1977

The use of discriminate function analysis in the identification of two species of larval smelt, Spirinchus thaleichthys and Hypomesus T. transpacificus, in the Sacramento-San Joaquin estuary, California: a thesis ...

Marilou Simonsen

University of the Pacific

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THE USE OF DISCRIMINATE FUNCTION ANALYSIS IN THE IDENTIFICATION OF TWO SPECIES OF LARVAL SMELT, SPIRINCHUS THALEICHTHYS AND HYPOMESUS T. TRANSPACIFICUS, IN THE SACRAMENTO-SAN JOAQUIN ESTUARY, CALIFORNIA

A Thesis
Presented to
the Graduate Faculty of the University of the Pacific

In Partial Fulfillment of the Requirements for the Degree Master of Science

by
Marilou Simonsen
May 1977
This thesis, written and submitted by

Marilou Simonsen

is approved for recommendation to the Committee on Graduate Studies, University of the Pacific.

Department Chairman or Dean:

F. B. Hunter

Thesis Committee:

Lee Christianson Chairman

Joe W. Miller

John E. Tucker

Dated 3 May, 1977
ACKNOWLEDGEMENTS

I would like to offer my sincere thanks to Lee Miller who helped me to initiate this investigation and whose guidance, encouragement and helpful criticism enabled me to complete the study. I wish to thank the California Department of Fish and Game, Bay-Delta Studies, in Stockton, California and especially Jim Orsi of the Neomysis Study for the use of their facilities and data, without which this project would have been impossible. I am indebted to John Horton of the California Department of Fish and Game and Dr. David Hughes for their help in computer programming and data analyzing.

I also wish to express my sincere appreciation to Dr. Lee Christianson for his invaluable time and assistance. I'm very appreciative to Sid Cook for his work on the illustrations. Dr. John Tucker made valuable suggestions too for which I am grateful. Thanks also to my parents and family for their constant encouragement and support.
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<td>b. <em>Hypomesus t. transpacificus</em> 10.4 mm</td>
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<td>28</td>
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INTRODUCTION

The proper identification of the larval stages of fish is important for gaining an understanding of fish populations in any region. However, there are few complete and comprehensive reports available on this subject. Eggs, larvae and postlarvae of many species of fish are undescribed or only partially described. Work on the early stages of marine fish has been done by Bigelow and Schroeder (1953), Alexander (1961), Mansueti (1962), Wade (1962), Mansueti and Hardy (1967), Moser and Ahlstrom (1970) and Lipps and Moran (1974). The developmental stages of many species of fresh water fish have been described also by Adams and Hankinson (1928), Fish (1932) and Taber (1969) among others.

Distributional and ecological studies of larval fish have sometimes been neglected because of the high cost of collecting samples and the difficulty of identifying larval fish. The number of these studies are now increasing because of the need for better information about fish populations due to environmental concerns.

In the Sacramento-San Joaquin Estuary no comprehensive study of early life history stages of fish has been done. An annual survey to determine the number of eggs and
larval striped bass during spring has been conducted by the California Department of Fish and Game since 1966 though. Striped bass eggs and larvae are the principle objects of the survey but other species are collected. Additional information that would make possible the accurate identification of the larval stages of other species would increase the knowledge gained from these surveys.

I chose to work with smelt (Family Osmeridae) because: 1) only two species spawn in the Sacramento-San Joaquin Estuary which somewhat simplifies identification, 2) they are relatively abundant and are easily obtainable with the sampling gear in use, and 3) relatively little is published about the early developmental stages of either species of fish.

McAllister (1963) published a monograph on smelt systematics but restricted it to the adult forms. Dryfoos (1965) and Moulton (1970) studied the life history of *Spirinchus thaleichthys* (Ayres) in Lake Washington but only briefly described the larval stages.

*Hypomesus transpacificus* transpacificus (McAllister) is found only in the Sacramento-San Joaquin Estuary and no life history study describing the larval stages has been published.

The objectives of this study are to identify the larval forms of the two species of smelt, *Spirinchus thaleichthys* and *Hypomesus transpacificus transpacificus*,...
and to better describe their spawning times in the Sacramento-San Joaquin Estuary.

