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Managing Water in the West

Dichotomous Key to Fish Eggs of the Sacramento-San Joaquin River Delta

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A dichotomous key was developed for fish eggs of the Sacramento-San Joaquin River Delta (Delta). The eggs were collected between 2002 and 2010 from the Tracy Fish Collection Facility (TFCF; Byron, California), the Delta and its tributaries, and reservoirs associated with the Delta. Eggs from less common fish species were obtained *in vitro* and through environmentally controlled spawning in large aquaria. The key was developed to aid TFCF operators and biologists identify spawning events and presence of fish species salvaged at the TFCF.

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Dichotomous Key to Fish Eggs of the Sacramento-San Joaquin River Delta

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by

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February 2011

U.S. Department of the Interior
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Fish photography by René Reyes, Tracy Fish Collection Facility, Tracy, California.
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EXECUTIVE SUMMARY

A dichotomous key was developed for fish eggs of the Sacramento-San Joaquin River Delta (Delta). The eggs were collected between 2002 and 2010 from the Tracy Fish Collection Facility (TCFC; Byron, California), the Delta and its tributaries, and reservoirs associated with the Delta. Eggs from less common fish species were obtained *in vitro* and through environmentally controlled spawning in large aquaria. The key was developed to aid TFCF operators and biologists identify spawning events and presence of fish species salvaged at the TFCF.

Key words: Fish eggs, embryo, yolk sac, dichotomous key, Tracy Fish Collection Facility, Sacramento-San Joaquin Delta

INTRODUCTION

The Tracy Fish Collection Facility (TFCF; Byron, California) is a fish salvage facility located on the intake channel of the C.W. "Bill" Jones Pumping Plant (JPP) and the Delta-Mendota Canal. These facilities are part of the U.S. Department of the Interior, Bureau of Reclamation (Reclamation) Central Valley Project, Delta Unit located in the southern region of the Sacramento-San Joaquin River Delta (Delta). The TFCF collects (salvages) and identifies over 50 species of fish, including threatened and endangered fishes such as delta smelt (*Hypomesus transpacificus*) and winter-run Chinook salmon (*Oncorhynchus tshawytscha*).

Eggs have been collected during routine fish salvage estimation (*i.e.*, 10-minute fish counts) and special larval osmerid sampling (*i.e.*, 30-minute fish counts). Although eggs are noted, they are not usually identified. This paper identifies and describes all the Delta fish eggs using a dichotomous key, several images of eggs, and a table summarizing egg size range. Very few early life stage taxonomic keys are available for the Delta; therefore, this key narrows that void.

METHODOLOGY

Eggs were collected from rivers and streams associated with the Delta with known species composition. For eggs collected from streams and shallow rivers, a fine mesh dip net (200 µm) was utilized similar to a Surber stream bottom sampler. Several eggs were collected from nests and from vegetation. For eggs collected from larger rivers and lakes, a plankton net (300 µm) was towed by a boat. Snorkel sampling was also implemented. Finally, eggs were collected from salvage operations at the TFCF.

Eggs of less common species were obtained through artificial means. This included photoperiod and temperature manipulation in aquaria, strip spawning, and induced spawning through hormone injections. Adults were sometimes collected from the wild and strip-spawned onsite.

Although the dichotomous key emphasized egg morphology, the method of reproduction and collection are important when identifying fish eggs (Matarese and Sandknop 1984). Therefore, ecological features such as salinity levels, type of substrate used for attachment, and parental care involvement were also included in the key as secondary characteristics.

Stereomicroscopy and Photography

Eggs were digitally photographed using a Leica™ DFC 420 digital camera attached to a Leica™ MZ7_5 stereomicroscope (Leica Microsystems, Bannockburn, Illinois).

Photographs were calibrated, labeled, and archived for future reference. Because of the accumulation of images over time, an image database of the eggs was created. Image-pro© version 6.2 (Media Cybernetics, Inc., Bethesda, Maryland), an imaging analysis software, was used to obtain measurements of egg.

Limitations

There are some limitations to the key. First, eggs used in the development of this key were alive or were freshly preserved in formalin; therefore, preserved eggs may not key out properly since preservation renders eggs opaque. Caution should also be used when identifying eggs from alcohol-based preservatives since eggs shrink in alcohol. Second, eggs of closely related species look more similar to each other than to more distantly related species. Thus, eggs of cyprinids, except Sacramento pikeminnow (*Ptychocheilus grandis*), osmerids, centrarchids, and ictalurids were not identified to species due to several overlapping characteristics within the family. In these cases, the eggs were identified to family level. Finally, a few species were excluded from the key: viviparous species or livebearing species such as tule perch (*Hysterocarpus traskii*) and western mosquitofish (*Gambusia affinis*) and rare species such as river lamprey (*Lampetra ayresii*) and western brook lamprey (*Lampetra richardsoni*).

RESULTS

Of the 50 oviparous fish species that spawn in the Delta, eggs from 38 species were collected. Except for green sturgeon (*Acipenser medirostris*) from Klamath River and Sacramento perch (*Archoplites interruptus*) from Abbotts Lagoon Point Reyes National Seashore (Marin County), all eggs were obtained directly from the Delta, its tributaries, and watershed. Eggs collected through artificial means were also from Delta broodstock. Species that were not collected, for example riffle sculpin (*Cottus gulosus*) and Pacific staghorn sculpin (*Leptocottus armatus*), were included in the key from descriptions in literature. Egg collection results showed that most eggs collected in the Delta were demersal and collected from substrate (Appendix 1). Spherical Delta eggs can be categorized into two size ranges: eggs ≤2.4 and eggs ≥2.5 (Appendix 2). Although an effective diameter in nonspherical eggs can be calculated using the lengths of major and minor axis (Coleman 1991), only the major axis (long axis) are reported in this study. For ease of identification, the dichotomous key is presented as a flow chart in Appendix 3.

