FISHERIES SCIENCE 2001; **67**: 811–817

Original Article

Daily growth increment formation and its lunar periodicity in otoliths of the myctophid fish *Myctophum asperum* (Pisces: Myctophidae)

AMANE HAYASHI,[1]* KOUICHI KAWAGUCHI,[1] HIKARU WATANABE[1a] AND MINORU ISHIDA[2]

[1]*Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164-8639 and* [2]*National Research Institute of Fisheries Science, Kochi, Kochi 780-8010, Japan*

ABSTRACT: The surface-migratory myctophid fish, *Myctophum asperum*, of the western North Pacific was found to have daily growth increments of its sagittal otolith, which also exhibited lunar periodicity in the deposition of increments. Daily deposition of the otolith increments was verified because the width of the marginal increment increased during the night and early morning between 20.00 h and 08.00 h and its growth stopped during the day. An autocorrelation analysis of the increment widths, which were measured consecutively in 11 specimens covering 33 synodic months, also showed a lunar periodicity in increment deposition. The mean increment widths during five days around the time of a full moon were significantly narrower than those around a new moon in 18 of the 33 full moon cases ($P < 0.01$: Student's t-test) and, on average, tended to be narrow in 29 cases. The cause of this tendency is thought to be slower growth caused by staying in deeper and colder habitat due to the suppression of diel vertical migration and/or lower food availability resulting from the possible dispersion of zooplankton during the full moon period.

KEY WORDS: **daily otolith increment, lunar periodicity, *Myctophum asperum*.**

INTRODUCTION

Aging techniques based on daily otolith increments are commonly used in ecological studies of various fish species. A prerequisite for adopting this technique is to prove that the increments are formed daily. However, this is difficult in deep-sea fishes, which are hard to rear. Gartner[1] verified daily increment formation for three myctophid species (*Benthosema suborbitale*, *Lepidophanes guentheri* and *Diaphus dumerilii*) by marginal increment analysis based on the 24 h sampling proposed by Brothers and McFarland[2] and established by Ré.[3] A daily increment in the otoliths of *D. kapalae* was also verified using the same method, although no daytime samples were examined.[4]

Giragosov and Ovcharov reported that otolith increment growth varies with the lunar cycle in the surface-migratory myctophid, *Myctophum nitidulum*.[5] They showed that a pair of opaque and translucent zones consists of 26.19 ± 8.17 (mean ± SD) increments and translucent zones are formed mainly during the full moon with no validation of the daily otolith increment. Linkowski also reported the presence of a lunar cyclic pattern in the width of incremental structures in the otoliths of *Hygophum benoiti* and *H. macrochir*.[6] Assuming that the increment is formed daily, he showed that the otolith increment width decreased during the new moon phase when these two myctophid species stop their diel vertical migration and remain in deep, cold habitats all day long.

Myctophum asperum is one of the most dominant surface-migratory myctophid fishes in the western tropical and subtropical North Pacific,[7–9] but no ecological study based on the verified aging technique has been made previously. The aims of the present study are to prove the daily formation of otolith increments in this species based on 24 h time series sampling and to confirm the presence

*Corresponding author: Tel: 81-3-5351-6477. Fax: 81-3-5351-6481. Email: ahayashi@ori.u-tokyo.ac.jp

[a]Present address: National Research Institute of Far Seas Fisheries, Shimizu, Shizuoka 424-8633, Japan.

Received 10 July 2000. Accepted 5 February 2001.

of a lunar periodicity in the increment growth based on both autocorrelation analysis of consecutive increment widths and comparison of the increment widths between full and new moon periods.

MATERIALS AND METHODS

Sampling data are shown in Table 1. Fish were collected in the Kuroshio and transitional waters of the western North Pacific during the cruises of the research vessels *Shunyo-maru* of the Japanese Fisheries Agency, and *Hakuho-maru* of the Ocean Research Institute, the University of Tokyo. Samples were frozen or preserved in 80% ethanol aboard the ship.

To prove the daily formation of otolith increments, the flat surface of the sagittal otoliths of 37 fish were mounted on glass slides with the proximal side down using epoxy cement. They were then ground and polished with 600–2000 grit wet sand papers and 4000 grit and 10 000 grit lapping films until the marginal increments could be observed clearly with a light microscope. Widths of the outermost three increments were measured either at 200× or 400× magnification with an image processing measuring system attached to the microscope. Increment width was measured from the outer margin of an opaque zone to that of the next outer opaque zone on a line perpendicular to the rostrum margin. Daily increment was proven by the marginal increment analysis following Gartner;[1] that is, the width of the outermost-growing increment was compared with the mean width of the two preceding inner increments using Student's *t*-test in time series samples. The distribution pattern was tested using skewness and kurtosis. Sampling time was defined as the halfway point of the sampling period.

