Appendix 8

Marine and Freshwater Resources Institute

Age estimates of two species of threadfin bream (*Nemipterus theodorei* and *N. aurifilum)*

Final Report to the

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Corey Green and Kyne Krusic-Golub

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Age estimation of two species of threadfin bream (*Nemipterus theodorei and Nemipterus aurifilum)*

Investigating unit: Central Ageing Facility
Address: Marine and Freshwater Resources Institute
P.O. Box 114
Queenscliff, Vic 3225
Tel: (03) 5258 0111 Fax: (03) 5258 0270
Email: corey.green@nre.vic.gov.au

SUMMARY

James Haddy from the Southern Fisheries Centre (Queensland Department of Primary Industries, QLD) approached the Central Ageing Facility (CAF), Marine and Freshwater Resources Institute, to provide annual age estimates of two species of threadfin breams (*Nemipterus theodorei* and *N. aurifilum*). A total of 314 and 186 *N. theodorei* and *N. aurifilum* sagittal otoliths collected from *N. theodorei* and *N. aurifilum* respectively, were sent for age estimation. Age estimates were determined by counting incremental structure on transversely sectioned otoliths. Estimates revealed that both species were relatively short lived. Ages ranged from 0 to 6 years for *N. theodorei* and 0 and 4 years for *N. aurifilum*. Repeated readings of otoliths from both species indicate a high level of precision.

METHODS

Samples

Initially, otoliths from a total of nine *N. theodorei* were sent to the CAF for examination to determine whether age estimates were possible. On the basis of the initial examination, the CAF was contracted to provide age estimates for a larger sample of *N. theodorei* and *N. aurifilum*. A total of 314 *Nemipterus theodorei* and 186 *N. aurifilum* sagittal otolith pairs were forwarded to the CAF for age estimation. Otoliths were stored dry in numbered envelopes with accompanying date of capture information. No other biological details were provided.. The samples arrived at the CAF and were registered in July 2002. Samples were allocate a unique identification code and registered according to Morison et al. (1998). Either the left or right otoliths were weighed to the nearest milligram, on the assumption that there was no significant difference between left and right otoliths.

Otolith preparation

Both *N. theodorei* and *N. aurifilum* were prepared using identical methods.
An initial examination of *N. theodorei* indicated that otoliths were best viewed when they were transversely sectioned as opposed to viewing them whole. The otoliths were embedded in clear polyester casting resin in rows of five. The resin blocks were oven cured at 55°C for 24 hours.

Otolith sections were cut using a Gemmasta™ lapidary saw fitted with a diamond-impregnated blade. From each row of otoliths, four sections were attained (approximately 350μm in thickness) to ensure the primordium of each otolith was included. Sections were cleaned using alcohol and stored in vials. For identification, each vial contained a numbered label.

Cleaned sections were mounted in polyester resin on glass slides (50 x 75 mm) under glass coverslips. Slides were oven cured at 30°C for a minium of 3 hours.

Reading Protocol

Sections were viewed using a dissecting microscope at a magnification of 10x (16x primary and 0.63x secondary objective) illuminated with transmitted light. Before attempting to assign age estimates the reader first became familiar with otolith structure by making a preliminary examination of the samples. Age was estimated by counting the number of complete zones (translucent – opaque sequence) on either the dorsal or ventral side; whichever displayed the highest clarity. A customised image analysis system (Morison et al., 1998) was used to mark and count increments along the ageing transect. The image analysis system was also used to measure the distance from the primordium to each of the increments. Measurement were made along a transect on the ventral side adjacent to the sulcus. The otolith margin was classified as either wide or narrow relative to the previously completed zone. Other information recorded in the Excel spreadsheet was a readability score. This is a subjective measure of the sample's readability based on the combination of the quality of the preparation and the clarity of the increments (Table 1). To avoid potential bias, all counts were made without knowledge of otolith weight.

Table 1. Interpretation of readability scores.

Score	**Interpretation**
1	Sample has excellent readability, increments exceptionally clear
2	Sample is unambiguous but not as clear as 1
3	Sample may be subject to two interpretations
4	Sample is subject to multiple interpretations
5	Sample is unreadable

As increment deposition and spawning duration usually occur over a period of months, a simple count of increments would most likely allocate a fish to an incorrect cohort. To assign an increment count to a particular age class, the following criterion was adopted.

