Timing of hyaline-zone formation as related to sex, location, and year of capture in otoliths of the widow rockfish, *Sebastes entomelas*

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Widow rockfish, *Sebastes entomelas*, are an important component of the west coast commercial groundfish fishery. Annual landings between 1983 and 1992 were 9,700 metric tons (Pacific Fishery Management Council[1]). In addition to widow rockfish, many other rockfish species are important targets of commercial and sport fisheries. Most stock assessments of rockfish are based on ageing methods (Pacific Fishery Management Council[1]). In most cases, rockfish are aged by the broken-and-burnt otolith method (Chilton and Beamish, 1982).

Age validation of several rockfish species has been conducted by marginal increment analysis. The following species have been validated: yellowtail rockfish, *S. flavidus* (Kimura et al., 1979); shortbelly rockfish, *S. jordani* (Pearson et al., 1991); chilipepper rockfish, *S. goodei* (Mellow[2]); and widow rockfish (Lenarz, 1987). In all four studies, the hyaline zone formed between December and April.

Marginal increment analysis relies on the assumption that if a hyaline zone is laid down once a year, there should be a clear pattern of periodic growth on the edge during the year. Marginal increment analysis is appropriate only if all fish in the population lay down the hyaline zone at the same time. An annulus would then consist of a single hyaline zone and an opaque zone. Conventionally, hyaline zones are counted for age determination purposes.

The hyaline zone is believed to form during periods of slow growth (Blacker, 1974; Schramm, 1989). Specifically, hyaline-zone formation has been attributed to reproduction (Hostetter and Munroe, 1993), water temperature (Thomas, 1983; Schramm, 1989), lack of food (Schramm, 1989), and environmental perturbation (Beckman et al., 1991). Schramm (1989), in a laboratory experiment with bluegill, *Lepomis macrochirus*, found that low temperature produced a hyaline zone. He was also able to induce the formation of hyaline zones by varying photoperiod and restricting food intake, but reported that the hyaline zones formed by these environmental agents were much fainter than those resulting from temperature.

If the hyaline zone is formed exclusively by one factor (i.e. reproduction), and if the factor occurs exactly once a year, at the same time each year, every year, and at the same time in all areas where fish are collected, then samples can be combined from multiple years and different areas. However, Thomas (1984) found that hyaline-zone formation in West African pilchard, *Sardinops ocellata*, otoliths varied between years, and false marks (checks) were laid down with some degree of periodicity. Given the large number of factors known to produce hyaline zones, it seems likely that timing of hyaline-zone formation is variable.

Rockfish are ovoviviparous; parturition occurs about one month after fertilization (Moser, 1967), and thus males expend energy on reproduction at a different time than do females. If spawning is important in the formation of the hyaline zone for rockfish, then it is possible that males do not lay down the hyaline-zone at the same time as females. Failure to account for differences among sexes in the timing of hyaline-zone formation could result in errors in age determination.

The purpose of this study was to examine the otoliths of an important rockfish species and to determine whether there were sexual, geographical, or annual variations in the time of hyaline-zone formation. Possible causes of variations in the time of hyaline formation are also discussed.

Methods

Sagittal otoliths of widow rockfish were obtained from California and Washington commercial groundfish port sampling programs for the years 1983–87. This interval was selected because in 1983 an El Niño event occurred (Glynn, 1988) and a sequential series of years with adequate sample sizes was desired. Otoliths were collected from widow rockfish caught in northern Califor-

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[1] Pacific Fishery Management Council. 1993. Status of the Pacific Coast groundfish fishery through 1993 and recommended acceptable biological catches for 1994. Report of the Pacific Fisheries Management Council, Portland, OR, 467 p.

[2] Mellow, J. 1993. Bodega Bay Field Office, California Department of Fish and Game, 1785 Highway 1, Bodega Bay, CA 94923. Personal commun.

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nia (lat. 40° to 42°N) and throughout the coast of Washington (lat. 46° to 49°N).

