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Early Development of Pacific Surf Smelt (*Hypomesus pretiosus*)

Abstract

We observed development in surf smelt (*Hypomesus pretiosus*) embryos from 24 hours post fertilization through hatch, at water temperatures of 12.52 °C (SD = 0.22) to 22.37 °C (SD = 1.14). Our system mimicked the tidal cycle by draining incubation chambers of water for 12 hours a day in concert with a photoperiod that mimicked a seasonal light/dark cycle of 16 hours of light and eight hours of darkness. The first detected heartbeat was documented at 62 degree days, partial hatch occurred by 187 degree days, and all remaining live eggs had hatched by 225 degree days. Developmental milestones were documented with micro photography and videography. Various developmental stages were illustrated in pen and ink for documentation. This work was used to gather developmental baseline data for the species.

Key Points:

- We documented in photos, video, and illustrations, the embryological development of surf smelt
- This lays the groundwork for future studies, including ecotoxicology, ocean acidification, and effects of shoreline modification

Keywords: ecotoxicology, embryology, forage fish

Introduction

In order to understand how environmental perturbations and pollutants can affect development of marine fish we must first have a firm understanding of what is normal. Pacific surf smelt (*Hypomesus pretiosus*) is an important native forage fish along the Pacific Coast of North America. These small (up to 203 mm) silvery fish range from Long Beach, California to Chignik Lagoon, Alaska (Russell 2022, WDFW 2023). They are abundant in nearshore waters throughout Puget Sound, an urban estuary in northern Washington State, and as obligate intertidal spawners (Harding et al. 2022) lay their eggs in mass spawning events on beaches in the upper tidal zone in the summer or fall/winter. Surf smelt are iteroparous (able to spawn repeatedly in their lifetime) and can live up to five years. Some fish mature and spawn at about one year of age, and all fish are reproductively mature by two years of age (Yap-Chiongco 1941, Eschmeyer and Herald 1983). Surf smelt provide food to seabirds, larger fish, marine mammals, and crustaceans, and smelt eggs are an important food source for many species including juvenile Dungeness crab *(Metacarcinus magister*) (Rice 2006, Penttila 2007). Surf smelt depend on access to upper tidal beaches; shoreline armoring impacts available spawning locations. Additionally, fishing pressures, pollution, climate change, shoreline development, non-native species, and sediment flow from rivers could cause surf smelt populations to decline (Rice 2006, Penttila 2007). Rice (2006) showed that surf smelt eggs on modified shorelines contained “approximately half” the number of live embryos when compared to eggs on natural beaches.

It is thought that surf smelt do not form large schools in open water (WDFW 2023), which makes them difficult to survey. The species is

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assumed to be abundant (Chow et al. 2020) and is commonplace in seining surveys (WDFW 2022). Surf smelt are targeted by recreational, tribal, and commercial fisheries. Historically, no license has been required to recreationally fish for surf smelt, and surf smelt recreational take was not tabulated by managers. Commercial landings since 2000 have been tabulated at about 95,000 pounds yearly, and in 2014 Washington State set the commercial take at 60,000 pounds yearly (WDFW 2023). Since 2005, Washington State has considered surf smelt a Species of Greatest Conservation Need (SGCN) under the State Wildlife Action Plan (WDFW 2015) due to their importance as a forage fish and the lack of data on species abundance. Because of this designation, their importance as a forage fish species, perceived vulnerability to climate change (Russel et al. 2022), and the lack of data on noncommercial landings, there is an increased call for a greater understanding on surf smelt life history.

Previous Studies on Surf Smelt Development

Information on the early development of surf smelt is limited (Yap-Chiongco 1941, Moulton and Penttila 2001, Langness et al. 2014). Information on temperature, substrate adherence, and effects of various salinities largely comes from investigations by Middaugh et al. (1987) and Morgan and Levings (1989) which exposed eggs and newly hatched larvae to contaminated dredged sediment. They found that surf smelt showed sensitivity to suspended sediment exposure but they did not publish photographs of development. To be confident in identifying abnormalities, proper documentation of normal development should be available as a reference.

