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Using pectoral fin rays as a non-lethal aging structure for smallmouth bass: precision with otolith age estimates and the importance of reader experience

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Abstract

We evaluated the potential utility of pectoral fin rays as non-lethal aging structures for smallmouth bass *Micropterus dolomieu*. We compared age estimates among three reading pairs, and against sectioned sagittal otolith age estimates for precision. Reading pair bias was not detected, although the highest rates of age estimate agreement occurred between reading pairs with high and moderate aging experience. Precision of otolith and fin ray age estimates were equivalent based on between-structure comparisons of average percent error and coefficient of variation. However, fin rays underestimated fish age compared to otoliths for older fish, especially when aged by less experienced readers. Pectoral fin rays may provide a non-lethal alternative to otoliths for aging smallmouth bass, particularly for younger fish (≤ age-4) or when experienced readers conduct aging. Additional evaluations of fin rays as smallmouth bass aging structures are needed, including validation using known-age fish and documentation of consistent annulus formation throughout a fish's life.

Keywords: age estimation; *Micropterus dolomieu*; reader experience; pectoral fin ray; otolith; precision

Introduction

Accurate and precise age estimation is essential for fish population assessment and subsequent management. Accurate age data coupled with other fisheries metrics can be used to estimate growth, mortality rates, population structure, and change in species abundance, biomass and yield. However, inaccurate age estimation can lead to gross errors in stock metrics (e.g., recruitment, growth, and mortality) and adversely affect management strategies (Mills and Beamish 1980; Beamish and McFarlane 1983; Lai and Gunderson 1987; Leaman and Nagtegaal 1987; Tyler et al. 1989; Reeves 2003; Yule et al. 2008). Therefore, it is crucial that fisheries managers and biologists use the best available methods to estimate age.

Fish hard-part structures (e.g., otoliths, fin rays, and scales) are used for age estimation in fishes because these structures contain annuli. Annuli are alternating bands of opaque and clear zones within a structure that represent periods of slow and fast growth (Casselman 1987). Formation of annuli in otoliths occurs annually during periods of slow growth (opaque zones; e.g., during winter), throughout a fish's lifespan (Hales and Belk 1992), and annuli formation in fin rays also form in a similar manner (Simkiss 1973; Shirvell 1981). Thus, fisheries biologists and managers are able to use these annuli of hard-part structures to estimate age of individual fish.

Otoliths are commonly used to estimate ages in a variety of fish species, and generally provide the most accurate (Heidinger and Clodfelter 1987) and precise (Long and Fisher 2001) estimates of age. However, a disadvantage in using otoliths is that their removal requires the undesirable sacrificing of perhaps an important sport or long-lived species (DeVries and Frie 1996). Alternative non-lethal aging techniques have been developed for numerous fish species using a variety of hard-part structures. Scales may be a viable alternative to otoliths in some species (Kruse et al. 1993; DeVries and Frie 1996), but in general scales tend to underestimate age in older fish (Welch et al. 1993; Long and Fisher 2001; Phelps et al. 2007). Age estimation from scales when compared to otoliths in smallmouth bass *Micropterus dolomieu* revealed that scales tended to underestimate age (Long and Fisher 2001), suggesting they may be a poor choice for aging smallmouth bass. Pectoral fin rays are another non-lethal alternative to otoliths, and evidence suggests that pectoral fin rays may yield age estimates that are more precise than scales and comparable to otoliths in some fish species (Howland et al. 2004; Walsh et al. 2008). However, fin rays may also underestimate age of relatively old fish for some species (Maraldo and MacCrimmon 1979; Beamish 1981; Sikstrom 1983; Rien and Beamesderfer 1994; Phelps et al. 2007; Stolarski and Hartman 2008).

No published studies have evaluated the precision of pectoral fin rays as a non-lethal technique for estimating the age of smallmouth bass. We sought to assess the potential size- and age-related bias in age estimates derived from smallmouth bass pectoral fin rays compared to age estimates obtained from otoliths. Aging fish can be difficult because of the possibility of false annuli and annuli compression, and an investigator should consider reader experience when aging hard-part structures. Thus, we included six readers with variable experience estimating age from otoliths and fin rays to determine how experience affects age-estimate bias.

