SPAWNING MIGRATION CHARACTERISTICS AND ECOLOGY OF EULACHON
(Thaleichthys pacificus)

By

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Abstract

Eulachon *Thaleichthys pacificus* has experienced dramatic reductions in their distribution and abundance along the west coast of North America. This prompted the listing of this species as “Threatened” under the U.S. Endangered Species Act (ESA) of 1973 for populations found in the southern portions of their range, but not in Alaska. Key gaps in knowledge of Eulachon spawning ecology exist that impede population monitoring efforts and habitat protection. Currently, many monitoring efforts ignore estuaries as possible spawning habitat leading to inaccurate estimates of population abundance and trends. Furthermore, estuaries are not designated as critical spawning habitat for Eulachon. This is important because a critical spawning habitat designation under the ESA provides for a regulatory framework on which to focus conservation and restoration efforts for some of the most imperiled aquatic habitats in North America. I hypothesized that Eulachon spawn in estuaries based on limited observations in other research and the close phylogenetic relationship between Eulachon and other smelts (Osmeridae) that can tolerate salinity. To test this hypothesis, I first studied the effects of salinity on the fertilization and hatching success of Eulachon in a laboratory setting to determine salinity tolerance. Second, I investigated estuary spawning in the Twentymile and Antler rivers, Alaska using radio telemetry and substrate surveys to confirm spawning areas. Third, I examined the relationship between adult spawning run intensity and the environmental variables of tide height, water discharge, and day or night to better inform future population monitoring efforts. My findings in the laboratory indicated that Eulachon can fertilize eggs and produce viable offspring in brackish water. These results were confirmed by egg areas observed in the estuaries of the Twentymile and Antler rivers. Furthermore, spawning run intensity increased in association with spring tides, but there were no clear relationships between spawning run
strength and freshwater discharge or day and night. Based on the results of my work, I recommend changes to population monitoring study design and designation of critical spawning habitat to include estuaries. Future research should focus on determining the lower limits of Eulachon spawning habitat to further improve population monitoring and habitat protection.
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CHAPTER ONE

General Introduction

Eulachon *Thaleichthys pacificus* (Richardson 1836) is a smelt from the family Osmeridae that spawns in rivers draining into the Pacific Ocean ranging from northern California to western Alaska (Hubbs 1925, Odemar 1964; Willson et al. 2006). The scientific name stems from Greek words thaleia for rich, ichthys meaning fish, and pacificus for the Pacific Ocean (Hart 1973). This name is appropriate because Eulachon has a very high fat content from 16.7% to 28.9% total body weight (Payne et al. 1999; Iverson et al. 2002; Vollenweider et al. 2011). This high energetic value contributes to its importance as a forage fish for the ecosystem and humans.

Because of its high energetic value and large abundance, Eulachon is important to the ecosystem (Payne et al. 1999; Hay and Boutillier 1999). Marine and estuarine ecosystems are highly influenced by seasonal pulses of resources that can have significant effects on populations and communities (Odum et al. 1995; Womble et al., 2009; Csepp et al. 2017) and the biomass of Eulachon returning to the estuaries and rivers annually can be substantial. The estimated Eulachon biomass returning to the Fraser River annually is estimated to range from 100 to 1600 MT (Hay et al 1997). Species such as gulls (*Larus* spp.) and the Bald eagle *Haliaeetus leucocephalus* rely heavily on pulses of energy-rich Eulachon and alter their movements and breeding seasons based on the Eulachon (Rogers et al 1990; Hinrichsen 1998). Numerous pinniped and whale species prey on Eulachon in estuaries and inlets as they return to spawn, including Steller sea lion *Eumetopias jubatus* (Marston et al. 2002), Harbor seal *Phoca vitulina* (Pitcher 1980), Northern fur seal *Callorhinus ursinus* (Clemens and Wilby 1967), Harbor
porpoise *Phocoena phocoena* (Jeffries 1984), White-sided porpoise *Lagenorhynchus obliquidens* (Morton 2000), Beluga whale *Delphinapterus leucas*, (Moore et al. 2000), and Humpback whale *Megaptera novaeangliae* (Marston et al. 2002). Additionally, Eulachon is consumed by a wide variety of fish species including Green Sturgeon *Acipenser medirostris* (Fry 1979), Pacific Halibut *Hippoglossus stenolepis* (Scott and Crossman 1973; Yang 1993), Pacific Cod *Gadus macrocephalus*, (Hart 1949; Yang 1993), and salmon *Oncorhynchus* spp. (Clemens and Wilby 1967; Barraclough 1964; Betts 1994). Clearly, Eulachon is a key species supporting the ecosystem.

Eulachon is important to humans both culturally and for food (Kuhnlein et al. 1982; Senkowski 2007; Patton et al. 2019). Native Indigenous people of the Pacific Coast coveted the Eulachon because of the high oil content (Kuhnlien et al 1982; Beveridge et al. 2020) and the fish provided an essential source of nutrition in the winter and spring when other high energy food resources were scarce. Fish and rendered oil were bartered with Native Tribal groups farther inland forming the “grease trails” of southeast Alaska and British Columbia (Hart 1973; Bartlett 1994; Hay et al. 1997). Today, Eulachon still support important subsistence and personal fisheries throughout the range of the fish where populations are robust.

Eulachon is anadromous and spends 98% of its life at sea. They feed primarily on copepods and euphausiids found on the continental shelf (Hay and McCarter 2000) and on the continental slope of the Gulf of Alaska in depths to 500 m in Alaska (Mueter and Norcross 2002). Eulachon reach sexual maturity at two to five years old before ascending the lower reaches of rivers to spawn in the winter and spring (Hay and McCarter 2000; Willson et al. 2006). Spawn timing and location
within rivers is highly variable and some rivers may experience more than one run in a year (Willson et al. 2006). Eulachon is semelparous and fecundity ranges from 7,000 to 67,000 eggs which are approximately 1 mm in diameter (Hay and McCarter 2000; Spangler 2002). Spawning occurs over sand or coarse gravel and eggs become adhesive after fertilization and stick to the substrate (Smith and Saalfield 1955). Hatching occurs after three to eight weeks depending on water temperature and the 4 to 9 mm larval eulachon drift out to the ocean. Trawl surveys in British Columbia indicate that larval Eulachon remain in the upper 15 m of the water column for the first months of life before dispersing to deeper water across coastal areas and the continental shelf within the first year (Hay and McCarter 2000; Csepp et al. 2011).

Eulachon populations from south of the Nass River in northern British Columbia to northern California have declined dramatically in the last 30 years (Schweigert et al. 2007; Gustafson et al. 2012). This prompted a petition and the ensuing review by the National Marine Fisheries Service leading to the listing of the southern distinct population segment as “Threatened” under the U.S. Endangered Species Act (ESA) of 1973 (United States Government 1988; Gustafson et al. 2012). Most Eulachon populations appear to be stable in Alaska while a few have experienced infrequent or poor runs (Moody 2008). For example, the Unuk River Eulachon fishery has been closed from 2005 to 2020 due to low returns (Susan Howell, personal communication, U.S. Forest Service, Ketchikan, Alaska).

Knowledge of the migration and spawning ecology of Eulachon is needed to protect sensitive habitat and to manage this important species. Currently, Eulachon is thought to only spawn in freshwater. However, evidence suggests the possibility that portions of Eulachon populations
spawn in the brackish water of estuaries. In both the Nass and Fraser rivers, eggs were found in the lower reaches influenced by the tide, but salinity was never assessed (Langer et al., 1977; Hay et al. 2003). If Eulachon spawn and produce viable offspring in the upper estuaries under the influence of saltwater, there would be important implications on the designation and protection of critical habitat and on the population monitoring studies used to guide researchers and managers. For example, if Eulachon spawns in estuaries below where monitoring is occurring, adjustments would need to be made in the monitoring sampling strategy.

Another aspect of Eulachon spawning ecology needed to support population monitoring is understanding of environmental variables that influence the spawn timing and strength. This knowledge could assist in the design of adult or larval population monitoring because sources of variation could be incorporated into the study design. For example, if Eulachon spawning peaks during spring tides and at night, adult sampling protocols should address tide height and light intensity as sample strata. The ability to determine the start, peak, and end of the run is also important for informing when to sample for larval fish drift. Spawn timing and strength correlates with larval density when accumulated thermal units are taken into account (Spangler 2002) and consequently timing of sampling can be determined, which would increase sampling efficiency. Therefore, quantifying factors influencing spawn timing and strength are important refinements to sampling strategies.

There are inconsistencies in how tidal phase, high tide height, and freshwater discharge influence anadromous fish migration behavior. Peak counts of Eulachon entering rivers coincide with the relatively high tide heights that occur in the Nass River (Langer et al. 1977) and high slack tides
in the Frasier River (Higgins et al. 1987) in British Columbia, but tide height did not influence Eulachon catch rates in the Susitna River (Barrett et al. 1984) or in Berners Bay, Alaska (Marston et al. 2002). However, none of these studies thoroughly evaluated the relationship between spawning run strength and tide. Another environmental characteristic thought to influence spawning migration of Eulachon is the volume of water flowing in the river channel, commonly known as river or freshwater discharge. River discharge increases in the early spring may prompt the start of the spawning run (Ricker et al. 1954; Smith and Saalfeld 1955; Langer et al. 1977), but little is known about the effect of river discharge on spawning migrations once a run begins.

Light intensity is a variable reported to affect the run strength of Eulachon. Peak catches of Eulachon occurred at night or at low light intensities in the Nass (Langer et al. 1977), Fraser (Higgins et al. 1987), and Skeena rivers (Lewis et al. 1997). Similarly, female Eulachon migrate into the Kemano River, British Columbia to spawn at night on high tides before retreating to the lower river (Lewis et al. 2002) and fish captured and held in the Cowlitz River, Washington always deposited eggs at night (Smith and Saalfeld 1955). However, little is known about the effects of day and night in glacially dominated rivers such as the Twentymile or Antler rivers.

In this study, I hypothesize that Eulachon is capable of fertilizing eggs and producing larvae in brackish water. Anadromous Eulachon probably evolved from a wholly marine ancestor (Dodson et al. 2009) and therefore it is probable that eggs and larvae can tolerate some salinity. Chapter 2 examines the effects of salinity on the fertilization and hatching success of Eulachon in a laboratory setting. I collected sexually mature Eulachon from the Twentymile River, Alaska
to spawn in the University of Alaska Fairbanks Seward Marine Center in Seward, Alaska. I tested fertilization and hatching success of Eulachon eggs challenged to salinities of 0, 12, 18, 24 and 30 Practical Salinity Units (PSU) to determine if Eulachon were physiologically capable of producing viable offspring in brackish water.

To verify whether spawning in estuaries occurs in the wild, in Chapter 3, I conducted field investigations to determine if Eulachon actually spawned in estuaries. Specifically, my objectives were to: map the salinity in upper estuaries in both the Twentymile and Antler rivers, locate areas of potential egg deposition by evaluating the movements of radio-tagged fish, search for eggs where radio-tagged fish congregated, and to characterize the salinity and substrate of identified egg areas.

In my examination of the relationship between adult spawning run intensity and environmental variables, I hypothesize that peak spawning migration occurs during the high tides associated with the spring tide phase and at lower freshwater discharge which would limit energy expenditure. Moreover, I hypothesize that Eulachon will not have a preference of migration when comparing day to night because the study streams are dominated by glacial run off and have a high level of turbidity that could provide concealment from predation. For Chapter 4, I investigated influence of tide height, freshwater discharge and day or night on the spawning migratory behavior of Eulachon. Environmental data were compared to catch data collected using traps, dip nets, and seine nets from the Antler and Twentymile rivers in Alaska to investigate which environmental variables appeared to influence spawning run strength.
To conclude, in Chapter 5, I recommend expansion of the definition of critical spawning habitat to include estuarine areas and propose modifications to population monitoring methods proposed based on my study results. Additionally, I describe the relationship between spawning run strength and environmental variables to assist researchers and managers in the development of future population assessment work.

References


CHAPTER TWO

The Effects of Salinity on the Fertilization and Hatching Success of Eulachon

*Thaleichthys pacificus* from the Twentymile River, Alaska

Abstract

Eulachon *Thaleichthys pacificus* is an anadromous smelt found along the North Pacific coast of North America that has suffered drastic reductions in distribution and declines in abundance over the past century. Currently, researchers and managers lack knowledge of its use of estuaries as spawning habitat, information that is vital to protect habitat and improve current population monitoring programs. Field observations suggest that Eulachon spawn primarily in fresh water, so I examined the effects of salinity on fertilization and hatching success to determine if Eulachon is biologically capable of producing viable larvae in estuaries. Fish were collected from the Twentymile River in southcentral Alaska and used in laboratory fertilization and egg hatching experiments. Fertilization of eggs was successful in water up to 24 Practical Salinity Units (PSU) when tested in static salinity concentrations of 0, 6, 12, 18, 24 and 30 PSU. To examine hatching success, fertilized eggs were exposed to the same salinities in two concurrent experiments: one holding eggs in 24 x 30-cm shallow glass trays and the other using 1000-ml beakers. Hatching success ranged from 4 to 40% with no success at or above 18 PSU. The results indicated that Eulachon is capable of producing viable larvae in the brackish water of estuaries.

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Introduction

Eulachon (*Thaleichthys pacificus*, Richardson 1836) is an anadromous smelt of the family Osmeridae inhabiting western North America. It is found from Kuskokwim Bay in the Bering Sea, Alaska, to Monterey Bay, California (Hubbs 1925; Odemar 1964; Hart 1973). Eulachon is considered important as a cultural (Kuhnlein et al. 1982; Patton et al. 2019) and commercial resource (Smith and Saalfeld 1955; Langer et al. 1977; Hay et al. 1997) as well as valuable food for many fish, birds, and marine mammals (Hay et al. 1997; Marston et al. 2002).

Adult Eulachon return from the ocean at age two to five to spawn in rivers characterized by a spring freshet or prominent spring flow event (Hay and McCarter, 2000). Spawning occurs between December and July with multiple spawning runs in the same year occurring in some rivers (Gustafson et al. 2010). Eulachon has a fecundity of 7,000 to 67,000 eggs, is thought to be semelparous (Hay and McCarter 2000; Spangler 2002), and usually spawns in the lower reaches of rivers (Smith and Saalfeld 1955; Samis 1977; Spangler 2002). After fertilization, eggs become sticky and adhere to the river bottom substrate where they incubate between two and eight weeks before hatching (Parente and Snyder 1970). Larval Eulachon are relatively small (4 to 9 mm) with little swimming ability and drift out to the estuary to develop. Juveniles move to the continental shelf within the first year of life and remain at sea until their spawning run (McHugh 1940; Hay and McCarter 2000).

Eulachon populations south of the Nass River in northern British Columbia (BC) to northern California have declined dramatically (Schweigert et al. 2007; Gustafson et al. 2012). This prompted a petition (Cowlitz Indian Tribe 2007), review by the National Marine Fisheries
Service, and subsequent listing of the southern population segment as “Threatened” under the U. S. Endangered Species Act (ESA) of 1973 (U.S. Government 1988; Gustafson et al. 2012). Most Eulachon populations appear to be stable in Alaska while a few have experienced infrequent or poor runs (Moody 2008). For example, the Unuk River Eulachon fishery has been closed from 2005 to 2020 due to low returns (Susan Howell, U.S. Forest Service, personal communication). The precipitous decline in eulachon abundance has prompted increased study by both Canadian and American researchers along the North Pacific Coast (Gustafson et al. 2010). Population monitoring has occurred in the Fraser River and other central BC rivers using larval sampling as an index of spawning strength (Hay et al. 2002; Moody 2008). This method uses larval density, fecundity, and sex ratio to calculate a biomass estimate (McCarter and Hay 2003). In some studies, larval monitoring efforts employ sampling of larvae within rivers upstream of estuaries (JCRMS 2005). If portions of some Eulachon populations spawn in estuaries, monitoring designs need revision. Furthermore, effective designation of critical habitat requires understanding of spawning requirements.

There is evidence to suggest that some Eulachon spawn in the brackish water of estuaries. Portions of the upper Columbia River estuary were reported to be important spawning areas for Eulachon (Smith and Saalfield 1955). Salinities of 30 Practical Salinity Units (PSU) have been recorded in more recent monitoring of the lower Columbia River in areas previously reported as possible spawning sites (Chawla et al. 2008). Similarly, spawning areas in lower reaches of the Fraser River experienced salinities of 24 PSU during high tides (Ward 1976). Moreover, significant numbers of viable embryonic eggs were found drifting downstream within the upper estuary of the Twentymile River (Spangler 2002) and in the lower Fraser River (Hay et al. 2002).
However, it is unknown how salinity affects fertilization and hatching success of Eulachon in these estuaries.

