Proceedings of the Pacific Region Workshop on Stomach Content Analyses, February 27-March 1, 2018, Nanaimo, British Columbia

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PROCEEDINGS OF THE PACIFIC REGION WORKSHOP ON STOMACH CONTENT ANALYSES, FEBRUARY 27-MARCH 1 2018, NANAIMO, BRITISH COLUMBIA

by

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Table of Contents

ABSTRACT

King, J., Boldt, J. and King, S. 2018. Proceedings of the Pacific Region workshop on stomach content analyses, February 27-March 1 2018, Nanaimo, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 3274: v + 55 p.

Fundamental to assessing and managing ecosystems (i.e. ecosystem-based fisheries management) is understanding the underlying trophic structure of ecosystems. This requires quantification of predator-prey dynamics and species interdependencies. Since species' interactions vary over time and by ecosystem, ongoing stomach content analyses is a central research priority. Recognizing this priority, the Strategic Program for Ecosystem-Based Research and Advice (SPERA) of Fisheries and Oceans Canada (DFO) funded a regional workshop on Fish Stomach Content Analyses, in Nanaimo, BC from February 27th to March 1st, 2017. The main objective of the workshop was to focus on current and future Pacific Region science needs for diet data and to recommend stomach content analyses protocols to meet those needs. The workshop was chaired by Jackie King and Jennifer Boldt from the Pacific Biological Station, DFO. The workshop was attended by 30 participants from DFO Pacific, Gulf, Quebec, Maritimes, and Newfoundland Regions as well as the US National Marine Fisheries Service and the University of British Columbia. Invited talks provided an overview of stomach content analyses protocols employed by two of the longest-standing trophic interactions programs: the Food Web Dynamics Program (Northeast Fisheries Science Center) and the Trophic Interactions Laboratory (Alaska Fisheries Science Center) of the US National Marine Fisheries Service. Invited DFO staff provided overviews of fish diet analyses and research conducted in other Regions. Staff within Pacific Region provided background on existing Regional diet research and needs for trophic interaction data. Group discussion facilitated the development of final recommended at-sea and laboratory protocols. These Proceedings summarise the workshop presentations and demonstrations, group discussion, a literature review on stomach content analyses protocols and statistical approaches for diet data, and the resultant at-sea and laboratory stomach content sampling protocols that are recommended for Pacific Region research programs and surveys.

RÉSUMÉ

King, J., Boldt, J. and King, S. 2018. Proceedings of the Pacific Region workshop on stomach content analyses, February 27-March 1 2018, Nanaimo, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 3272: v + 55 p.

La compréhension des réseaux trophiques et de la structure trophique sous-jacente des écosystèmes est fondamentale pour l'évaluation et la gestion des écosystèmes (c'est-à-dire la gestion écosystémique des pêches). Cela nécessite une quantification de la dynamique prédateur-proie et des interdépendances des espèces. Puisque les interactions entre les espèces varient au fil du temps et selon l'écosystème, le suivi du régime alimentaire des différentes espèces par l'analyse de contenus stomacaux constitue une priorité de recherche. Reconnaissant cette priorité, le Programme Stratégique de Recherche et d'Avis Fondés sur L'Écosystème (PSRAFE) de Pêches et Océans Canada (MPO) a financé un atelier régional sur l'analyse des contenus stomacaux de poissons, à Nanaimo, C.B., du 27 février au 1 mars 2017. L'objectif principal de l'atelier était de se concentrer sur les besoins scientifiques actuels et futurs de la région du Pacifique en matière de données sur l'alimentation et de recommander des protocoles d'analyse de contenus stomacaux pour répondre à ces besoins. L'atelier était présidé par Jackie King et Jennifer Boldt de la Pacific Biological Station, MPO. L'atelier a réuni 30 participants des régions du Pacifique, du Golfe, du Québec, des Maritimes et de Terre-Neuve du MPO, ainsi que du National Marine Fisheries Service des États-Unis et de l'Université de la Colombie-Britannique. Les conférences invitées ont donné un aperçu des protocoles d'analyse du contenu stomacal utilisés par deux des programmes d'interactions trophiques les plus anciens: le Food Web Dynamics Program (Northeast Fisheries Science Center) et le Trophic Interactions Laboratory (Alaska Fisheries Science Centre) du National Marine Fisheries Service des États-Unis. Le personnel du MPO a fourni un survol des analyses du régime alimentaire du poisson et des recherches menées dans d'autres régions. Le personnel de la Région du Pacifique a fourni des renseignements sur la recherche actuelle sur les régimes alimentaires régionaux et sur les besoins en données sur les interactions trophiques. Les discussions de groupe ont facilité l'élaboration des protocoles finaux recommandés en mer et en laboratoire. Ces comptes rendus résument les présentations et les démonstrations, la discussion de groupe sur les protocoles d'analyse du contenu stomacal et les approches statistiques pour les données d'alimentation, ainsi que les protocoles d'analyse en aval et en laboratoire qui sont recommandés pour les programmes de recherche et les relevés.