Methods

Smallmouth bass (n = 72, 125-483 mm total length) were collected using pulsed DC electro-fishing (~8-10 amperes) during October 2010 and March-May 2011 from four natural lakes, rivers and reservoirs in central and northern Illinois. Total length of each fish was measured to the nearest mm. Whole sagittal otoliths and the leading pectoral fin ray (cut as close to the body as possible) were removed from each fish and both structures were stored dry. Otoliths were embedded in epoxy and cut into 1.3-mm sections surrounding the nucleus in the transverse plane using a Buehler ISOMET™ low-speed saw (Buehler Inc., Lake Bluff, IL, USA). Leading pectoral fin rays were embedded in epoxy and sectioned at the articulating process (the widest portion at the base of the fin ray) to 1.3-mm sections using the low-speed saw. Otolith and pectoral fin ray sections were sanded with silicon carbide paper to reveal annuli, polished with lapping film, and mounted on glass microscope slides using double-sided tape (3M, St. Paul, MN, USA).

Both otolith and pectoral fin ray sections were viewed at 20-40x magnification under transmitted light. The translucent bands following opaque growth zones were considered annuli in both otolith and pectoral fin ray sections. The outer edge of otoliths and pectoral fin rays were counted as annuli for fish collected during October 2010; however, outer edges were not counted as annuli for fish collected in spring 2011 because of the opportunity for structural growth subsequent to deposition of the prior winter's annulus. Otolith and fin ray sections were examined independently by six readers (from low to high experience levels) who initially worked together to define criteria for identifying annuli. Readers were unaware of fish size and which structures corresponded to a particular fish within the sample population. Readers were paired to form three sets of readers with each pair representing a different level of experience; (high [3-4 years of fish aging experience], moderate [2 years of fish aging experience] and low [≤1 year of fish aging experience]). Each of the three reading pairs aged each structure (i.e., from all 72 fish) by coming to a consensus age with one another after each individual within a pair had independently estimated age.

Precision of smallmouth bass age estimates was assessed for each structure by calculating the average percent error (APE; Beamish and Fournier 1981) and coefficient of variation (CV; Chang 1982) of age estimates. Beamish and Fournier (1981) calculated APE in aging the *j*th fish as:

$$APE = 100 \times \left(\frac{1}{R} \sum_{i=1}^{R} \frac{\left| X_{ij} - X_j \right|}{X_j} \right)$$

where R is the number of times each fish was aged, X_{ij} is the *i*th age determination of the *j*th fish, and X_j is the mean age of the *j*th fish. Coefficient of variation was calculated for age estimates from each fish as: CV = 100 x SD/mean (Chang 1982). Student's *t*-tests were used to test whether mean APE and CV for age estimates derived from otoliths and pectoral fin rays differed significantly among structures. Linear regressions were used to assess relationships between APE and CV and fish total length for each aging structure to determine whether fish length had an influence on the precision of age estimates for each structure.

Reading pair bias was assessed using age-bias plots (Campana et al. 1995). Bias was determined for all possible pairwise comparisons among reading pairs by regressing fish age determined by reading pair *X* (higher level of experience) onto the mean age (± SE) for every fish aged by reading pair *Y* (lower level of experience). T-tests were used for each age-bias graph to test the null hypotheses that the intercept (β_0) was not significantly different from zero and the slope (β_1) was not significantly different from one (indicating 1:1 agreement in age estimates between reading pairs). Rejection of either hypothesis was interpreted as reading pair bias (Long and Fisher 2001). Percent agreement between reading pairs was also determined to assess the frequency of complete agreement and agreement within one year for otoliths and pectoral fin rays.

