Location Confirmation of Sockeye, Coho and Pink Salmon Species on the Coast of British Columbia

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Abstract:

Migration patterns of Pacific Salmon are changing, and previous literature has suggested that monitoring species location and abundance is essential in recording their dynamic patterns. Therefore we conducted a survey of salmon species using samples from Steveston Harbour, Richmond, B.C. to provide a snapshot of confirmed salmon species in various locations along the coast of British Columbia on October 28 2018. Our data is a preliminary step to creating a model tracking population abundance and migration patterns, which would require a compilation of many similar snapshots. Analysis of samples from fisherman using DNA isolation and PCR techniques yielded a broad geographical distribution. Sockeye (*Oncorhynchus nerka*) were found in Nanaimo and the Fraser River, chum (*Oncorhynchus keta*) were found in Nanaimo and the Lasqueti Islands, and pink (*Oncorhynchus gorbuscha*) were found in Haida Gwaii. This information can be used as preliminary data towards a larger scale projection of salmon populations and migration patterns.

Introduction:

Every year, millions of Pacific salmon return from the Ocean to their natal rivers and streams to spawn. Five main species can be found in British Columbia waters from late summer to early fall: chinook (*Oncorhynchus tshawytcha),* coho (*Oncorhynchus kisutch),* sockeye (*Oncorhynchus nerka*), chum (*Oncorhynchus keta*), and pink (*Oncorhynchus gorbuscha*) (Gende et al., 2002). Unfortunately, recent decades have seen a steep decline in the number of salmon stocks that can successfully spawn (Gende et al. 2002). Threats to salmon include warming ocean temperatures which prompt them to begin their migrations increasingly earlier in the year, during suboptimal river conditions (Quinn & Adam, 1996). Of the 91 Pacific salmon stocks being monitored in BC, only 28 were projected to return in healthy numbers during the 2018 season (FOC 2018).

Extensive research has been put into finding the best way to protect Pacific salmon as they return for spawning (Gende et al., 2002). One method involves implementing fishing bans or restrictions on highly threatened species at times of the year when they are most vulnerable (FOC 2018). For example, In the 2018 season the maximum catch is limited to 4 salmon of each species per day (FOC 2018). In order

to ensure that conservation measures such these fishing bans are actually protecting salmon, it is vital to individually monitor their populations.

One means of monitoring migrating populations is using place based survey techniques: collecting several "snapshots" of each species at a specific time and locations, and compiling the data to predict abundance and migration patterns (Gayeski et al., 2018). Each snapshot is created by collecting many samples of salmon at a given time to determine the current population status in each location. Since the majority of a salmon's life cycle is spent traveling in open ocean when tracking their movements is difficult, it is most effective to take samples as salmon approach their unique natal rivers (Gayeski et al., 2018). Commercial catch survey methods has been used in previous studies as a cost effective way of attaining many samples in a short period of time to monitor salmon populations (Gritsenko et al., 2000; Klovach et al., 2000).

Therefore, we took advantage of the catch brought in by local fishermen to attain a snapshot image of when and where salmon species were passing through coastal waters as they approached their spawning streams in late October 2018. By obtaining samples from fishermen, recording where they were caught, and then confirming the reported species by running DNA gel electrophoresis analysis, we were able to answer what specific localities salmon species could be found in late October 2018 as well as determining viability of different tissues for DNA isolation and confirming that fishermen accurately reported their catch.

Materials and Methods:

Sample Collection

Thirteen salmon samples were obtained from fishermen at Steveston Harbor in Richmond on October 28th, 2018. The species label on the salmon being sold as well as the the location and date of capture according to the fisherman were recorded. The tissue type and whether the sample was frozen or fresh were also recorded. One piece of tissue the size of a fingernail was cut from each sample and placed

into an individual 1.5 mL eppendorf tube using scissors. The scissors were cleaned with 70% ethanol between each sample preparation. Samples were kept frozen overnight prior to DNA isolation.

The following methods were adapted from the UBC Biology 342 lab manual. DNA was isolated, amplified using PCR, cut using RFLP restriction enzymes and run on an agarose gel in order to identify the species of each sample by differences in banding patterns.