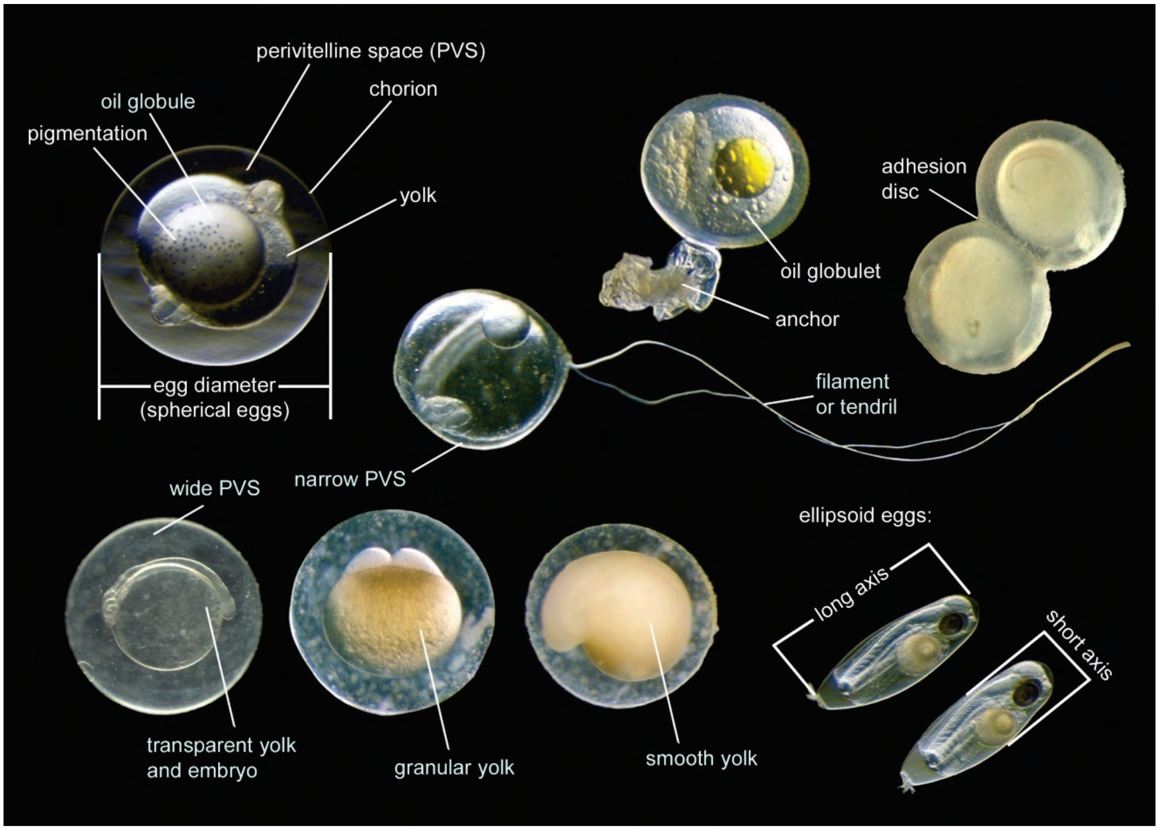

FIGURE 1.—Anatomical features and characteristics of eggs found in the Sacramento-San Joaquin Delta.

Dichotomous Key to Fish Eggs of the Sacramento-San Joaquin River Delta

Refer to Figure 1 for location of anatomical features of an egg.

1a. Egg is ellipsoid or football-shaped 2
1b. Egg is spherical 4

Ellipsoid egg

2a. Egg is long (>3 mm at the long axis; Figure 2); collected from polyhaline/mesohaline waters .... **yellowfin goby**
2a. Egg is short (<2 mm at the long axis); collected from freshwater/ oligohaline waters 3

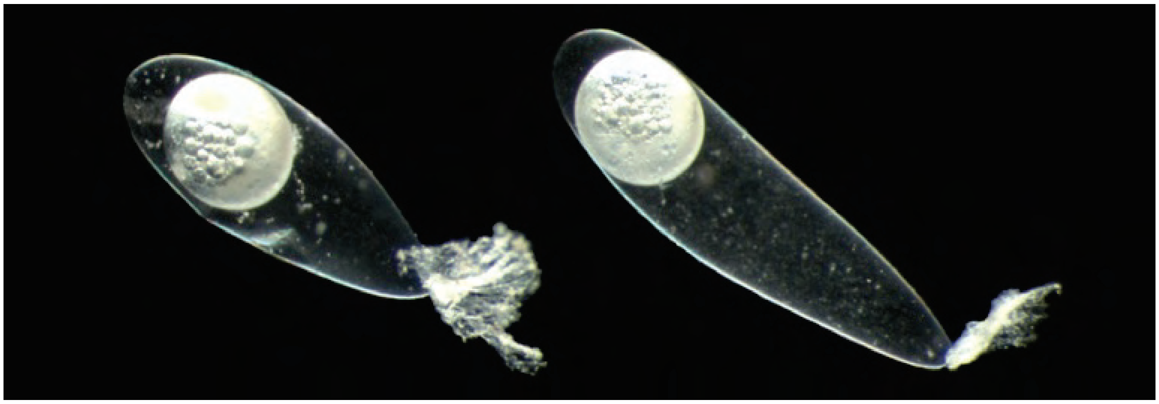

FIGURE 2.—Yellowfin goby eggs at 3 mm (left) and 3.6 mm (right).

3a. Distal tip is pointed (Figure 3a) ....**shimofuri goby**
3a. Distal tip is rounded (Figure 3b) ....**Shokihaze goby**

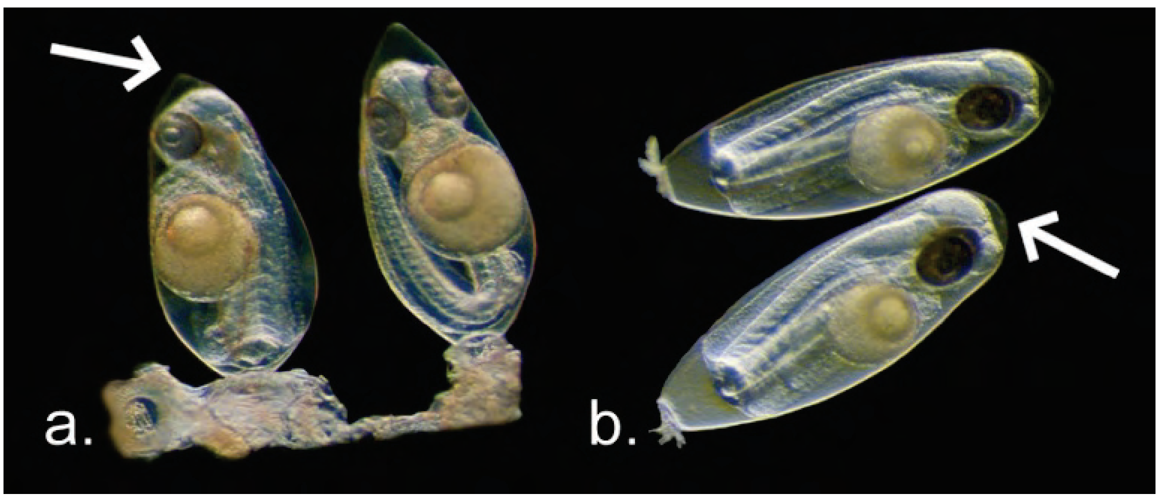

FIGURE 3.—(a) 1.3-mm shimofuri goby eggs and (b) 1.4-mm Shokihaze goby eggs. White arrow points to distal tip of the eggs.