Sacramento-San Joaquin Estuary Study Area

The Sacramento-San Joaquin Estuary is formed by the confluence of the Sacramento and the San Joaquin Rivers and their tributaries. The rivers vary in width from approximately 60 m to over 1.6 km wide. Depths vary from 3 m in some channels to 12 m in most channels with depths greater than 30 m in the Carquinez Straits. The Delta portion of the Estuary, east of Antioch, CA., is comprised of nearly 1127 km of anastomosing channels (Turner, 1966) created from marshland by constructing levees in the early 1900s (Fig. 1). Below the junction of the Sacramento and San Joaquin Rivers the water flows through Suisun Bay and San Pablo Bay before entering San Francisco Bay.

Outflow in the Estuary is determined by precipitation, snow melt and diversion rates. Diversions for irrigation and urban uses above the Delta have decreased the flow in the San Joaquin and Mokelumne Rivers (Kelley, 1966). Two large pumping plants near Tracy, CA. export water from the Delta to the central and southern portions of the state. One plant is operated by the Central Valley Project of the U.S. Bureau of Reclamation and the other by the State of California. These exports have decreased outflow an average of 34% in June and 35% in July since 1959 and, at times, have reversed the flow of water in the lower San Joaquin River and in the Old and Middle Rivers (Chadwick, Stevens
and Miller, M. S.).

In all but the dead-end sloughs fairly strong currents occur. Velocities of 0.75 m/sec on the ebb flow are common (Kelley, 1966). Tides also affect the flows and water levels, with a mean tide change of 0.6 to 1.0 m (Turner, 1966). At mean lower low tide 36% of Suisun Bay is under less than one meter of water (Kelley, 1966). Water may move 13 km downstream and back again in one tidal phase (Kelley, 1966). This tidal excursion can result in drastic environmental changes at a single location.

Although the Delta is virtually freshwater, there is about an 80 km salinity gradient from sea to freshwater starting at San Pablo Bay (Turner and Chadwick, 1972) or San Francisco Bay (Chadwick, 1964) depending on the outflow. Salinity seldom exceeds 1,000 ppm chlorides beyond a few kilometers above the junction of the Sacramento and the San Joaquin Rivers (Chadwick, 1964). Water released from dams above the Delta can repel the intrusion of saline water into the Delta.

There is hardly any stratification in the freshwater areas of the Delta due to tidal mixing and winds. Water temperatures, dissolved oxygen and conductivity change little with depth (Kelley, 1966). The waters are very turbid though, with Secchi disk readings seldom over 60 cm.

Many native fish populations in the Delta have declined due to alterations of their habitat or over-fishing. Some native fish surviving today are white sturgeon,
Acipenser transmontanus; green sturgeon, A. medirostris; Sacramento perch, Archoplites interruptus; Pacific lamprey, Entosphenus tridentatus; chum salmon, Oncorhynchus keta; king salmon, O. tshawytscha; coho salmon, O. kisutch; steelhead trout, Salmo g. gairdnerii; longfin smelt, Spirinchus thaleichthys; and delta smelt, Hypomesus transpacificus transpacificus. Many species of fish have been introduced to supplement the native populations too. Such introductions include striped bass, Roccus saxatilis; American shad, Alosa sapidissima; white catfish, Ictalurus catus; brown bullheads, I. nebulosus; black crappie, Pomoxis nigromaculatus; bluegill, Lepomis macrochirus; largemouth bass, Micropterus salmoides; and threadfin shad, Dorosoma petenense (Skinner, 1972). There are now over 150 species of fish in the Delta (Central Pacific Basins, 1967) and almost half of California's anadromous fish populations spend some part of their life in the Delta (Skinner, 1972). Some of the more important ones are salmon, Oncorhynchus sp.; steelhead trout, Salmo g. gairdnerii; American shad, A. sapidissima; striped bass, R. saxatilis; longfin smelt, S. thaleichthys and delta smelt, Hypomesus t. transpacificus (Central Pacific Basins, 1967).
METHODS

Field Procedures

Sampling in the field was done in conjunction with the Neomysis study of the California Department of Fish and Game in Stockton, CA. The purpose of their study is to index the numbers of Neomysis, primarily *N. mercedis*, since they are an important food source for many fish including smelt. Neomysis abundance is measured by sampling at forty-seven stations located from east of the Martinez-Benicia Bridge to near Rio Vista on the Sacramento River and to Stockton on the San Joaquin River. Other stations were sampled in the south Delta (Fig. 1). Stations were sampled once during every survey at slack water of the higher high tide in the order shown in Table 1.

