02-P31-FR_October2005_Delta_Smelt_Culture_and_Research-T4

Delta Smelt Culture and Research Program
Final Report: 2003-2005

by

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CALFED Bay-Delta Program
Project Title: Delta Smelt, *Hypomesus transpacificus*, Culture and Research Program
RA #: ERP-02-P31
Agreement #: 4600002881

October 2005

I. Executive Summary

The development of fish culture methodologies for the *threatened* delta smelt has improved markedly over the last few years. Within the current term of funding (2003-2005) significant improvements in spawning efficiency were realized. The culture of delta smelt is a technical and intensive process, but the methodologies are now reliable, resulting in a stable and predictable supply. The expanded facility has a capability of producing upwards of 20,000 adult fish annually from 2000 wild-caught delta smelt. The current bottleneck is no longer particularly difficult life stages, but funding.

Delta smelt are captured each fall from the wild to help prevent genetic divergence from the wild stock. As the numbers of delta smelt have declined sharply in the wild, the number of eggs recovered per female has continued to increase in the laboratory. The number of eggs produced and number of hatched larvae per female increased from 450 eggs and 223 hatched larvae per female in 2000-2002, compared to 640 eggs and 440 hatched larvae per female during 2004-2005. Excellent hatch rates were also obtained during 2004-05 (65-69%). Gains were due to the development of *in-vitro* fertilization for delta smelt and development of a new incubator design. *In-vitro* fertilization enables advanced planning of spawns and better management of the broodstock. This allows spawns to be restricted to relatively few days and eliminates a considerable amount of labor, which was necessary to clean numerous small batches of naturally spawned eggs. New eggs incubators tested this year also contributed to the high hatch rates

Fish produced with the support of CALFED funding (or previously with the support of IEP and DWR) were not earmarked for a particular group of researchers, but were available to the whole research community. The reliable supply of cultured delta smelt expedites the scientific process. Cultured delta smelt have been supplied to over 11 different research projects (1999-2003) with many of the projects requesting up to 1,000-2,000 juveniles for their studies. Fish are often requested for several consecutive years to complete the research.

With the rapid decline in the delta smelt abundance index for over the last three years we recommend continued support for a limited number of delta smelt (ca.5000 adults; equivalent to 10,000 40mm juveniles, 20,000 20 mm juveniles, or 40,000 newly-hatched larvae) to be produced each year for the research community. Cultured smelt, with known rearing history, are required for toxicology studies. Cultured smelt also represent a safeguard against the possibility of species extinction. Research efforts that require large numbers of delta smelt can plan for additional funding to support a dedicated supply. As of October 31, 2005, funding for the production of delta smelt for the general research community ends – there will no longer be a reliable supply of smelt to meet the various research needs, until new funding becomes available.

II. Project Objectives

The purpose of this project is to refine methods for the successful culture of delta smelt so that a reliable supply of all life stages is available to the research community. Culture of delta smelt is essential for the studies of reproductive and developmental biology, physiological ecology, toxicology, and conservation biology of this species. A decline of delta smelt abundance in the 1980's and lack of recovery in the '90s resulted in the listing of delta smelt as *threatened* (Moyle et al 1992). Abundance indices for delta smelt have declined sharply over the last three years and are at a record low for 2004. This decreasing population trend has also been observed for other pelagic species of the Delta over the same time period. Low abundance and *threatened* status limits the number of wild delta smelt available for research and there is concern that the abundance of this indicator species will further decline in the future (Bennett 2005). The development of reliable culture methods and a cultured supply of delta smelt represent a safeguard against the possibility of extinction. A continuing effort is made to retain genetic variation in the cultured fish by collecting broodfish each year from the Sacramento – San Joaquin Delta, rather than retaining and spawning first and second generation cultured fish.

The CALFED Bay-Delta Program and other agencies consider the recovery of this and other at-risk species a priority to ecosystem restoration. However, restoration goals are often hampered by lack of information on the life history of at-risk species. The CALFED Ecosystem Restoration Program (ERP) goals are met in the creation of a supply of all life stages of delta smelt for state and federal research programs. The current work addresses 3 of the 8 priorities of the CALFED ERP and PSP for the Delta and Eastside Tributaries Region:

1) Developing a better understanding of the life history of delta smelt.
2) Providing a unique stock of this native fish with known rearing history - required for delta contaminant studies (toxicological research).
3) Providing a supply of smelt to test methods for reducing the impact of water diversions in the Delta, such as new fish screen designs, louver efficiency, "10-minute count" screens.

III. Introduction

The Fish Conservation and Culture Laboratory (FCCL) is a research and development facility located in the south Delta on State Water Project land near Byron, CA. This project was supported by state and federal agencies (DWR, CALFED and IEP) over the last several years. The research program was initiated to develop a methodology for the culture of delta smelt to provide live animals for research, without further depleting the wild population. Captive smelt were sought by investigators working in a variety of areas, including fish screen design and testing contaminant toxicity and physiological tolerance. Culture represents the only reliable means to create a supply of these fish for research as the capture and survival of this delicate fish from the wild is difficult at the adult stage and at the juvenile and younger stages it is nearly impossible.

Experimentation over the past two years has increased our production capabilities and we have contributed information on the basic biology and life history of delta smelt. This knowledge improves predictability of smelt performance in culture and may aid in predicting smelt performance in the field as well. During the course of this project, over 75,000 delta smelt were provided to state and federal agencies in support of delta smelt research. Cultured delta smelt also serve as a safeguard against the possibility of extinction of this *threatened* fish endemic to the Delta. We are the first laboratory to close the life-cycle of smelt in captivity, rearing fish under controlled conditions of the environment and husbandry. Four generations of delta smelt have been reared on site to confirm feasibility of the ex-situ conservation.