Otoliths were examined only from fish less than 45 cm fork length because it is seldom possible to determine reliably the edge type of older, larger fish. On the basis of age-length relationships developed by Pearson and Hightower (1991), these fish ranged between four and twelve years. The dates of capture were not provided to the age reader; however, the reader did know the source (state) from which the otoliths were collected. The otoliths were broken and burnt according to the methods described by Chilton and Beamish (1982) and examined with reflected light with a dissecting microscope (20–30×). Edge types were classified as hyaline, narrow opaque (opaque area less than 1/2 of the previous opaque zone), and wide opaque (opaque area greater than 1/2 of the previous opaque zone). In about 1% of the fish (older fish), the edge could not be clearly identified owing to the narrowness of the rings. In this situation the otolith was omitted from the analysis. An attempt was made to obtain edge types of otoliths from 100 fish per month, per year, per state, but this was not possible in many cases (Table 1). A total of approximately 10,230 otoliths were initially examined, and edge types were ultimately determined for 6,384 fish.

The purpose of this study was to examine the timing of hyaline-zone formation. More than 95% of all otoliths had formed new opaque zones by July, and because sample sizes were small and patchy for August through December, these samples were not tested.

As some edge types were rare in certain months, edge types were omitted from some of the monthly analyses for both states as follows. When fewer than three otoliths had a given edge type, and when the edge type was uncharacteristic for that month, the otoliths were removed from the analysis. In January and February, narrow opaque edges were omitted; in April and May, wide opaque edges were omitted; and in July, hyaline edge types were omitted.

To determine whether significant differences in edge type existed between sexes, locations, and years, multiway contingency tables were examined by chi-square analysis with the CATMOD procedure, which has the log-linear option, in the SAS statistical analy-

Table 1

Numbers of widow rockfish, *Sebastes entomelas,* otoliths with identifiable edge types from Washington and California in 1983–87, by month. October, November, and December were omitted from the marginal increment study owing to small sample sizes.

State	Month	1983	1984	1985	1986	1987	Total
California	Jan	10	54	116	92	69	341
	Feb	75	17	111	101	63	367
	Mar	37	126	115	71	98	447
	Apr	45	54	107	66	102	374
	May	39	53	80	88	111	371
	Jun	24	89	56	70	52	291
	Jul	41	50	50	59	93	293
	Aug	39	66	54	18	137	314
	Sep	35	30	52	53	63	233
	Oct	15	14	57	18	82	186
	Nov	10	1	13	6	1	31
	Dec	2	1	1	1	1	6
	Total	372	555	812	643	872	3,254
Washington	Jan	97	98	88	100	39	422
	Feb	40	30	0	97	64	232
	Mar	99	67	93	100	50	409
	Apr	83	42	99	99	90	413
	May	34	81	0	68	48	232
	Jun	96	95	0	73	0	266
	Jul	67	59	77	69	25	297
	Aug	99	0	93	0	43	237
	Sep	0	28	0	0	95	126
	Oct	1	91	1	20	79	192
	Nov	1	14	57	67	1	140
	Dec	1	26	1	100	36	164
	Total	619	632	513	795	571	3,130

sis program (SAS Institute Inc., 1987). The log-linear option was used because edge types would not be normally distributed within the month. The analyses were used to test for interactions between edge type and each factor (sex, year, and location). In addition, two-way interactions were tested for the relationship of edge type to sex by year, sex by state, and year by state. Since there were no samples from Washington in February 1985, May and June of 1985, and June of 1987 (Table 1), the analyses of state, year by state, and state by sex, were not done for these months. Each test was conducted separately by month because edge type was known to vary between months (Lenarz, 1987) and because the purpose of this study was to examine differences among sexes, years, and areas.

Table 2

Chi-square values and probabilities of significance for variables associated with differences in edge type on widow rockfish, *Sebastes entomelas*, otoliths collected from California and Washington. Years used were 1983–87. * = significant difference ($P<0.05$).

Month	Variable	df	χ^2	P
January n=760	Year	4	22.79	0.0001*
	State	1	66.26	0.0001*
	Sex	1	3.28	0.0699
	Year × State	4	86.77	0.0001*
	Year × Sex	4	3.38	0.4964
	State × Sex	1	0.43	0.5138
February n=592	Year	4	163.49	0.0001*
	Sex	1	1.73	0.1887
	Year × Sex	3	12.50	0.0059*
March n=856	Year	8	43.12	0.0001*
	State	2	14.05	0.0009*
	Sex	2	20.95	0.0001*
	Year × State	3	42.64	0.0001*
	Year × Sex	8	23.56	0.0027*
	State × Sex	2	20.63	0.0001*
April n=731	Year	4	94.10	0.0001*
	State	1	126.52	0.0001*
	Sex	1	0.23	0.6307
	Year × State	4	44.12	0.0001*
	Year × Sex	8	23.56	0.0027*
	State × Sex	1	1.15	0.2836
May n=583	Year	4	113.53	0.0001*
	Sex	1	2.88	0.0898
	Year × Sex	3	9.53	0.0230*
June n=555	Year	8	300.22	0.0001*
	Sex	2	2.29	0.3177
	Year × Sex	4	4.43	0.3509
July n=575	Year	4	129.40	0.0001*
	State	1	73.72	0.0001*
	Sex	1	0.09	0.7704
	Year × State	4	69.78	0.0001*
	Year × Sex	4	6.49	0.1654
	State × Sex	1	.92	0.3376