Extensive ecotoxicology studies have been carried out on the effects of chemical exposure on developing fish embryos (Lema et al. 2007, Milinkovitch et al. 2013, Scholz and Incardona 2015). Studies on oil spills and urban stormwater runoff repeatedly find cardiotoxicity is a highly sensitive form of injury for teleost embryos (Incardona et al. 2009, Incardona et al. 2013, Scholz and Incardona 2015, McIntyre et al.2016). For example, Paine and Leggett (1992) found that capelin (*Mallotus villosus*) larvae that were exposed to oil had larger yolk sacs and smaller heads compared to control larvae, and as oil concentrations increased, larvae head size decreased.

While these studies have been conducted in a variety of species, the morphology related to the heart and yolk positions in the aforementioned species have been similar. In surf smelt, there is an anatomical difference in position of head, pericardium, and yolk sac when compared to many other forage fish larvae. Prior to this study, there were also no videos of normal surf smelt heartbeats to use as a baseline for normal cardiac activity in the species. Without this baseline information, designing and interpreting ecotoxicological studies that compare heart size and rhythm in embryos that have been exposed to environmental toxins to normal embryos is difficult. Additionally, having reliable culturing information such as temperature and timing is key to designing future experiments. This natural history study provides needed baseline data on an important forage fish species.

Methods

Experimental System

This study was conducted at the Northwest Fisheries Science Center's (NWFSC) Mukilteo Field Station in Mukilteo, Washington, USA (now permanently closed). Twelve small tanks (27.0 cm × 16.5 cm, depth 7.6 cm) were used for this study (Figure 1). Seawater filtered to 10 µm flowed into each tank at 0.66 L/min in a flow-through setup. Water temperature reflected ambient conditions as it was not changed after pumping it in from Puget Sound (at depth approximately 9 m from high tide line at Mukilteo [47.9445° N, 122.3046° W]). Water temperature was recorded in the sump with HOBO data loggers and temperatures ranged from 12.52 °C (SD 0.22) to 22.37 °C (SD 1.14).

Degree days (DDs) (Oliver 2019) are commonly used to calculate development in both plants and animals and there are numerous methods of calculating DDs (Neuheimener and Taggart 2007, Chezik et al. 2014a, PennState Extension 2023). We calculated DDs by using the formula:

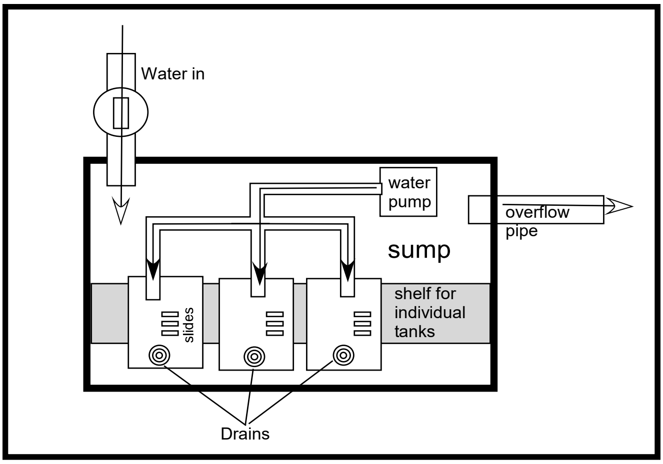

Figure 1. Bird's eye view of setup for replicates. Each small tank contained a stand that kept 6 slides vertical in the water column. There were twelve small tanks in total; the last tank in the row contained glass plates with adhered egg masses as opposed to the slides, which had individually placed eggs. All small tanks experienced water from a communal sump that was split equally with tubes and valves so that each tank experienced equal water flow.

$$DD = \frac{(T_{max}+T_{min})}{2} \times Hours \qquad (1)$$

where T_{max} was the daily maximum water temperature and T_{min} was the daily minimum water temperature. For Hours, we calculated actual number of hours from time of first sampling. This was necessary as we took samples at various times throughout the day and we wished to hone in on the number of hours to each stage of development. While the metric is called degree days, it is more accurately "degree hours." The rearing system was designed to mimic tidal and light cycle effects by setting overhead lights and the water pumps on digital timers. The tidal cycles were twelve hours each and the tanks were without water during the 12-hour low tide periods. The lights (Dengarden CFL Indoor Grow Lights, model 33156634) were set on a timer to mimic the diurnal light cycle of the season, 16 hours of light and eight hours of darkness.