The primary goal of this two year project was to improve efficiency of delta smelt culture protocols, in order to produce a reliable supply of all life stages for research. Low survival from hatch to a juvenile stage initially limited the production of delta smelt. However, survival rates have markedly improved in recent years due to advanced broodstock management, disease prevention, and larval rearing techniques (Swanson et al., 1996; Antonio et al., 2000; Baskerville-Bridges et al., 2001; Mager et al 2004). Continued support during 2004 and 2005 allowed the increase of survival from larval to the adult stage. This was largely achieved by improving husbandry techniques for larval and juvenile rearing. Production of delta smelt was also enhanced through improvements of broodfish management (in vitro fertilization techniques) and new incubator designs. In this report we summarize the improvements made during 2004 and 2005.

Each year, in addition to culturing delta smelt, we conduct studies to further our knowledge and improve culture production and efficiency. Over the current two-year funding term (2004-2005) we developed a new spawning technique, improved upon our egg incubation techniques, and conducted a weaning trial. In the weaning trial we measured growth and mortality as two size classes of larvae were switched from the live prey to a prepared commercial diet. The primary goal of this two year project was to improve efficiency of delta smelt culture to sustain a reliable supply of all life stages of the fish for research.

IV. Collection of broodfish

Sub-adult delta smelt were collected in 2004 and 2005 from the Sacramento-San Joaquin Estuary, in the area from Rio Vista to Chipps Island for rearing brood stock at the FCCL. Delta smelt tend to congregate from the late fall to mid-winter in the lower Sacramento-San Joaquin Estuary. To reduce collection mortality, collections were postponed until ambient water temperatures declined below 12^{o}C (typically November) and fish were 50-60 mm fork length. All fish were caught using a lampara net near Decker Island in the lower Sacramento River. We fished in open water (25-30' deep) targeting the surface waters while avoiding snags on the bottom. Catch was higher during the early morning hours and appeared to decrease in the afternoon with sunny conditions.

Delta smelt were retrieved from the lampara net using small dip nets. They were quickly processed and placed into large black carboys (120-L) at a density of 4/L. Salt (5-7 ppt) and "stress coat" (polymer used for water conditioning) were added to the water to reduce stress during transport (Swanson et al. 1996). The containers were oxygenated and then capped for transport back to the FCCL.

Delta smelt were collected each fall as sub-adults and over-wintered to spawn the following spring. A total of 2,325 delta smelt broodfish were collected from the field to support the CALFED project during 2004 (1,420 delta smelt collected in 2003) and 2005 (905 delta smelt collected in 2004; Table 1). Delta smelt are extremely sensitive to capture and handling stress. Survival after 72 hours acclimation was between 64-71% on average for the two years. Most of the initial fatalities were thought due to capture and transport stress as mortalities generally ceased within 72 hours. Survival has gone up each year with more experienced staff and improved methods. In 2004 the fish appeared to be scarcer in the wild and it was difficult to find areas with large numbers of delta smelt. The catch per unit effort in 2004 was half that of 2003 (22 vs. 44 delta smelt/set, respectively).

Table 1: Number of wild-caught delta smelt and 72 hour survival from Nov-Dec 2003 and Nov-Dec 2004. Delta smelt were collected in the lower Sacramento River near Decker Island (38:06:47.9N, 121:42:28.2W)

Collection year	Total collected	Survival after 72 hours	
		(#)	(%)
2003	1420	909	64
2004	905	642	71

All incidental catch was released as quickly as possible to minimize injury and stress. By catch consisted primarily of threadfin shad and American shad (92.5%) (Table 2). Silversides accounted for another 3.6% and the remaining species for 3.9% (longfin smelt, wakasagi smelt, Chinook salmon juvenile, shimofuri gobi, striped bass, largemouth bass).

Table 2: Abundance of by-catch caught in the net during 2003 and 2004 field collections for the 2004 and 2005 spawning season, respectively.

Species Collected	2003	2004
American shad	912	13
Chinook salmon juv.	0	2
Inland Silverside	36	25
Largemouth bass	2	0
Longfin smelt	6	45
Shimofuri gobi	0	1
Striped bass	1	2
Threadfin shad	401	251
Wakasagi smelt	3	5

Captive fish acclimated well to flow through tanks (raw delta water) at the FCCL and few failed to make the transition to a dry diet (<5%). These typically died during the winter months (Nov-Jan) leading up to spawning.

V. Maintenance of broodfish

Wild caught delta smelt were reared at the FCCL from November through May in flow through systems equipped with 1000-L tanks (1.5 m diameter; 0.6 m height). Water inflow was maintained at 8-10 L/min/tank and the delta smelt were exposed to a natural photoperiod (outdoor tanks) or simulated artificial photoperiod with fluorescent lighting (indoor tanks). The outdoor tanks with a natural photoperiod yielded higher production and better quality eggs, compared to indoor tanks with artificial lighting; fish held indoors also experienced a one month delay in spawning. Holding the broodfish outdoors simulates more natural conditions and is now being done exclusively.