A birthday of September 1 was assigned to *N. theodorei.* This date was chosen because it:

- confirms to the best knowledge of the timing of spawning (Rao, 1986)
- is also similar to the time of increment deposition (based on analysing increment width in relation to month of capture).

Plotting the month of capture against otolith weight collected from juvenile fish (<1yr) can give an approximate indication of the spawning period. A broad spawning period may be indicated by similar otolith weights distributed over an extended time interval (months).

The spawning time and increment formation for *N. aurifilum* was unknown so the same birthday allocated to *N. theodorei* (1st September) was used.

If a fish was caught between April and August with a recently formed increment (narrow edge), then Age Class = Increment Count -1 (as the fish was caught before the birthday and the increment was just formed).

If a fish was caught between April and August with a wide edge, then Age Class = Increment Count (as the fish was caught before the birthday).

If a fish was caught between September and March with a recently formed increment (narrow edge) then Age Class = Increment Count (as the fish was caught after the birthday with a recently formed increment).

If a fish was caught between September and March with a wide edge, then Age Class = Increment Count +1 (as the fish was caught after the birthday and an increment would be forming but not quite visible).

For otoliths that did not possess an increment and were presumed to be less than one year of age, a 0+ age class was assigned.

Using the above criteria, samples were assigned to the appropriate age class during ageing.

Comparison of age estimates

Repeated readings of the same otoliths provide a measure of intra-reader and inter-reader variability. They do not validate the assigned ages but provide an indication of the size of the error to be expected with a set of age estimates, due to variation in otolith interpretation. Beamish and Fournier (1981) have developed an index of average per cent error (IAPE), which has become a common method for quantifying this variation. The IAPE is calculated as:

$$IAPE = \frac{100}{N} \sum_{j=1}^{N} \left[\frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j} \right]$$

where N is the number of fish aged, R is the number of times fish are aged, X_{ij} is the ith determination for the jth fish, and X_j is the average estimated age of the jth fish. The index has the property that differences in age estimates for younger fish will contribute more to the final value than will the same absolute error for older fish (Anderson et al., 1992).

To establish confidence intervals to these estimates of precision, a bootstrap technique was employed on the individual error estimates following methods described by Efron and Tibshirani (1993). Five thousand samples of error estimates (each the same size as the original) were randomly taken with replacement from the repeat readings, and a new IAPE calculated for each. The mean of these replicate IAPE's is the mean bootstrap IAPE. The bootstrap procedure exaggerates any bias present in the original estimate, so it is necessary to correct for this by adding the difference between the original statistic and the bootstrap mean, to the original estimate. The bias-corrected bootstrapped IAPE is thus calculated as:

Bias-corrected IAPE = Original IAPE + (Original IAPE- Mean Bootstrap IAPE)

The 95% confidence interval was calculated as:
95 % C.I. = Bias-corrected IAPE ± (1.96 Standard deviation of Mean Bootstrap IAPE)*

According to CAF protocol, a minimum of 25% of samples were re-read by the same reader. Precision estimates were compared with the acceptable level of agreement between readings (Morison et al., 1998). An age bias plot (Campana *et al* 1995) was used to indicate any systematic bias in the repeated age estimates. The distribution of differences between repeat readings was also inspected as another indicator of ageing error, and bias between readings.

RESULTS

Nemipterus theodorei
Transverse sections of *N. theodorei* displayed relatively clear increments from the primordium to the edge of either the dorsal or ventral sides (Figure 1) The relative position of the first increment when compared to the position of primordium varied considerably between samples. The average readability score was 2.2.