Capture and Fertilization

Surf smelt gametes from both males and females were collected from ripe, wild Puget Sound fish, caught by recreational and commercial fishers on 15 July 2019. Preliminary work found that fertilization was most effective if done in the field immediately after gonad collection. Ripe females were identified by applying gentle pressure on the abdomen to determine if eggs would freely flow. If so, the female's pore was wiped with alcohol and 15–25 mL of eggs were collected. This process was done for three females and eggs from each female were kept separately in 50 mL tubes until males were found. While we did not water activate eggs until after fertilization, the eggs did not dry out as they were expressed with ample ovarian fluid to retain moisture. Three ripe males were then selected in the same manner. Because males often drip seawater into the container when expressing sperm, all eggs were collected first to delay water hardening. Milt from all the males was combined and 5 mL of milt was added to each tube with eggs, then seawater was added until the tube was full. All tubes were capped and gently inverted and lightly shaken until no clumps of eggs remained. Eggs were placed in a cooler to maintain temperature and transported back to the NWFSC's Mukilteo Research Facility and then placed in a 5 °C refrigerator overnight. We opted for fertilizing eggs at time of capture because of no success in previous studies with delaying fertilization until returning to the lab. Because we opted to beach fertilize, development started immediately and continued overnight in the holding fridge. The average temperature of the holding fridge was 5 °C, which is slightly cooler than the average winter water temperatures (6.7–9.6 °C) for Puget Sound (Seattle Sea Temperature, 2024).

The next morning, 16 July 2019, at 0700 PST, eggs were rinsed with filtered saltwater and allowed to adhere to either standard microscope slides (75 × 26 mm) with a 50 × 15 mm grid or three 127 mm diameter glass plates. For the slides, eggs were manipulated with probes into three rows of 10 eggs. Six slides were placed in each of the first 11 tanks, for a total of 66 slides, and the three glass plates were placed into the final tank. We were able to leave eggs in tubes overnight without danger of eggs adhering to the insides of the tubes because smelt eggs, in our experience, do not adhere to plastic.

Daily Observations and Photography

After being held overnight at 5°C in a refrigerator, some eggs appeared to have developed to the two to four cell stage. After all eggs settled on slides or plates, we took photographs to note the adhesion and development stages (16 July 2023, 0800). We then sampled 12 hours later and noted developmental stages. At this point most eggs appeared to be at the 8–32 cell stage. We then randomly chose slides daily to observe development (Table 1, Figures 2 and 3) until Day 5 when all eggs on all slides had died. As hatching approached, which was between 10 and 14 days per a pilot study, we agitated the plated eggs on a mini vortex machine (Heathrow Scientific Mini Vortex Mixer Model 120598) to mimic tidal action and stimulate hatching. This agitation process was performed twice daily, eight hours apart, on Days 10–14, by holding the plates on the vortex pad and gently pressing down. This allowed us to better anticipate the mass hatching event. After the hatching event, we used Sigma-Aldrich Tricaine mesylate (Lot #MKCJ6340, MS-222) to anesthetize the larvae to take photos and videos of the dorsal, ventral, and lateral views. Figure 4 is of the lateral view of a newly hatched surf smelt larva. We also took videos of the lateral view pre-anesthesia to note normal heart rhythms. Because of head morphology (large, protruding eyes) and the size of the larvae, anesthesia was necessary to obtain dorsal and ventral views to examine morphology of the entire pericardium. All larvae were maintained at 10 °C during imaging with temperature-controlled microscope stages (Brook Industries, Lake Villa, IL, USA).

To document the first detected heartbeat, we took still images of each egg in every grid of the randomly selected slides and of all the plates daily until the first heartbeat was seen with a 1.2 MP resolution video camera (Unibrain Fire-I 785c, Unibrain, San Ramona, CA, USA) mounted on a Nikon SMZ-800N stereomicroscope. Once the heart was visible, particular care was given to document gross morphology of the heart location in relationship to the yolk globule (Figures 4 and 5), development of the heart chambers (Figure 6), and the timing of heartbeat initiation. Upon completion of the study, larvae that were not moved to a grow-out tank were euthanized by MS-222 overdose.

