Appendix 2E

Delta Smelt Supplementation Strategy

DELTA SMELT SUPPLEMENTATION STRATEGY

Prepared by:

U. S. Fish and Wildlife Service
San Francisco Bay-Delta Fish and Wildlife Office

With technical assistance from:
U.S. Fish and Wildlife Service
Regional Office, Sacramento

California Department of Water Resources
Division of Environmental Services

U.S. Bureau of Reclamation
Bay-Delta Office

California Department of Fish and Wildlife
Water Branch and Fisheries Branch

University of California Davis
Department of Animal Science

21 October 2020

EXECUTIVE SUMMARY

The Supplementation Strategy provides a roadmap to transition from current management practices for delta smelt to population supplementation conducted under a formal set of Standard Operating Procedures (SOPs). This strategy will capitalize on an initial period of research, monitoring, and evaluation to test the efficacy and effects of production and release of cultured delta smelt, and within an adaptive process, institute science-based modifications to the developing supplementation SOPs. This approach will also serve to refine the research, monitoring, and evaluation (RM&E), within an adaptive management (AM) framework as supplementation begins sometime between 2022 and 2024 per the U.S. Fish and Wildlife Service's (Service) *Biological Opinion For the Reinitiation of Consultation on the Coordinated Operations of the Central Valley Project and State Water Project*, signed October 21, 2019 (2019 BiOp).

This Supplementation Strategy is based on adaptive management of an integrated hatchery model that will be guided by a carefully designed and reported monitoring and modeling program. The objective of this Supplementation Strategy is to provide the Service, Bureau of Reclamation (Reclamation) and the State of California Departments of Water Resources (DWR) and Fish and Wildlife (CDFW) with a roadmap explaining how to scientifically and adaptively deploy captive propagation as a conservation strategy to supplement the delta smelt population in the wild.

The Supplementation Strategy also includes a Regulatory Framework that describes the permitting steps needed for supplementation, and incorporates facility needs, and research, monitoring, and evaluation into a roadmap to initiate supplementation as provided in Reclamation's and DWR's Proposed Action in the Biological Assessment on the Reinitiation of Consultation on the Coordinated Long-term Operation of the Central Valley Project and State Water Project, dated October 2019 (2019 BA), and the 2019 BiOp.

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BACKGROUND

Status of Delta Smelt

The delta smelt is a small fish of the family Osmeridae. In the wild, very few individuals reach lengths over 3.5 inches (90 mm; Damon et al. 2016). At the time of its listing under the Endangered Species Act (ESA), only the basics of the species' life history were known (Moyle et al. 1992). In the intervening 26 years, enough has been learned about the delta smelt to support its propagation in captivity over multiple generations (Lindberg et al. 2013), to support the development of complex conceptual models of the species life history (IEP 2015), and mathematical simulation models of its life cycle (Rose et al. 2013a; Polansky et al. 2020). Much remains unknown, including aspects on reproductive behavior. The abundance of delta smelt has been trending down for as long as the species has been biologically monitored and was likely in decline prior to that (Nobriga and Smith 2020). The abundance of the population is currently so low that many long-term surveys are regularly returning zero catches and the U.S. Fish and Wildlife Service's (Service or USFWS) intensely-sampling Enhanced Delta Smelt Monitoring Program has been required to better document the species' abundance and distribution.

The Service proposed to list the delta smelt (*Hypomesus transpacificus*) as threatened with proposed critical habitat on October 3, 1991 (USFWS 1991). The Service listed the delta smelt as threatened on March 5, 1993 (USFWS 1993), and designated critical habitat for the species on December 19, 1994 (USFWS 1994). The State of California also listed the delta smelt as a threatened species in 1993 (under the California Endangered Species Act, CESA) and uplisted it to endangered in 2009. The delta smelt was one of eight fish species addressed in the Service's *Recovery Plan for the Sacramento–San Joaquin Delta Native Fishes* (USFWS 1996). A 5-year status review of the delta smelt was completed on March 31, 2004 (USFWS 2004). The review concluded that delta smelt remained a threatened species. A subsequent 5-year status review recommended uplisting delta smelt from threatened to endangered (USFWS 2010a). A 12-month finding on a petition to reclassify the delta smelt as an endangered species was completed on April 7, 2010 (USFWS 2010b). After reviewing all available scientific and commercial information, the Service determined that re-classifying the delta smelt from a threatened to an endangered species was warranted but precluded by other higher priority listing actions (USFWS 2010c). The Service reviews the status and uplisting recommendation for delta smelt during its Candidate Notice of Review (CNOR) process. Each year it has been published, the CNOR has recommended the uplisting from threatened to endangered. Electronic copies of these documents are available at https://ecos.fws.gov/ecp0/profile/speciesProfile?sId=321.

Refugial Population

Following its listing under ESA and CESA, CDFW identified the need for scientific research to support creation of a refuge population of delta smelt and for refining hatchery and production techniques (CDFW Publication 93-DS, p. 60). Intensive fish culture techniques were initiated and funded by DWR and the Service in 1993. Through cooperative efforts of several agencies since that time, refinement of these techniques has assisted in development of a captive refugial population as one level of security against species extinction, and in maintaining genetic diversity of the species and a reliable supply of captive-reared fish for research (Fisch et al. 2013; Lindberg et al. 2013).

The refuge population is currently housed in two locations. The primary population is maintained at the University of California (U.C.) Davis Fish Conservation and Culture Laboratory (FCCL) in Byron, California, where presently the facility rears 34-40 multi-family groups, producing over 20,000 adult fish or ~200,000 eggs annually (pers. comm. T. Hung, FCCL, May 1, 2020). The second (redundant) population, which uses replicate broodstock (i.e., approximately 6,800 fish from FCCL [200 fish/multifamily group]), is located at the Livingston Stone National Fish Hatchery (LSNFH) in Shasta Lake, California. The LSNFH population serves as a backup to minimize risk associated with catastrophic loss at either facility. The FCCL has: (1) developed reliable techniques for the capture of delta smelt from the wild and for the production of all life stages of delta smelt, (2) provided a source of animals for on-site and off- site research, and (3) maintained a genetically diverse captive population through genetic management of broodstock. Each year up to 100 (adult equivalents*) wild delta smelt are incorporated into the FCCL broodstock to maintain genetic diversity, reduce hatchery effects, and retain similarity of cultured delta smelt to the wild stock. The U.C. Davis Genomic Variation Laboratory (GVL) maintains broodstock histories and population pedigrees and conducts microsatellite genotyping for parentage reconstruction and to assess genetic diversity (Fisch et al. 2013).

The FCCL continues to develop and refine critical culturing techniques and technologies under their ESA §10(a)(1)(A) Recovery Permit. However, current permits do not allow for captive-reared or hatchery-propagated fish to be released back into the wild. The framework for necessary permitting and supporting documentation for supplementation to the wild is outlined in Appendix 1.

**Beginning in 2018, the Service has counted delta smelt take using the metric "adult equivalents", which uses life stage-specific weighting (Slater 2017), where earlier life stages are counted fractionally based on an underlying survivorship curve.*

Consideration of Supplementation

In July 2008, the CALFED Science Program hosted a workshop titled "The Use of Artificial Propagation as a Tool for Central Valley Salmonid and delta smelt Conservation." Given the precipitous decline of delta smelt documented in San Francisco Bay estuary since 2001, this workshop led to early discussions as to whether the controlled propagation program at the FCCL or another facility could be scaled up to enable supplementation of the wild population. The outcome of that workshop was a peer-reviewed paper (Israel et al. 2011) which advocated for scientifically defensible and ecologically based restoration programs—that adequately address limiting factors facing delta smelt in its estuarine habitat—before any attempt to supplement (augment) the wild population. Further, the authors stated "a mitigation [supplementation] hatchery for delta smelt should be expected to create all the same risks for the natural population as a salmonid hatchery (i.e., loss of genetic diversity, domestication selection, impairment of carrying capacity available to the natural population)" (Israel et al. 2011, p. 9).

In the years since Israel et al. (2011) published the conclusions of the 2008 workshop, continuing delta smelt decline forced a re-evaluation of risks related to supplementation. The much greater risk of imminent extirpation led to a second workshop in 2017 called "The Delta Smelt Culture Program: From Experiments to Supplementation" (Lessard et al. 2018). The participants in this second workshop advocated for *in situ* experiments using cultured delta smelt as a precursor to supplementation actions. Participants agreed that experimental and supplemental releases of cultured fish need to be conducted within an adaptive management program that is integrated with other conservation strategies, including habitat restoration, and concluded that there is now sufficient baseline information about delta smelt and the existing culture program to proceed with targeted field research that utilizes cultured fish. The purpose of this research is to fill critical knowledge gaps about delta smelt in order to increase the likelihood of successful supplementation strategies. Following this workshop, DWR developed a workplan which outlined the identified knowledge gaps, as well as priorities and feasibility of each knowledge gap (DWR 2018).

The Service's San Francisco Bay-Delta Fish and Wildlife Office (BDFWO) has been systematically moving toward a regulatory approach to implement important research, including the experimental use of cultured fish from FCCL in contained conditions (See Appendix 1: Regulatory Framework for Supplementation of Delta Smelt).

Relationship to the 2019 USFWS Biological Opinion

Supplementation of the wild delta smelt population with fish raised in captivity is a conservation measure proposed by Reclamation and DWR through the ESA §7(a)(2) consultation with the Service on the Coordinated Operations of the Central Valley Project and State Water Project (2019 BA). The first step in the process described was development of a Supplementation Strategy by the Service. The 2019 Service Biological Opinion (hereafter, 2019 BiOp; USFWS 2019) analyzed the effects of the Proposed Action from the 2019 BA, including the supplementation program, on delta smelt, delta smelt critical habitat, and several other federally-

listed species. The approaches, research, and experiments identified in the Supplementation Strategy are intended to increase the likelihood that the population of delta smelt will be sustained in the wild. Through the development of this Supplementation Strategy, the Service is articulating the need for augmentation of the wild population. The goal of the Supplementation Strategy is to provide a roadmap for how to increase delta smelt hatchery-production of life stages necessary to effectively augment the wild population and to continue to capture and maintain genetic diversity of the species. Successful implementation of this strategy will require the attention to *Facility Needs*, *Research Needs*, and *Monitoring and Evaluation* sections below.

Reclamation's and DWR's goal in proposing this conservation measure was to minimize the effects of long-term water operations and address the downward trend in abundance and distribution of delta smelt. Information about the life history and ecology of delta smelt, as well as its current status, can be found in the 2019 BiOp. Supplementation was proposed to address this trend by maintaining a genetic bank, alleviating effects of further population decline, bolstering the resilience of the population in poor recruitment years, and allowing the population to withstand stressful environmental conditions associated with recurring drought. The current status of delta smelt has reached a state where the Service considers population supplementation to be an essential element of any realistic recovery strategy to be enacted alongside ongoing habitat restoration, and water operations management. Reclamation and DWR proposed to continue supporting the FCCL in its ongoing efforts to capture and maintain existing genetic diversity and to expand the facility's rearing capacity. If necessary, other rearing facilities may be employed to produce approximately 125,000 delta smelt annually within three years of the issuance of the 2019 BiOp (2019 BA pp. 4-79 and 2019 BiOp pp. 171-172). The supplementation of delta smelt into the wild is expected to occur within 3-5 years from the issuance of the 2019 BiOp. This means that supplementation is anticipated to begin between October 2022 and October 2024.

Interagency Coordination on Delta Smelt Supplementation

The **Culture and Supplementation of Smelt (CASS)** is a critical coordination forum among four agencies (USFWS, USBR, CDFW, and DWR) that will be involved in supplementation implementation efforts. Each of the CASS agencies plays an important role in each step described in this strategy. Interagency coordination allows for focus on (1) the use of fish for research, (2) policy direction, and (3) identification and coordination on regulatory steps. As part of Reclamation's and DWR's Proposed Action in the 2019 BA, planning by at-large membership in the CASS for supplementation efforts, and inclusion of representatives from the FCCL in working groups is consistent with the Collaborative Planning approach.

Relationship to the Hatchery and Genetic Management Plan (HGMP)

A hatchery and genetics management plan (HGMP) for delta smelt is needed for hatchery operations and is a critical regulatory step for supplementation. A draft HGMP was developed by Dr. Daphne Gille (DWR) in collaboration with state and federal partners, most recently through the working groups and steering committee of the CASS. Aspects of this draft HGMP have been incorporated into this supplementation strategy where appropriate.

SUPPLEMENTATION STRATEGY

Objective of the Supplementation Strategy

The objective of this Supplementation Strategy is to provide the Service, Reclamation, CDFW, DWR, and FCCL with a roadmap explaining how to scientifically deploy captive propagation as a conservation strategy to support the persistence of delta smelt in the wild.

The Supplementation Strategy describes how Reclamation can facilitate an increase in captive delta smelt production to approximately125,000 individuals available for supplementation (facility needs), describes a conservation-oriented approach to meet this production target (adaptive management through IHM) and outlines associated monitoring to inform ongoing planning and implementation for a delta smelt supplementation program. This strategy also identifies key experiments needed before supplementation occurs, most of which are planned or underway, though the Service expects additional studies will be needed as the strategy evolves into a formal Supplementation Plan. A formal Supplementation Plan, which may take the form of adaptively implemented standard operating procedures (SOPs), will be based on the tenets of this Supplementation Strategy- i.e., a conservation-oriented IHM in which scientifically-defensible management decisions for supplementation will be based on RM&E as carried out under an adaptive management process that is described in a formal Adaptive Management Plan (TBD). Other details that could affect the success of a supplementation program, such as attempts to mitigate the causes of delta smelt decline, habitat restoration and water management actions that should occur, and granular details of adaptive management, broodstock management, and genetic management, etc. are beyond the scope of the Supplementation Strategy and will need to be evaluated as the supplementation program commences and enters into adaptive cycles of 'learning by doing, evaluating and responding.'

Roadmap to Supplementation

Development of a supplementation strategy benefits from following three critical steps: 1) identify a conceptual model of the supplementation program; 2) identify information needed to bring the model to fruition; and 3) construct a roadmap to implement supplementation under that model.

INTEGRATED HATCHERY MODEL

Integrated Program

Based on best-available science, an appropriate conceptual model for supplementation of delta smelt is a conservation-oriented integrated hatchery model (IHM; Figure 1) as generalized by the Hatchery Science Review Group (Columbia River, HSRG 2009; Pacific Northwest, HSRG 2014). An IHM is a hatchery program that specifically incorporates wild fish into broodstock to reduce the genetic effects of domestication on the wild population and to maximize the similarity of hatchery and wild stocks (HSRG 2009, 2014; Baskett and Waples 2012). This model was developed by a team of biologists and hatchery managers looking to improve the conservation viability of hatchery programs (Morbrand et al. 2005) and is recommended for use by hatchery managers. IHM have been successfully implemented in federal and state management of salmonids, notably Redfish Lake Sockeye Salmon (*Oncorhyncus nerka*, Kline and Flagg 2014). In the simplest IHM, the hatchery and supplemented (wild) stocks represent populations that comprise a single managed population. Interaction between these populations includes dispersal (via supplementation and by incorporation of wild fish back into the broodstock) and geneflow (subsequent reproduction).

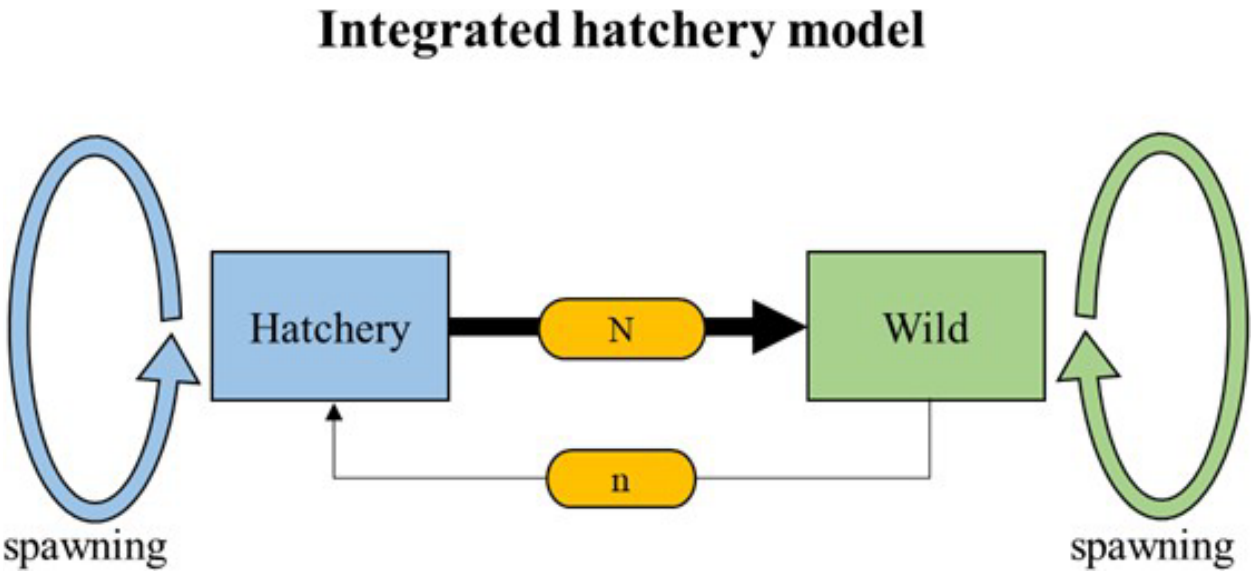

Figure 1. Conceptual rendering of an integrated hatchery model, IHM. Supplementation is represented by the bold arrow with N individuals released; incorporation of n natural-origin individuals into hatchery broodstock is represented by the thin arrow. The IHM incorporates research, monitoring, and evaluation (RM&E) to make scientifically-defensible management decisions through an adaptive management process where results of ongoing experimentation inform the need for changes in strategy over time.

Note: Integration of hatchery and natural-origin populations results in interconnected subpopulations (i.e., hatchery and wild stocks) that interact and thus have non-independent vital rates (e.g., fecundity, growth, and survival).

Integrated Program for Delta Smelt

For delta smelt, the IHM will include a supplemented population in the Bay-Delta as well as one or more hatchery-origin populations for production of the 1) refugial population; 2) research stock; 3) supplementation stock; and 4) potentially additional hatchery stocks maintained at separate facilities for grow out, additional production, or both. Management of an integrated population will require a set of mathematical models that can track and predict the effects of different supplementation strategies on the demographic and genetic status of the wild population. Monitoring and modeling of captive propagation and supplementation of delta smelt will be essential to successful implementation of the IHM.

Principles

The delta smelt IHM is founded on adaptation of guidelines and requirements for conservation hatcheries as described by the HSRG (2009, 2014), with imperatives driven by the need to maintain the natural population of delta smelt and its genetic resources. Three HSRG principles (2014; cf. pp. 2, 14), with text modified for application to delta smelt, guided development of this Supplementation Strategy:

- **Principle 1.** Develop clear, specific, quantifiable conservation goals for natural and hatchery populations of delta smelt within the context of habitat, hatcheries, and water operations. A comprehensive strategy will be used to coordinate habitat restoration, hatchery management, and water operations to best meet clearly defined management objectives for the supplementation program for delta smelt.

- **Principle 2. Design and operate the delta smelt hatchery program in a scientifically defensible manner.** The hatchery program will be developed based on an explicit scientific rationale, including assessment of benefits and risks and how the supplementation program expects to meet its stated conservation goals under a strategy consistent with up-to-date scientific knowledge.

- **Principle 3. Monitor, evaluate, and adaptively manage the delta smelt hatchery program.** Dynamic and complex interactions between the Bay-Delta ecosystem and the hatchery program are expected. For example, hatchery production may vary annually as might availability of wild fish for broodstock. New data will inform adaptive response to demographic and genetic effects of the hatchery program. This information will help determine how to flexibly and successfully execute the integrated program.

Development and Implementation

Development and implementation of the IHM (or 'program') for delta smelt will be evaluated based on the following criteria as modified from HSRG (2009) and HSRG (2014; cf. pg. 9). These questions will be evaluated at a regular interval (schedule TBD) and will incorporate input from the CASS.

1. Is management (including but not limited to formal adaptive management) responsive to the status of the naturally spawning population? This requires that management be able to recognize and implement scientifically-defensible adjustments to management actions and tactics (Bearlin et al. 2002).

2. Does the program promote natural selection over domestication selection? This may be achieved, for example, by employing hatchery management protocols that maximize PNI*, minimize effects of domestication selection on the integrated population, or both

3. Does the program design maximize survival of hatchery fish consistent with conservation goals?

4. Do hatchery practices increase the value of habitat restoration? For example, habitat restoration can be of greater value if hatchery practices effectively manage against inbreeding and other effects that reduce fitness in the wild.

5. Are the scientific elements of the IHM designed in a way to inform policy decisions in management of the integrated program?

** PNI is Proportionate Natural Influence (PNI) on a composite hatchery-origin/natural-origin population. PNI represents the percentage of time that genes of the composite population spend in the natural environment. PNI is calculated as pNOB/(pNOB + pHOS), where pNOB is the mean proportion of a hatchery broodstock composed of natural-origin adults each year, and pHOS is the mean proportion of natural spawners composed of hatchery-origin adults each year in the natural environment (HSRG 2014).*

Hatchery Science Examples under the IHM

Hatchery programs have benefits and risks to conservation through their efficacy and their effects on the viability of natural populations. Primary risks center on effects of supplemented fish on fitness and adaptive potential of the natural population, and how these demographic indicators relate to the effectiveness of habitat improvements and restoration (HSRG 2014).

Application of hatchery science to supplementation of delta smelt requires attention to the following areas (as adapted from the HSRG 2014; cf. Section 3, pp 36-48):

- Clearly define working hypotheses (theoretical foundation) to evaluate hatchery effects on the wild population of delta smelt

- Manage hatchery broodstock to balance conservation benefits and risks

- Manage fish health to reduce risk to wild and hatchery stocks

- Clearly define what it means to adaptively manage the integrated hatchery for conservation and recovery of delta smelt

- Integrate RM&E into management of the hatchery program

RESEARCH, MONITORING, AND EVALUATION

Categories and Requirements

Of the four stages of recovery identified by the HSRG (2014), this document provides an adaptive management roadmap for preservation (stage 1) and re-colonization (stage 2), as supplementation of delta smelt involves both stages simultaneously.

This program is further based on the following four categories and requirements for RM&E needed to support adaptive management (AM) of conservation programs (HSRG 2014; cf. Section 3.7, pp 92-113 for details; HSRG text in *italics* and USFWS modifications in **bold** font).

1. *Performance or implementation— monitoring hatchery program operations relative to plans and agreements, including in-hatchery survival, broodstock collection, pNOB, disease management, etc.*

2. *Status and trends— monitoring goals and objectives for natural production and* **conservation goals**.

3. *Effectiveness monitoring— evaluating the proximal outcomes of hatchery programs (e.g., survival,* **natural-origin contributions***, pHOS, etc.)*

4. *Research— hypothesis testing and parameter estimation related to the working hypotheses for hatchery programs and their conservation goals. Research programs tend to have global relevance and should therefore be subject to regional coordination and collaboration, e.g., via the CASS.*

Successful supplementation under IHM hinges on RM&E being integrated into a formal adaptive management program. Uncertainty over potential success of supplementation demands that adaptive management guide decision making; additionally, early stage (i.e., pre-supplementation) research and development is crucial to initiation of supplementation under the IHM. In the following section on structure of the Supplementation Strategy, *Adaptive Management* of delta smelt (1) is therefore treated first. Subsequent sections identify *Facility Needs* (2), *Research Needs* (3), and *Monitoring and Evaluation* (4) described in accordance with standards developed by the HSRG and to meet objectives for initiation of supplementation as provided in the Proposed Action in the 2019 BA and 2019 BiOp. Appendix 1 of this Supplementation Strategy describes the permitting steps and supporting documentation needed for supplementation.