DNA Isolation

DNA was isolated from each sample as the first step towards molecular species identification. Each sample was mashed in an eppendorf tube with a toothpick. Next, 300 µl of cell lysis solution with proteinase K was added to each tube. The samples were incubated at 65 °C for 15 minutes and then vortexed every five minutes until the solutions looked cloudy. The samples were placed on ice for five minutes, then 150 µl of protein precipitate reagent was added to each tube. The samples were vortexed for 30 seconds and centrifuged at maximum speed for 10 minutes. The supernatant was then transferred to a new tube and 500 µl of ice cold isopropanol was added. Next, the tubes were inverted before being centrifuged again at maximum speed for 10 minutes. The isopropanol supernatant was removed from each tube and disposed of. Finally, 500 µl of ethanol was used to rinse the pellets of any leftover salts before leaving the tubes open to dry overnight.

Polymerase Chain Reactions (PCR)

First, DNA pellets were suspended in 30 µL of TE buffer. Next, a master mix (MM) was created by multiplying the components for one PCR reaction by 15 (for 13 samples plus 2 extra tubes), as seen in Fig 1. The components were kept on ice at all times. 23.0 µL of MM were then pipetted into each PCR tube. The DNA was resuspended to ensure presence throughout the solution. 2.0 µl of each DNA sample was added to each PCR tube. The 13 tubes were placed in a PCR machine and placed at 95 °C for 5 minutes, then cycled through 50 °C for 40 seconds, 50 °C for 80 seconds, and 72 °C for 80 seconds for total of 35 cycles. Finally, the tubes were placed at 72 °C for 7 minutes before being kept at 4 °C overnight. When the PCR process was complete, the tubes were frozen for storage.

Figure 1. Master Mix recipe for 15 PCR reactions.

MM Component	Amount per 1 sample	Amount per 15 Samples
1. dH_20	11.5 µL	172.5 µL
2. 50% Glycerol	5.0 µL	75.0 µL
3. 10 µM Forward Primer	1.0 µL	15.0 µL
4. 10 µM Reverse Primer	1.0 µL	15.0 µL
5. 10 PCR Buffer	2.5 µL	37.5 µL
6. 10 µM dNTP	0.5 µL	7.5 µL
7. 25 µM $MgCl_2$	2.0 µL	30.0 µL
8. Taq Polymerase	0.5 µL	7.5 µL
9. Final Volume	23.0 µL	
10. Sample DNA	2µL	

Restriction Fragment Length Polymorphism (RFLP)

The amplified DNA was cut at specific sequences using two restriction enzymes. Two master mixes (MM) were made with components for 15 NIaIII RFLP reactions, and 15 Sau3AI RFLP reactions (Fig 2). 14.5 µL of NIaIII MM and 14.5 µL of Sau3AI MM were added to 13 eppendorf tubes each (26 in total). 5 µL of each DNA sample was added to an NIaIII tube, and an Sau3AI tube. 0.5 µL of Sau3AI and NIaIII restriction enzymes were then added to each of their respective 13 tubes.

Figure 2. Master Mix Recipe for *NIaIII and Sau3AI* RFLP components.

MM for enzyme	Distilled water	Buffer Type	Buffer Volume	Total Volume
NIaIII	12.5x15=187.5 µL	CutSmart	2x15=30.0 µL	217.5 µL
Sau3AI	12.5x15=187.5 µL	Bsp	2x15=30.0 µL	217.5 µL

Gel Electrophoresis

Gel electrophoresis was performed using two 3% agarose gels (3 g electrophoresis grade agarose per 100 mL of TAE buffer). 4 µl of 6X loading dye was added to each RFLP tube and mixed. 15 µl of each NIaIII product was loaded into the first gel, and 15 µl of the Sau3aI product was added to the second gel. The DNA ladder was then added. The gel was run for 90 minutes at 120V. The base pair lengths present in our samples were determined by comparison to the DNA ladder banding scheme (Fig 3). The observed band base pair lengths were compared to those expected in the flow chart (Fig 4) to determine the species of each sample.

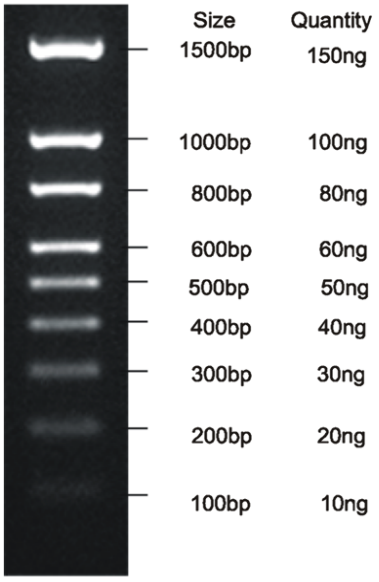


Figure 3. DNA Ladder banding scheme containing fragments of 100, 200, 300, 400, 500, 600, 800, 1000 and 1500 base pairs. Ladder provided from UBC Biol 342 Lab Manual.