The broodfish were fed a mixture of two dry diets (Lansy and Hikari plankton) at 1-2% body weight per day. The food was distributed every hour using vibratory feeders. All tanks were also hand fed to better monitor and adjust feeding rates as needed. The broodfish tanks were cleaned daily to remove uneaten food and debris; they were siphoned and wiped down thoroughly. Working with the fish on a daily basis allowed us to closely monitor their status so that we were aware of their spawning condition.

The male to female ratio among our wild broodstock has consistently been close to 1:1. Growth rates of fish during the past two seasons were also quite consistent (Fig. 1). Fish collected during November-December during 2003 and 2004 averaged 55 mm and 59 mm, respectively. By the end of the spawning season, fish grew to a fork length of 70 mm.

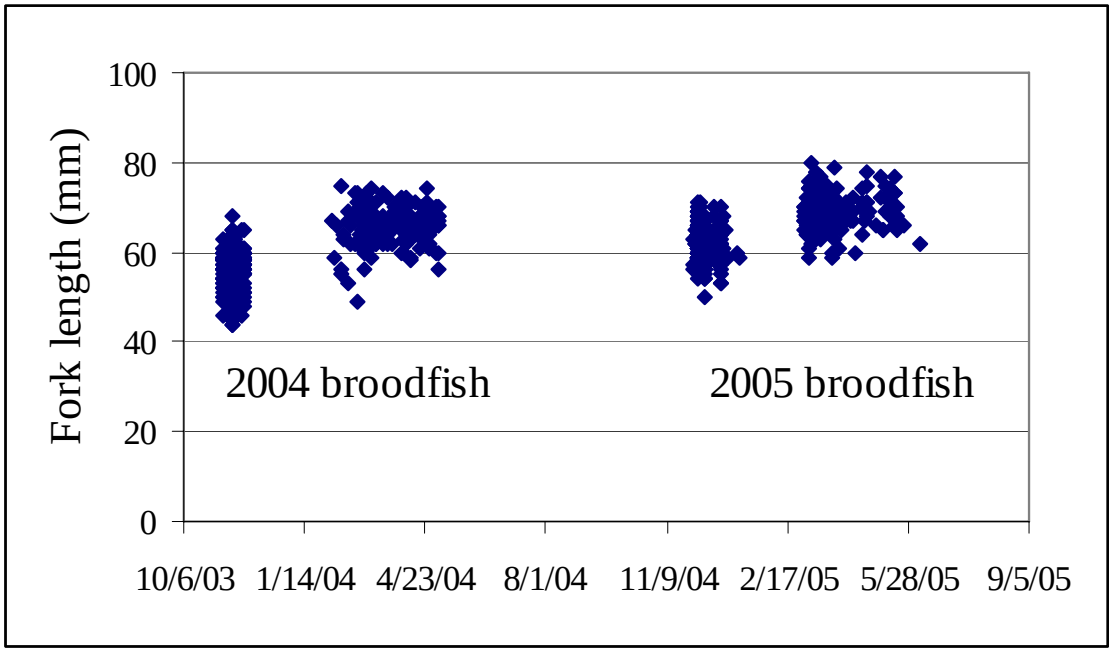

Figure 1: Fork length of wild broodfish from the 2004 and 2005 spawning seasons.

VI. Spawning

Spawning began in February when water temperature approaches 12-15^{o}C and fertilized eggs were consistently produced through April. Eggs collected later in the season were often poorer quality, resulting in lower hatch rates. Delta smelt broadcast their eggs along the bottom of the tank during the night and early morning hours. The eggs are always present as a single layer on the tank bottom and are deposited in areas associated with high flow (near the water inlet or the airstone). Eggs have not been observed on the walls of the tank. Naturally spawned eggs were collected each day by scraping them off of the tank floor; a total of 76,881 eggs were collected from the tanks during 2004 and 2005 seasons (Table 3). Percent hatch of naturally deposited eggs (36.2%) was considerably lower than that of strip spawned eggs (72.5%). Fish spawning naturally in the tanks may release eggs in response to one or more stressors, as we noted many of these egg batches were not fertilized. Overall the quality of eggs manually expressed from the females and fertilized *in-vitro* appears to be better than eggs spawned naturally in the tank. This technique is now the preferred method of spawning eggs for culture of delta smelt for reasons discussed in the next section.

Table 3: Percent hatch of delta smelt eggs in natural and in-vitro spawning during 2004 and 2005.

	Eggs	Larvae	% hatch
2004 Season			
Naturally spawned eggs	70,963	26,972	38.0
Strip-spawned eggs	197,388	148,410	75.2
Total	268,351	175,382	65.4
2005 Season			
Naturally spawned eggs	5,918	888	15.0
Strip-spawned eggs	218,917	153,502	70.1
Total	224,835	154,390	68.7

VII. *In-vitro* fertilization

A significant proportion of the captive wild-caught delta smelt do not spawn each year. Therefore, considerable effort has been spent over the last two years investigating the feasibility of manual expression of eggs and in-vitro fertilization. This was done in an attempt to improve egg harvest and better manage our limited broodstock. Brood fish were examined weekly for ripe eggs and running milt and sorted into two containers. Eggs from one female were expressed into a small plastic dish. They were fertilized in vitro with sperm from multiple males.

In-vitro fertilization improved our production of eggs and egg quality in the following ways:

1. Total egg harvest appeared to be higher when strip spawned, as many females tend to retain ripe eggs, resulting in over-ripeness

2. Strip-spawned eggs were cleaner. Naturally deposited eggs collected from the tank also contained food and feces, which is difficult to remove.