Structure-related bias in age estimates was assessed using methods described in Long and Fisher (2001). The modal age for each structure encompassing data from all reading pairs was computed for each fish and otolith modal age was plotted against pectoral fin ray mean modal age (mean age calculated for all fish of a given otolith modal age). T-tests were used to determine whether the intercept of the relationship between otolith modal age and pectoral fin ray mean modal age was significantly different from zero and if the slope of this relationship was significantly different from one. Rejection of either hypothesis was interpreted as structure-related bias in age estimates. Age bias plots comparing fin ray age and otolith age estimates for individual fish within reading pairs were also constructed and used to test for deviations from a 1:1 relationship between fin ray and otolith age estimates for each reading pair. All statistical analyses were performed using SAS 9.2 (SAS Institute, Inc. Cary, NC). P-values ≤ 0.05 were considered significant for all statistical tests.

Results

Precision of age estimates for smallmouth bass from sectioned otoliths and fin rays were equivalent based on comparisons of APE and CV between structures. Mean APE of age estimates from otoliths (20.14%) and pectoral fin rays (25.36%) were not significantly different ($t_{142} = -1.858$, $P = 0.066$). Likewise, mean CV of age estimates from otoliths (11.72%) and pectoral fin rays (12.85%) were not significantly different ($t_{142} = -0.805$, $P = 0.422$). There was no relationship between either APE or CV and fish total length for age estimates derived from otoliths (APE: $F_{1, 70} = 2.31$, $P = 0.132$; CV: $F_{1, 70} = 2.33$, $P = 0.131$) or pectoral fin rays (APE: $F_{1, 70} = 3.86$, $P = 0.053$; CV: $F_{1, 70} = 1.55$, $P = 0.217$).

Reading pair bias in estimating fish age from otoliths was not detected for any possible pairwise comparisons between reading pairs ($P \geq 0.75$ for all tests of $\beta_0 = 0$; $P \geq 0.27$ for all tests of $\beta_1 = 1$; Figure 1). Highest agreement in otolith age estimates between groups occurred between pair one (high aging experience) and pair two (moderate aging experience), where 49% of fish were assigned the same age and 94% of fish were aged within one year of each other. Agreement was lower between pair one and pair three (low aging experience), where 28% of fish were assigned equivalent ages and 76% of fish were aged within one year of each other. Agreement between pair two and pair three was also relatively low, as 31% of fish had exact agreement in age estimates and 90% of fish were aged within one year of each other.

Similar to otoliths, no reading pair bias in estimating age of smallmouth bass from sectioned pectoral fin rays was detected for any pairwise comparisons between reading pairs ($P \geq 0.81$ for all tests of $\beta_0 = 0$; $P \geq 0.16$ for all tests of $\beta_1 = 1$; Figure 2). The highest rate of agreement for pectoral fin ray age estimates occurred between pair one and pair two, as 54% of fish had exact agreement of age estimates and 92% of fish were aged within one year of each other. Agreement was lower between pair one and pair three, as only 26% of fish were assigned the same age and 58% of fish were aged within one year of each other. Exact agreement of age estimates between pair two and pair three occurred for 35% of fish, while agreement of age estimates within one year between these two groups was achieved for 86% of fish examined.

Structure-related bias in smallmouth bass age estimates was detected within and across reading pairs. The intercept of the relationship between fin ray mean modal age and otolith mean modal age encompassing data from all reading pairs was not significantly different from zero ($t = 1.22$, $P = 0.268$), but the slope of this relationship was significantly less than one ($F_{1, 6} = 10.46$, $P = 0.018$; Figure 3). The frequency of exact agreement between mean modal age estimates derived from pectoral fin rays and otoliths was 46% and the frequency of agreement of mean modal pectoral fin ray and otolith age estimates within one year was 92%. Slopes of relationships between fin ray and otolith age estimates for individual fish within reading pairs were all significantly less than one and y-intercepts of these relationships were all significantly different from zero ($P < 0.001$ for each test; Figure 4). The coefficient of determination (r^2) for the relationship between fin ray and otolith age estimates was higher for the most experienced reading pair than for the two less-experienced reading pairs. For the most experienced reading pair, 99% of fin ray age estimates were within one year of the age estimate obtained from the sectioned otolith from the same individual fish.

