

## First Progress Report

**Title:** *Histopathological Evaluation of Starvation and/or Toxic Effects on Pelagic Fishes*

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### A. Executive Summary

This progress report presents and interprets the preliminary results of histopathology studies conducted on adult delta smelt (*Hypomesus transpacificus*) collected at various locations in the Sacramento-San Joaquin Rivers, Suisun Bay and Suisun Marsh in 2005 as part of the Pelagic Organism Decline 2005 Workplan.

Delta smelt were collected by the California Department of Fish and Game during the Spring Kodiak Trawl headed by Kelly Souza and Randy Baxter. Eight sampling stations and between 10-16 fish per station in the vicinity of upper Sacramento River, at the confluence of Sacramento and San-Joaquin Rivers, Suisun Bay, and Suisun Marsh were randomly selected for histopathological evaluation (Table 1). In addition, stations 699 and 799 of 2003 delta smelt were selected for histopathological evaluation (Table 1). Overall, we examined 144 delta smelt livers.

Inland silverside and striped bass were collected by the Department of Water Resources as part of a microcystis sampling program headed by Peggy Lehman and Matt Nobriga. Fish have been processed for histopathological evaluation and results will be submitted in the next progress report.

Liver lesion scores were higher in stations 699 and 799 of delta smelt collected in March 2003. In addition, liver lesion scores were significantly different between station 716 and stations 609, 699, and 799 in delta smelt collected between January 26 and February 24 of 2005. However, there were no differences in liver lesion scores of delta smelt collected during the months of July 2005 between stations 602 and 519.

Glycogen depletion, lipidosis, and single cell necrosis were probably the most significant liver lesions observed in 2005 delta smelt. Although only two stations were evaluated, glycogen depletion, lipidosis, megalocytosis, foci of cellular alterations, and hepatic adenoma were the most significant lesions observed in 2003 delta smelt.

In summary, results indicate delta smelt collected from Station 609, 699, and 799 have a greater variety, higher prevalences, and more severe liver lesions when compared to fish from other stations. In addition, results indicate that delta smelt from these stations are adversely affected by toxic exposure and/or food limitation. Based on the current study, delta smelt collected from station 716 were the least affected when compared to fish from

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other stations.

Recommendations for future studies include: 1) continuous histopathological evaluation of delta smelt already processed in Swee Teh's laboratory to increase the number of sampling stations and sample size for statistical analysis; 2) integration of this study with the finding of William Bennett, Peggy Lehman, and Inge Werner to establish causality; 3) fish necropsy by a trained pathologist to expand organ sampling (e.g., gill, kidney and gonads) for histopathological evaluation and to archive tissue samples for organic and metallic chemical analyses; and 4) tissue body contaminant analysis to separate sublethal effects of toxic exposure from food limitation.

## **B. Materials and Methods**

Tissues from individual fish were assigned a random alpha-numeric identification code to perform a blind study. Processed tissues were embedded in paraffin and sectioned at 3-5 microns thickness. Tissue sections were mounted on glass slides and stained with hematoxylin and eosin (HE). Tissue slides were read and scored by Swee Teh. Severity scores were semiquantitative and based on a scale of 0 to 3 (0 = not present, 1 = mild, 2 = moderate, and 3 = severe).

## **C. Results**

Mean histologic scores for liver lesions are given in Table 2 and Figure 1. Lesions in delta smelt included: glycogen depletion (GD), macrophage aggregates (LMA), cytoplasmic inclusions (CI), lipidosis (LIP), eosinophilic droplets within hepatocytes (EPD), infiltration of inflammatory cells (INF), megalocytosis (KARYO) and single cell necrosis (SCN). (See appendix 1). Percentages (%) of liver lesions in delta smelt with severity scores of 2 (moderate) and 3 (severe) are shown in Table 3 and Figure 2.

Comparison of average lesion scores (Figure 1) among stations revealed that: 1) glycogen depletion was higher in all 2003 and 2005 stations except for station 716 ( $p < 0.05$ ), 2) Single cell necrosis was higher in stations 609, 699, and 799 of 2005 delta smelt, and 3) Lipidosis was higher in stations 606, 609, 699, and 799 of 2005 and stations 699 and 799 of 2003 delta smelt. Liver lesion scores were higher in stations 699 and 799 of delta smelt collected in March 2003. In addition, liver lesion scores were significantly different between station 716 and stations 609, 699, and 799 in delta smelt collected between January 26 and February 24 of 2005. However, there were no differences in liver lesion scores of delta smelt collected during the months of July 2005 between stations 602 and 519.