ADAPTIVE MANAGEMENT

This Supplementation Strategy contemplates adaptive implementation of the delta smelt supplementation program that will be guided by a carefully conceived monitoring and modeling program (HSRG 2014; see also *Monitoring and Evaluation* below). The HSRG (2014) emphasized the importance of having explicit working hypotheses to guide the management of a captive propagation program. A working hypothesis helps to assure that RM&E produce scientifically defensible information that enables accurate assessment of the direction(s) for a hatchery program chosen by policy makers. The HSRG (2014) also emphasized being explicit about transitions around which management strategies may need to change, but which must continue to proceed in a scientifically defensible manner. The main working hypothesis in this supplementation strategy is that captive-bred delta smelt will survive at sufficient rates to successfully reproduce in the wild.

Supplementation strategies may change at important transitions (HSRG 2014) based on the periodic evaluation of the *Development and implementation* criteria. The most important transition for this supplementation strategy is that once supplementation begins, the wild and hatchery populations will become a single entity per the IHM. There is an emphasis on the supplementation strategy's monitoring and modeling aspects because research has shown that adaptive management of a rare species re-introduction can be impossible when monitoring data are too noisy to determine if management targets have been met (Bearlin et al. 2002). This is of concern for delta smelt given the high uncertainty in abundance estimates (Polansky et al. 2019). Note, however, that the proposed monitoring program for the IHM involves extensive use of genetic techniques that should provide relatively robust data so long as delta smelt persist at an abundance that allows for annual capture of individuals to incorporate into broodstock. There is a long list of logistical and methodological unknowns for which this strategy offers a roadmap to lower uncertainty in the coming few years [See sections *Facility Needs*, *Research Needs*, and *Monitoring and Evaluation*]. These uncertainties are to be addressed adaptively through RM&E. The Service has identified this as the most effective model approach to address fundamental unknowns that need to be resolved as quickly and efficiently as possible. This approach is centered on adaptive management based on the following primary working hypothesis:

Adaptive Management Working Hypothesis: Captive-Bred Delta Smelt Will Survive and Reproduce in the Bay-Delta.

Delta smelt abundance has plummeted in the last 20 years (Polansky et al. 2018; 2019), recently reaching the lower limits of detectability in several long-term surveys. Going back further in time, delta smelt abundance was higher (Thomson et al. 2010) but was still low compared to other similar and co-occurring fishes, suggesting that this species may have been in decline for many decades (Nobriga and Smith 2020). There is a long list of stressors that have differing degrees of scientific support as factors affecting the viability of delta smelt, but scientific consensus is forming around a strong role for warm water temperatures having a negative effect on recruitment and survival. We do not imply that water temperature is the only important factor affecting delta smelt recruitment, only emphasize that multiple lines of evidence provide strong support that temperature is a key driver of population success and failure (e.g., Rose et al. 2013b; Komoroske et al. 2015; Brown et al. 2016). This support also transcends several models with variable formulations and falls into three probable mechanisms: i) duration of the spawning season in the spring, which affects how many eggs can be spawned and how well the eggs and larvae survive (Bennett 2005; Maunder and Deriso 2011; Rose et al. 2013b; Polansky et al. 2020; Smith et al. in revision); ii) energetic stress in the summer which likely has consequences for foraging behavior, disease susceptibility, and predation risk (Mac Nally et al. 2010; Maunder and Deriso 2011; Komoroske et al. 2015; Nobriga and Smith 2020; Teh et al. 2020); and iii) a cumulative lifetime effect of water temperature on growth rate that can affect the number of eggs adult females ultimately generate (Rose et al. 2013b). Other habitat stressors with reasonable scientific support are the effects of a less productive pelagic food web (Mac Nally et al. 2010; Maunder and Deriso 2011; Rose et al. 2013b; Hammock et al. 2015; Schreier et al. 2016; Hamilton and Murphy 2018; Kimmerer and Rose 2018; Polansky et al. 2020) and historically high entrainment loss (Kimmerer 2011; Smith et al. in revision), although recently applied OMR flow controls seem to have mitigated the latter to the extent reasonably possible (Smith et al. in revision).

Given the extensive modifications of the Bay-Delta system it is reasonable to ask: will captively-propagated delta smelt survive in the contemporary Bay-Delta environment and, if so, will they reproduce successfully? Some observers of the current status of delta smelt may believe the record low abundance reflects an inability of the ecosystem to support this species much longer, but extirpation is a near certainty without intervention. Thus, an adaptively implemented supplementation effort is warranted (Moyle et al. 2016; 2018; Hobbs et al. 2017). One particularly good reason to try supplementation is that delta smelt might be constrained by Allee effects, which are limitations on survival and reproduction that species face when abundance drops to levels that are too low for successful completion of the life cycle. For a small, shoaling fish such as the delta smelt, possible Allee-effect mechanisms include shoals that are too small to offer adequate (1) protection from predators; (2) opportunities to find enough mates to spawn successfully, or both, depending on circumstances. These mechanisms have been reported for other forage fishes (Liermann and Hilborn 2001). If Allee effects are occurring in the delta smelt population, aggressive supplementation may be able to remove the effect(s) by re-establishing more resilient population abundance levels.

FACILITY NEEDS

The dominant technical challenge of the Supplementation Strategy is to identify a pathway to overcome production constraints that currently preclude i) annual production of approximately 125,000 adult delta smelt for supplementation and ii) requisite conservation genetic management at that level of production. Overcoming this challenge requires identification of the infrastructure and staff required to meet production and hatchery management objectives as outlined in the 2019 BA and 2019 BiOp. The Service, in coordination with its partners in the CASS, has identified existing capabilities and necessary future expansion and operational needs, focused on the following:

- Initial production levels (number of individuals by life stage) for supplementation, as proposed by Reclamation in the 2019 BA (USBR 2019).
- Infrastructure and operations required to meet targeted production capacity and conservation genetic management for supplementation of delta smelt to the wild.
- Production constraints and capacities to meet target allocations of fish to the refuge population and to research and supplementation purposes.
- Role of LSNFH and staff, including coordination and communication structure between FCCL and relevant Federal and State agencies to determine a pathway to produce and maintain a full backup of the refugial population at LSNFH.

Identification of means to increase production capacity under best conservation genetic management practices is paramount. Facility needs to meet this challenge are described below in six (**I-VI**) points listed below.

Point I— describes current and planned capacities for delta smelt production at FCCL to outline the facility operations and infrastructure that will be required to achieve i) production goals of the 2020-2025 Reclamation contract to FCCL and ii) the obligations described in the 2019 BA and 2019 BiOp.

Points II-V— describe potential pathways- none mutually exclusive- to increase production capacities in the near term to begin supplementation no later than October 2024. These cover **II**) alternative spawning designs to increase production under a genetic management program at FCCL; **III**) operations and infrastructure to support genetic and physical tagging for broodstock and supplementation management; **IV**) Potential augmentation to FCCL production capacity via flexible spawning and release schedules or grow-out of delta smelt at other secondary facilities to increase survival and extend production capacity beyond what can be accomplished using only FCCL.

Points V-VI— consider opportunities over a longer time horizon but require early integration with design and implementation of a Supplementation Plan for delta smelt and thus are included here; **V** describes the capacity and role of LSNFH and **VI** summarizes status and the production potential of future expanded facilities.

I. Projected Capacity at FCCL

For current (2020) FCCL operations, up to 16,000 delta smelt are produced annually for the refuge population and about 10,000 adult equivalents are produced for external research, most of which are provided at pre-adult life stages. A new 5-year contract (2021-2025) between FCCL and Reclamation added a specific task to produce a minimum of 50,000 sub-adult (200 dph) delta smelt for supplementation by 2022, in complement to current levels of production which maintain the refuge population and research stock. This leaves a deficit of approximately 75,000 delta smelt needed to meet the supplementation target proposed in the 2019 BA.

Current facility capacities (footprint, infrastructure, and number of staff) are inadequate to meet production of approximately 125,000 genetically managed adult delta smelt. Quantification of the number of delta smelt produced per developmental stage for prior and future operations is shown in Table 1 (current production and facility infrastructure) and Table 2 (summary of current and projected capacity as proposed in the 2019 BA). Allocation of fish for research is discussed in the *Research Needs* section below. Production estimates are based on current mortality schedules and on the current paired-mating design used for the refuge population. Estimates of survival by life stage are based on mean survival and do not account for year-to-year variance, nor do they account for differential survival and reproduction according to multi-family group (MFG) or domestication index (Finger et al. 2018). Because survival to each life stage also has a compounding effect on number of delta smelt at subsequent life stages, production may differ from estimates provided in Table 1 and Table 2; nonetheless, these are the best estimates that can be generated at this time.

To increase the likelihood of successful supplementation and reduce risk of extinction in the wild, increased production under genetic management of delta smelt will adhere to best practices for fish quality and core tenets of conservation hatcheries as described under the HSRG (2009, 2014; also see concerns raised by George et al. [2009] and Bingham et al. [2014]). To facilitate this multifaceted goal, additional space, infrastructure, and trained staff will be required. The likelihood of successful supplementation may also be bolstered by increasing the number of available broodfish represented by wild delta smelt. This would require take to be increased from the current limit of 100 adult equivalents (FCCL's USFWS 10(a)(1)(A) permit; CDFW California Endangered Species Act (CESA) Memorandum of Understanding [MOU]), and is contingent on how many delta smelt can realistically be captured in the wild and what additional resources are available to capture and maintain.

Allocation to Research

Under current and projected production capacities cultured delta smelt will be allocated to three purposes under the integrated hatchery (refuge and backup population, research, and supplementation). Production of sufficient numbers of fish to consistently satisfy all three components will require a higher level of production that i) meets criteria specified in the 2019 BiOp and ii) can adapt to anticipated variability around expected means described in Table 1 and Table 2. Research and monitoring are core components of the IHM; allocation to research for supplementation may change based on new information and changing priorities identified through the CASS. Allocations to research may be larger during the period prior to supplementation (i.e., in the next several years), as some fish produced in the scale-up to the supplementation target also may be available for external research and other purposes during this transition period. A process for request and approval of fish for research is in development by the CASS Working Groups.

Table 1. Delta smelt Refuge population culture parameters at the UC Davis FCCL.

Tank/Life Stage	Ovulated Eggs	"Embryo (3 dpf)"	Hatch Rate	Larval		Juvenile	Sub-Adult	Spawning Adult*
General								
Cumulative Mortality rate (%)*	0%	20%	24%	62%	73%	87%	88%	89%
Target Number	150,0006	120,000	114,500	57,000	40,000	20,000	18000	16,000
Size (mm)	1	1		10 - 15 mm	15 - 25 mm	25 - 50 mm	50 - 55 mm	>55 mm
Age	0 dpf	3 dpf	0 dph	1 - 40 dph	40-80 dph	80 - 120 dph 120 - 200 dph	200 - 250 dph	>250 dph
Tank Parameters								
Size (L)	2	2		130	400	1,100 Indoor; 1100 Outdoor	1,100 Outdoor	1100 Outdoor
Diameter (cm)	43	43		61	91	152	152	152
Depth (cm)	51	51		46	61	61	61	61
Tank Water volume (L)	N/A	N/A		120	360	860	860	860
No. Tanks per Reuse System	N/A	N/A		20	16	12	12	12
Total # of Tanks	14	14		40	36	34	34	34
Stocking Rate (fish/tank)*	N/A	N/A		5,600	2,500	"1,000-1,500 (@120 dph) / 1,000 (@ 160 dph)"	600 (@200 dph)	200 (fish tagged)
Stocking Density (fish/liter)	N/A	N/A		46.6	6.9	1.7	0.7	0.2
Flow rate*	Static	Static		3 L/min	6 L/Min	8 L/min	8 L/Min	8 L/min
Make-up water (% Flow rate)*	Static	Static		10 - 20	10 - 20	10 - 20	10 - 20	10 - 20
Tank cover*	N/A	N/A		N/A	N/A	"Shade cloth @ <160dph / Awning + Shade cloth @ 160 dph Shade cloth only"	Shade cloth	Shade cloth
Water System	Static	Static		Recirculating	Recirculating	Recirculating	Recirculating	Recirculating
Water Quality								
Temperature	16	16		16	16	16	16	12-16
Turbidity (NTU)	<5	<5		5.5	5.5	0	0	0
Feed	Yolk sac	Yolk sac		"Rotifer & Artemia"	"Rotifer & Artemia"	Dry diet	Dry diet	Dry diet

**Cumulative Mortality rate, stocking rates (fish/tank), tank flow rate, make-up water rate, and tank cover type provided by FCCL. FCCL uses 700 eggs per cross to start and current capacity is between 280-320 crosses at the average egg production of 1,525/female. The program will target a maximum of 320 single-pair crosses that are combined into 40 multi-family groups (MFG), each made up of 8 of 8 full-sibling groups.*

Current Life-Stage Schedule

Current information on fecundity and survival per development stage (dph = days post hatch) is summarized below. Cultured females have an average fecundity of 2145 eggs/female; wild females produce an average of 1466 eggs/female when brought into FCCL. The mortality schedule across development cycle for cultured delta smelt is estimated as follows:

1. 80% of eggs produced per spawn are successfully fertilized
2. 95% of the fertilized eggs are successfully hatched
3. 50% hatched larvae survive to 40 dph
4. 70% 40-dph old larvae survive to 80 dph
5. 40%* 80-dph old larvae survive to 300-dph*survival in captivity is higher for adults (~80%, 200-300 dph) than for juveniles (~50%, 80-200 dph)

Based on the above mortality schedule, estimated production under the 2020-2025 contract would yield the numbers of delta smelt per development stage listed in Table 2; estimates are projected means and do not account for variance in survival due to changes in culture methods and stochastic events. These estimates will be updated annually as production increases to meet the supplementation target of approximately 125,000 delta smelt.

Table 2. Allocations by life stage and use (refuge, research, and supplementation) under increased production of delta smelt under the 2020-2025 Reclamation contract to FCCL, 2020-2022 and 2023-2025. Refuge and research allocations are held constant to recent allocations at FCCL. Increased production would require expansion of operations and infrastructure. For eggs, **s** is spawned unfertilized, **f** is fertilized, and **h** is hatched. All table numbers have been rounded up to the nearest 500.

Life stage	**FCCL (2020-2022)**				**FCCL (2023-2025)**			
	Refuge	*Research*	*Supplementation*	*Total*	*Refuge*	*Research*	*Supplementation*	*Total*
Eggs (s)	150,000	94,000	375,000	619,00	150,000	94,000	938,000	1,182,000
Eggs (f)	120,000	75,000	300,000	495,00	120,000	75,000	750,500	945,500
Eggs (h)	114,000	71,000	285,000	470,00	114,000	71,000	713,000	898,000
40	57,000	35,500	142,500	235,00	57,000	35,500	356,500	449,000
80 dph	40,000	25,000	100,000	165,00	40,000	25,000	250,000	315,000
200 dph	20,000	12,500	**50,000 ***	82,500	20,000	12,500	**125,000 ***	157,500
300 dph	16,000	10,000	40,000	66,000	16,000	10,000	100,000	126,000

**Per the contract, the supplementation production target number is for 200-dph fish*

II. Increased Production with Genetic Management

Successful supplementation depends in part on sufficient production underpinned by genetic management (George et al. 2009; Bingham et al. 2014). In 2020, FCCL completed an agreement with Reclamation to expand capacity to, within 3 years (2022; 2019 USBR PA), produce 50,000 sub-adult delta smelt annually for supplementation, in addition to continuing to produce the numbers needed for the refuge population and research stock. Under the current Reclamation contract to FCCL, production for supplementation would be based on paired-crosses (1 dam x 1 sire, or 1 x 1). Paired-crosses, however, are resource intensive (e.g., in personnel, time, and space) and impractical for the scale of production required for supplementation.

Production for supplementation requires stricter genetic management than what is described in the FCCL-Reclamation agreement. An alternative spawning strategy is needed to increase efficiency of production through increasing the number of breeders per spawn while maintaining reasonable genetic management of variance in reproductive success and family size. This entails identifying a new breeding design that is feasible with existing facilities and is logistically realistic to achieve. Conservation genetics outcomes must be quantifiable and of a sufficient diversity level (e.g., genetic diversity metrics, effective size of single releases) that genetic management goals can continue to be met.

These requirements preclude designs such as uncontrolled group spawns, which carry unacceptably high costs in reduction of effective population size, N_e, and consequent increased inbreeding, loss of adaptive genetic variation, reduction of fitness (LaCava et al. 2015), and increased domestication (Finger et al. 2018). Other strategies are not currently feasible as a spawning regimen for production to the scale needed for supplementation. For example, delta smelt has proved difficult to spawn unassisted under hatchery conditions typified by tank-based combinations of dams and sires in a more controlled group (Hung, pers. comm.). Thus, a 'natural spawning' strategy, while workable in principle, is excluded from consideration for the foreseeable future.

Alternative Spawning Design

Reclamation funded a study (Appendix 2) led by BDFWO, in collaboration with FCCL and GVL, to identify an alternative spawning design that will satisfy the production and genetic management requirements described in the 2019 BA and 2019 BiOp.

Following Gold et al. (2008) and equations adapted from Lacy (1989), we will evaluate the effects of number of dams and sires in each multi-family cross (MFC) that contributes to spawns, variance in family size among spawns of MFCs, and effects of individual and combined spawns (i.e., combined MFCs) on the potential pool of delta smelt slated for release (i.e., effective size of release populations, N_{eR}). Empirical data will be used in simulations to further investigate reasonable bounds for expected range of genetic effects of each potential alternative breeding design. From this arrangement, effects of variance in reproductive success and family size can be quantified to determine the best approach to simultaneously increase the number of spawns and improve genetic management of the spawns (Gold et al. 2008). Simulations can facilitate estimation of reasonable bounds within which genetic management of offspring can be improved.

Upon completion of the study in 2022, the following will be provided to Reclamation by the Service:

1. A preferred breeding design to improve production efficiency, maintain genetic variation, and predictions of average effective population size of average single releases.

2. Quantification protocol to measure variance in reproductive success and family size under the preferred spawning design.

3. Quantification protocol to measure average effective population size, N_e, of dams, sires, and single spawns.

4. Quantification protocol to measure expected improvements in average effective size N_{eR} of potential single releases under equalization and pseudo-equalization methods.

5. Quantify logistical and operational resources required to fully implement each alternative breeding design as an addendum to this Supplementation Strategy document.

III. Fish-marking Techniques

Genetic Tags

The Supplementation Strategy will require genetic tagging and monitoring of broodstock and supplemented delta smelt. Under an agreement funded by Reclamation (Appendix 3), BDFWO is leading a collaboration with FCCL, GVL, the Service's Abernathy Fish Technology Center (AFTC) to develop a genotyping-by-thousands sequencing (GT-seq) panel and establishment of a new GT-seq specific baseline for long-term genetic monitoring under an integrated hatchery model for supplementation of delta smelt. Ultimate use of the GT-seq panel will include parentage-based genetic tagging of broodstock used for production, for supplementation and annual analysis of hundreds of single nucleotide polymorphisms (SNP) for the thousands of samples from individuals that will be produced for supplementation and including individuals recaptured from the wild during monitoring surveys. In addition to high- throughput and cost-efficiency of GT-seq, other benefits include rapid generation of sequencing data for immediate dissemination to partners and replicability across labs. This study will utilize a combination of existing SNPs (Lew et al. 2015) and potentially identify new SNPs for incorporation into a GT-seq panel designed for long-term genetic monitoring for supplementation of delta smelt and for pedigree reconstruction used for production and management of the refuge population at FCCL.

Establishment of a genetic baseline for the combined wild-hatchery population will meet one of the most urgent needs for genetic management of delta smelt under the Supplementation Program. These products will increase the efficiency with which genetic data are collected, as well as increase the types of insights data can yield about broodstock management and the success of supplementation. The most salient product of this study will be the development of a novel genomic-era genotyping panel for application to long-term genetic monitoring called for in this Supplementation Strategy.

Physical Tags

If the supplementation program moves forward with stocking of post-larval life stages it will require physical tagging of hatchery-origin delta smelt to facilitate their visual distinction from wild-origin delta smelt captured in population monitoring surveys and fish salvage. The proposed use of physical tags in this strategy includes unique colors and combinations for identification of fish by cohort, hatchery of origin, and location and time of release. Physical tags will facilitate validation of parentage-based tagging (PBT) used in genetic monitoring and rapid visual identification of hatchery fish in the field; early life stage fish cannot be tagged physically. In addition, physical tags are needed when genetic tags identify fish to cohort but not to the hatchery used for grow-out. Physical tags also serve as a partial backup to genetic tags.

Under an agreement funded by Reclamation (Appendix 4), BDFWO is leading a collaboration with FCCL and the Service's Lodi Fish and Wildlife Office (LFWO) to establish i) methods for use of visible implant elastomer (VIE) tags in the supplementation program and ii) determine infrastructure, logistical, and personnel needs to scale tagging capacity to approximately 125,000 adult delta smelt per year. This will require development and testing of procedures to implant tags, and evaluation of post-tagging survival, growth, and tag retention.

When complete in 2023, the Service and its partners will know the best implant location on the fish and can propose tag colors and color combinations for use in the supplementation SOPs.

IV. Augmentation to FCCL Production Capacity

Production capacity at FCCL is limited primarily by available footprint (land area), which constrains outward expansion of facilities and infrastructure. To overcome this limitation, approaches such as proposed alternative spawning designs (see *Increased production with genetic management,* above) are likely necessary but not sufficient. To increase production capacity, additional measures- which could incorporate alternative spawning strategies- should be explored, including but not limited to:

i. *Flexible production and release schedule*. A flexible, or staged spawning and release schedule (within the natural spawning season) has been proposed by FCCL as a potential means of increasing annual production despite limited land area for production. FCCL plans to test the feasibility of this approach.

ii. *Grow-out at a secondary site*. Since there is insufficient space at FCCL for the targeted 125,000 delta smelt, use of secondary rearing sites could support increased annual production by providing off-site facilities for rearing delta smelt to size-at-release. Potential facilities and their infrastructure, staffing, and other operational needs warrant immediate investigation.

V. Livingston Stone National Fish Hatchery (LSNFH)

The delta smelt program at Livingston Stone National Fish Hatchery (LSNFH) is currently limited to maintaining a redundant backup of the FCCL Refugial population. Delta smelt infrastructure at LSNFH is at maximum capacity and requires infrastructure expansion to maintain sufficient backup of the Refugial population as FCCL increases the number of MFG produced annually. The Service is evaluating the feasibility and cost of expansion.

Note: Any fish that are transported to LSNFH could be considered to increase capacity for supplementation, research, or other purposes, should these fish no longer be needed as part of the fully redundant back-up population.

VI. Additional Facilities

Proposed new facilities at Rio Vista will not be available before supplementation is anticipated to begin. The Service, however, assumes that the supplementation program could eventually rely in part on these or other facilities. As envisioned, a new Delta Research Station would consist of two facilities: a Fish Technology Center (FTC) operated by the Service and a Rio Vista Estuarine Research Station (RVERS) that would be an interagency office and laboratory complex. The FTC would focus on the development and refinement of captive propagation techniques for native fish species and house a copy of the refugial population of delta smelt. It could also help produce delta smelt for the supplementation program.

