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A COMPARISON BETWEEN OTOLITHS AND SCALES FOR USE IN ESTIMATING THE AGE OF *DISSOSTICHUS ELEGINOIDES* FROM SOUTH GEORGIA

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Abstract

Age composition is fundamental to understanding the population dynamics and productivity of a fish stock. The use of scales to estimate age can result in large errors in age data for long-lived species, usually due to compression of scale circuli with age. Patagonian toothfish (*Dissostichus eleginoides*) is considered to be a long-lived species whose age has been estimated using both scales and otoliths.

We estimated the age of *D. eleginoides* caught off South Georgia using otoliths and scales. For scales, we made impressions on acetate slides; for otoliths, we used transverse sections prepared by baking and grinding the posterior and anterior sides. Using ANOVA, we compared data obtained from the two structures to test the hypothesis that otoliths and scales give the same age estimates, and compared the precision of age estimation for both structures and between readers.

Ages estimated using scales were significantly less than those estimated using otoliths. For scales, bias occurred for both readers between readings; for otoliths, only one reader was biased. Residual variances indicated one reader was relatively more precise than the other in estimating age using otoliths, but less precise using scales. This reflected the comparative experience of the two readers in estimating the age of *D. eleginoides* using otoliths and scales.

Résumé

La composition en âges est fondamentale pour la compréhension de la dynamique des populations et à la productivité d'un stock de poissons. L'utilisation des écailles pour estimer l'âge peut produire des erreurs importantes dans les données d'âge des espèces à vie longue, le plus souvent en raison de la compression des circuli avec l'âge. La légine australe (*Dissostichus eleginoides*) est considérée comme une espèce à vie longue dont l'âge a été estimé tant au moyen des écailles que des otolithes.

Nous estimons l'âge de *D. eleginoides* capturé aux large de la Géorgie du Sud au moyen des otolithes et des écailles. Pour ces dernières, nous avons pris des empreintes sur des lames d'acétate; pour les otolithes, nous avons utilisé des sections transversales chauffées et limées sur les faces postérieure et antérieure. Par une ANOVA nous avons comparé les données obtenues des deux manières différentes pour tester l'hypothèse selon laquelle les otolithes et les écailles donnent les mêmes estimations d'âge. Nous avons ensuite comparé la précision de l'estimation des âges obtenue par chaque méthode et pour chaque lecteur.

Les âges estimés au moyen des écailles sont nettement moins élevés que ceux estimés avec les otolithes. Par l'utilisation des écailles, le biais est apparent pour les deux lecteurs d'une lecture à l'autre; avec les otolithes, le biais n'apparaît que pour l'un des lecteurs. Les variances résiduelles indiquent que l'un des lecteurs est relativement plus précis que l'autre dans son estimation de l'âge par les otolithes, mais moins précis par les écailles. Les résultats reflètent l'expérience comparative des deux lecteurs relativement à l'estimation de l'âge de *D. eleginoides* par les otolithes et par les écailles.

Резюме

Возрастная струткура лежит в основе понимания динамики популяций и продуктивности запасов рыб. Использование чешуи для оценки возраста может привести к сильным искажениям данных по возрасту долгоживущих видов, обычно из-за сжатия возрастных колец на чешуе с возрастом. Патагонский клыкач (*Dissostichus eleginoides*) считается долгоживущим видом, возраст которого оценивается и по чешуе, и по отолитам.

Мы провели оценку возраста *D. eleginoides* из района Южной Георгии, используя и отолиты, и чешую. В случае чешуи были сделаны отпечатки на ацетатном слайде; в случае отолитов использовались поперечные срезы, подготовленные путем обжига и шлифования задней и передней частей. Чтобы проверить гипотезу о том, что полученные по отолитам и чешуе оценки возраста аналогичны, мы провели сравнение соответствующих данных, используя ANOVA, а также сравнили точность оценки возраста для обоих способов и различных считывателей.

Оценки возраста по чешуе были намного ниже оценок возраста по отолитам. В случае чешуи систематическая ошибка между считываниями была у обоих считывателей, а в случае отолитов - только у одного. Остаточная дисперсия указывает на то, что один из считывателей был относительно точнее при оценке возраста по отолитам, а другой - при оценке возраста по чешуе. Это отражает опытность считывателей в оценке возраста *D. eleginoides* по отолитам и чешуе.

Resumen

El conocimiento sobre la composición por edades del stock de peces es fundamental para entender la dinámica de la población. Las estimaciones de edad de las especies longevas derivadas de la lectura de escamas pueden ser muy imprecisas debido, en gran parte, a la compresión de los anillos de las escamas con la edad. De acuerdo a las estimaciones con escamas y otolitos, el bacalao de profundidad (*Dissostichus eleginoides*) es una especie longeva.

La edad de *D. eleginoides* capturado frente a Georgia del Sur fue estimada mediante otolitos y escamas. Para las escamas se prepararon moldes en una superficie de acetato, y para los otolitos se utilizaron secciones transversales preparadas cociendo y moliendo los lados posterior y anterior. Mediante el ANOVA se compararon los datos obtenidos con las dos estructuras para probar la hipótesis de que tanto los otolitos como las escamas generan las mismas estimaciones de edad; también se comparó la exactitud de las estimaciones derivadas de ambas estructuras y efectuadas por distintos lectores.

Las edades estimadas de las escamas fueron significativamente menores a las estimadas con otolitos. Para las escamas, ambos lectores tuvieron errores sistemáticos en varias de sus lecturas; para los otolitos, sólo uno de los lectores estaba prejuiciado. Las variancias residuales indicaron que un lector era más exacto que el otro en la estimación de la edad con otolitos, pero menos preciso en la lectura de escamas. Esto reflejó la experiencia relativa de los dos lectores en la estimación de la edad de *D. eleginoides* con otolitos y escamas.

Keywords: Southern Ocean, Patagonian toothfish, *Dissostichus eleginoides*,
age, estimation, otoliths, scales, bias and precision, CCAMLR

INTRODUCTION

Knowledge of the age composition of a fish stock is fundamental to estimating its growth and vital rates, and modelling its population dynamics and productivity. Fish age estimates are usually obtained using growth features in calcified structures, most notably otoliths and scales. Scales are easier to obtain and prepare than otoliths, and can be sampled several times during the life of a fish. However, scales may be reabsorbed and regenerated, their development delayed for several months after hatching, or annuli in older fish can be obscured by compression of the circuli as growth slows with age. As a result, true age can be underestimated, especially in long-lived species whose growth is concentrated in the early life history (Beamish and McFarlane, 1987; White, 1991).