Spherical egg

4a. Small egg (≤2.4 mm in diameter) 5
4b. Large egg (≥2.5 mm in diameter) 17

Eggs ≤2.4 mm in diameter

5a. Egg has no oil globule or globulets.. 6
5b. Egg has oil globule or globulets.. 8

6a. Egg is pelagic, with a finely wrinkled chorion; collected from seawater/polyhaline water.. **starry flounder**
6b. Egg is demersal, with a tough chorion; collected from freshwater............................ 7

7a. White or green smooth yolk (Figure 4a)...**Pacific lamprey**
7b. Clear to yellow granular yolk (Figure 4b) .... ***Cyprinidae* spp.**

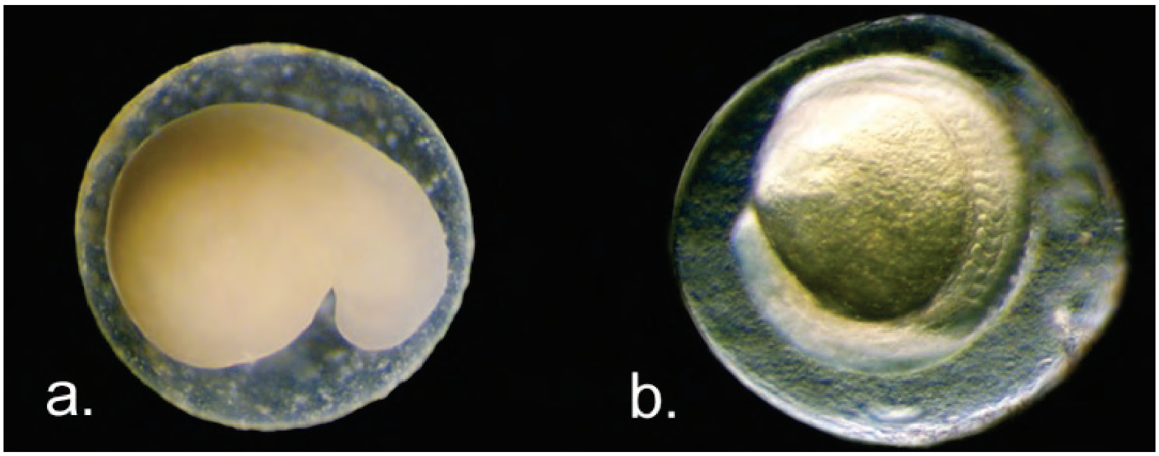

FIGURE 4.—(a) Pacific lamprey egg and (b) *Cyprinidae* spp.

8a. Egg has external attachment structures such as anchors or filaments........................ 9
8b. Egg has no external attachment structures.. 11

Egg with attachment structures

9a. Egg has adhesive anchor (Figure 5a) ....***Osmeridae* spp.**
9b. Egg has filaments (Figure 5b).. 10

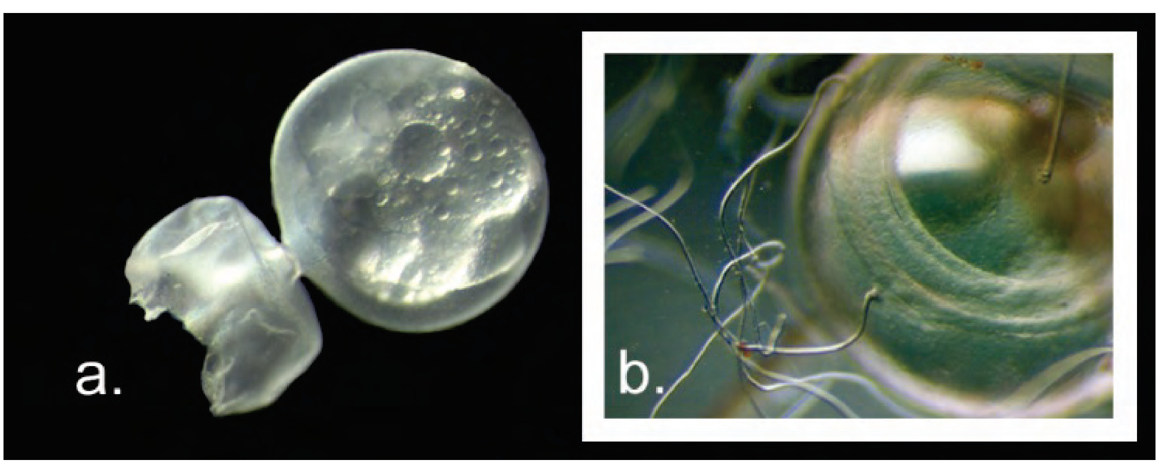

FIGURE 5.—(a) *Osmeridae* spp. egg with an adhesive anchor and (b) closeup of egg with filaments.

10a. Egg has fine filaments tangled around the chorion (Figure 6a) ...... **rainwater killifish**
10b. Egg has 1–20 long filaments or tendrils attached to chorion (Figure 6b) .... **inland silverside**

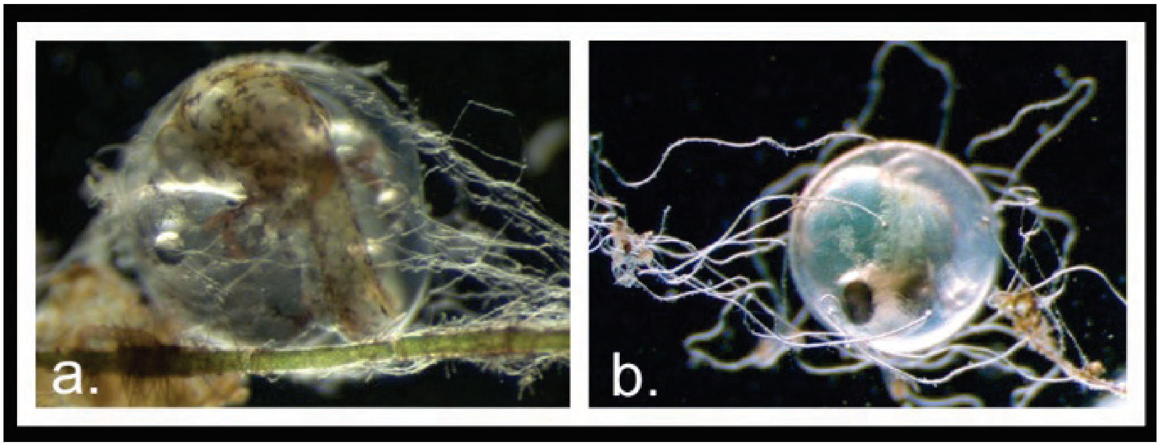

FIGURE 6.—(a) Rainwater killifish egg entangled by fine filaments and (b) inland silverside egg with chorion-attached filaments.