Salt tolerance by Eulachon eggs may affect several characteristics of their life history and influence population structure. The ability of fish to return to a particular stream depends heavily on olfaction (Hasler 1966; Hasler et al. 1978). As a consequence of variation in the geographic location of egg development (stream, estuary), Eulachon may not be able to “home” to a particular river because of the mismatch between olfactory development and residence in the stream system during the egg or early larval stage (Hay and McCarter, 2000). Thus, the ability for Eulachon eggs to be successfully fertilized and/or develop in estuaries will influence potential gene flow and level of population differentiation. To address the physiological ability of Eulachon to produce viable offspring in the brackish water of estuaries, I tested the effects of salinity on fertilization and hatching across a salinity gradient ranging from freshwater to full strength seawater.

**Methods**

Fifty adult pre-spawn males and fifty pre-spawn females were captured from the Twentymile River (60° 84’ N, 148° 99’ W) in southcentral Alaska on 21 May 2006 and transported to the University of Alaska Fairbanks Seward Marine Center. Eulachon were held in live wells for 2 to 5 days containing water from the capture site with a salinity of 0 PSU. To test the effects of salinity on fertilization, eggs were fertilized in water ranging from freshwater to high salinity seawater found in estuaries (0, 6, 12, 18, 24, and 30 PSU). Full strength seawater from Resurrection Bay was diluted with filtered freshwater to create the test salinity water; salinity was measured
with an YSI® salinity meter. Six 24 x 30-cm glass trays were filled with test salinity water. Ten ripe males and ten ripe females were randomly chosen from the live wells. Gametes were expelled by gently squeezing the abdomen. Equal amounts of eggs from one ripe female were broadcast in each of the six test salinities using a random tray to start. The procedure was repeated for each of 10 females. After eggs from all 10 females were expelled, the same method was completed for the 10 males within one minute. Gametes were mixed by swirling the water in the trays to ensure equal distribution and allowed to stay in contact with each other for five minutes. Each tray received an air stone for oxygen and circulation, and was placed in a climate control room at 6°C. After 24 hours, egg fertilization status was recorded. Fertilized eggs were identified by their light gray appearance whereas unfertilized eggs turned an opaque white color. The experiment was replicated three times concurrently.

I completed two experiments to test the hatching success of fertilized eggs exposed to the six test salinities (0, 6, 12, 18, 24, and 30 PSU). In the first experiment, eggs were incubated in the same 24 x 30-cm glass trays using methods described in the fertilization tests with eggs held in a climate-controlled room at 6°C. In the second experiment, I suspended 6 x 2-cm glass slides with eggs attached in 1000-ml beakers filled with the test salinity water as described by Carls et al. (1999). To control temperature, the beakers were placed in a bath of sea water pumped continuously from the ocean that ranged from 4 to 8°C. In both experiments, eggs were exposed to the same set of experimental salinity levels in which they were fertilized. The exception was the test using water with a salinity of 30 PSU. I wanted to test tolerance of hatching at this high salinity level despite eggs not being able to be fertilized at this salinity concentration. Therefore, I fertilized eggs at a salinity of 0 PSU and then immediately transferred them to a tray with
salinity of 30 PSU. In both experiments air stones were added for oxygen and water circulation, and eggs were treated with 30 PSU water for 30 minutes once every 48 hours to prevent infection (Edgell et al. 1993). Dead eggs and larvae were counted and removed from beakers once per day. Only hatched eggs were counted in the trays. It was difficult to determine the exact time when an egg had died as there was a time lag between death and when the eggs had changed color indicating they had died. Both experiments were replicated three times.

A generalized linear mixed model framework was used to analyze all fertilization and hatching data using the PROC GLIMMIX procedure in SAS version 9.2 (SAS Institute Inc. 2008). The outcome was modeled as binary (hatched or unhatched) and tray or beaker was treated as a random error effect within each salinity level for each respective experiment analysis. Contrasts with F-tests were used to compare hatching success among the test salinities.

Results

Salinity influenced fertilization rates observed in experimental laboratory trials. Eulachon eggs experienced high fertilization rates when exposed to salinity between 0 and 24 PSU, but fertilization fell to zero at salinity of 30 PSU. The mean observed fertilization rates were 98.6% in salinities of 0 PSU (SE=0.5) and 6 PSU (SE=0.6), 98.9% in salinities of 12 PSU (SE=0.4) and 18 PSU (SE=0.5), and 95.6% (SE=1.3) in a salinity of 24 PSU suggesting a strong threshold response in fertilization rate between 24 and 30 PSU (Table 2.1). There were no significant differences in the fertilization rate among the 0, 6, 12, and 24 PSU salinity treatments (Table 2.2).
The influence of salinity on the hatching success of eggs was more pronounced than observed in the fertilization trials. Hatching success for eulachon eggs incubated in glass trays was greatest in salinities of 0 PSU (mean, 39.6%; SE=7.1) and 6 PSU (mean, 44.7%; SE= 3.8), followed by a salinity of 12 PSU (mean, 3.5%; SE= 1.0; Table 2.3). There was no significant difference in hatching success between salinities of 0 and 6 PSU ($P=0.5676$); however, hatching success was significantly lower for eggs in a salinity of 12 PSU when compared to salinities of 0 and 6 PSU ($P=0.0002$, $P=0.0001$; Table 2.4). There were no eggs that hatched in salinities of 18, 24 or 30 PSU.

In beakers hatching success was greatest in a salinity of 0 PSU (mean, 24.4%; SE =3.8) followed by a salinity of 6 PSU (mean, 11.6%; SE =1.5), and a salinity of 12 PSU (mean, 7.9%; SE =1.6; Table 2.5). Significant differences were found between salinities of 0 and 6 PSU ($P =0.0003$) and 0 and 12 PSU ($P <0.0001$), but not between salinities 6 and 12 PSU ($P =0.1005$; Table 2.6). Similar to the tray experiment, no eggs hatched in salinities of 18, 24 or 30 PSU. The number of days at which 50% of the unhatched eggs died occurred generally decreased with increasing salinity, ranging from 26 days for a salinity 0 PSU and 8 days for a salinity of 30 PSU (Figure 2.2). Because of difficulty determining exact time of death, I expect that observations overestimate that the time of death by up to two days.

**Discussion**

Experimental observations of fertilization and hatching rates for Eulachon eggs exposed to a range of salinity demonstrate the ability of this anadromous fish to produce viable larvae in the brackish waters, such as those found in estuaries. Although the salinity of estuaries varies with
tide and time of year, these experimental results indicate Eulachon eggs can survive long-term exposure to a static salinity of 12 PSU and even periodic exposures to a salinity of 30 PSU during fungus prevention immersions. Although previous researchers did not monitor salinity at spawning locations, some assumed that Eulachon spawned in the intertidal lower reaches of rivers based on egg accumulations (Smith and Saalfeld 1955; Langer et al. 1977) or because some adults spend the duration of the spawning run in high salinity lower reaches of rivers (Spangler 2002). Therefore, it is likely that successful reproduction is occurring in the brackish water of estuaries.

My findings are the first reported records of Eulachon eggs surviving salinity up to 12 PSU, which is not surprising considering that confamilial species such as Surf Smelt Hypomesus pertiosus, Capelin Mallotus villosus and Night Smelt Spirinchus starski produce viable larvae in full strength sea water (Mecklenburg et al. 2002). Two other anadromous smelt occur in Alaska, Rainbow Smelt Osmerus mordax and Longfin Smelt Spirinchus thaleichthys, but salinity at spawning locations has not been investigated. However, in Russian and Japan, silver smelt (H. japonicas) spawn in areas with salinities ranging from 4 to 25 (Gritsenko and Churikov 1983). Delta smelt H. transpacificus and Wakasagi H. niponensis in California’s Sacramento-San Joaquin estuary spawn and rear in the estuary. Delta Smelt appears to be an estuary obligate species living in salinities ranging from 0 to 14 PSU (Swanson et al. 2000). Pond Smelt H. nippoensis lives and spawns in Lake Ogawara, Japan (Katayama and Okata 1995) where salinity averages 12 PSU at depths below 20 m where Pond Smelt are thought to spawn (Nishida and Susuki 2007). Given that other related smelt species spawn in brackish water, it is not surprising that Eulachon eggs are able to tolerate some salinity.
The ability to spawn in estuaries and across a salinity gradient can have several advantages. During some years, rivers found north of 60° latitude may have limited spawning habitat available early in the spawning season resulting in estuarine habitats providing the only spawning sites. In particularly cold years, anchor ice prevents spawning by Eulachon in rivers until late in the spring. For example, in the Twentymile River, the time of ice-out can vary from early April to mid-May (ADFG 1973) with Eulachon returning to spawn in the same time frame (Spangler 2002). Eulachon have been caught as early as March 15 in ice free water approximately 30 km downstream of the Twentymile River in Turnagain Arm (Jeff Urbanus, U. S. Forest Service, personal communication). While the upper river channel is dominated by anchor ice and overflow, the Turnagain Arm and lower channel in the Twentymile River experience tidal inundations that prevent freezing and keep the area open and available for spawning (personal observation). Another advantage to spawning in the estuary is that Eulachon can avoid freshwater predation by stenohaline freshwater and terrestrial predators that benefit from the low and clear conditions of rivers during the early spawning season.

There may be an energetic advantage to Eulachon spawning in estuaries. Eulachon is a poor swimmer in swift current and sustained water velocities >0.4 m/s may restrict its upstream passage (Lewis et al. 2002). The ability to spawn in the lower river, despite higher salinity, reduces the energetic costs associated with migrating up a river, making that energy available for gamete production and spawning. This can be particularly advantageous in years when ocean survival and growth is poor because less energy would need to be expended for migration. The mass-specific aerobic costs of fish have been shown to increase proportionally as size decreases (Goolish 1991) and therefore it costs more energetically on a per mass basis to swim upstream.
for small fish than for larger fish (Weihs 1974; Glebe and Leggett 1981; Leonard and McCormick 1999). There is some evidence in the Bella Coola and Fraser rivers to suggest a shift in spawning location by Eulachon to the lower reaches as populations have declined (Hay et al. 2002; Moody 2008). Therefore, the ability to spawn in estuaries could provide benefit to the reproductive fitness of Eulachon.

Eulachon may successfully hatch at higher salinities than our static salinity tests indicated. First, the daily salinity changes in an estuary would allow time for eggs to reach osmotic equilibrium after being stressed by short durations of high salinity water. Certainly, exposing eggs to a salinity of 30 PSU for 30 minutes every 48 hours for the duration of the experiment to prevent infection did not prevent hatching. Second, salinity tolerance could increase later in egg development. In other species such as Pacific herring Clupea pallasii eggs tolerate higher salinities later in their development compared with freshly fertilized eggs (Holiday 1965). Lastly, laboratory experimental conditions could be more stressful to developing eggs. The developing eggs of the perciform Smelt-whiting Sillago siham were able to withstand higher salinities when naturally spawned compared to artificially spawned (Lee et al. 1981).

If Eulachon is spawning in estuaries, it could have an influence on stock structure and population discreteness by decreasing reproductive isolation. For example, in Berners Bay Alaska, the Berners, Antler, and Lace rivers enter the Bay in close proximity to each other. At low tide, the freshwater portions of rivers remain separate, but become connected lower in the watershed in areas influenced by higher salinity water and tidal activity. Lack of barriers during spawning increases gene flow and homogenizes a population (Ryman and Utter 1987), explaining one
potential mechanism for the lack of genetic population structure observed among central BC Eulachon populations that share a common bay or inlet (Beacham et al. 2005; Flannery et al. 2013; Candy et al. 2015).

The observed salinity tolerance of eggs means that drifting eggs can be viable after being swept downstream into the brackish water of estuaries and inlets which could also partially explain the lack of genetic differentiation among Eulachon populations sharing the same bay or inlet. In the Fraser River, BC, embryonic eggs drifting from the river downstream to the estuary comprised as much as 25% of all eggs and larvae caught in plankton tow nets (Hay et al. 2002). Lower salinity water (0 to 12 PSU) was observed in the upper layers of estuarine and inlet areas adjacent to Eulachon spawning rivers off the coast of BC and where the majority of larval Eulachon were caught (McCarter and Hay 1999). Therefore, it is possible that drifting embryonic eggs could be retained in the lower salinity upper layer until hatching. If a significant proportion of Eulachon hatch while eggs drift in these coastal areas, it may be more difficult for them to return back to their natal river to spawn. Eulachon could lack the ability to imprint on particular rivers because of short freshwater residence time and lack of sensory development due to the small size of larval fish (McCarter and Hay 1999; Hay and McCarter 2000). Thus, the ability of eggs to tolerate salinity greater than zero can indirectly lead to lower population discreteness by increasing the rate of straying and subsequent gene flow among populations.

The ability to tolerate moderate salinity levels would allow incubating Eulachon eggs to drift through unproductive areas before hatching in more productive waters downstream in estuaries. Cook Inlet, for example, is highly unproductive in the upper reaches areas whereas the lower
reaches are very productive (Speckman et al. 2005). Upper Cook Inlet areas are dominated by water sediment transport processes that are driven by extreme tidal fluctuation (Bartsch-Winkler and Ovenshine 1984) and are highly turbid with water visibility as low as 2 cm during the summer (Shelden and Angliss 1995) when larval Eulachon are drifting downstream (Spangler 2002). Thus, in the uppermost limits of Cook Inlet where the Twentymile River flows into Turnagain Arm, it is likely that productivity is low and the highly turbid environment combined with high sediment loads mean fish have to travel far out into Cook Inlet before they encounter conditions favorable for feeding.

Recognition of Eulachon tolerance to salinity during spawning has important implications for researchers and managers. Management decisions regarding monitoring and priorities for habitat protection or restoration could change based on the recognition that Eulachon may be actively using the lower portions of stream systems for spawning. Eulachon is known to be highly variable in both spawning abundance and location among and within rivers (Hay et al. 2002; Hay et al. 2005; Willson et al. 2006). The potential for Eulachon spawning in estuaries complicates the design of many monitoring programs. Currently, the most reliable methods employ larval sampling as an index of spawning run strength (Hay and McCarter 2000; Spangler 2002; Moody 2008). However, monitoring protocols generally exclude the estuary from the sampling framework to avoid double counting of larval fish that could be entrained in estuarine circulation (JCRMS 2005). Estuaries are difficult to monitor because egg and larvae movements could lack the linear movement found in streams. Consequently, conventional monitoring may be under-sampling spawning Eulachon and the proportion of under-sampling is likely to differ across years.
In conclusion, I have shown that Eulachon is physiologically capable of spawning and producing viable larval fish in the brackish water of estuaries. Future research should focus on verifying estuary spawning in the field. Proof of estuary spawning would have important implications in the management and protection of this threatened species.

Acknowledgements

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Figure 2.1. Study area in southcentral Alaska
Figure 2.2. Mortality rate of unhatched Eulachon eggs in salinities of 0, 6, 12, 18, 24, and 30 Practical Standard Units (PSU).
Table 2.1. Fertilization success (%) of Eulachon tested in salinities of 0, 6, 12, 18, 25, and 30 Practical Standard Units (PSU) in three replicates.

<table>
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<tr>
<th>Salinity PSU</th>
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<th>Eggs not fertilized (n)</th>
<th>Eggs fertilized %</th>
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Table 2.2. Results of generalized linear mixed model contrasts for percent fertilization of Eulachon among the test salinities of 0, 6, 12, 18, and 24 Practical Standard Units (PSU).

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Table 2.3. Hatching success (%) of Eulachon tested in salinities of 0, 6, 12, 18, 25, and 30 Practical Standard Units (PSU) in three replicates in trays.

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<tr>
<th>Salinity (PSU)</th>
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Table 2.4. Results of generalized linear mixed model contrasts for hatching success of Eulachon in trays among the test salinities of 0, 6, and 12 Practical Standard Units (PSU).

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Table 2.5. Hatching success (%) of Eulachon tested in salinities of 0, 6, 12, 18, 25, and 30 Practical Standard Units (PSU) in three replicates in beakers.

<table>
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* Disease outbreak during experiment compromised beaker
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Table 2.6. Results of generalized linear mixed model contrasts for percent hatch of Eulachon in beakers among the test salinities of 0, 6, and 12 Practical Standard Units (PSU).

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<td>1</td>
<td>3.58</td>
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References

Alaska Department of Fish and Game (ADFG). 1973. Inventory and cataloging of sport fish and sport fish waters in the Cook Inlet Drainage. Alaska Department of Fish and Game 14: 53–54, 66–71.