The times of formation of the hyaline zones are discussed in light of sea-surface temperature data from northern California and southern Washington. Mean monthly sea-surface temperatures were obtained from National Weather Service marine buoys located off Point Arena (northern California) and the Columbia Basin (where many of the Washington fish were caught).

Results

There was a significant difference in edge type between the sexes for the month of March (Table 2). Examination of the data indicates this difference was due to a difference in the percentage of opaque zone types present. Males had a slightly smaller fraction of narrow opaque zones than did females (5.7% vs. 6.3%), and a slightly larger percentage of wide opaque zones (8.4% vs. 6.9%). There were no significant differences between the sexes for any other month.

There were significant differences in the timing of hyaline-zone formation between the states for all months that could be tested (Table 2). Both hyaline and opaque zones form earlier in Washington than in California (Fig. 1). By July, about 50% of all otoliths from Washington have a wide opaque zone whereas only about 20% of the otoliths from California have a wide opaque zone.

There were significant differences in edge types among years for all months (Table 2). Hyaline zones formed earlier in 1985 and 1987, at an intermediate time in 1986, and later in 1983 and 1984 (Fig. 2, A and B).

There were significant differences in edge type for all months for which the year-state interaction could be tested (Table 2). This indicates that the states do not vary to the same extent. Yearly differences are much more pronounced in California than they are in Washington (Fig. 2, A and B). In all cases, the annual variations are in the same direction; however, the changes are smaller in Washington (Table 2).

There were significant differences in edge types in February, March, and April for the year-sex interaction. There were no differences for this interaction for January, May, or June. These differences may be attributed in part to the strong effect of yearly variability. Examination of the frequency distributions also suggests that a somewhat higher fraction of females have hyaline zones in February, March, and April in 1983, whereas the fraction tends to be equal in other years.

There was a significant difference in edge types in March for the state-sex interaction (Table 2). There