To detect lunar periodicity, daily increment widths were measured in a frontal section through the core and rostrum. The otoliths of 11 fish, ranging 42.7–55.7 mm in standard length (SL), were embedded in epoxy cement, mounted on a glass slide, ground to the core alternately from both the dorsal and ventral sides and polished until the increments outside of the postlarval zone[1] were clearly visible under a light microscope. The prepared sections were photographed at either 10× or 25× magnification and the increments in the postmetamorphic zone[1] were counted and measured on an enlarged microphotograph using transmitted light (C, Fig. 2). Five-point running means of increment widths were calculated to delete daily random noise. To detect lunar periodicity, autocorrelation analyses[10] were made on the increment widths of 11 otoliths measured consecutively, which covered 33 synodic months. The presence of periodicity is indicated by peaks in the autocorrelation coefficient function, as the lag value corresponding to the peaks indicates the period or its multiple. To detect any difference in significance, Student's *t*-test was applied to the average increment width between the full moon period and the two closest new moon periods. Each period was defined as five days around the day of the full or new moon; that is, that day ± two days.

RESULTS

Daily increment

A total of 37 specimens, ranging from 15.2 mm to 42.5 mm SL, were used for marginal increment analysis. Widths of the three outermost complete increments of the 37 otoliths, excluding the outer-

Table 1 Sampling data of *Myctophum asperum* for the marginal increment analysis and observation of lunar periodicity in daily otolith increment formation

Sampling			No.	Used for*	
Date	Time	Locality	Inds.	D	L
June 14 1997	11:48–13:18	36°10′N 142°40′E	5	○	
June 14 1997	13:38–15:24	36°10′N 142°40′E	4	○	
June 14 1997	16:34–17:50	36°10′N 142°40′E	1	○	
Feb. 10 1996	19:24–19:34	33°00′N 136°00′E	5	○	
Feb. 11 1996	20:55–21:05	32°30′N 135°00′E	5	○	
Feb. 19 1996	22:51–23:01	30°20′N 130°20′E	3	○	
June 7 1997	00:17–01:01	39°30′N 144°30′E	5	○	
Feb. 26 1996	02:09–02:19	29°00′N 130°00′E	4	○	
June 9 1997	07:22–08:22	39°00′N 147°00′E	5	○	
Aug. 9 1994	19:55–20:25	39°00′N 147°00′E	11		○

* D, used for daily increment validation; L, used for lunar periodicity validation.

most growing increments, ranged between 1.92 μm and 19.20 μm. The mean percentage of the penultimate increment widths to the mean width of the preceding inner two increments was 105% (mainly 85.2–132.1%) and their distribution was not significantly different from that of a normal distribution ($P > 0.05$). As the 95% confidence interval for the mean percentages of penultimate increment widths was 99.9–110.2%, the width of the penultimate increment is not different statistically from the mean width of the preceding two increments in the present specimens. This means that no statistically significant abrupt change occurred in the growth of the three outermost increments; that is, change was gradual. This validates the adoption of marginal increment analysis. In Fig. 1, percentage of the outermost growing increment width to the mean width of the preceding two increments is plotted in relation to sampling time. In the afternoon and evening (12.00–20.00 h), percent widths were clearly separated into two groups: one less than 38% with a mean of 28%, and the other being more than 97% with a mean of 107%. Percent widths of the latter group fluctuated between 97% and 117% probably because of day-to-day differences in the growth rates of each fish. No growth in increments was observed in otoliths during the afternoon. In the latter group, percent width of the outermost growing increments was not significantly different from the mean percentage of the penultimate increment widths ($P > 0.10$), indicating that increment formation was completed. The percent width of the outermost growing increment width increased from 20.00 h until 08.00 h in the morning. After midnight, all values were more than 50%, suggesting that increment formation in the entire population had started by midnight. Consequently, it was concluded that one otolith growth increment is formed daily.