Comparison of percentages (%) of moderate and severe lesions in 2005 smelt indicated that markedly higher prevalence occurred in fish from stations 602, 606, 609, 519, 699, and 799 than from stations 704 and 716. Prevalence of moderate and severe lesions were highest in station 799 of 2003 smelt when compared to all of the stations collected in 2003 and 2005 (Table 3).

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Except for the presence of intersex fish (i.e., ovatestis) in station 799 (1 of 14 fish), 609 (1 of 15 fish) and 716 (1 of 11 fish), no foci of cellular alterations and tumors were observed in 2005 smelts. A 6.25% incidence of intersex, foci of cellular alterations, and hepatocellular adenoma were observed in 2003 smelt collected from Station 799.

#### **D. Conclusions and Recommendations**

Hepatocyte storage disorders (i.e., glycogen depletion and lipidosis) are non-specific lesions that indicate infectious, parasitic, or xenobiotic stress resulting in either depletion of hepatocellular glycogen and/or abnormal metabolism and mobilization of fat with subsequent deposition in the liver. Although neither GD nor LIP is specific for exposure to any particular toxicant, both are good general indicators of health. On the other hand, single cell necrosis (SCN) can result from adaptation of an organ to normal size following a period of cellular proliferation, but can also be a direct effect of contaminant exposure. In general, liver histopathology results revealed that fish collected from Station 716 were healthier than fish collected from Stations 609, 699, and 799. These results suggest that fish at this station are not likely to be affected by toxic exposure and/or food limitation.

The presence of intersex (i.e., ovatestis) in smelt can result from exposure to endocrine disrupting chemicals or occurred spontaneously. Since there is little information on endocrine disruption effects on delta smelt, additional laboratory evaluation is needed to identify the cause. The most significant lesions observed in 2003 delta smelt were foci of cellular alterations and hepatocellular adenomas. These preneoplastic and neoplastic lesions are consistent with exposure to a xenobiotic carcinogen(s) and or promoters. If additional studies are planned, contaminant body burden should be quantified and matched to fish age data since cancers are also an age-related phenomenon. Finally, it is warranted to integrate histopathology data with age and population studies from William Bennett's laboratory.

**Table 1.** Description of sampling locations included in the histopathological analysis of pelagic fishes

| <i>Location Description</i>                     | <i>Station Number</i> | <i>Sample Size</i>         |
|---|-----------------------|----------------------------|
| Upper Sacramento River                          | Station 704           | 13                         |
|   | Station 716           | 11                         |
| Confluence of Sacramento and San Joaquin Rivers | Station 699           | 14 (2005); 13 (2003)       |
|   | Station 799           | 14 (2005); 16 (2003)       |
| Suisun Bay                                      | Station 519           | 13                         |
| Suisun Marsh                                    | Station 602           | 14 (7/21/05); 10 (7/28/05) |
|   | Station 606           | 11                         |
|   | Station 609           | 15                         |
| <b>Total</b>                                    | <b>8 stations</b>     | <b>144</b>                 |

