



Western Washington University
Western CEDAR

WWU Graduate School Collection

WWU Graduate and Undergraduate Scholarship

Summer 2020

Investigating the effects of climate co-stressors on surf smelt energy demands

Megan Russell
meg83@hawaii.edu

Follow this and additional works at: <https://cedar.wwu.edu/wwuet>



Part of the [Biology Commons](#)

Recommended Citation

Russell, Megan, "Investigating the effects of climate co-stressors on surf smelt energy demands" (2020). *WWU Graduate School Collection*. 967.
<https://cedar.wwu.edu/wwuet/967>

This Masters Thesis is brought to you for free and open access by the WWU Graduate and Undergraduate Scholarship at Western CEDAR. It has been accepted for inclusion in WWU Graduate School Collection by an authorized administrator of Western CEDAR. For more information, please contact westerncedar@wwu.edu.

Investigating the effects of climate co-stressors on surf smelt energy demands

By

Megan Russell

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

ADVISORY COMMITTEE

Dr. Brady Olson, Chair

Dr. Brooke Love

Dr. Benjamin Miner

GRADUATE SCHOOL

David L. Patrick, Dean

Master's Thesis

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Megan Russell

June 30, 2020

Investigating the effects of climate co-stressors on surf smelt energy demands

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
Megan Russell
June 30, 2020

ABSTRACT

Surf smelt (*Hypomesus pretiosus*) are ecologically and economically important to the Pacific Northwest. They play a critical role in the food web and support numerous commercially important species and are an economically important baitfish. Surf smelt interact closely with the nearshore environment, utilizing approximately 10% of Puget Sound coastlines for spawning throughout the year. Surf smelt spawn at high tide and adhere fertilized eggs to beach sediment, causing their embryos to be exposed to air and seawater throughout embryonic development. Because of this unique life history, surf smelt may be susceptible to anthropogenic stressors including coastal development and climate change. However, very few studies have attempted to test the tolerance of surf smelt to climate change, including elevated temperature and ocean acidification. The purpose of this study was to examine the interactive effects of climate co-stressors ocean acidification and seawater warming on the energy demands of developing surf smelt. Surf smelt embryos and larvae were collected and placed into experimental basins under three temperature treatments (12°C, 15°C, and 18°C) and two total carbon treatments (ambient and elevated) for a period of 14 days for the embryos, and 4 days for the larvae. Increased temperature significantly decreased yolk size in developing surf smelt embryos and larvae. During this time, embryo yolk sacs in the high temperature treatment were on average 10.2% smaller than embryo yolk sacs in ambient temperature water. Larval yolk and oil globules mirrored this trend with larvae in the high temperature treatment having on average 32.5% smaller yolk sacs and 20.0% smaller oil globules compared to larvae in ambient temperature. While no effect of acidification as a singular stressor was observed, the interaction with temperature significantly increased surf smelt embryo heart rates by 5% above ambient conditions. These results indicate that near-future climate change scenarios are going to impact the energy demands of developing surf smelt, a result that may have a variety of potential

impacts including altered hatch times, larval deformities, and increased mortality, all of which will increase interannual variability in adult recruitment. The results of this study highlight the need to increase focus on studying surf smelt in the context of ecological and climate change research.

ACKNOWLEDGMENTS

I would like to start by thanking my advisor, Dr. Brady Olson, for his endless support and guidance throughout this project. I would also like to thank my committee member, Dr. Brooke Love, for answering my numerous chemistry questions, troubleshooting problems, and for letting me play with the fidget toys in your office during particularly stressful weeks. Thank you to Dr. Ben Miner, first and foremost for your humor and friendship, but also for allowing me to argue with you about statistics for a year and a half until I finally understood that you were right all along. Thank you all for never giving up on me.

Thank you to Dr. Brian Bingham for always making time to sit with me and discuss models. And to the incredible support staff at Shannon Point Marine Center for allowing my experiments to run smoothly. This project would not be possible without the support of Todd Sandell and the Washington Department of Fish and Wildlife Forage Fish Team. Thank you for helping me design this project and modify it when we needed to. Thank you to Dan Penttila, who's knowledge of everything surf smelt was critical to the adaptation and continuation of my research. I would like to acknowledge the invaluable support of the Northwest Climate Adaptation Science Center who funded this project and guided me throughout their fellowship program. The entire NW CASC team were supportive, inspirational, and kind.

Thank you to all of my friends, cohort, and undergraduate assistants for their help throughout my many experiments. A special thank you to Jake Lawlor for your guidance, graduate school wisdom, and for bringing me food during long experiment days. And to Nathan Guilford for being my sounding board and staying with me until 3 AM to count fish embryos so that I would not be there all night. And finally, none of this would be possible without the support of my truly amazing family who have always allowed me to pursue my dreams.

Thank you.

TABLE OF CONTENTS

Abstract.....	iv
Acknowledgements.....	vi
List of Figures.....	viii
List of Tables.....	ix
Introduction.....	1
Methods.....	9
Results.....	15
Discussion.....	26
Works Cited.....	36

LIST OF FIGURES

Figure 1: Schematic of experimental design.....	11
Figure 2: Temperature and pH effect on average surf smelt embryo yolk size.....	18
Figure 3: Temperature and pH effect on average surf smelt larval yolk size.....	19
Figure 4: Temperature effect on surf smelt larval yolk size.....	20
Figure 5: Temperature and pH effect on average surf smelt larval oil globule size.....	21
Figure 6: Temperature effect on surf smelt larval oil globule size.....	22
Figure 7: Temperature and pH effect on average surf smelt larval length.....	23
Figure 8: Embryo heartrate measurements at each treatment level.....	25

LIST OF TABLES

Table 1: Overview of experiment timelines and measurements.	9
Table 2: Water quality measurements for each experiment.	16
Table 3: Effect of climate change on surf smelt embryo yolk size.	17
Table 4: Effect of climate change on surf smelt larval yolk size.	19
Table 5: Effect of climate change on surf smelt larval oil globule size.	21
Table 6: Effect of climate change on surf smelt larval length.	23
Table 7: Effect of climate change on surf smelt embryo heart rate.	24

INTRODUCTION

Forage fish are small, schooling fish that represent a critical link in the marine food web by facilitating energy transfer from plankton to upper trophic levels, including numerous species of sea birds, marine mammals, and other commercially important fish (Therriault et al. 2009; Essington et al. 2016; Lindegren et al. 2017). In addition, forage fish support recreational and commercial fisheries as both a food resource and as a baitfish (Penttila 2007; Essington et al. 2016). An estimated 30% of the global fisheries landings by weight consists of forage fish, making them the largest fishery in the world (Alder 2008; Essington et al. 2016). Forage fish are globally distributed and occupy a prominent ecological role in the Puget Sound, the southern region of the Salish Sea (Penttila 2007; Greene et al. 2015). In the Puget Sound, the forage fish community is predominantly composed of three species: Pacific herring (*Clupea pallasii*; Valenciennes 1847), Pacific sand lance (*Ammodytes hexapterus*; Pallas 1814), and surf smelt (*Hypomesus pretiosus*; Girard 1854) (Penttila 2007). Given their importance in marine ecosystems, understanding the drivers in forage fish population dynamics is important.

Forage fish interact closely with their nearshore environments, specifically during spawning events (Penttila 2007). The three dominant species are widespread throughout the Puget Sound, and each use approximately 10% of the Puget Sound nearshore environment for spawning activity (Penttila 2007). Spawning behavior and selection of spawning location across the species varies and is dependent upon factors such as sediment grain size, tidal height, shading, and vegetation (Penttila 2007; Quinn et al. 2012). While Pacific herring utilize nearshore eel grass and other aquatic vegetation beds for spawning (Hay 1985; Penttila 2007), Pacific sand lance and surf smelt utilize high tides to adhere fertilized eggs to beach sediment (Penttila 2007).

Of the three species of forage fish in the Puget Sound, comparatively little is known about surf smelt, and their life history outside of their spawning grounds remains poorly understood. Surveys conducted in the 1970's found that Puget Sound surf smelt stocks consisted primarily of one- and two-year-old fish, suggesting that surf smelt rarely survive past the age of four (Penttila 2007). Despite most beaches in the Puget Sound being objectively well suited for surf smelt spawning (i.e. ample shading, correct grain size, and limited disturbance), only a small portion of these are used (Penttila 2007; Quinn et al. 2012), and how surf smelt locate and choose spawning sites or what cues trigger spawning events remains unknown (Penttila 2007).

Surf smelt are obligate beach spawners and require unaltered coastlines for successful spawning (Rice 2006; Quinn et al. 2012). Whereas Pacific herring spawn in distinct populations that vary in locations and timing, thus limiting genetic interchange between individual stocks, surf smelt appear to have no defined stock boundaries (Penttila 2007; Sandell et al. 2019). Surf smelt are also unique in how they use their spawning grounds (Penttila 2007; Quinn et al. 2012). Surf smelt spawn during high tides, utilizing the upper third of a beach's tidal range, typically in the +7 to extreme high-water range (Loosanoff 1937; Rice 2006; Penttila 2007). Eggs are adhered to substrate with preference for a sand-gravel mix with grain sizes between one and seven millimeters in diameter (Penttila 2007; Quinn et al. 2012). Once deposited, embryos are exposed to both air and seawater throughout their development. This unique spawning behavior exposes surf smelt embryos to a unique set of environmental stressors, including a broad thermal range, desiccation stress, exposure to terrestrial predators, etc. (Lee and Levings 2007; Quinn et al. 2012).

Surf smelt embryos develop by utilizing maternally provided stored yolk for roughly 14 to 21 days depending on temperature and sediment humidity conditions. When embryos are

fully developed and capable of hatching, hatch is triggered by physical disturbance, primarily resulting from tidal activity and wave action (Rice 2006; Lee and Levings 2007; Penttila 2007). After hatching, planktonic surf smelt larvae rely on their spawning sites to act as a nursery while they continue to utilize their remaining yolk for approximately one week. Once mouth parts have developed, surf smelt begin exogenous feeding (Penttila 2007). In addition to a yolk sac, surf smelt larvae also possess a lipid oil globule. Although the function of the oil globule has not been identified in surf smelt, in other species of marine fish it serves as an important source of energy post hatching (Avila and Jaurio 1987; Rønnestad 1998; Berkely et al. 2004). For example, in black rockfish, oil globule volume was a significant predictor of larval size, growth, and survival (Berkely et al. 2004).

Forage fish, in general, are declining across their ranges (Essington et al. 2016; Lindegren et al. 2017), and this is especially true in the Puget Sound (Penttila 2007; Sandell et al. 2019). Given their keystone importance in marine ecosystems, this is cause for concern. Of the three common species of forage fish in the Puget Sound, the status of Pacific herring populations have been the most closely monitored by management agencies (Sandell et al. 2019). Across their range in the Salish Sea, most Pacific herring spawning stocks are in population decline (Sandell et al. 2019). Further, some of the distinct Pacific herring spawning stocks in the Puget Sound are at the lowest abundance on record, and near extinction (Sandell et al. 2019). Unfortunately, the population status of surf smelt is unknown by management agencies due to a lack of methodology and funding to consistently quantify their annual spawning abundance as they do with Pacific herring (Penttila 2007). However, a pilot study in 2015 attempted to estimate the recreational harvest of surf smelt using a variety of methods including monitoring public access points and boat-based creel survey techniques (Lowry et al. 2015). This project reported that

recreational harvest might exceed previous estimated levels that have been assumed for management plans (Lowry et al. 2015). This unaccounted for exploitation of surf smelt might cause unnecessary stress on spawning populations. Another project synthesized 40 years of surface-trawling data and estimated that both Pacific herring and surf smelt populations have declined in the Central and South Puget Sound by up to two orders of magnitude over this time period (Greene et al. 2015). With little information about the population status of surf smelt, it is essential to improve the foundation of knowledge pertaining to this species.

What is causing forage fish decline across their ranges remains unknown, but forage fish in general are notorious for showing high interannual variability in spawning population recruitment (e.g. Schweigert 1995; Williams and Quinn 2000). After decades of research aimed at gaining understanding of this variability, there is growing appreciation for the ecosystem-level factors that drive recruitment each year (Houde 2008), which include trophic dynamics, climate variability, and maternal factors.

