Abstract: To estimate the live body length of marbled sole larvae from a preserved specimen, the notochord-length (NL) shrinkage due to preservation was investigated. In 5% formalin solution, significant shrinkage was observed in all NL classes after 30 days and 1.5 years preservation (5.2% and 6.3% in the 2.8-5.0 mm class; 3.3% and 3.8% in the 5.1-7.0 mm class; 3.8% and 4.1% in the 7.0-7.7 mm class, respectively). In 90% ethanol solution, significant shrinkage was observed only in the 2.8-5.0 mm NL class after both durations (4.9% and 6.3%, respectively). In both preservatives, the influence of preservation on shrinkage was stronger for small larvae than for large ones.

Key words: Marbled sole; Larva; Body shrinkage; Preservation

In the study of larval fish, accurate body-length data are indispensable for discussing size-distribution or growth, but the shrinkage of body length due to preservation must be noted. A correction factor accounting for shrinkage must be determined for each species, because the magnitude of shrinkage varies among species. Therefore, many studies have been reported about various species (e.g., Tucker and Chester; Yin and Blaxter; Hjörleifsson and Klein-MacPhee; Pepin et al.; Rosenthal et al.). Fukuhara examined red sea bream Chrysophrys major eggs, larvae, and juveniles preserved in 3, 5, and 10% formalin solutions, and found that the most shrinkage occurred during the first month of preservation. Thelacker reported that the relative shrinkage of northern anchovy Engraulis mordax larvae preserved in formalin solution is the same for all sizes, but in other species, it has been found that shrinkage is greater in smaller larvae. For flatfish larvae, only one study has examined the variation of shrinkage that occurs between size classes preserved in ethanol solution.

Marbled sole Pseudopleuronectes yokohamae (Günther) is a commercially important flatfish in Japan. Studies on the early life stages of this species in Hakodate Bay have examined the spatial and temporal distribution of the larvae and juveniles and feeding ecology. However, variations of shrinkage between size classes and between different preservatives have not been examined in the larva. In this study, we reared marbled sole larvae and investigated the shrinkage of body length in samples preserved in 5% formalin and in 90% ethanol solutions.

Adult marbled sole were captured in Hakodate Bay and transported to the Usujiri Fisheries Laboratory (the Field Science Center for Northern Biosphere, Hokkaido University) on 15 March 2001. Eggs from the fish were artificially fertilized by the dry method. The eggs and larvae were reared in 10 or more 5 I cylindrical tanks set in a 150 I water bath. The larvae were fed the rotifer Brachionus plicatilis (a mixture of L-type and S-type reared on chlorella and supplied by the Hokkaido Institute of Mariculture) during 3-20 days after hatch and newly hatched Artemia nauplii (originated in the Great Salt Lake, USA; Pacific Trading Co., Ltd.) from 15 days after hatch. Both rotifers and Artemia nauplii were enriched with PlusAquaran (BASF Japan Ltd.) before they were fed to the larvae. The mean water temperature was 16.0°C (range: 15.5-16.4°C) through the rearing period.

The larvae were sampled from rearing tanks with a metal spoon and anesthetized with a small amount of MS-222 (Ethyl m-Aminobenzoate Methanesulfonate) every few days. After anesthetization, the larvae were put on a slide glass and most water around each specimen was blotted. The notochord length (NL) was then measured under an objective microscope with a micrometer at 10x, 12.5x, 16x, and 20x magnification; the accuracy of measurement at each of these magnifications was ±0.05, 0.04, 0.03, and 0.02 mm, respectively. After measurement, specimens were preserved in 5% formalin solution or 90% ethanol solution individually, and the NL was re-measured under the same magnification used for live measurement 30 days and 1.5 years after the onset of preservation. Each period of preservation was representative of short and long term preservation. After preservation, some larvae exhibited a slight body curve. In those cases, the larvae were adhered to a slide glass and straightened using the method described in Hjörleifsson and Klein-MacPhee before they were measured. The shrinkage of the NL was analyzed using the t-test for paired comparisons that assumed the percent shrinkage was 0% (e.g. live NL - preserved NL). The difference in arcsine-transformed percent shrinkage among three size classes (≤5 mm, 5.1-7.0 mm, and >7 mm NL) was tested by one-way ANOVA. To look for trends in shrinkage through the whole NL range, the relationship between live NL and preserved NL was examined using linear-regression for each preservation period, and the difference in shrinkage between the two periods was analyzed by ANCOVA.