CHAPTER THREE

Examining estuarine spawning by Eulachon *Thaleichthys pacificus*, in the Twentymile and Antler rivers, Alaska

Abstract

Eulachon *Thaleichthys pacificus*, is an anadromous smelt found along the North Pacific coast of North America that has suffered drastic declines in distribution and abundance over the past century. Currently, researchers and managers lack knowledge of its use of estuaries as spawning habitat which is needed to prioritize habitat protection and to improve current population monitoring programs. The goals of this study were to determine if Eulachon use estuaries to spawn and to characterize habitat, in particular salinity and substrate, associated with spawning areas in two Alaskan rivers over several years. Salinity was measured to determine the upper limits of the estuary and in potential spawning locations in upper estuaries in both rivers. Radio telemetry was used to find aggregations of tagged fish and suspected spawning areas were sampled for the presence of eggs by walking, snorkeling, and dredging the substrate. My research findings indicate that not only are estuaries used for spawning by Eulachon, but more egg locations were found in the brackish water of estuaries than upstream in freshwater in both the Twentymile and Antler rivers. Salinity in estuary egg areas varied between 0 Practical Salinity Units (PSU) at low tide to a maximum of 25 PSU at high tide. Eggs were attached primarily to pebble (4–64 mm) substrates and secondarily to the cobble (64–256 mm), granule (2–4 mm), and in one case, boulders (>256 mm). Confirmation of Eulachon reproduction in

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estuaries necessitates a revision in the current designations of critical habitat and requires modifications to existing population monitoring programs.


**Introduction**

Eulachon, *Thaleichthys pacificus*, is an anadromous smelt from the family Osmeridae that ranges from northern California to the southeastern Bering Sea coast of Alaska (Odemar, 1964; Hay and McCarter, 2000; Willson et al., 2006). The Eulachon is considered to be important ecologically (Hay et al., 1997), commercially (Hay et al., 1999; Gustafson et al., 2010), and culturally (Mills, 1982; Kuhnlein et al., 1982; Betts, 1994). Significant declines in its distribution and abundance have occurred in California, Oregon and Washington where it has been listed as “Threatened” under the U.S. Endangered Species Act of 1973 (Schweigert et al., 2007; Gustafson et al., 2012). Most Eulachon populations appear to be stable in Alaska while a few have experienced infrequent or poor runs (Moody, 2008). For example, the Unuk River Eulachon fishery has been closed to fishing since 2005 due to low returns (Susan Howell, personal communication, U.S. Forest Service, Ketchikan, Alaska). The estuaries where Eulachon migrate and rear are under threat from direct and indirect anthropogenic influences, such as land development (Hall and Schreier, 1996; Marcoe and Pilson, 2017) and climate change (Hatcher and Jones, 2013).

Lowering standards on Clean Water Act environmental regulations by the United States Government (Federal Register, 1/20/2020) could exacerbate degradation of these key habitats that are thought to be important to Eulachon; however, little is known about their use of estuaries as spawning habitat.

Anadromous fishes spend most of their adult lives at sea, returning to freshwater to spawn. This strategy is used by a wide variety of fishes including the sturgeons (Acipenseridae), herrings (Clupeidae), salmons (Salmonidae), and smelts (Osmeridae). Most of the smelts are anadromous (Dodson et al. 2009) and at least two anadromous smelt species spawn in estuaries in addition to
freshwater: the Arctic Rainbow Smelt Osmerus eperlanus (Shpilev et al. 2005) and the Pond Smelt Osmerus olidus (Arai et al. 2006). It is therefore plausible that the related Eulachon could spawn in the brackish water of estuaries as well.

After spending two to five years in the ocean, Eulachon return to rivers in the winter and spring to spawn, laying demersal adhesive eggs that attach to the substrate (Smith and Saalfeld 1955; Gustafson et al., 2010). Although Eulachon spawn in the lower rivers near the estuaries, it is generally assumed that they only spawn in freshwater as there are no reports of eulachon spawning in brackish water (Willson et al. 2006; Gustafson et al. 2010). Substrates used for spawning range in size from silt to cobble and detritus (Barret et al. 1984; Vincent-Lang and Queral 1984; Smith and Saalfield 1955), but sand is most frequently associated with the presence of eggs (Langer et al. 1977; Samis 1977; Lewis et al. 2002). After 21 to 54 days, depending on temperature and location (Parente and Snyder 1970; Spangler 2002), the newly hatched larvae drift downstream to rear in the estuaries (Hay and McCarter 2000).

There is evidence that some Eulachon spawn in the brackish water of estuaries, but salinity levels have not been measured at spawning areas during spawning or egg incubation to confirm exposure to saltwater. In the Nass River, British Columbia, Eulachon eggs were found from river km 22 to km 52, which was considered the upstream limit of tidal influence though salinity was not assessed (Langer et al., 1977). Perhaps the most convincing data indicating spawning in upper estuarine areas comes from the Fraser River. At least two of the spawning areas found in the lower river (Hay et al. 2003) overlap with the location of the saltwater wedge (Ward, 1976; DFO, 2010); however, actual salinity conditions were not measured at time of spawning and
incubation. Additionally, Eulachon eggs have been found on the bottom of the lower reaches of the Fraser River, but there was uncertainty if the eggs were spawned there or if they had drifted in from another area (Samis 1977). In a laboratory study, Eulachon were capable of egg fertilization in water with a salinity of up to 24 Practical Salinity Units (PSU) and eggs hatched successfully after rearing in water of up to 12 PSU (Chapter 2). Therefore, it is likely that Eulachon is capable of producing viable offspring in brackish areas of estuaries.

Evaluating if Eulachon successfully spawn in brackish water will refine the understanding of potential spawning habitat and may have widespread implications for future population monitoring. Current population monitoring programs use either in-river spawning adult or egg and larval fish assessments to determine population status and trends (Hay et al., 1997; Spangler et al., 2003; Gustafson et al. 2010); yet, the inclusion of estuary spawning habitat is missing in many monitoring programs. The location and timing of spawning in rivers varies by year (Hay et al., 2003), which makes it even more important to consider all available spawning habitat in population assessment work. For example, in years when populations are low, the Eulachon is reported to migrate lower in the river than when populations are high (Stockley and Ellis, 1970; Hay et al., 2003; Moody, 2008). Downstream extension of existing monitoring areas would be proposed if estuary spawning is confirmed.

For this study, I am describing the estuary to include the uppermost limits of where saltwater is observed. This follows Pritchard’s (1955) definition of an estuary as “a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with freshwater derived from land.” Estuaries are dynamic and tidal phase,
height, and freshwater discharge all affect where and when the boundary exists between the freshwater river and the upper estuary.

The overall goal of this study was to determine if Eulachon spawn in estuaries. Specifically, my objectives were to: map the salinity in upper estuaries in both the Twentymile and Antler rivers, locate assumed areas of egg deposition by evaluating the movements of radio-tagged fish, search for eggs where radio-tagged fish congregated, and to characterize the salinity and substrate of identified egg areas. Proof of reproduction in estuaries would guide potential adjustments of existing monitoring programs and help efforts to catalogue habitat in need of protection in other river systems where they are found.

**Methods**

*Study area*

The Twentymile River (60° 84′ N, 148° 99′ W) is a glacial tributary of Turnagain Arm located in southcentral Alaska (Figure 3.1). The river is almost entirely in its natural state and has been minimally affected by land management activities (USDA Forest Service 2002). It has a watershed of approximately 362 km² (U.S. Geological Survey, 2016) and is silty with a high suspended-sediment load and low water visibility (Blanchet 1995). Tides in Turnagain Arm are semi-diurnal exceeding 11 m in range (NOAA tide data). These large tidal changes can create a bore tide wall of water up to 2 m high on the incoming tide (Bartsch-Winkler and Ovenshine 1984).
The Antler River (58° 47' N, 134° 58' W) is influenced by glacial melt and flows into Berners Bay in southeast Alaska (Figure 3.2). It has a watershed of approximately 760 km² with a high suspended-sediment load and is largely unaffected by ground disturbing anthropogenic influences (USDA Forest Service 2016). Tides in the area are semi-diurnal with a tidal range that exceeds 7 m (NOAA tide data).

Salinity to define upper limits of the estuary

In the Twentymile River, salinity measurements were taken twice each year in 2000, 2001 and 2002 at high tide and at low tide the same day. I collected data at sites spaced 1420 m to 1860 m apart in the lower river using an YSI™ water quality meter with a 500 g lead weight attached to the probe to ensure it stayed at the bottom of the river. The sites were established for collecting salinity data only. Site 1 was located near the confluence of the Twentymile River and Turnagain Arm (Figure 3.3). Site 2 was established approximately 2 km upstream of Site 1 and the location of Site 3 was determined by measuring salinity approximately every 200 m until 0 PSU was measured. Therefore, location of Site 3 changed within each year and among years depending on the salinity and represented the approximate upstream limit of saltwater and the upper limits of the estuary. Sampling occurred during spring tides to ensure measurement on the highest tidal height that the upper estuaries experienced during a lunar month when saltwater intrusion into the river was at its maximum. At approximately 30 minutes after high slack tide, salinity was measured starting at Site 1 followed by Site 2 and Site 3. The entire sampling event took approximately 1 hr and the process was repeated at low tide 6 hrs later. All salinity measurements were taken mid-channel and recorded in PSU. The high tide height was recorded
from the Anchorage NOAA Tide Table that corresponded with the tide during which the manual salinity measurements were taken.

Similar methods were applied in the Antler River in 2003, except salinity was collected at six sites spaced from 230 m to 560 m apart (Figure 3.4). Site 1 was located near the confluence of the Antler River with Berners Bay and the other sites were numbered sequentially moving upstream. The distance between sites was decreased between sites 3 to 6 because the salinity gradient was greater than that in the Twentymile River. All sites were sampled in numerical order starting at Site 1 near the mouth of the Antler River at high slack tide and finishing upstream at Site 6 approximately an hour later. Salinity was measured again at the six sites during low tide 6 hrs later. Site 6 (the upper limits of the estuary) was determined by measuring salinity approximately every 200 m until salinity was zero and therefore the location changed throughout the study. Data were collected approximately once per week throughout the five week spawning run for a total of three spring tides and two neap tides. At time of sampling, the high tide height was recorded from the Juneau NOAA Tide Table. To further characterize salinity within the estuary, automated salinity data were collected at Sites 1 and 4 within 2 m of the river shoreline using a Sea Bird Scientific™ SBE 911 Conductivity Depth and Temperature (CDT) automated data recorder. The CDTs, set to record data every 15 minutes, were placed on 24 April and removed 4 June 2003. This time span corresponded with the Eulachon spawning run duration (Chapter 4).
Radio telemetry tagging procedure

Adult Eulachon were caught using dip nets near the mouth of the Twentymile River (Figure 3.1) and with a beach seine net near the mouth of the Antler River (Figure 3.2). After capture, fish were examined to evaluate sex and spawning condition (Barret et al., 1984). Male Eulachon could be identified by tubercles, the large muscle mass along the lateral line, and long pelvic fins that often extended past the anus. Males were considered as pre-spawn condition if they were bright in appearance discharging a small amount of thick white milt when gently squeezed. Spawning males were darker in appearance than pre-spawn fish and freely expelled watery milt. Post-spawners were often even darker in color than spawning fish, essentially void of milt, and in poor condition with frayed fins. Females were smooth, lacked the mass of muscle along the lateral line, and had shorter pelvic fins than males. Female Eulachon were considered as pre-spawning condition when their bellies were robust and full in appearance and did not expel eggs freely, whereas spawning female Eulachon expelled eggs freely. Post-spawning female Eulachon were almost void of eggs and had a gaunt, dark appearance with frayed fins.

Only vigorous fish that were free of physical injury and in pre-spawning condition were selected for tagging. Gastric implantation was used for implanting tags because fish were not feeding and the procedure was much less invasive and quicker than surgical implantation. Before implanting the radio tag in the fish, the antenna was threaded through a straw leaving the radio transmitter at one end and the antenna protruding out the other. The straw was used as a handle to gently insert the tag into the esophagus while the fish was held dorsal side up. When slight resistance was felt, usually within 4 to 6 cm, the straw was removed, leaving the tag in place.
**Tag retention and mortality trials**

Experimental trials were conducted in the Twentymile River in 2000 and the Antler River in 2003 to determine if tags would be retained and if there were tagging effects on fish. In the Twentymile River, 30 fish were collected and 15 fish were implanted with dummy radio tags (9 males, 6 females) and 15 control fish (7 males, 8 females) were not tagged. Fish were marked with unique fin clipping combinations to allow for individual fish identification before they were transferred to a 1-m³ live-well holding tank anchored in the river near the tagging site where fish experienced environmental conditions of the study area. The fish were checked after three days and examined for expelled tags and mortalities. Strong tidal currents and accumulation of sediment in the Twentymile River precluded daily checks and longer holding times. This experiment was expanded in the Antler River with 48 fish. Twelve fish were randomly selected for each of the four treatments; male control, male tagged, female control and female tagged. All fish in the experiment were checked most days for tag retention and mortalities over an 11-day period.

**Radio tracking study**

Tagging and tracking of Eulachon occurred in the Twentymile River in 2000, 2001, and 2002 and in the Antler River in 2003. Eulachon were tagged with coded microprocessor radio tags. The radio tags (Lotek™ MCFT-3KM) weighed 1.4 g in air, were 18.0 mm in length, 7.3 mm in diameter and had an antenna length of 30.0 cm. The operational life of the transmitters was 14 days. In the Twentymile River, frequencies ranged from 149.60 to 149.68 megahertz (mHz) in 2000, from 148.60 to 148.74 mHz in 2001, and from 149.50 to 149.68 mHz in 2002. In the Antler River frequencies ranged from 149.50 to 149.70 mHz in 2003. All transmitters were
programmed with five second burst rates. Tags were placed in near equal percentages of males and females and released throughout the runs.

To track Eulachon, I used small maneuverable boats to access available habitat. In the Twentymile River a 4 m inflatable boat and in the Antler River a 6 m aluminum boat was used to track radio-tagged fish. Both boats were powered by jet outboard engines outfitted with shielded spark plugs to reduce electrical interference with the radio tags. A Lotek™ SRX_400 W5 receiver and a four-element Yagi© antenna mounted on a 360 degree pivot mount were used to find fish. Tracking of fish occurred daily from the first day of tagging until the transmitter for last fish tagged had reached the end of its operational life. In the Twentymile River, the route began 11 km above the tag release site and continued downstream to 1 km below the tag release site (Figure 3.1). In 2001, fish were monitored an additional 7 km downstream of the tagging site using a vehicle on the Seward Highway, which runs parallel to the shore of Turnagain Arm. The route in the Antler River started 7 km upstream of the tag release site and ended 1 km below the tag release site (Figure 3.2). The upper limits of the tracking routes were 2 km above the maximum reported upstream distribution in both the Twentymile River (B. Kitto, personal communication, U.S. Forest Service, Girdwood, Alaska) and the Antler River (M. Willson, personal communication, University of Alaska Southeast, Juneau).

A combination of gain and power level on the radio telemetry receiver was used to determine a fish location fix. The telemetry receiver was set to a gain level of 85 and when a signal was detected, the boat was maneuvered until the power reading reached 230 to 235, a non-dimensional unit of measure that indicated signal strength. A power reading of 235 was
approximately equal to 40 decibels of dynamic range (Lotek Engineering, Ontario, Canada) and the maximum strength that was detected. At the location fix, the GPS coordinates, date, time, frequency, and code were recorded using a Geo Explorer 3™ Geographic Positioning System (GPS) unit with data entry capability. Salinity was also measured at the fish location with an YSI™ water quality meter.

Surveys to determine presence of eggs

In both the Twentymile and Antler rivers, egg surveys were conducted where aggregations of radio-tagged fish were observed. Aggregations were defined as a location where a minimum of two radio-tagged fish were located within approximately 100 m of each other. Three different methods were used to find eggs: walking transects, snorkeling and dredging. Walking surveys were conducted in the estuary at low tide by walking transects spaced approximately 5 m apart from each other parallel to the shoreline. Eggs could be observed in water down to 0.5 m deep and 2 m from the water’s edge. Snorkeling took place early in the sampling season when the water was clear enough to see the substrate. For each survey area, I floated downstream with the current approximately 5 m from the river edge while looking for eggs. If the water was less than 1.5 m deep at 5 m from the shoreline, additional transects were added at approximately 5 m intervals into deeper water. A dredge was used to sample the substrate later in the run in the Twentymile River in 2002 and in the Antler River in 2003. The dredge (0.3 m by 0.3 m by 0.3 m) was attached to a cable and lowered off the bow of a 6 m jet boat to the river bottom to sample the substrate as the boat drifted downstream. The dredge was winched aboard after it came into contact with the bottom and sampled the substrate. A minimum of three transects
perpendicular to the current across a suspected egg site was used to sample at four equidistant points along each transect.