Habitat degradation in nearshore environments will likely have a detrimental effect on forage fish, specifically surf smelt given their usage of the upper-intertidal for spawning (Rice 2006; Lee and Levings 2007). Research comparing heavily modified beaches to natural beaches found that modified beaches had significantly higher substrate temperatures and significantly lower relative humidity (Rice 2006). In that same study they also observed a 50% decrease in the proportion of live surf smelt embryos found at modified beaches compared to natural beaches, indicating that embryo survival is coupled to spawning substrate temperature and humidity (Rice 2006). Further, surf smelt embryo mortality was observed to positively correlate with air temperatures and embryo viability declined outside of a very narrow humidity range (Lee and Levings 2007). However, these studies were limited to only studying the effect of air temperature

and did not investigate the possible impact of changes in seawater temperature on developing surf smelt. Surf smelt embryos are also submerged at various times during their development, the degree to which depends on how high in the intertidal they were deposited, and the tidal range to which they are subjected (Penttila 2007). Accordingly, a more holistic experimental design should also include environmental stress associated with water chemistry and motion.

Given the correlation between surf smelt mortality and elevated temperatures, it is worth considering the effects that climate change will have on surf smelt populations. With the world's oceans absorbing approximately 93% of the extra heat attributed to the greenhouse gas effect (IPCC 2014), the Puget Sound alone saw an increase in sea surface temperature by about 0.5°C to 1°C since the year 1900 (Mauger et al. 2015). Climate models predict that global air temperatures will continue to rise and sea surface temperatures in the northeast Pacific Ocean will increase by another 1.2 °C by the year 2040 (Mauger et al. 2015), and the Salish Sea specifically will increase by approximately 1.5°C by the year 2095 (Khangaonkar 2019). The effects of water temperature on fish biology are wide-ranging. Rising seawater temperatures can change marine fish spatial distributions, recruitment success, population abundance (Walther et al. 2002; Letcher 2015), and the timing of behaviors such as spawning and migration events (Lynch et al. 2016). Temperature can also affect the rate of fish development, and if temperature exceeds a fish's thermal tolerance, can be lethal (Alderice and Velson 1971; Kamler 2002). Research on the effects of temperature on surf smelt has been limited to the effects of air temperature and desiccation stress on mortality and hatching (Rice 2006; Lee and Levings 2007). Few studies have attempted to rear surf smelt in the laboratory and assess under what conditions surf smelt thrive (Garreton 1983; Middaugh et al. 1987). In one study it was concluded that surf

smelt possess physiological mechanisms to compensate for temperature changes in seawater between the range of 12°C to 22 °C (Garreton 1983).

In addition to elevated temperature, excess atmospheric CO₂ is also causing the oceans to acidify, a process termed ocean acidification (OA), and this stressor may also imperil surf smelt. Since the industrial revolution, the pH of the ocean's surface water has decreased by 0.1, with a hydrogen ion increase of 26% (IPCC 2014). Decreased survival, growth, abundance, and altered development are all documented effects of OA across multiple taxonomic groups (see review by Kroeker et al. 2013). Cold-water ecosystems like the Puget Sound are vulnerable to OA due to the ability of cold water to absorb atmospheric gasses more efficiently than warm water (Form and Riebesell 2012). Climate models predict that the pH of the Puget Sound will continue to decrease by 0.18 by the year 2095 (Khangaonkar 2019). Additionally, the Puget Sound is especially vulnerable to anthropogenic OA due to the added acidification by way of upwelling and strong tidal mixing, both of which introduces CO₂ enriched seawater into surface waters (Feely et al. 2010; Reum et al. 2011; Newton et al. 2014). This natural acidification coupled with anthropogenic acidification may have widescale implications for the Puget Sound marine ecosystem (Feely et al. 2010).

While adult fish are not universally immune to the effects of OA, research indicates that adult fish have the potential to be efficient acid-base regulators through the retention of bicarbonate anions and increased net acid excretion, thus alleviating acidosis stress (Heuer and Grossell 2014; review by Esbaugh 2018). Fish embryos and larvae, however, lack these physiological mechanisms at this stage of their development (reviewed by Kikkawa et al. 2003), thus making them potentially vulnerable to the effects of OA. Multiple studies have reported delayed hatching in fish embryos when exposed to low pH (e.g. Peteron et al. 1980; Nelson

1982; Parker and Mckeown 1987). Currently, no information about the effects of OA on surf smelt exists.

Examining the effects of climate stressors in isolation precludes the understanding of their synergistic impacts (Pimental et al 2014; Davis et al. 2018). Given that global warming and OA both, in part, result from the emission of anthropogenic CO₂, it is essential to consider both stressors when assessing organismal fitness under climate change. For example, elevated pCO₂ alone had no effect on the metabolic rate, heartrate, or ventilation rate for the Antarctic rockcod, *Trematomus bernacchii* (Davis et al. 2018). However, when combined with elevated temperatures, the synergy of these two climate variables caused an elevation in metabolic rate and ventilation (Davis et al. 2018). Research on the Atlantic flatfish, *Solea senegalensis*, found similar results, with a greater percentage of larval deformities being observed when elevated seawater temperature was combined with acidification (Pimental et al. 2014).

Marine fish often compensate for climate stress by adjusting their energy budget (Kamler 2002; Davis et al. 2018; Rangel and Johnson 2018). Fukuhara (1990) found that the rate of yolk utilization, a metric for energy demand, in four different larval marine fish was faster under elevated temperature, and it increased the variation in development rates. Temperature also had a significant effect on the rate of yolk usage in summer flounder larvae (Johns and Howell 1980). Other research indicates that juvenile fish preferentially select locations where thermal conditions are favorable in order to optimize metabolic efficiency and growth (e.g. Clark and Green 1991, Mortensen et al. 2007, Pörtner and Knust 2007; Reum et al. 2011). In surf smelt, increased air temperature has been linked to increased developmental rates (Rice 2006), which could be an indication of increased yolk utilization. Surf smelt embryos raised between 12°C and 22°C had yolk utilization rates three times faster than embryos raised at 6°C (Garreton 1983).

There was no reported difference in surf smelt yolk utilization in treatments between 12°C and 22°C (Garreton 1983), indicating that surf smelt may not be sensitive to this temperature range.

The purpose of this study was to investigate the combined effects of elevated seawater temperature and OA on the energy demands of surf smelt embryos and larvae. This was accomplished through measuring yolk and oil globule exhaustion in surf smelt embryos and larvae under three temperature treatments and two dissolved inorganic carbon (DIC) levels, resulting in a range of pH and pCO₂ concentrations when combined with temperature. It was hypothesized that energy demand, measured as yolk sac/oil globule exhaustion, for both embryos and larvae would increase, with elevation of seawater temperature and total DIC (i.e. acidification). It was also predicted that the highest energy usage would be observed under treatments with both increased temperature and elevated DIC, as developing fish need to consume more energy to maintain homeostasis while experiencing simultaneous co-stressors. This study provides a better understanding of the holistic effects of climate change on surf smelt and will help guide management practices under a changing climate.

METHODS

Experimental Overview

Three experiments were conducted over the summer of 2019 focusing on different aspects of developing surf smelt energy usage (Table 1). Surf smelt embryos were collected from Fidalgo Bay in Washington State, aged and placed into experimental basins. These basins were divided into three temperature treatments: ambient (~12°C), 14°C, and 18 °C, as well as two DIC levels: ambient and elevated, in a split-block design. Experiments were conducted for as long as embryos and larvae from each treatment remained viable.

Table 1. An overview showing the timeline for each experiment conducted starting with the date of collection and terminating with the last day of measurements. The response variables measured throughout the duration of each experiment are listed.

Experiment	Dates	Response Measurements
Larval Experiment	7/9/19 – 7/12/19	Larval yolk size, oil globule size, and larval length
Embryo Yolk Experiment	7/16/19 – 7/28/19	Total embryo size and yolk size
Embryo Heartrate Experiment	8/19/19 – 9/6/19	Embryo heartrate and development rate

Experimental Design

All experimental seawater was collected from the Salish Sea at the Shannon Point Marine Center (SPMC). This water was dispensed into a header tank that gravity fed seawater into six mixing basins. In each mixing basin, submersible powerhead pumps (Marineland® Maxi-jet

900) circulated water using magnetically driven impellers. Three mixing basins continuously received CO₂ gas from an 8 channel Masterflex® L/S Digital Drive peristaltic pump attached to a 20lb food grade CO₂ gas cylinder. The peristaltic pump was set at a rate of 19 mL/min and the feeds were placed directly into the intakes of the powerhead pumps, allowing the CO₂ to be broken into small bubbles by the impeller and associated turbulence, resulting in complete dissolution of the pure CO₂ bubbles (Jokiel et al. 2014). This created two total DIC levels, ambient and elevated. Each mixing basin delivered water to three experimental basins held at the different temperature treatments (ambient seawater ~12°C, 15°C, and 18°C). The tanks were heated using 100W Aqueon® submersible heaters attached to Inkbird® temperature regulators. The temperature regulators engaged the heaters anytime the temperature deviated -0.3°C of the set temperature. Treatment temperatures were chosen to represent ambient seawater temperatures during the summer surf smelt spawning period (12 °C) and predicted future climate conditions in shallow Salish Sea waters (15°C - 18°C). Due to the sensitivity of pCO₂ to temperature, the two levels of total DIC (ambient and elevated) result in a range of pCO₂ for each temperature level encompassing 600-2200 µatm, and a pH range of approximately 7.42-7.88. The pCO₂ treatment concentrations were chosen to represent pCO₂ levels representative of the ambient seawater system (600 µatm) at the SPMC, elevated concentrations currently observed in the Salish Sea during naturally occurring CO₂-enrichment (Feely et al. 2010), and highly-elevated levels that may result in the future when increased anthropogenic pCO₂ combines with natural acidification events. Experimental basins were covered with 5/8" acrylic sheets to limit gas exchange during experiments. Figure 1 shows a schematic of the experimental system used in this study.

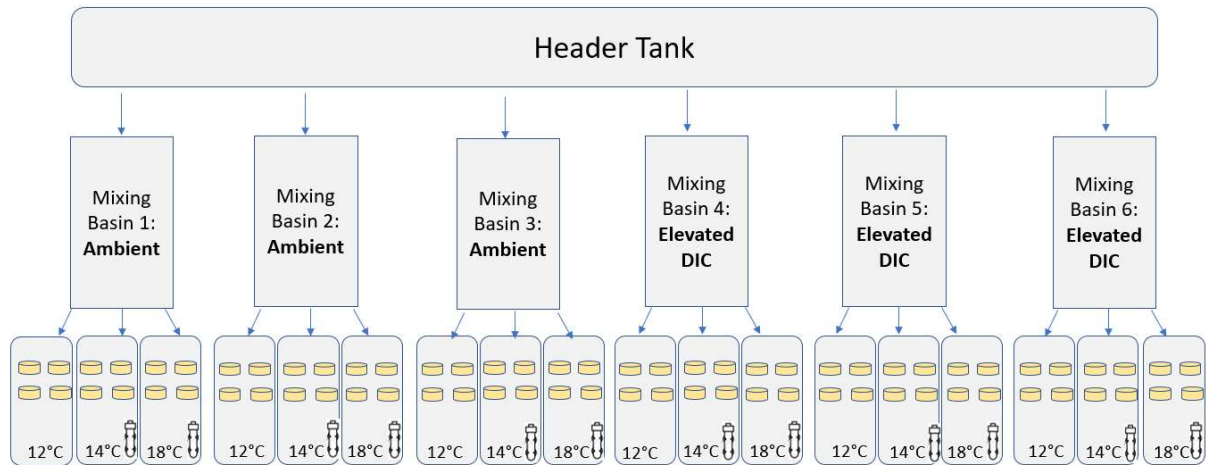


Figure 1. Schematic showing the experimental design. Seawater in the header tank came from the northern Puget Sound in the Salish Sea, and flowed into 6 mixing basins circulated with submersible powerhead pumps. A peristaltic pump delivered CO₂ gas into mixing basins 4, 5, and 6 at regulated output of 15 psi. The mixing basins delivered water to the treatment basins which were equipped with 100W aquarium heaters. In each treatment basin, there were 4, 200 mL bowls that contained surf smelt embryos or larvae. Bowls were covered by mesh netting which held organisms within the vessels, but also allows water flow in and out of bowls. Treatment basins were covered with 5/8” acrylic sheets to limit gas exchange.