Results

Our study documented surf smelt developmental milestones in still and video images. Within 12 hours of fertilization, we placed eggs on slides or glass plates and noticed that all eggs had reached the two to four cell stage (first and second cleavage). Eggs achieved some development overnight at approximately 5 °C; while we documented that most eggs had not experienced first cleavage when adhered to slides or glass plates on Day 1, there were a subset of eggs (uncounted) that had developed to the 2-cell stage overnight. For clarification, Day (e.g., 16 July 2019) refers to the calendar day that we photographed samples and degree days (DD) refers to the calculated time to that developmental stage. By Day 2 (15 DD), eggs on both the plates and the slides had reached gastrula. By Day 3, we started noticing a significant die-off of eggs on glass slides, with the exception of one batch of slides. By Day 5, all eggs on the slides had died and we discarded the slides and switched over to viewing only the plates. Surviving eggs developed optic vesicles and the first somites by Day 4 (48 DD), and the first heartbeat in the non-chambered tube-like heart was observed on Day 5 (62 DD). By Day 9 (131 DD), the pericardium was established, the heart was rhythmically pulsing, and embryos were observed moving within the eggs. Embryos initiated development of the circulatory system on Day 10 (148 DD). On Days 10 through 12 (148–174 DD), we observed melanophores appearing, the eyes were pigmented, and the embryos were actively moving within the egg. We saw a partial hatch on Day 13 (187 DD), along with fins becoming apparent; by Day 15 (225 DD) all larvae hatched. After the hatch, we microphotographed

Table 1. Average temperature, degree days, and developmental stages of larva. Many, but not all, eggs held overnight showed development to the two or four cell stage. YSL = yolk syncytial layer. We sampled twice on 18 July.

Day	Date	Avg. temp. ˚C	Degree days (DD)	Figure 3	Development
0	15 July	5	0	A	Eggs collected, held overnight in fridge; some eggs reached two to four cell stage
1	16 July	16.53	4	B	Two to four cell stage for remaining viable eggs
2	17 July	15.29	15	C	Blastula (128 cell cap)
3	18 July	15.18	30	D	Early gastrula, epiboly about 50%
3	18 July	15.18	40	E–G	Epiboly continues, YSL two layers thick, blastopore forms, bud formation
4	19 July	15.27	48	H–I	Optic vesicles forming, eyes and lenses white. First somites and pericardium appear. Embryo one complete wrap around yolk
5	20 July	15.09	62		First heartbeat detected, alimentary canal defined, eyes starting to turn grey
6	21 July	15.85	81	J	Eyes dark grey, lenses well defined, one and half wraps around yolk, skull well defined
7	22 July	16.02	98		Eyes black, heart is beating rhythmically
8	23 July	15.39	114		Embryos are coiled tightly, body is segmented, melanophores starting to appear
9	24 July	15.58	131		Eyes have a silvery sheen, lower jaw forming
10	25 July	15.78	148		Embryo highly motile in egg
11	26 July	15.93	163		Circulation evident, pectoral fins buds visible
12	27 July	15.49	174	K	Prehatch embryo, melanophores highly visible
13	28 July	15.30	187		Partial hatch when eggs agitated
14	29 July	15.71	207		Partial hatch continues
15	30 July	15.76	225	L	Full hatch

immobilized larvae (anesthetized and placed in an agar plate with a groove to hold larvae in place), viewed the pericardium, and documented dorsal, ventral, and lateral gross anatomy.

Discussion

The main objectives of this project were to document normal embryological development in surf smelt and to record the heart beating in pre-hatched and newly-hatched surf smelt as it correlated to temperature in a controlled environment. We accomplished this goal by taking ample microphotographs of developing embryos as well as videos of the beating heart to be used in subsequent studies. We tied the development to recorded average water temperatures and have a significant number of photographs that are time-stamped as reference material for future studies. Although there are numerous studies on development in surf smelt and similar species, we could not identify, at the time of this project, data that filled this research need. Yap-Chiongco (1941) provided background information and many developmental stage illustrations for surf smelt, however these stages were not correlated to temperature-based degree days. Romney et al. (2019) provided data on a similar species, delta smelt (*H. transpacificus*), and provided developmental stage and time as hours and days post-fertilization at 16 °C. This study was very close to our study on *H. pretiosus*, except for its lack of cardiac activity video. Middaugh et al. (1987) also looked at surf smelt, correlating development to stages outlined in Lagler et al. (1977) which described developmental stages based on a species of mummichog (*Fundulus heteroclitus*). This staging (Lagler et al. 1977) was homogenized to describe development in bony fishes as a group.