Beamish and McFarlane (1987) listed studies demonstrating species whose scales should not be used to estimate age for this reason. They described the case of sablefish (*Anoplopoma fimbria*) off the west coast of North America, where scales were used to age fish prior to 1981, indicating fish in the commercial fishery were between 3 and 8 years. However, a validated age method using otoliths demonstrated that fish in the commercial fishery in fact ranged between 4 and 40 years, indicating slower growth and a much less productive fishery. Sustainable exploitation rates should have been 20–30% of that based on age determinations from scales, in order to avoid overexploitation; management strategies using validated ages from otoliths resulted in a stable fishery (McFarlane et al., 1985).

Similarly, otolith-based age estimates of Pacific Ocean perch indicated fish were considerably older than the scale estimates used previously (Beamish and McFarlane, 1983). The resulting estimates of natural mortality were considerably lower, leading to a more conservative management strategy. However the loss of wholesale value due to under-estimated ages was calculated to be $4 million in 1981 Canadian dollars.

Stewart (1926) validated the yearly growth of scales in young white suckers, and subsequent studies using scales indicated white suckers grew quickly at ages 4–7 years, few surviving beyond an age of 9 years. Annual mortality was considered high after maturity even though active growth continued. By contrast, Beamish and Harvey (1969) used a method based on fin-rays that had been validated for the full age range, and demonstrated that the oldest age was 23 years. Large numbers of fish in unexploited populations survived after growth slowed or ceased. Yearly annuli on scales were difficult to identify after age 5, but were distinct on fin-ray sections; validation of older fish was necessary to prevent errors in age estimation and evaluate their importance as a component of the population (Beamish and McFarlane, 1983).

For Patagonian toothfish (*Dissostichus eleginoides*), Hureau and Ozouf-Costaz (1980) used scales with polarised light to estimate age. They gave brief criteria for interpreting age from scales and otoliths, and compared ages read for the same fish using otoliths and scales: these were considered to give good agreement, but data were presented from only two fish. Young et al. (1995) compared scales and otoliths from *D. eleginoides* caught off southern Chile. They found that ages obtained using scales were significantly different from ages using otoliths, while the precision of ages read by two readers was greater using scales than otoliths. There was evidence that for fish older than 16 years (estimated using scales), scales gave lower estimates for age than did otoliths. In contrast, Cassia (1998) compared age readings from scales and otoliths from *D. eleginoides* caught in FAO Subarea 48.3, and found agreement between otoliths and scales in 44% of cases for which she could obtain readings, and no significant difference in the other 56% of cases.

At its 1998 meeting the CCAMLR Working Group on Fish Stock Assessment considered age determination methods for *D. eleginoides* and, noting that otoliths and scales had been used for previous studies, encouraged members to undertake studies to determine which method would be the most effective (SC-CAMLR, 1998). The present study was undertaken in response to that request. We present the results of pairwise comparisons between age estimations using otoliths and scales taken from individual *D. eleginoides*.

MATERIALS AND METHODS

Preparation and Reading

The observer on board the longliner FV *Koryo Maru 11* took samples from catches made between April and June 1999 off South Georgia. Full biometric data were recorded; otoliths and scales were wiped clean and placed in paper envelopes to dry. A sub-sample of 133 fish was selected; comparatively few fish were obtained for the total length range larger than 100 cm, so the subsample was supplemented using 44 larger fish from the study by Ashford and Wischniowski (1998).

Otoliths were prepared by grinding to produce thick sections for viewing under reflected light. One of each pair of otoliths was selected randomly and baked in a furnace at 400°C for approximately two minutes, or until a light brown colour was obtained. Once cool, the anterior part of the otolith was removed by grinding: the anterior side was positioned manually to contact the grinding wheel of a Hillquist thin section machine, and the surface ground away until a predetermined internal mark, located consistently anterior to the nucleus, was revealed. The remaining otolith half was then affixed, ground surface down, to a 1.2 mm thick microscope slide using Loctite 349, an optically clear ultraviolet adhesive that requires a multiband UV-254/366NM catalyst to initiate curing. Once cured, the slide was mounted on the sectioning arm of the Hillquist thin section machine, and the otolith half ground from the posterior side until a transverse section of c. 0.5 mm thickness was left, which incorporated the nucleus and avoided crenellations. To improve the clarity of the exposed surface, the otolith section was polished on a Buehler Ecomet 3 grinder–polisher, using Mark V Laboratory 3M aluminium oxide polishing paper, and covered with Flo-Texx. Flo-Texx also served to seal and protect the otolith.

For each fish, four to eight scales of uniform size and having even margins, with no evidence of scale regeneration, were selected and cleaned by scrubbing with a small brush. Extruded clear 020 acetate sheets (25 x 75 mm) were cut into slide-size strips, and impressions of the selected scales from each fish made on a single acetate slide using a Carver laboratory heated press (model C). Scales were pressed at 12 000 psi at 75–80°C for 10 minutes. Otolith and scale slides were treated separately and randomly sorted. Otolith sections from 47 fish, taken from a reference collection of otoliths with ages previously estimated (Center for Quantitative Fisheries Ecology, unpublished data), were randomly incorporated into the otolith sample set to check for consistency in interpreting structures.

Scales and otolith sections were examined using a Leica MZ8 binocular microscope. Two readers read scales and otoliths twice each: each reading was completed in two days, and all readings occurred within two weeks of the first reading. All readings were made without auxiliary information or reference to any previous set of readings. The order of readings was determined randomly.

Criteria for Scale and Otolith Interpretation

Otoliths

Criteria were the same as for Ashford and Horn (1999) developed from the criteria outlined by Hureau and Ozouf-Costaz (1980). Terminology follows Everson (1980): an annulus consists of a hyaline and an opaque zone, and is not necessarily annual by definition. Hureau and Ozouf-Costaz (1980) divided the section into three regions: an easily recognisable nucleus, surrounded by a region consisting of seven large annuli, followed by a region consisting of a succession of narrower regular annuli.