I used the data collected in nine of the 1976 Neomysis surveys with the first starting on March 19, 1976. Each survey required five days of sampling. Bimonthly surveys were done in April, May, June and July (Table 2). Few smelt were caught after July, apparently because they were large enough to avoid the net. The surveys were conducted monthly during the winter months, November, 1976, through February, 1977. Smelt appeared again in the January and February surveys so the data from these two surveys were included with the 1976 data.

Tows were made with a conical two net attached to a steel towing frame. The net length was 1.48 m with a mouth
Table 1. The Order of Neomysis Stations Sampled During Each Survey

<table>
<thead>
<tr>
<th>First Day</th>
<th>Second Day</th>
<th>Third Day</th>
<th>Fourth Day</th>
<th>Fifth Day</th>
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<tr>
<td>98</td>
<td>78</td>
<td>62</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>C9</td>
<td>80</td>
<td>64</td>
<td>22</td>
<td>34</td>
</tr>
<tr>
<td>D28A</td>
<td>82</td>
<td>66</td>
<td>24</td>
<td>42</td>
</tr>
<tr>
<td>MD7</td>
<td>84</td>
<td>68</td>
<td>28</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>72</td>
<td>30</td>
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<tr>
<td></td>
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<td>90</td>
<td>76</td>
<td></td>
<td>36</td>
<td>50</td>
</tr>
<tr>
<td>92</td>
<td>D11</td>
<td></td>
<td>38</td>
<td>52</td>
</tr>
<tr>
<td>104</td>
<td>D14</td>
<td></td>
<td>40</td>
<td>54</td>
</tr>
<tr>
<td>D15</td>
<td></td>
<td>S42</td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>D19</td>
<td></td>
<td>MD10</td>
<td></td>
<td>58</td>
</tr>
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<td></td>
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Table 2. Dates of the Neomysis Surveys of 1976-1977

<table>
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<tr>
<td>1</td>
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<td>2</td>
<td>April 5, 6, 7, 8, 9</td>
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<td>3</td>
<td>April 19, 20, 21, 22, 23</td>
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<td>May 4, 5, 6, 7, 10</td>
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<td>5</td>
<td>May 17, 18, 19, 20, 21</td>
</tr>
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<td>6</td>
<td>June 2, 3, 4, 8, 9</td>
</tr>
<tr>
<td>7</td>
<td>June 16, 18, 21, 22, 23</td>
</tr>
<tr>
<td>8</td>
<td>July 1, 2, 5, 6, 7</td>
</tr>
<tr>
<td>9</td>
<td>July 15, 16, 19, 20, 21</td>
</tr>
<tr>
<td>10</td>
<td>Jan. 14, 17, 18</td>
</tr>
<tr>
<td>11</td>
<td>Feb. 14, 15, 16</td>
</tr>
</tbody>
</table>
area of 0.065 m$^3$. The mesh size was .505 mm.

The depth was measured with a fathometer. Depths ranged from 3.0 to 12.5 m. Diagonal bottom to surface tows were made by letting out suitable cable length for the depth at each station. The ten minute towing time was divided into the appropriate time intervals for retrieving the cable in ten foot segments. The engine was kept at about 1000 RPM to maintain a boat speed of 0.7 m/sec.

A digital flow meter (General Oceanics, Inc. M.20301) was mounted on the net to measure the amount of water sampled. The collecting bucket was a polyethylene jar with one side screened with stainless steel bolting cloth of the same mesh as the net.

After retrieval the sample was preserved with 10% formalin and stored in a one liter sample bottle. Rose bengal dye was used to stain the specimens.