Anatomy of the Teleostian Heart

Our study had two purposes: first, to document the embryological development of surf smelt; and sec-

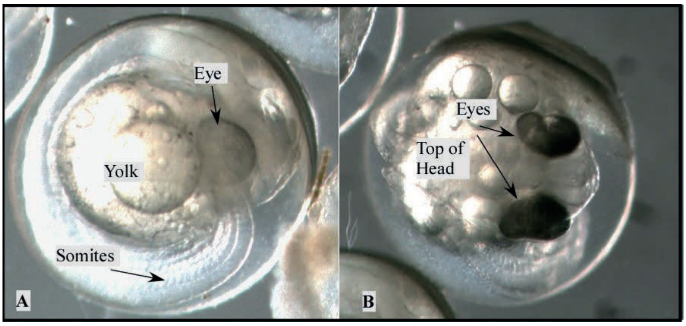

Figure 2. Microphotograph of surf smelt embryos. A. Early segmentation. B. Pharyngula stages. The embryos wrap around the yolk. Average egg size was 1.1 mm.

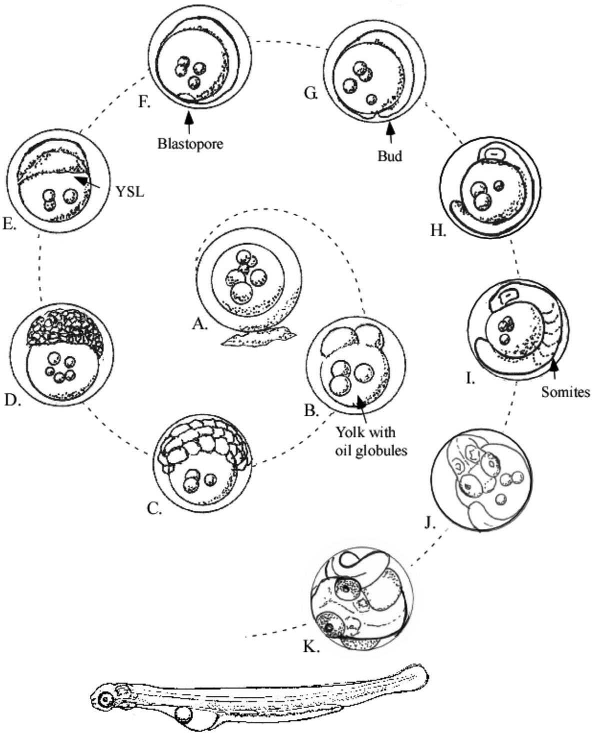

Figure 3. Developmental milestones for surf smelt at approximated average water temperature and Degree Days (DD). Development was initiated at time of fertilization and retarded overnight at 5 °C. Eggs had developed to the two or four cell stage when placed on glass the morning after fertilization. Developmental stages A–L are described in Table 1.

ond, to microphotograph and video record hearts and heart beats. Gaining this information was this impetus for this project as surf smelt heart morphology and cardiac rhythms have not been closely studied. To better understand the cardio morphology of surf smelt, we reviewed studies on morphology of various fish species to familiarize ourselves with the teleost heart. Fish hearts consist of the sinus venosus (SV), one atrium (A), one ventricle (V), and the bulbus arteriosus (BA) (Figure 5). Fish have a single circulatory system, which is well suited for aquatic living where the atmospheric oxygen pressure is low. In a single-pass circulatory system, blood flow from the body passes through the sinus venosus and collects in the atrium; it then enters the ventricle which pumps it through the bulbus arteriosus and back to the gills to be oxygenated. This differs from non-fish, as most non-teleost vertebrates have a double circulatory system in which deoxygenated and oxygenated blood have separate pathways in and out of the heart (Godinho 1970, Lagler et al. 1977, Moriyama et al. 2016).