1. INTRODUCTION

The Strategic Program for Ecosystem-Based Research and Advice (SPERA) of Fisheries and Oceans Canada (DFO) supports research projects and scientific tool development aligned with national priorities for managing ecosystems. One of those priorities is quantifying predator-prey dynamics and species interdependencies which is required to understand the food webs and underlying structure of ecosystems. Predator-prey relationships change over time due to variations in relative abundance of prey or predators based on changes in fishing mortality and environmental drivers. Species' interactions can vary by ecosystems, depending on the mechanisms linking physical processes to biological productivity which affects species' abundance and availability. Fundamental to quantifying these predator-prey relationships, particularly if they vary over time or by ecosystem, are ongoing stomach content analyses.

Despite its importance, stomach content data are not collected on many existing surveys in the Pacific Region. When applied, diet data are collected at varying taxonomic scales, metrics (e.g. prey volume vs. prey number) and precision. Recognizing these limitations, several researchers in the Pacific Region identified the need for a regional standard protocol to be applied by field-based programs. To help address these limitations, SPERA funded a dedicated workshop with the following objectives:

1. Review historic and current approaches for stomach content analyses conducted by DFO Pacific and other Regions
2. Review programs from other jurisdictions and from academia that have extensive, ongoing stomach content analyses projects
3. Identify advantages and short-falls of various approaches for stomach content analyses
4. Identify current and future needs for diet data of Pacific Region ecosystem, food-web, and predator-prey dynamics research
5. Recommend protocols in stomach content analyses to meet those needs.

The workshop was held February 27 to March 1, 2018, at the Pacific Biological Station in Nanaimo, B.C (Appendix A). The workshop was attended by 30 participants from DFO Pacific, Maritimes, Gulf, Quebec and Newfoundland/Labrador Regions; three US National Marine Fisheries Science Service (NMFS) laboratories (Alaska Fisheries Science Center, Northeast Fisheries Science Center and Northwest Fisheries Science Center), the University of British Columbia and private consultants (Figure 1, Appendix B). Prior to the meeting a literature review on stomach content analysis protocols and statistical approaches was prepared and distributed to participants (Appendix C). The meeting was co-chaired by Jackie King and Jennifer Boldt. Stephanie King was the rapporteur. Presentations by invited participants covered methodologies for stomach content analyses employed by other DFO Regions, and long-standing fish diet investigation laboratories. Presentations from Pacific Region staff focused on current stomach content analyses conducted. Discussion periods addressed the Pacific Region's current and future diet data needs, statistical approaches and data management. On the second day, laboratory demonstrations on different protocols were conducted with preserved stomachs and participants had an opportunity for hands on evaluation of various protocols. On the final day, participants recommended at-sea and laboratory stomach content enumeration protocols for the Pacific.