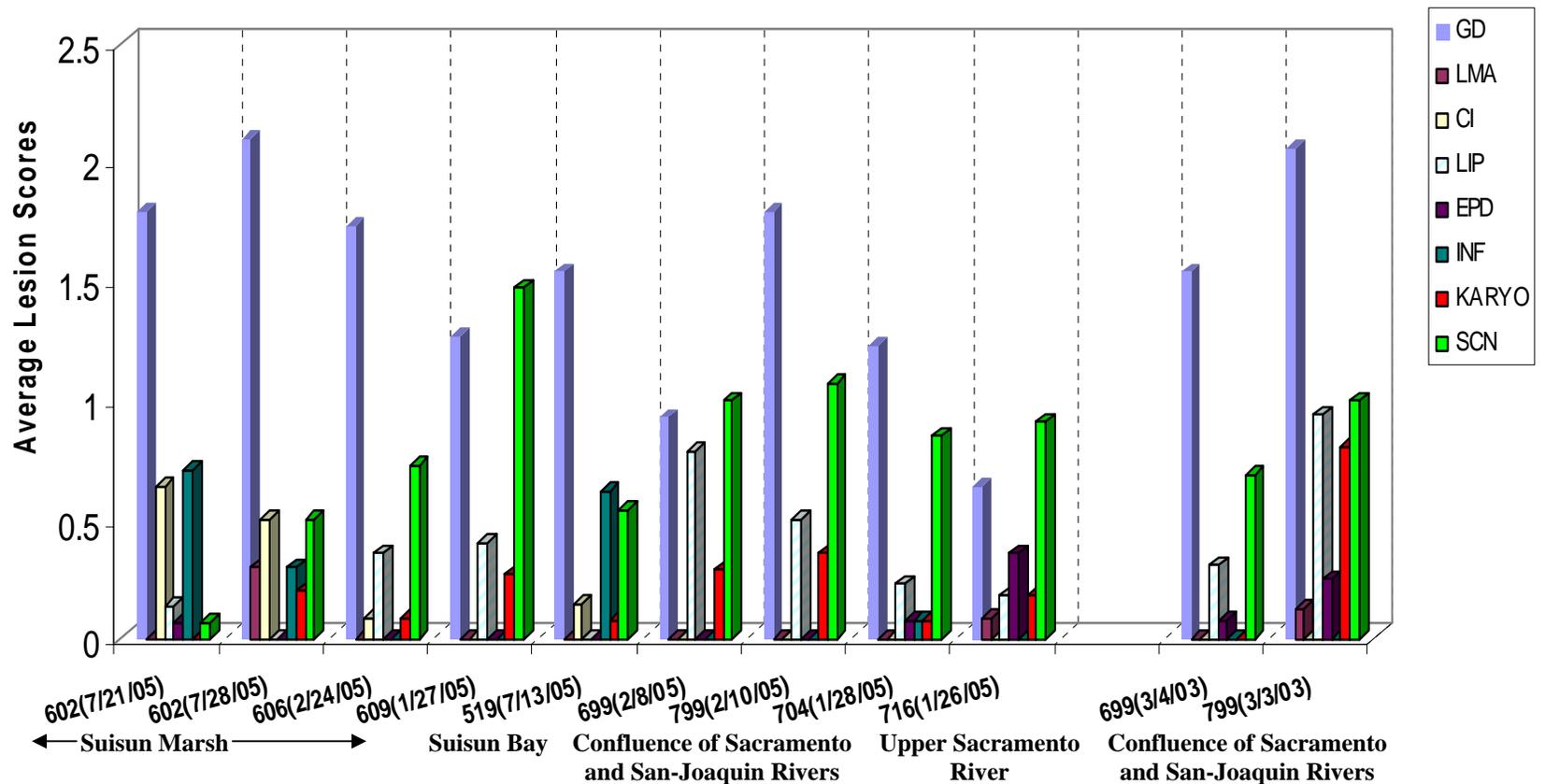
**Table 2.** Average Liver Lesion Scores of Delta Smelt

| Station #    | GD   | LMA  | CI   | LIP  | EPD  | INF  | KARYO | SCN  | Sum of Average lesion Scores |
|--------------|------|------|------|------|------|------|-------|------|------------------------------|
| 602(7/21/05) | 1.79 | 0.00 | 0.64 | 0.14 | 0.07 | 0.71 | 0.00  | 0.07 | 3.42                         |
| 602(7/28/05) | 2.10 | 0.30 | 0.50 | 0.00 | 0.00 | 0.30 | 0.20  | 0.50 | 3.90                         |
| 606(2/24/05) | 1.73 | 0.00 | 0.09 | 0.36 | 0.00 | 0.00 | 0.09  | 0.73 | 3.00                         |
| 609(1/27/05) | 1.27 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.27  | 1.47 | 3.41                         |
| 519(7/13/05) | 1.54 | 0.00 | 0.15 | 0.00 | 0.00 | 0.62 | 0.08  | 0.54 | 2.93                         |
| 699(2/8/05)  | 0.93 | 0.00 | 0.00 | 0.79 | 0.00 | 0.00 | 0.29  | 1.00 | 3.01                         |
| 799(2/10/05) | 1.79 | 0.00 | 0.00 | 0.50 | 0.00 | 0.00 | 0.36  | 1.07 | 3.72                         |
| 704(1/28/05) | 1.23 | 0.00 | 0.00 | 0.23 | 0.08 | 0.08 | 0.08  | 0.85 | 2.55                         |
| 716(1/26/05) | 0.64 | 0.09 | 0.00 | 0.18 | 0.36 | 0.00 | 0.18  | 0.91 | 2.36                         |
| 699(3/4/03)  | 1.54 | 0.00 | 0.00 | 0.31 | 0.08 | 0.00 | 0.00  | 0.69 | 2.62                         |
| 799(3/3/03)  | 2.06 | 0.13 | 0.00 | 0.94 | 0.25 | 0.00 | 0.81  | 1.00 | 5.19                         |