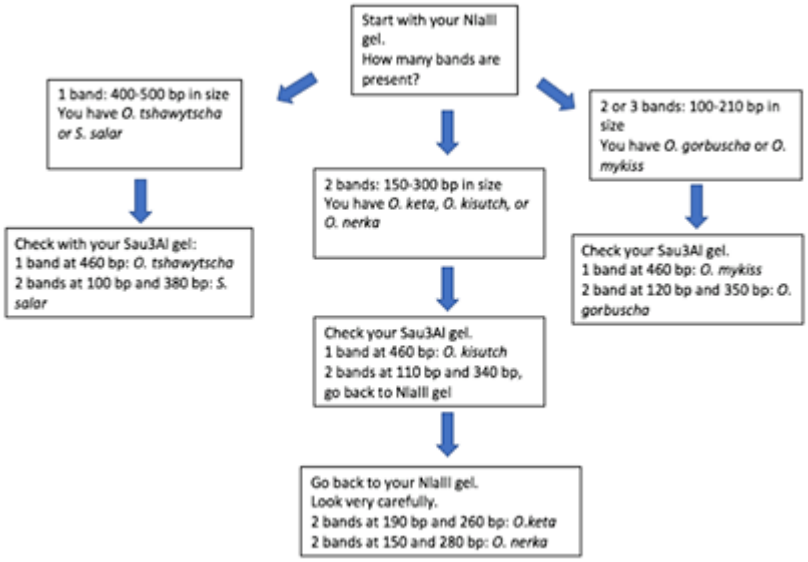
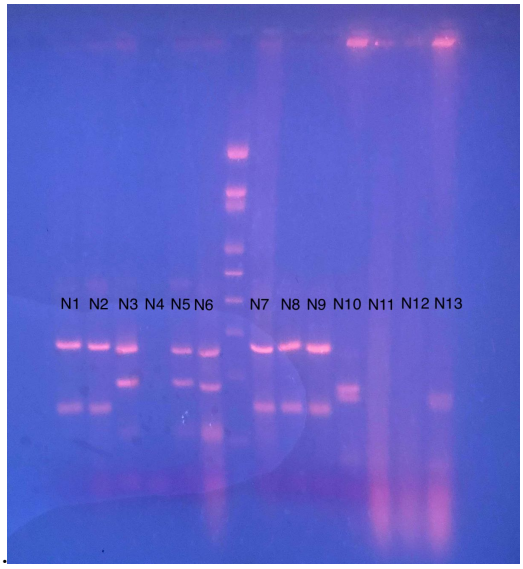


Figure 4. Salmon identification flow chart modified from Rasmussen, Morrissey and Walsh, 2010. After the two restriction enzymes have acted and samples have ran through the gel, this flowchart is looked at for identification of the sample salmon species.

Results:

We were successful in confirming locations of three salmon species in British Columbia in late October. Based on differences in banding patterns, we were able to identify the species of 10 of the 13 samples.

Samples 1, 2, 7, 8 and 9 were found to be sockeye as determined by a banding pattern of two bands on the NIaIII gel at 150 and 280, and two bands on the Sau3AI gel at 110 and 340. Samples 3, 5, and 6 were found to be chum, on account of these samples having two bands at 190 and 260 on the NIaIII gel and 2 bands at 110 and 340 on the Sau3AI gel. Samples 10 and 13 were found to be Pink, seeing that these samples had 2 bands at 180 and 190 on the NIaIII gel and two bands at 120 and 350 on the Sau3AI gel. Sample 4 had no bands in both the NIalll and the Sau3AI gels. Samples 11 and 12 had long streaks of RFLP product with no distinct bands. For these reasons, we were unable to identify the species of those samples (*Fig 5)*.

A. 

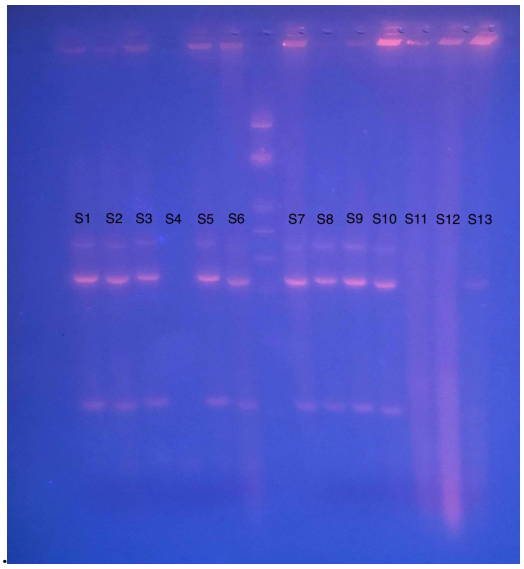
B. 