3. Scheduled removal of gametes streamlines egg harvest and provides control of egg production (once per week).

4. Managing the broodstock resulted in the ability to produce large batches of eggs on a pre-determined day, rather than collecting daily small batches of eggs.

Each week all of the broodfish were checked to examine their degree of ripeness; to estimate the stage of ova development. Females are generally easy to identify by their larger body size and distended abdomen. Males are more slender and should be ripe with milt by the end of January. The best sexual characteristic to distinguish males from females is spawning tubercles on males. These are visible on close examination as minute elevated tubercles located on top of the head region and along the sides and back of males. The fish can be netted into small dishes and hand sorted. Females that are close to spawning were held together in a single tank, to spawn in the near future. When eggs were easily expressed with gentle pressure, the females were saved in a black bucket. Males were also collected ahead of time, and held in a black bucket, to fertilize the eggs.

A. Procedure for egg removal and in vitro fertilization

A ripe female was removed from the holding bucket, anaesthetized (100 mg/L MS-222), and dried gently with a paper towel. Her eggs were gently expressed into a small plastic dish. Milt from two to three males was also expressed into the dish. Water was added to activate the sperm and the dish was quickly mixed so that the eggs separated and spread out along the bottom of the dish. After 10 minutes, the incubation dishes were rinsed to remove excess milt, refilled, and placed into water baths and maintained at 15^{o}C.

Incubation dishes were rinsed daily by pouring off the water and refilling with clean water. On the fourth day, the eggs were de-adhered by gently rubbing the eggs. Dead eggs were removed (picked) and a volumetric count was made to determine the quantity of eggs collected. The volumetric measuring device was made using a pipette and can measure up to 10 mL eggs. There are approximately 990 $\pm$ 3.4 eggs per/mL (mean $\pm$ standard error). On average 1,280 $\pm$ 34 healthy larvae were obtained from each "strip spawned female"; note not all females spawned during the season.

VIII. Production of delta smelt

Growth rate and survival of delta smelt have increased substantially over the past several years. Over the previous term (1998-2003, with support from CALFED) we were able to determine the best rearing temperatures (Baskerville-Bridges et al 2004) resulting in faster growth rates as compared to initial efforts (1992-1997) and current growth rates are more comparable to the wild population. Since then, we have been able to manipulate temperature to increase or slow growth rates if necessary. Most of the larval and juvenile tanks however, are maintained at moderate temperatures (15-17^{o}C) throughout the year to minimize the incidence of disease. Growth, in terms of length (hatching to 20 mm – measured total length; fish >20 mm – measured fork length), is best illustrated by a linear relationship and has been consistent over the past couple of years (Fig. 2).

Ambient temperatures at the FCCL fluctuate drastically throughout the year and are manipulated at the facility to prevent rapid temperature spiking. During the summer months, the temperature can approach 30^{o}C (July). Fish held at ambient water temperatures (slowly warming) tolerated elevated temperatures reaching 28^{o}C when the change was not too drastic. These fish were held at low stocking densities to prevent compounded stress. However, chronic mortality was observed when temperatures increased past 28^{o}C.

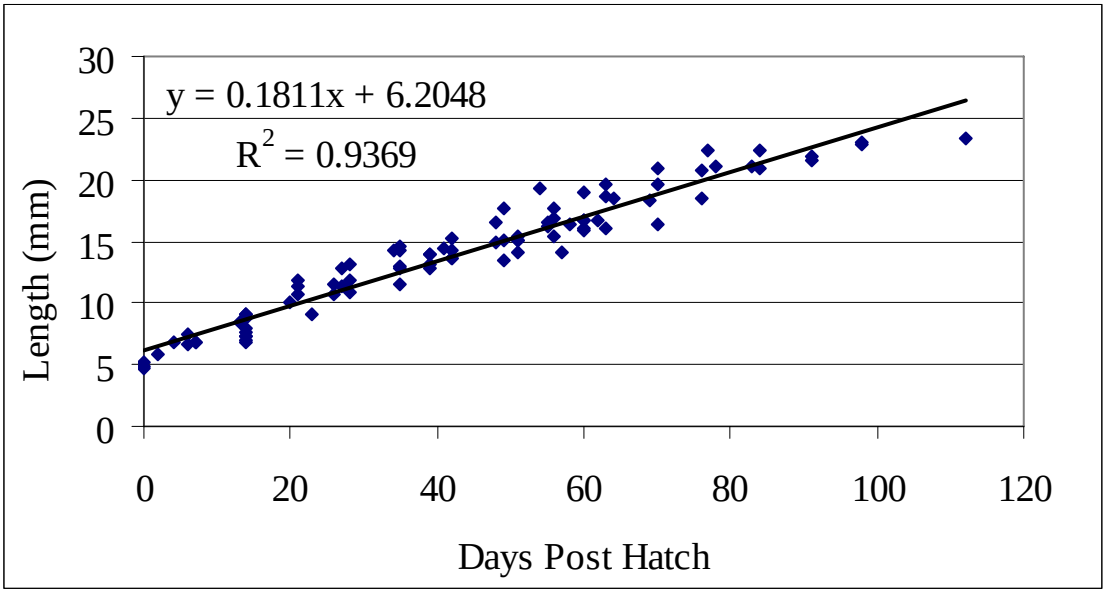

Figure 2: Linear relationship between size of delta smelt (length) and age (days post hatch). Total length was measured from hatching to 20 mm. Fork length was measured on fish greater than 20 mm.