After 30 days preservation in 5% formalin solution, significant shrinkage was observed in all NL classes (Table 1; 3.3-5.2%), and the amount of shrinkage did not differ significantly among size classes (one-way ANOVA; P=0.07). After 1.5 years preservation, significant body shrinkage was observed in all classes, and mean body shrinkage differed significantly among the three size classes (one-way ANOVA; P=0.01). A previous study of body-length shrinkage of winter flounder Pleuronectes americanus preserved in formalin solution reported that the average shrinkage of the smallest size class larvae was 15.0% and decreased with increasing size to 11-12% in >5 mm larvae. In this study, a similar difference among size classes was observed in marbled sole larvae preserved in formalin solution after 1.5 years, and shrinkage was greater in the <5.0 mm NL class (6.5%) than in the larger NL classes (3.8% and 4.1%, Table 1). Because the intercepts of the original regression lines did not significantly differ from 0 (regression analysis; P=0.25 for 30 days and P=0.09 for 1.5 years), regression lines of preserved NL on live NL without intercepts were calculated (30 days: Preserved NL=0.861 live NL, P<0.001; 1.5 years: Preserved NL=0.958 live NL, P<0.001; Fig. 1). The slopes of these lines differed significantly (ANCOVA; P=0.01), indicating that shrinkage was greater after 1.5 years preservation than after 30 days, and suggesting that shrinkage continues after more than 30 days in 5% formalin. After preservation in 90% ethanol for both 30 days and 1.5 years, mean body shrinkage differed significantly among the three size classes (one-way ANOVA; P<0.001; Table 1), and significant body shrinkage was observed only in the ≤5 mm NL class (P<0.001). The regression lines were estimated as Preserved NL=1.06 live NL-0.37 for 30 days (P<0.001; Fig. 2) and Preserved NL=1.05 live NL-0.41 for 1.5 years (P=0.001). Neither the intercepts nor slopes differed significantly

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decalcification16). In 90% ethanol solution, little shrinkage
References

Table 1. Results of body shrinkage of marbled sole P. yokohamae larvae after 30 days and 1.5 years preservation

<table>
<thead>
<tr>
<th>Preservative</th>
<th>Duration of preservation</th>
<th>Size class</th>
<th>Number of specimens</th>
<th>Mean body shrinkage and SE (%)</th>
<th>t-test for the hypothesis that no shrinkage occurred</th>
<th>Significance of one-way ANOVA among size classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% formalin solution</td>
<td>30 days</td>
<td>2.8-5.0mmNL</td>
<td>13</td>
<td>5.2±0.66</td>
<td>P=0.001</td>
<td>P=0.07</td>
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<tr>
<td></td>
<td>1.5 years</td>
<td>5.1-7.0mmNL</td>
<td>10</td>
<td>3.3±0.49</td>
<td>P=0.001</td>
<td>P=0.001</td>
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<tr>
<td></td>
<td></td>
<td>7.1-7.7mmNL</td>
<td>14</td>
<td>3.8±0.4</td>
<td>P=0.001</td>
<td>P=0.001</td>
</tr>
<tr>
<td></td>
<td>5% formalin solution</td>
<td>30 days</td>
<td>2.8-5.0mmNL</td>
<td>11</td>
<td>6.3±0.61</td>
<td>P=0.001</td>
</tr>
<tr>
<td></td>
<td>1.5 years</td>
<td>5.1-7.0mmNL</td>
<td>10</td>
<td>3.8±0.61</td>
<td>P=0.004</td>
<td>P&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td>7.1-7.7mmNL</td>
<td>13</td>
<td>4.1±0.44</td>
<td>P=0.001</td>
<td>P=0.001</td>
</tr>
<tr>
<td>90% ethanol solution</td>
<td>30 days</td>
<td>2.8-5.0mmNL</td>
<td>30</td>
<td>4.9±0.63</td>
<td>P=0.001</td>
<td>P=0.001</td>
</tr>
<tr>
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<td>1.5 years</td>
<td>5.1-7.0mmNL</td>
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<td>0.5±1.02</td>
<td>P=0.62</td>
<td>P=0.001</td>
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<td></td>
<td>7.1-8.7mmNL</td>
<td>10</td>
<td>1.1±0.7</td>
<td>P=0.14</td>
<td>P=0.001</td>
</tr>
</tbody>
</table>

Fig. 1. Relationship between live notochord length (NL) and preserved notochord length (Fig. 2). (ANCOVA; P=0.051 and P=0.77, respectively). Thus, the shrinkage after 1.5 years did not differ from that after 30 days in 90% ethanol preservation, and we concluded that body shrinkage was quite small after 30 days. Hjörleifsson and Klein-MacPhee reported that preservation in ethanol causes significant body shrinkage in small (<5 mm) P. americanus larvae but not in larger (>5 mm) larvae. In this study, a similar phenomenon was observed. A likely cause of shrinkage is osmotic water loss from larvae. Since the water content of larvae decreases with age and length, it is expected that smaller larvae will shrink relatively more than larger larvae.

Preservation in formalin solution is widely used in ichthyoplankton investigations, but specimens preserved in formalin solution cannot be used in otolith studies because of decalcification. In 90% ethanol solution, little shrinkage occurred in larvae >5 mm NL, suggesting that ethanol is a better overall preservative, particularly for larger fish larvae.

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