Working out from the nucleus, the annuli were largest in the dorsal axis, and compressed on the medial and proximal sides (Figure 1a). The dorsal axis then became compressed, and the annuli widest on the proximal sides. In the regular region, the narrowest annuli on the proximal side were considered to be annual. The yearly annuli tended to diminish in width towards the edge of the otolith, although exhibiting some variation in width. In the region of large annuli, heavily calcified zones were interspersed with narrower zones consisting of bundles of narrow micro-increments: these were considered to be the opaque and hyaline zones of yearly annuli respectively. They tended to occur at decreasing intervals but were very variable in contrast along the count path and between fish. The nucleus consisted of a central core, strongly marked by regular micro-increments, surrounded by a region with less defined micro-increments forming a dorsal protrusion.

The count path followed the large annuli along the dorsal axis, moving to the regular annuli along the proximal dorsal axis as the dorsal axis became compressed. Structures occurred at different scales in all regions: in the regular region, the narrowest annuli were considered annual as long as they persisted clearly either side of the count path. Annuli that did not persist far to either side of the count path or occurred irregularly at a lower scale were considered false checks. In the region of large annuli, distinguishing between yearly annuli and checks was more difficult: annuli were considered to be larger, have stronger contrast between opaque and hyaline zones, and to persist either side of the count path notably into the compressed medial region. Checks tended not to persist or vary considerably in clarity. In the nucleus, a discontinuity was observed running diagonally between the core and the dorsal protrusion. The edge of the nucleus was defined as the inner border of the first hyaline zone, which was typically clearer than the succeeding hyaline zones. As the hatch date of *D. eleginoides* is not known, the nucleus may not represent a full year's growth, so the outer edge of the nucleus was considered as year 0. The birthday of all fish was taken to be 1 July, so that the outer annulus was counted as one year if the fish was taken after 1 July but not before.

Scales

Typically, annuli on scales are determined by 'crossing-over' features (e.g. Mosher, 1969; Jearld, 1983; Penttila and Dery, 1988; Yole, 1989; Almeida et al., 1992), where the compressed circuli zone of an annulus interrupts the circuli of the proximal annulus. Crossing over is most evident on the lateral margins near the posterior/anterior interface of the scale; typically the annulus will protrude partially into the ctenii of the posterior field.

We found this feature occurred very infrequently in *D. eleginoides*, and crossing over alone could not be used for scale criteria. Furthermore, little if any information could be extracted from the posterior region of the scale due to lack of readable structure. Hureau and Ozouf-Costaz (1980) considered annual structures to be marked by a thickening of the scale combined with circuli that were closely spaced and corresponded to periods of slow growth. Zones corresponding to annual periods of fast growth were characterised by thinning of the scale and widely spaced circuli. We found that the compressed circuli could be seen in the lateral margins of the *D. eleginoides* scale, and followed as a semi-circular pattern around the focus of the scale (Figure 1b). They remained continuous, revealing no discontinuous or segmented circuli associated with the usual scale criteria; and, when viewed with non-filtered transmitted light under a dissecting microscope (8–15x), were most easily seen where the annuli bisected the scale radii. Under polarised light, the zone appeared dark where compressed circuli gave way to expanding circuli (Hureau and Ozouf-Costaz, 1980).

For estimating age, the acetate slide was positioned with the impression facing upwards. One of the four to eight acetate scale impressions was selected for age estimation, based on: (i) uniform shape and size consistent with the other scales; and (ii) no evidence of regeneration or resorption. From the focus, located just above the posterior/anterior interface, the reading plane followed a 45° angle along the distal radii towards the outer edge of the scale. The zones of compressed circuli were most easily identified when bisecting the radii. Compressed circuli marking the end of the first annulus were often found close to the focus, following a semi-circle around the focus without disruptions, from the posterior/anterior interface of one side of the scale to the other. Once the first annulus was determined, the radii were followed out to the next set of compressed circuli. Again, if these continued undisrupted around the focus, the compressed and intervening circuli were considered to form an annulus. The process was repeated to the outer edge of the scale. To aid in the identification of annuli, readers alternated between non-polarised and polarised light.

Analysis

Data were analysed using Lotus 1-2-3 for Windows and SAS Version 4.0. To test the hypothesis that otoliths and scales give the same age estimates, an ANOVA randomised complete blocks design was used with a single replicate of each treatment per block (Ashford, 2001). The blocking factor was individual fish, considered randomly drawn from the wider population. Each reading was considered a separate fixed treatment, with a single replicate in each cell. The treatment and block effects are assumed to be additive, with no interaction. Let y_{ij} be the ith reading on the otolith from the jth fish. Under the assumptions of the mixed effects model:

$$y_{ij} = \mu + \tau_i + b_j + \varepsilon_{ijk} \qquad (1)$$

$$i = 1, 2, \ldots t; j = 1, 2, \ldots r.$$

where μ is the general mean; τ_i is the effect of the ith level of the factor reading; b_j is the effect of the jth level of the blocking factor (fish); and ε_{ijk} is the experimental error.

The random effect blocking factor b_j and random error ε_{ijk} were assumed to be independent, normally distributed random errors with mean 0 and variances σ_b^2 and σ^2 respectively. τ_i was a measure of the effect due to each reading: where it was found significant using the conventional F-test, the individual means were tested using the Tukey Honestly Significant Difference (HSD) test and the Student-Newman-Keuls (SNK) multiple range test pairwise treatment comparisons. The difference between the estimated general mean and estimated treatment mean $(y_{..}-y_{i.})$ was used as an estimate of the reading bias. MSE is an unbiased estimate for σ^2 within the design, however it is dependent on how the degrees of freedom are structured. Instead, the variance of the residuals was used as an estimate of relative precision.

Table 1: Total length and mean age estimated for each fish using scales and otoliths.