Survival of each life stage is estimated by sub-sampling several “batches” of spawned eggs and following them through the egg, larval, and juvenile life stages. Each batch of eggs is tracked for percent hatch, and larval and juvenile growth and survival while each “batch” is being transferred to consecutively larger tanks. These designated batches of fish are sampled at regular intervals to estimate growth and survival of the population of fish the sub-sample represent. From these population sub-samples we can estimate the number of fish of each life stage produced (Fig. 3). Survival from hatch to 20 mm is typically close to 50%. Many of the smaller larvae die at an early stage of development and are already broken down within 24 hours. Survival from 20-40 mm is further reduced by 50%. During this phase it is crucial to reduce the density of fish, as they do not tolerate the high stocking densities that can be used during the larval period. If they are not thinned, fish are likely to become diseased, resulting in a loss of the majority of fish affected. *Columnaris flexibacter* has been identified at the cause of this “white head” disease and is devastating to 20-40 mm fish. Preventative maintenance is the best way to deal with the problem. Careful monitoring of the fish and maintaining proper water quality is imperative. Mortality after 40 mm is minimal and usually results from human error or system failure. Production of delta smelt has increased dramatically over the past several years. The number of newly hatched larvae has gone up each year along with an increase in the survival of each life stage (Fig. 3).

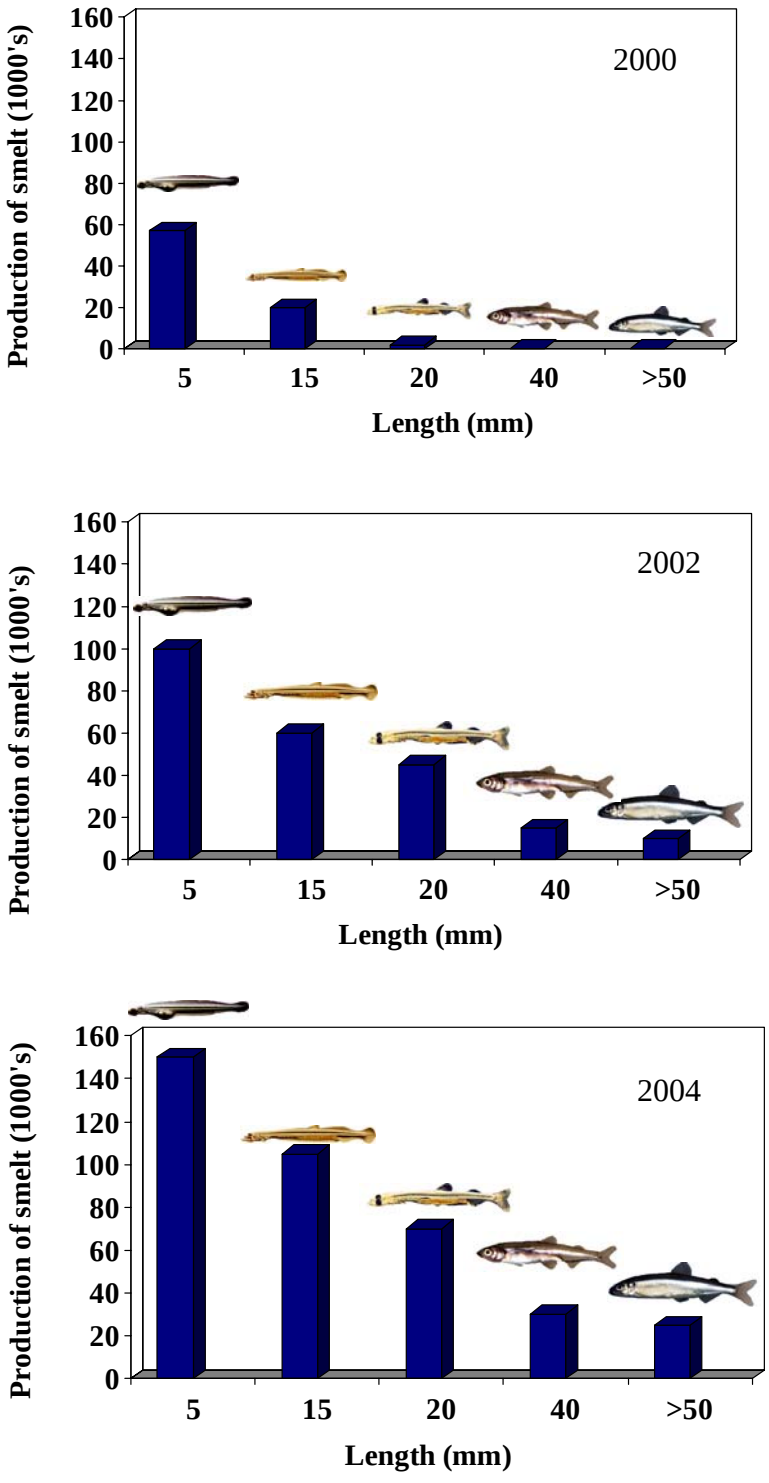

Figure 3: Production of delta smelt during 2000, 2002, and 2004 research seasons.

IX. Use of cultured smelt in research 1999 - 2005

The FCCL has provided live and preserved delta smelt of all life stages to numerous state and federal agencies over the past several years. They have been used in studies to provide valuable information on topics ranging from management studies to basic science. Below is a list of many of the projects that received smelt from our facility during the previous grant period (1999-2003).