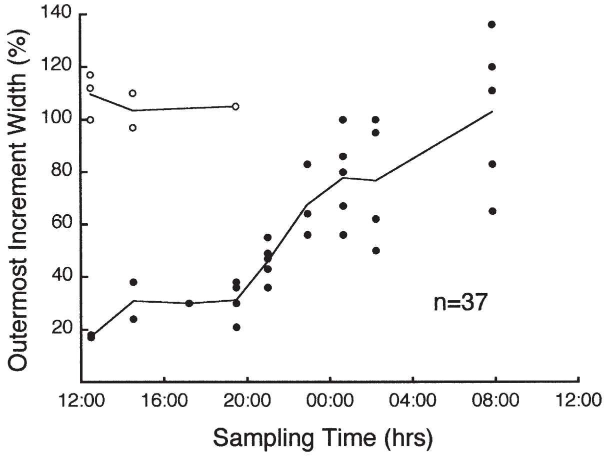

Fig. 1 Percentages of the outermost-growing increment width to the mean width of the preceding two increments in relation to sampling time of *Myctophum asperum*. (○) Otoliths in which the outermost increment width formation was almost completed, although their percent widths fluctuated between 85.2% and 132.1% due to daily fluctuations in growth.

Lunar periodicity

A microphotograph of the frontal section of a 51.7 mm SL fish otolith is shown in Fig. 2, in which the three zones were clearly recognized. The innermost zone (A, Fig. 2) is characterized by fine increments in the core area; the outermost zone (C) is made up of well-defined increments; and the middle zone (B) consists of dark and light bands. These three zones can be correlated with the larval zone forming during the larval period; the postmetamorphic zone forming during the juvenile to adult periods; and the postlarval zone forming during metamorphosis period, respectively, as defined by Gartner.[1]

The number of daily increments in the postmetamorphic zone and the range of increment widths are shown in Table 2. The 5-point running

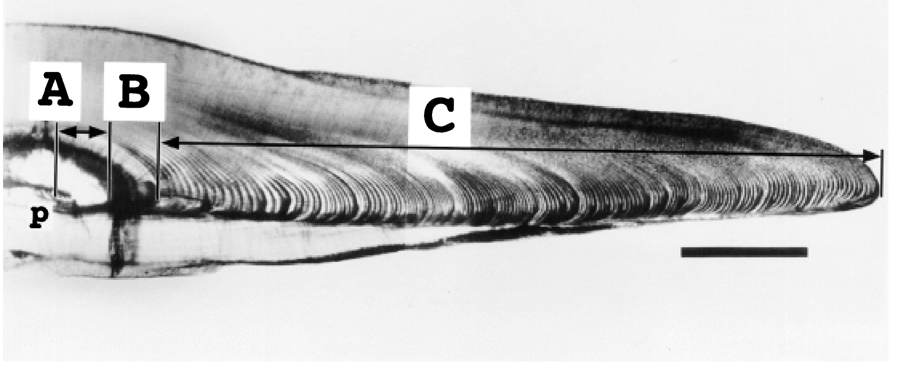

Fig. 2 Photomicrograph of a frontal section of the sagittal otolith of *Myctophum asperum* (51.7 mm standard length). A, larval zone; B, postlarval zone; C, postmetamorphic zone; p, primordium. Bar = 200 μm.

Table 2 Number of increments in the postmetamorphic zone, range of increment widths, and the lag and correlation coefficient for the first peak of the autocorrelation function for the increment widths of *Myctophum asperum*

Specimen no.	SL (mm)	Increment No.	Increment Width (μm)	Lag (day) corresponding to the first peak
1	44.0	108	3.4–20.0	28
2	54.5	132	1.0–15.5	31
3	54.3	131	0.0–21.5	29
4	52.9	129	3.9–24.6	59
5	51.7	163	2.5–15.6	59
6	51.6	126	0.7–15.3	29
7	50.0	136	2.1–19.1	24
8	55.7	157	1.8–13.3	32
9	47.4	136	3.5–13.3	55
10	49.4	129	2.1–21.3	26
11	42.7	102	4.5–15.8	16

One synodic month is equivalent to 29.53 days; two synodic months are equivalent to 59.06 days.