A more long-term solution to increase numbers of cultured delta smelt would be the construction and operation of a new conservation hatchery as described by Reclamation in the BA (Reclamation 2019 pg. 4-80). RM&E and AM described in this strategy, and resultant learning from early supplementation work, would help guide development and design of the new conservation hatchery.

RESEARCH NEEDS

Studies and research to obtain information needed to design, implement, evaluate, and adaptively manage a supplementation program are critical for successful supplementation. Under the IHM, research is an essential component of management (see RM&E). Initial research and other studies inform decisions about facilities management and provide information needed before the Service and its partners can proceed to supplementation. Experimental studies, informed by simulation studies and models where applicable, are critical to the hypothesis driven foundation of (and continued management under) the IHM (Figure 4). The status of delta smelt does, however, require that allocation of cultured delta smelt for research (Table 2) gives priority to studies that address primary information needs related to the supplementation program, decisions that will be vetted by the CASS and its associated work groups.

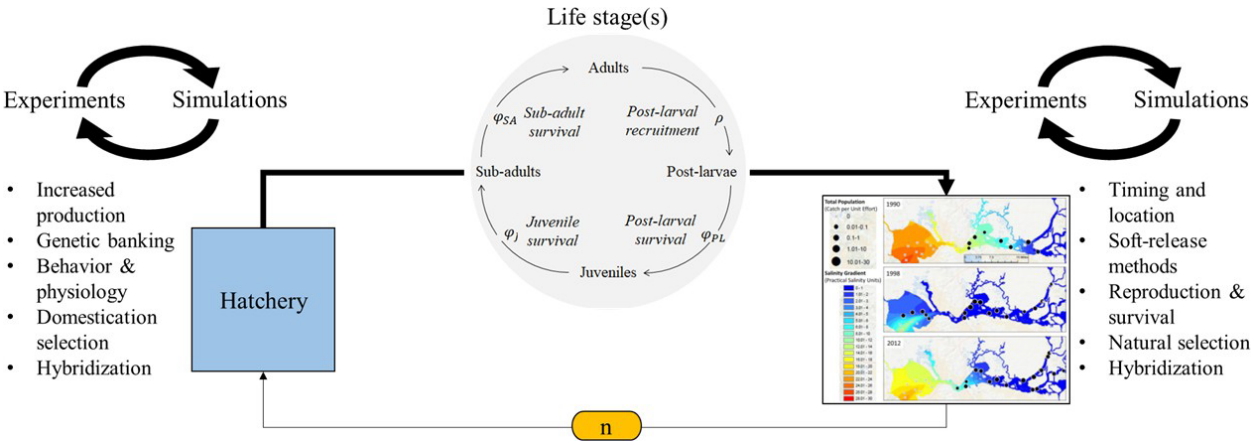

Figure 2. Conceptual illustration of research needs under the IHM. Research is conducted for hatchery (left) and natural (right) populations to inform decisions about best approaches for supplementation, including life stage(s) to release (middle). Experiments and models (demographics and genetics) inform each other in an adaptive management framework.

Measurement of Success and Information Needed

Successful supplementation depends not on the number of hatchery origin releases but on i) the number that survive to reproduce; ii) the relative contribution (relative reproductive success) of those releases to spawning in the natural population (HSRG 2014); and iii) effects on viability (demographic and genetic) of the natural population. Thus, RM&E will include a set of models and studies to quantify the effects of supplementation on the Bay-Delta population of delta smelt and an adaptive program implementation to adjust to new information when needed.

A combination of simulation studies and field experiments are needed to better gauge benefits and costs of release by life stage. A combination of these approaches is needed because models allow rapid assessment to test hypotheses but rely on data from experimental observations to provide useful estimates for management; in turn, experiments are needed to obtain information most relevant to models, and models help circumscribe and prioritize experiments to test hypotheses most pertinent to management. In an adaptive management framework, models and empirical experiments sequentially inform each other to best estimate efficacy of alternative supplementation strategies and their interaction with spatio-temporally varying habitat and environmental or ecological conditions.

Rigorous application of RM&E is crucial to address management uncertainties that can only be rigorously evaluated once fish begin to be released (supplemented) into the estuary.

Rigorous field experiments, and complementary laboratory studies, will accelerate progress towards needs of the Service and its partners to address fundamental unknowns as effectively and efficiently as possible through RM&E within an adaptive IHM program.

Production and Allocation to Research

Under current and projected production capacities, allocation to the three components (refuge population, research, and supplementation) of the integrated program will require a higher level of production that i) meets the level identified in the 2019 BA; and ii) can adapt to expected variability in production that differs from the projected mean production described in Table 1 and Table 2. Current allocation to research should be maintained, and it could be bolstered when allocation to the refuge population and to supplementation targets are met. Allocations to research may be larger (i.e., above that currently allocated to external research; Table 2) during the period prior to supplementation (i.e., 2022-2024), as production focused on supplementation targets may be available for other purposes during this interim.

The studies included as appendices to this Supplementation Strategy are those needed to implement the annual supplementation of the wild delta smelt population with propagated fish by no later than 2024. These studies emphasize increasing production and identification of optimal life stages and conditions for release of cultured delta smelt to the wild. The CASS Research Working Group (RWG) is developing a more expansive list of studies they have identified as high priority for supplementation (Appendix 5). A second group of studies, tracked by the RWG, are currently underway or in the advance-planning stage (Appendix 6). The CASS is developing a process for request of fish for research.

For the Supplementation Strategy to inform a Supplementation Plan, the following priority activities and study areas are critical (Figure 4): **I**) life stage(s) to use for supplementation; **II**) field experiments; **III**) timing and location of releases; **IV**) soft-release methods; **V**) domestication selection; **VI**) cryopreservation of milt; **VII**) hybridization; and risk aversion measures (**VIII**).

I. Which Life Stage(s) Should Be Used for Supplementation?

Worldwide, captively-propagated fishes of all life stages are released into natural waterways. This variability in release strategy stems from the need in some cases to make decisions based on economics, whereas in other cases ecological limitations can require de-emphasis of strategies that might otherwise make the most economic sense. There are two perspectives about which delta smelt life stage would be the ‘best’ one to supplement – eggs or sub-adult/adults. One fundamental research question for the IHM is which strategy will have the biggest positive impact on the population growth rate across generations? It is generally assumed that supplementing juvenile fish during the physiologically challenging summer months is not likely to be a preferred strategy so we recommend that consideration of a juvenile fish release option be foregone until other life stages believed to have a better chance of post-release survival have been tried and vetted through the adaptive management process.

Whether delta smelt are supplemented at the adult life stage, the egg stage, or both, the eggs will ultimately need to survive at comparable rates to wild fish and each successive new generation of fish must be able to successfully reproduce (i.e., priority adaptive management hypothesis). With regard to facility needs and space to grow delta smelt, there is not likely to be much difference between either an egg-release or sub-adult/adult release strategy since producing enough eggs to match what may be produced by stocked adults would require a similar number of adult fish in the hatchery. The key unknowns are (1) how well captively- propagated adult delta smelt will be able to select suitable spawning sites in the estuary and exhibit suitable spawning behaviors; if eggs were supplemented, they could be released at times and in places that existing information indicates would optimize their chance of survival (e.g., water temperature of 16°C and in turbid locations away from the south Delta) versus (2) captively-propagated subadult/adults would likely be well fed and have low contaminant body burdens, which might enable the released fish to have higher fecundity than the wild fish and higher frequency of multiple spawns (based on influences on the refractory period; UCD, FCCL, & USFWS, unpublished) in years when the duration of the spawning window allows for it. Thus, it is hypothesized that if captively-propagated sub-adults/adults are successful spawners in the wild, they may be able to generate more eggs per generation than could be produced in culture.

II. Field Experiments

Additional field experiments are needed to continue progress on understanding performance of cultured delta smelt in the Bay-Delta. Recent experiments by scientists at DWR, UCD, and the Service deployed species-specific cages in several field trials (Baerwald et al., unpublished.). In each case, cultured delta smelt reared at FCCL were tested under different natural conditions.

The studies showed that in enclosures deployed for specific periods cultured fish are capable of surviving all but the most extreme environmental conditions tested thus far. Extreme high temperatures at different locations had a marked effect on survival; otherwise, survival was not significantly different among fish in cages constructed out of different materials. Such studies can inform understanding of relative performance of cultured fish under natural and hatchery conditions (see Domestication selection studies below), inform models for studies of supplementation (life stages to release, timing, location, and methods of release, etc.), and be used to reduce the list of reasons why wild fish are surviving at such low rates.

Point of Emphasis
Among field-based studies, enclosure experiments and experimental releases are among the most critical off-site research to conduct with cultured delta smelt between now and 2024. The operational goal is to produce 50,000 200-dph subadults for supplementation by 2022, and subadults could be used for experimental release, actual release, or other experimentation.

Experimental release-recapture to measure performance of cultured fish in the wild would advance information gained from enclosure studies and other experiments prior to initiation of supplementation.

III. Timing and Location of Releases

Stressors
To maximize survival and decrease the chance of thermal stress, which is a major concern for delta smelt (Komoroske et al. 2015; DWR, unpublished), releases of fish ≥ 200 dph should occur between the late fall and early the following spring when water temperatures are lowest. Fish become ripe and spawning can therefore occur at the FCCL from January to June. This period includes possible release windows between November and April for sub-adults and adults, and typically between March and April for release of fertilized eggs.

Habitat
A strong predictor of successful supplementation is whether habitat identified as supportive of the species is used to inform choice of release locations (Cochran-Biederman et al. 2014).

Release locations for delta smelt, therefore, should meet as many of the following criteria as feasible (Swanson et al. 2000; Sommer and Mejia 2013; Slater and Baxter 2014; Bever et al. 2016): adequate food resources; low salinity (<6 psu); adequate turbidity (>12 NTU); cool water temperatures (< 20 Celsius for older fish; circa 16 Celsius for eggs) moderate tidal flow; areas that are protected from extreme flow and disturbance. Releases also should occur at a secure distance from the south Delta; within tidal wetland; in or near restored habitat; within areas that are accessible by boat; and should be monitored regularly. Supplementation of adults and fertilized eggs at locations where wild fish have historically been most reliably collected should improve opportunities for mating and recruitment.

North Delta Arc
To meet these criteria, we expect that delta smelt will be released in the North Delta Arc (see Hobbs et al. 2017), a large expanse of inter-connected habitat in the north and west Delta that contains habitat of acceptable quality for delta smelt, benefits from managed flow actions (NDFA and SMSCG), and is far removed from the south Delta and risk of entrainment of releases. The North Delta Arc extends from the Cache-Slough complex, through the lower Sacramento River, and westward through Suisun Bay and Marsh. The North Delta Arc is preferred for delta smelt population supplementation activities for the following reasons. The FCCL predominantly captures wild broodfish within this geographic area, most often near Decker Island along the Sacramento River (Baskerville-Bridges 2005). Habitat restoration projects that have been completed, are pending, or are planned in the North Delta Arc (CNRA 2016, 2017) are anticipated to improve foraging opportunities for released delta smelt (e.g., Hammock et al. 2019; Hartman et al. 2019). Specific sites for population supplementation are not listed because conditions in the Bay-Delta are highly variable. Instead, water quality and biological data collected by monitoring programs and *in-situ* gauges should be evaluated as releases are being readied each year. Release location will therefore be managed in real-time.

IV. Soft-Release Methods

The spawning work-flow at FCCL depends almost entirely on the natural timing of female fish reaching sexual maturity and is therefore spread out over a period of four to five months.

Consequently, pair crosses spawned in the same brood year can also differ in age by the same amount. Therefore, simultaneous release of all fertilized eggs or adult fish (<300 dph) is not possible under current hatchery management practices.

The introduction of adult fish to the natural environment should be gradual and follow a soft release protocol. Here, a soft release refers to holding fish in an enclosure at the release site to allow for predator-free acclimatization to the wild habitat prior to liberation, as opposed to a hard release in which no acclimatization occurs (Brown and Day 2002). The benefits of soft release methodologies have been well documented in cultured fish and include improved fitness, growth, survival, and reduced stress (Linley 2001, Brown and Day 2002; Brennan et al. 2006; Billman and Belk 2009; Bice et al. 2013). Species-specific cages should be deployed at release sites as per Baerwald et al. (unpublished). Delta smelt should be released into cages at a to-be-determined density (fish/cage) for 3-7 days. At the end of this period, the lid to the cage should be opened and the cage should be tipped to allow the fish to swim out on their own. Other release aspects such as releasing during the night, or into certain habitats or conditions (turbid water), may maximize survival.

Serial releases of adult fish aged ~250 to 280 dph (but no more than 300 dph), at a schedule to-be-determined, could allow the FCCL to rear all fish for supplementation and research on-site rather than relying on an alternative facility for grow out (see *Facility Needs*). Adult fish should transition to live prey before release and transportation methods should be the same as those used for transfer of fish to LSNFH. Several weeks prior to each release, a subset of adult fish should be sent to the California-Nevada Fish Health Center for pathogen screening (see *Risk aversion measures* for additional details).

For release of fertilized eggs, one of the following two approaches would be needed depending upon staff and resource availability: (1) eggs are fertilized in the hatchery and transported on the same day to the release site and deployed on hatching frames in hatching boxes or (2) eggs and milt are stored for up to a to-be-determined number of days in the hatchery, transported to the release site, fertilized in the field, and immediately deployed on hatching frames in hatching boxes. Fertilized egg release-frequency is contingent upon which method is selected.

Points for Further Consideration

- The optimal time to hold adult fish in cages prior to release and the optimal fish density are unknown. Pilot experiments in these areas should be conducted prior to 2022 if possible and at the latest should be completed by 2024.

- Studies have shown that the selection differential between cultured and wild fish can be reduced by rearing fish in an enriched hatchery environment (e.g., varied food availability and spatial cues such as rocks and plants; Berejikian et al. 2000, Braithwaite and Salvanes 2005), ensuring rearing density is not too crowded (Thompson and Blouin 2015, Christie et al. 2016), or by providing life skills training (e.g., predator avoidance; Berejikian 1995; Tetzlaff et al. 2019). The benefit of these rearing practices could be evaluated and, if appropriate, attempted at FCCL or LSNFH.

- All adult fish (or a representative subsample) that are to be released could be genotyped to confirm that they originate from at least a minimum number of families necessary to meet genetic management objectives; minimum number would be determined through the RM&E and AM processes. A preliminary examination of the numbers of families currently represented in extraneous fish produced by FCCL (i.e., the ~13,000 thinned fish) should be performed to check that this target number of families exists and is possible to achieve.

- Although performed in unique instances in the past, there is no formal protocol for the transportation of fertilized or unfertilized eggs. Various egg transportation methods should be trialed and written into the delta smelt hatchery operations manual (as one of the SOPs).

- Deployment of hatching frames and boxes common in lakes and estuaries in Japan but has never been attempted in the Bay-Delta. Several deployment techniques (e.g., in cages or mesocosms or anchoring with weights) should be tried using empty hatching frames and boxes or a surrogate species prior to the first release.

V. Domestication Selection Studies

Every year at FCCL, some pair crosses do not survive or are recovered in very low numbers (Finger et al. 2018). The GVL monitors the recovery and potential loss of genetic diversity of pair crosses. Recovery refers to the identification of genetically-tagged offspring from pair crosses from the previous generation of adult fish. When, during the spawning season, some pair crosses in an MFG were found to have not been tagged, hatchery staff conduct focused tagging of fish in that tank in order to increase the likelihood of data recovery from their offspring. In some cases, individuals from a pair cross may be late-maturing and will therefore not be tagged until near the end of the season.

One hypothesis for poor recovery of some families is competition and varying levels of fitness among offspring from the eight pair crosses that are combined into a single tank to form each MFG. Some families may be inherently better equipped to survive in a hatchery setting.

Such domestication selection is an inevitable hatchery effect (Allendorf 1993, Frankham 2008). Family size is equalized at the egg and larval stage to reduce reproductive variance among families and thereby slow the rate of genetic adaptation to captivity. Despite this practice, and as expected, domestication selection appears to have occurred (Finger et al. 2018). For example, survival and growth of cultured delta smelt at FCCL increased after the first two years of the program (Lindberg et al. 2013); following handling and transport stress, stress response markers were distinctly reduced in first generation cultured fish (crosses with one wild and one cultured parent) compared to fish born in the wild (Afentoulis et al. 2013); and body condition and upper temperature tolerance was greater in later generations of hatchery crosses compared to early generations (Davis et al., unpublished). Furthermore, Finger et al. (2018) showed that more offspring are recovered from pair crosses with higher indices of hatchery ancestry and that this trend has increased over time. These studies indicate that delta smelt are adapting to FCCL yet the rate and extent of domestication remains unknown, including its effects on physiology and behavior across generations in cultured delta smelt. The FCCL, in partnership with researchers at UC Davis and federal and state agencies, is actively investigating the basis of domestication in delta smelt and operational changes that can be made to mitigate it. More robust understanding of effects of domestication selection could benefit from identification of domestication-linked SNPs to incorporate into the GT-seq panel.

Enclosure Studies

- Scientists at DWR, UCD, and the Service have developed species-specific cages to study cultured delta smelt in the field (DWR et al. unpublished). In 2021 cages with cultured fish that show low and high indices of domestication will be deployed in the Bay-Delta. Domestication index (DI) is an additive measurement calculated by the program PMx (Lacy et al. 2012) that quantifies the length of time (generations) that the genome of an individual fish has spent in the hatchery. Finger et al. (unpublished) found in-hatchery survival of cultured delta smelt with low DI to be much lower than that of high DI fish. The objective of the new study is to compare survival of these two groups in the natural environment to inform population supplementation strategies.

- Scientists at UCD, DWR, CDFW, and BDFWO are in the process of collecting genomic, transcriptomic, and epigenetic data from groups of FCCL-reared delta smelt with high and low DI to elucidate the molecular basis of domestication selection. This project began in 2020 and has a three-year tenure.

VI. Cryopreservation of Milt

The Service recommends banking of cryopreserved milt from wild and cultured delta smelt because this could have numerous benefits for species conservation, including i) preservation of genetic variation for future generations; ii) protection against inbreeding and inbreeding depression; iii) the crossing of genetically diverse fish at will; iv) flexibility in spawning time; v) the ability to spawn when broodfish are scarce; vi) reduction of milt waste from important individuals; and vii) ease of milt transport and spawning among geographically distant locations. These elements are important for conservation hatchery efficiency and could facilitate the increased production of cultured delta smelt. The Service currently is exploring technical and logistical aspects of cryopreservation of delta smelt milt.

VII. Hybridization Studies

Hybridization between delta smelt and wakasagi (*Hypomesus nipponensis*) is insufficiently understood in terms of current and potential risk posed to delta smelt. May (1996) and Trenham et al. (1998) genotyped eight allozyme loci in the upper estuary's three osmerids, namely delta smelt, wakasagi, and longfin smelt (*Spirinchus thaleichthys*). The purpose of both studies was to assess the extent of wakasagi invasion and hybridization with delta smelt, and species misidentification at the state and federal fish facilities. Results indicated that hybridization of wakasagi and longfin smelt with delta smelt occurred at low levels and that species identification based upon morphological characteristics alone can sometimes be problematic. To improve resolution and gain a greater understanding of more recent patterns of hybridization, Fisch et al. (2014) screened nine microsatellite loci and 16 species diagnostic SNPs and sequenced a region of the mitochondrial cytochrome *b* gene in delta smelt, wakasagi, and longfin smelt collected from numerous sites in the Bay-Delta in 2003, 2005, 2007, and 2009. As in previous studies, the authors found that hybridization among the three species was infrequent and that 11% of morphologically ambiguous fish captured during monitoring surveys were hybrids. For all hybrids detected, delta smelt was the male parent. Benjamin et al. (2018) expanded upon genetic tools for measuring hybridization among osmerids by developing SNP assays from RAD-seq data that could distinguish pure species, first generation hybrids, and backcrosses (the offspring of a hybrid with one of the parent species). The authors used these assays to test hybridization in wild fish caught between 2010 to 2016 in the Yolo Bypass and discovered several delta smelt and wakasagi F_1 hybrids and backcrosses, again all with delta smelt as the male parent.

Additionally, 32.7% of species field-identifications, which had relied on morphology, were inaccurate and overestimated the numbers of delta smelt. Misidentifications in this study were attributed to morphological variance, particularly in isthmus pigmentation, in larval and juvenile delta smelt and wakasagi, and degradation of fish specimens.

Studies to date suggest a sex-biased asymmetry in hybridization between delta smelt and wakasagi. If so, this could stem from, for example, rarity, behavioral influence (choice, co-location of sexes), cross-specific genetic incompatibility, differential post-hatch survival, or some combination. There is clear need to sufficiently characterize the spatio-temporal dynamics of hybridization between delta smelt and wakasagi. This information could improve screening of incoming wild fish to identify and exclude hybrids from incorporation into the broodstock, influence choice of release sites, and facilitate monitoring of hybridization in the wild.

Genomics-based study of hybridization will benefit from incorporation of species-specific SNPs (Benjamin et al. 2018) into the GT-seq panel. Studies by Carson and colleagues (Carson and Dowling 2006; Carson et al. 2012) provide a framework for experimental design and monitoring and evaluation of hybridization, including genetic introgression (Carson and Dowling 2006), phenotypic diversity (Tobler and Carson 2010; Dowling et al. 2016), and relationships between genetic and environmental variation over space and time (Carson et al. 2008, 2012).

VIII. Risk Aversion Measures

If the wild population remains extant when supplementation begins, every effort must also be made to defend this population against potentially deleterious effects of hatchery-origin population supplementation. As above, incorporation of wild broodfish and intensive genetic management by the GVL has resulted in cultured fish that closely resemble the wild in terms of co-ancestry and levels of genetic diversity. The risk of swamping wild population alleles with hatchery alleles or a genotype-environment mismatch is therefore low (Waples and Do 1994; Tringali and Bert 1998; Lynch and O'Hely 2001; George et al. 2009; Evans et al. 2019).

Supplementation, however, can reduce fitness by increasing inbreeding if relatively few broodstock contribute disproportionately to reproduction in the wild (Ryman and Laikre 1991). To maintain high levels of genetic diversity, a sufficient number of pair crosses need to be represented among fertilized eggs and adult fish that would be released across the season. It is possible, due to hatchery rearing, that cultured adult fish will not behave or respond to the natural environment (e.g., foraging, migration, reproduction) in the same way as wild fish and that cultured fish could compete with wild fish for resources. To minimize these differences, delta smelt could be released at the fertilized egg stage or, as adult fish reared in an enriched environment, trained in predator avoidance, transitioned to live prey before release, or using a soft release strategy. Domestication selection is an inevitable outcome of hatchery rearing that should continue to be investigated in delta smelt so that effects of domestication can be ameliorated in the future. To avoid inadvertent transfer of pathogens into the wild, a subset of delta smelt (n = 60) from each release cohort should be sent to the Service's California-Nevada Fish Health Center for pathogen screening at least one month in advance of release. If validated in delta smelt before supplementation, disease monitoring should be expanded to include the high throughput DNA assay developed by Miller et al. (2016) and Teffer et al. (2017) and testing the same subsets of fish as well as environmental samples (e.g., water and tank scrapings) from the hatchery and from release sites prior to supplementation to test for pathogens that could negatively impact program success. Finally, meta-analysis studies of correlates of population supplementation and reintroduction success note that neglecting to mitigate the cause of decline was the top reason for program failure (Fraser 2008; Cochran-Biederman et al. 2014). Habitat alteration and loss are believed to be the most important factors in delta smelt decline; improvement and restoration of habitat and ecological conditions to support delta smelt is essential to long-term success of management of this species. Releasing cultured delta smelt in or adjacent to restored habitat where resources will be most plentiful may be most beneficial.