1. J. Kozlowski (Jones and Stokes): 2003. Fish bypass system at the Banta-Carbona Irrigation District's fish screen bypass system.

2. L. Grimaldo (DWR): 2000 and 2001. Development of a diagnostic key for discriminating Osmerid larvae

3. Tracy Fish Collection Facility (US Bureau of Reclamation): 2000, 2001, 2002, and 2003. Development of a methodology for tagging test fish

4. B. Bennett (BML, UCD): 2001 and 2002. Predation of delta smelt by Inland Silversides on Delta Smelt

5. B. Bennett (BML, UCD): 2002 and 2003. Feeding rate of delta smelt larvae on copepod nauplii

6. J. Cech (UCD): 1999, 2000, 2001, and 2003. Fish treadmill study

7. B. Bennett (BML, UCD): 2001, 2002, and 2003. Role of Contaminants in the Decline of Delta Smelt in the Sacramento-San Joaquin Estuary

8. Tracy Fish Collection Facility (US Bureau of Reclamation): 2000, 2001, 2002, and 2003. Testing of fish friendly pumps

9. Tracy Fish Collection Facility (USBR): 2002 and 2003. Louver efficiency

10. B. Finlayson (CDFG): 2002 and 2003. Toxicity testing of herbicide monitoring program

11. J. Cech (UCD): 2003. Trash rack study

Requests for cultured delta smelt continued in 2004 and 2005 and over 75,000 fish were provided for research (Table 4). Fish were used at the TFCF and some were shipped to Denver, CO for testing in models. The Pelagic Organism Decline (POD) working group (organized by Interagency Ecological Program, IEP) also requested fish this year and the FCCL provided juveniles for toxicity work during 2005. The toxicity studies tested whether water samples from the Delta, taken from regions where chemicals were

sprayed, were toxic to delta smelt (Inge Werner, University of California at Davis). This group will also investigate whether there is a relationship between the toxic effects and the presence of *Microcystis* toxins.

Table 4: Total number of each life stage of delta smelt supplied for research during 2004 and 2005.

Project	Agency	Larvae (<20 mm)	Juveniles (20-50 mm)	Adults (>50 mm)	Total
2004					
10-min count screen study	USBR/TFCF	0	1,184	0	1,184
Transport study	USBR/Denver	5,200	0	0	5,200
Fish screen design	USBR/TFCF	0	5,218	4,832	10,050
Total		5,200	6,402	4,832	16,434
2005					
Phototonic tagging study	USBR/TFCF	0	0	800	800
Full facility evaluation	USBR/TFCF	0	0	3,347	3,347
Fish screen design	USBR/TFCF	56,514	0	0	56,514
Toxicity studies	UCD	0	619	0	619
Total		56,514	0	4,147	61,280
Grand Total					**77,714**

X. Summary of culture protocols

The primary goal for this program was to develop a reliable and technically feasible method for culturing all life stages of delta smelt. The methodology was developed over several years and is continually updated. Below is a brief summary of the care needed for raising newly hatched larvae to the adult stage. A more detailed explanation is available in the updated version of the Delta Smelt Culture Manual (Baskerville-Bridges et al. 2005).

Newly hatched larvae are placed into 120-L tanks (black circular tanks) at a stocking density of approximately 40/L (5,000 larvae/tank). The larvae are reared in recirculation systems, which provide more control over the water quality and temperature. Water temperature is maintained at 15-17^{o}C with an exchange rate of 2-L/min and gentle aeration. Each larval tank is fitted with a spray bar over the water surface and water flow is directed almost straight down for the first 30 days, after which it is redirected to create a gentle current. The larvae will quickly swim into a gentle current. Flow is reduced if the larvae are unable to make headway against the current.

The larvae are fed rotifers starting on 4 days post hatch (dph) until 40 dph at a prey density of 10/L. Newly hatched *Artemia* nauplii are fed to the larvae on 10 dph and are switched to enriched *Artemia* nauplii at 20 dph (4 nauplii/L). The larvae are fed five times throughout the day to ensure that sufficient prey organisms are available: (0800, 1000, 1200, 1400, and 1600 h). Concentrated alga paste (Premium 3600 Nannochloropsis; Reed Mariculture, San Jose, CA) is added to the tanks to maintain turbid conditions. Tanks with first- feeding larvae are kept more turbid (25 NTU) than those for older larvae (ie. 15 mm, 10 NTU). Between 50 and 60 dph the larvae are transferred to the juvenile system; they should be approximately 15-18 mm. Water to water transfer is important during this stage, as they do not tolerate handling with a dip net.

Juvenile tanks (400-L black circular tanks) are stocked at much lower densities (10/L). Juveniles are maintained in these larger tanks until they reach a size of 30 mm (120 dph). They should be actively feeding on Artemia and collectively swimming into the current. Fish larger than 30 mm can be graded and transferred to the adult facility. Soft dip nets work well to catch the larger juveniles. A turbidity of 5-10 NTU is maintained to ensure that they are all feeding well.

The adult facility is equipped with black fiberglass tanks (800-L circular tanks). Juveniles are weaned at a fork length of 30 mm to a dry diet. They are maintained in these tanks until they are raised to an adult size (>50 mm). At this stage of development, the fish will feed at very low turbidities and it is not necessary to add algae to the systems. Adult fish are hardier and can be moved to the flow-through systems or holding facility (raw-delta water) where they are maintained until needed for various research programs.