Collection Procedure

Surf smelt eggs containing embryos and their attached sediment were collected throughout the summer months of 2019, with experiments starting on 7/9, 7/16, and 8/19. Collected embryos were transported to the SPMC where they were examined via stereomicroscopy to determine age. Embryo experiments began if the eggs were determined to be spawned and fertilized within 24 hours prior to collection. Age was determined using the guidelines of Penttila and Moulton (2006). After aging, eggs containing embryos were dispensed into experimental treatment basins. Approximately 50 embryos were added to each of the 72, 200 mL glass bowls. These bowls were divided evenly into 18 treatment basins (4 bowls per basin).

If the collected embryos were near hatch, then the embryos and attached sediment were placed into 12 L bins where they were gently oscillated for 1-minute increments to simulate wave disturbance and initiate hatching. Hatched larvae were collected, and the bins were oscillated again until no additional hatch was observed. Approximately 15 freshly hatched larvae were collected and distributed into each of the 72, 200 mL glass bowls which were divided evenly into 18 treatment basins (4 bowls per basin).

Water Chemistry Measurements

Temperature measurements from each basin were taken every 30 minutes by Onset HOBO® data loggers to verify that temperature remained consistent throughout the experiments. Daily pH measurements from treatment water within basins were taken using a hand-held Orion Star A329 pH conductivity meter, calibrated with NBS-buffers prior to use each day. Seawater samples stored in 20 mL plastic scintillation vials were also collected three times per week throughout the experiment for carbonate chemistry analyses. Water samples for carbonate chemistry analysis were poisoned with 20 μ L mercuric chloride (HgCl_2) to arrest microbial metabolism and stored for later analysis. Samples were analyzed for total DIC using the Apollo SciTech AS-C3. Sample salinity was measured using a refractometer, and salinity values and temperature were used to convert DIC measurements from $\mu\text{mol/L}$ to $\mu\text{mol/kg}$. Measurements of DIC were calibrated against a standard curve created from reference material (CRM, Batch 179, Dickson, Scripps Institute of Oceanography). CO2SYS (Lewis and Wallace 1998) was used to calculate all non-measured components of the carbonate system, using K_1 and K_2 equilibrium constants refit by Millero et al. (2006) and the sulfate dissociation constant by Dickson.

Embryo Yolk Measurements

To assess the effects of climate co-stressors on surf smelt embryo yolk usage, each day over a 14-day experiment (Table 1), 3 embryos from each of the bowls within each experimental basin were haphazardly selected and placed into a 6 well plate labeled with the corresponding treatment it was removed from. Embryos were photographed using a Leica M125 stereoscope attached to a Leica MC170 camera networked to Leica Suite software. After being photographed, embryos were removed from the experiment. Photos were later analyzed using the software ImageJ to determine the egg and total yolk area. These measurements were used to calculate the ratio of yolk area to total egg area. This ratio was used to account for initial differences in egg size.

Larval Energy Reserves Measurements

The effects of climate change on larval energy usage were assessed through a 4-day experiment (Table 1), during which 2 larvae were haphazardly selected each day from each bowl within each experimental basin. Larvae were anesthetized using tricaine following the methods of Masee et al. (1995). This method limits larval movement and allows still images of the larvae to be taken. Leica software and a stereo microscope were used to photograph the anesthetized larvae. After being photographed, larvae were removed from the experiment. Photographs were later analyzed using ImageJ to determine yolk area, oil globule area, and larval total length.

Heart Rate Measurements

To assess the effects of climate change on another metric representative of surf smelt embryo energy demand, heartrate measurements were made. Heart rate is affected by fish developmental stage, so to normalize heart rate measurements, an identifiable stage of

development was selected, and embryo heart rates were measured within treatments when this developmental stage was reached. An easily identifiable stage occurs when the eye spots of developing embryos darken (Penttila and Moulton 2006). When embryos were removed from glass bowls to be photographed for yolk measurements, if the eye spots had darkened since the previous day, the embryos were also video recorded for 10 seconds. The videos were used to count heart beats over the 10 second time frame.

Statistical Analysis

Yolk sac and oil globule exhaustion, embryo and larval size, and heart rates were analyzed using linear mixed effect models with DIC level, temperature, and their interaction as fixed factors, and mixing basin and day as random factors. To account for the nesting in the experimental design and the random effects associated with each of the experimental and mixing basins, random intercepts were assigned for temperature measurements nested in mixing basin, and mixing basin nested in each day. For each response variable measured, models were built with and without the DIC:temperature interaction term based on a combination of p-value significance ($\alpha= 0.05$) and AIC comparisons to choose the most appropriate model. Interaction terms that were insignificant ($\alpha> 0.05$) but lowered the AIC value and therefore increased the predictability of the model, were left in the model.

RESULTS

Treatment Conditions

Incubation temperatures remained consistent throughout the duration of the experiments, varying on average by ± 0.19 °C. Temperature variability was greater in the ambient temperature basins that were not controlled by temperature regulators and fluctuated in accordance with the source seawater (Table 2). During the embryo experiments, the medium temperature treatment was experimentally adjusted 1°C higher to provide greater separation between temperature treatment levels, as summer warming elevated the seawater temperature in the low temperature treatment. DIC additions created pH treatments distinct from one another, and average pH variability was similar between low and high DIC additions at comparable temperatures (± 0.03 pH units). Concentrations of DIC were consistent throughout the experiments, with standard deviations ranging from ± 1.87 $\mu\text{mol kg/SW}$ to ± 17.39 $\mu\text{mol kg/SW}$. The elevated DIC treatment was on average 42 $\mu\text{mol kg/SW}$ higher than the ambient DIC level (Table 2). Because pCO_2 is derived from temperature, pH, and DIC, the variability in all three measurements results in relatively high variability in pCO_2 estimates. The ambient tanks without added CO_2 had an average standard deviation of ± 50.58 $\mu\text{atm pCO}_2$ (Table 2). The elevated pCO_2 tanks had considerably higher variability, with an average standard deviation of ± 275.89 μatm (Table 2).

Table 2: Average seawater temperature and carbonate chemistry parameters for the three experiments. Data are shown as time-averaged means \pm 1 SD of (n) measurements. pH and temperature were measured daily, while DIC was measured 3 times per week. $p\text{CO}_2$ was derived from temperature, pH, and DIC measurements.

Experiment	Conditions (DIC + °C)	<i>In-Situ</i> Measurements		Discrete Samples	
		pH (NBS Scale)	Temperature (°C)	$p\text{CO}_2$ (μatm)	DIC ($\mu\text{mol kg}^*\text{SW-1}$)
Embryo Yolk Experiment	ambient +12	7.88 \pm 0.01 (36)	13.77 \pm 0.24 (36)	793.85 \pm 28.59 (15)	2015.06 \pm 10.44 (15)
	ambient +15	7.87 \pm 0.03 (42)	14.89 \pm 0.03 (42)	749.37 \pm 141.69 (15)	2013.21 \pm 16.53 (15)
	ambient +18	7.83 \pm 0.02 (39)	17.94 \pm 0.23 (39)	872.25 \pm 133.73 (15)	2010.92 \pm 14.86 (15)
	elevated+12	7.56 \pm 0.09 (39)	13.44 \pm 0.24 (39)	1695.41 \pm 337.05 (15)	2049.61 \pm 11.56 (15)
	elevated+15	7.56 \pm 0.06 (39)	15.02 \pm 0.03 (39)	1617.05 \pm 324.02 (15)	2050.43 \pm 15.9 (15)
	elevated+18	7.53 \pm 0.03 (39)	17.93 \pm 0.23 (39)	1695.41 \pm 368.98 (15)	2047.64 \pm 17.34 (15)
Larvae Experiment	ambient +12	7.85 \pm 0.01 (9)	13.22 \pm 0.35 (9)	828.75 \pm 25.25 (6)	2032.91 \pm 11.06 (6)
	ambient +14	7.83 \pm 0.01 (9)	14.15 \pm 0.08 (9)	888.25 \pm 25.49 (6)	2028.65 \pm 12.21 (6)
	ambient +18	7.79 \pm 0.01 (9)	17.42 \pm 0.15 (9)	992.22 \pm 39.38 (6)	2022.67 \pm 14.04 (6)
	elevated+12	7.54 \pm 0.07 (9)	13.43 \pm 0.43 (9)	1759.74 \pm 316.37 (6)	2067.31 \pm 16.84 (6)
	elevated+14	7.51 \pm 0.06 (9)	14.15 \pm 0.23 (9)	1787.97 \pm 247.35 (6)	2077.95 \pm 17.39 (6)
	elevated+18	7.51 \pm 0.06 (9)	17.32 \pm 0.11 (9)	1907.61 \pm 309.72 (6)	2065.77 \pm 16.79 (6)
Embryo Heartrate	ambient +12	7.86 \pm 0.01 (9)	13.56 \pm 0.26 (9)	839.89 \pm 1.03 (3)	2020.46 \pm 7.10 (3)
	ambient +15	7.84 \pm 0.01 (9)	15.04 \pm 0.08 (9)	884.78 \pm 4.89 (3)	2015.65 \pm 8.51 (3)
	ambient +18	7.84 \pm 0.02 (9)	17.98 \pm 0.19 (9)	940.38 \pm 55.21 (3)	2011.86 \pm 3.41 (3)
	elevated+12	7.43 \pm 0.04 (9)	14.10 \pm 0.32 (9)	2243.73 \pm 203.91 (3)	2066.24 \pm 1.87 (3)
	elevated+15	7.42 \pm 0.04 (9)	15.10 \pm 0.19 (9)	2342.09 \pm 239.96 (3)	2067.92 \pm 16.48 (3)
	elevated+18	7.46 \pm 0.04 (9)	17.98 \pm 0.07 (9)	2209.26 \pm 135.67 (3)	2057.98 \pm 8.30 (3)

Table 3: Summary of results of linear mixed effect model examining the effect of temperature, DIC treatment level, and temperature:DIC treatment interaction on the size of surf smelt embryo yolk sacs relative to total egg size over a 13-day time period. All values are compared to the intercept at the ambient DIC level.

	<i>Value</i>	<i>Std. Error</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>
<i>Intercept</i>	0.6011	0.0263	2607	22.8527	<0.0001
<i>Temperature</i>	-0.007	0.0012	173	-5.9183	<0.0001
<i>Elevated DIC</i>	0.0261	0.0295	12	0.8825	0.3948
<i>Temp:DIC</i>	-0.0009	0.0018	173	-0.4896	0.6250

Effects of Climate Change on Embryo Yolk Absorption

Embryo yolk size relative to total egg size (Y:E) decreased throughout the duration of the experiment in all treatments (Figure 2). Temperature had a significant, negative effect on Y:E throughout the 13-day experiment, and for every 1°C increase in temperature, the Y:E decreased by 0.007 (Table 3; Figure 2). The average Y:E decreased by 21.6% after 13 days of incubation in ambient temperature seawater compared to a 28.2% decrease in the high temperature treatments. This equates to Y:E being 10.2% smaller in the high temperature treatment compared to the low temperature treatment. There was no effect of DIC change on Y:E (Table 3).