MONITORING AND EVALUATION

Implementation of a Supplementation Program will require a monitoring plan for captive propagation (i.e., hatchery population), supplementation of delta smelt (i.e., supplemented wild population), and the demographic and genetic responses of the combined wild-hatchery population. The objective is to develop demographics and genetics monitoring plans to evaluate growth, survival, reproduction and recruitment, and changes in genetic diversity of delta smelt under the integrated program (IHM). This requires i) establishment of baselines for population abundance, genetic variation, and hatchery production; ii) an integrated population and genetic monitoring plan that is based on a conservation IHM and is informed as described in sections on *Adaptive Management*, *Facility Needs*, and *Research Needs*; iii) reliance on a modified EDSM for representative sampling across the spatial and temporal distribution of delta smelt in the Bay-Delta; and iv) incorporation of information about annual hatchery broodstock and hatchery production for supplementation (numbers, times, and locations).

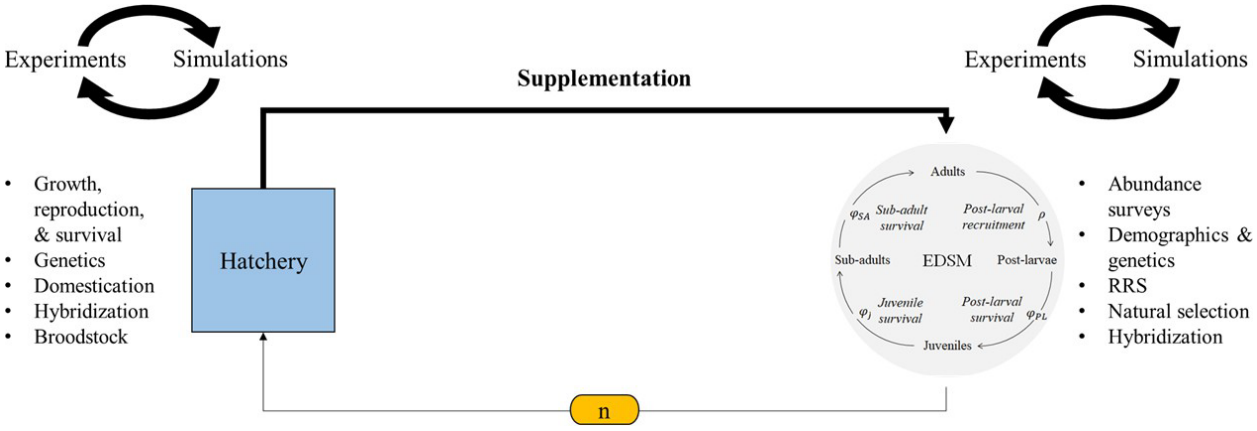

Figure 3. Conceptual illustration of monitoring and evaluation under the IHM. M&E is conducted for hatchery (left) and natural (right) populations. Experiments and models (demographics and genetics) inform adaptive management of the integrated program.

Monitoring under an Integrated Hatchery Program

Measuring success of a conservation-oriented integrated hatchery program requires robust monitoring and evaluation. This requires that management recognizes that hatchery and wild populations of delta smelt will be connected- and thus will interact- through movement of fish and their genes. For delta smelt, this entails monitoring that informs captive propagation, genetic management of natural and cultured stocks, broodstock management and breeding design, production and releases. This includes tracking status and trends in hatchery and natural populations, and requires tight coordination of population surveys and sampling for genetic and demographic estimation of effects of supplementation on both populations (natural and hatchery). In the case of delta smelt, there is uncertainty about population abundance, life stage to use for supplementation, necessary growth rate in wild, effects of domestication, among others. For these reasons, the supplementation monitoring program must rely on complementary

interdependence on models (conceptual, mathematical, genetic) and empirical studies (experiments) to inform adaptive implementation of supplementation (Figure 5). Below, sections describe priority components of demographic and genetic monitoring and relevant models.

Monitoring Plan

RM&E and AM under the IHM will require integration of population modeling and monitoring (demographic and genetic) with genetic management of hatchery-origin delta smelt. USFWS will work closely with partners as BDFWO leads modeling and monitoring of supplementation under the IHM.

Demographic and genetic monitoring will follow strategies recommended for conservation IHM and as described under Section 3 of HSRG (2014) and adapted to circumstances unique to delta smelt management. Structure of the monitoring plan is drawn from the following sub-sections:

1. Hatchery effects on the viability of natural populations (Section 3.1, pp. 36-48)
2. Hatchery broodstock management (Section 3.2, pp. 49-58)
3. Role of hatcheries in conservation and recovery (Section 3.3, pp. 59-70)
4. Fish health (Section 3.5, pp. 76-81)
5. RM&E and AM (Section 3.7, pp. 92-101)

Importance of Status and Trends

Effective monitoring is designed to capture status and trends (see **II. Population monitoring** below) in response of supplementation and other management actions and programs. Below sections consider needs for incorporating robust surveys and methodologies to design and implement a rigorous Monitoring Plan for supplementation of delta smelt within an integrated program. The first section describes modifications to Enhanced Delta Smelt Monitoring (EDSM) to provide requisite samples for estimation of demographic and genetic responses of the species to supplementation. Nested within this section are considerations for empirical and model-based (simulations) elements of the monitoring program, including relationship to RM&E and adaptive management under the IHM. Monitoring of hatchery populations and broodstock(s) is described briefly and as pertinent to the IHM; more comprehensive treatment of the hatchery stocks will be described in the delta smelt HGMP, which is in development (see *Relationship to the HGMP*).

I. Fish-Marking

Tracking of natural origin and hatchery origin fish and their offspring is critical to monitoring and evaluation of effects and effectiveness of supplementation. In an integrated program, tracking of hatchery and wild populations and their interaction requires a monitoring plan designed to i) identify (distinguish) origin of fish sampled (hatchery-origin, natural-origin) and assess their relative reproductive success (RRS) and ii) to track changes within individual population components (hatchery, wild) and synthesize this information to monitor changes in the global (total) population (i.e., all population components, including hatchery broodstock(s) and offspring, refuge population, and the Delta population for delta smelt). Use of genetic and, when possible, physical tags (described under *Facility Needs*) are vital to this monitoring design.

II. Population Monitoring

Quantitative assessments of managed populations rely on monitoring and modeling of population dynamics and genetics. The former depend on estimates of vital rates of reproduction, survival, and movement, and the latter rely on estimates of genetic diversity and effective population size. Monitoring is most effective when demographics and genetics monitoring are conducted together and used to inform each other.

Modifications to Enhanced Delta Smelt Monitoring (EDSM)

Technical and logistical modifications to the Enhanced Delta Smelt Monitoring program (EDSM) will be needed to support robust demographic and genetic monitoring of the integrated Bay-Delta population of delta smelt. Modifications to EDSM will require coordination with LFWO. Examples of modifications that are anticipated include revisions to their SOP to i) keep all fish caught and ii) preserve all fish returned to the laboratory in a manner that allows for genetic analyses to identify parentage (PBT). It is unclear at this time if fundamental design changes to EDSM are required.

If delta smelt are collected by other monitoring programs and can be preserved in a manner that allows them to inform the integrated hatchery program, then those fish may also be used to increase sample size for PBT efforts.

Demographics Models and Monitoring

For delta smelt, vital rates have been estimated in several modeling frameworks, using observations of abundance and entrainment over time (Rose et al. 2013a,b; Polansky et al. 2020; Smith et al. 2020). So far, delta smelt population models have only needed to keep track of the wild population, but if the wild stock is supplemented with hatchery-origin fish in the future, delta smelt population models will need to simultaneously track the dynamics of wild- and hatchery-origin fish.

Because delta smelt recruitment and survival rates depend on different environmental factors throughout the year, assessments of how best to supplement in relation to timing, amount, and conditions of salient environmental factors is needed. What is "best" is a complicated concept involving hatchery program operations, maintenance of both the wild and hatchery origin stock's genetic integrity, and short and long-term changes in abundance. The Service and DWR are working on modifications to the Service's Delta Smelt Life Cycle Model (Polansky et al. 2020) that will provide preliminary quantitative insight into relative benefits of releasing egg and sub-adult to adult life stages. This quantitative modeling may then be fed into a structured decision-making effort that can weigh expected ecological outcomes with economic, logistic, and other relevant considerations. Unless the structured decision-making effort is fairly unambiguous, the only way to confirm the modeled expectations may be to try both release strategies and empirically evaluate the relative merits of each. We caution, however, that doing this in a scientifically rigorous manner may be time and resource intensive.

Supplementation Life Cycle Model

A framework for potential approaches to integrate demographic modeling is described in Appendix 7. Briefly, Section 1 presents some simple examples of how supplementation questions could be approached from a population modeling perspective. Section 2 uses a previously fit life cycle model of delta smelt to estimate needed levels of supplementation to reverse past cohort-specific declines, and to simulate future abundances under different scenarios of simulation strategies (i.e., numbers of fish released and at what life stage). The first two sections are likely unrealistic in that hatchery origin fish are assumed to have the same vital rates (reproduction and survival rates) as wild origin fish. Section 3, therefore, describes a conceptual framework along with some initial mathematical description to allow integration of vital rate differences between natural origin and hatchery origin fish into Delta and hatchery populations. Additional development of the model will incorporate demographic estimates of effective population size, *Ne*, to complement genetic models (see next section) that estimate this parameter, which is critical to RM&E and AM of supplementation of delta smelt.

Genetic Models and Monitoring

Genetic management has been effective in conserving genetic diversity in the refugial population of delta smelt (Fisch et al. 2013; Finger et al. 2018) and will be instrumental to monitor population supplementation. Use of parentage-based tagging (PBT) will be used to track hatchery origin releases and their offspring. With PBT, all hatchery broodfish are genotyped, which allows identification of their progeny by parentage assignment (Anderson and Garza 2005, Steele et al. 2013), estimation of survival and recruitment of cultured delta smelt families released into the wild population, and monitoring of changes in genetic diversity following release (Schwartz et al. 2007). Use of PBT is a powerful tool because it can be applied at any life stage and there is no risk of physical loss of tags or electronic detection error (e.g., coded wire tags; Ebel 1974, Anderson and Garza 2005); accurate hatchery records and other operations particulars, however, remain critical to successful use of PBT. Validation of PBT also is necessary and is best approached through comparison to data obtained from physically-tagged

hatchery broodstock and production data. The GT-seq panel (as described in *Facility Needs)* for PBT will include SNPs developed by Lew et al. (2015). Transition to a SNP-based GT-seq panel for parentage assignment will ensure cross-lab reproducibility of data for broodstock management of the refuge population and captive-bred fish released into the Bay-Delta.

Genetic monitoring and PBT are instrumental to integrated hatchery models for conservation programs. Fin clips from delta smelt collected in population surveys, including wild broodfish captured by FCCL staff, will be preserved in ethanol and genotyped by AFTC for monitoring by BDFWO and GVL to support ongoing genetic management of the refuge population and the future genetic management of the integrated population.

Methods, metrics, and design of the long-term genetic monitoring also will be adapted to an IHM model and, as applicable, follow well-established genetic monitoring programs, including for conservation integrated programs (Redfish Lake Sockeye, Kline and Flagg 2014); monitoring and evaluation of relative contribution of hatchery-origin releases to natural-origin populations (red drum, Karlsson et al. 2008; Carson et al. 2009; Carson et al. 2014); for monitoring status and trends over space and time (razorback sucker, Dowling et al. 2014; Carson et al. 2016; and Rio Grande silvery minnow, Osborne et al. 2012; Carson et al. 2020), and from monitoring of delta smelt (Finger et al. (2017, 2018). Changes in genetic metrics and their relationship to census population size and hatchery stocks will be assessed regularly under the RM&E and AM framework of the integrated supplementation program for delta smelt.

Supplementation Genetics Models

A framework for potential approaches to integrate genetic modeling is described in the delta smelt Population Genetics Model, DSPGM (Carson 2019). The DSPGM is an individual-based population genetics model developed to investigate potential population genetics effects of supplementation of delta smelt. The model simulates demographic decline of the natural sub-population and establishment of a refuge population, followed by initiation of an integrated program (IHM) for hatchery-origin supplementation of the natural-origin population. A multifactorial framework is presented to evaluate population genetics responses across combinations of i) a plausible range of post-supplementation changes to population growth rate in the wild and ii) strategies for hatchery broodstock management under the IHM. The model uses a range of variance in reproductive success (VRS) of broodstock, where VRS spans from low (conservation hatchery) to high (production hatchery). Further development and application of the model will inform RM&E and AM of delta smelt supplementation.

III. Evaluation and Management

The Supplementation Strategy provides a roadmap to transition from current management practices to supplementation conducted under a formal Supplementation Plan. This transition strategy will capitalize on an initial period of RM&E to test the efficacy and effects of production and release of cultured fish, and through an adaptive management process to institute science-based modifications to the developing supplementation SOP. This approach also will serve to refine the RM&E and adaptive management process for its continuation once supplementation begins. A summary of some primary information and metrics, and processes to be evaluated and adaptively managed are summarized in Table 3.

Table 3. This is a draft list of metrics that will need to be tracked to implement this supplementation strategy. Sub-table A. Project management. Sub-table B. Research and Monitoring. Sub-table C. Quantitative metrics. Acronyms and metrics include terms as described and defined in HSRG (2014).

A. Project Management

Target	Metric to be Monitored	Management Application
Integrated program	Integration of multiple facility operations, RM&E of IHM and EDSM	Facilities and Monitoring
Infrastructure, operations, and staffing	Capacity to meet facility, research, and supplementation needs	Facilities
Increase production under genetic management	Number of fish produced and associated conservation genetics thresholds	Research --> Facilities
Broodstock management	Genetic metrics (see below), proportion wild fish in broodstock, and as described in HGMP	Facilities
Backup population	Full broodstock redundancy	Facilities
Production for flexible release schedule	Number of fish available for release at desired times	Facilities
Grow-out facility	Production capacity	Facilities
New facilities	Status	Facilities
Genetic tags	GT-seq panel composition	Research --> Facilities
Physical tags	Methodology and scaling personnel effort to production	Research --> Facilities
Fish availability for Refugial population, research, and supplementation	Capacity increase success over timeline and production mean and variability by life stage	Facilities
Research allocation	Availability to priority and current studies	Facilities (rolling)
EDSM	Catches of delta smelt	Population status and trends and hypothesis testing

B. Research and Monitoring

Target	Metric	Management Application
Proposed studies	Review, approval, and status	Facilities and hypothesis testing
Field and laboratory experiments	Track progress and data availability for assessment	Hypothesis testing
LCM/Life stage modeling studies	Vital rates and anticipated population trajectories	Hypothesis testing
Life stage field studies	Status, performance of cultured fish in hatchery and Delta, information for modeling studies	Hypothesis testing
Genetic simulations	Status of molecular performance metrics	Facilities; Hypothesis testing; population surveys;
Domestication selection studies	Status, performance of cultured fish in hatchery and estuary	Facilities and Hypothesis testing
Fish availability for RP, R, and S	Capacity increase over timeline and production mean and variability by life stage	Facilities
Research allocation	Availability to priority and ongoing studies	Facilities (rolling)

C. Quantitative Metrics

Acronym	Evaluation Metric	Management Application
HOB	The number of hatchery-origin fish used as hatchery broodstock.	Facilities and Monitoring
HOR	Fish of hatchery origin. As a variable, it is total number of Hatchery-Origin Recruits from a hatchery program	Facilities and Monitoring
HOS	The number of hatchery-origin fish spawning naturally	Monitoring
HOS_{census}	The number of hatchery-origin adults in the spawning population	Monitoring
NOR	Refers to a fish of Natural-Origin (a product of natural spawning). When used as a variable, it is the total number of Natural-Origin Recruits from a population.	Monitoring
NOS	The number of natural-origin fish spawning naturally	Monitoring
NOB	The number of natural-origin fish used as hatchery broodstock	Facilities and Monitoring
pHOS	Mean proportion of natural spawners composed of hatchery origin adults in a population each year	Monitoring
$pHOS_{census}$	Total Hatchery-Origin fish in the spawning population	Monitoring
$pHOS_{eff}$	Effective pHOS given reduced spawning success of hatchery-origin fish in wild (RRS* $pHOS_{census}$)	Monitoring
PNI	Proportionate Natural Influence on a composite hatchery-origin/natural-origin population. PNI is a measure of the percentage of time that genes of a composite population spend in the natural environment	
pNOB	Mean proportion of a hatchery broodstock composed of natural-origin adults each year	Facilities and Monitoring
R/S	Recruits per spawner	Monitoring
RRS	Reproductive success of first generation hatchery-origin adults relative to natural-origin adults	Monitoring
N	Population census size	Monitoring
N_b	Effective number of breeders	Monitoring
N_e	Effective population size, including global and local estimates (cf. Ryman et al. 2019)	Facilities and Monitoring
H_o	Observed heterozygosity	Facilities and Monitoring
H_e	Gene diversity (expected heterozygosity)	Facilities and Monitoring
N_A	Number of alleles	Facilities and Monitoring
AR*	Allelic richness	Facilities and Monitoring
NeI	Inbreeding effective population size	Facilities and Monitoring
NeV	Variance effective population size	Facilities and Monitoring
Kinship	Relatedness	Facilities and Monitoring

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Appendix 1

Regulatory Framework

REGULATORY FRAMEWORK FOR SUPPLEMENTATION OF DELTA SMELT

This document describes a pathway to implement supplementation of wild fish by hatchery-produced delta smelt under the basic framework as outlined in the October 2019 Biological Assessment (BA). The conservation measure outlined within the BA may require additional permitting steps at the federal and state level, and will involve a review process within the overall CASS interagency team designed for efficient and coordinated approval of research and work activities.

BACKGROUND

Status of 'Conservation Propagation' for delta smelt

The California Department of Fish and Wildlife (CDFW) identified in their 1993 Candidate Species Report the need for scientific research on creating a refuge population of delta smelt and for refining hatchery and production techniques (CDFW Publication 93-DS, p. 60). Intensive fish culture techniques were initiated and funded by California Department of Water Resources (DWR) and the U.S. Fish and Wildlife Service (Service, or FWS) in 1993 in response to the federal and state listing; through cooperative efforts of several agencies since that time, refinement of these techniques have assisted in development of a captive refugial population as one level of security against species extinction (Fisch *et al*. 2013; Lindberg *et al*. 2013), and in maintaining genetic diversity of the species and a reliable supply of captive fish for research. These activities are currently permitted via ESA §10(a)(1)(A) recovery permit to Dr. Tien Hung at the University of California-Davis (UCD) Fish Conservation and Culture Laboratory (FCCL).

Augmented each year with up to 100 (adult equivalents[1]) wild-caught delta smelt, the refuge population is currently housed in two locations. The primary population is maintained at the FCCL in Byron, California, where presently the facility rears 34-40 multi-family groups (8 families/tank, depending on numbers of available mature fish), producing over 20,000 adult fish or ~200,000 eggs annually (pers. comm. T. Hung, May 1, 2020). The second (redundant) population is a subset of the FCCL population composed of 200 fish/multifamily group and is located at the Livingston Stone National Fish Hatchery (LSNFH) in Shasta Lake, California. The LSNFH population serves as a backup to minimize risk. The FCCL, operated by researchers and technicians from UCD, has: (1) developed reliable techniques for the capture of delta smelt from the wild and for the production of all life stages of delta smelt (egg through to adult spawners), (2) provides a source of animals for numerous (~5–10) research programs including on-site and off-site research, and (3) is currently maintaining a genetically and demographically robust captive population. The UCD Genomic Variation Laboratory, Department of Animal Sciences on campus assists in the maintenance of broodstock histories, population pedigrees, and microsatellite genotyping using a combination of molecular and pedigree-based genetic methods (Fisch *et al*. 2013).

[1] Beginning in 2018, the Service has counted delta smelt take within the Interagency Ecological Program Programmatic BiOp using life stage-specific weighting (Slater 2017), such that earlier life stages are counted fractionally, based on an underlying survivorship curve. This metric is termed "adult equivalents."

The FCCL continues to develop and refine critical culturing techniques and technologies under their ESA §10(a)(1)(A) Recovery Permit. However, current permits do not allow for captive-reared or hatchery-propagated fish to be released back into the wild.

In July 2008, the CALFED Science Program hosted a workshop titled "The Use of Artificial Propagation as a Tool for Central Valley Salmonid and delta smelt Conservation." Given the precipitous decline of delta smelt documented in San Francisco Bay estuary since 2001, this workshop has led to early discussions as to whether the controlled propagation program at the FCCL or another facility could be scaled up if and when augmentation of the wild population becomes a desired recovery action. The outcome of that workshop is a peer-reviewed paper published in San Francisco Estuary and Watershed Sciences in April of 2011 (Israel *et al.* 2011) that advocates for scientifically defensible and ecologically based restoration programs- which adequately address limiting factors facing delta smelt in its estuarine habitat- before there is any attempt to supplement (augment) natural populations. Further, the authors state "a mitigation [supplementation] hatchery for delta smelt should be expected to create all the same risks for the natural population as a salmonid hatchery (*i.e*., loss of genetic diversity, domestication selection, impairment of carrying capacity available to the natural population)" (Israel *et al*. 2011).

A succeeding workshop in winter 2017 (Lessard *et al.* 2018) identified key issues for potential future use of cultured delta smelt for research and management. Participants at this workshop advocated for *in situ* experiments using cultured delta smelt as a precursor to supplementation actions. Participants agreed that experimental and supplemental releases of cultured fish need to be conducted within an adaptive management program that is integrated with other strategies, including habitat restoration; and concluded that there is sufficient baseline information about delta smelt and the existing culture program to proceed with targeted field research that utilizes cultured fish.

The BDFWO has been systematically moving toward a regulatory approach to implement important research, and currently experimental use of cultured fish from FCCL in *contained* conditions is allowed through the existing FCCL permit, and the accompanying December 7, 2018 *Programmatic Biological Opinion on the Amendment of Recovery Permit for U.C. Davis Fish Conservation and Culture Laboratory (TE-027742-6) Pursuant to Section 10(a)(1)(A) of the Endangered Species Act for Actions Involving the Use of Cultured Delta Smelt During Contained Study in the Natural Environment*. Release of cultured delta smelt to the wild will require an amendment to the existing FCCL recovery permit, or a novel recovery permit to the applicant, along with requisite steps for State permitting.

Next Steps Toward Large-scale Captive Propagation (a 'Conservation Hatchery')

The 2019 BA included a program for near term supplementation of wild delta smelt. The first step in the process described was development of a Supplementation Strategy by USFWS. The goal of the Strategy is to increase delta smelt hatchery-production to a number and the life stages necessary to effectively supplement the wild population and to capture and maintain genetic diversity of the species. This requires identification of studies to develop necessary information to begin a supplementation program, ongoing genetic management of hatchery delta smelt and expansion of production capacity of the FCCL, development of a monitoring program, and identification of a plan to acquire necessary permissions.

The intent of this program is to begin supplementation of delta smelt in the wild with fish captive-produced by the FCCL within 3-5 years from the issuance of the 2019 USFWS Biological Opinion (BiOp). Reclamation proposes to continue supporting the FCCL in its ongoing efforts to capture and maintain existing genetic diversity and to expand rearing capacity at the FCCL, and other sites if necessary, to annually produce up to approximately 125,000 adult delta smelt within three years from the issuance of the 2019 BiOp (BA pp. 4-79 and BiOp pp. 171-172).