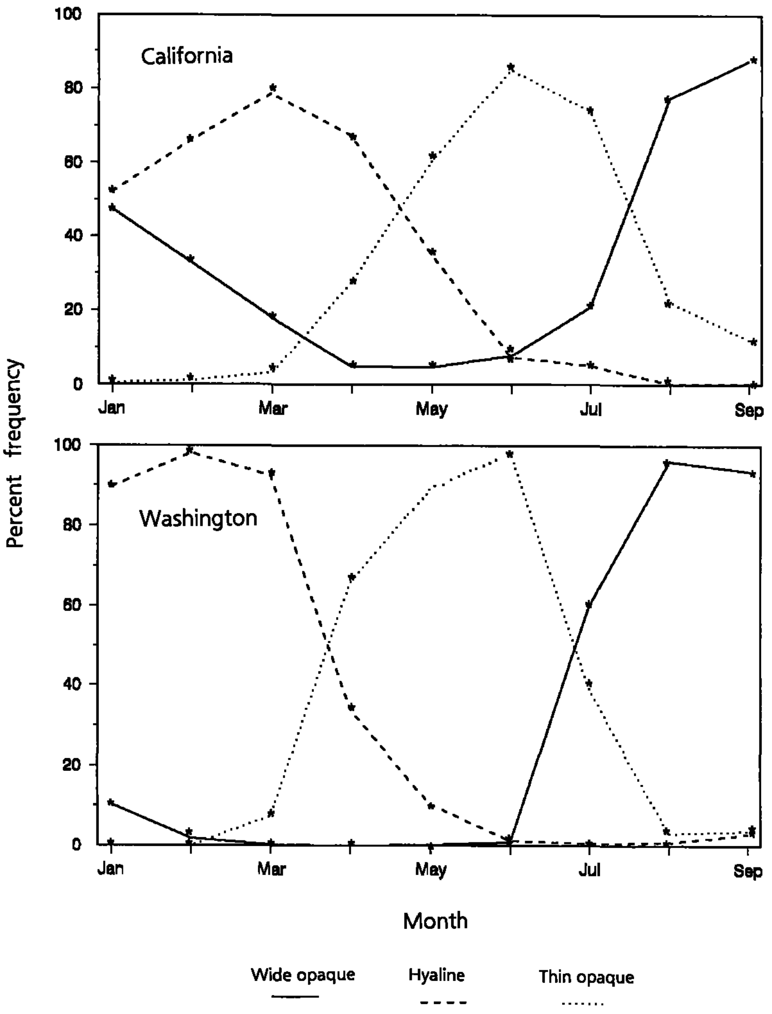

Figure 1

Marginal increment comparison for widow rockfish, *Sebastes entomelas*, between Washington and California for the years 1983–87 combined (unweighted means) and sexes combined. Large opaque is defined as an opaque edge >1/2 the size of the previous opaque zone; small opaque is defined as an opaque edge <1/2 the previous opaque zone.

were no differences in edge types for the months of January, April, or July. This difference appears to be due to the tendency of females in California to have a smaller portion of their population with hyaline edges and more of both late opaque and early opague edges in March. Males tended to be similar among states.

Discussion

Significant differences in the time of hyaline-zone formation exist among years and states and may have important ramifications for age determination. It is important to consider whether differences in the seasonal expression of the hyaline zone might cause errors in age determination and, if so, what type of errors might occur.

By convention all fish are considered to be born on 1 January (Chilton and Beamish, 1982) to assure proper year-class identification. In addition, if the edge appears hyaline before January, it is not counted, and if it is opaque after December, a hyaline edge is assumed to be present and therefore counted.

The results of this study indicate that the hyaline zone forms earlier in otoliths from fish in Washing-