Figure 5. 3% agarose gels loaded with 13 salmon RFLP products each. Gel set at 120 Volts for 90 minutes. Samples identified as sockeye (1, 2, 7-9), Chum (3, 5, 6), and Pink (10, 13) based on their correspondence to known banding pattern for these species. *A*. Gel loaded with NIaIII RFLP product. *B*. Gel loaded with Sau3AI RFLP product.

Each of the 10 identified samples were consistent with the species reported by fishermen. Sample tissues collected included tail fin, gonad, bone, and muscle from the head. Samples were caught fresh on October 28th, 2018 from Nanaimo, Lasqueti Island, and Haida Gwaii. The Fraser River sample was caught September 2018 and frozen (Fig 6).

Figure 6. Sample number corresponding to actual vs expected sample identities, tissue types, location of capture and frozen vs fresh. Nanaimo samples: 1-5, Lasqueti Island sample: 6, Fraser River Samples: 7-9, Haida Gwaii samples: 10-13. All Samples collected in 2018.

Sample number	Actual Species	Expected Species	Location of Capture	Date of Capture	Tissue Type	Frozen or Fresh Upon Collection
1	Sockeye	Sockeye	Nanaimo	Last week of October	muscle	Fresh
2	Sockeye	Sockeye	Nanaimo	Last week of October	muscle	Fresh
3	Chum	Chum	Nanaimo	Last week of October	gonad	Fresh

4	N/A	Chum	Nanaimo	Last week of October	gonad	Fresh
5	Chum	Chum	Nanaimo	Last week of October	gonad	Fresh
6	Chum	Chum	Lasqueti Island	Last week of October	Tail fin	Fresh
7	Sockeye	Sockeye	Fraser River	Last week of September..	muscle	Frozen
8	Sockeye	Sockeye	Fraser River	Last week of September	muscle	Frozen
9	Sockeye	Sockeye	Fraser River	Last week of September	muscle	Frozen
10	Pink	Pink	Haida Gwaii	Last week of October	bone	Fresh
11	N/A	Pink	Haida Gwaii	Last week of October	Tail fin	Fresh
12	N/A	Pink	Haida Gwaii	Last week of October	Tail fin	Fresh
13	Pink	Pink	Haida Gwaii	Last week of October	Tail fin	Fresh

The three identified salmon species - Sockeye, Chum and Pink - were matched up with their locations of capture, and confirmed sites for each salmon were visually described (Fig 7). Sockeye were mapped in Nanaimo and the Fraser River, chum were mapped in Nanaimo and off of Lasqueti Island, and pink were mapped in Haida Gwaii.