The approaches, research, and experiments identified in the Supplementation Strategy are intended to increase the likelihood that the population of delta smelt will be sustained in the wild by achieving a robust, genetically-diverse captive population. Implementation of the Strategy is intended to increase the likelihood for delta smelt to survive and reproduce in the wild, to boost population numbers and maintain distribution throughout the species range, and for the population to be able to withstand the multiple factors that have led to its decline, including entrainment and associated predation resulting from seasonal operations of the Banks and Jones facilities.

Following issuance of the 2019 USFWS BiOp, preparation for development of the Strategy has included progress on a suite of research studies, life cycle and population genetic models, and the establishment of the multi-agency committee on Culture and Supplementation of Smelt (CASS). The supplementation program consists of two broadly defined phases: an interim phase, which is 3-5 years following the signing of the BiOp (~October 2022-2024) and will focus on expansion of the existing FCCL capacity, demographic and genetic and monitoring, and critical research necessary to help define the specific approach for implementation of the full augmentation strategy. The full supplementation program will likely be implemented through a production-scale conservation hatchery (capable of full production to produce the numbers and desired life stages/genetic composition of the supplementation stock), which may be using expansion of existing infrastructure, or include new facilities, if necessary.

Interim Phase

The following premises are working assumptions within the regulatory framework as we progress from this Strategy through to an eventual Supplementation Plan:

- Anticipate no release to the wild in the near-term (initial research will focus work within contained facilities/structures).
- Existing USFWS December 7, 2018 Programmatic Biological Opinion on the Amendment of Recovery Permit for U.C. Davis Fish Conservation and Culture Laboratory (TE-027742-6) Pursuant to Section 10(a)(1)(A) of the Endangered Species Act for Actions Involving the Use of Cultured Delta Smelt During Contained Study in the Natural Environment can accommodate these planned research activities.

- Additional permitting will be facilitated through the CASS workteam planning process to include:
    - Initial Screening of Fish Requests for regulatory discussion (red flag review)
    - Coordination between PI's and RCWG designees/permitting staff with FWS/DFW to resolve any questions and stock availability/production review by CPWG and issues before technical merit review by RWG
    - RWG review team merit review of proposals approved for screening by initial RCWG and CPWG screening
    - Recent discussions suggest (for feasibility purposes and planning at FCCL) that this process may require an annual workplan process analagous to IEP SMT
- Additional adult equivalents (AE) for FCCL broodstock collections will require amendment of their current §10(a)(1)(A) permit and CESA MOU.
- Research projects that involve intentional release to the wild will require amendment of FCCL §10(a)(1)(A) permit and a CESA MOU and active participation by CDFW personnel.

Implementation Phase

It is intended that the current Supplementation Strategy provides the roadmap towards the eventual completion of a **Supplementation Plan** intended to be an interagency document submitted to the Service (and CDFW, where appropriate) in consideration for a §10(a)(1)(A) permit for full implementation of supplementation under the adaptive, science-based framework developed through the research and coordination conducted during the interim phase.

A complete application package for the recovery permit/State MOU (if required) to conduct the full supplementation program should include:

a. A Captive Propagation (Husbandry) Plan
    i. Details of capture, handling, transport
    ii. Holding/feeding
    iii. Expressing/fertilizing/incubating
    iv. Rearing etc.
    v. Scaling up of facilities and/or technologies

b. A final Hatchery Genetic Management Plan (HGMP)
    i. Integration of data on the distribution of genetic diversity with historical and current ecological data;
    ii. A robust discussion as to the recommendation that delta smelt continue to be managed as a single, unstructured population in order to focus efforts on maintaining effective population size (N_e), as opposed to maintaining/augmenting isolated subpopulations throughout the Delta to prevent local extinction (Fisch 2011, pp. 75–92);
    iii. Inclusion of a pedigree analysis to attempt to equalize family contribution (i.e., minimize variance in family size) and breeding schemes that minimize kinship and variance in reproductive success in the population (Fisch *et al.* 2013, p.102); and

iv. Development of a genetic risk assessment in order to set priorities and desired outcomes of the augmentation/reintroduction program.

c. A final Health Management Plan (HMP)

i. What disease screening entails
ii. SOPs for screening during facility operation and pre-release
iii. Contingency planning for outbreaks

d. A Release Programmatic Plan

i. Initial or Phases of plans for releases

1. Season/life stage
2. Locations
3. Intervals
4. Transport and technology
5. Release protocols

e. An Adaptive Management (Implementation) Plan

i. Includes demographic and genetic performance metrics and success benchmarks
ii. What monitoring and research will continue to evaluate success and improve
iii. Describes the feedback loop (interval of review cycle, oversight and forum)
iv. Independent review?

f. Secured commitment to funding.

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Appendix 2

Increased Production with Genetic Management

GENETIC MANAGEMENT OF INCREASED PRODUCTION OF DELTA SMELT FOR SUPPLEMENTATION

PI: Evan W. Carson[1]

Co-PIs: Amanda Finger[2], Angela Eads[3], and Tien-Chieh Hung[3]

[1] U.S. Fish and Wildlife Service, Bay-Delta Fish and Wildlife Office
[2] UC Davis Genomic Variation Laboratory
[3] UC Davis Fish Conservation and Culture Laboratory

BACKGROUND

The 2019 Biological Opinion (BiOp) for delta smelt (USFWS 2019) has a provision for supplementation that stipulates the twin mandate to, within 3-5 years of issuance of the BiOp, i) increase the production at FCCL to 125,000 delta smelt for supplementation and ii) conserve genetic diversity through genetic management of captive propagation of delta smelt for supplementation. The FCCL is finalizing an agreement with U.S. Bureau of Reclamation (USBR) to expand the capacity to, within 3 years, produce 50,000 adult delta smelt annually for supplementation; this is in addition to production of the refuge population maintained at the FCCL. The production method for supplementation requires stricter genetic management than that proposed in the pending agreement. Achieving robust genetic management brings logical and operations demands above those described in the pending agreement to triple current production at the FCCL. Therefore, a separate collaborative agreement is proposed to identify an alternative breeding strategy that will satisfy production and genetic management mandates under the BiOp.

A challenge for scaling production of delta smelt for supplementation is to develop a spawning design that is efficient and meets imperatives for genetic management. Under the pending agreement, production for supplementation would be based on paired-crosses (1 dam x 1 sire, or 1 x 1) from broodstock obtained from among 32-34 multi-family groups (MFG) used first for production of the refuge population of delta smelt maintained at FCCL. Paired-crosses are resource intensive (e.g., in personnel, time, and space) and impractical for the scale of production required for supplementation. Alternative designs that prioritize efficient production, such as uncontrolled group spawn, carry unacceptably high costs in reduction of effective population size, Ne, (increased inbreeding, loss of adaptive genetic variation, and reduction of fitness (LaCava et al. 2015) and further increased domestication (see Finger et al. 2018).

Alternative spawning strategies need to be considered to increase efficiency of production through increasing the number of breeders per spawn and through genetic management of variance in reproductive success and family size. Delta smelt, however, have proved difficult to spawn naturally through spawning designs typified by tank-based combinations of dams and sires in a more controlled group (Hung unpublished), and thus is not a feasible application to a spawning regimen for production scale needed for supplementation.

An alternative approach is a 'pseudo-communal spawn' design, similar to management of salmonids, for which strip-spawning is used to conduct controlled crosses. Multi-family crosses (MFC) can be performed quickly by pooling eggs from multiple female fish and adding milt from multiple males to create a greater number of family groups from a larger pool of breeders. However, MFCs can result in offspring with a higher relatedness index than single cross families, as there will be many half-siblings. Furthermore, resulting offspring from each family are unlikely to be evenly represented due to gamete inequalities (e.g. fecundity differences) or gamete interactions (e.g. sperm competition, compatibility, unequal fertilization). To manage against these negative effects in production and in released populations (i.e., hatchery-origin releases), management of MFCs requires genetic management for selection of breeders, quantification of variance in reproductive success and family size, and estimation of Ne of individual and combined spawns (batched multi-family crosses) that will be used released to the wild.

OBJECTIVE

The goal of the proposed work is to develop an alternative breeding design and spawning method necessary to meet the 2019 BiOp twin-mandate to upscale captive propagation for supplementation and simultaneously conserve genetic diversity of delta smelt. This entails identifying a sufficient breeding design for which facility and operations resource demands (e.g., logistics, infrastructure, and operations) and expected conservation genetic outcomes (e.g., genetic diversity metrics, effective size of single releases) can be quantified. End products will be two-fold:

1. Develop a SOP for captive propagation and genetic management for supplementation.
2. Develop a cross-selection application (algorithm) for efficient captive propagation under genetic management for supplementation.

PROJECT ACTIVITIES

We propose to develop and experimentally identify from among alternative strategies a multi-family spawning design that is:

1. Feasible given non-synchronous maturity (irregular availability) of potential broodstock from MFGs for use in MFCs.
2. Quantifiable in effect on genetic diversity and Ne of single and combined spawns.
3. Quantifiable in logistical demands and operational resources.
4. Amenable to inclusion of an extended sire-pool banked under the cryopreservation program (currently in development between FCCL and USFWS).
5. Meets the twin mandate of production and genetic management for supplementation.

Application of theoretical framework to quantification of efficacy of spawning designs

Following the framework of Gold et al. (2008) and equations adapted from Lacy (1989), we will evaluate the effects of number of dams and sires in each a multi-family group that contributing to spawns from that group, variance in family size among spawns of multi-family groups, and effects of individual and combined spawns (i.e., combined MFCs) on potential pool of delta smelt slated for release (i.e., effective size of release populations, NeR). Empirical data will be used in simulations to further investigate reasonable bounds for expected range of genetic effects of each alternative breeding design. From this arrangement, effects of variance in reproductive success and family size can be quantified separately to determine the best approach to improve genetic management of spawns though such measures as pseudo-equalization of reproductive success, family size, or both (Gold et al 2008). Simulations can facilitate estimation reasonable bounds within which genetic management of offspring can be improved.

Additional experimental testing to determine the impact of gamete inequalities on fertilization and resulting offspring family ratios

MFC results in unequal gamete contributions of parents. In addition to use of pedigree and genotypic information to address this expected outcome, this study also will use gamete staining techniques to quantify the effects of egg availability and sperm competition on resulting offspring ratios.

Quantification of logistical and operational resources

Personnel testing of alternative breeding designs will be used to measure efficiencies in situ to identify procedural bottlenecks or methodological constraints on increased production.

Relevance of artificial fertilization and genetic banking by cryopreservation

Advancements in artificial fertilization (stripping of gametes, cryopreservation) will allow more broodstock individuals and genetically diverse gametes to be accessible for optimum multi-family crosses. While it is impractical to count or separate eggs before they quickly become unusable, and it is preferable that all eggs are fertilized to maximize production, milt volume can be standardized, pooled among males, and added simultaneously to maximize the resulting family diversity. Milt cryopreservation offers the opportunity for the long-term storage of sperm to assist when milt volume may be limiting or to access genetic material for use across generations, for example, to cross between generations that do not mature at the same time or preserve milt with a particular genetic background. Males can then be selected according to pedigree from the broodstock population or from a genetic library in storage and used to fertilize pooled eggs to minimize inbreeding.

Quantification Framework for Evaluating Efficacy of Alternative Spawning Designs

1. Identify an 'optimal' breeding design to improve production efficiency, maintenance of genetic variation, and average effective population size of average single releases.
2. Quantify variance in reproductive success and family size among alternative pseudo-communal spawn designs.
3. Quantify average effective population size, Ne, of dams, sires, and single spawns.
4. Quantify expected improvements in average effective size NeR of potential single releases under equalization and pseudo-equalization methods.
5. Statistical comparison of performance of alternative breeding designs.
6. Quantify logistical and operational resources required for each alternative breeding design.

Expected Results, Outcomes, and Benefits

FCCL will determine trade-offs between increasing production output with a potential decrease in genetic diversity due to performing mass spawnings with pooled gamete procedures. Expected benefits include identification of a preferred spawning design, as determined by experiments designed to quantify the relationship between production, genetic diversity, and effective population size. Further, this study will quantify logistical and operation resource-demands for production under preferred and alternative spawning designs tested. An SOP will be developed for implementation of the preferred spawning design, and an application (algorithm) will be developed to increase efficiency of cross-selection. These will products will support implementation of the Supplementation Strategy, which requires satisfaction of the 2019 BiOp twin-mandate to i) upscale production for supplementation and ii) manage production to conserve genetic diversity of delta smelt.

Schedule of Deliverables

1. Identification and initial testing of experimental design – October 2021
2. Identification of preferred and alternative breeding design- October 2022
3. Report describing alternative breeding designs and SOP for captive propagation and genetic management for supplementation- October 2023
4. Peer-reviewed publications describing methods and application to supplementation of delta smelt- December 2023

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Appendix 3

GT-seq Panel for Genetic Tagging and Monitoring

DEVELOPMENT OF GT-SEQ PANEL AND GENETIC BASELINE FOR DELTA SMELT SUPPLEMENTATION STRATEGY

PI: Evan W. Carson[1]

Co-PIs: Amanda Finger[2], Justin Bohling[3], and Christian Smith[4], Tien-Chieh Hung[4]

[1] U.S. Fish and Wildlife Service, Bay-Delta Fish and Wildlife Office
[2] UC Davis Genomic Variation Laboratory
[3] U.S. Fish and Wildlife Service, Abernathy Fish Technology Center
[4] UC Davis Fish Observation and Culture Laboratory

BACKGROUND

Genetic information is an important tool used by the U. S. Fish and Wildlife Service (Service) and our partners to address a suite of threats to aquatic species and evaluate the success of hatchery programs attempting to restore at-risk species. A prerequisite for most applications of genetic data to conservation and management is the existence of a reference data set, commonly referred to as a genetic baseline. The baseline consists of data collected from specimens of known origin, frequently including broodstock founders, analyzed with the same set of genetic markers used for a target application, such as long-genetic monitoring of supplemented populations, and parentage analysis, among others. Recent advances in genetic technology have provided entirely new methods for identifying and genotyping markers based on massively parallel DNA sequencing. "Genotyping-in-Thousands by sequencing" (GT-seq) is one such method, which provides a cost-effective method for quickly and efficiently sequencing large numbers of samples (>1,000) for hundreds of genetic markers (Campbell et al. 2015; Meek and Larson 2019).

For the Supplementation Strategy for delta smelt, increasing the efficiency with which data can be collected, and sharing updated baselines with partners will provide the greatest conservation benefit given limited resources. GT-seq is especially favored for applications that demand large-scale sampling, high throughput of 100s of SNPs, and long-term monitoring (Meek and Larson 2019), as will be required for genetic monitoring of the integrated hatchery model (HSRG 2009; Baskett and Waples 2013) for delta smelt supplementation program. Briefly, the USFWS 2019 Biological Opinion (BiOp) for delta smelt (USFWS 2019) requires completion of a Supplementation Strategy within one year of issuance of the BiOp, and that within 3-5 years of issuance that i) 125,000 delta smelt will be produced for supplementation and ii) captive propagation and genetic management ensure conservation of genetic diversity. The BiOp further stipulates that within 10 years of issuance the program is transitioned to a full-scale supplementation program supported by a new FTC-based conservation hatchery.

Development and use of genomic-based markers for high-throughput, cost-efficient genetic monitoring of delta smelt thus are essential to help meet the mandates of the BiOp for production and conservation of genetic diversity. Updating baselines with this new class of markers will improve resolution of genetic analyses, add new functionality, and substantially reduce per-genotype costs relative to traditional methods such as microsatellite analysis and other genomics-

based methods. GT-seq has been identified by the Service as appropriate to accomplish this mandate, and has been supported by substantial investment in staff training and technology sharing among Conservation Genetics laboratories and the BDFWO.

This study will support the Supplementation Strategy through collaboration with the Genomic Variation Lab at UC Davis to develop a GT-seq panel and establishment of a new GT-seq specific baseline for long-term genetic monitoring under an integrated hatchery model for supplementation of delta smelt. Ultimate use of the GT-seq panel will include parentage- based genetic tagging of broodstock used for production for supplementation and annual analysis of 100s of SNPs for the 1000s samples of individuals anticipated for long-term genetic monitoring for supplementation of delta smelt. In addition to high-throughput and cost- efficiency of GT-seq, other benefits include rapid generation of sequencing data for immediate dissemination to partners and replicability across labs. This study will utilize a combination existing SNPs (Lew et al. 2015) and potentially identification of new SNPs for incorporation into a GT-seq panel designed for long-term genetic monitoring for supplementation of delta smelt. This panel will complement the separate use of a SNP panel to be used for pedigree reconstruction of delta smelt used by the GVL for production and management of the refuge population at FCCL.

OBJECTIVE

The goal of the proposed work is to develop novel genetic markers for a GT-seq panel and update baselines for Delta Smelt for genetic monitoring under the Supplementation Program for Delta Smelt. Similar to the large-scale parentage identification program for the SCS program in San Joaquin River Restoration Program (SJRRP), the objective is to facilitate quick analysis and reproducibility for the genetic monitoring program. The end products will be twofold:

5. Develop a GT-seq panel from genomic data obtained for delta smelt.
6. Establish a new genetic baseline for delta smelt genotyped with these markers.

PROJECT ACTIVITIES

Activities will occur in two phases. The first phase will generate genome-level data from which GT-seq marker panels will be developed and optimized; existing SNPs will be used as available The second phase will re-genotype existing samples to develop a new genetic baseline for delta smelt.

Phase 1: GT-seq Marker Development for delta smelt

1. Identify variable regions in the genome that can be used for marker development.
2. Test markers to develop species-specific panel(s).

Phase 2: Genetic Baseline Development for delta smelt

1. Identify appropriate baseline samples to genotype.
2. Prepare baseline samples for DNA sequencing.
3. Estimate statistical power of baselines for management applications.
4. Write report summarizing results. Send report and baseline data to partners.

Study Plan for Phases 1 and 2

1. Year 1. Use study individuals for Tasks 1 (Alternative breeding designs) and 2 (VIE tagging).
2. Year 2. Pre-supplementation genotyping of broodstock for supplementation and for wild delta smelt in years 1-3.
3. Year 3. Establish new baseline from samples from 2020-2023 for genetic monitoring of delta smelt supplementation

EXPECTED RESULTS, OUTCOMES, AND BENEFITS

Establishment of a genetic baseline from genomic-based markers used in a GT-seq panel will meet urgent needs for genetic management of delta smelt under the Supplementation Program. These products will increase the efficiency with which genetic data are collected, as well as increase the types of insights these data can yield about supplementation and broodstock management of delta smelt. Measurable goals will include: development of a novel genomic-era genotyping panel for application to long-term genetic monitoring under the Supplementation Strategy for delta smelt; the numbers of markers incorporated into the panel; reduced per-genotype costs of data collection; an R-script to evaluate genetic metrics of success identified in the Supplementation Strategy; and increasing the novel conservation and management questions that can be addressed in management of delta smelt.

This work will advance implementation of science studies identified in the Supplementation Strategy, and will be conducted in coordination with two of those studies as included in this IA: Task 1(increased production) and Task 2 (physical tagging). Finally, this work will have additional benefit of application for use in studies of survival, growth, and other performance metrics for hatchery delta smelt used in enclosure experiments and other research by partner agencies, and in population abundance and genetic modeling by the BDFWO.

PRODUCTS AND DELIVERABLES SCHEDULE

1. GT-seq panels for Delta Smelt – September 2021
2. Reports describing new markers that will be shared with partners – September 2022
3. Genomic sequences submitted to publically accessible databases – September 2022
4. Peer-reviewed publications describing new marker panels and comparisons to previous genetic baselines – September 2023

DATA MANAGEMENT SUMMARY

Baseline data of genotypes will be submitted to publically accessible databases where available (e.g., fishgen.net). They will also be mirrored across local servers of all participating laboratories, and shared with partners and the public upon request. Additionally, the original genomic data will be stored by FTCs and publically available databases (e.g., the NCBI Sequence Read Archive). Genetic IDs will be established and available for access through the USFWS Progeny database. Reports summarizing this work will be made publically available, shared with partners, and hosted on Service websites. A data and tissue curation storage plan will be finalized and fully annotated R-scripts used for genetic analysis will be provided. Database accessible to propagation managers for state, federal, and UC facility managers.

DELIVERABLES

A study plan will be written for the development of SNPs and GT-seq panel along with guidelines for long-term data analysis. A data and tissue curation storage plan will be finalized and fully annotated scripts used for genetic analysis provided. Database accessible to propagation managers for state, federal, and UC facility managers.

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Appendix 4

Visible Implant Elastomer (VIE) Tags for Monitoring

Draft Scope of Work

EVALUATION OF VISIBLE IMPLANT ELASTOMER TO INFORM SUPPLEMENTATION NEEDS FOR DELTA SMELT

PI-Evan Carson[1]

Co-PIs- Gonzalo Castillo[2] and Tien-Chie Hung[3]

[1] US Fish and Wildlife Service, Bay Delta Office
[2] US Fish and Wildlife Service, Lodi Office
[3] UC Davis Fish Conservation and Culture Laboratory

INTRODUCTION

As part of the Delta Smelt supplementation Strategy under development, the USFWS is proposing to conduct a tagging study using cultured Delta Smelt to validate the use of Visual Implant Elastomer (VIE) in this species. This three-year study will concurrently enable to augment the production capacity of cultured subadult and adult Delta and will guide potential supplementation in future years.

The tagging work is expected to occur from December to February 2020-21, 2021-22 and 2022-23 at the UC Davis Fish Culture and Conservation Laboratory (FCCL), with additional work required to implement the study, validate the tagging method and increase the production capacity of cultured fish occurring in the remaining months each year.

This preliminary scope of work was developed in coordination between the USFWS and FCCL. This proposed study will enable tagging and tracking an increasing number of cultured Delta Smelt in the laboratory, which could be used in mesocosm-level cage experiments in the Delta. This proposed tagging study will inform additional interagency coordination required for conducting future cage experiments or other field evaluations and will serve as a research and management baseline to guide the envisioned supplementation strategy for this species.

Visible implant elastomer (VIE) tags have been used to mass mark fish using a combination of colors and body areas, enabling batch marking a variety of fish species in both marine (Willis and Babcock 1998; Malone *et al.* 1999) and freshwater habitats (Blankenship and Tipping 1993; Dewey and Zigler 1996; Halls & Azim 1998). The VIE tags may provide advantages to tag small fish like Delta Smelt because tags are small, inexpensive and allow repeated detection without sacrificing individuals to retrieve tags (Sanford *et al.* 2019). Tagging is performed by injecting a liquid elastomer into a transparent tissue that sets to form a permanent, biocompatible mark (Griffiths 2002). Previous reviews of this method have highlighted the need of validating VIE tagging carefully for any species and study (Jungwirth *et al.* 2019). Hence, the effectiveness of VIE tagging as an identification tool for Delta Smelt supplementation purposes should be validated under a broad range of scenarios prior to large-scale applications of VIE tags.