Egg with no attachment structures

11a. Egg has hard chorion, yellow to orange yolk, and deposited in a cluster 12
11b. Egg has soft chorion, colorless yolk, and often deposited singly or small cluster 14

12a. Egg is large (≥2.0 mm diameter).. **riffle sculpin**
12b. Egg is small (1.3–1.5 mm diameter).. 13

13a. Egg collected in freshwater (Figure 7).. **prickly sculpin**
13b. Egg collected in mesohaline/polyhaline water .... **Pacific staghorn sculpin**

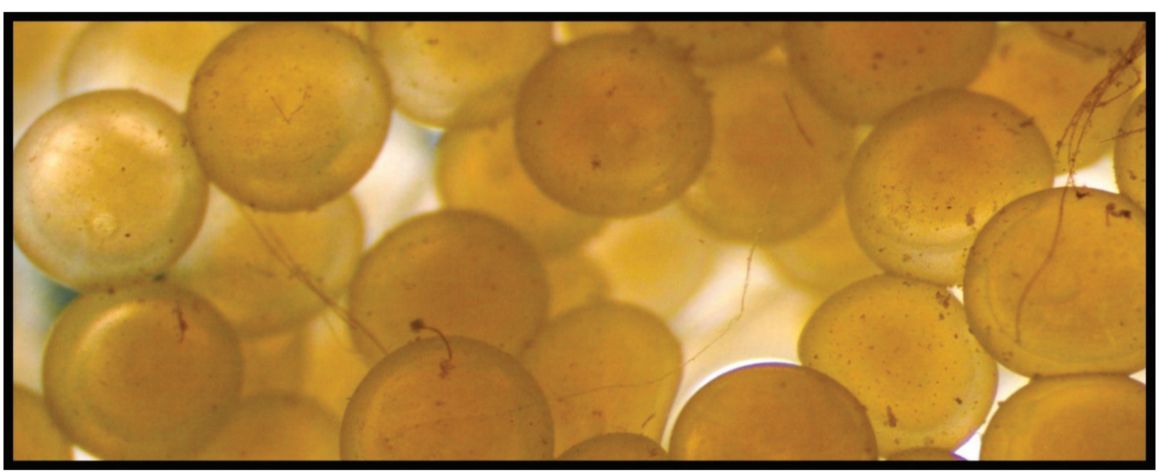

FIGURE 7.—Prickly sculpin egg cluster collected from freshwater.

14a. Egg has small oil globule (0.1–0.2 mm in diameter; Figure 8a)...........**threadfin shad**
14b. Egg has large oil globule (≥0.3 mm in diameter; Figure 8b).................................. 15

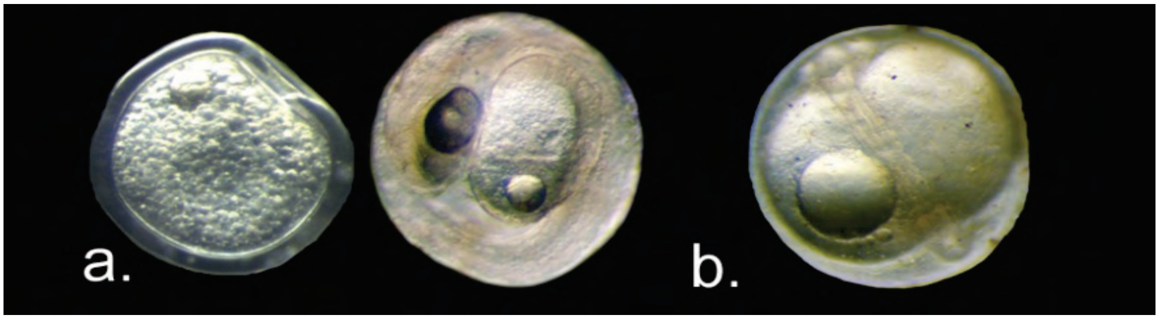

FIGURE 8.—(a) Threadfin shad with small oil globule and (b) egg with large oil globule.

15a. Embryo has no pigments; eyes never pigment (Figure 9a)............***Centrarchidae* spp.**
15b. Embryo has pigments on body and eyes (Figure 9b)... 16

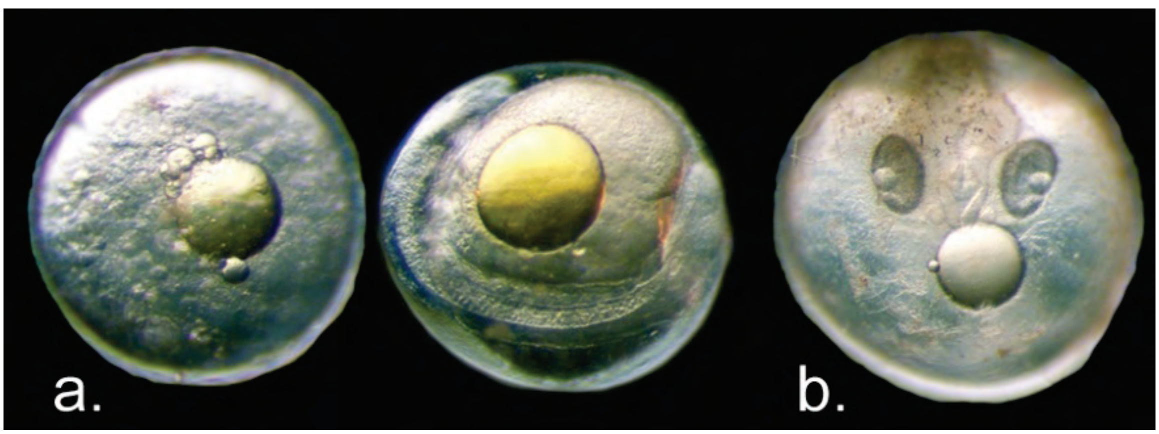

FIGURE 9.—(a) *Centrarchidae* spp. with no pigments and (b) egg with pigmenting eyes and body.

16a. Oil globule with globulets present at the early stage; pigmentation throughout body, yolk sac, and eyes at later stage (Figure 10a) ....**threespine stickleback**
16b. One oil globule with no globulets present at early stage; pigmentation is less at later stage (Figure 10b) ....**bigscale logperch**

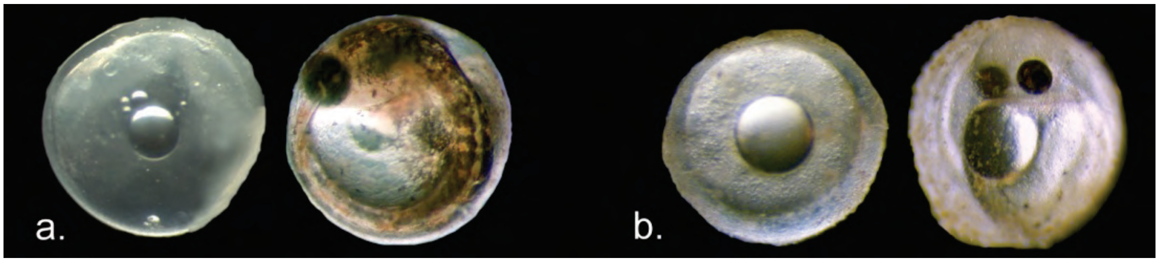

FIGURE 10.—(a) Threespine stickleback egg with several oil globulets at the zygote stage and heavy pigmentation on advanced embryo; (b) bigscale logperch showing one consolidated oil globule at the zygote stage and lighter pigmentation on advanced embryo.

