INTRODUCTION

It was discovered that two groups of pond smelt Hypomesus nipponensis coexist in Lake Ogawara,1,2 where egg transplantation of pond smelt has not been conducted at any time. Microstructural and microchemical analyses of otoliths revealed that the large and small fish demonstrate anadromous and resident life history styles.3,4 No significant differences in genetic characteristics were detected between the two groups.1 It is known that pond smelt hatch during late April to late May, and the seaward migration of anadromous fish occurs in June–August.3,4 However, the mechanism of divergence of the resident and anadromous fish remains unknown. This is because hatching locality and periodicity and the early growth and development of the sea-run migrants and residents have yet to be explored.

To examine the divergence mechanism, the present study aimed to disclose: (i) the life history style of spawners caught from the heterogeneous spawning grounds that we found previously in the lake and its inflowing rivers;1,2 and (ii) the size, dry weight, and water content of eggs and fecundity of resident and anadromous fish. This information will help us to understand the relationship between the co-occurrence of the two groups and their population dynamics, reproductive isolation, and contribution to the next generation.

MATERIALS AND METHODS

Sample collection

Lake Ogawara is a brackish lake located on the Pacific side of northernmost Honshu, Japan, and covers an area of 63.3 km² (Fig. 1). The northern
Pond smelt is a semelparous fish that sheds once and whose oocytes display a normal distribution. The maturational stages of pond smelt ovaries can be identified by observing their external color and separativeness which varies for each stage. Mature ovaries containing hydrated oocytes were characterized by their translucent exterior, and unseparated oocytes were identified after fixation in formalin. Post-spawning ovaries were thread-like and contained empty follicles. Ovaries under spawning were identified by their partial shedding.

A total of 146 mature fish and 46 spawning fish were caught. All mature ovaries were used for egg analyses. The sagittae of 46 randomly resampled mature fish and all 46 spawning fish were removed to estimate growth and to discriminate their life history style. Thus, 46 mature females were used to compare the egg characteristics between anadromous and resident fish, and 46 spawning females were used to examine the relationship between life history styles and spawning grounds.

Discrimination of life history style

Sagittae were mounted in polyethylene and ground from both the anterior and posterior edges to reveal a sagittal section through the core. Daily increments were counted and radii were measured along a transect from the core to the dorsal edge. Radii of otolith and increments were measured with an image processing analysis system (Quantimet 600; Leica Cambridge Ltd, Cambridge, UK) at Tohoku University, Japan. Body lengths were back-calculated by regression. Measurements of otolith radii from the core to the dorsal edge of specific increments were converted to fish size according to a second-order polynomial fit of the relationship between standard length \( Y, \text{mm} \) and otolith width \( X, \text{mm} \):

\[
Y = 3.23X^2 + 33.59X + 3.66.
\]

Specific mean growth rates (body length increase in mm/day) for fish aged 40–100 days were calculated. The bimodal frequency distribution of the mean growth rates was divided into two \( \chi^2 = 12.78, P < 0.05; \text{Fig. 2} \) by a method for analyzing polymodal frequency distributions as normal distributions, which was proposed by Tanaka and Akamine. Based on Mahalanobis' generalized distance, pond smelt having a mean growth rate of >0.426 mm/day were considered anadromous and those with a mean growth rate of <0.426 mm/day were considered resident. Average specific mean growth rates ± SD were 0.520 ± 0.057 mm/day \( (n = 28) \) for anadromous fish and 0.329 ± 0.058 mm/day \( (n = 64) \) for resident fish. Their SLs were 76.8 ± 11.3 mm and 53.2 ± 8.57 mm, respectively, and have a discriminant function of 63.8 mm.
Egg measurements

Mature ovaries were taken out, weighed, and placed in 5% neutralized formalin for several weeks. Fixation in formalin causes a reduction in the dry weight and diameter of eggs. Egg diameters and dry weights were measured under these conditions a few weeks after sampling.

The ovaries were weighed to the nearest 0.1 mg on an analytical balance. We weighed and counted oocytes that were subsampled again from the central part of the previously subsampled and fixed ovary, which showed a smaller egg diameter variance than other parts. More than 200 hydrated oocytes were retaken, and their diameters were measured. Because of the possibility of deformation by fixation, the equivalent circle diameter was estimated with the image processing analysis system (Quantimet 600; Leica). The specific mean diameter of hydrated oocytes was used as the egg diameter ($MD$). The fecundity ($FC$) of pond smelt is equal to the total number of oocytes in the left and right leaves, because the fish is semelparous and spawns almost all eggs in the one spawning run. Fecundity was estimated by a gravimetric method. Counts from subsamples were standardized to the total ovary weight.

The residual ovaries, from which the subsamples were removed, were weighed and then dried at 120°C for 24 h for reweighing. The water content (WC) was derived from the formula $(1 – \text{dry/wet weight}) \times 100\%$. The dry weight of the whole ovary was divided by $FC$ to estimate the mean dry weight per oocyte ($DW$).

Egg characteristics and $FC$ were related to body length through a regression analysis. The inflection point of the FC–SL relationship was detected using Akaike’s Information Criterion. Using Student’s $t$-test, egg characteristics were compared between resident and anadromous fish, which were discriminated by otolith analysis.