METHODS

Delta Smelt used in the tagging studies will be produced at the University of California Davis Fish Conservation and Culture Laboratory (FCCL; Byron, California). Detailed system setup, fish care, and maintenance procedures will follow Lindberg et al. (2013). Tagging will be performed using subadult and adult fish and the number of fish to be used in VIE tagging experiments will be 5000 (year 1), 20,000 (year 2) and 50,000 fish (year 3). The tagging period is expected to occur between December and February each of the 3 years while other tasks will take place the remaining months each of the three study years for Lodi USFWS staff (Table 1) and UC Davis FCCL (Table 2). The supplies for VIE tagging will be acquired from Northwest Marine Technologies (NMT). Subadult and adult fish will be held in black-interior, insulated fiberglass tanks (1,100 L). Fish will be screened prior to tagging and individuals less than 45 mm FL will be excluded to facilitate subsequent fish handling and tagging. Fish will be inspected prior to tagging to ensure consistent appearance in each test (i.e., no lesions, lordosis, distended abdomen, emaciation, exopthalmia, or severe operculum deformity). We will test four tag colors which have proven to result in the best tag identification in previous studies in Silvery Minnow (red, green, yellow, white) at various areas of the fish body. The general tagging setup will consist of a table with four people working in pairs in an indoor area or shaded outdoor area to minimize light-induced stress.

GENERAL TAGGING PROCEDURES

Tagging for Delta Smelt in this study will adapt the tagging procedures used to VIE tag Rio Grande Silvery Minnow *Hybognathus amarus*, given their similar size at the time of tagging and the use of tagged fish for supplementation purposes (USFWS 2016, Figure 1). The staff assigned to VIE tag Delta Smelt (taggers) will follow proper tagging procedures adapted for Delta Smelt based on the Standard Operating Procedures for tagging Rio Grande Silvery Minnow (Appendix) and general VIE tagging guidelines (NMT 2017). Taggers will perform at last one practice tagging session before conducting formal tests. Taggers will practice using preserved fish before tagging live fish. Preserved and live fish in the practice session will be concurrently examined and photographed by independent observers to ensure VIE tags are properly inserted and that tags are within the required tag length (appendix). Post-tagging survival of practice fish and control untagged fish will be compared at the end of a week. If necessary, further adjustments in the tagging process and environment factors (temperature, DO, ambient light) will be considered to improve tag retention, tag visibility and fish survival when compared to results achieved in this species using alpha numeric tags (Sandford et al. 2019).

Fish will be transported from the holding tank to 1-gallon anesthesia black pans containing tricaine methanesulfonate (MS-222; 0.1 g/L) to reduce stress and facilitate tagging. Fish will be individually handled once they are mostly anaesthetized so they can be grabbed while still floating. Fish will then be individually picked by each of the taggers and will be tagged using a manual elastomer injector (Figure 2). Both water temperature and DO will be monitored at regular intervals to keep optimal water quality prior and during tagging (temperature < 13 °C, DO > 4 mg/L).

Figure 1. Rio Grande Silvery Minnow showing VIE tags (USFWS 2016)

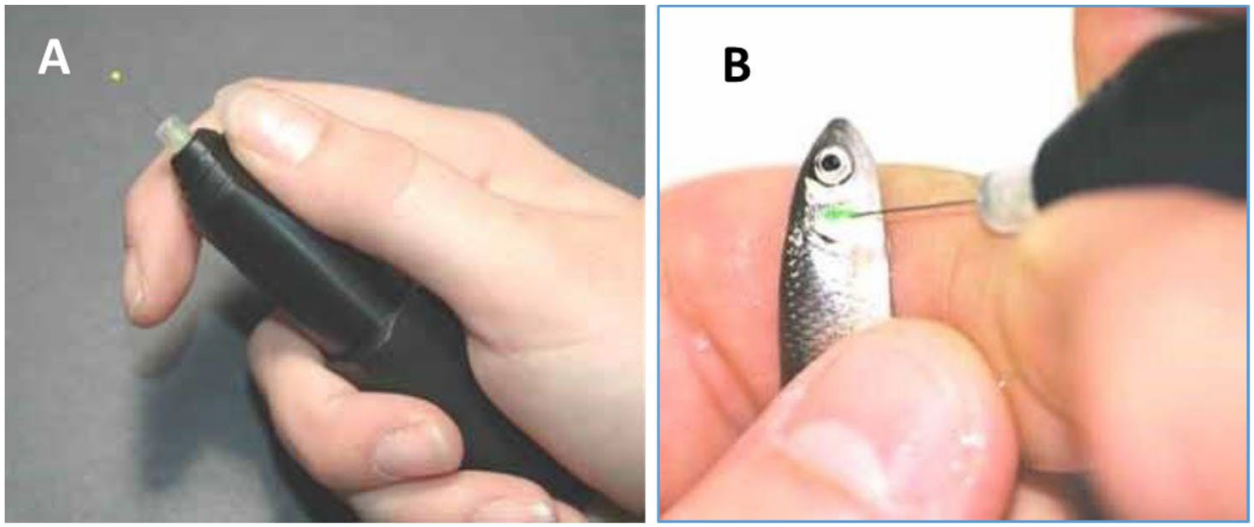

Figure 2. A: Manual Elastomer Injector, B: Small cyprinid being injected with a VIE tag (NMT 2017)

Year 1- Test 1: Suitable body areas to VIE tag Delta Smelt

Initial will be conducted to refine VIE tagging method to identify the body areas most appropriate for VIE tagging subadult delta Smelt. Treatments will consider potential body locations for VIE tagging including, the operculum area (OP) and areas adjacent to the fins: forward anal fin (FN); rear anal fin (RN) caudal fin (CD); forward dorsal fin (FD); mid dorsal fin (MD); rear dorsal fin (RD); forward adipose fin (FA); rear adipose fin (RA); pectoral fin (PT) and pelvic fin (PV) (Figure 3). Each selected treatment will include c.a. 40 fish and include the same number of fish tagged by each tagger. Taggers will be considered sub-treatments to account for potential differences in the length of VIE tags, tag retention and fish survival per treatment. To distinguish sub-treatments, each tagger will only use a particular VIE color across treatments. Three replicated controls (each including c.a. 40 fish) will include individuals exposed to the same anesthesia and handling process but will remain untagged). Following tagging, fish in each treatment and control will be transferred to separate holding tanks and treated with antibiotics to limit potential infections due to VIE tagging. Fish will also be exposed to a 1% salt solution for 4 h to improve survival (Knight 2006). Fish survival will be monitored daily in treatments and controls, and tag visibility and retention will be monitored every two week intervals for at least one month.

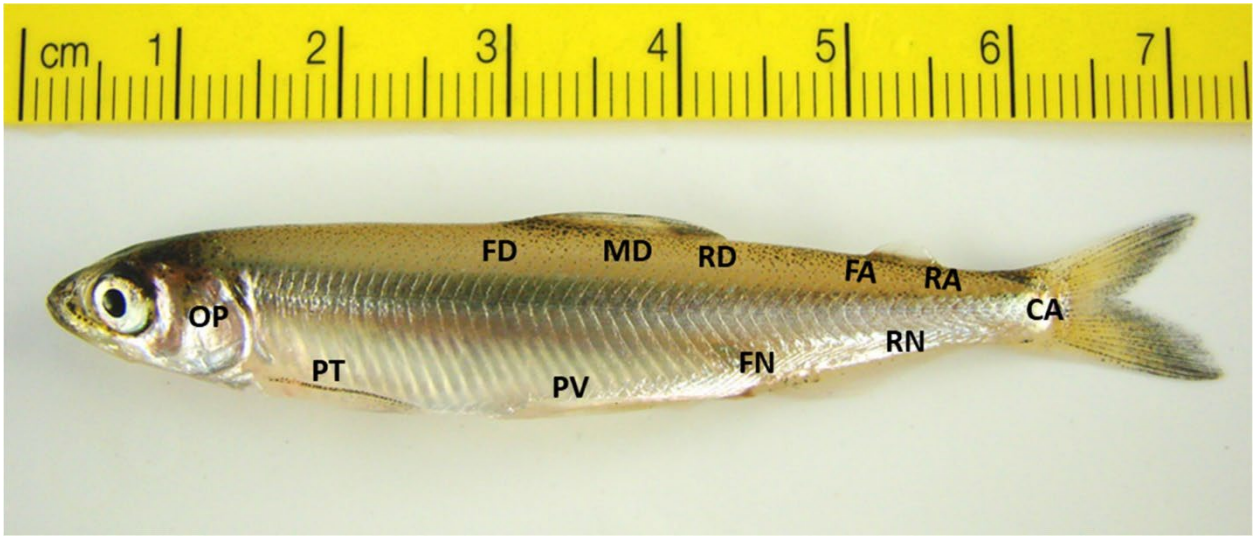

Figure 3. Left side of an adult delta smelt showing potential locations for VIE tags.

Year 1- Test 2: Influence of fish size on VIE tagged Delta Smelt

This test will help to evaluate potential differences in tag quality and fish survival across fish lengths. Based on the result obtained in test 1, we will tag fish ranging between 45 mm and c.a. 90 mm FL and will select body areas showing the best tag retention and use only one tag per fish. Whenever possible we will tag a similar numbers of fish across a broad range of fish lengths to attain a similar representation of fish numbers across length intervals (i.e., 40 fish for each 10 mm length interval). We will record the fork lengths of all tagged fish and will adjust the sample size for particular fish lengths as needed using random stratified sampling. Unless sub-treatments in test 1 show significant differences in tag retention, tag quality or fish survival among taggers (i.e., tagger effect), sub-treatments will be assigned to VIE tag colors rather than taggers in test 2 and subsequent tests. If no tagger effects are apparent, each tagger will use a variety of VIE colors per treatment rather than a single color. Otherwise, taggers will be considered sub-treatments. Fish survival will be monitored daily and tag retention, tag visibility and fish growth will be monitored monthly over a period of 3 to 7 months.

Year 1- Test 3: Influence of ambient light on VIE tagged Delta Smelt

This test will help to evaluate whether the range of ambient light levels potentially experienced by delta smelt across habitats could result in different post-tagging survival, tag retention, tag visibility and fish growth. Experimental design of test 3 will be refined based on test 2 results and will consider two light treatment (Castillo et al. 2019). Tag visibility will be evaluated qualitatively (1: low, 2: average, and 3: high) after examining at least 30 tags per VIE color. Post-tagging survival, tag retention, tag visibility and fish growth will be monitored at a monthly interval. As in previous tests, test 3 will consider one tag per fish unless tag retention is consistently low among body areas and fish survival is high.

Year 2: Potential differences in predation for untagged and VIE tagged Delta Smelt

Based on results in year one, replicated predation tests will be conducted separately for subadult and adult delta smelt under laboratory conditions. Predation test will be based on the experimental design described by Castillo et al. (2014) and will consider a consistent size of predators and prey among trials. Predation experiments will enable to determine potential differences in vulnerability to predation associated with VIE tag location, VIE color, life stage and fish length. If results show that predation depends on tag location and/or color of VIE tags, predation experiments will further provide valuable data to limit predation risk. Potential differences in predation vulnerability between tagged and untagged fish irrespective of tag location and color could further suggest systematic behavioral differences between tagged and untagged fish.

Year 3: Number of VIE tag codes to batch tag Delta Smelt

We will estimate the number of distinct VIE tag codes that can be reliably used to batch tag subadult and adult delta smelt. We will consider year 1 and 2 results to inform detailed experimental design of tagging test to be conducted in year 3. The number of unique VIE tag codes (#) is calculated as:

$$\# = [L! / (L - N)! N!] C^N$$

where: C = number of colors used, L= number of body locations and N = number of tags per animal. For example, 3 body locations, 4 colors and 3 tags per fish provides unique codes to potentially distinguish 64 groups of fish. Because more than one VIE tag per fish could potentially reduce post-tagging survival while greatly increasing the effort required to tag fish for large-scale supplementation purposes, we will consider double tagging fish if it is justified based on post-tagging survival, tag visibility and tag retention observed in years 1 and 2. Provided certain body locations result in higher tag quality (i.e., higher tag visibility, tag retention and fish survival) based on results in years 1 and 2, VIE tags in year 3 could be sub-categorized into left and right to optimize the quality of VIE tags and the number of VIE codes (Knight 2006). To estimate an appropriate number of unique VIE codes, we will also consider the anticipated research and management needs to track distinct batches of VIE tagged delta smelt in future years, as determined by the supplementation strategy needs for Delta Smelt.

STATISTICAL ANALYSES

We will use parametric test whenever possible and transform data if required to meet statistical assumptions. Different ANOVA designs will be considered to account for potential differences among treatments, sub-treatments and controls. To evaluate the influence of fish size on tag retention, tag visibility and fish survival we will use ANCOVA to determine whether the regression lines of response variables (tag retention, tag visibility and fish survival) as a function of fish length across treatments differ in slope or intercept. Detailed description of statistical analyses will be informed as experimental designs are refined based on initial test results.

DELIVERABLES

Each of the three proposed study years will include quarterly updates and an annual report. Presentations will be given each year at professional conferences and will include oral and/or poster presentations. A manuscript including results from year 1 and/or year 2 will be submitted to a peer- reviewed journal by the third year of the study. A second manuscript on the use of VIE tag for supplementation purposes will be submitted to a peer-reviewed journal within a year following completion of this three-year study.

BUDGET

A preliminary total budget of $818,074 covers the VIE tagging component for subadult and adult delta smelt for the three years. This budget supports the tagging related tasks for the Lodi USFWS and the UC Davis FCCL (Tables 2 and 3).

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Table 1. Lodi USFWS tasks for the Delta Smelt VIE Tagging Study. Work is planned for FY21 (Oct 2020-Sep 2021), FY22 (Oct 2021-Sep 2022) and FY23 (Oct 2022-Sept 2023)

	Months (FY21-FY23)											
LFWO Staff	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Project Co-lead (GS-12)	1	1	1	1	1	1	1	1	1	1	1	1
Fish biologist (GS-9)	2	2										
			3	3	3							
						4	4	4	4	4	4	
	5	5				5	5	5	5	5	5	5
Fish biologist (GS-5)			6	6	6							

LFWO Tasks

1. *Directs LFWO tagging effort:* Directs LFWO tagging study in coordination with Bay Delta FWS Office, LFWO, and UC Davis FCCL. Updates SOW and reviews study updates
2. *Purchases equipment & supplies:* Acquires tagging equipment + supplies
3. *Leads and conducts fish tagging:* Leads and conducts tagging work in coordination with LFWO and UC Davis FCCL
4. *Leads and conducts post-tagging work:* Leads and conducts post-tagging work in coordination with LFWO and UC Davis FCCL to monitor fish survival, growth, tag shedding
5. *Data analyses, interpretation, reporting:* Analyzes and interprets test results, presents updates (IEP PWT, conference) and prepares annual report
6. *Fish tagging:* Conducts VIE tagging in coordination with LFWO and UC Davis FCCL

Table 2. UC Davis FCCL tasks for Delta Smelt Tagging Study. Work is planned for FY21 (Oct 2020-Sep 2021), FY22 (Oct 2021-Sep 2022) and FY23 (Oct 2022-Sept 2023)

	Months (FY21-FY23)											
UC Davis FCCL Staff	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Director FCCL	1	1	1	1	1	1	1	1	1	1	1	1
LTE (limited term employees)	2	2	2	2	2	2	2	2	2	2	2	2
			3	3	3							

FCCL Tasks

1. *Directs tagging study at FCCL:* Directs VIE tagging study in coordination with LFWO, Bay Delta FWS Office and UC Davis FCCL
2. *Daily fish maintenance and monitoring:* Maintains cultured fish and monitors fish survival and water quality on a daily basis
3. *Conducts fish tagging:* Conducts VIE tagging at UC Davis FCCL in coordination with LFWO

Table 3. Preliminary Budget: Evaluation of Visible Implant Elastomer to Inform Supplementation Needs for Delta Smelt

	FY 2021		FY 2022		FY 2023	
Items	**hours**	**Amount $**	**hours**	**Amount $**	**hours**	**Amount $**
Salaries Lodi FWS staff*						
Project Co-lead (GS-12)	200	$16,199	200	$17,009	200	$17,859
Fish biologist (GS-9)	2000	$161,988	2000	$170,088	2000	$178,593
Fish biologist (GS-5)	40	$3,240	160	$13,607	400	$35,719
Salaries UC Davis FCCL staff**						
Director	100	$7,273	100	$7,273	100	$7,273
LTE (limited term employees)	1000	$19,270	3000	$57,810	4000	$77,080
Supplies & Equipment						
VIE tags (6ml pack)		$765		$3,060		$7,650
VIE Trial pack		$45		$180		$450
Large refill syringes		$60		$240		$600
MS-222		$35		$160		$400
Antibiotics		$910		$3,640		$9,100
O2 tank & aeration supplies		$120		$300		
Plastic containers to tag fish		$80				
Total per year		$209,984		$273,367		$334,724
Total 3 years						**$818,074**

* 35.9% indirect cost already assumed by Bay Delta FWS Office
** Include 17.5% indirect cost

APPENDIX

Preliminary VIE tagging Procedure for Delta Smelt (adapted from USFWS 2016)

1. After the fish have been transferred from holding tank to the anesthesia bin, wait until fish start to lose equilibrium (rolling over), then retrieve one fish at a time for tagging.

2. Position the fish in your hand in a way that is consistent, comfortable and without holding on to the fish very tightly.

3. Insert the tagging needle at as shallow an angle as possible

    a. After the needle is past the scales, angle the needle so you are inserting it parallel to the skin.

    b. Insert the needle without approaching too close to the head, or putting undue pressure on the fish while allowing an optimum tag length of about 5 to 7 mm (minimum-maximum VIE tag length range: 3-9 mm).

    c. Begin squeezing the plunger on the syringe as you are retracting the needle straight out of the original insertion point.

    d. Stop squeezing the plunger 1-2mm before the needle exits the fish, so the VIE tag is not protruding from the fish.

    e. Lightly swipe over the entrance wound from front to back to help seal the wound.

4. Place the tagged fish in the recovery bucket.

5. For each batch of fish (same tag-color and same body area tagged) tally each fish on a counter after you put the fish in the recovery bucket. Exact counts of fish are important, as post-tagging fish survival, tag retention, and growth will be regularly monitored.

Appendix 5

Priority Research

Appendix 5. Initial list of priority studies, as identified by CASS, for supplementation of delta smelt.

Study Category	Relevance to	High Priority Studies (identified to date)	Priority for Supplementation Strategy (next 2-4 years)*	Needed over (timeframe)	When Fish Needed	Fish Request Needed
Hatchery practices		Study of viability of embyros trasferred from FCCL to LSNFH (maybe, Javier is not sure this is research)-CPWG study need [Planned trial Nov-Jan 20/21]	High	Short-term		Y
	HGMP	Trends in relative survival of individual pair crosses in each multi-family group at each life stage. Track which families are better suited to the hatchery environment. Is it possible to achieve release of 100 different families?	High	Short-term		N
	HGMP	Develop protocols for the transportationof un/fertilized eggs.	High	Short-term		Y
		ID morphological, behavioral, or physiological effects of observed domestication to determine how it may imapact wild survival by life stage [several studies are addressing aspects of this]	High	Short-term		Y
	HGMP	Benefits of rearing cultured fish with varied food (diet study) to prepare for release	Low	Long-term	pre-supplementation	Y
	uncertainty around SOP, so any deviations of the SOP or improvements need studies of those impacts	How to foster life history diversity (FW residents vs migratory) through SOPs and broodstock collection [RWG notes, this data is not available in the short term for supplementation, longer term research issue]	Low	Long-term		Y
	HGMP	Predator avoidance training study	Low	Long-term	pre-supplementation	Y
	HGMP	Benefits of rearing cultured fish in enriched environments (e.g., varied food availability and spatial cues such as rocks and plants)	Low	Long-term	pre-supplementation	Y
		FTC design to reduce domestication that has been observed in FCCL (e.g., more natural-based laboratories)	Low	Long-term		N
		FCCL experimental operational changes to reduce domestication that has been observed	Low	Long-term		Y
		How to foster life history diversity and reduce domestication effects via new facility design	Low	Long-term		N
Monitoring Studies	In-facility monitoring	Genetic Diversity-software to optimize crosses on a given day	High	Short-term		N
	Ex-facility monitoring	Effectiveness monitoring - on a 3 yr timeline, need studies of IDing released fish, use of traditional surveys and NEW directed sampling	High	Short-term		N
	Ex-facility monitoring	Parentage Based Tagging Studies--useful for each type of life stage released	High	Short-term		N
	In-facility monitoring	Pathogen panel optimized for delta smelt specific pathogens	Medium	Medium-term		Y
	In-facility monitoring	Study of non-lethal pathogen screening methods (maybe via swabs and in tandem with eDNA methods)-dependent on above study	Medium	Medium-term		N
	In-facility monitoring	Pathogen panel screening methods to scale up (using two methods?)-dependent on panel study	Medium	Medium-term		Y
	Ex-facility monitoring	Entrainment/Salvage--genetics and behavioral differences that impact risk of entrainment	Low	Long-term		Y
	Ex-facility monitoring	eDNA [underway but on-going]	Low	Long-term		N
Ecosystem Restoration Studies		effectiveness of restoration and managed flow actions [ongoing DWR cage studies]	High	Short-term		Y
	HGMP	Metabarcoding study to evaluate species diversity in restored areas for release site prioritization. [tabled]				

Study Category	Relevance to	High Priority Studies (identified to date)	Priority for Supplementation Strategy (next 2-4 years)*	Needed over (timeframe)	When Fish Needed	Fish Request Needed
Supplementation Implementation Studies	gets at performance monitoring at certain places and for certain objectives; HGMP	Evaluation of acclimation and release methods (e.g., number of days to hold fish for soft release); also predator dynamics due to cage location	High	Short-term	pre-supplementation	Y
		Evaluation of release locations/timing [field trial of modeled patterns]	High	Short-term	pre-supplementation	Y
		Release demographics (more fish at one location or less fish at many locations)	High	Short-term	pre-supplementation	Y
	HGMP	Evaluation of release methods for fertilized eggs and early life stages; also mesocosms.	High	Short-term	pre-supplementation	Y
	HGMP	Conduct field trials of hatching frames and boxes with and without fertilized eggs.	High	Short-term	pre-supplementation	Y
Fundamental Physiology and Biology research		disease	Low	Long-term		Y
		behavioral studies	Low	Long-term		Y
		predator/prey relationships	Low	Long-term		Y
Modeling	Modeling and/or studies focused on key assumptions of the models	Life Cycle Modeling - genetic modeling - demographic modeling	High	Short-term		N
	HGMP	Use Life Cycle Model to determine which life stage would have the greatest likelihood of survival. [exisiting prototyping tool from USBOR may address this question]	High	Short-term		N
	HGMP	Use Life Cycle Model to estimate extinction risk and/or time to extinction	Low	Long-term		N

Appendix 6

Supporting Studies

Appendix Table AX. Study tracking tool.