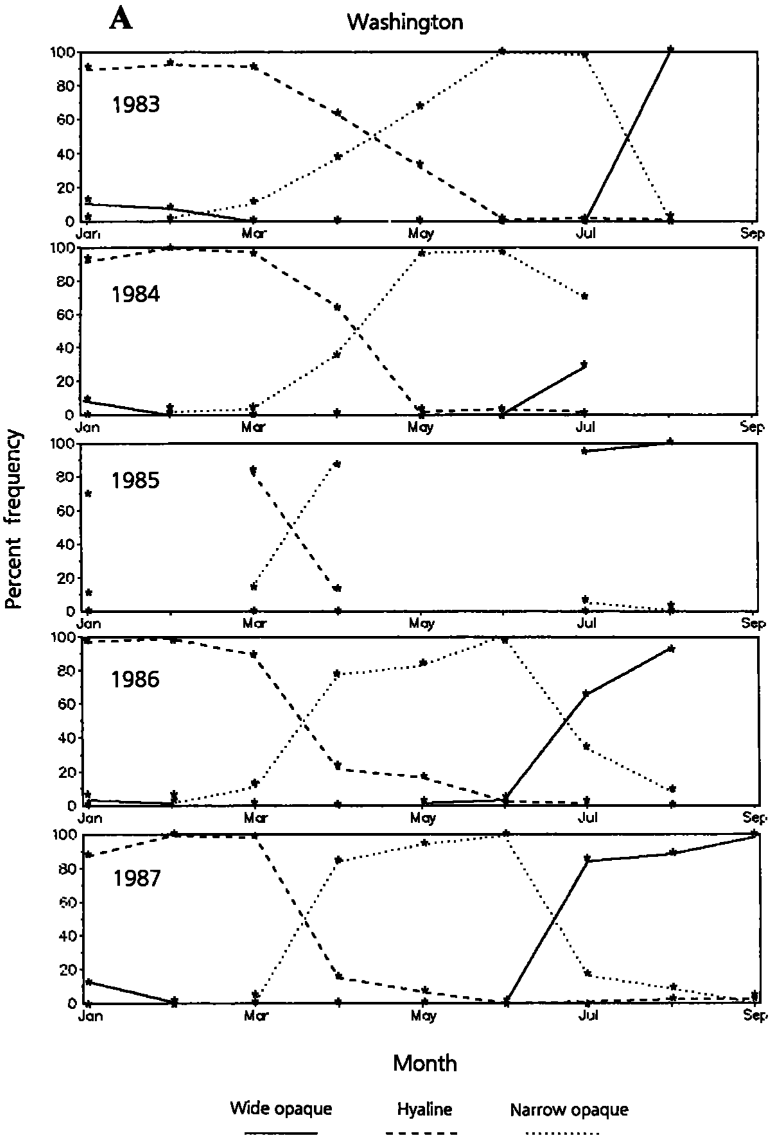

Figure 2

Marginal increment comparison for widow rockfish, *Sebastes entomelas*, between states and years 1983–87. Refer to Table 1 for sample sizes.

ton than in those from fish in California. In addition, there are annual differences in the timing of hyaline-edge formation. Problems in age determination may arise when the forming annulus is small relative to preceding annuli. In this case, a fish collected in March might be mistakenly aged as one-year younger. This would occur because the reader might consider the completed opaque zone to be new growth for the current year. This problem is more likely to occur in young fish where the relative size of the annuli is comparatively large.

The later time of hyaline-zone formation in 1983 (Fig. 2) suggests a possible explanation for the observation that the opaque zone formed that year was smaller than those formed in preceding and subsequent years (Woodbury[3]). As the opaque zone formed later in 1983, particularly in California (Fig. 2), there was less time for the opaque zone to form, and therefore a smaller opaque zone was visible for that year.

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[3] Woodbury, D. P. 1994. National Marine Fisheries Service, 3150 Paradise Drive, Tiburon, CA 94920. Unpubl. data.

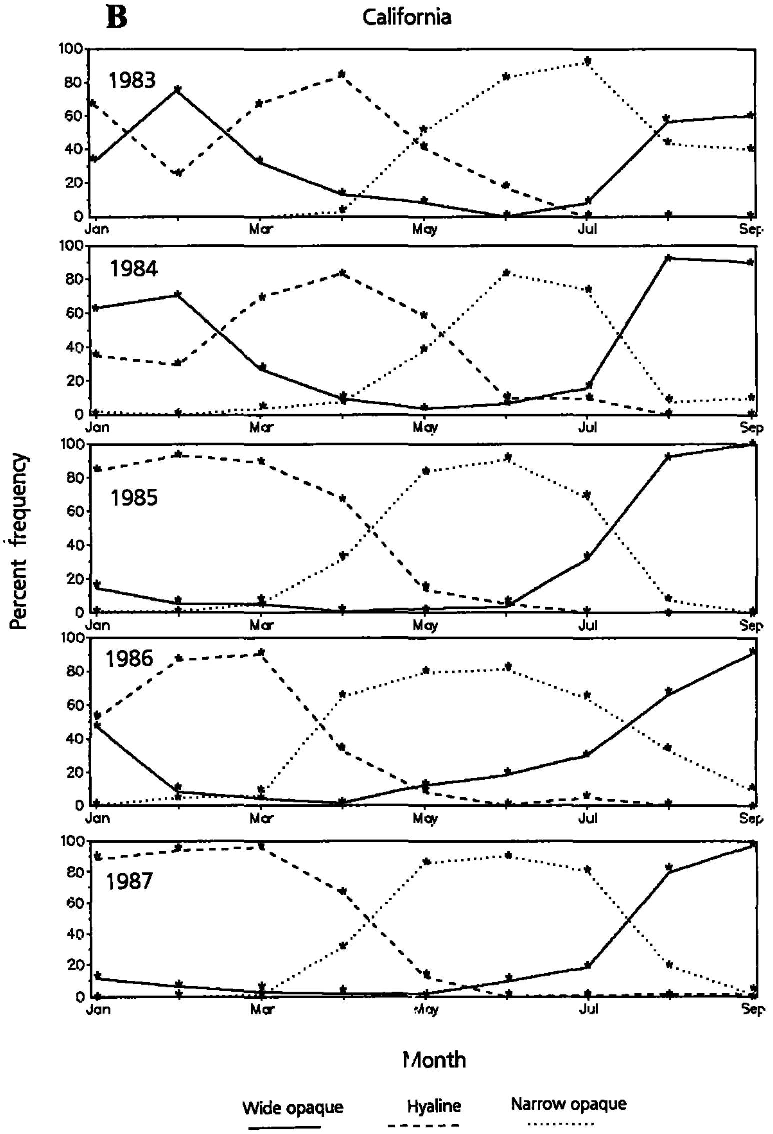

Figure 2 (continued)

The late onset of opaque-zone development may be related to ocean temperature.