Table 3 Comparison of the mean increment widths for the five days around the full moon with those for the five days around the previous and next new moons in *Myctophum asperum* otoliths

Specimen no.	June FM (μm)	June NM (μm)	June Ratio	May FM (μm)	May NM (μm)	May Ratio	April FM (μm)	April NM (μm)	April Ratio	March FM (μm)	March NM (μm)	March Ratio
1	6.8	9.3	0.72*	10.6	11.5	0.92						
2	6.4	7.8	0.82*	7.5	8.2	0.92	7.4	8.2	0.90			
3	7.0	7.5	0.93	7.4	8.9	0.83*	9.3	11.0	0.95*			
4	6.2	7.5	0.84*	8.4	9.8	0.86	12.1	12.6	0.96			
5	5.7	6.3	0.91*	7.1	6.1	1.16*	4.4	5.9	0.75*	6.9	8.9	0.78
6	7.4	9.0	0.82*	7.6	8.9	0.86*	12.5	9.7	1.28*			
7	5.2	6.7	0.77*	6.7	8.6	0.78*	9.4	10.2	0.93			
8	4.2	6.5	0.65*	7.4	7.6	0.98	9.7	9.0	1.08	8.8	11.0	0.80*
9	6.1	7.8	0.77*	5.7	7.0	0.81*	7.7	8.0	0.96			
10	6.4	5.6	1.14	6.1	6.9	0.86	9.5	11.6	0.96			
11	6.7	8.5	0.81*	9.1	9.5	0.96						

* Significant ($P < 0.01$).
The date of the full moon was back-calculated based on the daily increment.
FM, mean increment width of five days around full moon day; NM, mean increment width of five days around previous and next new moon days.

means of increment widths for six specimens are shown in Fig. 3, as well as the increment numbers and the back-calculated lunar phase. A periodic change in the increment width was predicted because the increment width tended to decrease around the time of the full moon period in 11 of 24 cases (Fig. 3), but in two or three of 23 cases during the new moon period.

Four typical autocorrelograms are plotted in Fig. 4. All of the 11 lag values (day) corresponding to the first peaks are shown in Table 2. Lags for the first peak were close to one synodic month of 29.53 days in seven of 11 otoliths (range 24–32, mean 28.4), and were close to two synodic months of 59.06 days (range 55–59, mean 57.7) in three otoliths. These results strongly suggest the presence of a lunar cycle in the growth of daily otolith increments of *M. asperum*. Ratios of the average increment width during the full moon period to that during the closest two new moon periods are shown in Table 3. The mean widths during the full moon tended to be narrower than those of the previous and next new moon in 29 of 33 cases, although the level of significance was $P < 0.01$ in 16 cases. This lunar periodicity is supposed to be occasionally masked by other factors such as weather conditions, as discussed later. Taking this into account, it was concluded that lunar periodicity was present in daily otolith increment growth.