Discussion

Precision of age estimates from sectioned otoliths (as measured by APE or CV) was similar to a previous study investigating precision of age estimates from smallmouth bass otoliths (Long and Fisher 2001). Additionally, precision of age estimates from smallmouth bass otoliths was similar to that of other species such as largemouth bass *Micropterus salmoides* (Long and Fisher 2001), spotted bass *Micropterus punctulatus* (Long and Fisher 2001), and striped bass *Morone saxatilis* (Welch et al. 1993). However, some studies have indicated far better precision of age estimates using otoliths (e.g., white bass *Morone chrysops*, Soupir et al. 1997 and channel catfish *Ictalurus punctatus*, Michaletz et al. 2009). Annuli formation in otoliths of smallmouth bass has been validated (Heidinger and Clodfelter 1987) indicating that annuli are accrued consistently throughout the aging process. The sectioning and polishing process may have had an influence on precision of otolith age estimates, although the otolith core appeared visible in all samples. However, some annuli near the edge appeared faint in some otoliths. Burning and then sectioning otoliths may help increase precision of age estimates, as burning may help faint annuli become more visible (Buckmeier et al. 2002). Most published studies suggest that age estimates from sectioned otoliths are more precise than those obtained from whole otoliths (Hoyer et al. 1985; Iserman et al. 2003). However, Howells (1994) and Long and Fisher (2001) suggested that whole otoliths may provide more precise age estimates than sectioned otoliths for largemouth bass and smallmouth bass.

Precision of pectoral fin ray age estimates for smallmouth bass was comparable to or lower than precision (as measured by APE or CV) of age estimates from published studies that used pectoral or pelvic fin rays to age non-centrarchid species. Precision of age estimates from rainbow smelt *Osmerus mordax* (Walsh et al. 2008) pectoral fin rays and muskellunge *Esox masquinongy* (Brenden et al. 2006) pelvic fin rays were similar to pectoral fin ray age estimates for smallmouth bass. However, age estimates derived from pectoral fin rays were more precise for carpsuckers, *Carpiodes* spp., (Spiegel et al. 2010), pallid sturgeon *Scaphirhynchus albus* (Hurley et al. 2004) and white sturgeon *Acipenser transmontanus* (Rien and Beamesderfer 1994) compared to pectoral fin ray age estimates in the present study. Failure to identify the first annulus is a common occurrence when aging fish with pectoral fin rays due to central lumen expansion and obliteration of the first annulus (Buckmeier et al. 2002). For long-lived and large individuals there is a tendency for fin ray annuli to become compressed due to reduced growth (Rein and Beamesderfer 1994; Buckmeier et al. 2002). However, there was no relationship between fish length and precision of pectoral fin ray age estimates for smallmouth bass, suggesting that fin ray annuli were consistently recognized and counted across fish sizes.

Lower precision of age estimates for smallmouth bass using pectoral fin rays or otoliths in comparison to some prior studies may have been due in part to differences in reader experience among studies. Statistical evidence for reading pair bias in estimating smallmouth bass age using a particular aging structure was not detected. It should be noted that our highest experienced readers are still relatively young compared to some fisheries professionals that have decades of reading experience. Nonetheless, percent agreement between reading pairs for both otolith and pectoral fin ray age estimates was highest for the high and moderate experience groups, suggesting some effect of experience level on smallmouth bass age estimation. Rates of exact agreement between reading pairs for otolith age estimates were generally lower than those reported for other fish species, but rates of agreement within one year were similar to values reported in published studies (Welch et al. 1993; Iserman et al. 2003). Rates of exact agreement between reading pairs and agreement within one year for pectoral fin ray age estimates were within the ranges of values reported in published studies for other fish species (Sikstrom 1983; Welch et al. 1993; Rien and Beamesderfer 1994; Spiegel et al. 2010). Several studies have reported an effect of reader experience on precision of otolith or fin ray age estimates (Campana and Moksness 1991; Ross et al. 2005; Brenden et al. 2006), whereas other studies found no difference in precision of otolith or fin ray age estimates with experience level (Howland et al. 2004; Vandergoot et al. 2008). Reader experience should be considered when using otoliths or pectoral fin rays to age smallmouth bass, as inexperienced readers may produce less precise estimates of fish age and may have a greater tendency to underestimate age of older fish using pectoral fin rays.