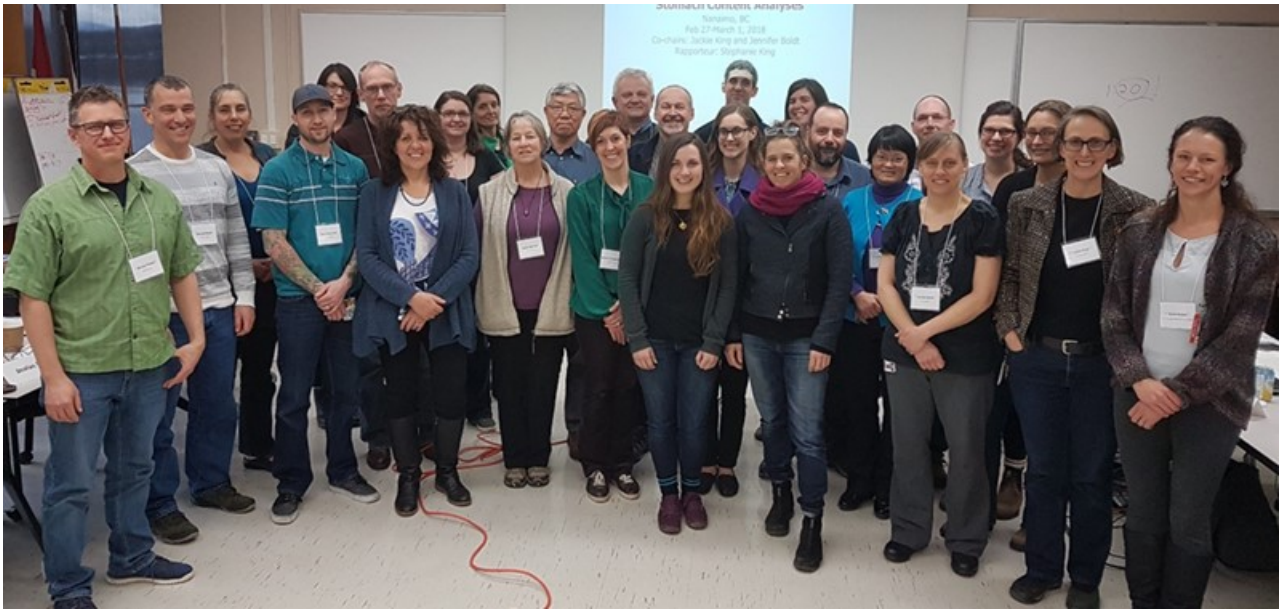

Figure 1. Participants at the February 27 – March 1, 2018 Pacific Region workshop on stomach content analyses.

2. INVITED PRESENTATIONS AND CONTRIBUTED TALKS

The first day of the meeting was a series of 20 to 30 minute presentations given by experts describing stomach sampling programs from other jurisdictions and for current Pacific Region projects or surveys. Two external experts were invited to give an overview

2.1. Long-term Stomach Content Analyses Programs

2.1.1. Food Web Dynamics Program, Northeast Fisheries Science Center, NMFS

Brian Smith, the Program Leader for the Food Web Dynamics Program (FWDP) at the Northeast Fisheries Science Center (NEFSC) in Woods Hole, MA, was invited to give an overview of the program. The FWDP's objectives are:

1. Quantify fish trophic interactions of the NE U.S. continental shelf
2. Estimate predation mortality, and model species interactions that influence the status of commercial fish stocks
3. Relate diet variability to changes in population- and community-level processes

The diet sampling program has been ongoing since 1973, with sampling in the spring, fall, and occasionally the winter and summer. The surveys are bottom trawls which follow a depth-stratified, random design and cover a larger area with 5 regions based on stock structure. The station density is about 1 station every 200nm^2; the number of stations were proportional to stratum size. There are currently 60 predators of interest and about 15000-20000 stomachs are sampled annually. The number of samples collected depends on the predator species, size and the length distribution of the tow (one fish per 1, 5, 10, 20 cm size classes).

Sampling at sea is done by two people, one is the 'cutter' (i.e. fish sampler) and other is the recorder. Recording is done on a touch screen using the Fisheries Scientific Computer System (FSCS). Each stage has a time stamp so they know exactly how long everything takes. Diet adds 10 minutes per fish to sampling per station (Link et al. 2008). Currently, a typical cruise would have 15 scientists on the ship and 7 people on each 12-hour watch. Briefly, the at-sea (macroscopic) protocols are as follows:

1. Eviscerate stomachs of individual sample
2. Estimate total prey volume (used to convert to weight in the lab) (see "wind chimes" in Figure 5)
3. Prey taxa separated, estimate % of each group
4. Prey digestion noted (Fresh, Partial, Well). This can be used to filter the data later.
5. Prey abundance estimated (only for important species, e.g. fishes, crabs and squids).
6. Prey lengths measured for key prey (estimated for prey that are not fully intact and recorded as estimated length)
7. Prey comments (parasites, trawl feeding).
    a. Don't count parasites but make a note of them.
    b. Trawl feeding is bypassed if sure, or noted if not sure.
    c. Auditing back at the lab, comments used for quality control.
    d. Empty marked as zero and move on.