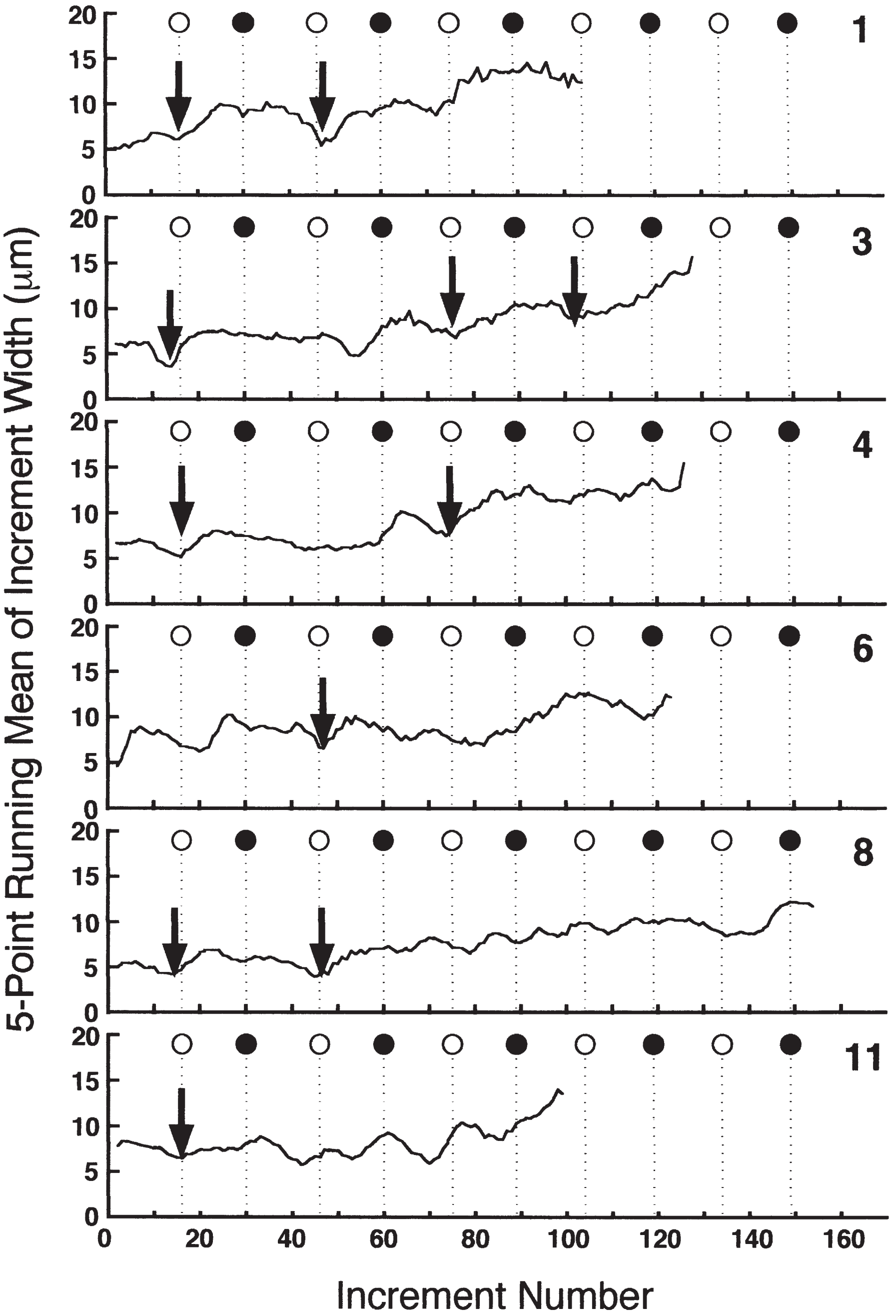

Fig. 3 Changes in the 5-point running mean of increment widths from juvenile to adult stages in relation to back-calculated lunar phase in six specimens of *Myctophum asperum*. (●) New moon and (○) full moon. Arrows indicate narrower increment widths during the full moon period. Number in each figure indicates specimen number.

DISCUSSION

Daily increments

Gartner reported that deposition of a new increment begins around 20.00 h and is completed by around 06.00 h in the three myctophid species *B. suborbitale*, *L. guentheri* and *D. dumerilii* from the eastern Gulf of Mexico.[1] In *D. kapalae* collected in the southern Coral Sea, approximately 20% of the outermost-growing increment is formed before 19.00 h and almost 100% is formed by 03.00 h[4] which, together with the present data, support Gartner;[1] that is, increments are formed at night. In the case of *M. asperum*, a thin light zone, which looks like a new increment, was often seen at otolith margins from 12.00 h, but their width did not change for 8 h until 20.00 h, suggesting the possibility that this might be a virtual increment induced by optical diffraction, as reported in *Sardina pilchardus* by Ré;[3] however, this does not affect our conclusion. The deposition of new increments also began at night, at approximately 20.00 h, and finished by 08.00 h the next morning.