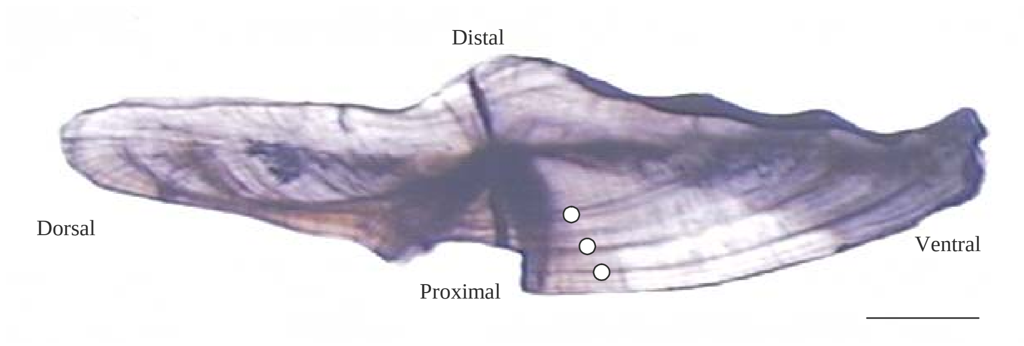

Figure 1. A transverse section of a *N. theodorei* otolith viewed with transmitted light. White circles indicate position of presumed annual increments. Scale bar =1mm

Age estimates were attained for all samples. Age classes from 0+ to 6+ years were present in the sample. The modal age class was 2+ with 97% of the entire sample less than 3 years (Figure 2).

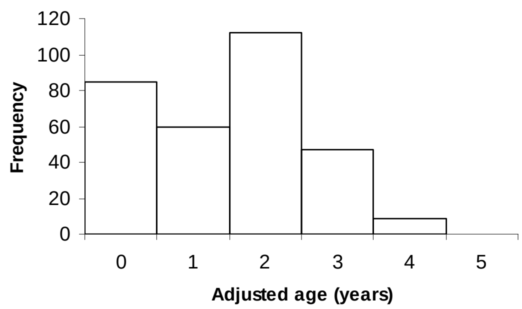

Figure 2. Age frequency distribution of *N. theodorei*. N=314

The distribution between otolith weight and month of capture is depicted in Figure 3. The figure indicates that juvenile fish with otolith weights approximately 0.01g were caught between September and February.

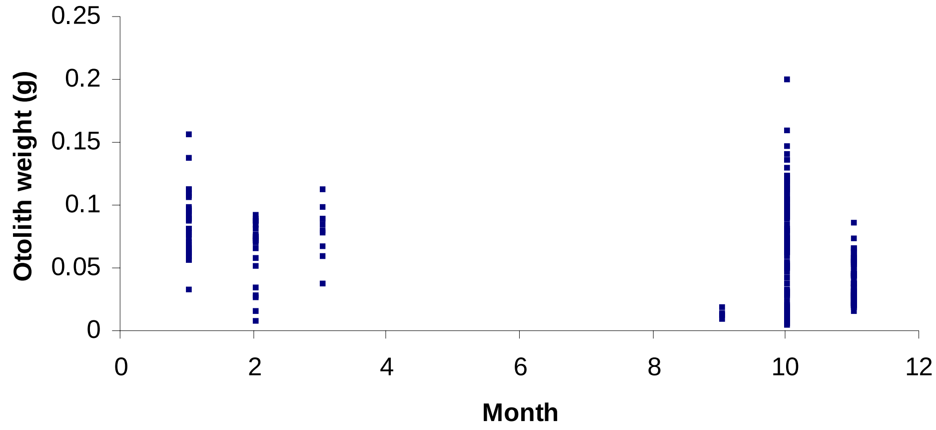

Figure 3. The distribution of otolith weight by month for *N. theodorei*.

A total of 80 preparations were re-aged to determine the level of precision surrounding the estimates. The bias corrected bootstrap mean IAPE was 3.63% (C.I. 1.53% – 5.74%). The distribution of differences between first and second readings is represented in Figure 4.