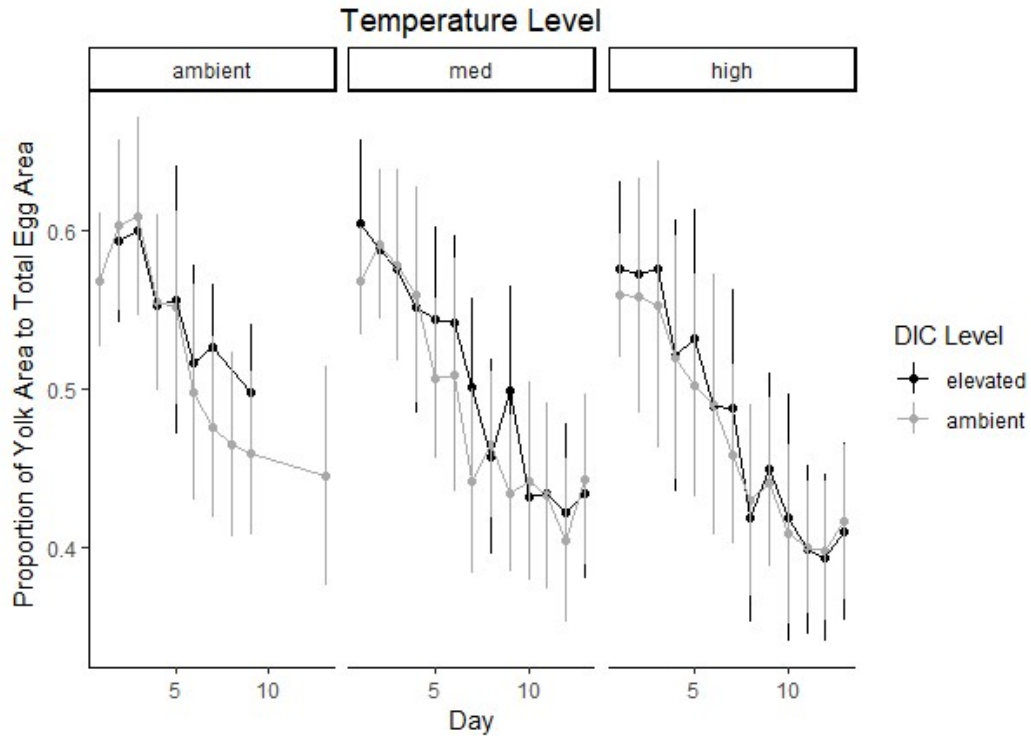


Figure 2: Proportion of yolk area to total egg area (Y:E) of surf smelt embryos (n=216/day) that were submerged in treatment water for 13 days. Each point is an average measurement representative of the temperature treatment, DIC treatment, and day with standard deviation shown as whiskers.

Effects of Climate Change on Larvae Yolk Absorption

Larval yolk size decreased over time in all treatments (Figure 3). Temperature had a significant, negative effect on larval yolk size throughout the duration of the experiment (Table 4; Figure 4) and for every 1°C increase in temperature, the average larval yolk size decreased by 0.011 mm² (Table 4). The average size of larval yolk sacs decreased by 27.1% in the ambient temperature basins and 49.8% in the high temperature basins over the duration of the experiment. The average larval yolk size in high temperature treatments was 32.5% smaller than average larval yolk size in ambient temperature treatments (Figure 4). Larval yolk size was not significantly affected by DIC treatment level (Table 4; Figure 3).

Table 4: Summary of results of a linear mixed effect model of temperature and DIC level on the size of surf smelt larvae yolk sacs over a 3-day time period. All values are compared to the intercept at the ambient DIC.

	<i>Value</i>	<i>Std. Error</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>
<i>Intercept</i>	0.2841	0.0235	356	12.1089	<0.0001
<i>Temperature</i>	-0.0112	0.0011	40	-10.2221	<0.0001
<i>Elevated DIC</i>	0.0098	0.0039	2	2.5389	0.1264

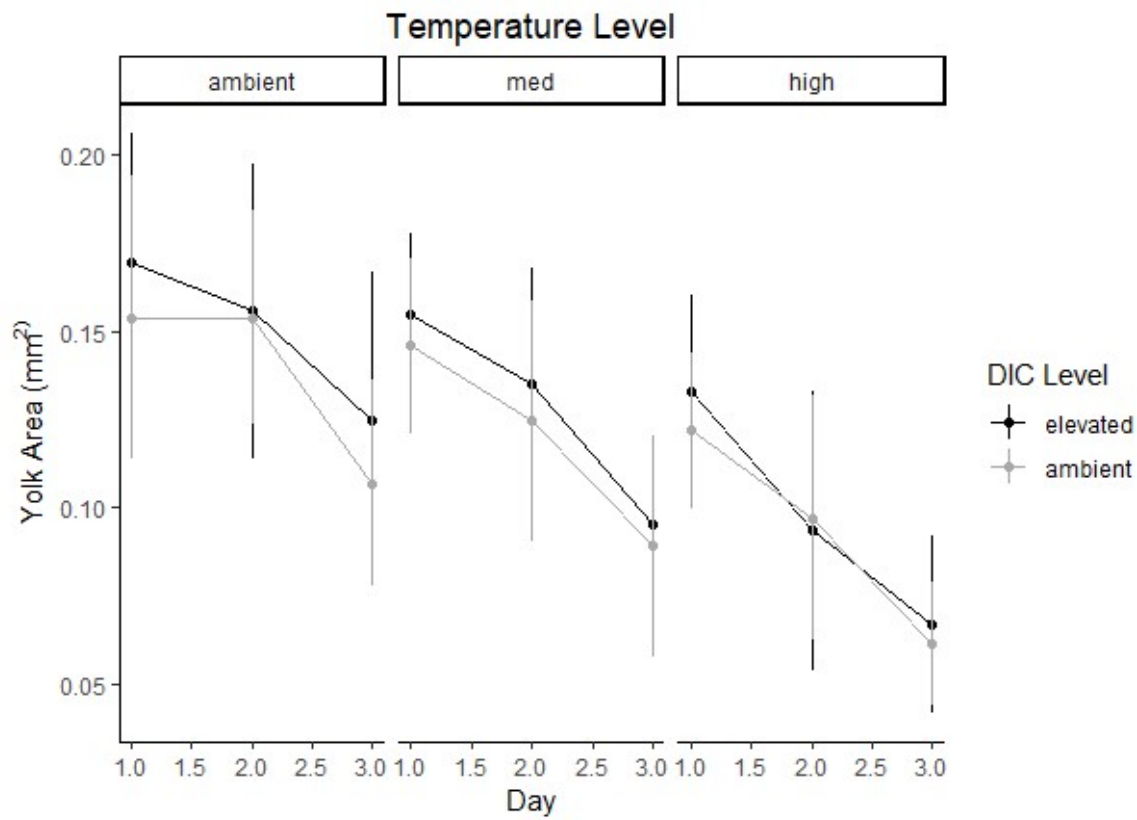


Figure 3: Yolk sac area of surf smelt larvae (n=144/ day) that were submerged in treatment water for 3 days. Data are averages representative of the temperature treatment, DIC treatment, and day with standard deviation shown as whiskers.

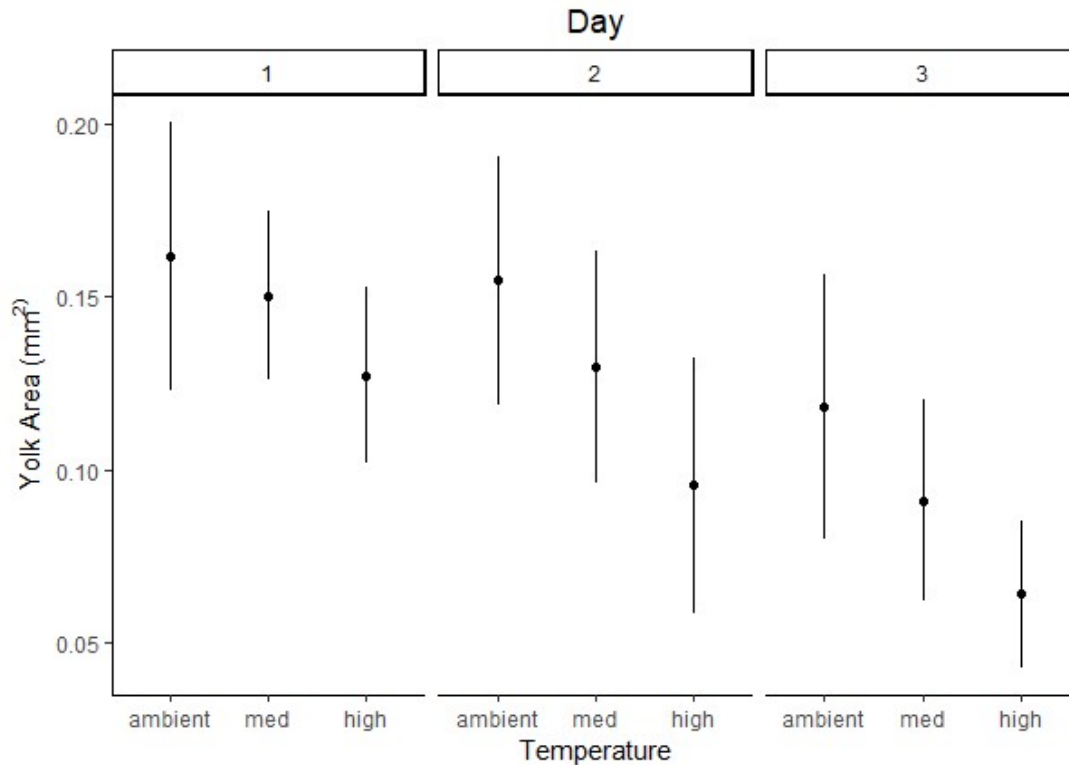


Figure 4: Surf smelt larval yolk sac area (n=144/day) that were submerged in treatment water for 3 days. Data are averages representative of the temperature treatment, with no separation by DIC treatment, and standard deviation shown as whiskers.

Effects of Climate Change on Oil Globule size

Surf smelt oil globule size decreased over time in all treatments (Figure 5). Temperature had a significant negative effect on the size of larval oil globules and for every 1°C increase in temperature, the average oil globule area decreased by 2.1 μm^2 (Figure 6; Table 5). Over the three-day incubation, oil globule size decreased by 25.7% in ambient temperatures compared to 43.2% in high temperature treatments. On average, oil globules in the high temperature treatments were 20.0% smaller than oil globules in the ambient seawater (Figure 6). Treatment DIC level had no significant effect on oil globule size (Table 5).

Table 5: Summary of results of linear mixed effect model examining the effect of temperature and DIC treatment on the size of surf smelt larvae oil globules over a 3-day time period. All values are compared to the intercept at the ambient DIC treatment.

	<i>Value</i>	<i>Std. Error</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>
<i>Intercept</i>	0.0687	0.0068	356	9.9617	<0.0001
<i>Temperature</i>	-0.0021	0.0003	40	-5.8537	<0.0001
<i>Elevated DIC</i>	-0.0002	0.0013	2	-0.1571	0.8896

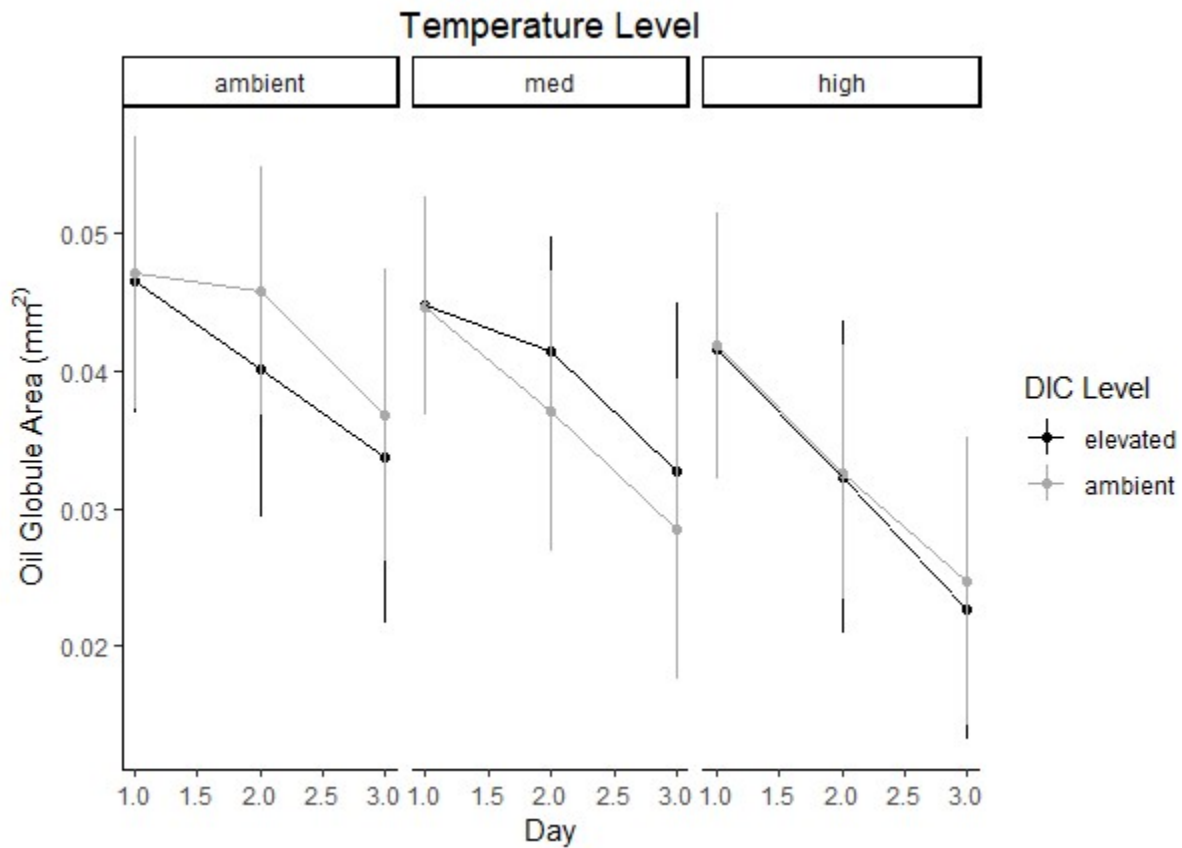


Figure 5: Oil globule area of surf smelt larvae (n=144/day) that were submerged in treatment water for 3 days. Each point is an average measurement representative of the temperature treatment, DIC treatment, and day with standard deviation shown as whiskers.