TL	Scale	Otolith	TL	Scale	Otolith	TL	Scale	Otolith	TL	Scale	Otolith	TL	Scale	Otolith
56	3	2.5	69	6.25	7	84	6.25	8.5	93	9	9.25	104	9.75	17.25
60	4.25	4.25	66	3.5	4.75	81	6.75	8	96	9.5	11.5	102	7	36
56	4.25	2.5	71	7	8	88	7.25	7.25	95	6.25	8	103	10.5	29
59	4.25	4.25	78	7.75	7.5	81	6.25	8.75	100	9	25.75	107	9.5	29.25
60	4.75	2.75	77	7.5	8.5	82	7.25	9.75	94	5.75	10	106	10.75	17
56	3.5	2.75	79	8	6.25	82	7.75	8.75	95	9.75	11		8.75	0
60	4.5	5.75	77	5	8	88	7	8	91	9	9.25	105	10	15.5
53	2.5	2.5	78	6.5	7.25	90	6.75	11.75	94	9.25	15.25	105	9.75	13.25
66	5.25	6.5	74	6.5	7.75	85	6	12.25	95	8	15.25	108	11	30.25
69	7.25	7.25	74	6.75	7.25	87	8.75	11.5	96	6.25	13.25	106	10.25	13.25
67	6.25	7.5	79	7	8	89	8	11.75	98	8.75	12.5	110	12.25	27.5
69	7	7.75	73	6.5	8.25	81	6.75	7	101	7	8.25	118	10.5	14
69	7.25	5.25	77	7.5	6.5	87	6.75	8.75	104	8	20.5		9.25	0
70	7	8.5	80	5.75	9.75	90	9.25	9.25	110	9.5	11.75	112	12	21.5
69	6	7.5	78	5.75	10	86	7.75	14.25	102	8	11	115	10	27.25
69	5.5	7.25	75	8	7.5	82	7	12.75	103	7.75	17.75	120	11	25.75
69	5.25	7.75	73	7.25	7.25	90	7.75	13	106	9.25	22	111	16.25	24.5
69	4.75	7.75	79	5.5	13	88	5.5	11	110	9.5	37	122	11.75	13.75
65	7.5	7.5	80	7.25	15	85	7.75	11	103	10	12.5	123	13	32.25
66	4.75	6.25	72	6.25	8.5	83	6.5	6	112	9	24	127	12.75	25
63	2.5	6.25	73	4.75	7.75	85	9	14	115	9.75	27.5	127	12.25	31.25
68	5.25	5.25	71	7	8.75	82	7.25	13.75	119	10.75	24.75	129	11.75	36.5
62	5.25	7	75	6	5.5	85	7.25	11	121	15	29	127	11.75	20.5
65	7	6.75	73	7	7.5	84	8.25	8	129	13.5	34.75		13.25	0
67	4.5	5.5	77	6.75	11.5	81	6	7.25	145	10	37.75	123	10.75	33.75
60	3	3	74	8.25	10	82	8.5	11.5	105	10.75	18	129	14.5	24.25
65	3.5	3.5	78	9	9.75	96	10	17.5	105	11.25	10.25	121	9.75	30.25
67	3.5	4.75	79	7.75	13.75	91	10	12.25	105	11.5	14.75	139	14.25	20
70	5.25	7	80	7.5	7.25	98	7.25	16.5	105	11.25	17.75	140	13.5	35.5
70	5.25	7.25	78	6.25	8.75	99	6.25	8	101	11.75	28.75	139	9.5	30.5
65	4.25	6.5	80	6	7	93	8.25	10.25	107	12.5	12.5	170	12.5	42.25
67	6.25	6	79	6.5	8	91	7.75	8.5	102	10.25	10	146	19	36
62	4	3.5	83	7.25	7.5	99	11.5	15.25	104	12.25	16.75	145	14.25	23.75
66	4.25	3.75	83	4.5	7.25	92	5	9.5	103	8	22			
61	3.5	4	84	8	15.5	94	7.75	7.5	105	10.75	27.25			
68	4.25	7.75	83	9.25	9.5	93	7	12.25	104	8.75	24.5			

Table 2: Results from randomised block ANOVA, used to test for differences between repeated age estimations using scales and otoliths by two readers, for *Dissostichus eleginoides* sampled off South Georgia.

Source	df	Sum of Squares	Mean Square	F	Pr>F
Total	1 391	73 375.2			
Fish	173	43 716.6	252.7	15.9	0.0001
Treatment	7	10 439.8	1 491.4	94.0	0.0001
Error	1 211	19 218.8	15.9		

Table 3: Results from randomised block ANOVA, used to detect bias and estimate precision variability for repeated age estimates by two readers, for Patagonian toothfish (a) using otoliths, (b) using scales.

	Source	df	Sum of Squares	Mean Square	F	Pr>F
(a)	Total	695	56 624.8			
	Fish	173	55 359.8	320.0	138.8	0.0001
	Treatment	3	68.7	22.9	9.9	0.0001
	Error	519	1 196.3	2.3		
(b)	Total	695	6 458.6			
	Fish	173	5 604.8	32.4	21.7	0.0001
	Treatment	3	79.3	26.4	17.7	0.0001
	Error	519	774.4	1.49		

Table 4: Residual variances and mean CV for (a) readings using otoliths and scales, (b) readings by Reader 1 and Reader 2 for otoliths and scales.

	Readings	Variance	Mean CV
(a)	All otoliths	1.72	11.8
	All scales	1.11	14.4
(b)	Reader 1 otoliths	0.73	7.0
	Reader 2 otoliths	1.10	7.8
	Reader 1 scales	0.48	9.2
	Reader 2 scales	0.45	10.2

To test for differences between readings using scales and otoliths, the ANOVA model was used to examine all treatments together. To test for differences between readers, the model was used to examine scale and otolith treatments separately. To test for consistency by each reader, the model was used to examine readings by each reader for each structure separately. Mean coefficient of variation (Chang, 1982) was also calculated for all treatments, for scale and otolith treatments separately, and for each reader for each structure, and used as an estimate of relative precision.

RESULTS

Total length frequency for all sampled fish is shown in Figure 2, and mean age estimated for each fish using scales and otoliths is given in Table 1. Plots of pairwise readings from otoliths and scales by the same reader indicated substantial differences in the ages estimated (Figure 3). Plots of individual readings by each reader for each structure are shown in Figure 4. Comparisons of readings between readers for otoliths and scales are shown in Figure 5.