**Laboratory Procedures**

Specimens were sorted in the lab according to station and date. From this collection specimens in good condition and no marked body curvature were selected for morphometric measurements. An ocular micrometer and dissecting scope were used to obtain these measurements. The characteristics chosen were those found most useful in identifying the larvae in the preliminary identification. Measurements were made to the nearest 0.1 mm except for eye width which was measured to the nearest 0.01 mm.
The twelve measurements taken are defined as follows with the first seven taken from Moser and Ahlstrom (1970):

Body length = in early-stage larvae and in those larvae undergoing notochord flexion it is the distance from the tip of the snout to the tip of the notochord. After the notochord is fully flexed and the caudal fin is formed the usual standard length is measured, i.e., the distance from the tip of the snout to the posterior margin of the hypural elements.

Snout to anus = distance along the midline of the body from the tip of the snout to a vertical from the anus.

Interorbital width = width of the fleshy tissue dorsal to the eyes.

Body depth = measured just posterior to the pectoral fins.

Eye width = maximum width of the pigmented portion of the eye in an horizontal plane.

Snout to anal fin = distance along the midline of the body from the tip of the snout to a vertical from the anterior end of the anal fin.

Snout to dorsal fin = distance along the midline of the body from the tip of the snout to a vertical from the anterior end of the dorsal fin.

Snout to pelvic fin = distance along the midline of the body from the tip of the snout to a vertical
from the anterior end of the pelvic fin.
Gut length = length of the gut from the anterior point of the pectoral fin to the anus.
Gas bladder width = the maximum width of the gas bladder.
Gas bladder depth = the maximum depth of the gas bladder.
Gas bladder to pelvic fin = the distance from the anterior end of the gas bladder to the anterior end of the pelvic fin.

Fry (1973) reported Spirinchus migrating into the Sacramento-San Joaquin Estuary in winter and spawning from midwinter to early spring. Hypomesus is reported to spawn in late winter and spring. Based on this information I selected smelt from the beginning of the year and from late spring and compared them. By working from the larger, more easily identified fish backwards to the smaller, unknown fish, I was able to identify the smaller smelt and describe the characteristics that I used to do so. Spirinchus was found in the first surveys and were later replaced by Hypomesus. Although overlap in their spawning times did occur there was enough separation to assure getting just Spirinchus at the beginning of the year and just Hypomesus of 20 mm or less in late spring.

Twelve characteristics were measured on the identified fish and a discriminant analysis was run on Burroughs B6700 at the University of California at Davis using the
stepwise discriminant analysis computer program (Nie et al., 1975).

The discriminant analysis separates two or more groups that cannot be distinguished by a single variable by combining two or more variables into a discriminant function. The discriminant functions are calculated to maximize the ratio of the variance between species to that within species. Each variable is weighted by the discriminant function coefficient according to how good a discriminator it is. The absolute value is a measure of the relative discriminatory power of the variable. The higher the absolute value of the discriminant function coefficient the better a discriminator it is.

The discriminant scores (D) are computed as follows:

\[ D = a_1 \text{var}_1 + a_2 \text{var}_2 + \cdots + a_n \text{var}_n + \text{constant} \]

for \( n \) variables. \( \text{var}_1 \) through \( \text{var}_n \) are the raw data scores for the characteristics 1 through \( n \) and \( a_1 \) through \( a_n \) are the associated discriminant function coefficients.

For a more detailed description of discriminant analysis see Rao (1952), Groves (1963) or Blackith and Reyment (1971).

Because some of the characteristics measured were not present on all sizes of fish, some specimens lacked a complete set of measurements. Since the computer will delete any specimen with missing data in the discriminant analysis, running the program with all the specimens either greatly reduced the number of characteristics available to
those common to all the fish, or restricted the analysis to larger fish that had a complete set of characteristics. For example, when distances from the snout to dorsal and anal fins were included, only **Spirinchus** larva 12 mm and larger could be used while **Hypomesus** larva of 9 mm and larger were used. When gas bladder measurements, width and depth, were used only **Hypomesus** of 15, 16 or even 17 mm could be included since their gas bladders were smaller, developed at a slower rate and, hence, were not measureable until a larger size. Thus, in some of these runs the smallest larvae, from 6 to 8 mm, were dropped by the computer making identification of the remaining fish easier due to their larger size.