Eggs ≥2.5 mm in diameter

17a. Egg has oil globule 18
17b. Egg has no oil globule 20

18a. Egg has large oil globule (≥0.5 mm) and wide perivitelline space, pelagic (often collected from water column; Figure 11a) .... **striped bass**
18b. Egg has small oil globule and narrow to no perivitelline space, demersal (collected in benthic zone such as gravel; Figure 11b) 19

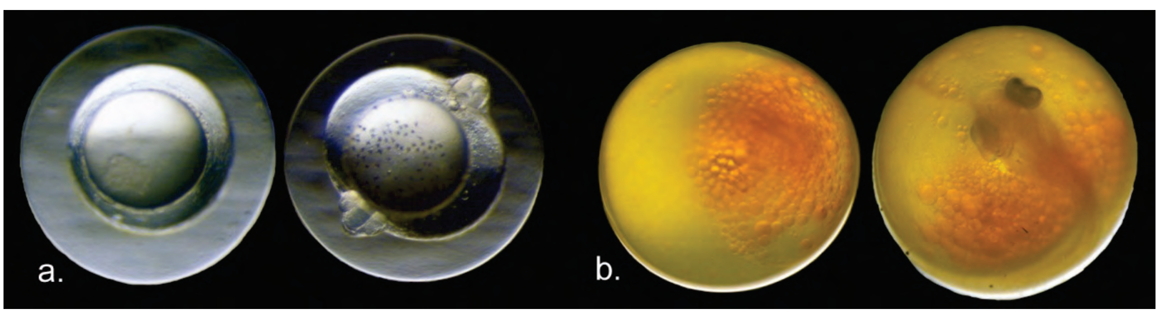

FIGURE 11.—(a) Striped bass egg (early and advanced stages) with large oil globule and wide perivitelline space; (b) egg (early and advanced stages) with small oil globules and globulets and very narrow perivitelline space.

19a. Larger egg (6–9 mm in diameter); reddish and orange yolk coloration (Figure 12a) .... **Chinook salmon**
19b. Smaller egg (3–6.2 mm in diameter); yellow and cream yolk coloration (Figure 12b) .... **steelhead**

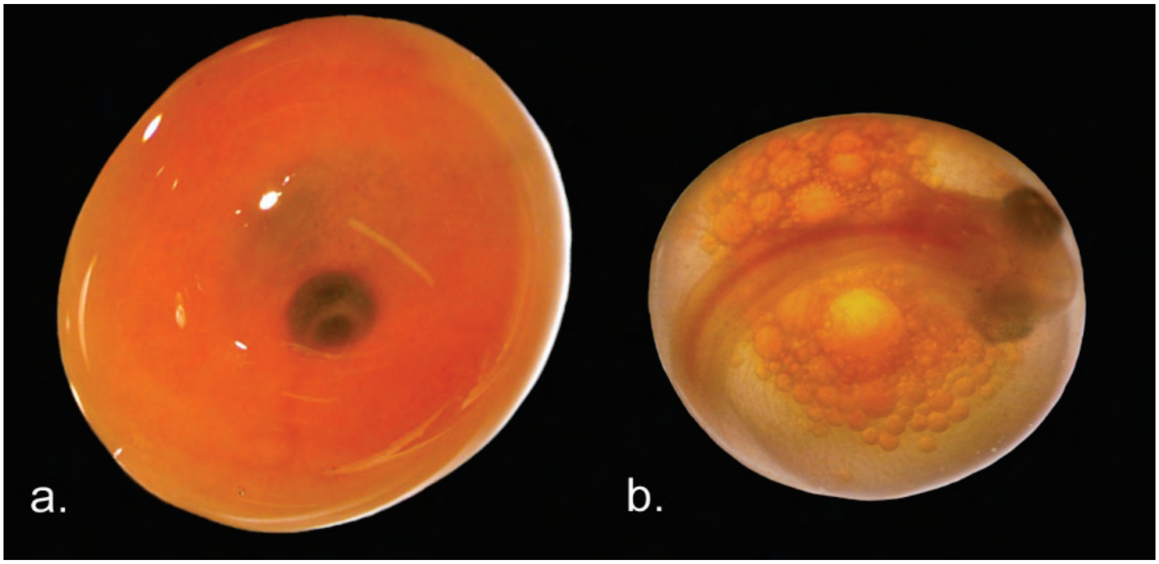

FIGURE 12.—(a) Chinook salmon egg and (b) steelhead egg.

20a. Egg looks solid and marbled (Figure 13) 21
20b. Egg has soft chorion and does not have marbled look... 22

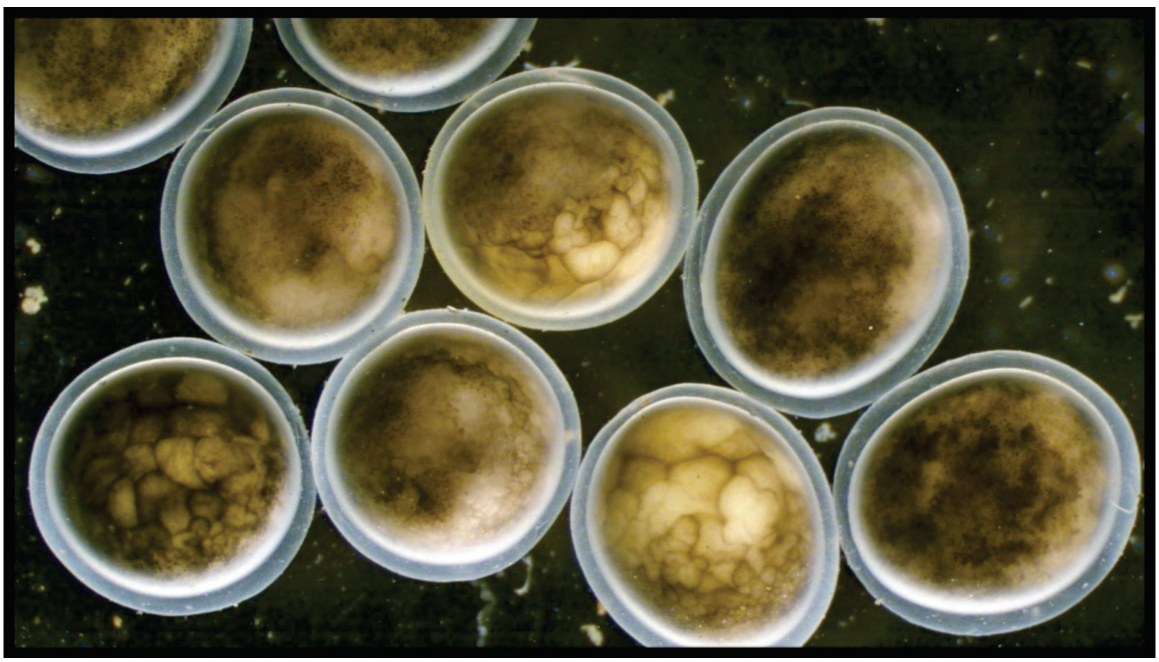

FIGURE 13.—Solid and marbled eggs.