For all three methods, confirmed egg areas had to contain live eggs attached to the substrate. This prevented false identification of an area by counting live eggs that were not attached to the substrate that could have been transported from upstream by the river current. Eggs were light gray in color and conspicuous against the dark substrate. Dead or unfertilized eggs were almost white whereas live fertilized eggs were light grey (Chapter 2). Only presence or absence of live eggs was recorded for all surveys because differences in efficiencies and application prevented comparisons of abundance among sampled areas or methods. Confirmed egg areas were tracked and labeled to allow comparisons among years.

*Salinity and substrate type at egg locations*

Salinity measurements closest to egg areas were used to describe saltwater exposure. In the Twentymile River, salinity data were collected less than 500 m in distance from the egg areas and in the Antler River the distance was 200 m. Salinity data were collected manually within two weeks after the eggs were located. The exception was in the Antler River where salinity was recorded continuously with CDTs for egg areas near Sites 1 and 4.

In both rivers, the substrate was evaluated to determine the dominant and subdominant particle sizes where eggs were found. Areas located using walking or snorkeling surveys were assessed for substrate size at three random locations using a circular weighted hoop with a 5 cm x 5 cm grid and an area of 0.2 m². The dominant and subdominant substrate particle sizes at each of the
20.5 cm x 5 cm intersections was measured using a gravelometer (Hey and Thorne 1983) and was classified based on a modified Wentworth (1922) scale for sediment grain size (Table 3.1). The dredge contents were emptied into a 0.6 m x 0.6 m x 0.3 m tub and particles were measured and categorized using the same technique. The dominant (most common) and sub-dominant (second most common) sizes were assessed for each egg area based on frequency of the substrate particle sizes observed after adding the number of substrate particles in each size category.

Data analysis

Salinity data collected manually to establish the upper limits of the estuary were summarized by day, year, and river. Salinity at each egg area was characterized using data from the nearest salinity measuring site. Salinity data collected with the CDT were used to describe the salinity level and duration of saltwater exposure for nearby egg areas.

Fish mortality in the controlled trials and radio-tagged fish movement data were summarized by river and year. Fish mortality data were analyzed to determine differences in the survival rates between tagged and control fish and between males and females for both the Twentymile and Antler rivers. Fisher’s Exact tests were conducted to test differences in male and female survivorship among treatments starting six days after tagging.

Individual fish travel trajectories created using fish movement data were compared to the upper estuary limit. A fish was categorized as an estuary-only fish if all tracking positions were downstream of the estuary limit or it was categorized as an upstream fish if it was observed upstream of the estuary limit on at least one occasion. Fish that remained at the tagging site or
only moved downstream after tagging were eliminated from the study due to possible tagging effects. Chi-square tests were used to compare the number of estuary-only fish with the number of upstream fish. When sample sizes were too small (n<5), statistical comparisons were not made. Fisher’s Exact tests were conducted to evaluate differences in the proportions of males and females using estuaries. Mann-Whitney U tests were conducted to determine differences in the median upstream distance traveled from the radio tagging release site between males and females, between years within a river, and between rivers. A Bonferroni correction was calculated to control the error rate for multiple comparisons. All statistical comparisons were calculated using Program R (R Core Team, 2020).

Egg substrate data were summarized by dominant and subdominant substrate for each egg area found. For example, if the dominant substrate was pebble for two out of three samples taken within an egg area, that egg area was assigned with a dominant substrate of pebble. This same technique was applied for the subdominant substrate type.

Results

Salinity to define upper limits of the estuary

For both the Twentymile and Antler rivers, the influence of saltwater was confined to relatively short sections of the lower river. Based on manual salinity measurements taken in the Twentymile River, the maximum upstream limits of the estuary were 4.2 km in 2000, 3.6 km in 2001, and 2.9 km in 2002 from the confluence with Turnagain Arm (Figure 3.3). The estuary in the Antler River extended up to 2.8 km above the confluence with Berners Bay based on salinity
measured at the six data collection sites (Figure 3.4). Salinity in the estuaries oscillated between brackish water at high tide to freshwater at low tide (Table 3.2).

Radio tag retention and mortality trials

There was no tag regurgitation by Eulachon in either the Twentymile or Antler rivers (Table 3.3). After a holding period of three days in the Twentymile River, one tag pulled out of the fish because the antenna caught on hardware in the live-well and only one fish died, a control female. There were no significant differences in survival between control and tagged fish \((P=1)\) or between males and females \((P=1)\) at day 3 when the trial ended. In the Antler River, survival was significantly higher for males than females on day six \((P=0.023)\), nine, ten, and eleven \((P<0.0001)\) in both the control and tagged groups.

Tracking detection

Although sample sizes were small for statistical comparison, fish detection rates were greater in the Twentymile River (range 68\% to 86\%; 3-year mean, 81\%) than in the Antler River (47\%). In the Twentymile River a total of 23 fish (16 male, 7 female) were tagged in 2000 (Table 3.4). Of the 23 fish tagged, 22 were detected for a success rate of 96\%. In 2001 a total of 108 fish (54 male, 54 female) were tagged and 85 fish (79\%) were detected. Of the 34 fish (15 male, 19 female) tagged in 2002, 23 (10 male, 13 female) were detected for a success rate of 68\%. For the Twentymile River, there were no significant differences between male and female detection rates in any year \((2000, P=0.304; 2001, P=1.000; 2002, P=1.000)\), or for all years combined \((P =0.804; \text{Table 3.4})\). Similarly, there was no significant difference between the detection rates of males and females in the Antler River \((P=1.000)\).
Fish movement

While the proportion of estuary-only Eulachon versus those upstream Eulachon varied by river and year, significant proportions of estuary-only Eulachon were detected (Table 3.5). For the Twentymile River, there was no significant difference between the number of estuary-only Eulachon versus upstream Eulachon in 2000 ($\chi^2 = 1.80$, df=1, n=20, $P=0.180$). However, in 2001, significantly more estuary-only Eulachon were found than upstream Eulachon ($\chi^2 = 4.651$, df=1, n=86, $P=0.031$). In 2002, the sample sizes were too small (n=4) for meaningful comparisons. The differences in the Antler River in 2003 were more dramatic, where no upstream Eulachon were found ($\chi^2=17.143$, df=1, n=15, $P<0.001$). The number of estuary-only Eulachon was significantly higher in the Antler River when compared with the Twentymile River (Fisher’s Exact test, $P=0.002$). There were no significant differences between the number of males and females found in the estuary compared with upstream (Fisher’s Exact test, $P=1.000$) in the Twentymile or Antler rivers.

Overall, Eulachon did not migrate very far up either the Twentymile or Antler rivers. In the Twentymile River, the maximum distance upstream of the tagging site was 7.424 km by a male in 2000 and 6.619 km for a female in 2002 (Table 3.6). There were no significant differences in the median distance traveled upstream from the tagging site between males and females in 2001 ($P=0.166$), or in 2002 ($P=0.337$). The low sample size for females (n=4) in the Twentymile River in 2000 precluded statistical comparison. In the Antler River the maximum distance upstream from the tagging site was 0.973 km by a male and 0.867 km by a female. There was no significant difference between the median distance traveled upstream by males and females.
nor was there any significant differences among years in the Twentymile River (Table 3.8).

_Egg locations_

In both the Twentymile and Antler rivers, eggs were not discovered at all locations where clusters of radio-tagged fish were found. Eggs were found both on the edges of the river and in the deeper portions of the river channel. In the Twentymile River, eggs were present in one of four areas identified by radio-tagged fish in 2000, two out of four in 2001 and four out of eight in 2002 (Figure 3.3). Eggs were found at area B in all three years sampled, and at area C for two of three years (Figure 3.3). Eggs were found primarily in estuaries, with only three egg areas (C, D, E) found upstream of the estuary in freshwater in 2002. In the estuary egg areas, salinity oscillated between freshwater and brackish water. At low tide all egg areas had a salinity of 0 PSU. At high tide the highest recorded salinity was 19 PSU near site B in 2001 (Table 3.9; Figure 3.3). In the Antler River, eggs were detected in four out of six surveyed radio tag cluster areas and all were located in the estuary (Figure 3.4). All egg areas had a salinity of 0 PSU at low tide and the highest measured salinity at any egg site was 25 PSU at high tide at site A. The CDT data indicated that salinity peaked at 30 PSU 480 m below egg area A and experienced saltwater intrusions of water on 23 of 41 days lasting from 0.25 hr to 6.5 hr each day that the saltwater entered the estuary (mean, 2.8 hr, SD=1.6; Figure 3.5). Salinity measured at Site 4 located 150 m below egg area C peaked at 23 PSU and saltwater intrusion occurred on four days of 41 days lasting from 1.25 to 2.5 hr each day (mean, 1.9 hr, SD=0.5). During the neap tides, saltwater did not intrude into the estuary with one exception on 8 May 2003 (Figure 3.5).
The dominant substrate type found at all discovered egg areas (100%) was pebble (4–64 mm) in both the Twentymile (n=12) and Antler rivers (n=7; Table 3.9). The subdominant substrate in the Twentymile River egg areas was mostly granule (2–4 mm; 75%) followed by cobble (64–254 mm; 17%) and boulder (> 256 mm; 8%). In the Antler River the subdominant substrate was cobble (100%). Egg areas A, B and C in the Twentymile River had large areas of silt and sand, but I only found eggs in the areas of pebble-sized substrate. In the Antler River eggs were found exclusively in areas with a dominant substrate of pebble and a subdominant substrate of cobble. However, the dredge had difficulty sampling substrate particles larger than 75 mm and therefore sampling was biased against larger particles.

Discussion

My results clearly demonstrate that Eulachon spawn partially in estuaries of two river systems in Alaska. It was not a one-time event but rather occurred in every year sampled. To my knowledge, this is the first research documenting eggs in brackish water as prior to this research Eulachon were reported to spawn only in freshwater (Lewis et al. 2002; Gustafson et al. 2010). My observations of Eulachon spawning in estuaries are corroborated by laboratory experiments in which I demonstrated that the Eulachon is capable of fertilizing eggs in salinities up to 24 PSU and hatched successfully in static salinities up to 12 PSU (Chapter 2). Furthermore, I used treatments of 30 PSU water for 30 minutes every 48 hrs to control disease when rearing the fertilized eggs in that experiment. Consequently, it is likely that Eulachon eggs can tolerate periods of salinity over 12 PSU given the daily salinity changes in the estuary. Daily exposure to lower salinity that occurs at low tide would give eggs time to recover and reach osmotic equilibrium.
It is likely that Eulachon were spawning even farther downstream in the estuaries than I was able to detect because of methodology limitations. While radio telemetry is useful for documenting the movements of Eulachon, salinity attenuates the radio signal making tracking difficult as salinity increases (Neizgodal et al. 1998). Although I was able to time radio telemetry surveys during low tide when salinity was low, I was unable to track fish after the saltwater entered the estuary. Combined, these lines of evidence support successful reproduction lower in the estuaries with higher salinity than I report.

The ability of Eulachon to spawn in estuaries is not surprising given the evolutionary history of osmeriforms. Currently accepted hypotheses of evolutionary relationships indicate stomiiforms (dragonfishes, lightfishes, loosejaws, marine hatchetfishes, and viperfishes) and osmeriforms (northern and southern smelts, and noodlefishes) are closely related groups (Betancur et al. 2017). As these groups of fishes contain all marine species, anadromy might have evolved from marine origins, i.e., following a “safe site hypothesis” (Dodson et al. 2009). In this hypothesis, osmerids started with an exclusive marine life cycle but then began exploiting estuaries and freshwater seeps to place eggs in safe areas where predation was reduced (Frank and Legget 1982). The euryhaline environment of estuaries provides refuge from predators for many marine fish species (Moyle and Cech 2004). Over time, the euryhaline wanderer developed anadromy as selection for migration into freshwater occurred. For Eulachon, the lack of genetic differentiation among populations (Beacham et al. 2005; Flannery et al. 2013; Candy et al. 2015) and short duration in freshwater (Hart and McHugh 1944) support the hypothesis that they evolved from marine origins. As such, it is likely they have retained the ancestral trait of spawning in saltwater-influenced areas.
Proposed estuary spawning in this study is supported by the location and the lack of mobility of large substrates to which that eggs were attached in brackish water. The pebble and cobble substrates in the estuaries where I found eggs were too large to have been moved by the current during the brief spawning and incubation period. Furthermore, if the pebble and cobble substrates had moved downstream with the current, the tumbling action of the substrate likely would have removed the eggs. In contrast, previous research documented loose eggs or eggs stuck to smaller particles such as sand, silt, or organic debris in the lower part of the Frasier River which led to the assumption that Eulachon had spawned there (Samis 1977). However, sand, silt and fine organic debris are easily transported by the river current compared with the pebble and cobble substrates where I found eggs. Eulachon spawn in many rivers just before or during spring runoff as river discharge is increasing (Hay et al. 2002). As river discharge increases, shear stress increases scouring the substrate and mobilizing fine particles that could have eggs attached. In another study conducted in the Twentymile River, live eggs attached to sand were caught in the water column after the freshwater discharge had increased rapidly (USGS Gaging station 15272380; Spangler et al. 2003). Therefore, it is possible that loose eggs or eggs attached to small particles were transported from upstream and/or recirculated during tidal incursions in the previous studies.

The observed distribution of eggs and the eggs’ unique adhesive properties provided added proof of localized spawning in estuaries countering the assumption that eggs drifted from upstream. First, if fertilized eggs had drifted a significant distance downstream from freshwater before attaching to the substrate in brackish water, the eggs would have had a dispersed and uniform distribution on the substrate. Conversely, I observed a patchy distribution of eggs in areas of the
river where flow and substrate were relatively uniform. This is evidence that spawning occurred in the immediate vicinity. Second, it is also unlikely that eggs drifted downstream significant distances before attaching to the substrate because the egg adhesive layer becomes ineffective after a relatively short period of time. Although the precise time when the adhesive layer becomes impaired is not known, I discovered fertilized eggs lost their ability to attach to glass slides after approximately 10 to 15 minutes in the laboratory (Chapter 2). Nonetheless, this is enough time for eggs to be transported some distance depending on the current speed of the river. Even though studies of egg adhesiveness have not been conducted on smelt, the time for the adhesive layer to become ineffective is somewhat less than the 20 minute time interval observed in Starry Sturgeon Acipenser stellatus (Gorbacheva 1977). However, environmental conditions in glacial rivers likely degrade the adhesive layer more quickly than in the filtered water of the laboratory. Glacially dominated rivers such as the Twentymile and Antler have extremely high rates of erosion and transport high concentrations of clay and fine sediments (Matmon and Haeussler 2020). Clay is used in the aquaculture of Shishamo Smelt Spirinchus lanceolatu, (Mizuno et al 2004) to remove the adhesiveness of fish eggs and prevent them from sticking together reducing the likelihood of disease. The fine clay particles bind to the adhesive jelly coat layer formed by the secretions and transformation of follicular epithelium cells (Shelton 1978; Siddique et al. 2014). Taken together, these lines of evidence provide a strong case for spawning where eggs were documented, rather than transportation to these sites.

Knowledge of Eulachon spawning limitations is necessary to correctly catalog critical spawning habitats in need of protection both locally in the Twentymile and Antler River and for other rivers across the species’ range. In Alaska, the proposed highway realignment at the mouth of
the Twentymile River and the Kensington Gold Mine near the Antler River in Berners Bay threaten estuary spawning habitat. Any breach of the mining waste from the retaining lake (Alaska DNR, 2005) would dump toxic water into Berners Bay that could be transported to known estuarine spawning areas. Outside Alaska, the estuaries in California, Washington, and Oregon are some of the most highly modified on the Pacific coast of North America due to anthropogenic influences (Bilby et al., 2007; McCreary et al. 2008). The decline in Eulachon in southern portions of its range has led to changes in the critical review of habitat threats in these states. Broadening the definition of critical habitat to include spawning assist in protection of Eulachon.

The findings of this study also necessitate a change in the designation of Eulachon critical habitat under the ESA. In 2011 the National Oceanic and Atmospheric Administration (NOAA) issued a final rule that designated critical habitat for the southern Distinct Population Segment (DPS) of Pacific Eulachon pursuant to section 4 of the ESA (NMFS, 2011). The ESA mandates that critical habitat be designated and a recovery plan developed to move the species towards recovery. However, under the current critical habitat designation, estuaries are considered nursery areas or migration corridors and not as actual spawning habitat. The critical habitat designation under the ESA provides for Recovery plans must contain descriptions of "site-specific management actions" and "objective, measurable criteria" to conclude whether a species has recovered and can be delisted on that basis. Without knowledge of the spawning habitat and requirements of Eulachon, recovery plans are less informed and effective and it is difficult to recommend site-specific management actions. This is even more important as it appears that during low run return years, Eulachon do not migrate as far upstream to spawn (Hay et al. 2003;
Moody (2008) meaning a high proportion will be spawning in the estuary. Consequently, a greater proportion of the spawning population may be affected by adverse environmental activities in the estuary.