Precision of age estimates from smallmouth bass pectoral fin rays was similar to precision of age estimates derived from otoliths. However, structure-related bias in age estimation was detected, with pectoral fin rays tending to underestimate age of older fish compared to otoliths. The tendency to assign lower ages to older fish using fin rays than when using otoliths was most pronounced in the less experienced reading groups, suggesting that reader experience should be taken into account when selecting an aging structure for smallmouth bass. Underestimation of fish age through use of pectoral fin rays in comparison to otolith ages (especially in older individuals) has frequently been reported across a variety of fish species (Maraldo and MacCrimmon 1979; Rien and Beamesderfer 1994; Sylvester and Berry 2006; Phelps et al. 2007; Stolarski and Hartman 2008). Few published studies have shown pectoral fin ray age estimation to be a comparable and reliable alternative to use of otoliths across all sizes and ages of fish examined (but see Howland et al. 2004; Walsh et al. 2008). Pectoral fin ray underestimation of age in older individuals was likely attributed to the aforementioned effects of failure to identify the first annulus, expansion of the central lumen, or annuli compression.

Sectioned or whole otoliths (Long and Fisher 2001) are recommended for estimating smallmouth bass age when sacrificing fish is not a concern. Annulus formation in smallmouth bass otoliths has been validated (Heidinger and Clodfelter 1987) and otoliths appear to provide the most precise and least biased estimates of age among potential aging structures based on this study and Long and Fisher (2001). Pectoral fin rays appear to provide a suitable, non-lethal alternative to use of otoliths for aging smallmouth bass, particularly for younger fish (≤ age-4) or when experienced readers are aging fish. However, precision and accuracy of fin ray age estimates can differ among fish from different populations or latitudes (Brenden et al. 2006). Additional evaluation of fin rays as aging structures for smallmouth bass is recommended, particularly for fish from geographic areas that are distant from those sampled in this study. Validation of fin ray aging techniques and confirmation of consistent formation of fin ray annuli for smallmouth bass using known-age fish are also recommended.

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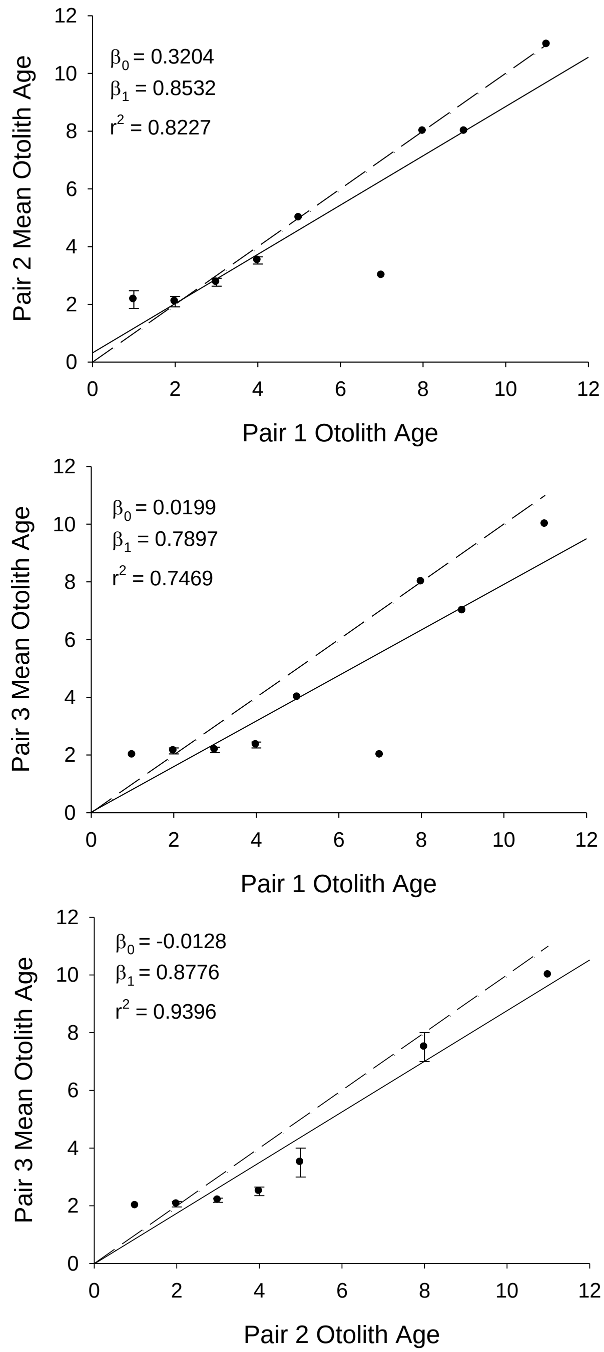
Figure Captions