21a. Egg is smaller (3.6–5.6 mm) and darkly pigmented (Figure 14a) .... **white sturgeon**
21b. Egg is larger (4.0–7.0 mm) and lightly pigmented (Figure 14b) .... **green sturgeon**

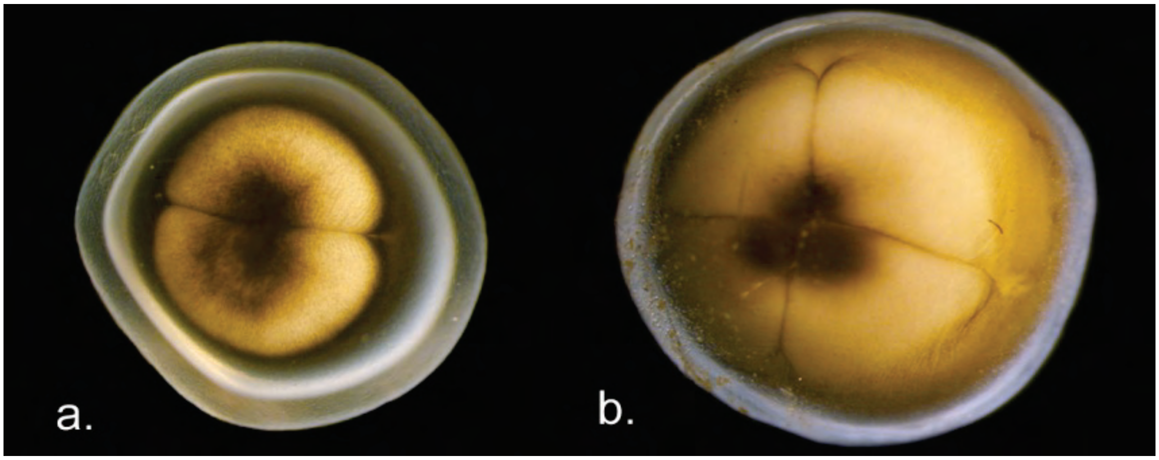

FIGURE 14.—(a) White sturgeon and (b) green sturgeon.

22a. Eggs attached to each other and deposited in clusters, parental care observed (Figure 15) .... ***Ictaluridae* spp.**
22b. Egg is deposited singly, no parental care observed 23

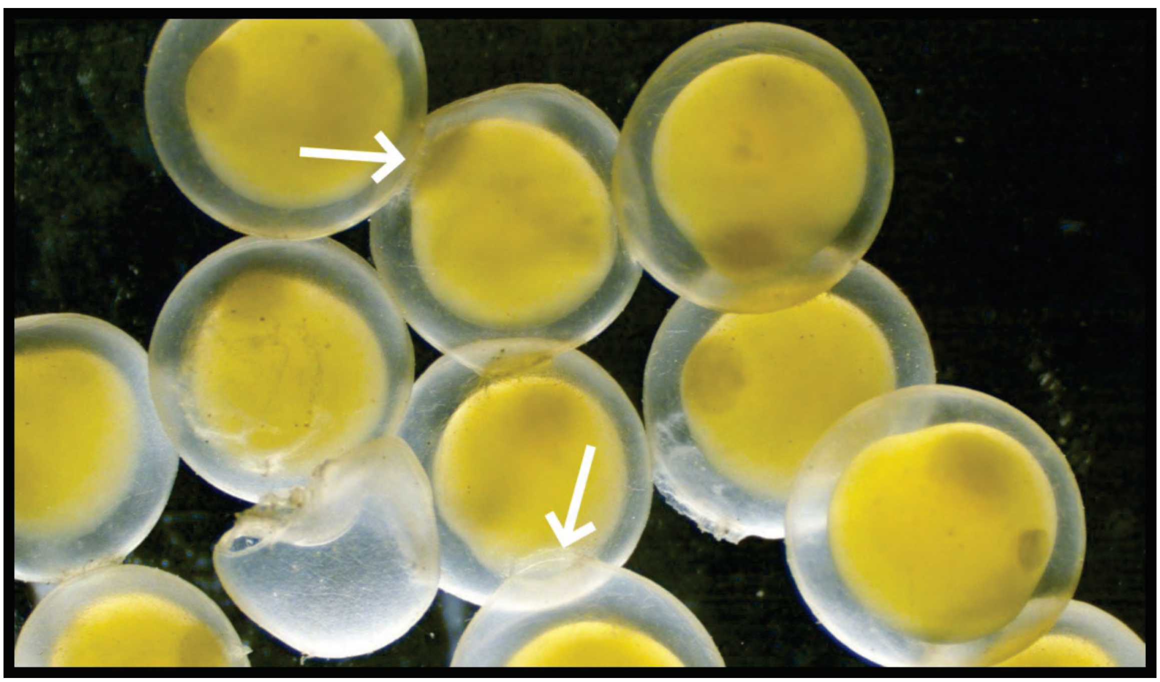

FIGURE 15.—Cluster of eggs of an *Ictaluridae* spp. Arrows point to where eggs adhered to each other.

23a. Yolk sac and embryo are pigmented but transparent, wide
perivitelline space (Figure 16) ....**American shad**
23b. Yolk sac and embryo are not pigmented and opaque 24

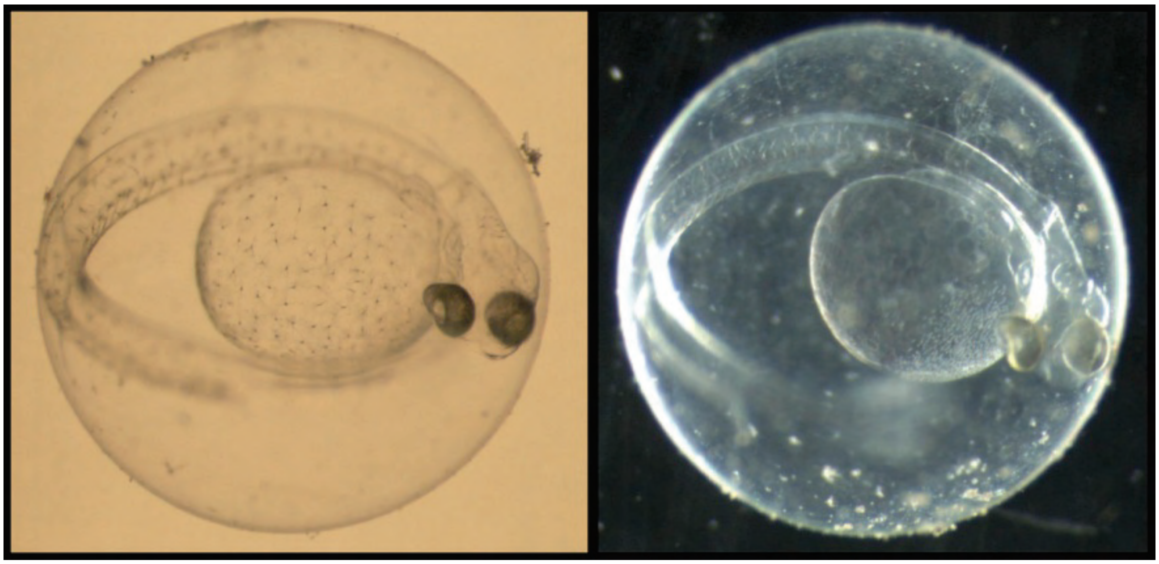

FIGURE 16.—Bright field image of American shad egg showing pigmentation on the yolk sac and the embryo (left) and dark field image of the same egg (right).