Because brackish water spawning is newly described in this study, revision to current Eulachon population monitoring designs are needed. A common method for monitoring populations uses the density of larvae in rivers with fecundity and sex ratio to infer the size of the spawning population (Hay et al., 1997; McCarter and Hay, 2003). However, some studies do not take into account estuary spawning and instead only sample in the freshwater of rivers. For example, in the Columbia River, sampling for larval Eulachon occurs at Price Island located approximately 56 km upstream of the river mouth (JCRMS 2005) whereas the saltwater wedge is only reported to move up to 45 km upstream (Bisson et al. 2000). Given that Eulachon eggs can tolerate brackish water, it is highly likely that Eulachon spawn below this location in the Columbia River. In this study, sampling for larval Eulachon in the Twentymile River occurred in an area that was influenced by brackish water at high tide (Spangler et al. 2003), but previous larval fish sampling occurred exclusively upstream of a spawning site I found in this study. Population metrics such as abundance and density estimates would be biased low because the estuary portion of the spawning population would be missed.

Existing monitoring programs based on larval fish assessments could be improved through additional site specific study. An array of CDTs appropriately spaced set to collect data throughout the estuary during Eulachon spawning and egg incubation would be useful for creating salinity maps of possible spawning habitat. Additionally, data would need to be
collected during a wide range of river discharge flows and high tide heights in the estuary because of the influence these environmental variables have on the estuary (Pritchard 1952, Hansen and Rattrey 1965; Prandle 2009). The goal would be a dynamic salinity map of the estuary based on a range of freshwater discharge and high tide heights. The salinity map could be compared with the salinity tolerances for fertilization and hatching (Chapter 2) to describe where Eulachon is capable of spawning. Locations for larval sampling would be moved downstream of the possible spawning areas; however, questions remain about the characteristics of larval fish drift.

Probably the most critical question regarding larval fish drift sampling bias is determining the risk of double counting due to recirculation of larvae during the flood tide. In shallow well-mixed estuaries such as the Turnagain Arm larval Eulachon could be pushed directly back upstream by the tidal current or entrained in the saltwater wedge in highly-stratified estuaries such as Berners Bay. Larval Eulachon are small (4–9 mm) weak swimmers unable to swim against the river current and it has been suggested that that larval fish could be recirculated subject to the tidal currents pushing them back upstream (Hay et al 1997; Spangler 2003). However, this is unlikely following reasons. Newly hatched larval Eulachon would not live long if they were continually recirculated in the highly turbid and unproductive areas of the Turnagain Arm as an example. It is more likely that larval Eulachon developed behavioral strategies to ensure their transport to productive waters downstream. Although the movements of larval Eulachon in estuaries have not been studied, vertical migration by Rainbow Smelt in a turbid glacial estuary was used to move longitudinally and remain in areas with high prey biomass (Laprise and Dodson 1989; Dauvin and Dodson 1990). Because most larval Eulachon are found
in the upper 15 m or water (McCarter and Hay 1999) it is probable that they avoid advection upstream in the saltwater wedge of highly stratified estuaries.

The problem of counting recirculated larva can also be addressed by timing surveys to coincide with low tide. For example, Spangler (2002) sampled 6 hrs after high slack tide to avoid double sampling larval Eulachon that had been pushed back upstream during the flood tide. Although this technique may not work as well in highly complex estuaries such as the Fraser River where archipelago islands create large slack water areas in the estuary, timing retention of tidal water could prove useful in small and simple estuaries such as the Turnagain Arm or Berners Bay. The tidal water residence time can be calculated using the distance the tidal water moved upstream and the average speed of the current moving downstream after high tide (Spangler, 2002). For example, the upstream limits of tidal pooling in the Twentymile River on a spring tide is approximately 7.5 km upstream of the river mouth and the average current speed is 4 km/hr. Therefore, it is likely that the tidal water and any associated larval Eulachon should have retreated after several hours.

In conclusion, it is important that the Eulachon is spawning in estuaries. First, many estuaries where Eulachon spawn are highly degraded, sensitive, and rare aquatic habitats. Any Eulachon populations that depend on threatened estuarine habitats for spawning could face more extreme reductions in population size and decreases in their long-term probability of persistence. As the human population and urban development increase it is likely these habitats will become more imperiled. Second, current population assessment work has missed evaluating the estuary
component of spawning populations, adding an unknown bias to estimates and therefore methods must be revised.

Acknowledgements

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Figure 3.1. Study area map of the Twentymile River, Alaska with the Eulachon radio telemetry tagging release site, and the beginning and ending of the radio telemetry tracking route.
Figure 3.2. Study area map of the Antler River, Alaska with the Eulachon radio telemetry tagging release site, and the beginning and ending of the radio telemetry tracking route.
Figure 3.3. Eulachon locations determined using radio telemetry, maximum measured salinity in Practical Salinity Units (PSU), and the observed upstream limits of the estuary in the Twentymile River 2000–2002. Letters indicate fish aggregation areas where eggs were found.
Figure 3.3. Continued
Figure 3.4. Eulachon locations determined using radio telemetry, identified spawning areas, the maximum measured salinity in Practical Salinity Units (PSU), and the observed upstream limits of the estuary in the Antler River 2003. Letters indicate fish aggregation areas where eggs were found and unlabeled red circles indicate areas that were searched but no eggs were found.
Figure 3.5. Maximum daily salinity (Practical Salinity Units, PSU), measured with Sea Bird ScientificTM SBE 911 at sites 1 and 4 in the Antler River, 2003. The bars indicate the maximum salinity observed on each day (m/dd) and the lines represent the time duration in hours (hrs) each day that salinity was >2 PSU. The lighter bars and lines represent the data from Site 1 found near the river mouth and the darker gray bars and lines represent the salinity at Site 4 upstream.
Table 3.1. Substrate size scale used to categorize substrate with attached Eulachon eggs according to Wentworth (1922).

<table>
<thead>
<tr>
<th>Grain size</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;256 mm</td>
<td>Boulder</td>
</tr>
<tr>
<td>64–256 mm</td>
<td>Cobble</td>
</tr>
<tr>
<td>4–63.9 mm</td>
<td>Pebble</td>
</tr>
<tr>
<td>2–3.9 mm</td>
<td>Granule</td>
</tr>
<tr>
<td>&lt;1.9 mm</td>
<td>Fines (Sand, Silt, Clay)</td>
</tr>
</tbody>
</table>
Table 3.2. Salinity levels in Practical Salinity Units (PSU) measured at sample sites, in the Twentymile (2000–2002) and Antler (2003) rivers within 90 min of high slack tide. Salinity was 0 at low tide for all samples taken. Salinity sites were oriented where 1 is closest to the ocean and the highest number is farthest upriver and correspond to sites in Figures 3.3 and 3.4. A blank space denotes no data recorded. The high tide height was recorded from the Anchorage and Juneau NOAA Tide tables that corresponded with the sampled tide. In the Twentymile River, all samples were taken during spring tides. In the Antler River, salinity was measured on three spring tides (May 1, 8, and 18) and on two neap tides (May 13 and May 26).

<table>
<thead>
<tr>
<th>River</th>
<th>Year</th>
<th>Date</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>15</td>
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<tr>
<td></td>
<td></td>
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<td>0</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>2001</td>
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<td>19</td>
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<td></td>
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<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
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<td></td>
<td></td>
<td>June 10</td>
<td>10</td>
<td>4</td>
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<tr>
<td>Antler</td>
<td>2003</td>
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<td>27</td>
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<td>12</td>
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<td>0</td>
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<td>19</td>
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<td>May 26</td>
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<td>0</td>
<td>0</td>
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Table 3.3. Cumulative mortalities by day of Eulachon in the radio telemetry tag retention test and conducted in the Twentymile River in 2000 and Antler River in 2003 (n = number, FL = fork length, SD = standard deviation). A blank space denotes no data recorded.

<table>
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<th>River</th>
<th>Treatment</th>
<th>Sex</th>
<th>n</th>
<th>Mean FL</th>
<th>Expelled tags</th>
<th>Cumulative Mortalities by day</th>
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</thead>
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<td>Twentymile</td>
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<td>m</td>
<td>7</td>
<td>211</td>
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<td></td>
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<td>13 0</td>
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<tr>
<td></td>
<td>Tagged</td>
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<td>11 1</td>
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<tr>
<td></td>
<td></td>
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<td>6</td>
<td>214</td>
<td>10 0</td>
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<tr>
<td>Antler</td>
<td>Control</td>
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<td>201</td>
<td>8 0</td>
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<td>198</td>
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<td>8 0</td>
<td>1 3 4 8 8 11</td>
</tr>
</tbody>
</table>
Table 3.4. Number (n) of Eulachon tagged with radio telemetry tags and the percent detected 24 hours after tagging for the Twentymile River (2000–2002), and the Antler River in 2003. Results from Fisher’s Exact test comparing males to females within river, year and between rivers.

<table>
<thead>
<tr>
<th>River</th>
<th>Year</th>
<th>Tagged fish</th>
<th>Detected fish</th>
<th>Detection males vs females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>Twentymile</td>
<td>2000</td>
<td>16</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>54</td>
<td>54</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>15</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>Antler</td>
<td>2003</td>
<td>18</td>
<td>18</td>
<td>36</td>
</tr>
</tbody>
</table>

All years

0.874
Table 3.5. Number and percent of adult Eulachon implanted with radio transmitters that stayed in the estuary during all tracking compared to those that moved upstream into freshwater in the Twentymile River (2000–2002) and the Antler River (2003).

<table>
<thead>
<tr>
<th>River</th>
<th>Year</th>
<th>Total fish</th>
<th>Estuary only</th>
<th>Upstream Estuary</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>male</td>
<td>female</td>
<td>n</td>
</tr>
<tr>
<td>Twentymile</td>
<td>2000</td>
<td>20</td>
<td>10</td>
<td>77</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>86</td>
<td>26</td>
<td>49</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>22</td>
<td>3</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>Antler</td>
<td>2003</td>
<td>15</td>
<td>9</td>
<td>60</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 3.6. The minimum, maximum, and median distance in kilometers (km) that adult Eulachon swam upstream of the tagging site in the Twentymile River (2000–2002; Mdn = median).

<table>
<thead>
<tr>
<th>River</th>
<th>Year</th>
<th>Sex</th>
<th>n</th>
<th>Min.</th>
<th>Max.</th>
<th>Mdn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twentymile</td>
<td>2000</td>
<td>male</td>
<td>16</td>
<td>0.159</td>
<td>7.424</td>
<td>1.571</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>4</td>
<td>0.052</td>
<td>5.659</td>
<td>1.155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>both</td>
<td>20</td>
<td>0.052</td>
<td>7.424</td>
<td>1.456</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>male</td>
<td>43</td>
<td>0.155</td>
<td>6.404</td>
<td>2.540</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>43</td>
<td>0.140</td>
<td>6.407</td>
<td>2.270</td>
</tr>
<tr>
<td></td>
<td></td>
<td>both</td>
<td>86</td>
<td>0.140</td>
<td>6.407</td>
<td>2.310</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>male</td>
<td>10</td>
<td>0.167</td>
<td>6.271</td>
<td>3.930</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>13</td>
<td>0.079</td>
<td>6.619</td>
<td>1.430</td>
</tr>
<tr>
<td></td>
<td></td>
<td>both</td>
<td>23</td>
<td>0.079</td>
<td>6.619</td>
<td>3.178</td>
</tr>
<tr>
<td>Antler</td>
<td>2003</td>
<td>male</td>
<td>8</td>
<td>0.174</td>
<td>0.973</td>
<td>0.668</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>7</td>
<td>0.301</td>
<td>0.867</td>
<td>0.531</td>
</tr>
<tr>
<td></td>
<td></td>
<td>both</td>
<td>15</td>
<td>0.174</td>
<td>0.973</td>
<td>0.564</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>River</th>
<th>year</th>
<th>n</th>
<th>U value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twentymile</td>
<td>2001</td>
<td>86</td>
<td>811.5</td>
<td>0.166</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>23</td>
<td>49</td>
<td>0.337</td>
</tr>
<tr>
<td>Antler</td>
<td>2003</td>
<td>15</td>
<td>26.0</td>
<td>0.865</td>
</tr>
</tbody>
</table>
Table 3.8. Results of Mann Whitney U tests comparing the median distance (km) traveled upstream for all tagged Eulachon (males and females combined) between year and river. * indicates significance at $P$-value $< 0.0083$ with Bonferroni correction.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>n</th>
<th>U value</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twentymile 2000 vs 2001</td>
<td>106</td>
<td>643</td>
<td>0.145</td>
</tr>
<tr>
<td>Twentymile 2000 vs 2002</td>
<td>43</td>
<td>176</td>
<td>0.289</td>
</tr>
<tr>
<td>Twentymile 2001 vs 2002</td>
<td>109</td>
<td>906</td>
<td>0.541</td>
</tr>
<tr>
<td>Antler vs Twentymile 2000</td>
<td>35</td>
<td>67</td>
<td>0.006*</td>
</tr>
<tr>
<td>Antler vs Twentymile 2001</td>
<td>101</td>
<td>186</td>
<td>$&lt;0.001^*$</td>
</tr>
<tr>
<td>Antler vs Twentymile 2002</td>
<td>46</td>
<td>56</td>
<td>$&lt;0.001^*$</td>
</tr>
</tbody>
</table>
Table 3.9. Presence (+) and absence (−) of eggs by survey date and method in the Twentymile (2000–2002) and the Antler (2003) rivers. Letters A–E indicate areas checked for eggs where radio-tagged fish were aggregated and correspond to egg area labels in Figures 3.3 and 3.4. Dominant substrate sizes to which eggs were adhered are: Gr=granule, P=pebble, C=cobble, B=boulder. A blank space denotes no survey conducted or no eggs found. Substrate types bolded are dominant substrate with attached eggs.

<table>
<thead>
<tr>
<th>River</th>
<th>Year</th>
<th>Date</th>
<th>Method</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twentymile</td>
<td>2000</td>
<td>May 1</td>
<td>walk</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>May 20</td>
<td>walk</td>
<td></td>
<td></td>
<td>+P B</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>April 30</td>
<td>snorkel, walk</td>
<td>+Gr P</td>
<td>+Gr P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 1</td>
<td>walk</td>
<td>+Gr P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>May 25</td>
<td>dredge, walk</td>
<td>+Gr P</td>
<td>+Gr P</td>
<td>+Gr P</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 10</td>
<td>dredge</td>
<td>+Gr P</td>
<td>+Gr P</td>
<td>+Gr P</td>
<td>+P C</td>
<td>+P C</td>
</tr>
<tr>
<td>Antler</td>
<td>2003</td>
<td>April 26</td>
<td>snorkel, walk</td>
<td>+P C</td>
<td></td>
<td>+P C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>May 6</td>
<td>walk</td>
<td>+P C</td>
<td>+P C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>May 17</td>
<td>dredge</td>
<td>P C</td>
<td>+P C</td>
<td>+P C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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CHAPTER FOUR

Tidal flow, river discharge, and diel effects on Eulachon *Thaleichthys pacificus* spawning migration in Alaska.

Abstract

Eulachon *Thaleichthys pacificus* is an anadromous smelt found on the North Pacific coast of North America that has suffered drastic declines in distribution and abundance over the past century. To assist fishery managers with population assessments and to learn more about the spawning migration ecology of Eulachon, I evaluated the relationship of catch-per-unit effort (CPUE) with environmental variables commonly thought to influence the migration of Eulachon. Specifically, I tested the hypotheses that adult Eulachon peak spawning migration occurs during spring tides, low river discharge, and at night in the Antler and Twentymile rivers in Alaska. Eulachon spawning runs were quantified using data collected with traps (2004–2008) and seine nets (2002 and 2003) in the Antler River and with dip nets in the Twentymile River (2000–2003). My results support the hypothesis that peak migration of Eulachon occurs with the highest tide heights associated with the spring tide phase when tidal incursions push far up into the estuaries and lower reaches of rivers. I was able to confirm the hypothesis that migration was greater during lower freshwater discharge in the Antler River, but not in the Twentymile River. Timing migration with the spring tide phase and during lower freshwater discharge has energetic benefits, increases access to spawning areas, and limits predation on Eulachon, all of which likely increases their likelihood of survival and successful reproduction. Contrary to previous

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findings, I found no significant differences between day and night catches in either the Antler or Twentymile Rivers. Eulachon may not need to migrate at night in the turbid water of the Antler and Twentymile Rivers because the low water clarity provides enough visual cover from predators. Here, I report evidence to suggest future population assessment work should consider the relationships of catch rates with tide height and freshwater discharge.
Introduction

Eulachon *Thaleichthys pacificus* is an anadromous smelt that ranges from northern California to the southeastern Bering Sea coast of Alaska (Odemar 1964; Hart 1973; Hay and McCarter 2000; Willson et al. 2006). Eulachon is a forage fish considered to be important ecologically (Hay et al. 1997), commercially (Smith and Saalfeld 1955; Langer et al. 1977; Hay et al. 1997; 1999), and culturally (Mills 1982; Kuhnlein et al. 1982; Betts 1994). Eulachon populations south of the Nass River in northern British Columbia to northern California have declined dramatically (Schweigert et al. 2007; Gustafson et al. 2012). This prompted a petition (Cowlitz Indian Tribe 2007), review by the National Marine Fisheries Service, and subsequent listing of the southern population segment as “Threatened” under the U. S. Endangered Species Act (ESA) of 1973 (Gustafson et al. 2012). Most Eulachon populations appear to be stable in Alaska while a few have experienced infrequent or poor runs (Moody, 2008). For example, the Unuk River Eulachon fishery has been closed since 2005 due to low returns (Susan Howell, personal communication, U.S. Forest Service, Ketchikan, Alaska).