Results for the ANOVA comparing all treatments (Table 2) showed significant differences between treatments, and treatment comparisons using SNK and HSD showed separation between scale readings and those using otoliths. Residuals were not normally distributed, showing kurtosis, but the assumptions were considered to hold due to the averaging effect under the central limit theorem with large numbers of experimental units. The hypothesis that otoliths and scales give the same age estimates was therefore rejected, with scales giving significantly lower age estimates than otoliths.

Examining otoliths and scales separately (Table 3), within-otolith treatments were significantly different, and treatment comparisons using SNK and HSD showed separation between Reading 1 by Reader 1 and the three other readings. The bias for Reading 1 by Reader 1 was estimated to be 0.49 years. For scales, Reading 2 by Reader 1 and Reading 1 by Reader 2 were not significantly different, but both were significantly different from both the other two readings. The bias for Reading 2 by Reader 1 was 0.51 years; the bias for Reading 2 by Reader 2 was -0.43 years.

Residual variances indicated that precision variability was lower for scales (Table 4); in contrast, mean CV for otoliths was lower than for scales. Residual variances were similar for each reader for each structure; but somewhat higher for Reader 2 when reading otoliths compared to Reader 1, and lower when reading scales. Mean CV was lower for Reader 1 for both structures.

DISCUSSION

These results clearly demonstrate that ages estimated using scales were significantly less than those estimated using otoliths. This may be due to the compression of circuli with age in scales, or the lack of clear 'crossing-over' features usually used for estimating age in scales. Age estimation has not been fully validated for *D. eleginoides*, but there is broad consensus among several laboratories on how otoliths should be read (Horn, 1999; Ashford and Horn, 1999): these ages were underestimated consistently using scales.

We found mean CV for otoliths was lower than for scales, but the estimate included error due to bias as well as error due to precision, whereas residual variances only included error due to precision (Ashford, 2001). Additionally, variability increased little with age, so that CV decreased, and the lower mean CV for otoliths reflected the higher age estimates produced rather than relative precision (Ashford, 2001). Residual variances were therefore better measures of relative precision. Reader 2 was more experienced reading scales than Reader 1, and this was reflected by lower residual variance than Reader 1 when reading scales. However, the age estimates by Reader 2 using scales were still significantly less than his age estimates using otoliths. We also found that bias occurred for both readers between readings using scales, while only Reader 1 was biased using otoliths.

Taken together, these results indicate that scales underestimate age compared to otoliths, even with increasing reader experience. We therefore recommend that *D. eleginoides* ages should be estimated using otoliths and not scales. In addition, otoliths are not reabsorbed or metabolically re-worked (Campana and Neilsen, 1985), are not as exposed to damage as external scales, and contain chemical traces taken up from the water column, which can be used to place a fish retroactively in space and time (Thorrold et al., 1998). There are thus considerable advantages to using otoliths over scales for estimating age in *D. eleginoides*.

However, although otoliths should be used for estimating age in *D. eleginoides*, a validation test is needed to assess the accuracy of the age estimates, and therefore whether the age data underpinning estimation of growth and vital rates, and modelling of production and population dynamics, are reliable. But, in a quantitative design to test for significant differences between real and estimated ages, the variability of the estimated ages will be critical: if variability is high, statistical power may be too low to conclude that estimated ages are accurate and no different from real ages, even if no significant differences are found. Therefore, precision needs to be measured prior to any quantitiative validation test. This study provides estimates of precision achieved by readers at the Center for Quantitative Fisheries Ecology using the methodology outlined. With these estimates, a validation test can be designed with sufficient power to test the accuracy of ages estimated from toothfish otoliths.

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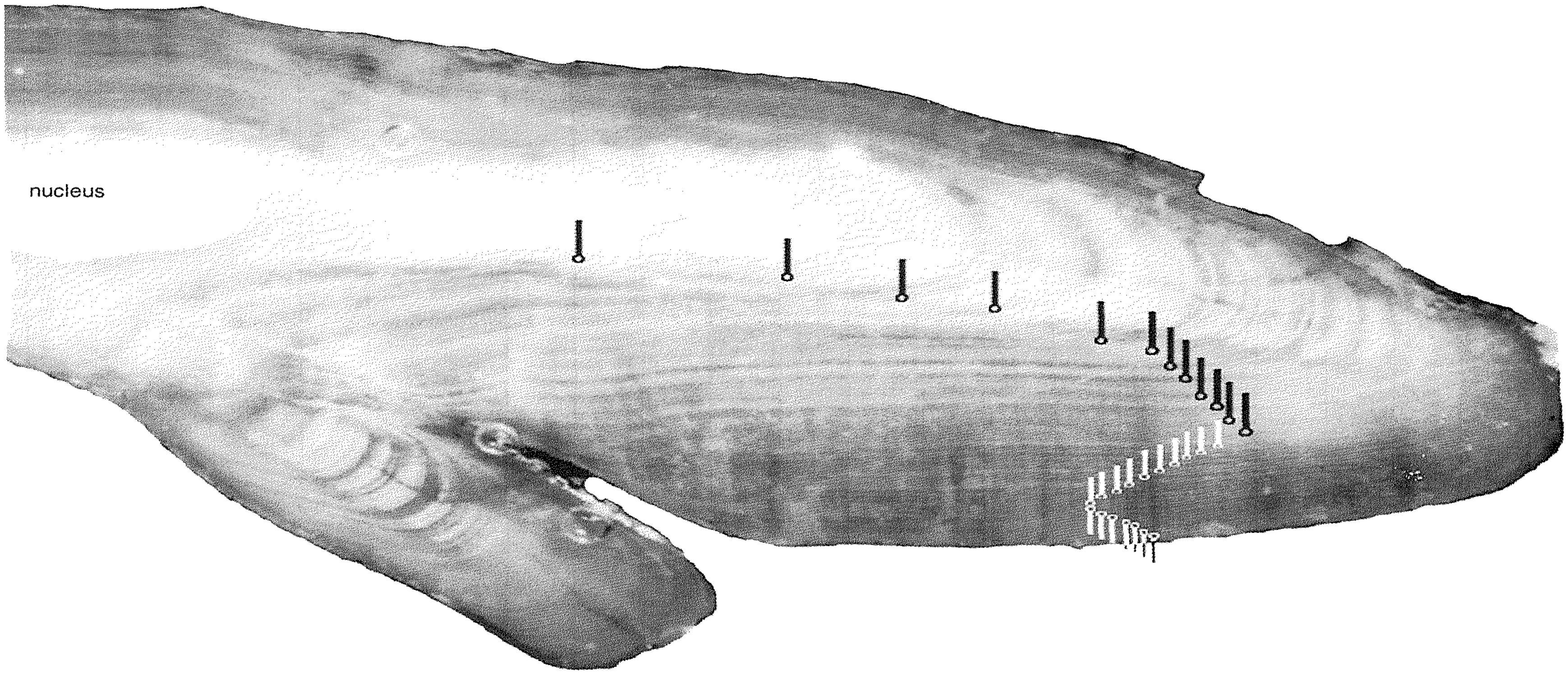