To circumvent this problem of missing measurements, specimens were arranged into five size groups each having a complete set of data for its grouping. The first size group of 6 to 8 mm larvae were tested using body length, snout to anus length, interorbital width, body depth, eye width and gut length. The second group, 9 to 10 mm larvae, had snout to anal fin length added to the above characteristics. The 11 to 12 mm group included snout to dorsal fin length as well as all the characteristics mentioned above. The fourth group, 13 through 16 mm larvae, added gas bladder width and depth to the list. The final group, 17 to 25 mm larvae, were analyzed using all twelve characteristics.
RESULTS AND DISCUSSION

Spawning Times and Larval Distribution

Longfin smelt, Spirinchus thaleichthys, were collected in the Neomysis tows from January through May. Taking into account the size of the larvae, I would estimate that they spawn from late December through April. Delta smelt, Hypomesus t. transpacificus, were caught from April through July with their spawning occurring from April to June. These results agree with Fry (1973) who reported longfin smelt spawning from midwinter to early spring and delta smelt spawning in late winter and spring.

Not only were the spawning times different but the distribution of the larvae differed too. Spirinchus were consistently found further downstream in more saline water. Eight millimeter larvae were caught off Antioch and Pittsburg and below, whereas only one Hypomesus was ever found below the junction of the Sacramento and San Joaquin Rivers and it was 20 mm long. As larvae, Hypomesus appear to remain in fresher water longer than Spirinchus, which are more tolerant of saltwater. This is consistent with the differences in environments of the adult forms since Hypomesus remain in brackish water whereas Spirinchus migrate to the ocean (McAllister, 1963).

Discriminant Function Analysis

The results of the discriminant function analysis are presented in Tables 3 to 11. Only those characteristics found
Table 3. Results of the Discriminant Function Analysis of Interorbital Width and Eye Width of Smelt Larvae (All Size Groups Included)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interorbital Width b</td>
<td>0.7 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>-4.64</td>
</tr>
<tr>
<td>Eye Width</td>
<td>0.44 ± 0.16</td>
<td>0.59 ± 0.25</td>
<td>4.24</td>
</tr>
<tr>
<td>Constant</td>
<td>111</td>
<td>110</td>
<td>0.61</td>
</tr>
<tr>
<td>% Correctly Ident.</td>
<td>85.6%</td>
<td>90.0%</td>
<td></td>
</tr>
</tbody>
</table>

aDiscriminant function coefficient
bIn millimeters with ± one standard deviation

Table 4. Results of the Discriminant Function Analysis of Eye Width, Interorbital Width, Body Length and Gut Length of Smelt Larvae (All Size Groups Included)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Width b</td>
<td>0.44 ± 0.16</td>
<td>0.59 ± 0.25</td>
<td>7.99</td>
</tr>
<tr>
<td>Interorbital Width</td>
<td>0.7 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>-1.42</td>
</tr>
<tr>
<td>Body Length</td>
<td>13.4 ± 4.9</td>
<td>13.1 ± 4.3</td>
<td>-0.44</td>
</tr>
<tr>
<td>Gut Length</td>
<td>7.6 ± 2.4</td>
<td>7.3 ± 1.9</td>
<td>0.31</td>
</tr>
<tr>
<td>Constant</td>
<td>111</td>
<td>110</td>
<td>0.17</td>
</tr>
<tr>
<td>% Correctly Ident.</td>
<td>91.9%</td>
<td>94.5%</td>
<td></td>
</tr>
</tbody>
</table>

aDiscriminant function coefficient
bIn millimeters with ± one standard deviation
to be significant for each run are listed. They are listed in decreasing order of discriminatory power.

In the first run, using all the fish with the variables interorbital width and eye width, (Table 3) sixteen larvae originally identified as *Spirinchus* were misidentified; thirteen were in the size group of 6 to 8 mm and three were 9 mm. Eleven, originally identified as *Hypomesus*, were also misidentified; with eight in the 10 to 11 mm size group plus two 12 and one 15 mm larvae. The discriminant scores for the 12 and 15 mm fish were so close to zero (0.009, 0.094 and 0.007, respectively) that the probability of them being either species is about equal. Because fish 12 to 15 mm in length can be identified using these two characteristics, interorbital width and eye width, in conjunction with other characteristics that will be discussed later, such as gas bladder size, I would tend to keep the original identification. By using only these two characteristics it was faster and easier to make identifications but it also reduced the reliability.