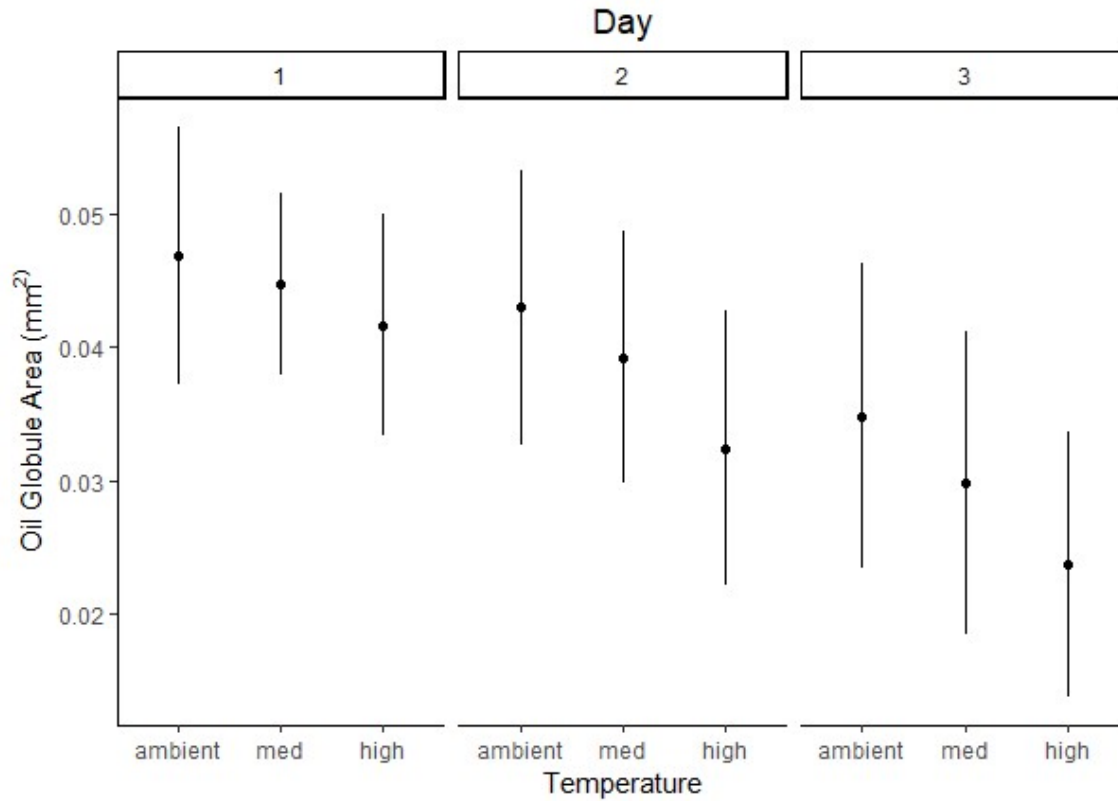


Figure 6: Oil globule area of surf smelt larvae (n=144/day) that were submerged in treatment water for a total of 3 days. Data are averages representative of the temperature treatment with standard deviation shown as whiskers.

Effects on Larval Length

Larval lengths in general increased after three days (Figure 7). Model results found no temperature or DIC effect on larvae length (Table 6).

Table 6: Results of a linear mixed effect model examining the effect of temperature and DIC treatment on the total length (mm) of surf smelt larvae over a 3-day time period. All values are compared to the intercept at the ambient DIC treatment.

	<i>Value</i>	<i>Std. Error</i>	<i>Df</i>	<i>t-value</i>	<i>p-value</i>
<i>Intercept</i>	5.5613	0.3985	316	13.9537	<0.0000
<i>Temperature</i>	0.0052	0.0237	36	0.2189	0.8279
<i>Elevated DIC</i>	0.0054	0.0855	2	0.0631	0.9554

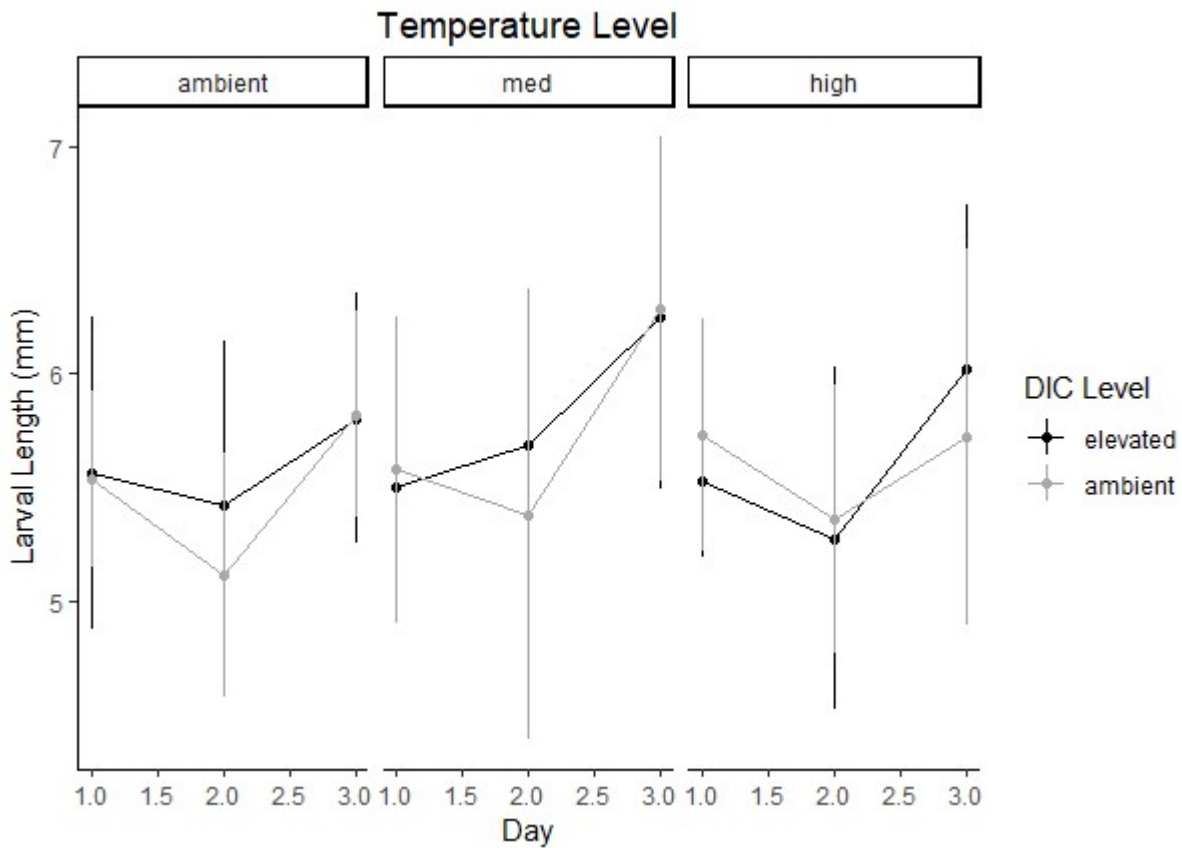


Figure 7: Average larval length of surf smelt larvae (n=144/day) that were immersed in treatment water for 3 days. Each point is an average measurement representative of the temperature treatment, DIC treatment, and day with standard deviation shown as whiskers.

Table 7: Linear mixed effect model results of temperature and DIC level on the heartrate (total beats/10 seconds) of surf smelt embryos at comparable stages of development. All values are compared to the intercept at the ambient DIC treatment.

	<i>Value</i>	<i>Std. Error</i>	<i>df</i>	<i>t-value</i>	<i>p-value</i>
<i>Intercept</i>	18.935	3.8181	62	4.9263	<0.0000
<i>Temperature</i>	-0.4314	0.242	26	-1.7682	0.0867
<i>Elevated DIC</i>	-11.714	5.912	2	-1.981	0.1861
<i>Temp:DIC</i>	0.8224	0.3735	26	2.202	0.0367

Effects on Embryo Heartrate

While embryonic heart rates were not affected by temperature or pH level as singular stressors at comparable stages of development, there was a significant interactive effect of temperature and DIC treatment on heart rates (Table 7; Figure 8). Surf smelt embryo heartrate in ambient temperature treatments remained consistent across DIC treatments with an average percent difference of 3.4% (Figure 8). However, as temperature increased, the average heartrate per DIC treatment diverged, with percent differences of 8.1% and 19.6% for the medium and high temperature treatments, respectively. Embryo heart rate increased by 7.7% between ambient and high temperature treatments when experiencing elevated DIC. Comparatively, embryo heart rates declined by 15.9% between ambient to high temperature treatments when at ambient DIC (Figure 8).

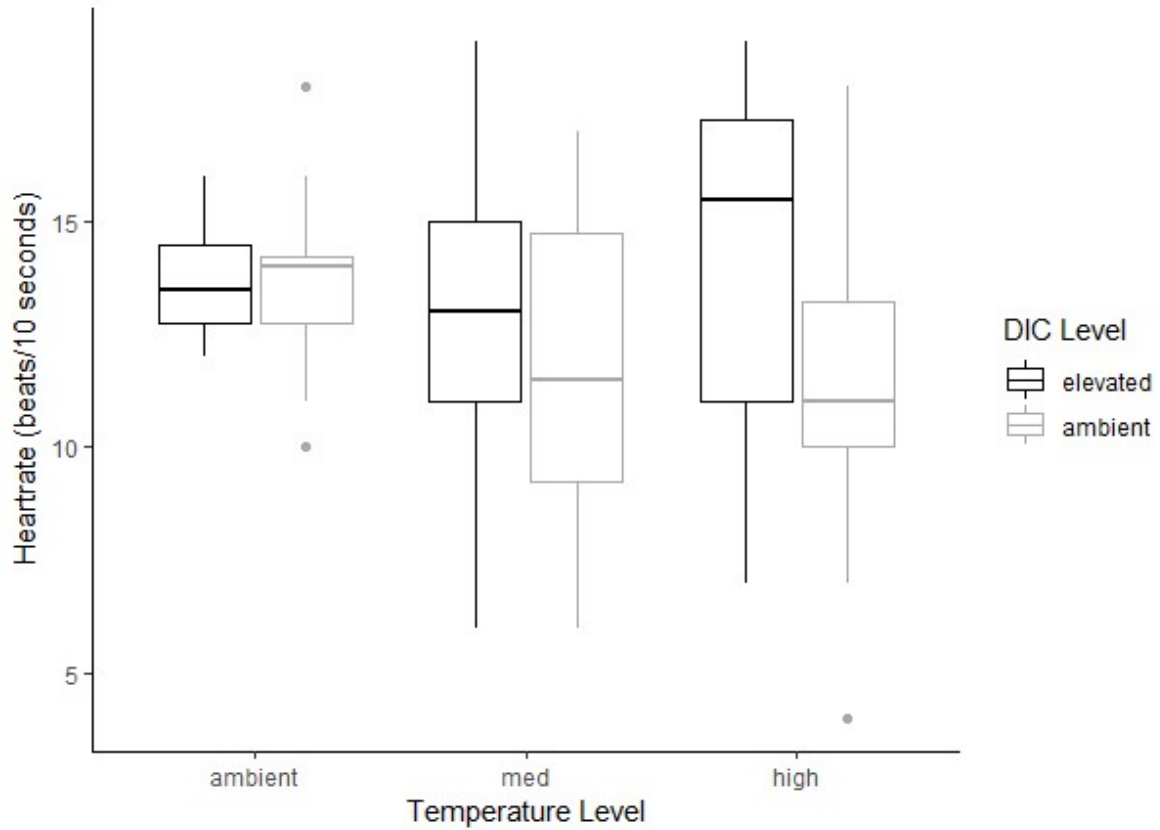


Figure 8. The number of heart beats from embryos (n=96) per ten seconds from each treatment. Whiskers extend from the upper and lower quartiles to 1.5 times the interquartile range. Data outside of this range are shown as points.