Figure 1(a): Example of age count for transverse section of otolith from *Dissostichus eleginoides* using criteria given by Hureau and Ozouf-Costaz (1980). The edge of each yearly annulus is marked by the open circle of each symbol; the first symbol marks the edge of the first annulus. The change from dark to light symbols marks the boundary between the region of large clear yearly annuli and the region of regular yearly annuli. Age = 29 years.

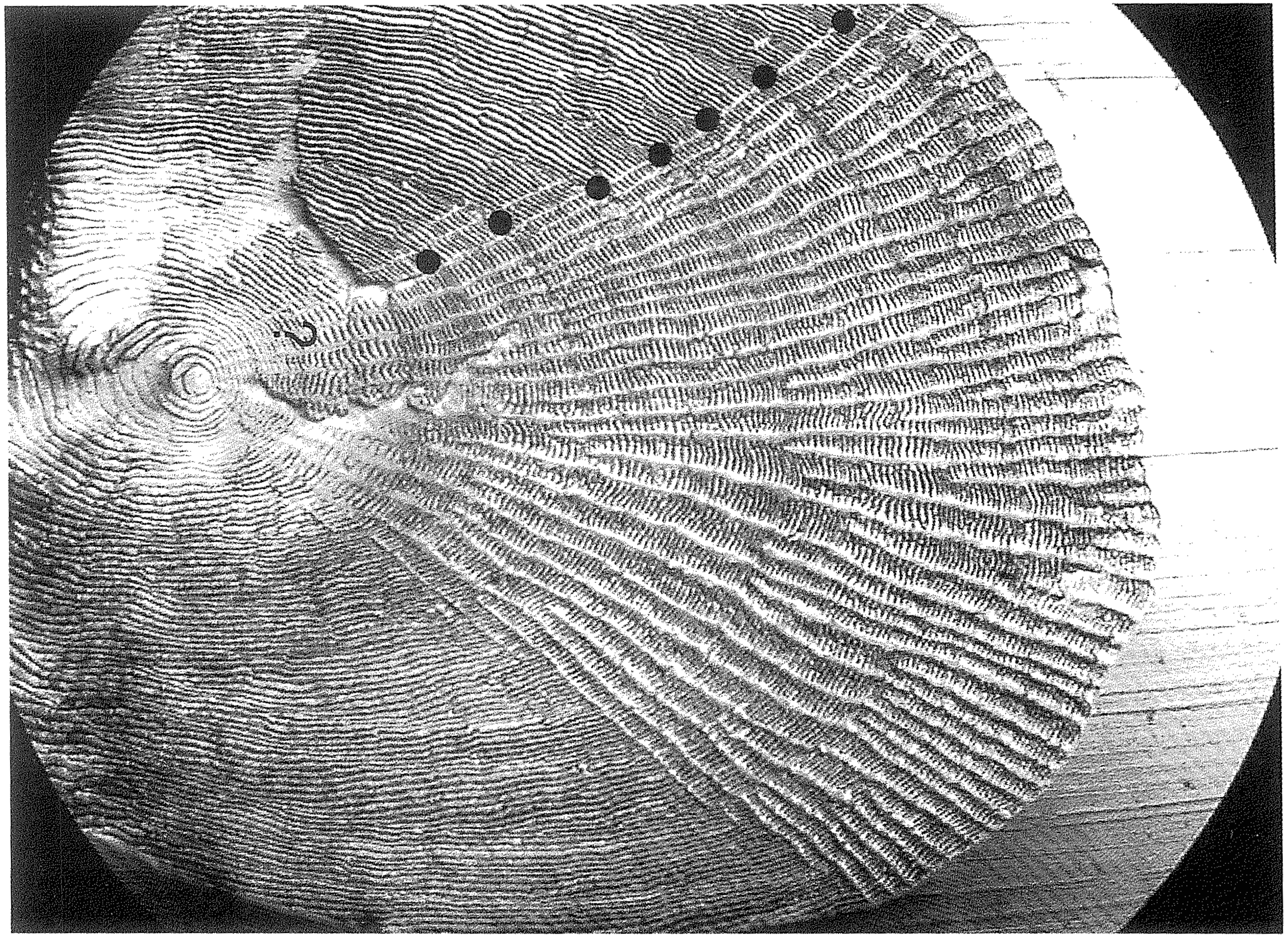
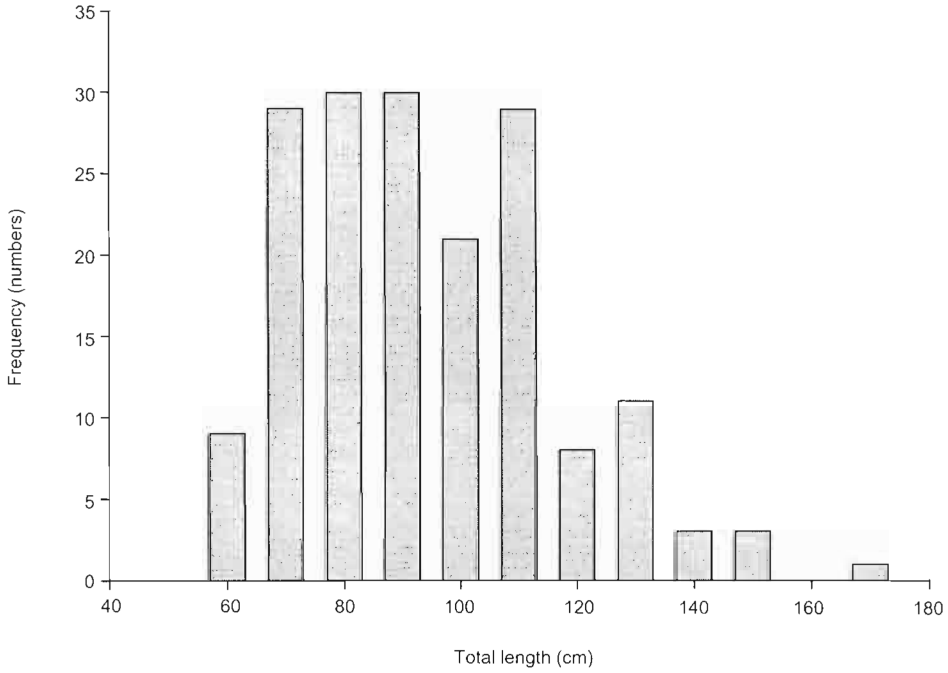