**Table 3.** Percentages (%) of liver lesions in delta smelt with severity scores of moderate and severe

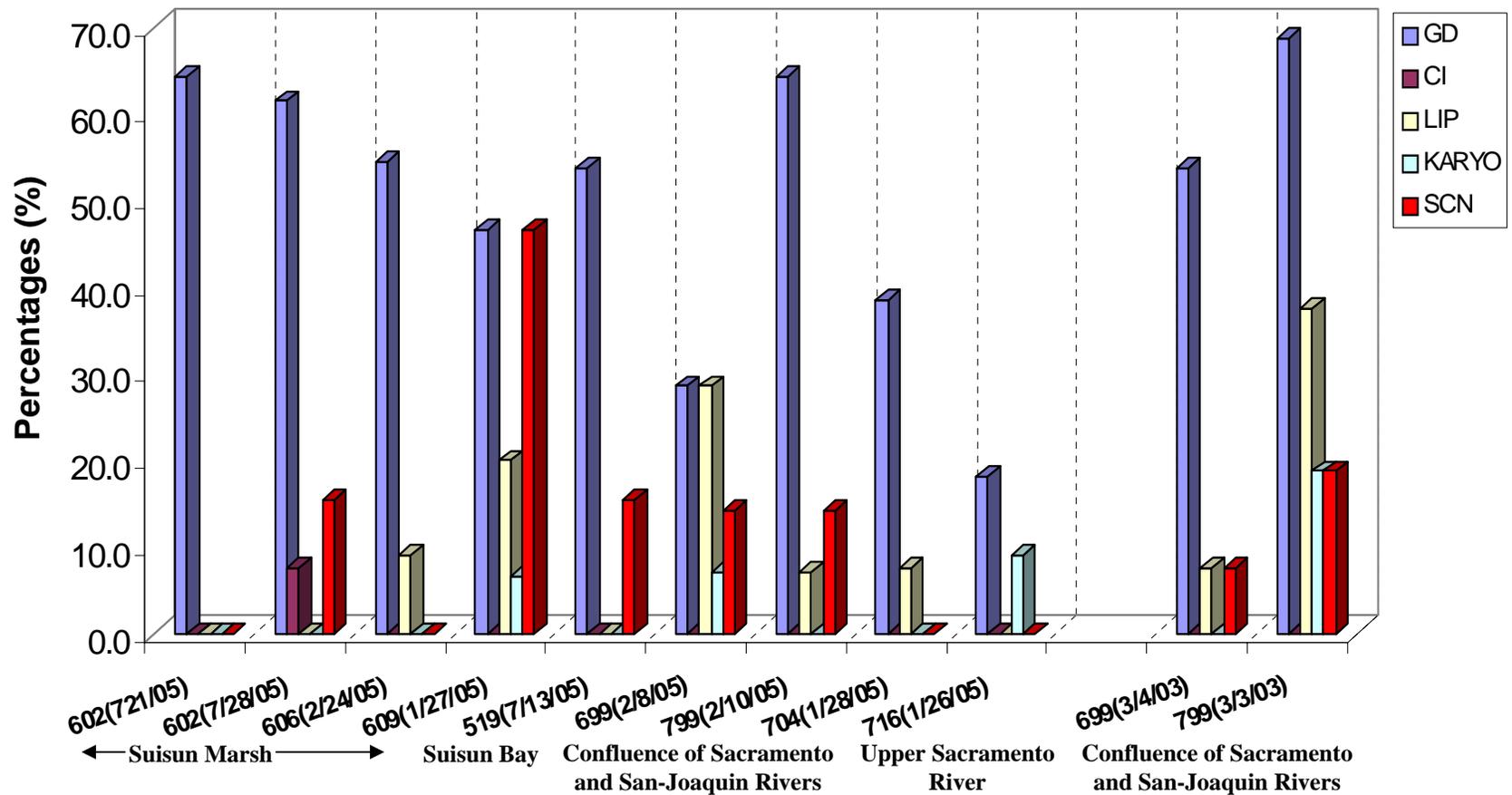
| Station#     | GD    | CI   | LIP   | KARYO | SCN   |
|--------------|-------|------|-------|-------|-------|
| 602(721/05)  | 64.29 | 0.00 | 0.00  | 0.00  | 0.00  |
| 602(7/28/05) | 61.54 | 7.69 | 0.00  | 0.00  | 15.38 |
| 606(2/24/05) | 54.55 | 0.00 | 9.09  | 0.00  | 0.00  |
| 609(1/27/05) | 46.67 | 0.00 | 20.00 | 6.67  | 46.67 |
| 519(7/13/05) | 53.85 | 0.00 | 0.00  | 0.00  | 15.38 |
| 699(2/8/05)  | 28.60 | 0.00 | 28.60 | 7.10  | 14.30 |
| 799(2/10/05) | 64.29 | 0.00 | 7.14  | 0.00  | 14.29 |
| 704(1/28/05) | 38.46 | 0.00 | 7.69  | 0.00  | 0.00  |
| 716(1/26/05) | 18.18 | 0.00 | 0.00  | 9.09  | 0.00  |
| 699(3/4/03)  | 53.80 | 0.00 | 7.70  | 0.00  | 7.70  |
| 799(3/3/03)  | 68.80 | 0.00 | 37.50 | 18.80 | 18.80 |