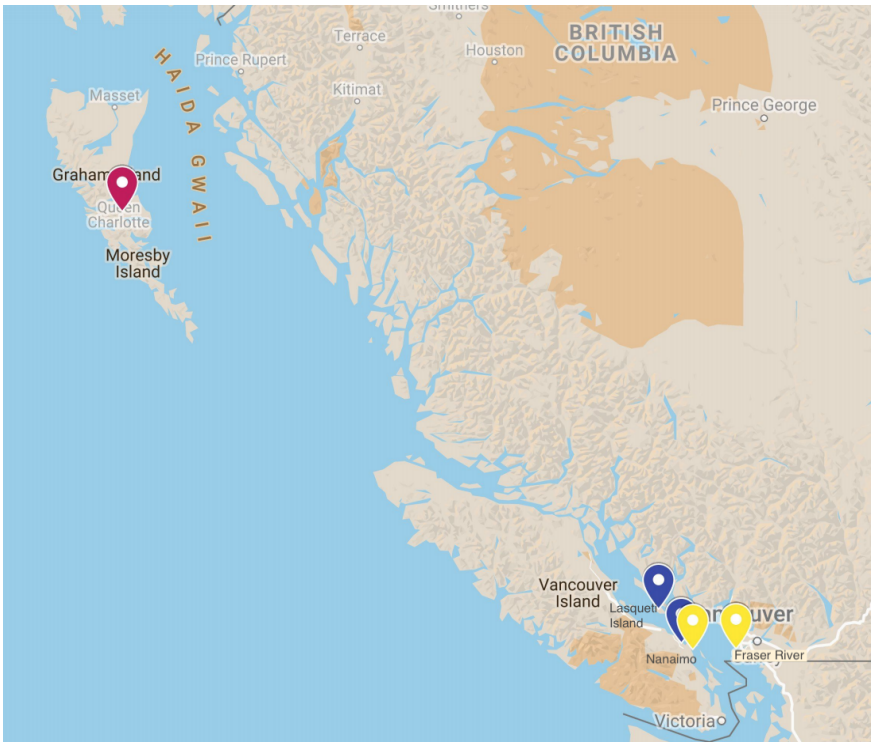


Figure 7. Confirmed locations of sockeye, chum and pink salmon on the coast of British Columbia.

Discussion:

This study aimed to map the presence of various salmon species in locations along the coast of British Columbia. The constant movement of salmon migrations in open ocean has posed limitations to standard population monitoring models, however using place based studies and commercial catch survey methods as the fish return to predictable spawning locations resolves this problem (Gayeski et al. 2018). By using samples obtained from fishermen, data regarding species type, location, and time of arrival can be mapped out.

One use of the map we created was confirming the presence of species at specific locations and looking at them in conjunction with Fisheries and Oceans Canada projections of salmon return (FOC 2018). We confirmed two samples of pink near Haida Gwaii where they were projected to have low returns, two samples of sockeye caught near Nanaimo where they were projected to have low returns, and three total samples of chum were found between Nanaimo and Lasqueti Island where they were projected

to have near target returns (FOC 2018). Although our small sample size means we cannot specifically see if those projections were correct, it is still useful to confirm if and when certain species under threat are arriving at all.

Currently there are three salmon species that have been placed on the species at risk act: sockeye, chinook and coho (FOC 2018). This means that special regulations and careful monitoring of these species is conducted to ensure that commercial and recreational fisherman use risk averse fishing management. There are also site specific restrictions that have been placed on many salmon species and which could affect the makeup of species we collected in the study samples. While Lasqueti Island, Haida Gwaii and Nanaimo are areas in which all salmon species have been deemed catchable, the Fraser River has currently banned the fishing of sockeye or pink salmon (FOC 2018). Although we did attain samples of sockeye form the Fraser River, the fish were caught in earlier in September when the ban was not in place. Due to their earlier date of catch, we did not include the Fraser sockeye in our species location snapshot for late October.

In our study we were only able to confirm banding patterns, and thus species, in 10 out of 13 samples. We can discount sample tissue type as the reason for the failed banding pattern because successful bands were achieved using DNA from all of the fin, gonad, bone, and muscles tissue types in other samples. The most likely cause of unsuccessful banding in sample 4 is failure to properly resuspend DNA before PCR. A distinct DNA pellet was observed after isolation, but the gel lane for sample 4 was empty, indicating the DNA may have been lost before the PCR step. The undistinguishable, highly streaked banding patterns of samples 11 and 12 were likely the result of protein contamination.

With its small sample size and single date of catch, our study represents a single snapshot, the preliminary step in what would be a much larger study that relies on analyzing multiple catches through a prolonged period of time, ideally lasting years (Rasmussen 2010). An expanded study would take each season's compilation of snapshots from commercial catch and, taking into account sources of error such as the accuracy of reported catch location and the limitations of fishing bans and restrictions, be able to

successfully project population parameters such as species abundance and migration timing for that year (Myers et al., 2007).

Ultimately, the goal for our project was to exemplify a model for larger scale survey techniques that could aid in conservation. It's impossible to create a plan to protect a species if it is not known where they can be found and which places are most vital to protect. For salmon, the weeks during which they migrate back to their natal streams are some of their most vulnerable and most important in terms of ensuring a future generation. Current studies tell us that salmon runs are returning in below average numbers year after year, threatening a major sector of the BC economy and its ecological stability, so it is essential that salmon populations continue to be watched closely in the coming years. The results of survey studies like ours will determine what direction conservation agencies and the government will take in advocating for new protective measures like increased fishing restrictions in the future, literally spelling out the fate for salmon in BC.

Conclusion

We were able to successfully run gel electrophoresis on DNA from fin, gonad, bone, and muscle tissue of commercial salmon samples. We were able to confirm the identification of the following species and their location of catch: sockeye near Nanaimo (and earlier in the year, the Fraser River), chum near Nanaimo and Lasqueti Island, and pink near Haida Qwaii. From this data we were able to compile a snapshot map which has possible future uses as preliminary data for projecting migration patterns and population abundance models.

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