DISCUSSION

Elevated Seawater Temperature

Temperature was the primary climate driver affecting early life history stages of surf smelt in this study. Elevated temperatures negatively affected yolk size in both surf smelt embryos and larvae, indicating that elevated temperatures might cause an increase in energy demand in developing surf smelt. The results here agree with findings from numerous other fish species, which showed increased energy usage in response to increasing temperatures (e.g. review by Fukuhara 1990; Flynn et al. 2015), likely in response to the increased cost of metabolism (Rangel and Johnson 2018). Increased energy usage from elevated metabolism results in trade-offs in growth, performance, and reproduction (Burel et al. 1996; Brown et al. 2006; Arnott et al. 2006). One previous study found no difference in energy consumption of developing surf smelt between the temperatures 12°C- 22°C (Garreton 1983). However, there were many differences in the experimental design between the two experiments, including their use of calorimetry as a measure of energy demand, which hinder direct comparisons (Garreton 1983).

In addition to trade-offs in fitness, an increase in energy demand can elevate embryo and larval mortality. Previous research showed that temperature increased mortality in developing surf smelt (Rice 2006; Lee and Levings 2007), concluding that desiccation stress was the primary cause of embryo mortality (Rice 2006). This research suggests an additional mechanism may be, in part, responsible for declines in embryo viability due to elevated temperature. Surf smelt deposit their eggs during high tides typically in +7 or above range, and as such, require an equally high tide after embryo development to trigger hatching (Rice 2006; Penttila 2007). An increase in energy usage could increase development rate and cause earlier hatch that is no

longer synchronized to this tidal cycle, potentially stranding larvae on beaches (Rice 2006). An increase in development rate was not directly observed here, but the only metric to assess that was duration to embryo eye spots darkening. In this study that was generally observed after only 5 days post-fertilization and, as such, likely too soon to observe a treatment difference in developmental rate. Further, using time of hatching to assess development rate was complicated by a lack of viable larvae left in each treatment at hatch age. Regardless, larvae that do survive hatching will have reduced yolk mass, putting them at a disadvantage as larvae by limiting their pre-feeding energy reserves (Stanley 1977). Larval fish are not able to feed directly after hatching, making energy reserves during this time crucial to survival (Alderice and Hourston 1985; Rønnestad et al. 1998).

This research also shows that in addition to embryos, surf smelt larvae also consume their energy reserves more rapidly with increasing temperature, exacerbating the stress on the already limited yolk reserves and decreasing the time they have before they need to begin exogenous feeding (Garreton 1983; Penttila 2007). A limitation to this available time also constrains the distance they can travel as planktonic drifters to feeding sites (Penttila 2007). Not all hatch sites will have high prey availability, and if larvae are unable to find prey patches with appropriately sized organisms and density in this time, they will not be able to survive (Penttila 2007).

In addition to decreased yolk area, a significant decrease in oil globule size with elevated temperature was observed in this study. While fish embryos use free amino acids found in their yolk as their primary energy source prior to hatching, they switch to using fatty acids from their oil globule post hatching (Rønnestad et al. 1998). Thus, the negative effect of temperature on oil globule size could inhibit larval development and survival, ultimately decreasing population

abundance and adult recruitment. This assumption is qualitatively supported by data generated in this study, where spinal deformities in larval surf smelt were observed, though not quantitatively analyzed. Further research is needed to determine if these deformities are directly related to increased temperature and/or oil globule size. The importance of the oil globule in larval fish development was promoted by Berkely et al. (2004), who showed that large oil globule size was highly correlated with larval performance and survival (Berkely et al. 2004). In black rockfish, larvae with the largest oil globules had growth rates up to three times faster and double the survival rate of larvae with the smallest oil globules (Berkely et al. 2004). In rabbit fish, the yolk is exhausted within the first 24 hours post hatching in order to develop a digestive tract, leaving the larvae almost entirely dependent on the oil globule for the duration of larval development prior to initiation of exogenous feeding (Avila and Jaurio 1987). The rapid use of the oil globule in surf smelt experiencing temperature stress may be detrimental to larval performance and survival.

It might be expected that because yolk and oil globule energy reserves were used faster at higher temperatures, there would be resultant variation in larval size across treatments. Although a significant temperature effect on larval yolk utilization was observed, this did not translate to differences in larval length, indicating that elevated yolk utilization was not being used for somatic growth expressed in length. A similar result was observed by Garreton (1983), who found only at 22 °C was surf smelt larval size affected, reducing larval length by on average 23% compared to all other temperature treatments. The stunted growth observed in Garreton (1983) could be due to the reduced energy available for growth from costly metabolic trade-offs (Garreton 1983; Rangel and Johnson 2018). As 22°C is exceptionally warm, and well above the highest temperature treatment (18°C) used in this, this may explain why a temperature effect on

larval length was not observed here. Another study found that temperature significantly impacted larval summer flounder yolk usage, with larvae in high temperatures absorbing yolk faster (Johns and Howell 1980). However, there was again no observed effect on larval size (Johns and Howell 1980). The authors attribute that result to larval fish appearing to have complex physiological mechanisms such as enzymatic adjustment and biochemical restructuring that help them acclimate to temperature change, allowing fish to continue to grow despite temperature stress (Crawshaw 1977; Johns and Howell 1980; Kubb et al. 1980; Garreton 1983). Supporting this notion, Hochachka and Lewis (1969) demonstrated that cold and warm water acclimated trout used two kinetically distinguishable versions of the enzyme citrate synthase to reduce temperature sensitivity of the enzymatic reaction. Similar mechanisms could be present in developing surf smelt that would explain their stability in size across variable temperatures.

Ocean Acidification

Surf smelt embryo and larval yolk and oil globule size were not directly affected by variation in pCO₂ and DIC. It is important to note that the pCO₂ levels in these experiments were higher than was anticipated due to variation in source water for these parameters. Although testing surf smelt tolerance to average global ocean pCO₂ levels (~400 µatm) was not achieved in this study, this research tested moderate pCO₂ similar to model predictions for pCO₂ in the Puget Sound by the year 2100, as well high pCO₂ levels currently observed in seasonally upwelled water into the Puget Sound (Feely et al. 2010; Mauger et al. 2015).

The absence of a DIC treatment effect in this study does not support the hypothesis that juvenile fish are ineffective acid-base regulators (reviewed in Kikkawa et al. 2003). Although acid-base regulation has not been definitively demonstrated in surf smelt, there are many

possible pathways that they might utilize to alleviate stress from internal acidosis. For example, Strobel et al. (2012) found that Antarctic notothenioid fish can compensate for long-term high pCO₂ exposure through the intracellular accumulation of bicarbonate ions throughout their tissues. Additionally, there is evidence that fish can establish new metabolic equilibria under high pCO₂ conditions allowing them to meet the energy demand required for acid-base regulation (Strobel et al. 2012; Esbaugh 2018).

The absence of a DIC treatment effect on the surf smelt metrics measured here could also result from the unique spawning behavior of surf smelt. Exposed to both air and seawater throughout their development (Penttila 2007), it would follow that surf smelt embryos would have adaptations to survive in highly variable conditions. In general, marine fish that spawn in the intertidal possess broad tolerance to environmental variability, which allows them to spawn and develop in highly dynamic environments (Demartini 1999; Snyder and Martin 2002; Rice 2006). Recent research on Pacific herring found that embryo and larval yolk supplies were not affected by pCO₂ as a singular stressor (Villalobos et al. 2018). While research on how OA affects other forage fish in the Puget Sound is limited, one can look at related species for clues. Elevated pCO₂ was found to have no effect on Atlantic herring larvae in terms of total length, dry weight, and yolk sac area (Franke and Clemmesen 2011). However, Atlantic herring larvae showed increased tissue damage and decreased growth and development rates under acidic conditions (Frommel et al. 2014). Atlantic herring spawn in locations that allow their embryos to be submerged for the duration of development (Haegeler and Schweigert 1985), making their embryos potentially more susceptible to changes in pCO₂. As this was not observed here, it is reasonable to assume that developing surf smelt have adapted robustness to changes in pCO₂.

The results presented here agree with numerous other studies that found no effect of OA on various metrics of fitness in marine fish (e.g. Munday et al. 2009; Frommel et al. 2013; Esbaugh 2018). A review of the effects of OA on marine fish metabolic rate found that 50% of studies showed no detrimental impact, whereas 21% found a consistent decline in metabolic rate in response to OA (Esbaugh 2018). Congruent with no change in energy demand, larval length was unaffected by elevated pCO₂ and DIC treatment level in this study. Larval growth is another fitness metric that does not appear to be consistently affected by pCO₂ with studies showing negative, positive, and no effect of OA (Munday et al. 2009; Frommel et al. 2016; review by Esbaugh 2018). The lack of consistent response in marine fish to OA may be attributed to differences in experimental design, including larval densities within treatments, duration of experiments, and food availability (Esbaugh 2018). In addition to experimental differences, there are species specific and parental effects that play a role in environmental tolerances (Miller et al. 2012; Esbaugh 2018), thus limiting our ability to generalize about fish tolerance to ocean acidification.

While elevated DIC did not affect surf smelt as a lone stressor, the interaction with temperature caused an increase in embryo heartrate. Surf smelt embryos experiencing increased temperature stress alone exhibited a decline in embryo heartrate. Decreased heartrate with increasing temperature has been demonstrated in other species of cold-water fish (Aho and Vornanen 2001), as well as the ability for fish to maintain heartrate independent of temperature change (Franklin et al. 2001). While not always linked to metabolic rate, elevated heart rates nonetheless are associated with an increase in the consumption of stored energy (Reviewed in Thorarensen et al. 1996). As such, this is another mechanism by which climate co-stressors may elevate surf smelt energy demands. Results obtained here agree with other studies on the

interactive effects of temperature and OA stress on juvenile fish. Antarctic rockfish did not respond to single climate stressors yet increased their metabolic rate and ventilation in response to combined acidification and temperature stress (Davis et al. 2018). Pacific herring showed increased embryo mortality in response to the temperature:pCO₂ interaction, and heart rates were significantly elevated in the highest temperature:pCO₂ treatment compared to all other treatments (Villalobos et al. 2018). A candidate mechanism for this response is that temperature can alter an organism's ability to acclimate to high pCO₂. For example, Enzor et al. (2013) found that while species of rockcod were able to acclimate to high pCO₂, determined by way of resumption of respiration rates to control levels, the addition of temperature stress significantly extended acclimation time, most likely due to energetic trade-offs between metabolism and other cellular functions. Altering the ability of larval fish to effectively acclimate may increase the energy needed to maintain internal acid-base homeostasis (Strobel et al. 2013). It will also inherently affect an organism's aerobic scope, i.e. the total energy available for reproduction, growth, and performance (Clarke et al. 2013; Esbaugh 2018; Rangel and Johnson 2018).