XI. Weaning experiment

A weaning experiment was conducted to determine how early delta smelt could utilize a dry diet. Early weaning would reduce our dependence on live prey which is expensive and labor intensive to rear. The experimental data would also provide insight regarding the digestive capabilities of delta smelt larvae and juveniles.

Delta smelt larvae were added to nine larval rearing tanks (120-L) in a recirculation system. After acclimating for three days, the experiment was initiated with a stocking density of 2.5 larvae/L (300 larvae/tank; 51 dph; 15 mm). Water exchange rates were adjusted to 2-L/min and gentle aeration was administered using small airstones. Water temperature was maintained at 17^{o}C throughout the experiment.

The nine tanks were randomly assigned to one of three weaning strategies (n=3). In the first treatment, three tanks were fed a dry diet from the start (early weaning; 51 dph). The second group of tanks was weaned later (late weaning; 95 dph) and the remaining group of tanks was fed a live diet, consisting exclusively of *Artemia*. At weaning, *Artemia* were co-fed with the dry diet for three days and then discontinued. The quantity of *Artemia* decreased each day by 25% (Day 1, 75%; Day 2, 50%, and Day 3, 25%), relative to the live prey controls. This gradual weaning technique is used to facilitate transition to the new diet type. The live-prey control groups were fed *Artemia* nauplii five times per day (0800, 1000, 1200, 1400, and 1600 h). Sufficient prey was fed to satiate the larvae (3-5 nauplii /mL).

Samples were taken for length measurements on 51 dph, 67 dph, 95 dph, 107 dph, and 129 dph to investigate the effects of weaning time on growth of delta smelt. All individuals were measured and preserved for future examination. All tanks were siphoned every two days to remove uneaten food, debris and mortalities. Mortalities were recorded from each tank to calculate percent survival over time. At the end of the experiment all fish were counted to verify that all fish were accounted for.

Larvae were similar in size for the first 20 days of the experiment, but growth rates began to diverge by 95 dph; larvae weaned at 15 mm were larger than the other two groups (Fig. 4). By the end of the experiment, the live prey control caught up with this group and fish from both treatments were larger, compared to the late weaning treatment (weaned at 20 mm).

High mortality was observed when the diet was introduced early (weaned at 15 mm; Fig. 5). Although the fish appeared to be ingesting the diet (diet appeared to be palatable), few larvae (5%) survived to the end of the 129 day experiment. Survival of fish in the late weaning tanks (weaned at 20 mm) was much better (38%) and was more comparable to the live prey control (48%). Early weaning may not be suitable for delta smelt, given the current diets available.

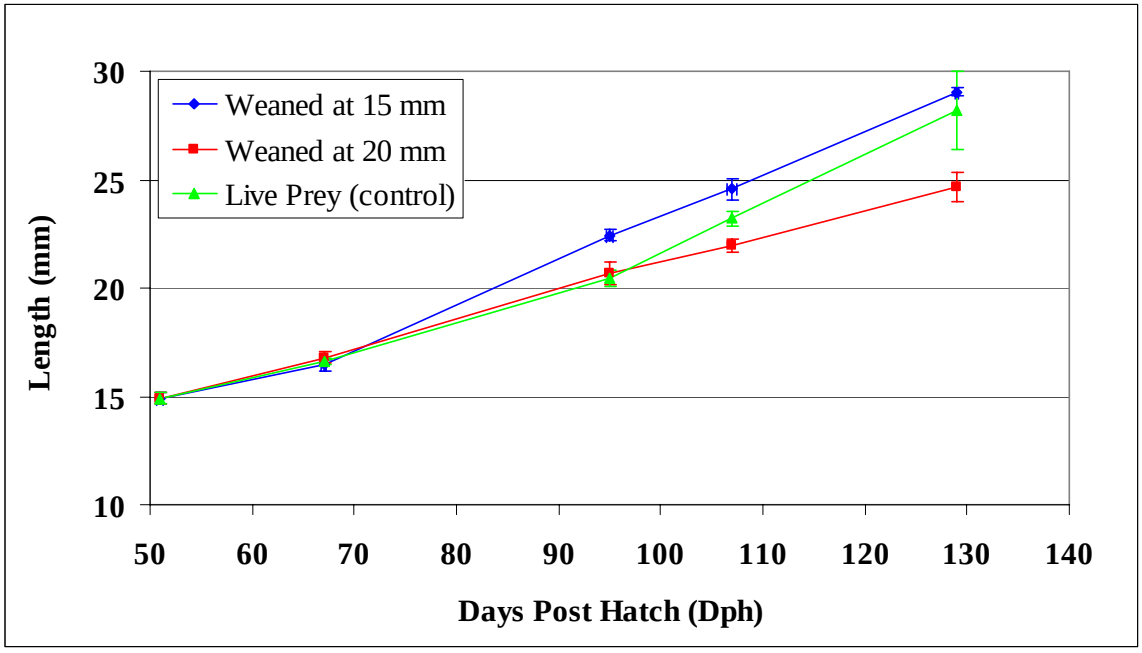

Figure 4: Length of delta smelt weaned at 15 mm, 20 mm, or fed exclusively on a live prey control.