24a. Smaller egg (2.5–3.2 mm), single large yolk sac, cream
yolk (Figure 17a)...**Sacramento pikeminnow**
24b. Larger egg (3.0–3.9 mm), large yolk sac often with three
segments, yellow yolk (Figure 17b) ....**Sacramento sucker**

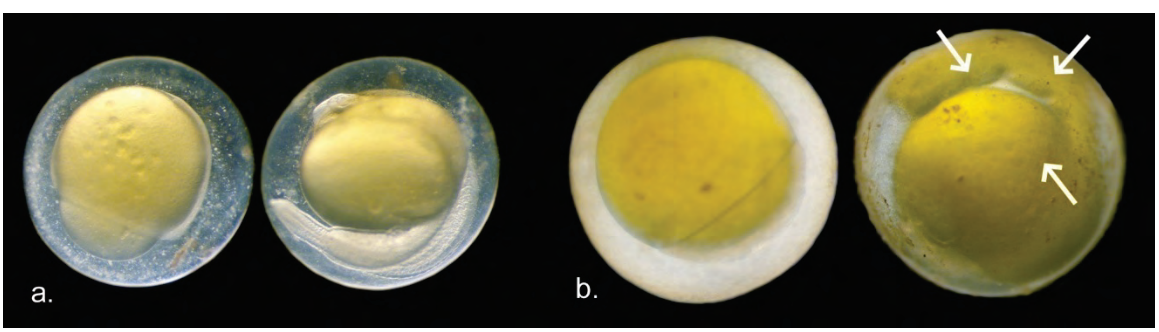

FIGURE 17.—(a) Sacramento pikeminnow and (b) Sacramento sucker egg with three yolk sac segments (white arrows).

DISCUSSION

The majority of eggs collected from the Delta and its watershed were demersal (negatively buoyant) although a few were pelagic (positively buoyant). Demersal eggs often have denser yolks with varied colors, have different types of attachment mechanisms, are deposited in various substrates, and are sometimes clustered. Pelagic eggs are transparent, have clear yolks, and have large perivitelline space (Ahlstrom and Moser 1980, Miller and Kendall 2009). Because of the various characteristics in Delta eggs, visual identification is possible.

Demersal eggs have dense yolk. Varying amounts of protein and carotenoid (for respiration) in the yolk produce visible coloration from light yellow in *Cyprinid* spp. to the bright red in *Salmonid* spp. Eggs buried in the gravel, observed in Sacramento sucker (*Catostomus occidentalis*), *Salmonid* spp., and *Acipenser* spp., were often large with large yolks that have visible vascular system and vitelline circulation. In general, larvae from these larger eggs live longer on yolk reserves (Blaxter 1988). Within the yolk sac of some Delta eggs are a single oil globule or several smaller oil globulets which contain primarily triglycerides, a specialized form of nourishment (Blaxter 1988, Heming and Buddington 1988). The presence or absence of these structures aids in species or, at least, family identification.

Attachment structures are diagnostic features for the eggs of *Gobiidae* spp., inland silverside (*Menidia beryllina*), rainwater killifish, and *Osmeridae* spp. Besides having an ellipsoid shape, the egg of the *Gobiidae* spp. has an adhesive foot on one pole made of bundle of threads or filaments. The eggs of inland silverside have filaments attached to the chorion. The rainwater killifish have very fine adhesive fibrils enveloping the chorion that are believed to aid in reducing water loss of the eggs at low tide (Brummett *et al*.1985). In *Osmeridae* spp., the outer adherent membrane of the egg inverts itself giving rise to an adherent stalk (Yamada 1963) almost immediately after fertilization (Mager *et al*. 2004). This anchoring stalk results from the rupturing and inversion of the chorion during spawning (Yamada 1963, Kunz 2004).

Egg clustering and grouping were observed in species that employed parental care. This is evident in families that are bottom dwellers, particularly the *Gobiidae*, *Cottidae*, and *Ictaluridae*, but also in littoral dwelling species such as *Centrarchidae* and a single species of cyprinid, the fathead minnow (*Pimephales promelas*). Clustering and grouping of eggs enables the guardian, usually the male (Gross and Shine 1981), to easily protect and aerate the eggs. The presence of a guarding male also significantly reduces rate of fungal and/or bacterial infection of eggs (Knouft *et al.* 2003).

Three Delta species produce pelagic eggs: striped bass (*Morone saxatilis*), American shad (*Alosa sapidissima*), and starry flounder (*Platichthys stellatus*). Like most pelagic eggs, they are transparent (Kunz 2004), spherical (Ahlstrom and Moser 1980), and have thinner lighter-colored chorion (Miller and Kendall 2009). Since these eggs are spawned in open water, transparency protects the eggs from radiation (Breder 1962) and probably aids in cloaking the eggs from predators. Striped bass have eggs that are slightly buoyant enabling it to “bounce” downstream especially during high riverflows. High flows aid in spawning and generally yield a strong striped bass year class (Stevens 1977). Striped bass eggs have an extremely large oil globule which helps the eggs be neutrally buoyant in freshwater (Mangor-Jensen *et al.* 1993). Striped bass and American shad eggs have wide perivitelline space which not only protects the developing embryo during the prehatching critical formative stages, but also acts as insulation and “liquid cushion” (Laale 1980) for the embryo during the downstream journey of the egg. Starry flounder spawn in polyhaline (18–30 ppt) waters (Wang 1986); therefore, their eggs are unlikely to be collected in the freshwater parts of the Delta.

Finally, there is a wealth of information for identifying fish eggs using scanning electron microscopy techniques and biochemical genetics. Scanning electron microscopy enables the observer to locate egg surface patterns that a light microscope cannot locate. Genetic identification can be used to verify the correctness of morphological identification (Shao *et al*. 2002, Saitoh *et al*. 2009). These techniques can aid in identifying eggs of species with overlapping morphological characteristics, especially species from the *Centrarchidae* and *Cyprinidae* families.

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Reclamation’s TFCF laboratory has been collecting information on the early life history and life stages of every fish species found in the Sacramento-San Joaquin River Delta and surrounding watershed for the past decade. This paper is a product of that continuous effort. Acknowledgments go to Dr. Johnson Wang for his invaluable comments to the improvement of the key, TFCF supervisory biologist Brent Bridges, Zachary Sutphin, Brandon Wu, Ronald G. Silva, and Reclamation librarian, Alba Scott. Finally, acknowledgments go to various individuals who provided fish eggs for the project including John Hannon (Reclamation), Brian Deason (Reclamation), John Pedretti (USFWS), Chris Miller (Contra Costa Mosquito and Vector Control), Joel Van Eenennam (UC Davis), Dr. David J. Ostrach (UC Davis), Dr. Bradd Baskerville-Bridges (UC Davis’ Fish Culture and Conservation Laboratory; FCCL), Luke Ellison (FCCL), Galen Tigan (FCCL), Dr. Joan Lindberg (FCCL), Bobby Renschler (FCCL), Peter Struffenegger (Stolt Sea Farm), James Rexroth (San Joaquin County Parks and Recreation), Donovan Ward (CDFG), Nick Cuevas (CDFG), Steve Slater (CDFG), Jerry Morinaka (CDFG), and Carol Raifsnider (Tenera Environmental).