When body length and gut length were included with eye width and interorbital width using all the larvae, nine *Spirinchus* were shown to be misidentified; with seven in the 6 to 8 mm group and two in the 9 to 10 mm group. Only six of the *Hypomesus* had been misidentified; two 6 mm larvae and four in the 10 to 12 mm group. Three of the discriminant scores were so close to zero again (0.002, 0.048 and 0.065) that they would be considered marginal cases with the final identification resting on other characteristics. The 15 mm larvae
Table 5. Results of the Discriminant Function Analysis of Eye Width, Interorbital Width, Body Depth, Snout to Dorsal Fin and Snout to Anal Fin of Smelt Larvae (All Size Groups Included, Individuals with Incomplete Data Omitted)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Width</td>
<td>0.55 ± 0.14</td>
<td>0.68 ± 0.23</td>
<td>2.34</td>
</tr>
<tr>
<td>Interorbital Width</td>
<td>0.9 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>-2.06</td>
</tr>
<tr>
<td>Body Depth</td>
<td>1.4 ± 0.7</td>
<td>1.2 ± 0.5</td>
<td>1.32</td>
</tr>
<tr>
<td>Snout to Dorsal Fin</td>
<td>3.9 ± 1.7</td>
<td>7.3 ± 1.5</td>
<td>-1.03</td>
</tr>
<tr>
<td>Snout to Anal Fin</td>
<td>12.5 ± 2.4</td>
<td>10.8 ± 2.3</td>
<td>0.27</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td></td>
<td>3.34</td>
</tr>
<tr>
<td>N</td>
<td>56</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>% Correctly Ident.</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

aDiscriminant function coefficient  
bIn millimeters with ± one standard deviation

Table 6. Results of the Discriminant Function Analysis of Eye Width, Gas Bladder Depth and Interorbital Width of Smelt Larvae (All Size Groups Included, Individuals with Incomplete Data Omitted)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Width</td>
<td>0.47 ± 0.15</td>
<td>0.97 ± 0.21</td>
<td>4.65</td>
</tr>
<tr>
<td>Gas Bladder Depth</td>
<td>0.6 ± 0.4</td>
<td>0.5 ± 0.3</td>
<td>-1.50</td>
</tr>
<tr>
<td>Interorbital Width</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>-0.50</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td></td>
<td>-1.35</td>
</tr>
<tr>
<td>N</td>
<td>97</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>% Correctly Ident.</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

aDiscriminant function coefficient  
bIn millimeters with ± one standard deviation
Table 7. Results of the Discriminant Function Analysis of Eye Width and Gut Length (6 to 8 mm Smelt Larvae)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Width b</td>
<td>0.27 ± 0.03</td>
<td>0.31 ± 0.05</td>
<td>32.14</td>
</tr>
<tr>
<td>Gut Length</td>
<td>4.3 ± 0.6</td>
<td>4.4 ± 0.6</td>
<td>-1.54</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td></td>
<td>-2.43</td>
</tr>
<tr>
<td>N</td>
<td>23</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>% Correctly Id.</td>
<td>69.6%</td>
<td>73.9%</td>
<td></td>
</tr>
</tbody>
</table>

aDiscriminant function coefficient

bIn millimeters with ± one standard deviation

Table 8. Results of the Discriminant Function Analysis of Eye Width and Snout to Anus (9 to 10 mm Smelt Larvae)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Width b</td>
<td>0.34 ± 0.03</td>
<td>0.42 ± 0.03</td>
<td>18.59</td>
</tr>
<tr>
<td>Snout to Anus</td>
<td>7.7 ± 0.7</td>
<td>7.6 ± 0.5</td>
<td>-0.38</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td></td>
<td>-4.09</td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>% Correctly Id.</td>
<td>94.4%</td>
<td>93.8%</td>
<td></td>
</tr>
</tbody>
</table>

aDiscriminant function coefficient

bIn millimeters with ± one standard deviation
Table 9. Results of the Discriminant Function Analysis of Eye Width and Snout to Anus (11 to 12 mm Smelt Larvae)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C.(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Width(^b)</td>
<td>0.39 ± 0.03</td>
<td>0.52 ± 0.09</td>
<td>14.77</td>
</tr>
<tr>
<td>Snout to Anus</td>
<td>9.0 ± 0.5</td>
<td>9.2 ± 0.9</td>
<td>-1.12</td>
</tr>
<tr>
<td>Constant</td>
<td>16</td>
<td>17</td>
<td>3.42</td>
</tr>
<tr>
<td>% Correctly Id.</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Discriminant function coefficient