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**Figure 1.** Average liver lesion scores of delta smelt at stations sampled by Department of Fish and Game during the 2003 and 2005 Spring Kodiak Trawl. GD = glycogen depletion; LMA = Macrophage aggregate; CI = cytoplasmic inclusions; LIP = fatty vacuolar degeneration or lipidosis; EPD = eosinophilic protein droplets; INF = infiltration of inflammatory cells; KARYO = Hepatocyte megalocytosis; and SCN = single cell necrosis.

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**Figure 2.** Percentages (%) of lesion severity score of moderate and severe in delta smelt at stations sampled by Department of Fish and Game during the 2003 and 2005 Spring Kodiak Trawl. GD = glycogen depletion; CI = cytoplasmic inclusions; LIP = fatty vacuolar degeneration or lipidosis; KARYO= Hepatocyte megalocytosis; SCN = single cell necrosis. Note delta smelt collected from station 716 had significantly lower prevalence of moderate and severe lesion scores than those collected from station 602, 609, 699, and 799.

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## **Appendix 1. Definition of Liver Histopathological Lesions**

1. GD = glycogen depletion, characterized by decreased size of hepatocytes, loss of the 'lacy', irregular, and poorly demarcated cytoplasmic vacuolation typical of glycogen, and increased cytoplasmic basophilia (i.e., blue coloration);
2. LMA = Macrophage aggregate, characterized as a cluster of macrophages packed with coarsely granular yellow-brown pigment;
3. CI = cytoplasmic inclusions, characterized by accumulation of unknown material within the cytoplasm of hepatocytes;
4. LIP = fatty vacuolar degeneration or lipidosis, characterized by excess lipid appears as clear, round, and well demarcated cytoplasmic vacuoles;
5. EPD = eosinophilic protein droplets, characterized by the presence of proteins appears as refractile, eosinophilic (pink coloration), round, and well demarcated cytoplasmic vacuoles;
6. INF = infiltration of inflammatory cells, characterized by infiltration of the hepatic parenchyma with a mixed population of mononuclear inflammatory cells; macrophages, and lymphocytes;
7. KARYO = Hepatocyte megalocytosis, characterized primarily by nuclear enlargement or karyomegaly. Hepatocytes were not considered megalocytes unless nuclei were at least 2-3X the average size in the section; and
8. SCN = single cell necrosis, characterized by cells having eosinophilic (i.e., pink coloration) cytoplasm with nuclear pyknosis and karyorrhexis.

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