Calculating Degree Days

We examined multiple methods of calculating DDs and selected our formula based on the work of Chezik et al. (2014a,b) which provided a formula used extensively in calculating DDs. However, we modified it because surf smelt do not have a minimum recorded temperature for development. Chezik et al. (2014a,b) applied the following formula:

$$DD = \frac{(T_{max}+T_{min})}{2} - T_{base} \qquad (2)$$

where T_{max} was the daily maximum water temperature, T_{min} was the daily minimum water temperature, and T_{base} is the accepted base temperature at which no development is seen. We initially set T_{base} at 5 °C, as that was the post-fertilization incubation temperature, but we decided against this T_{base} as we saw development during that temperature. Alternatively, we could have set T_{base} at an arbitrary temperature, for example 1 °C, which has been done for several other species (Pawiroredjo et al. 2011) but we decided against this too as we did not have conclusive data that this would be an accurate base temperature. A future study to document smelt T_{base} is warranted. We feel that including information about this unused formula (Chezik et al. 2014a,b) is of value because future studies that set T_{base} could provide a greater understanding of developmental timing by providing an accurate species specific T_{base} temperature.

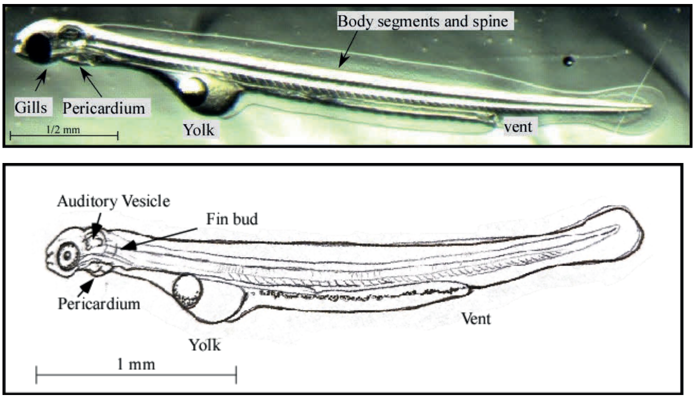

Figure 4. Microphotograph and illustration of surf smelt, lateral view. Average length was about 3 mm. Note pericardium in relationship to yolk sac.

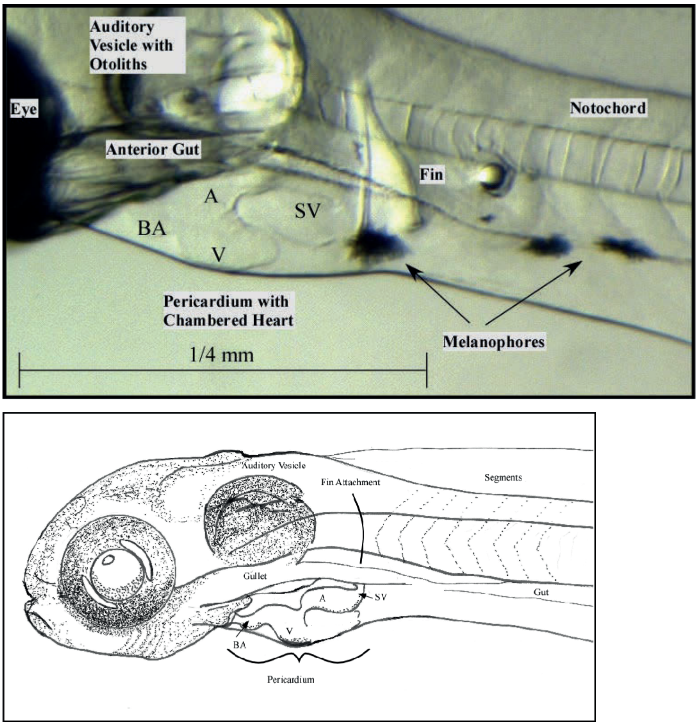

Figure 5. Normal pericardium on newly hatched surf smelt. The major components of the teleost heart are the sinus venosus (SV), the bulbus arteriosus (BA), the auricle (A), and the ventricle (V). The BA is considered novel to neoteleosts (Moriyama et al. 2016).