Figure 1(b): Example of age count for scale. The zone of compressed circuli is marked by a circle. Age = 7 years.



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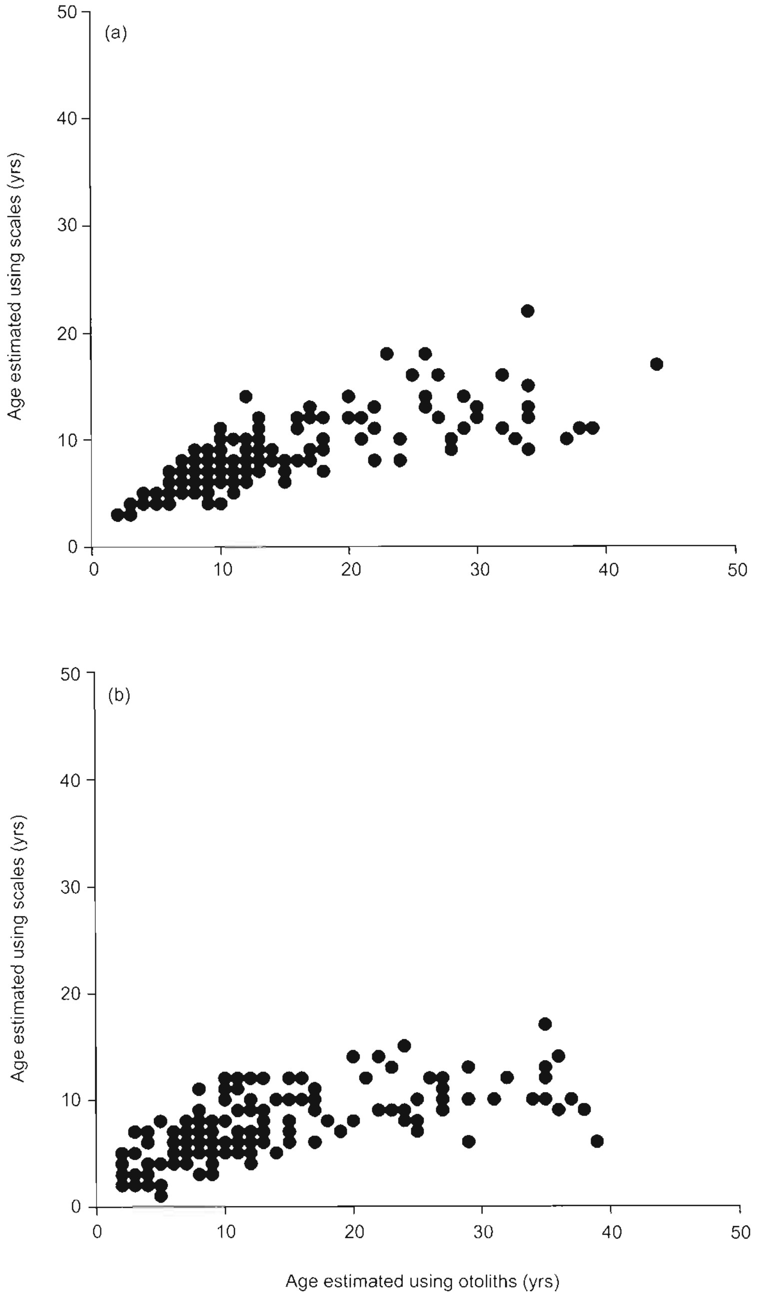


Figure 3: Pairwise age estimates using otoliths and scales from individual fish. Estimated by (a) Reader 1, (b) Reader 2, using second readings for each structure.