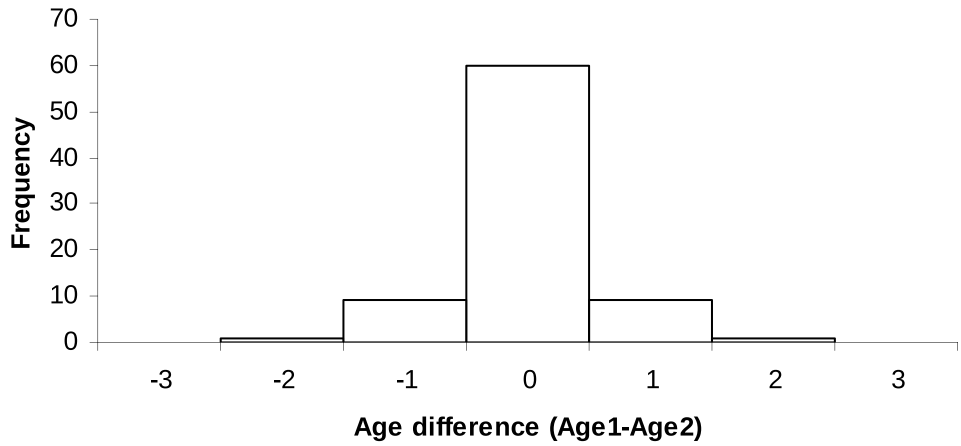

Figure 4. Distribution of differences between first and second age estimate on a sub-sample of 80 *N. theodorei* otoliths.

The level of agreement between first and second age estimates was high. Seventy-five per cent of second age estimates agreed with the first estimates obtained. Ninety-seven per cent were within one year and 100% were within two years (Table 2).

Table 2. Frequency of differences between repeated age estimates by first age estimate for *N. theodorei*

	Age1						
Age1-Age2	0	1	2	3	4	Total	%
-2		1				1	1%
-1	3	1	3	2		9	11%
0	**17**	**16**	**25**	**2**		**60**	**75%**
1		4	2	2	1	9	11%
2			1			1	1%
Total	20	22	31	6	1	80	

The relationship between otolith weight and adjusted fish age is Figure 5.

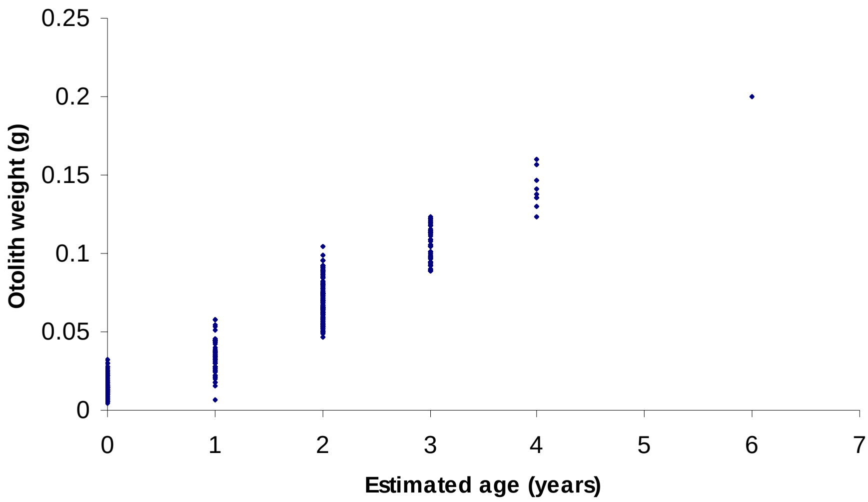

Figure 5. The relationship between adjusted fish age and otolith weight for *N. theodorei*.

Nemipterus aurifilum

Transversely sectioned otoliths from *N. aurifilum* were similar to *N. theodorei* in incremental clarity and morphological structure. Otolith structure and morphological characteristics are shown in Figure 6. This figure indicates a 'kink' in the sub-cupular meshwork fibre zone (SMF) and the change in direction of otolith growth along the ventral-distal surface used to help determine the position of the first increment. A feature known as the subcupular meshwork fibre zone has been useful for identifying the relative position of the first increment in snapper (Francis. et al. 1992), and can be seen in other species.

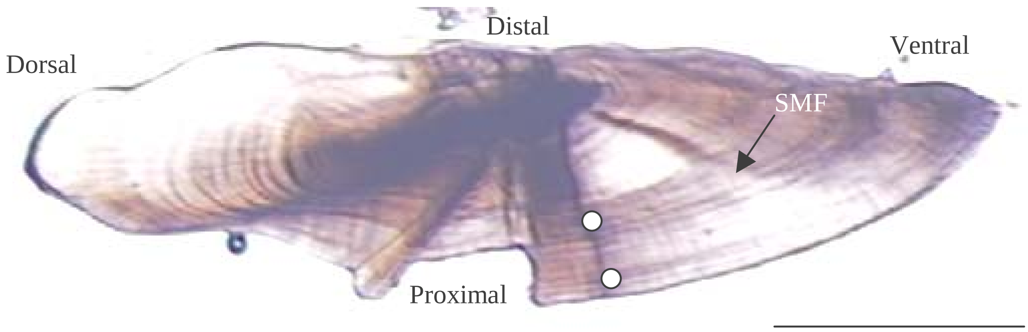

Figure 6. A transverse section of a *N. aurifilum* otolith viewed with transmitted light. White circles indicate position of presumed annual increments. Scale bar =1mm

