.B.M contributed data generation and manuscript preparation. E.P.P contributed ideas, manuscript preparation and funding. N.A.F contributed ideas, data generation, manuscript preparation and funding.

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