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Appendix 1

Method of Egg Collection and Number of Eggs Measured from the Sacramento-San Joaquin River Delta, 2002–2010

APPENDIX 1.—Method of egg collection and number of eggs measured from the Sacramento-San Joaquin River Delta, 2002–2010. Substrates where eggs were collected are in parenthesis.

Species name	**Method of egg collection**	***n***
Green sturgeon *Acipenser medirostris*	strip spawning [1]	60
White sturgeon *Acipenser transmontanus*	strip spawning	329
Inland silverside *Menidia beryllina*	aquatic vegetation [2] (*Egaria densa*, filamentous algae)	145
Sacramento sucker *Catostomus occidentalis*	net [3] (gravel, cobble)	107
Black crappie *Pomoxis nigromaculatus*	—	—
Bluegill *Lepomis macrochirus*	nest [4] (cobble, riprap)	156
Green sunfish *Lepomis cyanellus*	laboratory [5] (gravel, wood)	104
Largemouth bass *Micropterus salmoides*	nest (cobble, riprap)	110
Pumpkinseed *Lepomis gibbosus*	—	—
Redear sunfish *Lepomis microlophus*	nest (cobble, riprap)	113
Sacramento perch *Archoplites interruptus*	laboratory (filamentous algae, Spawntex™)	127
Smallmouth bass *Micropterus dolomieu*	—	—
Spotted bass *Micropterus punctulatus*	—	—
Warmouth *Lepomis gulosus*	laboratory (gravel)	146
White crappie *Pomoxis annularis*	—	—
American shad *Alosa sapidissima*	net (crevices of rocks, cobble)	105
Threadfin shad *Dorosoma petenense*	aquatic vegetation (filamentous algae)	50
Prickly sculpin *Cottus asper*	substrate supplied [6] (PVC pipes)	162
Riffle sculpin *Cottus gulosus*	—	—
Pacific staghorn sculpin *Leptocottus armatus*	—	—
California roach *Hesperoleucus symmetricus*	net (gravel)	150
Common carp *Cyprinus carpio*	aquatic vegetation (*Egaria densa*)	147
Fathead minnow *Pimephales promelas*	laboratory (PVC pipes)	135
Golden shiner *Notemigonus crysoleucas*	laboratory (plastic plants/hair grass)	117
Goldfish *Carassius auratus*	aquatic vegetation (filamentous algae, *Egaria densa*)	142
Hardhead *Mylopharodon conocephalus*	—	—
Hitch *Lavinia exilicauda*	—	—
Red shiner *Cyprinella lutrensis*	laboratory (Spawntex™)	139
Sacramento blackfish *Orthodon microlepidotus*	aquatic vegetation (curly leaved pondweed, cobble)	147
Sacramento pikeminnow *Ptychocheilus grandis*	net (gravel, cobble)	67
Splittail *Pogonichthys macrolepidotus*	strip spawning	149
Rainwater killifish *Lucania parva*	laboratory (filamentous algae, submerged aquatic plants)	105
Threespine stickleback *Gasterosteus aculeatus*	aquatic vegetation, laboratory (eggs enclosed in housing made with dead leaves and debris)	200
Shimofuri goby *Tridentiger bifasciatus*	laboratory (PVC pipes, porcelain cups)	61
Shokihaze goby *Tridentiger barbatus*	laboratory (PVC pipes, porcelain cups)	44
Yellowfin goby *Acanthogobius flavimanus*	laboratory (PVC pipes)	10
Blue catfish *Ictalurus furcatus*	—	—

Species name	**Method of egg collection**	***n***
Black bullhead *Ameiurus melas*	substrate supplied (20-cm PVC pipe)	128
Channel catfish *Ictalurus punctatus*	substrate supplied (75-L plastic barrel)	183
White catfish *Ameiurus catus*	substrate supplied (75-L plastic barrel)	272
Striped bass *Morone saxatilis*	strip spawning	92
Delta smelt *Hypomesus transpacificus*	strip spawning	88
Longfin smelt *Spirinchus thaleichthys*	strip spawning	200
Wakasagi *Hypomesus nipponensis*	aquatic vegetation (feathery, dead vegetation)	230
Pacific lamprey *Entosphenus tridentatus*	nest (gravel, cobble)	350
Bigscale logperch *Percina macrolepida*	roots of submerged vegetations	165
Chinook salmon *Oncorhynchus tshawytscha*	strip spawning	20
Steelhead *Oncorhynchus mykiss*	strip spawning	20
Starry flounder *Platichthys stellatus*	—	—

[1] Strip spawning: eggs and sperm fertilized *in vitro.*
[2] Aquatic vegetation: fertilized eggs attached to aquatic plants.
[3] Net: fertilized eggs collected by fine mesh dip net or plankton tow net.
[4] Nest: fertilized eggs collected from redds or nest.
[5] Laboratory: fertilized eggs collected through aquaria environmental manipulation.
[6] Substrate supplied: fertilized eggs collected by providing suitable substrate such as barrels and PVC.
— Eggs were not collected.
n = Number of eggs measured.

Appendix 2

Range of Egg Size from the Sacramento-San Joaquin River Delta Collected from 2002–2010

APPENDIX 2.—Range of egg size from the Sacramento-San Joaquin River Delta collected from 2002–2010.

≤2.4 mm in diameter:

Species	size range (mm)
I. Silverside	0.8–1.1
Centrarchidae spp.	0.8–1.7
Pacific herring	1.3–1.6
Threadfin shad	0.9–1.2
Prickly sculpin	1.3–1.5
Riffle sculpin	2.0–2.2 *
P.S. sculpin	1.4–1.5
Cyprinidae spp.	1.9–2.2
Rainwater killifish	1.2–1.3
T. stickleback	1.5–1.8
Osmeridae spp.	1.0–1.1
Pacific lamprey	1.4–1.6
Bigscale logperch	1.3–1.5
Starry flounder	0.9–1.3**

* Wang (1986)
** Orcutt (1950)

≥2.5 mm in diameter:

Species	size range (mm)
Green sturgeon	4.0–7.0
White sturgeon	3.5–5.6
S. sucker	3.0–3.9
American shad	2.5–4.4
S. pikeminnow	2.5–3.2
Black bullhead	3.1–3.7
Channel catfish	3.5–4.9
White catfish	3.9–4.1
Striped bass	2.5–4.2
Chinook salmon	6.0–9.0
Steelhead	3.0–6.2

Appendix 3

Flowchart for Identifying Eggs from the Sacramento-San Joaquin River Delta

APPENDIX 3.—Flow chart for identifying eggs from the Sacramento-San Joaquin River Delta.