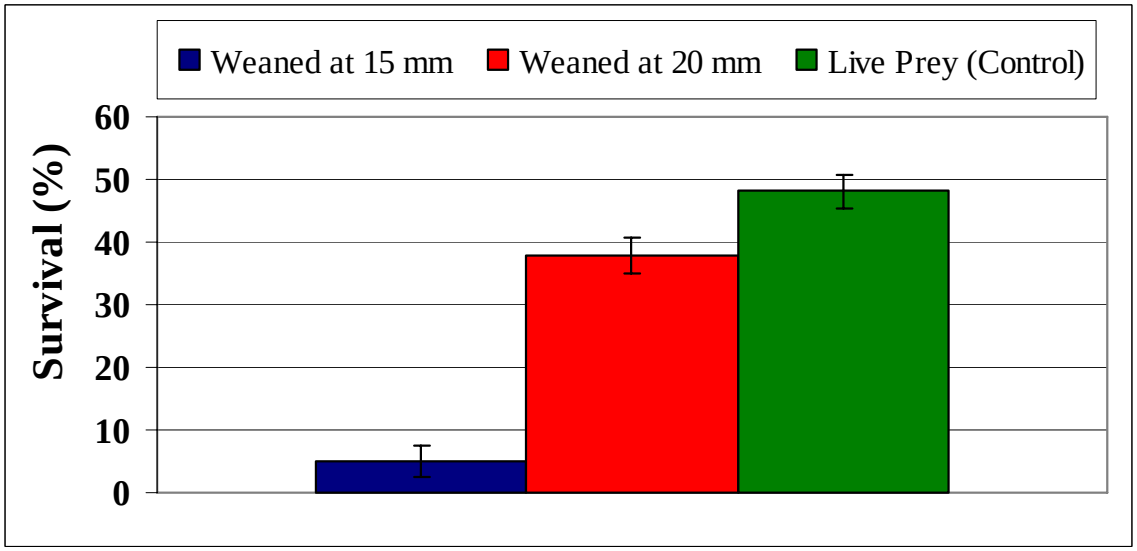

Figure 5: Survival of delta smelt weaned at 15 mm, 20 mm, or fed a live prey control.

XII. Conclusions

With the support of CALFED we were able to meet and exceed the stated objectives at the Fish Conservation and Culture Laboratory. We have improved the reliability and efficiency of delta smelt culture considerably over the two-year funding term. The development of a successful strip spawning (in-vitro fertilization) technique is perhaps the single most important fish production improvement over the recent term. The FCCL is equipped to handle higher production of all life stages and the fish are being reared more efficiently making many thousands of fish available for research each year.

All proposed goals for this project have been completed:

1. Information was gained related to the early life history of delta smelt. Digestive capabilities were investigated through a series of weaning trials. Work was performed to investigate their dependence on turbid conditions throughout ontogeny. Observations were recorded regarding the ability of juveniles to tolerate elevated temperatures.

2. Fish of known rearing history have been produced for toxicological studies. The POD working group is scheduled to use fish during 2006 (if funding becomes available).

3. Over 50,000 test fish were made available to researchers investigating methods for reducing the impact of water diversions in the Delta. Investigations included whole facility evaluations, louver efficiency testing, and analysis of the 10-minute count screen.

The support of CALFED, IEP, and DWR has enabled the development of culture techniques for delta smelt. Now that reliable culture techniques have been developed it is important to ensure a stable supply of all life stages of this *threatened* fish for research. The 2004 delta smelt abundance index was at a record low and it is alarming that the other pelagic species of the Delta are also in rapid decline. The IEP is leading an effort to evaluate several possible causes for the decline. One line of inquiry involves toxicity testing of several life stages of the delta smelt. A pilot test indicates that juvenile delta smelt are a sensitive indicator species to copper concentrations (more sensitive than juvenile striped bass for example; Inge Werner, UC – Davis), and therefore may make a good test animal for: the new pesticide (pyrethroids), for substances derived from *Mycrosistus*, and for the effect of unknown toxic agents in delta water samples. The cultured supply of delta smelt, with known rearing history, is required for toxicity testing. Large numbers of delta smelt are also being requested by state and federal water diversion facilities to improve fish screening procedures and evaluate fish passage and survival through the fish screening process. In addition the culture of delta smelt provides a safeguard against extinction.

XIII. Recommendations

We recommend the development of a consistent funding source for delta smelt culture to ensure a continued supply of this valuable resource for relatively small scale studies. The ready supply of delta smelt expedites science. In the past, support from State and Federal agencies (CALFED, IEP, and DWR) enabled the development of a reliable and technically feasible delta smelt culture program. The availability of all life stages of smelt has allowed many scientists to develop a research question, request the smelt, and receive the animals in a timely manor (within several days to a few months). Without continued support, the planning phase for smelt research would often extend to a timescale of years - to include the time delay between planning the experiment, receiving grant funding, and producing the fish. Continued funding would be used to eliminate big time delays, and to ensure a relatively small number of animals (ca. 5000) are produced each year to meet the needs of the research community. Research programs that require large numbers of animals (5,000 – 10,000 adults, or more) usually involve a longer planning phase, and can include the cost of rearing the required fish in their total research budgets. As of October 31, 2005 funds will no longer be available to produce a cultured supply of delta smelt for the smaller research programs; beyond this date funds are only available to produce animals for a designated research program (which has supplied funding for the required fish).

XIV. Acknowledgment

Thanks to the CALFED Bay-Delta Program for continued funding of this ongoing program (RA #: ERP-02-P31; Agreement #: 4600002881). We would like to thank the Department of Water Resources and the staff on site at the State Water Project who have continually supported our project. Thanks also to the US Bureau of Reclamation, who have helped our program collect wild broodstock each year. Special thanks also to FCCL employees Luke Ellison and Julie Belo who were instrumental to the success of this program.

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