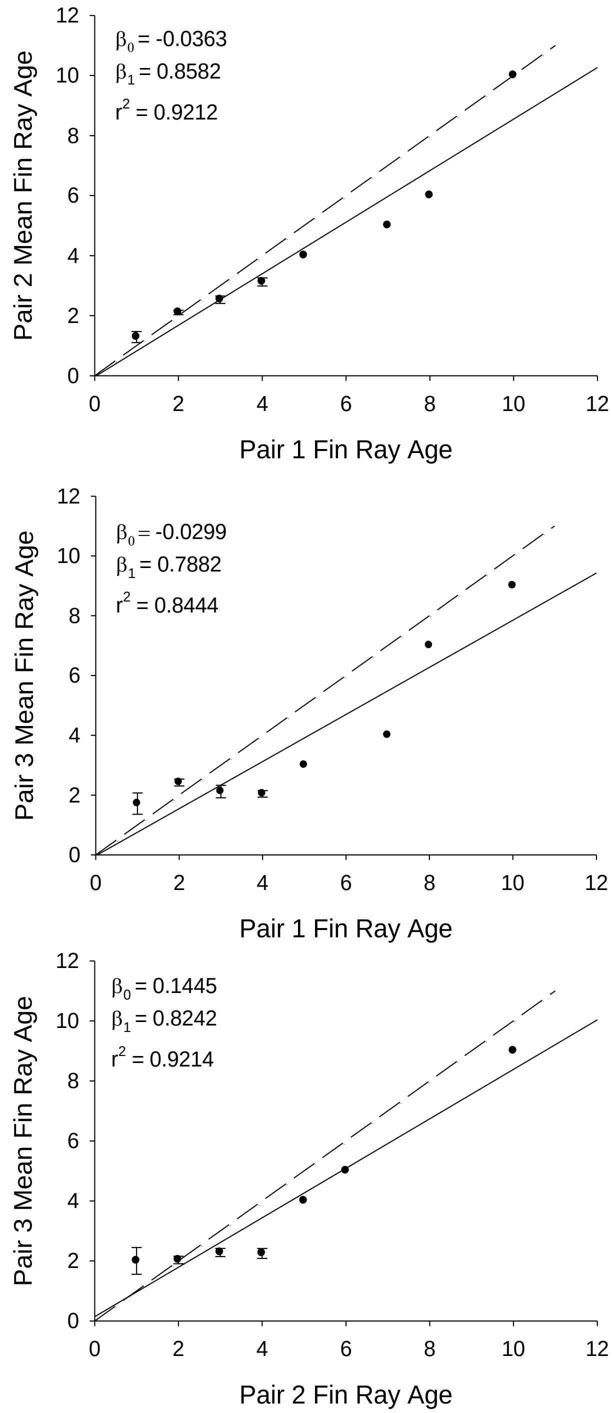
Figure 1. Age bias graphs for smallmouth bass age estimates from sectioned otoliths (N = 72) for all possible combinations of the three reading pairs. Dashed lines indicate 1:1 agreement in age estimates between reading pairs. Solid lines are least squares regression lines. Error bars are ± 1 SE around the mean age assigned by reading pair *Y* for every fish assigned an age by reading pair *X*. Asterisks indicate a significant difference from zero for the intercept (β_0) or a slope (β_1) that is significantly different from one.