Ecological Implications

In the Puget Sound, where surf smelt spawn over broad spatial scales, approximately one third of the coastline has been modified by humans (PSWQAT 2002). Human activities and development alter surf smelt spawning grounds by reducing nearshore vegetation and the shading it provides, causing a significant increase in beach gravel temperature and concurrent decreases in gravel humidity (Rice 2006). A survey of modified and natural beaches in the Puget Sound found that modified beaches had an average substrate temperature of 18.8°C, 4.7°C higher than the average temperature of natural beaches (Rice 2006), and a temperature known to cause increased surf smelt embryo mortality (Rice 2006). The predicted temperature increase

caused by shoreline modification is represented by the high temperature treatment (18°C) in this study. Both surf smelt embryos and larvae utilized significantly more energy in the high temperature treatment than in the ambient treatment, potentially leading to deleterious effects on surf smelt development and the timing of hatching. Furthermore, larvae that can successfully hatch will likely have less energy reserves and decreased fitness in an already vulnerable life stage, adding to the increased surf smelt mortality from desiccation. The high number of already developed beaches in the Puget Sound poses an immediate threat as surf smelt rely on a relatively small proportion of beaches for spawning each year, and disturbance to any of them could have major population implications (Quinn et al. 2012). This emphasizes the need for careful planning when it comes to future shoreline development projects, and habitat restoration efforts for spawning sites already heavily altered.

In addition to urbanization and its effect on shoreline integrity, findings here suggest that global climate change will negatively affect surf smelt development. Climate models predict that Puget Sound seawater temperatures will increase by approximately 1.2 °C by the year 2040 and will decrease in pH by 0.14 - 0.32 by the year 2100 (Mauger et al. 2015). The experimental design in this study captured this expected range in temperature and pH and extended beyond these ranges to more extreme endpoints. The medium temperature condition in this experiment was approximately 1.5 - 2.0 °C higher than ambient seawater depending on the time of day. On average, larval yolk size in the medium temperature group was 12.1% smaller than larval yolk size in the ambient temperature treatment. Larval yolk size in the high temperature (18°C) was on average 32.5% smaller than larvae in the ambient treatment, more than double the effect of the medium treatment. This indicates that small temperature changes that we will see by 2040 will increase surf smelt energy demand, an effect that will only magnify as temperatures

continue to rise. Given the estimated temperature increase, surf smelt will start experiencing temperature stress in this century.

Methodological constraints do limit the extent that these findings can be extrapolated to ecological implications. Specifically, surf smelt here were not raised under periodic exposure to air and wave disturbance. Because of that, future studies could build on these results by including site based ecological studies monitoring surf smelt development across known spawning sites where environmental parameters vary. In addition to these field surveys, future laboratory experiments would improve upon the information provided here by rearing surf smelt larvae in the lab for a longer time period, as this experiment ended after only four days of larval incubation due to high mortality across treatments. With a longer experiment, larval deformities and developmental rates could be more closely monitored and assessed. It is also important to note that mortality was not quantitatively measured in these experiments due to time constraints and the prioritizing of daily yolk measurements.

Conclusions

This study highlights the importance and urgency of including surf smelt in ecological and climate change research given the evidence provided here that surf smelt will be negatively affected by stressors associated with near-present climate change and under current nearshore urban development. Despite this, they remain one of the truly understudied forage fish in the north Pacific ecosystem. Given their keystone role in marine ecosystems, reductions to forage fish fitness and population abundance has the potential to cause ecosystem-wide effects. For example, ecosystem models show that Puget Sound seabird populations are negatively affected by reduction in forage fish biomass, with a substantial impact on juvenile seabirds (Therriault et al. 2009; Busch et al. 2013). This trend could also extend to other functional groups that rely on

forage fish such as piscivorous fish and marine mammals, potentially leading to trophic cascades (Penttila 2007; Therriault et al. 2009). Commercial and recreational fisheries would also experience a major loss from the decline in surf smelt populations (Essington et al. 2016). The continuation of ecosystem-based management of surf smelt populations is recommended given that their population success is so intimately linked with the health of the ecosystem. The critical role of surf smelt in marine ecosystems and as an economic resource makes their presence for future generations invaluable.

WORKS CITED

- Aho, E. and M. Vornanen. "Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (*Oncorhynchus mykiss*)." *Journal of Comparative Physiology B* 171 (2001): 173-179.
- Alder, J., B. Campbell, V. Karpouzi, K. Kaschner, and D. Pauly. "Forage Fish: From Ecosystems to Markets." *Annual Review of Environment and Resources* 33, no. 1 (2008): 153–66.
- Alderdice, D. F., and A. S. Hourston. "Factors Influencing Development and Survival of Pacific Herring (*Clupea harengus pallasii*) Eggs and Larvae to Beginning of Exogenous Feeding." *Canadian Journal of Fisheries and Aquatic Sciences* 42, no. S1 (1985): s56–s68.
- Alderdice, P., and F. Velsen. "Some Effects of Salinity and Temperature on Early Development of Pacific Herring (*Clupea pallasii*)." *Journal of the Fisheries Research Board of Canada* 28, no. 10 (1971): 1545–1562.
- Arnott, Stephen A., Susumu Chiba, and David O. Conover. "Evolution of Intrinsic Growth Rate: Metabolic Costs Drive Trade-Offs Between Growth and Swimming Performance in *Menidia menidia*." *Evolution* 60, no. 6 (2006): 1269–78.
- Avila, E. M., and J. V. Juario. "Yolk and Oil Globule Utilization and Developmental Morphology of the Digestive Tract Epithelium in Larval Rabbitfish, *Siganus guttatus* (Bloch)." *Aquaculture* 65, no. 3 (1987): 319–31.
- Berkeley, Steven A., Colin Chapman, and Susan M. Sogard. "Maternal Age as a Determinant of Larval Growth and Survival in a Marine Fish, *Sebastes melanops*." *Ecology* 85, no. 5 (2004): 1258–64.
- Brown, N. P., R. J. Shields, and N. R. Bromage. "The Influence of Water Temperature on Spawning Patterns and Egg Quality in the Atlantic Halibut (*Hippoglossus hippoglossus*)." *Aquaculture* 261, no. 3 (2006): 993–1002.
- Burel, C., J. Person-Le Ruyet, F. Gaumet, A. Le Roux, A. Sévère, and G. Boeuf. "Effects of Temperature on Growth and Metabolism in Juvenile Turbot." *Journal of Fish Biology* 49, no. 4 (1996): 678–92.
- Busch, D. Shallin, Chris J. Harvey, and Paul McElhany. "Potential Impacts of Ocean Acidification on the Puget Sound Food Web." *ICES Journal of Marine Science* 70, no. 4 (2013): 823–33.
- Clark, Donald S., and John M. Green. "Seasonal Variation in Temperature Preference of Juvenile Atlantic Cod (*Gadus morhua*), with Evidence Supporting an Energetic Basis for Their Diel Vertical Migration." *Canadian Journal of Zoology* 69, no. 5 (1991): 1302–7.

Clark, Timothy D., Erik Sandblom, and Fredrik Jutfelt. "Aerobic Scope Measurements of Fishes in an Era of Climate Change: Respirometry, Relevance and Recommendations." *Journal of Experimental Biology* 216, no. 15 (2013): 2771–82.

Crawshaw, L. I. "Physiological and Behavioral Reactions of Fishes to Temperature Change." *Journal of the Fisheries Research Board of Canada* 34, no. 5 (1977): 730–34.

Davis, B. E., E. E. Flynn, N. A. Miller, F. A. Nelson, N. A. Fanguie, and A. E. Todgham. "Antarctic Emerald Rockcod Have the Capacity to Compensate for Warming When Uncoupled from CO₂-Acidification." *Global Change Biology* 24, no. 2 (2018): e655–70.

DeMartini, E. E. "Intertidal spawning." *Intertidal fishes: life in two worlds* (1999): 143-164.

Enzor, L. A., M. L. Zippay, and S. P. Place. "High Latitude Fish in a High CO₂ World: Synergistic Effects of Elevated Temperature and Carbon Dioxide on the Metabolic Rates of Antarctic Notothenioids." *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 164, no. 1 (2013): 154–61.

Esbaugh, Andrew J. "Physiological Implications of Ocean Acidification for Marine Fish: Emerging Patterns and New Insights." *Journal of Comparative Physiology B* 188, no. 1 (2018): 1–13.

Essington, T.E. P. E. Moriarty, H. E. Froehlich, E.E. Hodgson, L. E. Koehn, Kiva L. Oken, Margaret C. Siple, and Christine C. Stawitz. "Fishing Amplifies Forage Fish Population Collapses." *Proceedings of the National Academy of Sciences* 112, no. 21 (2015): 6648–52.

Feely, R. A., S. R. Alin, J. Newton, C. L. Sabine, M. Warner, A. Devol, C. Krembs, and C. Maloy. "The Combined Effects of Ocean Acidification, Mixing, and Respiration on PH and Carbonate Saturation in an Urbanized Estuary." *Estuarine, Coastal and Shelf Science* 88, no. 4 (2010): 442–49.

Flynn, E.E., B. E. Bjelde, N. A. Miller, and A.E. Todgham. "Ocean Acidification Exerts Negative Effects during Warming Conditions in a Developing Antarctic Fish." *Conservation Physiology* 3, no. 1 (2015): cov33.

Form, A. U., and U. Riebesell. "Acclimation to Ocean Acidification during Long-Term CO₂ Exposure in the Cold-Water Coral *Lophelia pertusa*." *Global Change Biology* 18, no. 3 (2012): 843–53.

Franke, A., and C. Clemmesen, "Effect of Ocean Acidification on Early Life Stages of Atlantic Herring (*Clupea harengus*)." *Biogeosciences* 8, (2011): 3697-3707.

Franklin, C., M. Axelsson, and W. Davison. "Constancy and control of heart rate during an increase in temperature in the Antarctic fish *Pagothenia borchgrevinki*" *Experimental Biology Online* 6, (2001): 1-8.

Frommel, A. Y., A. Schubert, U. Piatkowski, and C. Clemmesen. “Egg and Early Larval Stages of Baltic Cod, *Gadus morhua*, Are Robust to High Levels of Ocean Acidification.” *Marine Biology* 160, no. 8 (2013): 1825–34.

Frommel, A. Y., R. Maneja, D. Lowe, C. K. Pascoe, A. J. Geffen, A. Folkvord, U. Piatkowski, and C. Clemmensen. “Organ Damage in Atlantic Herring Larvae as a Result of Ocean Acidification.” *Ecological applications* 24, no. 5 (2014): 1131-1143.

Frommel, A. Y., D. Margulies, J. B. Wexler, M. S. Stein, V. P. Scholey, J. E. Williamson, D. Bromhead, S. Nicol, and J. Havenhand. “Ocean Acidification Has Lethal and Sub-Lethal Effects on Larval Development of Yellowfin Tuna, *Thunnus albacares*.” *Journal of Experimental Marine Biology and Ecology* 482 (2016): 18–24.

Fukuhara, O. “Effects of Temperature on Yolk Utilization, Initial Growth, and Behaviour of Unfed Marine Fish-Larvae.” *Marine Biology* 106, no. 2 (1990): 169–174.

Garreton, Marta S. *Bioenergetics of Yolk Utilization in Embryos and Yolk-Sac Larvae of the Surf-Smelt Hypomesus pretiosus (Girard, 1855) under Different Incubation Temperatures*. Oregon State University, 1983.

Greene, C., L. Kuehne, C. Rice, K. Fresh, and D. Penttila. “Forty Years of Change in Forage Fish and Jellyfish Abundance across Greater Puget Sound, Washington (USA): Anthropogenic and Climate Associations.” *Marine Ecology Progress Series* 525 (2015): 153–170.

Haegle, C. W., and J. F. Schweigert. “Distribution and Characteristics of Herring Spawning Grounds and Description of Spawning Behavior.” *Canadian Journal of Fisheries and Aquatic Sciences* 42, no. S1 (1985): s39–55.

Hay, D. E. “Reproductive Biology of Pacific Herring (*Clupea harengus pallasii*).” *Canadian Journal of Fisheries and Aquatic Sciences* 42, no. S1 (1985): s111–s126.

Heuer, R. M., and M. Grosell. “Physiological Impacts of Elevated Carbon Dioxide and Ocean Acidification on Fish.” *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 307, no. 9 (1): R1061–R1084.