Cardiac Edema

It has long been known that exposure to crude oil, including during spill cleanups, can affect the cardiovascular health of humans (Marris et al. 2020, Denic-Roberts et al. 2022) but until recently there has been little research on the effects of oil spill events on the developing larvae of aquatic species. Research on oil exposure during embryology and larval stages of marine species has been increasingly investigated since the 1989 Exxon Valdez spill in Prince William Sound, Alaska, and the 2010 Deepwater Horizon Spill in the Gulf of Mexico (Sørensen et al. 2017). There are various hypotheses as to how an oil spill event can impact early development including ingestion by larvae and coating of the chorion, which increases potential uptake of toxic compounds (Sørhus et al. 2015). Damage to larvae can present as various skeletal malformations and yolk sac and heart edema (Sørhus et al. 2016).

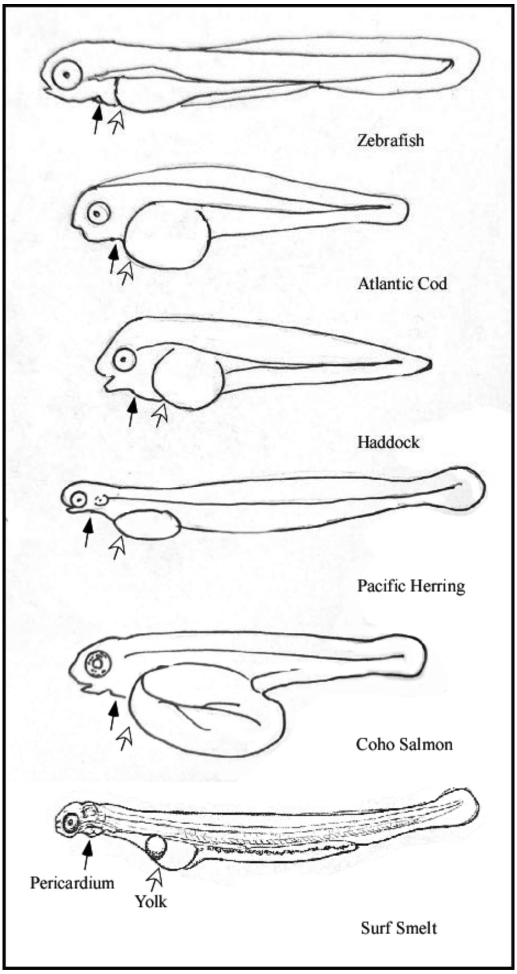

Figure 6. Comparative positioning of the heart and pericardium (black arrow) to the yolk sac (white arrow) in zebrafish (*Danio rerio*) (von Hellfeld et al. 2020), Atlantic cod (*Gadus morhua*) (Hall et al. 2004), Atlantic Haddock (*Elanogrammus aeglefinus*) (Jordan and Everman 1900), Pacific herring (*Clupea pallasii*) (Jordan and Everman 1900), coho salmon (*Oncorhynchus kisutch*), and surf smelt (drawings based on author's personal photo collection). All species have been subjected to toxicology studies.

Previously studied species including cyprinids, clupeids, perciforms, and gadids share a common morphology with the yolk sac adjacent to the heart (Scholz and Incardona 2015, Incardona and Scholz 2016, Sørensen et al. 2017), but in surf smelt, the yolk is placed more posteriorly (Figure 6), so standard measurements based on other species do not apply. The position of the heart relative to the yolk sac is important in that the cardio edema that is present with injured, abnormal, or weakened hearts is harder to detect in smelt as there is more room for the edema to spread out between the pericardium and the yolk sac (J. Spromberg, NWFSC, personal communication). We collected videos that show normal heart rhythm chamber timing in the developing heart, and the position of the heart in the larva; videos are available at: https://doi.org/10.6084/m9.figshare.25286056.v1.