Project_ID	Current Projects Using Cultured Delta Smelt from the FCCL:	PI	Partners	Date added	Status	Notes
1	Contaminant effects on two California fish species and the food web that supports them	Connon			Active	600 Adults
2	Delta Smelt refuge population management	Hung/Finger			Active	
3	Determination of Delta Smelt spawning behavior using cultured fish to inform future spawning habitat restoration	Hung			Active	
4	Clean Water Loop System: debris removal and its effects to juvenile Delta Smelt	Reyes				
5	Whole Facility Efficiency of larval and juvenile Delta Smelt at the TFCF	Reyes				
6	Feasibility of using Environmental DNA at the Tracy Fish Collection Facility	Reyes				
7	Prop 1 Embryo toxicity study	Teh				
8	Discovering genetic loci associated with wild, early, middle, and late hatchery ancestry in FCCL fish.	Finger			Active	
9	Contaminant effects on Delta Smelt with water from different location in Delta	Teh			Active	
10	Species/hybrid ID of FCCL wild Delta Smelt spawners	Finger			Active	As needed
11	Development of eDNA protocols (may use FCCL fish)	Finger/Baerwald			Active	
12	Estimating effective population size of the wild population (uses wild FCCL fish as part of the samples)	Finger			Active- pairs with genome sequencing	Every wild fish taken by FCCL
13	Pathogen screening of wild and cultured Delta Smelt, environmental samples	Gille/Baerwald/Connon			Active	Planning
14	Sequencing the genome of Delta Smelt	Finger			Active	
15	Finding genetic loci associated with sex determination in Delta Smelt, possibly developing an assay	Finger			Active- pairs with karyotype/genome	
16	Assessing Smelt responses to holding pen designs under variable environmental conditions	Fangue				
17	Laboratory testing of Delta Smelt enclosures	Fangue/Gille				
18	Assessment of domestication selection in captive populations	Hung/Carson			Active	
19	Thinning strategy of Delta Smelt culture	Hung			Active	
20	Field testing of Delta Smelt enclosures	Schreier/Baerwald			Active	
21	Aquarium of the Pacific	Trautwein			Active	
22	Tagging/pre-screen loss	Wilder				
23	An evaluation of sublethal and latent pyrethroid toxicity across a salinity gradient in two Delta fish species	Brander/Hladik/Connon			Active	12000 embryos
24	Improving the survival of delta smelt larval single-family groups	Hung/Carson			Active	
25	Wakasagi hatching frame study with Delta Smelt eggs	Hung/Carson			Active	
26	Quantifying genetic and epigenetic variation in delta smelt that may enable adaptation to future environments	Whitehead/Fangue/Hung			Active	
27	Continuation of sex determination study - karyotype study	Finger		1/1/2020	Active	Roughly 50 cultured fish
28	examining variation associated with domestication	Finger/Baerwald/Gille/Carson		1/1/2020	Active	Made crosses at the FCCL. Sacrificed offspring.
29	pairing otolith data on life history type with RAD sequencing/WGS	Finger			Active	
30	Delta smelt refractory period	Teh/Carson/Smith				
31	MFG representation in the 2-year olds that survive in LSNFH	Hung/Finger		5/27/2020	Active	

Project_ID	Current Projects Using Cultured Delta Smelt from the FCCL:	PI	Partners	Date added	Status	Notes
32	Effectve population size, sex markers	Finger		5/27/2020		
33	SNP panel for parentage, changing from microsatelites to SNPs	Finger		5/27/2020		
34	The significance of turbidity in safeguarding Delta Smelt from predation: growth, development, behavior and predation	Todgham/Fangue/Connon/Carson			Active	Planning
35	Development of propagation method of Delta Smelt for reintroduction	Hung/Carson				
36	Cryopreservation of Delta Smelt milt	USFWS		5/27/2020		USFWS starting in June 2020
Pending.x	**Planned Projects Not Yet Funded that will use Cultured DS:**					
P.2	Environmental Stressor Effects on Delta Smelt	Fangue				
P.3	Impacts of Multiple Stressors on Physiological Performance and Growth of Smelt Species	Fangue				
P.4	Thermal preference of fish species based on previous thermal acclimation history	Fangue				
P.5	Water depth utilization under varying photo-phases, turbidity, and light intensities	Fangue				
P.6	Sprint swimming speed of smelt	Fangue				
P.7	Strontium isotope study	Hobbs				
P.9	Development of enclosures for early life stages of Delta Smelt and density studies	Fangue				

Appendix 7

Supplementation Life Cycle Model (Technical Note 52)

DSM TN 52. Supplementing the wild delta smelt population with hatchery origin stock: a life cycle model perspective

Leo Polansky[1] and Evan W. Carson[2]

July 9, 2020

Overview

This document outlines possible approaches to integrate population modeling with genetic management and supplementation of hatchery origin delta smelt. Section 1 starts off with some simple examples of how supplementation questions could be approached from a population modeling perspective. Section 2 uses a previously fit life cycle model of delta smelt to estimate needed levels of supplementation to reverse cohort specific declines in the past, and to simulate future abundances under different scenarios of simulation strategies (numbers added and at what life stage). The first two sections are likely unrealistic in that hatchery origin fish are assumed to have the same vital rates (reproduction and survival rates) as wild origin fish. In Section 3, a conceptual framework along with some initial mathematical description is provided to allow integration of vital rate differences between wild and hatchery origin fish into Delta and hatchery populations.

1 The basic setup and some motivating toy examples

A summary of the symbols and their meanings used throughout is provided in Table 1.

To start with, assume there are four life stages, post-larvae (*PL*), juveniles (*J*), sub-adults (*SA*), and adults (*A*). Denote the cohort t specific wild origin (*W*) abundances by $N_{PL,W,t}$, $N_{J,W,t}$, $N_{SA,t}$, and $N_{A,W,t}$, respectively. The annual growth rate of the population is

$$\lambda_{W,t} = N_{A,W,t+1}/N_{A,W,t-1} = s_{SA,W,t}(s_{J,W,t}(s_{PL,W,t}(r_{W,t}(N_{A,W,t-1})))) \quad (1)$$

where $r_{W,t}(N_{A,W,t-1})$ is the reproduction function, and $s_{i,W,t}(N_{i,W,t})$ are survival functions.

Example 1. Assume $s_{\cdot,W,t}(N_{\cdot,W,t}) = \varphi_{\cdot,W,t}N_{i,W,t}$, where $\varphi_{\cdot,W,t} \in (0, 1)$. Is it better to add 5,000 post-larvae, or 1,000 juveniles? It will be better to add 5,000 post-larvae if $\varphi_{PL,W,t}5{,}000 > 1{,}000$, or $\varphi_{PL,W,t} > 0.2$, assuming $\varphi_{PL,W,t} = \varphi_{PL,H,t}$ and $\varphi_{SA,W,t} = \varphi_{SA,H,t}$ ■

Example 2. Assume the composite function determining the population growth rate is a constant λ. What is the effect of adding 30,000 post-larvae each year given a starting adult abundance of 10,000? Figure 1 illustrates the projected abundances for two different choices of post-larval survival while holding the other vital rates constant. ■

[1] leo_polansky@fws.gov, San Francisco Bay-Delta Fish and Wildlife Field Office
[2] evan_carson@fws.gov, San Francisco Bay-Delta Fish and Wildlife Field Office

Table 1: Symbols and their meanings used throughout the text.

Symbol	Meaning
A	Abundance threshold below which decreases in abundance lead to declines in the growth rate.
a	Intrinsic rate of population growth in the model $dN/dt = rN$.
$f_{\cdot,H,t}$	Equal to the number of hatchery origin abundances of life stage divided by the prior cohort abundance of wild adults.
g_H	Fraction of the hatchery origin portion of the population that reproduces with individuals of the same ancestry.
g_W	Fraction of the wild portion of the population that reproduces with individuals of the same ancestry.
$\lambda_{W,t}$	Annual growth rate of entirely wild origin population cohort t. For the life cycle model, $\lambda_t = s_{SA,t}(s_{J,t}(s_{PL,t}(\rho_t(N_{A,t-1})$
$N_{\cdot,t}$	The number of fish for life stage $\cdot$ in cohort t, including both wild and hatchery origin.
$N_{\cdot,H,t}$	The number of hatchery origin fish for life stage $\cdot$ in cohort t.
$N_{\cdot,W,t}$	The number of wild fish for life stage $\cdot$ in cohort t.
$r_{W(i)H(j)}$	Recruitment function of fish that have ancestry of i generations and hatchery origin of j generations.
$r_{\cdot,t}$	Recruitment, wild (W) or hatchery origin (H) as a function of the spawning adult abundance for cohort t.
$\rho_{\cdot,t}$	Realized wild (W) or hatchery origin (H) recruitment value of post-larvae per adult for cohort year t.
$s_{\cdot,\cdot,t}$	Life stage specific survival function of wild (W) or hatchery origin (H) and cohort t.
$\varphi_{\cdot,\cdot,t}$	Realized life stage specific wild (W) or hatchery origin (H) survival value for cohort t.

Example 3. Assume a density-dependent recruitment rate subjected to an Allee effect, e.g. such that for very low adult abundances recruitment success is hindered by individuals being able to locate others during the spawning season. A simple per capita growth rate model of this is $dN/Ndt = a(N/A - 1) =$ for which an discrete time recruitment function can be approximated by[3] $N_{P\,L,W,t} = N_{A,W,t-1}\exp(N_{A,W,t-1}/A - 1)$. For hatchery supplementation, the recruitment function is $r_t(N_{A,W,t-1},\ N_{A,H,t-1}) = N_{A,W,t-1}\exp(N_{A,W,t} + N_{A,H,t})/A - 1)$ Given survival rate functions $S_{\cdot,W,t} = \varphi_{\cdot,W,t}N_{\cdot,W,t}$, what adult supplementation is needed to achieve $\lambda_t \geq 1$? If $N_{A,H,t}$ are the number of hatchery origin adults added to the population,

$$\lambda_t = \phi_{SA}\phi_J\phi_{PL,t}r_t(N_{A,W,t-1} + N_{A,H,t-1}) \geq 1$$

implies that

$$N_{A,H,t-1} \geq \left(\ln(\frac{1}{\phi_{SA,t}\phi_{J,t}\phi_{PL,t}}) + \alpha\right)\frac{A}{\alpha} - N_{A,W,t-1}.$$

[3] See page 55 in the monograph "Complex Population Dynamics: a theoretical and emprical synthesis" by Peter Turchin. The discrete time analog to the continuous time equation can actually be solved analytically, often the preferred choice, but the resulting equation is unsuitable for population modeling in general because it can predict infinite population growth in finite time even for sensible parameter values for low abundance dynamics.

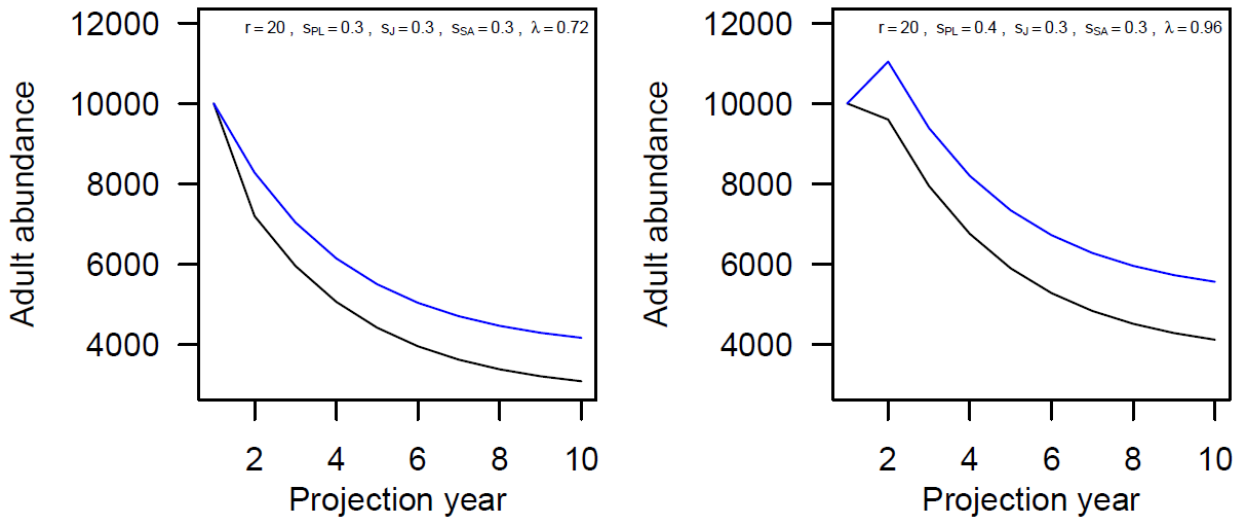

Figure 1: Projected abundance without supplementation (black lines) and with supplementation (blue lines) of 30,000 post-larvae per year. Panels differ by the vital rates used, shown at the top of each figure. While supplementation can have short term effects, abundances over time asymptote to a value dependent on both the growth and supplementation rates.

The above examples are not realistic in at least two ways: i) life stage and cohort specific recruitment and survival rates are likely to vary both deterministically and stochastically, and ii) hatchery origin fish may not have the same recruitment and survival rates as wild ones. Further, it is clear that to evaluate the effects of supplementation, future vital rates also must be known (within reasonably constrained limits), yet these are generally difficult to predict. Section 2 applies estimates of past recruitment and survival to examine supplementation rates needed to achieve a growth rate of one for the cohort, while Section 3 outlines a theoretical framework to simulate population abundances while allowing for variability of vital rates to occur because of time and ancestry.

Remarks:

- Management actions have potential to alter $s_{P\,L,W,\cdot}$ or $s_{SA,W,\cdot}$, such as through timing and location of releases (within life stages) or ecosystem-level approaches. These actions could increase or reduce survival of releases at each life stage.

- Vital rates could be non-constant if domestication selection accrues (likely) and confers fitness costs in wild (also likely); see also Figure 8.

2 Using estimated recruitment and survival rates to describe hatchery needs and effects

Estimates of past cohort specific recruitment and survival rates for three life stages are available in Polansky et al. (2020)[4]. These were used to calculate the supplementation needed for a given life stage in order to achieve a growth rate of one (Section 2.1), and to estimate the distributions of abundances given different scenarios of supplementation (Section 2.2).

2.1 Estimates of needed supplementation for $\lambda_{W,t} = 1$ based on historical estimates of recruitment and survival

The equations are as follows.

Case 1. Post-larval supplementation.

$$N_{A,W,t} = \phi_{SA,W,t}\phi_{J,W,t}\phi_{PL,W,t}(\rho_{W,t}N_{A,W,t-1} + N_{PL,H,t}) \geq N_{A,W,t-1} \tag{2}$$

$$\iff N_{PL,H,t} \geq \frac{N_{A,W,t-1}}{\phi_{SA,W,t}\phi_{J,W,t}\phi_{PL,W,t}} - \rho_{W,t}N_{A,W,t-1} \tag{3}$$

$$\iff f_{PL,H,t} \geq \frac{1}{\phi_{SA,W,t}\phi_{J,W,t}\phi_{PL,W,t}} - \rho_{W,t} \tag{4}$$

where $f_{PL,H,t} = N_{PL,H,t}/N_{A,W,t-1}$ is a multiplier relating hatchery origin post-larvae numbers to the abundance of the prior cohort's wild-origin adult numbers used for normalizing the estimate of supplementation effort.

Case 2. Juvenile supplementation.

$$N_{A,W,t} = \phi_{SA,W,t}\phi_{J,W,t}(\phi_{PL,W,t}\rho_{W,t}N_{A,W,t-1} + N_{J,H,t}) \geq N_{A,W,t-1} \tag{5}$$

$$\iff N_{J,H,t} \geq \frac{N_{A,W,t-1}}{\phi_{SA,W,t}\phi_{J,W,t}} - \phi_{PL,W,t}\rho_{W,t}N_{A,W,t-1} \tag{6}$$

$$\iff f_{J,H,t} \geq \frac{1}{\phi_{SA,W,t}\phi_{J,W,t}} - \phi_{PL,W,t}\rho_{W,t} \tag{7}$$

where $f_{J,H,t} = N_{J,H,t}/N_{A,W,t-1}$ is a multiplier relating hatchery origin juvenile numbers to the abundance of the prior cohort's wild-origin adult numbers used for normalizing the estimate of supplementation effort.

[4] Polansky, L., K. B. Newman, and L. Mitchell. 2020. Improving inference for nonlinear state-space models of animal population dynamics given biased sequential life stage data. Biometrics. DOI: 10.1111/biom.13267

Case 3. Sub-adult supplementation.

$$N_{A,W,t} = \phi_{SA,W,t}(\phi_{J,W,t}\phi_{PL,W,t}\rho_{W,t}N_{A,W,t-1} + N_{SA,H,t}) \geq N_{A,W,t-1} \tag{8}$$

$$\iff N_{SA,H,t} \geq \frac{N_{A,W,t-1}}{\phi_{SA,W,t}} - \phi_{J,W,t}\phi_{PL,W,t}\rho_{W,t}N_{A,W,t-1} \tag{9}$$

$$\iff f_{SA,H,t} \geq \frac{1}{\phi_{SA,W,t}} - \phi_{J,W,t}\phi_{PL,W,t}\rho_{W,t} \tag{10}$$

where $f_{SA,H,t} = N_{SA,H,t}/N_{A,W,t-1}$ is a multiplier relating hatchery origin sub-adult numbers to the abundance of the prior cohort's wild-origin adult numbers used for normalizing the estimate of supplementation effort.

Case 4. Adult supplementation.

$$N_{A,W,t} = \phi_{SA,W,t}\phi_{J,W,t}\phi_{PL,W,t}\rho_t(N_{A,W,t-1} + N_{A,H,t-1}) \geq N_{A,W,t-1} \tag{11}$$

$$\iff N_{A,H,t-1} \geq \frac{N_{A,W,t-1}}{\phi_{SA,W,t}\phi_{J,W,t}\phi_{PL,W,t}\rho_{W,t}} - N_{A,W,t-1} \tag{12}$$

$$\iff f_{A,H,t-1} \geq \frac{1}{\phi_{SA,W,t}\phi_{J,W,t}\phi_{PL,W,t}\rho_{W,t}} - 1 \tag{13}$$

where $f_{A,H,t} = N_{A,H,t}/N_{A,W,t-1}$ is a multiplier relating hatchery origin adult numbers to the abundance of the prior cohort's wild-origin adult numbers used for normalizing the estimate of supplementation effort.

Distribution of minimum needed supplementation estimates and $f_{\cdot,H,\cdot}$ multipliers are shown in Figure 2. The means of median values over years are shown in Table 2. For a particular example, taking the j^{th} sample of the posterior distribution for which the observed abundance of $N_{A,W,2014}$ is closest to model predicted one (based on the posterior mean) provides the (rounded) estimates of abundance as $N_{A,W,2014}$ = 125029, $N_{P\,L,W,2015}$ = 555886, $N_{J,W,2015}$ = 111035, $N_{SA,W,2015}$ = 48691, and $N_{A,W,2015}$ = 44441. The corresponding (rounded) vital rate estimates are $\rho_{W,2015}$ = 4.45, $\varphi_{PL,W,2015}$ = 0.20, $\varphi_{J,W,2015}$ = 0.44, $\varphi_{SA,W,2015}$ = 0.91, and a population growth rate of $\lambda_{W,2015}$ = 0.36, i.e. a year in which summer survival was poor, winter survival was high, and the overall growth rate was below one. Applying equations 3 and 4 gives $N_{PL,H,2015}$ = 1, 008, 020 and $f_{PL,H,2015}$ = 8.06. (Note that for adult indices and multipliers, because the t refers to cohort, it is one less than the calendar year of the adult abundance.)

Table 2: Mean over years of median values of minimum supplementation ($N_{\cdot,H}$) and the multiplier $f_{\cdot,H}$ relating the minimum supplementation to the prior adult cohort abundance for each life stage.

Life	$N_{\cdot,H}$	$f_{\cdot,H}$
Post-larva	38,888,510	17.07
Juvenile	17,309,260	5.80
Sub-adult	1,725,102	1.14
Adult	3,537,560	1.72

Remarks:

- The larger the abundance, the more supplementation is required to offset a decline. But at higher abundance the immediate- to near-term risk of extinction is lower (with or without supplementation). Some effort to explore estimation of Allee effect models with empirical data to estimate A in order to identify a lower target to keep the population above may be warranted. In the absence of density dependence, populations subject to random walk dynamics have a risk of extinction when abundances get low.

- $N_{\cdot,H}$ and $f_{\cdot,H}$ in Table 2 appear to be heavy weighted by pre-POD influence (see Figure 2).

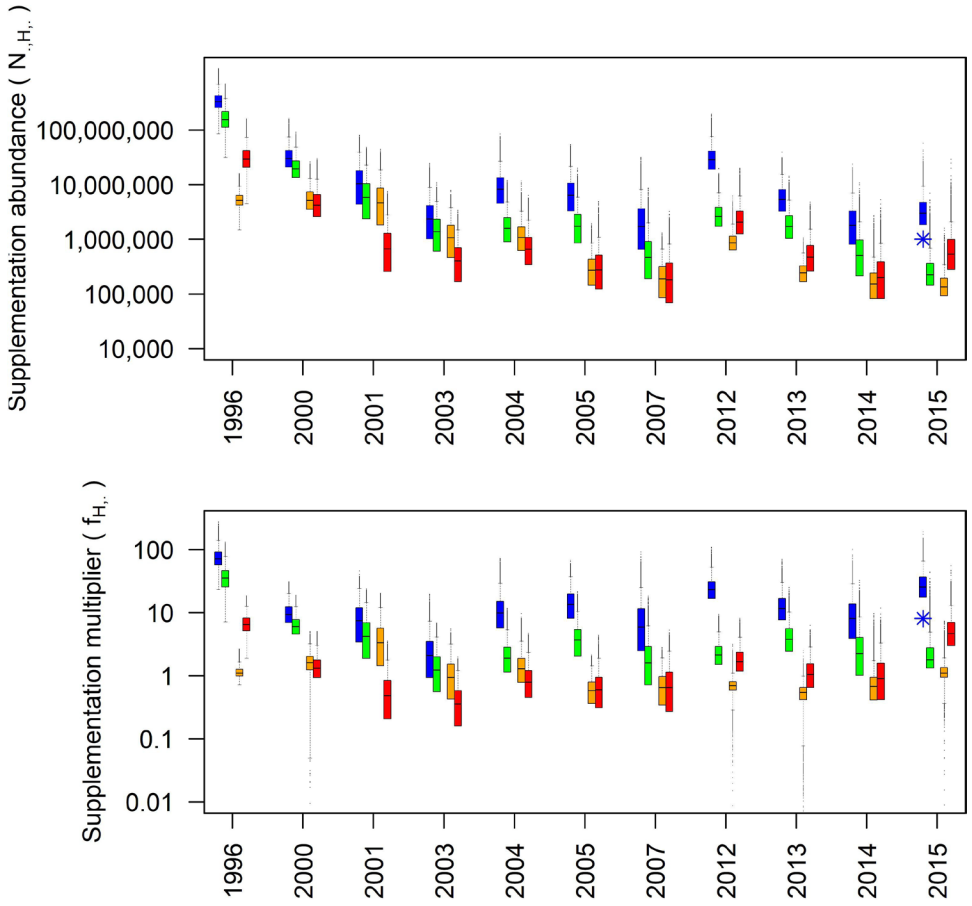

Figure 2: Tukey boxplots of total supplementation (top panel) and supplementation multipliers (bottom panel) needed to achieve a population growth rate of one based on posterior distribution estimates of abundance and vital rates for cohorts with growth rates less than one. Colors correspond to post-larvae (blue), juveniles (green), sub-adults (orange), and adults (red). The adult estimates are in reference to the adult abundance for the cohort immediately preceding the cohort for which a supplementation based growth rate increase is computed. The blue asterisks are at the values of $N_{PL,H,2015}$ = 1, 008, 020 (top panel) and $f_{PL,H,2015}$ = 8.06 (bottom panel) to illustrate the example described in the text.

- Supplementation can reduce demographic risk by increasing abundance yet inadvertently increase genetic risk by elevating inbreeding and, consequently, reducing fitness (Ryman-Laikre Effect[5]).

- The supplementation multiplier being relatively constant within a life stage reflects that accomplishing a minimum population growth rate of one does not require a relative increase or decrease in supplementation relative to the spawning adult cohort size preceding the cohort of interest.

- The relatively large values of $N_{SA,H,t}$ even when sub-adult survival is high suggest that early life stage losses necessitate continued large supplementation values to achieve a non-declining population.