Hochachka, P. W., and J. K. Lewis. “Enzyme Variants in Thermal Acclimation Trout Liver Citrate Synthases.” *Journal of Biological Chemistry* 245, no. 24 (1970): 6567–73.

Houde, E. D. “Emerging from Hjort’s Shadow.” *Journal of Northwest Atlantic Fishery Science* 41 (2008): 53–70.

IPCC. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, (2014): 151 pp.

- Johns, D., and W. Howell. "Yolk Utilization in Summer Flounder (*Paralichthys dentatus*) Embryos and Larvae Reared at Two Temperatures." *Marine Ecology Progress Series* 2 (1980): 1–8.
- Jokiel, Paul L., Keisha D. Bahr, and Ku'ulei S. Rodgers. "Low-Cost, High-Flow Mesocosm System for Simulating Ocean Acidification with CO₂ Gas: Ocean Acidification System." *Limnology and Oceanography: Methods* 12, no. 5 (2014): 313–22.
- Kamler, Ewa. "Ontogeny of Yolk-Feeding Fish: An Ecological Perspective." *Reviews in Fish Biology and Fisheries* 12, no. 1 (2002): 79–103.
- Khangaonkar, T., A. Nugraha, W. Xu, and K. Balaguru. "Salish Sea Response to Global Climate Change, Sea Level Rise, and Future Nutrient Loads." *Journal of Geophysical Research: Oceans* 124, no. 6 (2019): 3876–3904.
- Kikkawa, T., A. Ishimatsu, and J. Kita. "Acute CO₂ Tolerance during the Early Developmental Stages of Four Marine Teleosts." *Environmental Toxicology* 18, no. 6 (2003): 375–382.
- Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajo, G. S. Singh, C. M. Duarte, and J. P. Gattuso. "Impacts of Ocean Acidification on Marine Organisms: Quantifying Sensitivities and Interaction with Warming." *Global Change Biology* 19, no. 6 (2013): 1884–96.
- Kubb, R. N., J. R. Spotila, and D. R. Pendergast. "Mechanisms of Heat Transfer and Time-Dependent Modeling of Body Temperatures in the Largemouth Bass (*Micropterus salmoides*)." *Physiological Zoology* 53, no. 2 (1980): 222–239.
- Lee, C. G., and C. D. Levings. "The Effects of Temperature and Desiccation on Surf Smelt (*Hypomesus pretiosus*) Embryo Development and Hatching Success: Preliminary Field and Laboratory Observations." *Northwest Science* 81, no. 2 (2007): 166–171.
- Lewis, E., and D. W. R. Wallace. 1998. Program Developed for CO₂ System Calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.
- Letcher, Trevor M. *Climate Change: Observed Impacts on Planet Earth*. Elsevier, (2015).
- Lindgren, M., M. Van Deurs, B.R. MacKenzie, L.W. Clausen, A. Christensen, and A. Rindorf. "Productivity and Recovery of Forage Fish under Climate Change and Fishing: North Sea Sandeel as a Case Study" *Fisheries Oceanography* 27, no. 3 (2018).
- Loosanoff, V. L. "The spawning run of Pacific surf smelt, *Hypomesus pretiosus* (Girard)". *Internat'l Revue der gesamter Hydrobiologie und Hydrographie* 36, 1-2 (1937): 170-1 83.

Lowry, D., K. Stick, A. Lindquist, and Y. W. Cheng. "Evaluation of Creel Survey Methods to Estimate Recreational Harvest of Surf Smelt in Puget Sound, Washington." *North American Journal of Fisheries Management* 35, no. 3 (2015): 403–17.

Lynch, A. J., B. J. E. Myers, C. Chu, L. A. Eby, J. A. Falke, R. P. Kovach, T. J. Krabbenhoft. "Climate Change Effects on North American Inland Fish Populations and Assemblages." *Fisheries* 41, no. 7 (2016): 346–61.

Mauger, G.S., J. H. Casola, H.A. Morgan, R.L. Strauch, B. Jones, B. Curry, T.M. Busch Isaksen, L. Whitely Binder, M.B. Krosby, and A.K. Snover. "State of Knowledge: Climate Change in Puget Sound. Report prepared for the Puget Sound Partnership and the National Oceanic and Atmospheric Administration." Climate Impacts Group, University of Washington, Seattle. (2015).

Massee, K. C., M. B. Rust, R. W. Hardy, and R. R. Stickney. "The Effectiveness of Tricaine, Quinaldine Sulfate and Metomidate as Anesthetics for Larval Fish." *Aquaculture* 134, no. 3 (1995): 351–59.

Middaugh, D., M. Hemmer, and D. Penttila. "Embryo Ecology of the Pacific Surf Smelt, *Hypomesus pretiosus* (Pisces: Osmeridae)." *Pacific Science* 41, no. 1–4 (1987): 44–53.

Miller, G. M., S. Watson, J. M. Donelson, M. I. McCormick, and P. L. Munday. "Parental Environment Mediates Impacts of Increased Carbon Dioxide on a Coral Reef Fish." *Nature Climate Change* 2, no. 12 (2012): 858–61.

Millero, F. J., T. B. Graham, F. Huang, H. Bustos-Serrano, and D. Pierrot. "Dissociation Constants of Carbonic Acid in Seawater as a Function of Salinity and Temperature." *Marine Chemistry* 100, no. 1 (2006): 80–94.

Mortensen, A., O. Ugedal, and F. Lund. "Seasonal Variation in the Temperature Preference of Arctic Charr (*Salvelinus alpinus*)." *Journal of Thermal Biology* 32, no. 6 (2007): 314–20.

Munday, P. L., J. M. Donelson, D. L. Dixson, and G. K. Endo. "Effects of Ocean Acidification on the Early Life History of a Tropical Marine Fish." *Proceedings of the Royal Society B: Biological Sciences* 276, no. 1671 (2009): 3275–83.

Nelson, J. A. "Physiological Observations on Developing Rainbow Trout, *Salmo gairdneri* (Richardson), Exposed to Low PH and Varied Calcium Ion Concentrations." *Journal of Fish Biology* 20, no. 3 (1982): 359–72.

Newton, J. A., Constance Amanda Sullivan, Terrie Klinger, Richard A. Feely, Michael O'Donnell, Jude K. Apple, and Salish Sea Ecosystem Conference Seattle. "Carbon Chemistry Observations in the Salish Sea: Evidence for Upwelling Influence and Implications of Sills for

Ocean Acidification Effects.” Digital content made available by University Archives, Heritage Resources, Western Libraries, Western Washington University, 2014.

Parker, D. B., and B. A. Mckeown. “The Effects of Low PH on Egg and Alevin Survival of Kokanee and Sockeye Salmon, *Oncorhynchus nerka*.” *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* 87, no. 2 (1987): 259–68.

Penttila, Dan. “Marine Forage Fishes in Puget Sound:” Fort Belvoir, VA: Defense Technical Information Center, March 1, 2007.

Penttila, D. E., and L.L. Moulton. “Field Manual for Sampling Forage Fish Spawn In Intertidal Shore Regions” 2001.

Peterson, R. H., P. G. Daye, and J. L. Metcalfe. “Inhibition of Atlantic Salmon (*Salmo salar*) Hatching at Low PH.” *Canadian Journal of Fisheries and Aquatic Sciences* 37, no. 5 (1980): 770–74.

Pimentel, M. S., F. Faleiro, G. Dionísio, T. Repolho, P. Pousão-Ferreira, J. Machado, and R. Rosa. “Defective Skeletogenesis and Oversized Otoliths in Fish Early Stages in a Changing Ocean.” *The Journal of Experimental Biology* 217, no. 12 (2014): 2062–2070.

Pörtner, H. O., and R. Knust. “Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance.” *Science* 315, no. 5808 (2007): 95–97.

Puget Sound Water Quality Action Team (PSWQAT). “2002 Puget Sound Update: Eighth Report of the Puget Sound” Ambient Monitoring Program. Puget Sound Water Quality Action Team. Olympia, Washington. (2002).

Quinn, T., K. Krueger, K. Pierce, D. Penttila, K. Perry, T. Hicks, and D. Lowry. “Patterns of Surf Smelt, *Hypomesus pretiosus*, Intertidal Spawning Habitat Use in Puget Sound, Washington State.” *Estuaries and Coasts* 35, no. 5 (2012): 1214–1228.

Rangel, R. E., and D. W. Johnson. “Metabolic Responses to Temperature in a Sedentary Reef Fish, the Bluebanded Goby (*Lythrypnus dalli*, Gilbert).” *Journal of Experimental Marine Biology and Ecology* 501 (2018): 83–89.

Reum, J., T. Essington, C. Greene, C. Rice, and K. Fresh. “Multiscale Influence of Climate on Estuarine Populations of Forage Fish: The Role of Coastal Upwelling, Freshwater Flow and Temperature.” *Marine Ecology Progress Series* 425 (2011): 203–15.

Rice, Casimir. “Effects of Shoreline Modification on a Northern Puget Sound Beach: Microclimate and Embryo Mortality in Surt Smelt (*Hypomesus pretiosus*).” *Estuaries and Coasts* 29, no. 1 (2006): 63–71.

- Rønnestad, I., W. Koven, A. Tandler, M. Harel, and H. J. Fyhn. "Utilisation of Yolk Fuels in Developing Eggs and Larvae of European Sea Bass (*Dicentrarchus labrax*)." *Aquaculture* 162, no. 1 (1998): 157–70.
- Sandell, T. Lindquist, A.P., Dionne, P., and D. Lowry. "2016 Washington State Herring Stock Status Report." *Species Status Reports*, (2019).
- Schweigert, J.F. 1995. "Environmental effects on long-term population dynamics and recruitment to Pacific herring (*Clupea pallasii*) populations in southern British Columbia." In R.J. Beamish [ed.] *Climate change and northern fish populations. Can. Spec. Publ. Fish. Aquat. Sci.* 121 (1995): 569-583.
- Smyder, E. A., and K. L M. Martin. "Temperature Effects on Egg Survival and Hatching during the Extended Incubation Period of California Grunion, *Leuresthes tenuis*." *Copeia* 2002, no. 2 (2002): 313–20.
- Stanley, R. D. "Effect of Egg Size on Size and Viability of Newly Hatched Medaka (*Oryzias latipes*) and Surf Smelt (*Hypomesus pretiosus pretiosus*)." The University of British Columbia, 1977.
- Strobel, A., S. Bennecke, E. Leo, K. Mintenbeck, H. O. Pörtner, and F. C. Mark. "Metabolic Shifts in the Antarctic Fish *Notothenia rossii* in Response to Rising Temperature and P CO₂." *Frontiers in Zoology* 9, no. 1 (2012): 28.
- Strobel, A., M. Graeve, H. O. Poertner, and F. C. Mark. "Mitochondrial Acclimation Capacities to Ocean Warming and Acidification Are Limited in the Antarctic Nototheniid Fish, *Notothenia rossii* and *Lepidonotothen squamifrons*." *PLOS ONE* 8, no. 7 (2013): e68865.
- Therriault, T. W., D. E. Hay, and J. F. Schweigert. "Biological Overview and Trends in Pelagic Forage Fish Abundance in the Salish Sea (Strait of Georgia, British Columbia)," *Marine Ornithology*, no. 37 (2009): 3–8.
- Thorarensen, H., P. E. Gallagher, and A. P. Farrell. "The Limitations of Heart Rate as a Predictor of Metabolic Rate in Fish." *Journal of Fish Biology* 49, no. 2 (1996): 226–36.
- Villalobos, C., B. Love, B. Olson. "Interactive effects of Ocean Acidification and Warming on Pacific Herring (*Clupea pallasii*) early life stages." *Salish Sea Ecosystem Conference*, (2018), Seattle Washington.
- Walther, G., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. "Ecological Responses to Recent Climate Change." *Nature* 416, no. 6879 (2002): 389–95.

Williams, E. H., and T. J. Quinn. "Pacific Herring, *Clupea pallasii*, Recruitment in the Bering Sea and North-East Pacific Ocean, II: Relationships to Environmental Variables and Implications for Forecasting." *Fisheries Oceanography* 9, no. 4 (2000): 300–315.