Age was estimated for 98 % of the sample examined. Estimates for *N. aurifilum* ranged between 0+ and 4+ years. The age frequency distribution (Figure 7) indicates a modal age class of 1+ with the majority of the fish between 0+ and 2+ years.

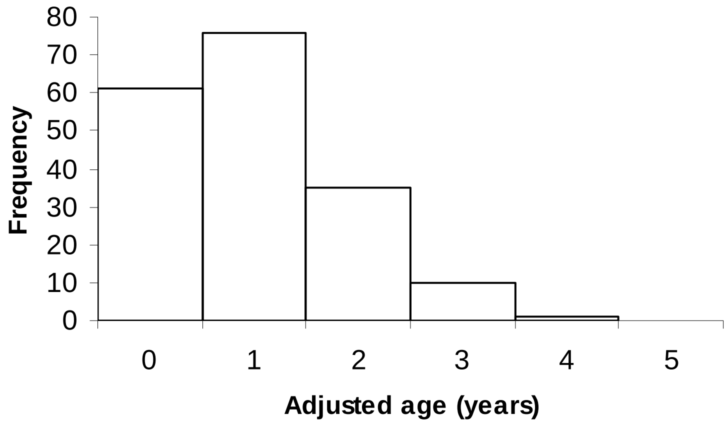

Figure 7. Age frequency distribution of *N. aurifilum*. N=183.

Figure 8. illustrates the distribution between otolith weight and month of capture. Samples were caught throughout the year with similar sized small otoliths (<0.01g otolith weight) represented. Since it was difficult to set a birthday for this species based on date of capture and spawning time, the birthday that was allocated to *N. theodorei* was used (1st September)

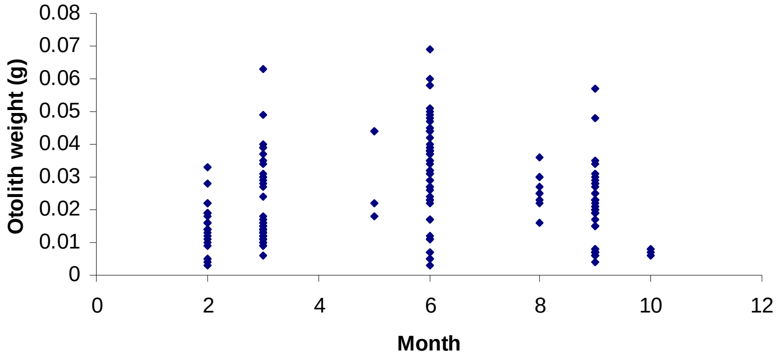

Figure 8. The distribution of otolith weight by month for *N. aurifilum*

A total of 54 *N. aurifilum* preparations were re-aged to determine the level of precision. The distribution of differences is illustrated in (Figure 9). Second age estimates agreed with the first estimates obtained for 78% of samples re-aged. All re-aged preparations were within one year. The bias corrected bootstrap mean IAPE was 5.09% (C.I. 2.01% – 8.18%).