2.2 Predicted abundances for different levels of post-larval and sub-adult supplementation given different scenarios of growth rates

A simulation experiment was carried out to evaluate how different choices of when and how much to supplement impacted abundances over a 10 year span. Starting with an adult abundance of $n_{A,W,t0}$ = 5, 000, supplementation at the post-larval and sub-adult life stages was considered by adding either 0, 30,000, 50,000, or 100,000 individuals to either or both life stages during a stochastic simulation that updated the initial adult abundances, using vital rates estimated from Polansky et al. (2020). Supplementation of 500,000 post-larvae also was included, though current and projected production capacities for supplementation are below that level after accounting for production required for maintaining the refuge population and research stock (Table 3).

Table 3: Estimated FCCL production of delta smelt by life stage (egg to 300 days post-hatch [dph]) under current capacity and projected additional production of 50,000 and 125,000 sub-adults for supplementation. All estimates include production for the refuge population and research stock.

Life stage	Current	+50,000	+125,000
Egg	221,803	691,729	1,396,616
0 dph	168,571	525,714	1,061,428
40 dph	84,286	262,857	530,714
80 dph	59,000	184,000	371,500
300 dph	23,600	73,600	148,600

[5] Ryman, N., and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. Conservation Biology 5:325-329.

For a given set of years (discussed next), the simulation study first samples a year, then samples a set of estimated vital rates given the sampled year, and then uses these vital rates to update abundances through the life stages, adding to the population the supplementation value at the appropriate life stage. This was repeated for 10 years, with the adult abundances recorded, and the entire process was done 10,000 times.

The first set of years used to sample vital rates were from 2008 to 2015. This set was chosen to reflect recent Delta environmental and management conditions impacting delta smelt. The second collection was from 1995 to 2015, further separated into dry years and wet years. Dry years were assigned to any year with classification below (drier than) normal, and wet years were assigned to any year with classification of above (wetter than) normal; there were no normal years from 1995 through 2015.

Using growth rates from 2008 through 2015, simulations result in increased abundances greater than half the time over the 10 year period in any scenario, in contrast with declines without supplementation (Table 4, Figure 3). Supplementing at only the sub-adult life stage will generally be better than supplementing at only the post-larval life stage, even when post-larval supplementation is 100,000 individuals per cohort and sub-adult supplementation is 30,000, with the only exception based on a comparison of supplementing 500,000 post-larvae vs 30,000 sub-adults (Figure 4).

Two other explorations were considered to help assess differences in predictions in Figure 3. Assuming a post-larval supplementation at a given level as a baseline scenario, Figure 5 shows the relative benefit of additional supplementation of sub-adults at different levels. Similarly, assuming a sub-adult supplementation at a given level as a baseline scenario, Figure 6 shows the relative benefit of additional supplementation of post-larvae at different levels.

Table 4: Fraction of 10,000 simulations with positive population growth after 10 years based on growth rates from the 2008-2015 cohorts given different levels of supplementation. Compare with Figure 3.

	Sub-adult supplementation			
Post-larva supplementation	0	30,000	50,000	100,000
0	0.20	1.00	1.00	1.00
30,000	0.71	1.00	1.00	1.00
50,000	0.85	1.00	1.00	1.00
100,000	0.96	1.00	1.00	1.00
500,000	1.00	1.00	1.00	1.00

Although supplementing at the sub-adult life stage is generally preferable, the relative improvements are specific to water year type. When the water year type is dry, supplementing at the sub-adult life stage has much greater impact than supplementing at the post-larval life stage (Figure 7). This reflects the dependence of post-larval survival on summer outflow (Polansky et al. 2020). Even when the earlier post-larva life stage has substantially higher additions, smaller median estimates occur only for the most extreme difference (of 30,000 sub-adult supplementation vs. 500,000 post-larvae supplementation), and the relative improvements remain specific to water year type (Figure 7).

Remarks:

- Medians and interquartile ranges (winsoring in a sense) were used here to summarize prediction trends rather than means and 95% prediction intervals because very large outlying predictions resulting from a lack density dependence in the model constraining the occasional very large prediction resulted in difficult interpretations of the later.

- Supplementation is expected to result in increased abundance in any scenario considered because the supplementation is larger than the starting population size and hatchery origin fish were assumed to have the same vital rates as wild origin ones.

- Increased abundance due to supplementation does not imply sustained positive growth; in fact, given the lack of density dependence in the model, the population would be expected to decline immediately upon cessation of supplementation. Nonetheless, increased abundance illustrates the utility of supplementation for lowering risk of extinction from demographic stochasticity in a vulnerable population.

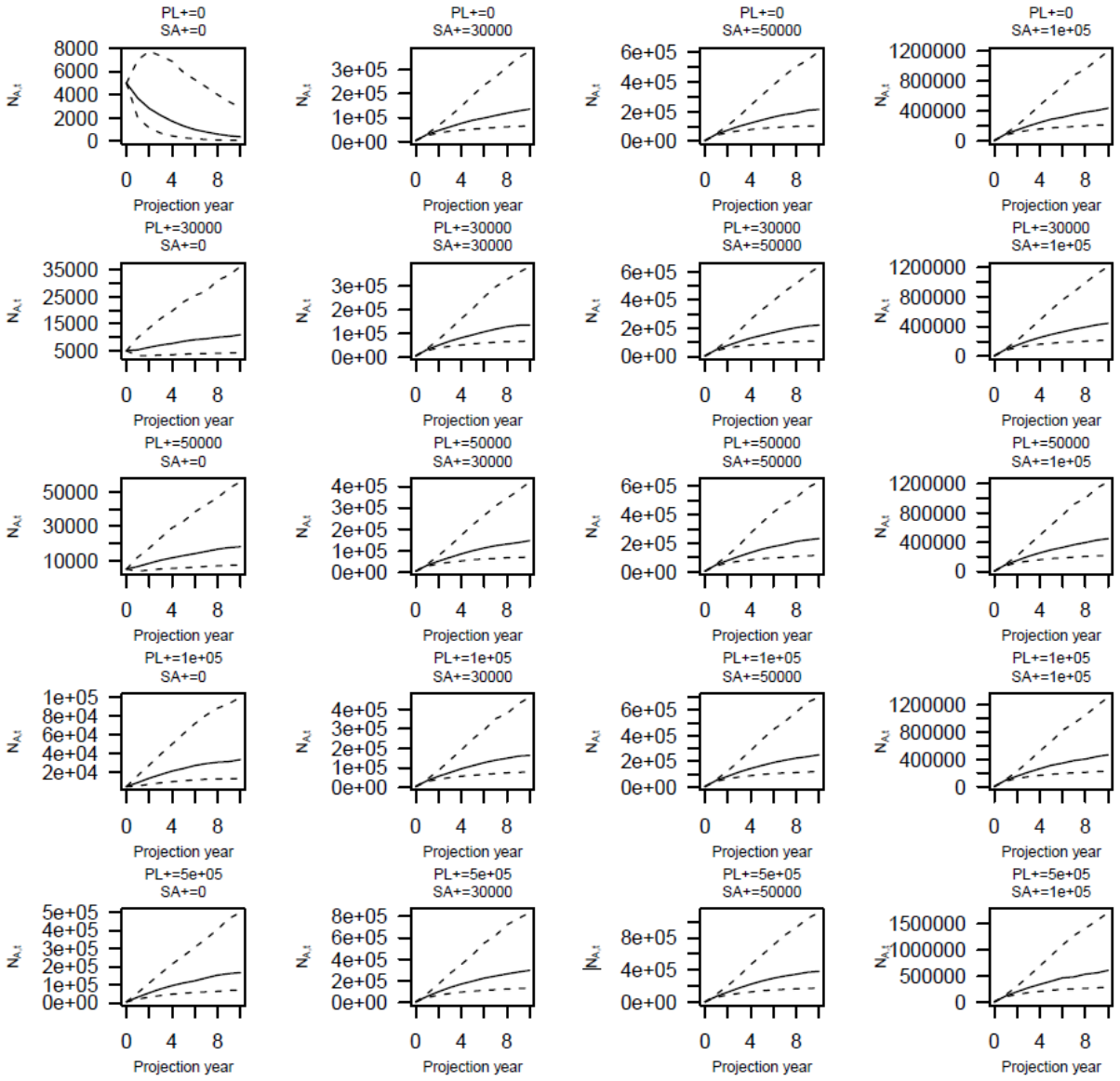

Figure 3: Median abundance estimates with the interquartile range denoted by dashed lines for 10,000 simulations based on different combinations of adding post-larvae or sub-adults, as indicated in the panel titles.

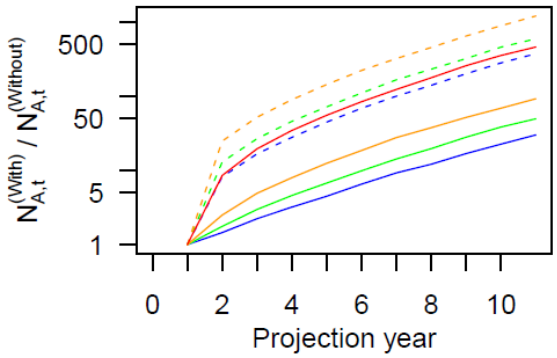

Figure 4: Ratio of median abundance estimates based on a supplementation scenario to the one with no supplementation (top left panel in Figure 3). The supplementation scenarios included either the post-larval (solid lines, also see left panel column in Figure 3), or sub-adult (dashed lines) life stages dashed lines, also see top panel row of Figure 3), for different values of supplementation: 10,000 (blue), 30,000 (green), 100,000 (orange), or 500,000 (red, *PL* only).

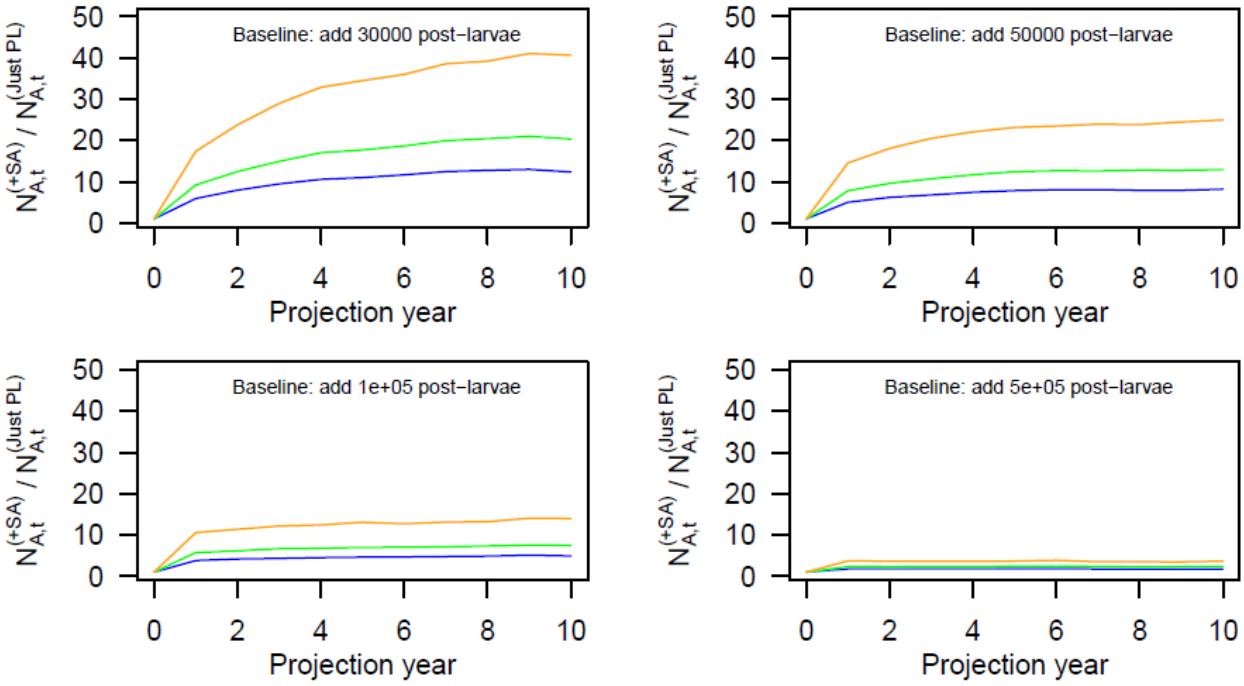

Figure 5: Ratio of median abundance predictions for a given baseline level of post-larval supplementation indicated by text in the panel augmented by an additional level of sub-adult supplementation to median abundance predictions without the further augmentation. Colors correspond to additional *SA* supplementation of 10,000 (blue), 30,000 (green), and 100,000 (orange).

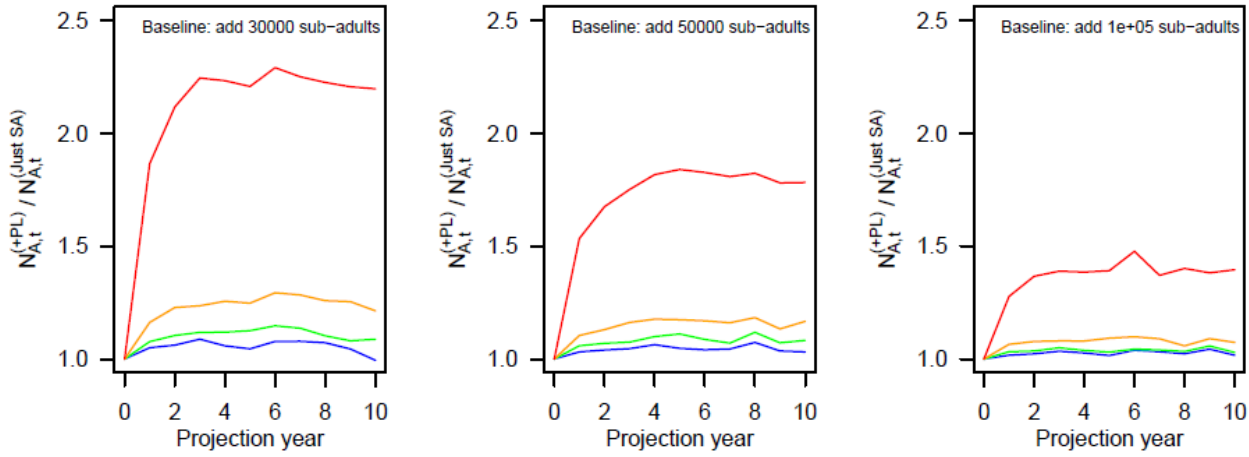

Figure 6: Ratio of median abundance predictions for a given baseline level of sub-adult supplementation indicated by text in the panel augmented by an additional level of post-larval supplementation to median abundance predictions without the further augmentation. Colors correspond to additional *PL* supplementation of 10,000 (blue), 30,000 (green), 100,000 (orange), and 500,000 (red).

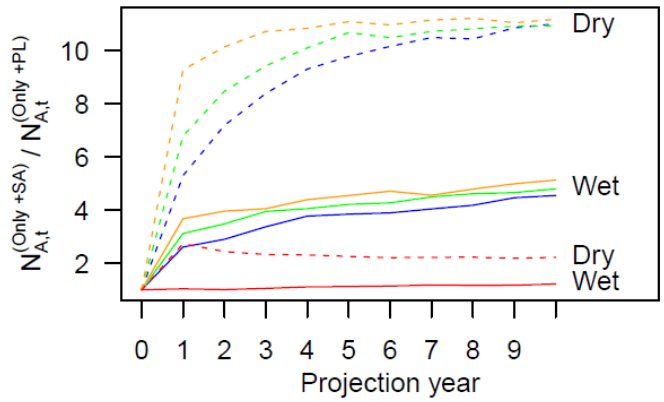

Figure 7: The ratio of median adult abundance at projection year *t* between predictions with only sub-adult supplementation to only post-larval supplementation, when vital rate estimates are obtained from dry or wet years. Supplementation for both was at either 30,000 (blue), 50,000 (green), or 100,000 (orange), fish per cohort. Red lines show ratios of median adult abundance predictions when supplementation is of 100,000 sub-adults to predictions with supplementation of 500,000 post-larvae. In all cases adding sub-adults results in higher adult abundances than adding post-larvae, but the relative improvements are more pronounced for dry season population processes than wet season ones.

3 A framework for simulating supplementation when wild and hatchery origin fish have different vital rates

The previous two sections were potentially unrealistic in assuming wild and hatchery origin fish have the same vital rates. To account for this, an allowance of (possible) differences in vital rates, and ancestry tracking is needed. A conceptual overview of the linkages making the feedback loop between the wild and hatchery populations (for an integrated hatchery model[6]) and pertinent considerations for the process is shown in Figure 8.

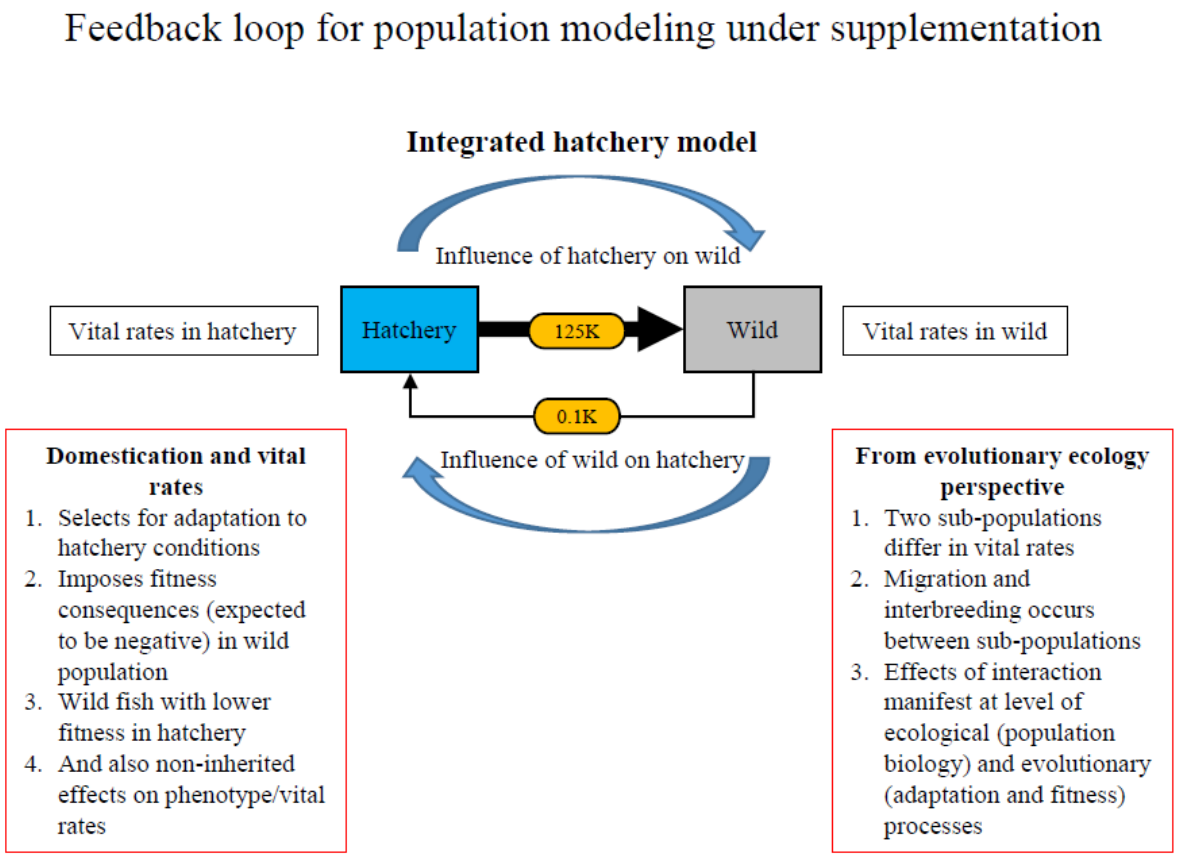

Figure 8: Conceptual rendering of population modeling as applied to an integrated hatchery model to illustrate influences of interaction (migration and interbreeding) between hatchery and wild sub-populations on vital rates of each sub-population. Supplementation is represented by the bold arrow, with an example of n = 125,000 individuals released; incorporation of wild individuals into hatchery broodstock is represented by a fine arrow, with an example of n = 100 wild individuals added to the hatchery stock. Flexed blue arrows represent direction of influence of each sub-population on vital rates of the other sub-population. Effects of domestication (hatchery conditions) are shown in the box at lower left, and those from an ecological-evolutionary perspective are shown in the box at lower right.

[6] In an integrated hatchery model, wild-origin fish are incorporated into broodstock used for production for supplementation.

To provide some mathematical structure to the problem, as before first assume that supplementation of a specific life stage occurs in sync with the life stage of the wild population (i.e. post-larvae are placed in the Delta at approximately the same time as the wild post-larvae begin their survival process to become juveniles). Further assume supplementation for the first time occurs at the post-larval life stage. Hatchery origin abundances and vital rates include additional superscripts to denote how many vital rate processes have previously occurred to a given group of hatchery origin fish. If $n_{PL,H^{(0)},t}$ hatchery origin post-larva fish are added to the Delta, these undergo a hatchery specific survival rate to produce hatchery origin juveniles, which in turn survive to become hatchery origin sub-adults and these in turn survive to become hatchery origin adults.

The equations updating the abundances through time are given by

$$N_{J,H^{(1)},t} = \phi_{PL,H^{(0)},t} N_{PL,H^{(0)},t} \tag{14}$$

$$N_{SA,H^{(2)},t} = \phi_{J,H^{(1)},t} N_{J,H^{(1)},t} \tag{15}$$

$$N_{A,H^{(3)},t} = \phi_{SA,H^{(2)},t} N_{SA,H^{(2)},t}. \tag{16}$$

$$\tag{17}$$

The adult abundance vector consisting of wild and hatchery origin abundances is

$$\boldsymbol{N}_{A,t}^{\intercal} = (N_{A,W,t}, N_{A,H^{(3)},t}). \tag{18}$$

$$\tag{19}$$

Recruitment to the next cohort will depend on wild and hatchery origin recruitment functions,

$$\boldsymbol{N}_{PL,t+1}^{\intercal} = (N_{PL,W,t+1}, N_{PL,W\times H^{(4)},t}, N_{PL,H^{(4)},t+1})^{\intercal} \tag{20}$$

$$= \begin{bmatrix} r_{W,t+1} & 0 & 0 \\ 0 & r_{WH^{(3)},t+1} & 0 \\ 0 & 0 & r_{H^{(3)},t+1} \end{bmatrix} \begin{bmatrix} g_W & 0 \\ (1-g_W) & (1-g_H) \\ 0 & g_H \end{bmatrix} \begin{bmatrix} N_{A,W,t} \\ N_{A,H^{(3)},t} \end{bmatrix} \tag{21}$$

where g_W and g_H are the fraction of the wild and hatchery origin populations that reproduce with individuals of the same ancestry, respectfully, and the subscript on the recruitment functions $r_{\cdot,t}$ allows for different functions for the different ancestries.

Remarks:

- The notation is clearly cumbersome but essentially the model is tracking ancestry and abundances and allows cohort and ancestry specific vital rates.
- Application to monitoring supplemented populations includes assessment of i) the relative contribution of supplemented fish to reproduction and recruitment and ii) the demographic and genetic consequences of (i).
- Note: In early generations of supplementation, vital rates may differ between wild and hatchery-origin delta smelt, and among offspring of wild x wild, hatchery-origin x hatchery-origin, and wild x hatchery-origin crosses. How this manifests over time will depend on a variety of influences, including conditions in the wild, rate and extent of domestication selection, rate of supplementation, and contribution of wild-origin and hatchery-origin fish to reproduction, among others.