Despite the importance of Eulachon, relatively little is understood about its spawning migration. Commonly accepted migration theory is that organisms will make optimal use of temporal and spatial variability of environmental conditions to enhance their reproductive success (Dingle 1996). The energetic cost of anadromous spawning migration is an important factor influencing reproductive strategies, as energy used for migration decreases the amount available for gamete production and metabolic maintenance (Glebe and Leggett 1981; Lambert and Dodson 1990). Anadromous female fish spend up to six times more energy on gonad development than males (Johnson et al. 1997; Fleming 1996) and therefore may employ different behavioral strategies to
increase reproductive success. It is widely accepted that anadromous fishes, such as Eulachon, adjust migratory behavior based on environmental cues to maximize reproductive success and reduce predation (Beer et al. 2006; Keefer et al. 2008). Examples of predictable environmental signals for fishes include lunar cycles (Forsythe et al. 2012), water temperature (Quinn and Adams 1996), tidal phase, river discharge (Anderson and Beer 2009; Forsythe et al. 2012) and daylight (Hartman et al. 1967).

There is disagreement about the influence that tide stage (ebb vs. flood) and tide phase (neap vs. spring) have on anadromous fish migration behavior. Peak counts of Eulachon entering rivers coincide with flood tides in the Frasier River (Higgins et al. 1987) but with the flood tides during the spring tidal phase in the Nass River (Langer et al. 1977) in British Columbia. However, tide stage and phase did not influence Eulachon catch rates in the Susitna River (Barrett et al. 1984) or in Berners Bay, Alaska (Marston et al. 1997). This variation in the association between tide phase, tide stage, and catch rates has been observed for other anadromous fishes. Atlantic Salmon *Salmo salar* use the upstream flow of the flooding high tides to assist their migration through the estuaries to rivers (Brawn 1982; Potter 1988; Priede et al., 1988), but overall, there is little agreement about the relationship between tide stage or tide phase and river entry. For example, Atlantic Salmon have been shown to enter rivers during the flood tide stage (Hayes, 1953), the flood and ebb tide stages (Priede et al., 1988), or with no clear association with tide stage or phase (Potter, 1988; Potter et al., 1992). For Eulachon, a more thorough evaluation of the relationship between high tide height and peak counts is necessary to better understand their reproductive ecology.
Another environmental characteristic thought to influence spawning migration of Eulachon is the volume of water flowing in the river channel, commonly known as river or freshwater discharge. River discharge increases in the early spring may prompt the start of the spawning run (Ricker et al. 1954; Smith and Saalfeld 1955; Langer et al. 1977), but little is known about the effect of river discharge on spawning migrations once a run begins. Greater river discharge increases turbidity due to mobilization of fine sediments in glacially-influenced drainages such as the Antler and Twentymile rivers (Wada et al. 2011). Turbidity has been shown to influence predator avoidance behavior in Chinook Salmon *Oncorhynchus tshawytscha* (Gregory 1993; McElroy et al. 2018) and therefore Eulachon may act differently in turbid rivers such as the Antler and Twentymile compared to less turbid rivers such as the Kemano River, in British Columbia where Eulachon almost exclusively spawn and migrate at night (Lewis et al. 2002).

The diel light cycle is another environmental variable that is known to influence the migratory behavior of Eulachon. Peak catches of Eulachon occur at night or at low light intensities in the Nass (Langer et al. 1977), Fraser (Higgins et al. 1987), and Skeena rivers (Lewis et al. 1997). Additionally, Eulachon prefer spawning at night compared to the day in the relatively clear water tributaries to the Columbia River in Oregon and Washington (Smith and Saalfield 1955). Female Eulachon migrate into the Kemano River, British Columbia to spawn at night on high tides before retreating to the lower river (Lewis et al. 2002) and fish captured and held in the Cowlitz River, Washington always deposited eggs at night (Smith and Saalfeld 1955). Predator avoidance was suggested as an explanation for this night spawning behavior (Spangler 2002). However, little is known about diel migration behavior in small highly turbid glacial streams such as the Antler or Twentymile Rivers of Alaska when compared with the larger and more
studied rivers such as the Fraser or Columbia. Higher turbidity levels can affect the visibility of fish to predators and therefore, the turbid glacial water may provide enough concealment for upstream movement so Eulachon do not need to migrate at night.

An understanding of how tidal phase, high tide height, freshwater discharge and the light cycle affect the migration behavior is essential to design effective monitoring strategies. Effective monitoring strategies are essential to produce reliable population estimates. Thus, the overall goal of this study was to investigate the influence of these variables on the spawning migratory behavior of Eulachon. Here I test the hypotheses that adult Eulachon peak spawning migration occurs during the higher tide heights associated with spring tides, low river discharge, and at night.

**Methods**

**Study Areas**

The Antler River (58° 47' N, 134° 58' W) is influenced by glacial melt and flows into Berners Bay in southeast Alaska (Figure 4.1). It has a watershed of approximately 760 km², is characterized by a high suspended sediment load, and is largely unaffected by ground disturbing anthropogenic influences (USDA Forest Service 2016). Tides in the area are semi-diurnal with a tidal range that exceeds 7 m (NOAA tide data). The sample site was located in the lower river where tidal incursions created high water depths of between 4 to 6 m at high slack tide.

The Twentymile River (60° 84' N, 148° 99' W) is a glacial tributary of Turnagain Arm located in southcentral Alaska (Figure 4.2). Similar to the Antler River, it is largely undisturbed by
anthropogenic activities (USDA Forest Service 2002) and has a watershed of approximately 362 km² (U.S. Geological Survey, 2016). This river is characterized as silty with a high-suspended sediment load and low water visibility (Blanchet 1995). Tides in Turnagain Arm are semi-diurnal and can exceed 11 m in range (NOAA tide data) resulting in large tidal changes that can create a wall of water, or bore tide, up to 2 m high on the incoming tide (Bartsch-Winkler and Ovenshine 1984). The sample site was located in the lower river where tidal incursions created high water depths of between 5 to 7 m at high slack tide.

Data Collection

I examined environmental factors known to correlate with catch data, including tide height, freshwater discharge, and diel light intensity. For trap catch data in the Antler River, daily maximum high tide height data were taken from the Juneau National Oceanic and Atmospheric Administration (NOAA) Tide Table Station 9452210 located 53 km southeast of the sampling area which represented the time of the highest high tide in a 24 hr period. The high tide height values from the Juneau NOAA Tide Table corresponding to the time of sample were used for the seine net catch data in the Antler River. For example, if sampling occurred on the first high tide of the day and the NOAA Tide Table had a maximum value of 12.6 m for that tide, then that value was used. This same method was applied to the Twentymile River dip net catch data. For the Twentymile River, tidal data were collected by referencing the Anchorage NOAA Tide Station 9455920 located 50 km west of the sampling area. Because of the distances between the sample sites and the tide station data, the actual time and tide height at the sample site were not necessarily the same as the tide station data. Water clarity was measured with a 20-cm Secchi
disk mounted on a line. Water clarity was considered low if visibility was between 0 and 0.99 m, medium between 1.00 m and 1.99 m and high over 2.00 m.

The daily maximum river discharge and day or night data were recorded for both the Antler and Twentymile rivers. The Antler River discharge data (m$^3$/sec) were collected at the USGS river gauging station located approximately 17 km upstream from the confluence of the Antler River with Berners Bay (Figure 4.1). In the Twentymile River, the United States Geological Survey (USGS) river gauging station was located approximately 6 km from the confluence of the Twentymile River with Turnagain Arm (Figure 4.2). Instruments at the gauging stations recorded stream flow every 15 minutes, 24 hours per day. I used the single highest value in a 24 hour period as the daily discharge since it was the maximum current to which a fish would be subjected on a given day. For diel light intensity comparisons, samples were categorized as day when they were taken from 30 minutes before sunrise to 30 minutes after sunset. Samples were categorized as night when they were taken from 31 minutes after sunset to 31 minutes before sunrise. The time boundaries between day and night categories changed daily with the changing times of sunrise and sunset. Daily times of sunset and sunrise were downloaded from the National Weather Service, NOAA for the day of year in Juneau and Anchorage for the Antler and Twentymile Rivers respectively.

In the Antler River, fish were caught using traps from 2004 to 2008 and with seine nets in 2002 and 2003 (Table 4.1). The same location was used near the mouth of the Antler River for both the trap and the seine nets (Figure 4.1). For the years when traps were used, the start of the run was determined by fishing with a seine net and/or making observations of the presence/absence
of gulls (Laridae), Steller sea lions *Eumetopias jubatus*, or harbor seals *Phoca vitulina*, as aggregations of predators can serve as an indicator of the presence of staging Eulachon in Berners Bay (Marston et al. 1997). Field visits were made once per week starting approximately April 8 each year. When fish were detected, the traps were placed in the river and removed when few or no fish were caught for three days after it appeared that the peak spawning migration had occurred. In 2005, the field crew did not start sampling until April 24 and in 2007 sampling was terminated before the run tapered off.

Swim-in traps deflected and guided fish through a small opening into the trap holding box. The trap had a height of 0.95 m, width of 0.87 m, and length of 1.85 m (Appendix 4.1). The frame was aluminum with 1.25 cm mesh size Aquamesh® panels. The deflector panels, constructed of the same materials, were 4.8 m long on each side of the trap and angled downstream to guide fish into the trap. The trap was checked every other high tide and fished continuously throughout the entire run.

Sampling with seine nets for adult migrating fish in the Antler River began when the river ice had melted at the mouth of the river, and sampling continued throughout the spawning run until no fish were caught for a 7-day period. In 2002 and 2003, sampling was conducted approximately once per day at the time of the daily highest high tide as determined from the Juneau NOAA Tide Table. Additionally, 59% of the second daily high tides were sampled in 2002, and 29% of them in 2003. There were several days during both years when sampling was not conducted due to equipment issues or personnel shortages. Sampling began 1.5 hrs before the high slack tide. Three persons (rower, boat net handler, and bank net handler) deployed a
seine net (30-m long and 3-m depth, 10-mm mesh size) off the bow of a 6-m boat (Appendix 4.1). While the bank handler held both the float and lead lines of the net, the rower maneuvered the boat directly across stream perpendicular to the river current as the boat net handler fed the net from the bow of the boat to ensure no tangles. Once most of the net was deployed, the boat was rowed downstream while the bank net handler walked slowly downstream 110 m to the end of the gravel bar with the ends of the net to increase sampling time and keep the lead line on the bottom. Once the end of the gravel bar was reached, the boat operator rowed to shore, anchored the boat and all three people pulled the float and lead lines tight. A total of four seine net samples were taken for each sampled tide and it took 1.0 to 1.5 hours to complete the four seine net sets depending on how many fish needed to be sorted and counted.

Fish were caught with dip nets in the Twentymile River from 2000 to 2003 (Table 4.1; Appendix 4.1). The sampling site was located 0.8 km upstream from its confluence with Turnagain Arm (Figure 4.2). Sampling for adult migrating fish commenced in the spring when the river ice had melted at the mouth of the river and continued throughout the spawning run until no fish were caught for a 14-day period. A total of 10 high tides (5 day and 5 night) were randomly selected from the NOAA tide prediction tables within each two-week sampling period for 38% of the available high tides. Tides were chosen before the spawning migration and sampling began.

Dip net sampling started two hours before the high slack tide. Two people standing approximately 20 m apart fished concurrently for 30-min periods in 2000 and for 90-min periods in 2001, 2002, and 2003 to sample each selected high tide. The dip nets were made of aluminum with a handle 4.1-m long, a net opening of 0.50 x 0.40 m, bag depth of 0.70 m, and net mesh of 5
mm. Personnel fished the dip nets by sweeping the net downstream slightly faster than the river current to keep the net bag open while holding the net near the bottom. Care was taken not to hit the bottom as this tended to scare fish. The average number of downstream sweeps varied from 640 to 750 sweeps per hour varying with river velocity.

All Eulachon caught with traps, seines, and dip nets were sorted and classified live (Barrett et al. 1984). Male Eulachon could be identified by tubercles, the large muscle mass along the lateral line, and long pelvic fins that often extended past the anus (Langer et al. 1977). Males were considered as pre-spawn and spawning condition if they were bright in appearance and discharged white milt when gently squeezed. Post-spawners were often darker in color than spawning fish, essentially void of milt, and in poor condition with frayed fins. Females were smooth with robust bellies, lacked the mass of muscle along the lateral line, and had shorter pelvic fins than males. Female Eulachon were considered as pre-spawning and spawning condition when their bellies were robust and full in appearance. Post-spawning female Eulachon were almost void of eggs and had a gaunt, dark appearance with frayed fins. All post-spawn fish were not included in the analysis as they would bias analyses designed to evaluate the response of upstream migrating pre-spawning and spawning fish. For the traps, catch-per-unit-effort (CPUE) was calculated by summarizing the total number of fish caught per 24 hr sampling period. For seine nets, CPUE was expressed as the total number of fish per net set. For dip nets, CPUE was calculated as the aggregate total number of fish caught from all nets divided by the number of hours fished.
Statistical Analysis

I analyzed CPUE values from three different sampling methods (traps, seine and dip nets) separately due to differences in metrics, sampling efficiency, and timing. First, I visually identified the peaks of each run by visually examining the peaks of each run, where a “peak” was defined as the highest cumulative CPUE in any consecutive five day time period. Second, I quantitatively examined relationships between environmental factors, CPUE and sex composition by river and year using Generalized Linear Models (GLM, Agresti 1990; McCulloch and Searle 2001; Wickham 2016; R Core Team, 2018). I modeled CPUE data as a GLM with Poisson errors variable using with high tide height, freshwater discharge, and day or night as explanatory variables. The proportion of females was modeled as a GLM with quasi-Poisson binomial errors variable. To determine the best fit model, a stepwise regression with backward elimination was used. Starting with all possible environmental variables in the model, variables were incrementally removed that did not significantly contribute to the goodness of fit. I selected models for further analysis whenever removing additional variables would not improve the goodness of fit. I also tested the assumptions of all fitted models, specifically looking at the data’s normality, homoscedasticity, dispersion, residuals versus leverage plots (Cook’s distance), correlation, autocorrelation, and multicollinearity.

Results

Run timing

Overall, there was not a large difference in the run start date by year in the Antler River (mean, April 23 or day 113, SD=4 days) or the Twentymile River (mean, April 19 or day 109, SD=4
days). However, there was considerable variation in the run duration depending on the river, sampling methods and year. In the Antler River, the run duration ranged from 14 to 22 days (mean, 19, SD=3 days) between 2004 and 2008 using traps (Table 4.1). When the sampling was conducted with seine nets in 2002 and 2003, the run lasted 43 and 67 days respectively (mean, 55, SD=12 days; Figure 4.2). One interesting observation was that the peak of the run was later by almost two weeks in the Antler River in 2007 and 2008 (mean, May 11 or day 113, SD=3 days) when compared with runs from 2001 to 2006 (mean, April 27 or day 116.8 SD=3 ± days; Figures 4.3, 4.4). Additionally, sampling was terminated while the run was still occurring in 2007 so end date was likely even later. The run duration in the Twentymile River ranged from 44 to 58 days (mean, 34.5, SD=6 days) and the peak ranged from May 16 (day 136) in 2001 to June 1 (day 152) in 2002 (Figure 4.5).