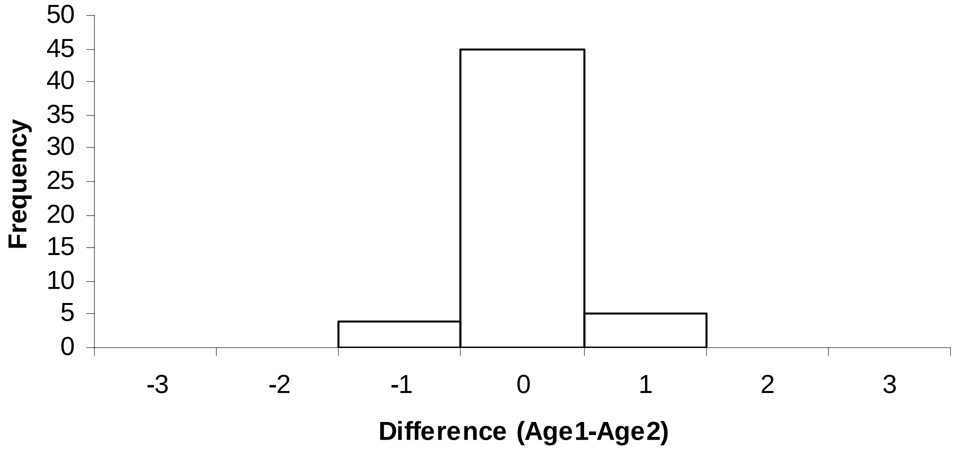

Figure 9. Distribution of differences between first and second age estimate on a sub-sample of 54 *N. aurifilum* otoliths.

Table 3. Frequency of differences between repeated age estimates by first age estimate for *N. aurifilum.*

	Age1					
Age1-Age2	0	1	2	3	Total	%
-1	3	3	1		7	13%
0	**19**	**15**	**7**	**1**	**42**	**78%**
1		5			5	9%
Total	22	23	8	1	54	

The relationship between otolith weight and adjusted and estimated fish age is depicted in Figure 10.

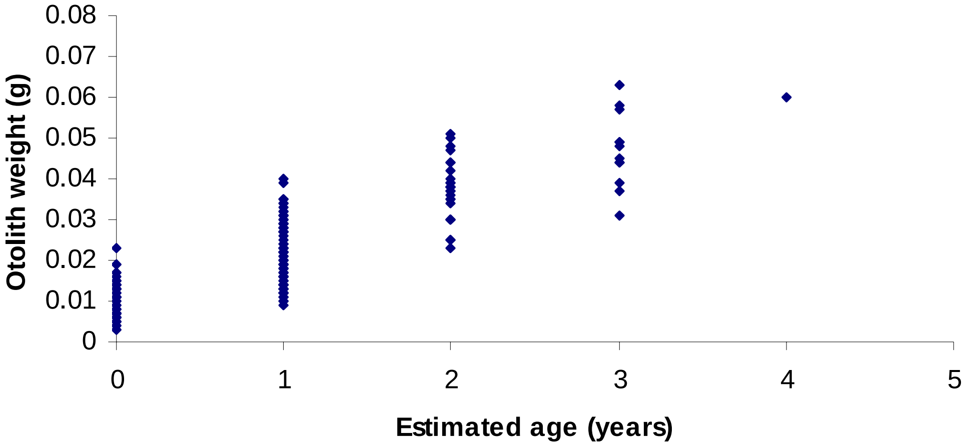

Figure 10. The relationship between estimated fish age and otolith weight for *N. aurifilum*.

DISCUSSION

This study indicates that *N. theodorei and N. aurifilum* are a relatively short-lived species. Ages are an estimate only and are un-validated for this species. Incremental structure and morphological characteristics were similar for both species. One of the major difficulties with ageing these species from thin otolith sections was determining the position of the first increment. The distance between the primordium and the first increment varied considerably between individual samples. For some preparations, the distance to the first increment was similar to the position of the second increment in other specimens. Assuming that increment formation occurs over a short time period (approximately 3 months), the relatively high distance from the first increment may suggest either a broad or multiple spawning period. Analysing the distribution between month of capture and otolith weight (Figure 3 and Figure 8) also supports this hypothesis. The distribution of fish with small otoliths (<0.01g, 0+ age class) extends over several months.

The distribution between otolith weight and age was relatively linear for both species. This suggests that unlike fish length, otoliths continue to grow as the fish age. Although a linear relationship clearly existed, the approximate otolith weight at each age class varied between species. *Nemipterus theodorei* exhibited a higher otolith mass growth rate compared to *N. aurifilum*.

The index of average per cent error (IAPE) was 3.6% and 5.06% and the average readability score was 2.22 and 2.4 for *N. theodorei and N. aurifilum* respectively. The otolith structure of *N. aurifuilum* was considered to be more complex than *N.theodori*. This was reflected in the higher IAPE and higher average readability score. Age difference plots and frequency distributions indicated that there was little evidence of a bias to over or under-estimate age for both these species.

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