Figure 2. Age bias graphs for smallmouth bass age estimates from sectioned pectoral fin rays (N = 72) for all possible combinations of the three reading pairs. Dashed lines indicate 1:1 agreement in age estimates between reading pairs. Solid lines are least squares regression lines. Error bars are ± 1 SE around the mean age assigned by reading pair *Y* for every fish assigned an age by reading pair *X*. Asterisks indicate a significant difference from zero for the intercept (β_0) or a slope (β_1) that is significantly different from one.

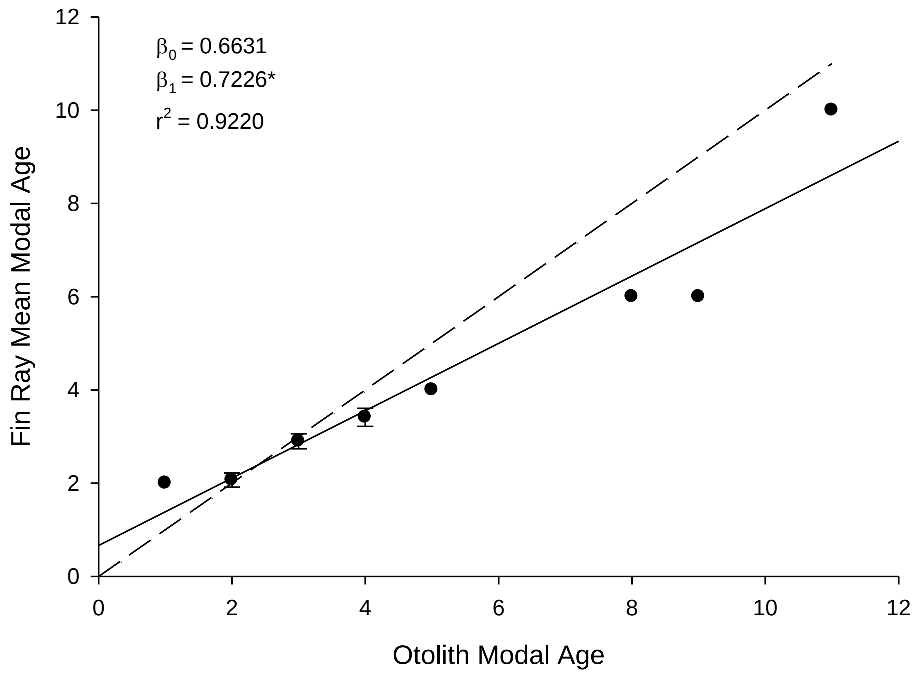
Figure 3. Age bias plot comparing otolith modal age and fin ray mean modal age (± SE). Dashed line indicates 1:1 agreement between aging structures. Solid line is the least squares regression line. Error bars are ± SE around the mean modal age assigned by fin rays for every fish assigned a modal age for otoliths. Asterisks indicate a significant difference from zero for the intercept (β_0), or a slope (β_1) that is significantly different from one.