The 1983 El Niño event began early in the year (Fig. 3). During the spring of 1983, sea-surface temperature was the warmest of all spring temperatures from 1983 through 1987. In addition, the minimum temperature off California occurred later in 1983 than in other years. These environmental changes coincide with the late formation of the hyaline zone in 1983.

The temperature-hyaline zone formation relationship is supported by the fact that the timing of the spring sea-surface temperature minimum in Washington is more constant than in California (Fig. 3). This is similar to the pattern observed in hyaline-zone formation in otoliths between the two states. Finally, the temperature declines earlier in Washington than it does in California, which is consistent with the earlier onset of hyaline-zone formation in otoliths from fish in Washington. Although the link between temperature and hyaline-zone formation is not conclusive, it is suggestive. It is possible that some factor, related to temperature, is responsible for formation of the annulus (e.g. food availability or nutrient content of prey).

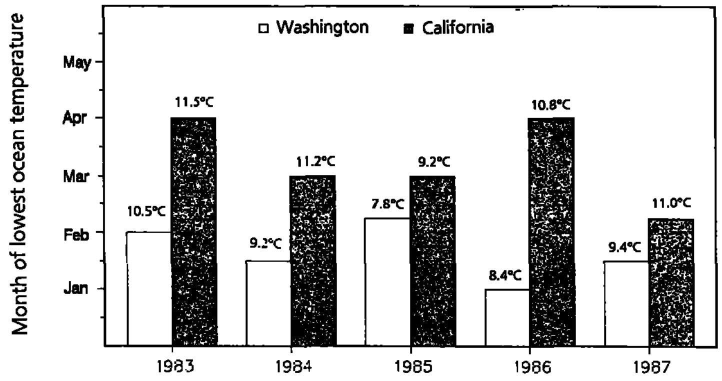

Figure 3

Month of minimum sea-surface temperatures in Washington and California from 1983–85. Source: Pacific Fisheries Environmental Group, National Marine Fisheries Service, P.O. Box 831, Monterey, California 93942, unpubl. data, January 1984.

Reproduction is unlikely to play a significant role in the time of hyaline-zone formation. The only difference among the sexes in timing of hyaline-zone formation occurred in March. In that month it appeared that females were more likely to have either wide or narrow opaque zones. It should be noted, however, that 81% of the females had hyaline zones (88% of males had hyaline zones). Males expend energy on spermatogenesis from October through January, whereas females expend most of their energy on reproduction later, with parturition in February (Wyllie Echeverria, 1987).

In some studies, maturity has been shown to be related to the time of annulus formation (Chugonova, 1931; Konstantinova, 1958). Although maturity was not recorded, about half of the fish from both areas in this study should have been sexually mature on the basis of maturity-at-length relationships (Barss and Wyllie Echeverria, 1987). If immature fish had failed to lay down a hyaline zone, the differences should have been evident in Figure 2; that is to say, a large fraction of the fish would show no hyaline-zone at any time of the year. If the timing of hyaline-zone formation had been markedly different between mature and immature fish, this would have been reflected as a second mode in the month of hyaline-zone formation.

Several different methods of marginal increment analysis have been used to validate ages. Beckman et al. (1991) used four edge types in a classification designed to characterize more precisely when the hyaline zone is formed. Many researchers have measured the width of the edge (Nelson and Manooch, 1982; Barger, 1985; Maceina et al., 1987; Manooch and Drennon, 1987; Bullock et al., 1992; Hyndes et al., 1992; Hostetter and Munroe, 1993). The measurement approach has an advantage in that it should be possible to plot growth of the edge over time to verify that only a single hyaline mark is laid down each year. However, in some species, it can be difficult to determine a consistent location to measure on the otolith because of the inherent variability of their otoliths. In addition, a great deal of time is required to process the otoliths. The classification of edge types into three or more categories is one way to qualitatively judge the width of the edge (usually relative to the preceding opaque zone). This approach has an advantage over simply classifying the edges as either hyaline or opaque in that it indicates whether the opaque zone has just formed or whether a new hyaline zone may be ready to form.

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