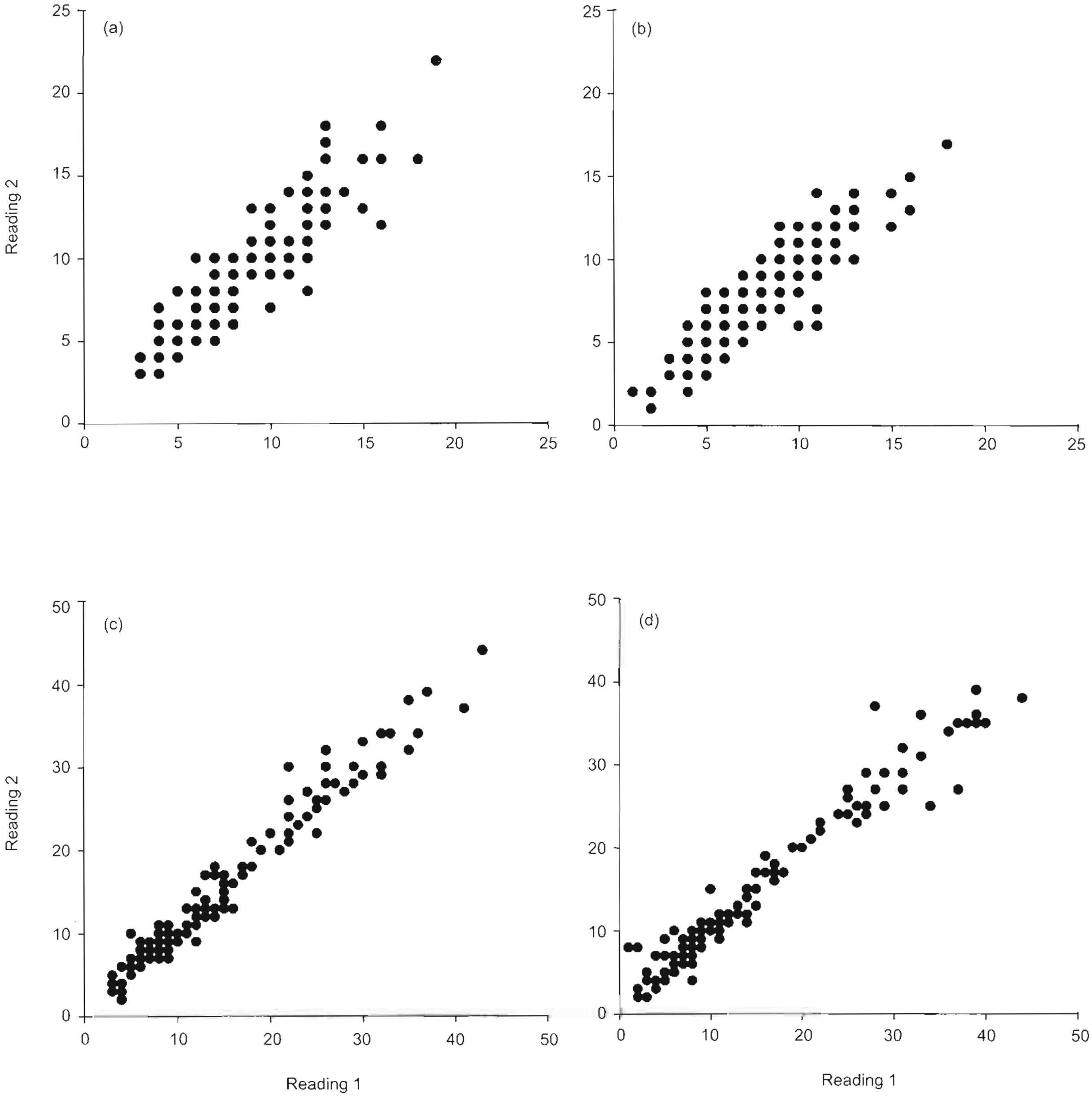
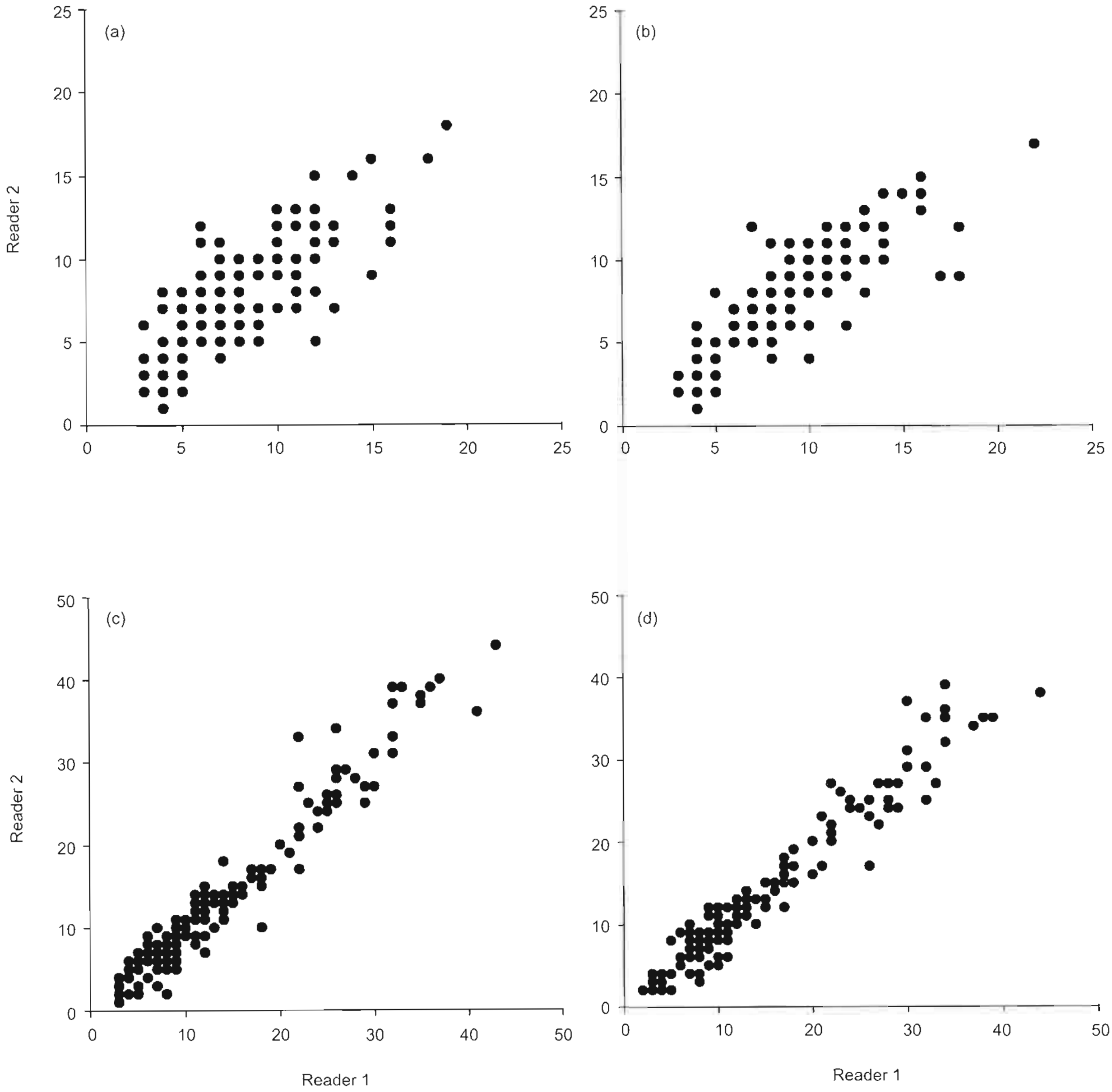


Figure 4: Pairwise age estimates from individual fish: (a) comparing readings by Reader 1 using scales; (b) comparing readings by Reader 2 using scales; (c) comparing readings by Reader 1 using otoliths; and (d) comparing readings by Reader 2 using otoliths.



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