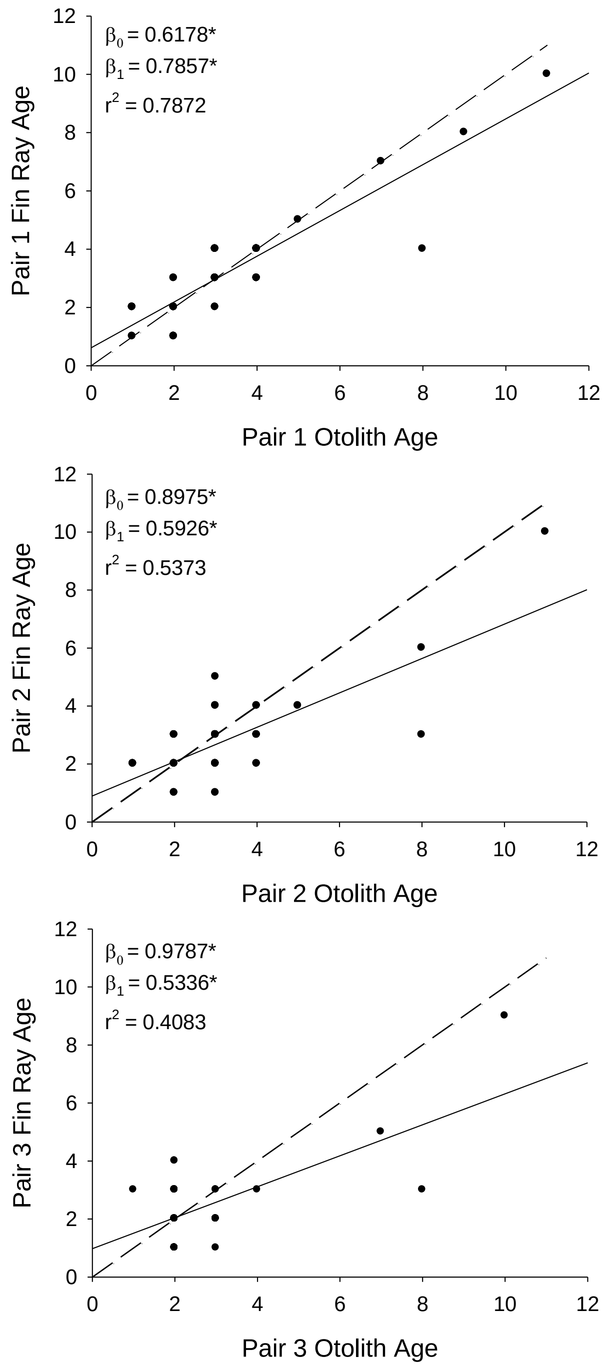
Figure 4. Age bias plots comparing pectoral fin ray age and otolith age for individual fish within each of the three reading pairs. Dashed lines indicate 1:1 agreement between aging structures. Solid lines are least squares regression lines fit to data. Asterisks indicate a significant difference from zero for the intercept (β_0), or a slope (β_1) that is significantly different from one. N = 72 fish for each plot. Note that some data points plot in identical locations.


β0 = 0.3204
β1 = 0.8532
r2 = 0.8227
Pair 2 Mean Otolith Age
Pair 1 Otolith Age
β0 = 0.0199
β1 = 0.7897
r2 = 0.7469
Pair 3 Mean Otolith Age
Pair 1 Otolith Age
β0 = -0.0128
β1 = 0.8776
r2 = 0.9396
Pair 3 Mean Otolith Age
Pair 2 Otolith Age



β_0 = -0.0363
β_1 = 0.8582
r^2 = 0.9212
Pair 2 Mean Fin Ray Age
Pair 1 Fin Ray Age
β_0 = -0.0299
β_1 = 0.7882
r^2 = 0.8444
Pair 3 Mean Fin Ray Age
Pair 1 Fin Ray Age
β_0 = 0.1445
β_1 = 0.8242
r^2 = 0.9214
Pair 3 Mean Fin Ray Age
Pair 2 Fin Ray Age


β0 = 0.6631
β1 = 0.7226*
r2 = 0.9220
Fin Ray Mean Modal Age
Otolith Modal Age
0
2
4
6
8
10
12
0
2
4
6
8
10
12


β0 = 0.6178*
β1 = 0.7857*
r2 = 0.7872
Pair 1 Fin Ray Age
Pair 1 Otolith Age
β0 = 0.8975*
β1 = 0.5926*
r2 = 0.5373
Pair 2 Fin Ray Age
Pair 2 Otolith Age
β0 = 0.9787*
β1 = 0.5336*
r2 = 0.4083
Pair 3 Fin Ray Age
Pair 3 Otolith Age