\(^b\)In millimeters with ± one standard deviation

Table 10. Results of the Discriminant Function Analysis of Eye Width, Interorbital Width, Snout to Dorsal Fin and Snout to Anal Fin (13 to 16 mm Smelt Larvae)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C.(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Width(^b)</td>
<td>0.46 ± 0.05</td>
<td>0.65 ± 0.06</td>
<td>5.26</td>
</tr>
<tr>
<td>Interorbital Width</td>
<td>0.7 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>-1.66</td>
</tr>
<tr>
<td>Snout to Dorsal Fin</td>
<td>7.8 ± 0.4</td>
<td>7.2 ± 0.6</td>
<td>-1.12</td>
</tr>
<tr>
<td>Snout to Anal Fin</td>
<td>10.9 ± 0.7</td>
<td>10.9 ± 0.8</td>
<td>0.45</td>
</tr>
<tr>
<td>Constant</td>
<td>30</td>
<td>31</td>
<td>1.62</td>
</tr>
<tr>
<td>% Correctly Id.</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Discriminant function coefficient

\(^b\)In millimeters with ± one standard deviation
Table 11. Results of the Discriminant Function Analysis of Eye Width, Interorbital Width and Gas Bladder to Pelvic Fin (17 to 25 mm Smelt Larvae)

<table>
<thead>
<tr>
<th></th>
<th>Spirinchus</th>
<th>Hypomesus</th>
<th>D.F.C.(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye Width(^b)</td>
<td>0.73 ± 0.07</td>
<td>1.04 ± 0.19</td>
<td>3.14</td>
</tr>
<tr>
<td>Interorbital Width</td>
<td>1.1 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>-1.99</td>
</tr>
<tr>
<td>Bladder to Pelvic</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.6</td>
<td>-0.63</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>% Correctly Ident.</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Discriminant function coefficient

\(^b\) In millimeters with ± one standard deviation
incorrectly identified in the last run was correctly identified this time. Once more the greatest chance of error was in the smaller fish.

Eye width was the best discriminator among the twelve characteristics tested, it always had a high discriminant function coefficient. Eye width is also a good diagnostic characteristic because it is available for all sizes of larvae and is easily located and measured. Hypomesus has a larger eye with the difference in eye width size increasing with age (Figs. 2-4).

Interorbital width is another useful characteristic which was a significant variable several times. Because of the larger eye size of Hypomesus the interorbital width is correspondingly smaller.

Other useful identifying characteristics (Tables 7-11) that were used often were the distances from the snout to the dorsal fin and the snout to the anal fin. When used in conjunction with eye width and interorbital width they gave good consistent results with fish of 13 mm or larger. Gut length was also useful when used with the two eye characteristics just mentioned in fish up to 9 mm. Snout to anus distance proved useful in conjunction with eye width in the 9 to 10 and 11 to 12 mm groups.

An additional useful characteristic was the size and initial appearance of the gas bladder. Its value was not demonstrated by the discriminant analysis because the gas bladder wasn't measured on the Hypomesus until after they
had reached 17 mm or more. However, the gas bladder in *Spirinchus* is conspicuous in larvae of 9 to 10 mm. The gut in *Spirinchus* curves down around the gas bladder as it deepens (Figs. 2-4). This difference in gas bladder formation is a valid characteristic for identification.

Another difference that did not show up on the discriminant analysis was that the pelvic fin developed at 15 mm on *Hypomesus* and not until 18 mm on *Spirinchus*. This suggests an inverse relationship between the development of the gas bladder and the pelvic fins.