There were notable differences between the catch rates of males and females in both the Antler and Twentymile rivers. First, the sex ratio was highly skewed towards males in both rivers and all sample years ranging from 77% to 88% in the Antler River and 61% to 87% in the Twentymile River (Table 4.2). Secondly, females tended to migrate earlier than males. In the Antler River trap data, 95% of the total female CPUE in 2005 was caught in the first half of the total run time duration for that year and similar results were recorded for 2006 (93%) and 2008 (87%), but not in 2004 (37%; Figure 4.3). The 2007 data could not be used because sampling was terminated early. A similar trend was observed in the Antler River seine data (2002, 54%; 2003, 73%; Figure 4.4). In contrast, the proportion of females in the first half of the run in the Twentymile River varied considerably (2000, 58%; 2001, 54%), 2002, 8%; and 2003, 19%; Figure 4.5).
Tide Height

Tide height appeared to influence the migration of Eulachon in both the Antler and Twentymile rivers. The 5 day peaks of the runs for both the CPUE and the proportion of females were associated with the high spring tides in the Antler and Twentymile rivers (Figures 4.6–4.8). There was an exception in the Antler River in 2004 when peak counts of Eulachon were associated with the neap tide phase. I observed a significant increase in both the total Eulachon CPUE ($\chi^2 (1) =11.24, P=0.008$) and the proportion of female Eulachon ($Z=3.90, SE=0.12, P<0.001$) in the Antler River trap data as the maximum daily tide height increased (Tables 4.3, 4.4). However, there were no significant relationships between high tide height and total Eulachon CPUE (Table 4.5) or percent female (Table 4.6) for the Antler River seine or Twentymile River dip net data (Tables 4.7, 4.8).

In most years, the start of the run began during the neap tide phase for both the Antler and Twentymile rivers. Exceptions were 2005 (Figure 4.6) and 2003 (Figure 4.7) for the Antler River and in 2000 (Figure 4.8) for the Twentymile River. In 2005, the sampling crew started late with fish detected on the first day of sampling in the traps. Therefore, it is likely the 2005 run actually started a few days earlier that year.

Freshwater Discharge and Water Clarity

In both rivers and all years sampled, freshwater discharge was low and water clarity was medium to high when the Eulachon run started. However, as the runs progressed, freshwater discharge dramatically increased and water clarity decreased. In the Antler River, freshwater discharge
ranged from 0.4 m$^3$/sec to 4.1 m$^3$/sec (mean, 2.1, SD=1.3 m$^3$/sec) at the start of the run. At the end of the run discharge ranged from 4.8 m$^3$/sec to 6.8 m$^3$/sec (mean, 6.2, SD= 0.6 m$^3$/sec).

Water clarity in the Antler River was only measured in 2002 and 2003, but ranged from 3.14 m to 3.30 m (mean, 3.22, SD=0.08 m) at the start of the run and had decreased to a range from 0.22 m to 0.27 m (mean, 0.25, SD=0.03 m) at the end of the run. In the Twentymile River, water discharge ranged from 3.2 m$^3$/sec to 8.7 m$^3$/sec (mean, 6.6, SD=2.4 m$^3$/sec) at the start of the run increasing to a range of 52.1 m$^3$/sec to 77.9 m$^3$/sec (mean, 66.4, SD=10.7 m$^3$/sec) at the end of the run. Water clarity ranged from 1.90 m to 2.29 m (2.1, SD=0.16 m) at the start of the run and decreased by the end of the spawning run ranging from 0.29 to 0.42 m (mean, 0.36, SD=0.07 m).

When comparing the 5 day peak of the run to freshwater discharge in the Antler River trap data, there were no obvious associations observed. The peak migration corresponded with periods of low freshwater discharge in three (2004, 2006, and 2008) out of five years, while in the other two years (2005, 2007), migration peaked with periods of rapidly increasing daily maximum freshwater discharge (Figure 6). However, when analyzing the data from all years combined using the GLM, the overall Eulachon catch increased significantly ($\chi^2=4.55$ (1), $P=0.03$) with freshwater discharge for the Antler River trap data (Table 4.3), whereas the percentage of female Eulachon decreased significantly ($Z=30.20$, SE=0.04, $P<0.001$) later in the run as discharge increased (Table 4.4).

In the Antler River seine data, visual analysis of the peak in total CPUE indicated that the percentage female occurred when freshwater discharge was low (Figure 4.7). Results of the GLM indicate that overall, there was a statistically significant increase ($\chi^2=7.2$ (1), $P=0.007$) in
the total CPUE with freshwater discharge. However, the percentage of females increased significantly ($\chi^2=5.79$ (1), $P=0.007$) with discharge in the Antler River (Tables 4.5, 4.6). There was a significant interaction ($\chi^2=23.33$ (1), $P<0.001$) between discharge and year for the percentage of females, indicating that the effect of discharge on the proportion of females was highly dependent on the sampling year (Table 4.5). For example, the proportion of females increased with discharge in 2002 but only slightly in 2003.

In the initial visual analysis of the Twentymile River dip net data, the peaks in total CPUE or percentage of females did not appear to have an overall (all years combined) direct relationship with freshwater discharge, but I did observe positive relationships within each year (Figure 8). When total CPUE was compared to discharge for in the GLM there was a positive significant relationship ($\chi^2=14.53$ (1), $P<0.001$; Table 4.7). Moreover, there were no significant relationships other than the year and discharge interaction, meaning the relationship between discharge and proportion of females varied significantly between years (Table 4.8).

*Daylight (or Time of day)*

Time of day made no difference in the overall catch or the percentage of females in either the trap and seine net data from the Antler River (Tables 4.3–4.6) or the dip net data from the Twentymile River (Tables 4.7, 4.8). There was an interaction effect between year and day or night for the proportion of females in the Antler River seine ($\chi^2=6.00$ (1), $P=0.014$) meaning the relationship varied significantly between the two sampling years (Table 4.6). As the fish caught in the Antler River trap were only counted once per day, day and night could not be compared.


**Discussion**

My findings from the trap and seine data in the Antler River support the hypothesis that peak Eulachon migration occurs during the spring tide phase. These results corroborate observations of Eulachon in the Nass (Langer et al. 1977) and Fraser (Higgins et al. 1987) rivers, British Columbia; and in the Chilkat River, Alaska (Bishop et al. 1989). Peak migration of another anadromous osmerid, Rainbow Smelt *Osmerus mordax* occurs during spring tides as well (Clayton 1976). During spring tides, the salt wedge pushes farther upstream into the Antler and Twentymile Rivers than during neap tides (Chapter 4) and Eulachon likely use the upstream flow of water to assist their passage through the estuary to the river mouth. Timing migration with the spring tides has obvious energetic benefits for Eulachon.

The energetic benefits of assisted upstream migration can be significant. Energy cost of fish migration can be reduced by up to 40% when swimming in the middle of the of the water column with the direction of the tide according to theoretical calculations using Sockeye Salmon and Haddock *Melanogrammus aeglefinus* swimming performance data (Weihs 1974; 1977). Hence, migration during spring tides when the directional flow persists for a longer time period and pushes farther upstream that during neap tides would be an energetic benefit for migrating Eulachon. This is particularly important for Eulachon as they are thought to be primarily semelparous (Hay and McCarter 2000; Clarke et al. 2007) allocating all their reproductive energy to a single event. Most anadromous semelparous fishes end their migration with their energetic reserves fully depleted and therefore energy economy is important for successful reproduction (Bernatchez and Dodson 1987). Just as important as overall energy savings is the ability to budget energy to stay within their metabolic scope during migration (Priede 1985).
Swimming for prolonged periods of time at a high metabolic rate can increase the likelihood of death due to exhaustion and failure of homeostasis (Priede 1977). Animals avoid functioning at high metabolic rates to increase fitness and lower mortality (Priede 1985). Migrating during spring tides would lower risk of death and save energy for migration and reproduction.

Female Eulachon may exhibit different migration behavior than males to increase their overall fitness. In this study females were more likely to migrate during spring tides earlier in the spawning run when freshwater discharge was low. Migrating during spring tides and low freshwater discharge could have additional energetic benefits for females. Although there are no published studies on the migration and reproductive energetics of Eulachon or other anadromous smelts, studies on other anadromous fishes indicate a higher energetic cost of reproduction for females than for males. The total reproductive energy for female Sockeye Salmon is higher than for males even when considering the energetic costs of male secondary sexual development and fighting (Hendry and Berg 1999). Additionally, anadromous females spend significantly more energy than males for gamete production. Atlantic Salmon females invest more energy in gonad development accounting for approximately 20–25% of their weight, whereas anadromous males invest approximately 3–6% (Fleming 1996). Such a high investment of energy in gonads leaves less energy available for migration (Jonsson et al. 1997). Moreover, demands of migration may be greater for females compared with males due to their smaller average size. Eulachon is sexually dimorphic with males averaging 5 to 15 mm longer than females at ages 3 to 5 (Hay and McCarter 2000; Moffitt et al. 2002; Spangler 2002). The mass-specific aerobic costs of fish have been shown to increase proportionally as size decreases (Goolish 1991) and therefore it costs more energetically on a per mass basis to swim upstream for small fish than for larger fish.
If female Eulachon partition energy similarly to other anadromous species, it is likely that the cost to reach the spawning areas is much higher for females than for males. Therefore, migrating during spring tides and low freshwater discharge may be important for females to increase reproductive success.

Another potential benefit for Eulachon migrating during the spring tide phase is increased access to spawning areas. Eulachon is a weak swimmer (Langer et al. 1977) and appears to be restricted to mean water velocities less than 1.2 m/s (Triton, 1991). Water velocities exceed 2 m/sec² in the Antler and Twentymile rivers and 5 m/s² in the Turnagain Arm (Ezer et al. 2008). European Smelt Osmerus operlanus migrate on spring tides to overcome high river velocity in the lower river during the ebb tide (Lyle and Maitland, 1997). Given swimming performance and distance travelled, if Eulachon used neap tides instead of spring tides to migrate upriver, energy expenditures would be greater which could result in lower fitness and a possible inability to ascend the estuary and lower river to important spawning habitat.

Additionally, Eulachon likely reduce the risk of predation from birds by migrating at higher tidal heights associated with spring tides when the water in tidally influenced portions of the river is deep. During neap tides the river channel is narrower and shallower than during spring tides. This reduction in width and depth at low tide exposes Eulachon to increased predation risk from birds such as gulls (Laridae) and Bald Eagles Haliaeetus leucocephalus (Marston et al. 1997; Spangler et al. 2003). However, during the spring tide phase, the high tide water depth increases by up to 3 m in the Twentymile River over the maximum high tide water depth during the neap
tide phase. This increase in water depth limits the availability of Eulachon as prey to birds. Bald eagles avoid foraging in estuarine areas where water depths exceed 3 m (Thompson et al. 2005) and therefore, migrating in deep water likely makes it more difficult for birds to capture Eulachon.

The start of each Eulachon spawning run almost always coincided with the neap tides, but males and females were not ripe enough to spawn. A possible explanation is that Eulachon may be staging near the river mouth before running upstream with the spring tides to spawn when they are ripe. When fish were collected from the Twentymile River to spawn in the laboratory they had to be held from two to five days for gametes of both sexes to mature before they could be spawned (Chapter 2). Synchronization of gonadal development and spawning with the lunar cycle occurs in many species of fish (Johannes 1978; DeVries et al. 2004; Takemura et al., 2004) including Surf Smelt Hypomesus pretiosus (Loosanoff 1938; Taylor 1984) that overlaps in geographic range with the Eulachon. Surf Smelt (Talyor 1984), Grunion Leuresthes sardina, (Thompson and Muench 1975) and Eulachon use spring tides to access spawning habitat and therefore these species have likely evolved to time gamete maturation with the spring tide phase.

I observed highly male-biased sex ratios similar to the results of other studies (Smith and Saalfeld 1955; Higgins et al. 1987; Spangler et al. 2003). However, these studies were conducted in rivers. When Eulachon samples were collected in the marine environment of Lynn Canal approximately 30 km from the Antler River, the sex ratio was nearly 1:1 (M. Sigler, NOAA, personal communication). I attribute the male-biased sex ratio in part, to differences in fish availability for capture caused by dissimilar migratory behavior between the sexes. One
obvious explanation for the observed differences is that freshwater residence time is shorter for females than males (Barrett et al. 1984, Spangler et al. 2003). Another reason is that males live longer in freshwater than females (Spangler 2002). Males are simply more available for capture because they spend more time in the sampled habitats. Another possible explanation may be that females migrate in a different part of the river than the one sampled. Dip nets and traps were both fished from the bank without sampling in the middle of the channel and therefore females migrating in the middle of the river would have been missed. However, this is likely not the case as seine nets were fished over half-way across the channel and skewed sex ratios were found in those data as well.

Traps would likely be superior to dip or seine nets for future adult population assessment work because they can be fished continuously and are very efficient at capturing fish. In contrast, seine nets and dip nets could not be fished continuously because of high labor costs. To reduce labor costs, both seine and dip nets were used to subsample each flood tide. Migrating Eulachon looked like they moved in pulses and sub-sampling effort may not always have coincided with passing schools of fish. The changing environmental conditions and capture efficiencies created additional challenges when sampling with seine and dip nets. Increased freshwater discharge water brought logs, stumps, and debris downstream to the sample site sometimes interfering with sampling and making seine nets more difficult to deploy the net from the boat. The efficiency of the seine net is susceptible to spatial differences in physical environment, such as sublittoral topography and substrate type (Parsley et al., 1989), abundance of macrophytes, and presence of submerged obstructions (Pierce et al., 1990; Bayley and Herendeen, 2000; Macbeth et al., 2005). A limitation of using dip nets is that they rely on higher densities of fish to be present because of
low catch efficiency. However, they were easy to deploy. Temporal differences in fish behavior and water turbidity (Allen et al., 1992) influences net efficiency as well. Turbidity increased during the spawning run each sample year due to snow melt and increasing freshwater discharge in both the Antler and Twentymile rivers. If net visibility changed over time, it could lead to added variation in catch. The trap took more effort than the two different net methods to set up, but once established, it required little maintenance and could be checked or adjusted by fewer personnel. Therefore, the trap should be strongly considered for future studies.

Water clarity could be influencing Eulachon migratory behavior. The Antler and Twentymile rivers are very turbid and the lack of water clarity could be providing enough cover from predators for Eulachon to permit migration during daylight hours. Additionally, Eulachon migrating in turbid rivers were not found associated with any of the clear tributaries, preferring instead to stay within the turbid river channel (Antler River, Marston et al. 1997; Suisitna River, Vincent-Lang and Queral 1984). In contrast to turbid rivers, migration in clear rivers such as the Kemano River, British Columbia (Lewis et al. 2002), or the Situk and Hooligan Rivers in Southeast Alaska, occurs at night and Eulachon take cover in the deepest holes of the river during the day (D. Gillikin and T. Tisler, USFS personal communications). Another smelt species, the Delta Smelt moves to areas of higher turbidity which is thought to be a behavioral adaptation to prevent predation in the Sacramento River (Bennett and Burau 2015). Although the role of turbidity in predation of Eulachon is not known, turbidity plays a role in the predator avoidance behavior of other fish such as juvenile Chinook Salmon (McElroy et al. 2018). If the visual cover from predators provided by turbid water is sufficient, the Eulachon may not need to migrate at night. Another possible explanation for the lack of preference between day and night
migration is the reduced few hours of night available in northern latitudes in the late Spring and early Summer. During the peak spawning migration, there are only approximately six hours of nighttime in the Antler River and fewer than 30 minutes in the Twentymile River.

In this study, I tested the hypotheses that adult Eulachon peak spawning migration generally occurs during the spring tides, low river discharge and at night. I was able to confirm peak spawning migration during spring high tides, but the evidence supporting peak migration during low river discharge was only found for female Eulachon in the Antler River trap data. I rejected the hypothesis that day or night influenced the migration of Eulachon in the turbid waters of the Antler and Twentymile rivers. In summary, future monitoring studies should incorporate tide height in the sampling strategy.

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Figure 4.1. Map of the Antler River, Alaska, Eulachon sampling site, and U.S. Geological Survey (USGS) gauging station.
Figure 4.2. Map of the Twentymile River, Alaska, Eulachon sampling site, and U.S. Geological Survey (USGS) gauging station.
Figure 4.3. Antler River (2004–2008). Daily total Eulachon catch-per-unit-effort (CPUE; fish per day) caught with traps. Day 110 = April 20, Day 120 = April 30, and Day 130 = May 10.
Figure 4.4. Antler River (2002–2003). Daily total catch-per-unit-effort (CPUE; fish per net set) of Eulachon caught by seine net. Day 110 = April 20, Day 120 = April 30, and Day 130 = May 10.
Figure 4.5. Twentymile River (2000–2003). Daily total catch-per-unit-effort (CPUE; fish per hour) of Eulachon caught by dip net. Day 120 = April 30, Day 140 = May 20, Day 160 = June 9.
Figure 4.6. Antler River (2004–2008). High tide height, daily maximum freshwater discharge, daily catch-per-unit-effort (CPUE; fish per day), and percent female Eulachon caught in trap. Day 110 = April 20, Day 120 = April 30, and Day 130 = May 10. Shaded bar represents highest five day cumulative count of fish.
Figure 4.7. Antler River (2002 and 2003). Daily maximum high tide, daily maximum freshwater discharge, total catch-per-unit-effort (CPUE; fish per net set) and percent female Eulachon caught by seine net). Day 110 = April 20, Day 130 = May 10, and Day 150 = May 30, m = meters, m$^3$/sec = cubic meters per second. Shaded bar represents highest five day cumulative count of fish.
Figure 4.8. Twentymile River (2000–2003). Maximum height of the sampled tide, daily maximum freshwater discharge, total catch-per-unit-effort (CPUE; fish per hour) and percent female caught by dip net. Day 120 = April 30, Day 140 = May 20, Day 160 = June 9, m = meters, m$^3$/sec = cubic meters per second. Shaded bar represents highest 5 day cumulative count of fish.
Table 4.1. Capture method, beginning and ending dates of sampling, and number of samples (n) for Eulachon caught in Alaska for each river and year.