Lessons Learned

We had two hypotheses on why eggs on slides died but the eggs on the plate did not. No eggs that had been placed on slides developed past the blastula stage. They may have died due to desiccation during the time when we mimicked low tides by clearing the sump of water, thus leaving the eggs to dry out, or they died due to mechanical insult from being placed onto the slides with the probes. We discounted mechanical stress because we did see eggs develop past second cleavage on many of the slides before perishing. We posit that the issue with gridded slides appears to be that the eggs were not clumped together. As gridded eggs and plated eggs were all subject to same environmental conditions, it appears that desiccation played a large role in single egg mortality. We surmise that clumped eggs retained a minute amount of moisture, which perhaps prevented complete desiccation and resultant death during low tide. A recent study by Harding et al. (2022) pursued this idea and showed that desiccation was the probable cause of egg mortality on slides.

We have noticed that surf smelt eggs that are placed on lower sides of tidal zone rocks nearer to the bottom survive while eggs that adhere to the top of rocks, and are exposed to full sun, perish. This proposed theory that eggs cannot survive drying out led us to wonder why a species would evolve to spawn on the beach. We note that unlike herring (*Clupea pallasii*), surf smelt eggs have only one sticky disk that adheres exclusively to rocks or sand granules and does not attach to organic matter. While smelt may lay eggs below the low tide zone, those eggs will not adhere well to surfaces that have a layer of biofilm and thus are often washed away and are easier for predators to consume. Intertidal rocks have far less algae and biofilm on them compared to rocks in the low tide zone, so have more surface area for egg adherence. Additionally, we have observed that there are often schools of crabs (various species) and shiner perch (*Cymatogaster aggregate*) that follow spawning smelt and pick off eggs as soon as they spawn. When smelt lay eggs at the highest tide, it gives eggs a chance to stick to rocks and when the tide goes out, perch and other predatory fish abandon foraging. Field observations show that during the next incoming tide, predatory fish do not necessarily return to the zone in which eggs were laid, suggesting that spawning on shore provides a modicum of protection from predators. As air temperatures climb due to climate change and anthropogenic development (including seawalls, docks and marinas, removal of native vegetation, runoff, and dredging) alters shorelines, this issue with egg desiccation could select against summer spawning, when temperatures and sun exposure are higher than in fall/winter spawning events (Lee and Levings 2007).

Future Work

This study successfully documented timing of developmental landmarks, the first heartbeat and hatch, at an average water temperature of 15.61 °C. Future work to further elucidate the surf smelt life cycle could include studies of shaded versus non-shaded beaches to better understand how exposure to sun affects embryo development and survival. Human development of coastal shorelines removes vegetation that provides shade for spawning areas. Saltwater associated with sea level rise may also inundate and kill certain types of shoreline vegetation (Dunagan 2016). We also suggest development stage work at a variety of temperatures to learn about the upper limit of thermal tolerance for Pacific surf smelt. This information would provide managers with tools to better identify important zones for surf smelt spawning.

Finally, in ecotoxicology studies, there are also morphometric changes to bony structures, such as the jaw (Sørhus et al. 2016, von Hellfeld et al. 2020) and the spine (Li et al. 2022, Taslima et al. 2022). According to Sørhus et al. (2016), is appears that crude oil exposure depletes intracellular calcium which disrupts development along several developmental pathways. While spinal deformities are frequently seen in cultured fish, knowing what is normal during development and being able to contrast with larvae that underwent laboratory challenges for a non-cultured wild species is important to the study of ecotoxins and their influences on developing embryos (Sørhus et al. 2016). Future work should include clearing and staining smelt larvae to improve understanding of skull and jaw development.

Acknowledgments

The authors wish to thank Dr. Julann Spromberg (NWFSC) for expertise with imaging and assistance in the lab.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Care and Use

This research was conducted under a Washington Department of Fish and Wildlife (WDFW) collection permit and Section 10 Endangered Species Act (ESA) Bycatch permit. Animal care followed the University of Washington vertebrate animal welfare protocols for laboratory animals (https://oaw.uw.edu/).

Data Availability Statement

Video of a surf smelt heartbeat is available at: https://doi.org/10.6084/m9.figshare.25286056.v1.

Author Contributions

MT acquired gametes, built rearing system, and provided knowledge on all aspects of husbandry. KN initiated study, wrote proposal, acquired grant, took data, prepared manuscript, and created all drawings.

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