RESULTS

Life history styles of spawners in the lake and inflowing river

Figure 3 shows the compositions of the various gonadal maturation stages of pond smelt that were caught from the spawning grounds of Lake Ogawara and Sadoro River. Compositions are presented for three classes of body lengths: $SL > 80\, \text{mm}$, $SL < 60\, \text{mm}$, and $60\, \text{mm} < SL < 80\, \text{mm}$, which correspond closely to anadromous, resident, and a mixture, respectively. Fish $< 60\, \text{mm}$ were not caught in the river, but all body length classes were present in the lake. Males dominated, and spent fish were of the majority in the lake. Spawning females covered all body length classes: two females $> 80\, \text{mm} SL$, 29 females $60–80\, \text{mm} SL$, and nine females $< 60\, \text{mm} SL$. In the river, $> 80\%$ of fish were male. Spawning females numbered five $> 80\, \text{mm} SL$ and one $60–80\, \text{mm} SL$.

Compositions of the life history style of spawning females in the lake and river are shown in Table 1. Spawners in the lake consisted of resident and anadromous females, although there were more resident fish. Only anadromous spawners were found in the river.

<table>
<thead>
<tr>
<th>Spawning ground</th>
<th>Life history style</th>
<th>Resident</th>
<th>Anadromous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Ogawara</td>
<td></td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>Sadoro River</td>
<td></td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1 Frequency occurrence of the life history style of spawning female pond smelt caught from the spawning grounds of Lake Ogawara and Sadoro River

Egg characteristics

The relationship of log-transformed $FC$ to log-transformed $SL$ had an inflection point at $63.8\, \text{mm}$ (Fig. 4). Data conformed well to two linear regressions: $FC = 1.13 \times 10^{-4} \times SL^{4.66}$ ($n = 78$, $r = 0.886$, $P<0.01$) for $40\, \text{mm} < SL < 63.8\, \text{mm}$, and $FC = 2.16 \times 10^{-3} \times SL^{3.39}$ ($n = 68$, $r = 0.822$, $P<0.01$) for $63.8\, \text{mm} < SL < 100\, \text{mm}$.

Figure 5 shows that $MD$ (mm), $DW$ (mg), and WC...
dent and anadromous females. No significant difference was detected for MD. The DW of resident fish was lower and WC was higher than those of anadromous fish (Table 2).

**DISCUSSION**

The composition of life history styles of spawning female pond smelts in the Lake Ogawara indicated a mixture of anadromous and resident fish. This suggests that anadromous and resident spawning groups share a common spawning ground. These fish spawn during almost the same period, from mid March to early May.8 Spawning locality and periodicity are not differentiated between the two groups, which suggests that reproductive isolation
does not occur. This also indicates that the anadromous and resident life history styles are ecological variations in a single population. In contrast, only anadromous fish spawned in the inflowing river. In other lakes of Japan, spawning grounds are formed in lakes or inflowing rivers, varying among lake; however, the selection mechanism of spawning grounds is yet to be studied.

Several variations in egg characteristics have been reported. Egg size has been found to increase with female size, and with age, and even to decrease with female size, but not to vary with body length. For pond smelt in Lake Ogawara, although the mean egg diameter increased slightly with female body length, no significant difference was detected between the anadromous and resident fish. However, the DW of anadromous females was obviously greater and the WC was lower than those of resident females. Several pieces of evidence suggest that the dominant effect of salinity on egg size arises during oogenesis. The mechanism responsible for the effect of salinity on egg size follows from the fact that the pre-ovulated eggs are more permeable to water and salts than ovulated hydrated eggs. However, the anadromous pond smelt of Lake Ogawara grow somatically and sexually in the adjacent sea and then migrate to the lake, where they quickly accelerate their final maturation stages and the hydrogenation of oocytes. Both the anadromous and resident females complete their final maturation and hydrogenation in the lake. Therefore, salinity seems to have little influence on the differences in egg characteristics between them. In addition, the relationship between FC and SL was discontinuous, with an inflection point at 63.8 mm SL, which coincides
Table 2  Comparison of the mean diameter, dry weight, and water content of eggs between resident and anadromous fish (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Resident fish</th>
<th>Anadromous fish</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Egg diameter (mm)</td>
<td>0.800±0.068</td>
<td>0.807±0.061</td>
<td>0.068 &gt; P (0.05)</td>
</tr>
<tr>
<td>Dry weight (mg)</td>
<td>0.0467±0.0062</td>
<td>0.0517±0.0100</td>
<td>P (0.01) &lt; 2.04 &lt; P (0.05)</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>75.0 ± 1.4</td>
<td>72.5 ± 1.4</td>
<td>5.49 &lt; P (0.01)</td>
</tr>
</tbody>
</table>

with the discrimination of large anadromous spawners from small resident spawners only. This relationship shows that the large anadromous female has a lower FC at a given body size than a small resident female. These results suggest that the anadromous fish would value egg quality more than egg quantity in reproductive allocation. In general, water content is known to be inversely related to the lipid content in fish.26 Egg characteristics influence larval size, mortality, activity, feeding, the swim bladder, and growth.27–31 The differences between anadromous and resident pond smelt in the DW and WC of eggs may influence larval condition, mortality, and the growth of their progenies.

Sea-run migration occurs mainly in June–August at 40–80 days of age.3 Unlike Arctic char and masu salmon, body size would be responsible for the divergence mechanism of resident and anadromous pond smelt.4 There were no differences in the spawning grounds of Lake Ogawara between resident and anadromous pond smelt, so hatching area seems not to be a conclusive factor in the life history style.

The differences in egg characteristics described in the present study may provide reasons for the variations in the developmental processes of their progenies up to anadromous migration. To determine the divergence mechanism between the progenies of sea-run migrants and lake residents, early growth records of both anadromous and resident pond smelts need to be examined and compared.

ACKNOWLEDGMENTS

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REFERENCES

5. Sato R. Biological observation on the pond smelt, Hypomesus olidus (PALLAS), in Lake Ogawara, Aomori Pref., Japan.