<table>
<thead>
<tr>
<th>River</th>
<th>method</th>
<th>year</th>
<th>begin</th>
<th>end</th>
<th>(n)</th>
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</thead>
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<tr>
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<td>April 24</td>
<td>May 14</td>
<td>21</td>
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<tr>
<td></td>
<td></td>
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<td>22</td>
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<td>April 26</td>
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<td>2008</td>
<td>April 30</td>
<td>May 13</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>seine net</td>
<td>2002</td>
<td>April 22</td>
<td>May 20</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2003</td>
<td>April 19</td>
<td>June 7</td>
<td>67</td>
</tr>
<tr>
<td>Twentymile</td>
<td>dip net</td>
<td>2000</td>
<td>April 24</td>
<td>June 21</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>April 21</td>
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<td>2003</td>
<td>April 14</td>
<td>May 28</td>
<td>30</td>
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Table 4.2. Percent (%) male and female Eulachon caught by method and year in the Antler and Twentymile rivers, Alaska.

<table>
<thead>
<tr>
<th>River</th>
<th>method</th>
<th>year</th>
<th>% male</th>
<th>% female</th>
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<tbody>
<tr>
<td>Antler</td>
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<td>77</td>
<td>23</td>
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<tr>
<td></td>
<td></td>
<td>2003</td>
<td>83</td>
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</tr>
<tr>
<td></td>
<td>trap</td>
<td>2004</td>
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<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2005</td>
<td>85</td>
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<tr>
<td></td>
<td></td>
<td>2008</td>
<td>83</td>
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<tr>
<td>Twentymile</td>
<td>dip net</td>
<td>2000</td>
<td>87</td>
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<td></td>
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<td></td>
<td>2003</td>
<td>68</td>
<td>32</td>
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Table 4.3. Antler River (2004–2008). Effect of year, height of highest daily tide, and freshwater discharge, on the daily catch-per-unit-effort (fish per day) of Eulachon caught in the trap tested with a generalized linear model and quasi-Poisson distribution. (LR $\chi^2 =$ Likelihood ratio Chi squared), df = Degrees of freedom. Variables that did not contribute to the goodness of fit were not included.

<table>
<thead>
<tr>
<th>Source</th>
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<th>df</th>
<th>$P$ - value</th>
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<td>0.004</td>
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<td>Tide</td>
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<td>0.008</td>
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<tr>
<td>Discharge</td>
<td>4.55</td>
<td>1</td>
<td>0.033</td>
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<tr>
<td>Year x tide</td>
<td>18.27</td>
<td>1</td>
<td>0.001</td>
</tr>
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Table 4.4. Antler River, Alaska (2004–2008). Effect of height of highest daily tide and freshwater discharge on the proportion of female Eulachon caught in the trap tested using a generalized linear mixed effects binomial model with random intercepts for day and year, and fixed effects model for maximum daily tide and maximum daily discharge. Significance determined at $P<0.05$ level.

<table>
<thead>
<tr>
<th>Source</th>
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<th>Std. Error</th>
<th>Z - value</th>
<th>P - value</th>
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</thead>
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<tr>
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<td>21.12</td>
<td>&lt;0.001</td>
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<tr>
<td>Tide</td>
<td>3.90</td>
<td>0.12</td>
<td>32.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Discharge</td>
<td>7.38</td>
<td>0.04</td>
<td>30.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tide x discharge</td>
<td>-1.10</td>
<td>0.04</td>
<td>30.18</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 4.5. Antler River Alaska (2002, 2003). Effects of high tide height at time of sample, freshwater discharge, and day or night on the catch-per-unit-effort (fish per net set) of Eulachon caught with a seine net tested using a generalized linear model (LR $\chi^2 = \text{likelihood ratio chi squared, df} = \text{degrees of freedom}$). Significance determined at $P<0.05$ level.

<table>
<thead>
<tr>
<th>Source</th>
<th>LR $\chi^2$</th>
<th>df</th>
<th>$P$ - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>25.48</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tide</td>
<td>0.43</td>
<td>1</td>
<td>0.501</td>
</tr>
<tr>
<td>Discharge</td>
<td>7.200</td>
<td>1</td>
<td>0.007</td>
</tr>
<tr>
<td>Day or night</td>
<td>0.07</td>
<td>1</td>
<td>0.786</td>
</tr>
</tbody>
</table>
Table 4.6. Antler River (2002–2003). Effects of high tide height at time of sample, freshwater discharge, and day or night on the proportion of females caught with a seine net tested with a generalized linear model (LR $\chi^2 = $ likelihood ratio chi squared, df = degrees of freedom). Significance determined at $P<0.05$ level.

<table>
<thead>
<tr>
<th>Source</th>
<th>LR $\chi^2$</th>
<th>df</th>
<th>$P$ - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>0.90</td>
<td>1</td>
<td>0.343</td>
</tr>
<tr>
<td>Tide</td>
<td>2.32</td>
<td>1</td>
<td>0.127</td>
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<tr>
<td>Discharge</td>
<td>5.7</td>
<td>1</td>
<td>0.016</td>
</tr>
<tr>
<td>Day or night</td>
<td>0.48</td>
<td>1</td>
<td>0.489</td>
</tr>
<tr>
<td>Year x discharge</td>
<td>12.87</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Year x day or night</td>
<td>6.00</td>
<td>1</td>
<td>0.014</td>
</tr>
</tbody>
</table>
Table 4.7. Twentymile River (2000–2003). Effects of high tide height at time of sample, freshwater discharge, day or night, the interaction of year and tide, and the interaction of year and discharge on total catch-per-unit-effort (fish per hour) caught with dip nets tested using a generalized linear model and a quasi-Poisson distribution (LR $\chi^2$ = likelihood ratio chi squared, df = degrees of freedom, $P$ = probability). Significance determined at $P<0.05$ level.

<table>
<thead>
<tr>
<th>Source</th>
<th>LR $\chi^2$</th>
<th>df</th>
<th>$P &gt; \chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>7.60</td>
<td>1</td>
<td>0.022</td>
</tr>
<tr>
<td>Tide</td>
<td>0.85</td>
<td>1</td>
<td>0.355</td>
</tr>
<tr>
<td>Discharge</td>
<td>14.53</td>
<td>1</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Day and Night</td>
<td>2.05</td>
<td>1</td>
<td>0.152</td>
</tr>
<tr>
<td>Year x Tide</td>
<td>23.33</td>
<td>1</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Year x Discharge</td>
<td>44.69</td>
<td>1</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>
Table 4.8. Effects of year, high tide height at time of sample, freshwater discharge, and day and night, and the interaction of year on the proportion of females caught with dip nets in the Twentymile River (2000–2003) tested with a generalized linear binomial model (LR $\chi^2$ = likelihood ratio chi squared, df = degrees of freedom, $P$ = probability). Significance determined at $P<0.05$ level.

<table>
<thead>
<tr>
<th>Source</th>
<th>LR $\chi^2$</th>
<th>df</th>
<th>$P&gt;\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>0.25</td>
<td>2</td>
<td>0.880</td>
</tr>
<tr>
<td>Tide</td>
<td>1.07</td>
<td>1</td>
<td>0.302</td>
</tr>
<tr>
<td>Discharge</td>
<td>3.48</td>
<td>1</td>
<td>0.062</td>
</tr>
<tr>
<td>Day and Night</td>
<td>0.70</td>
<td>1</td>
<td>0.403</td>
</tr>
<tr>
<td>Year x Discharge</td>
<td>17.13</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
References


Appendix 4.1. Trap, seine and dip nets used to capture Eulachon in the Antler River, Alaska.
Appendix 4.1. Continued
CHAPTER FIVE

General Conclusions

Overall this study has provided important advances in the understanding of Eulachon. I have gained unique insights into the migration behavior and spawning ecology that will assist managers and researchers in the conservation of this species. This new information can be used to map and protect critical spawning habitat and improve population monitoring assessment work, both of which are paramount to recover Eulachon currently listed as “Threatened” under the U. S. Endangered Species Act of 1973 (U.S. Government 1988).

In Chapter 2, I investigated the effects of salinity on the fertilization and hatching success of Eulachon in a laboratory setting to determine if the species is physiologically capable of producing viable offspring in brackish water. I found that eggs were fertilized between 0 and 24 PSU, but not at 30 PSU. This means that Eulachon eggs can be fertilized in relatively high salinity levels. I discovered that eggs hatched successfully in a salinity of up to 12 PSU and while eggs did not tolerate static salinities at or over 18 PSU, these results demonstrate that Eulachon is physiologically capable of producing viable larval fish in the brackish water of estuaries. As tests were conducted using static salinities, it is likely eggs could possibly tolerate higher salinity levels for shorter periods in the wild. In the laboratory, Eulachon eggs survived brief exposure (30 minutes every 48 hours) to full strength seawater that was applied to provide resistance to disease (Edgell et al. 1993). Salinity in estuaries changes more frequently than my laboratory studies because of the tide and other environmental conditions such as freshwater discharge. The daily salinity fluctuations between freshwater and brackish water in estuaries
would give the eggs time to reach osmotic equilibrium after exposure to salty water. This contrasts with my laboratory study where eggs were tested with static salinity concentrations.

In Chapter 3, I studied the locations of Eulachon spawning habitat in the Twentymile and Antler rivers using radio telemetry. Where aggregations of radio-tagged fish were located, I was able to infer spawning locations, characterize the substrate where eggs were attached, and monitor the salinity. I discovered that Eulachon were spawning in the brackish water of estuaries. The spawning sites varied in salinity from 0 PSU at low tide to a maximum of 24 PSU at high tide, the same range in which eggs were fertilized in the lab. These findings are significant because this is the first confirmed occurrence of estuary spawning by Eulachon.

In Chapter 4, I investigated the relationship between spawning migration and tide height, freshwater discharge, and day or night to learn more about the spawning ecology of Eulachon. I found that peak Eulachon spawning migration occurs during the spring tide phase. During the spring phase, the water depth at high tide increases by 2.3 m in the Antler River and by 3 m in the Twentymile River over the water depth at high tides during the neap phase resulting in a saltwater wedge that pushes far upstream. Eulachon likely use the additional upstream flow and depth increase of water to assist their passage to spawning habitat. I proposed that timing up-river spawning migration with spring tides has benefits for Eulachon. First, there are obvious energetic benefits in using the spring tides to assist upstream movement. Spawning migration is arduous and energy saved could be allocated for gamete production, mating, or other behaviors that increase reproductive success. Second, migrating during the spring tide phase can increase access to spawning areas. Some rivers where Eulachon spawn are swift and mean velocities can
exceed the swimming capabilities of fish and in some cases, there may be physical barriers such as falls that prevent migration during neap tides. Lastly, migrating during the spring tides could reduce predation from avian predators because the water is deeper than during neap tides. Bald eagles avoid foraging in estuarine areas where water depths exceed 3 m (Thompson et al. 2005) and therefore, migrating in deep water likely makes it more difficult for birds to capture Eulachon.

This study was subject to a few methodology limitations that could be used to guide future research. In the laboratory, I was unable to emulate the daily salinity variation that occurs in the natural environment where Eulachon spawn. Although I was able to determine that Eulachon eggs did hatch in brackish water, it is likely that eggs could hatch in higher salinities in a natural setting due to the approximate 6 hr recovery time afforded at low tide when salinity is zero. Therefore, the laboratory results I report could be a minimum salinity level for hatching as my tests were conducted using static test salinities. However, other than the full strength seawater used to control disease, I did not test acute exposure to more frequent high salinity water. While radio telemetry proved to be a useful tool for finding aggregations of fish and suspected spawning areas, I only tracked fish during low tide when the salinity of the estuary measured near zero because saltwater attenuates the radio signal (Neizgodal et al. 1998). This method worked well in the upper estuary where salinity was near zero at low tide, but was not able to be used in the lower estuary where salinity was too high to detect the radio signal. To determine the lower limits of spawning in the estuary other methods need to be employed such as the use of acoustic tags that can operate in saltwater. Lastly, I had fish capture method limitations when investigating the effects of tide, river discharge, and day or night on Eulachon spawning.
migration. I recommend using passive traps with wing deflectors to capture fish versus seine or dip nets. Traps were efficient and collected data almost continuously and therefore likely provide more representative samples of the spawning population than dip or seine nets.

Based on my findings, I recommend expansion of designated Eulachon critical habitat under the ESA. Under the current critical habitat designation, estuaries are considered nursery areas or migration corridors and not as spawning habitat. Under the ESA, protections are afforded to designated critical habitats. Therefore, estuaries should be included as critical spawning habitat for Eulachon as recovery plans developed from this broadened definition would provide the regulatory background needed to focus mitigation and restoration projects to protect Eulachon. This is important because the estuaries where Eulachon migrate, spawn and rear are under threat from direct and indirect anthropogenic influences, such as land development (Hall and Schreier 1996; Marcoe and Pilson 2017) and climate change (Hatcher and Jones 2013). Given the nexus of critical habitat and human disturbance, it is all the more important to document the full range of habitat use by Eulachon.

The results from this study should be used to revise current Eulachon population assessment work. A common method for monitoring populations uses the density of larvae in rivers with fecundity and sex ratio to infer the size of the spawning population (Hay et al., 1997; McCarter and Hay 2003). However, some monitoring plans do not take into account estuarine spawning and instead only sample in the freshwater of rivers (JCRMS 2005). Salinity maps created using data from automated salinity measuring devices set in an array distributed across the estuary would be useful in describing possible spawning habitat. The salinity map could be compared
with the salinity tolerances for fertilization and hatching to describe where Eulachon is capable of spawning. Locations for larval sampling should be expanded downstream of the possible spawning areas.

Given the drastic reductions in the distribution and abundance of Eulachon, protection of spawning habitat and accurate population monitoring is critical to the conservation of the species. If spawning habitat is not protected in estuaries, Eulachon could suffer further reductions in distribution and declines in abundance. Likewise, inclusion of estuarine habitat in any monitoring program is crucial to understanding the status and trends of this threatened species.

References


Appendix 5.1. Institutional Animal Care Use Committee (IACUC) original approval and renewal from the University of Alaska Fairbanks.

University of Alaska Fairbanks

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Dr. Erich H. Follmann, Chair
212 West Ridge Research Building
PO Box 757270,
University of Alaska Fairbanks
Fairbanks, Alaska 99775-7560

(907) 474-7800
IACUC Web Page: http://www.uaf.edu/iacuc
IACUC e-mail: fyiacuc@uaf.edu

Subject: IACUC annual review of Assurance of Animal Care form 04-27

Dear Dr. Norcross,

The University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) received the request for annual renewal of the following Assurance of Animal Care (Assurance) using vertebrates. This renewal request has been approved.

IACUC Protocol Number: # 04-27

Investigator/Instructor: Brenda Norcross, Ph.D.
Appendix 5.1. Continued

Title of Project/Course: Effects of salinity on the hatching success of eulachon eggs 
(*Thaleichthys pacificus*) in Alaska

Original Approval Date: May 20, 2004

Please note this assurance will permanently expire May 20, 2007

*This Assurance will be valid through May 20, 2005* and must be kept current with respect to new methods or techniques as they evolve. Please note: All lab animals and captive wildlife used under this Assurance of Animal Care Form *must* be identified with the assigned IACUC number by using cage cards, door cards, or some ready method of identifying pens or paddocks with this Assurance.

Erich H. Follmann, Ph.D.
Chair of the UAF IACUC
Appendix 5.1. Continued

May 1, 2006

To: Brenda Norcross, PhD
   Principal Investigator

From: Erich H. Follmann, PhD
      IACUC Chair

Re: IACUC Continuing Review

On behalf of the University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) I have reviewed the request for renewal of the following assurance. This renewal request has been approved.

Protocol: #04-27

Title: Effects of salinity on hatching success of eulachon eggs (Thaleichthys pacificus) in Alaska

Received: May 1, 2006
Approved: May 1, 2006
Next Due: May 20, 2007

This Assurance is valid through May 20, 2007, but must be kept current with respect to new methods, techniques and personnel. This is the first of two possible renewals for this Assurance.

Thank you for keeping your IACUC Assurance up to date.