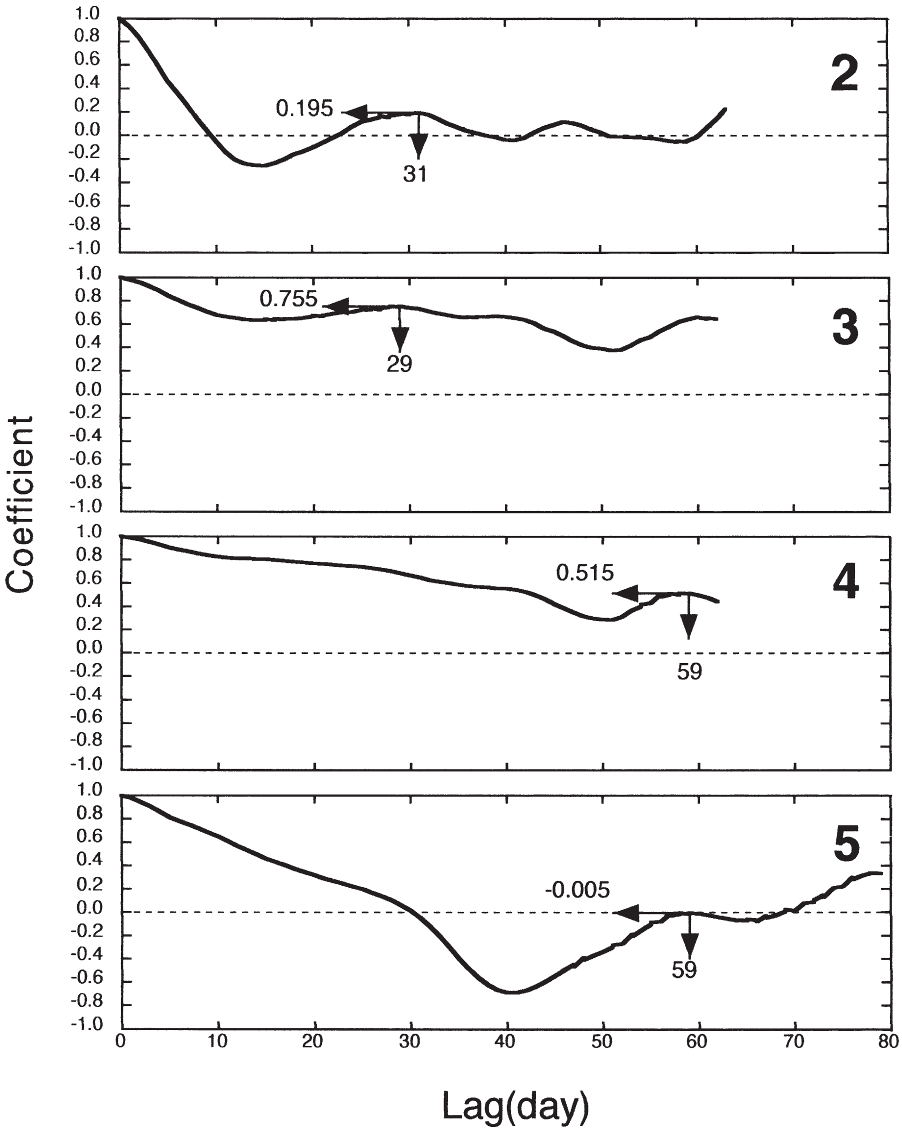

Fig. 4 Four typical autocorrelograms showing the relationship between lag and coefficient of autocorrelation analysis for consecutive increment widths in *Myctophum asperum*. Arrows indicate lag and coefficient for the first peak. Number in each figure indicates specimen number.

The fishes move into warm surface waters at night, which is when deposition of the new increment occurs. During the day, when fishes are at depth (cold water), lesser or no deposition occurred.

Lunar periodicity

Linkowski described how increment growth is suppressed in cold, deep waters during the new moon phase in *H. benoiti* and *H. macrochir* because both species show a tendency to cease vertical migration during the new moon period.[6] Clarke also found this vertical migration pattern in their congeners, *H. proximum* and *H. reinhardti*, in the central Pacific.[11] In contrast, Giragosov and Ovcharov showed that increment growth is reduced during the full moon in *M. nitidulum*,[5] as was observed in the present study in its congener *M. asperum*. Clarke described that most myctophid species, except *Hygophum* spp., are found approximately 50 m deeper than their migratory depth during full moon nights.[11] The same phenomenon probably occurs in *M. asperum* and *M. nitidulum*, suggesting that reduced increment growth is a result of slower growth caused by staying in deeper and colder habitats during the full moon period. However, in the Gulf of Mexico, the upper limits of the diel vertical migration of most myctophid species are reported to be unaffected by the lunar phase.[12]

Another explanation for the lunar cycle in otolith increment might be the reduced availability of food resulting from the possible dispersion of zooplankton in the surface layer in response to the light of the full moon. The light of the full moon underwater would be greatly affected by the weather and/or sea conditions, which would probably explain why the full moon did not always cause a narrow growth increment (Fig. 3) and explain the fluctuations in periodicity in some fishes (Table 2). During March–June 1994, 42.2% of the half days (Japan Meteorological Agency routinely describes weather reports every 12 h; i.e. from 06.00 h to 18.00 h and 18.00 h to the next 06.00 h) at Ofunato city, which is located approximately 425 km west of the sampling station, were cloudy or rainy throughout a 12 h period.[13–17] This suggests that during approximately 40% of full moon periods, light levels in the study area were affected by cloud or rain. Considering these weather conditions, the presence of the not significantly narrower increment in 15 of 33 full moon cases would probably be affected. Two synodic month cycles might also be explained by the masking of full moon light by weather conditions. We cannot back-calculate the affected lunar increments to these weather events because the locality of the fish one or two months before sampling is uncertain.

ACKNOWLEDGMENTS

We thank Captains H Shimojima and H Tanaka, and the officers and crew of the research vessels *Shunyo-maru* and *Hakuho-maru* for their assistance in the field sampling. We also thank Mr T Mitani and Drs S Uehara and S Kosaka of Nansei National Fisheries Research Institute for providing samples. Special thanks to Dr Jack V Gartner, Jr of St Petersburg Junior College for his critical review of this manuscript.

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