Melanophores or pigmentation were also helpful in identification. *Spirinchus* has two to three melanophores anterior to the gas bladder, one at the site of the gas bladder and a row of six to ten melanophores to the anus ending in a large circumanal melanophore. The caudal melanophores were more difficult to count but usually numbered three to five. These findings agree with Dryfoos (1965) who counted two melanophores anterior to the gas bladder, one at the gas bladder, eight to ten melanophores from the gas bladder to the anus and a maximum of seven caudal melanophores.

*Hypomesus* had longer dash-like melanophores that were often paired with one on either side of the gut. There were four to twelve paired melanophores with a maximum of eight small dot-like melanophores on the bottom of the gut. They also had the large circumanal melanophore.

However, utilizing pigmentation as an identifying characteristic should be done cautiously. It is often
variable within a species and melanophores often fade under preservation (Lippson and Moran, 1974).

The best overall criteria for visual identification in the lab are eye width, interorbital width, gas bladder size and melanophore pattern. If time permits, dividing the specimens into size groups and using the characteristics that the discriminant analysis chose as the best discriminators for each size group gives the most consistent and reliable results. The second best method is to take the body length, eye width, interorbital width and gut length and calculate the discriminant score. In either case the gas bladder and melanophores should be taken into account.

Specimens 9 to 10 mm have a 94% probability of being identified correctly. Larvae 11 mm and larger have a 100% probability of correct identification. The separation of the discriminant scores (Figs.5-13) becomes progressively larger as the size of the fish increases. This indicates that the two species become more differentiated and distinct as they grow larger. However, smelt in the 6 to 8 mm group, because of their small size and lack of distinguishing characteristics, cannot be positively identified unless further measurements such as myomere and vertebrae counts are made.
SUMMARY

1. The purpose of this study was to identify the larval forms of the two species of smelt, *Spirinchus thal- eichthys* and *Hypomesus t. transpacificus* and to better describe their spawning times in the Sacramento-San Joaquin Estuary, California.

2. *Spirinchus* was found to spawn from late December through April. The larvae are more tolerant of saltwater and are found further downstream than *Hypomesus* larvae. *Hypomesus* spawned from April to June.

3. Twelve characteristics were measured on the larval fish and analyzed in a discriminant function analysis program. The characteristics were body length, snout to anus, interorbital width, body depth, eye width, snout to anal fin, snout to dorsal fin, snout to pelvic fin, gut length, gas bladder width, gas bladder depth and gas bladder to pelvic fin.

4. Eye width was the best discriminator with *Hypomesus* having the larger eye size.

5. Interorbital width was another significant characteristic with *Hypomesus* having the smaller interorbital width due to their larger eye size.

6. Distances from the snout to the dorsal fin and
the snout to the anal fin were useful on fish of 13 mm or larger when used with the two eye characteristics, eye width and interorbital width.

7. Gut length was useful in discriminating between the species in larvae up to 9 mm. The distance from the snout to the anus was useful in fish of 9-12 mm.

8. The size and initial appearance of the gas bladder was very important in identifying the fish. The gas bladder started forming in *Spirinchus* at 9-10 mm and deepened with growth whereas the gas bladder in *Hypomesus* started forming later and wasn't readily visible until 17 mm or more. It was a narrower gas bladder too.

9. Melanophore patterns differed between the species. *Spirinchus* had two to three melanophores anterior to the gas bladder, one at the gas bladder, a row of six to ten melanophores to the anus, a large circumanal melanophore and three to five caudal melanophores. *Hypomesus* had four to twelve paired dash-like melanophores, one on either side of the gut, with a maximum of eight small dot-like melanophores on the bottom of the gut and one circumanal melanophore. Pigmentation and melanophores may vary within a species and will fade under preservation so care must be taken in using them as characteristics.

10. The discriminant function coefficients for the various size groups and the mean for each significant characteristic are given for use in future identification of smelt larvae. The discriminant scores for each group are also given.
11. Smelt larvae of 9-10 mm have a 94% probability of being correctly identified with those larger having 100% probability. However, 6-8 mm smelt cannot be positively identified unless further measurements such as myomere and vertebrae counts are made.
LITERATURE CITED


Central Pacific Basins. 1967. Effects of the San Joaquin master drain on water quality of the San Francisco Bay and Delta. Federal Water Pollution Control Administration, San Francisco, California.