Prey are generally identified to Class or Order for most invertebrates, and to Genus or Species for most fish, but it depends on what the analyst is comfortable with. Training on identification is done in a 2 hour lecture and another 1.5 hour session in the lab. They provide these courses once a year.

Microscopic examination is done in the lab on individuals <12.5 cm to reduce challenges at sea, and allow for the identification of smaller prey. About 500-600 fish are preserved at sea seasonally for further examination in the laboratory. Stomachs are preserved in formalin and the analysis is done by a NMFS biologist. Prey taxa are separated, weighed, and the proportion and total weight are calculated.

From 2004 to 2010, fish from every 25th station were preserved and brought back to lab for QA/QC monitoring. Overall, the at-sea data collected were considered acceptable. Brian noted differences between at-sea and in-lab data including: there are fewer empty stomachs in the lab data and the taxonomic resolution is higher in the lab.

Other topics covered included data gaps in the current sampling program (not all seasons and areas are sampled, e.g. less inshore). The rationale for protocols reflect changes in funding and program objectives; albeit, a core group of 20-30 species have maintained adequate sampling throughout out most of the time series. Brian described lessons learned as the program evolved including:

- The importance of sampling all fish in the ecosystem and not just the commercially important species,
- The trade-offs between at-sea vs. in-lab analyses. Many factors come into play when deciding on the best approaches for sampling programs, yet both approaches rely on visual examination thus they have similar challenges identifying digestion state.
- Diet variably and uncertainty: prey switching, preference, and abundance all play roles in diet variability, particularly when modeling. This variability translates into uncertainty and may not be received well by assessment models, but is also not required in single species assessments.
- The need to find a happy medium between what is logistically feasible to accomplish and the level of data quality or density in order to provide the best science available.

After the data are analyzed they are stored in Oracle. There are a number of statistical analyses, but they depend on the users who include people from a range of internal and external programs. Brian concluded with the following points:

- Maintaining a diet sampling program is challenging, but possible with clear mission objectives and support from field and IT staff
- For an ecosystem-understanding of continental shelves, monitoring predator-prey interactions is critical
- The FWDP has one of the largest fish diet databases available

Following the presentation, there was some discussion about the preservation methods. Evgeny Pakhomov noted that when using formalin, the specimens can lose up to 30% of the dry weight in the first two weeks. This is important if you want to calculate the gut fullness index but is not as important for proportions. Ethanol is used more frequently for genetic analysis. Rehydration increases prey weights by more than 60% when preserved with ethanol. The FWDP does not account for changes in volume due to preservation and have found that it's not a major issue when compared to measurements made at sea.

Answering additional questions, Brian clarified that the onboard sampling is set up so that groups can work simultaneously. Also, sampling time required on the cruises is not an issue and usually all of the sampling is accomplished. Brian noted that a study on the additional time required to collect stomach contents indicated that on average, only an additional 10 minutes per tow were required (Link et al., 2008). When asked, Brian noted that they had also compared day and night bottom trawl sampling, but that they didn't find much difference in diets for a limited number of species.

A number of training materials can be found on the NEFSC website at:
https://www.nefsc.noaa.gov/femad/pbb/fwdp/

2.1.2. Resource Ecology and Ecosystem Modeling Program, Alaska Fisheries Science Center, NMFS

Mei-Sun Yang, Fisheries Biologist with the Trophic Interactions Laboratory in the Resource Ecology and Ecosystem Modeling Program at the Alaska Fisheries Science Center (AFSC) in Seattle, WA, was invited to give an overview of the program and its protocols. The program began as the Gut Shop in 1982. The lab uses the protocols described in Livingston et al. (2017). Their sampling program covers three areas with bottom trawl surveys: 1) the Bering

Sea with 380 fixed stations and an additional 91 stations on the slope in some years; 2) the Gulf of Alaska with 510 randomly selected stations; and 3) the Aleutian Islands with 420 randomly selected stations. The focus species for sampling are five core commercially important species which vary depending on the area. In the Gulf of Alaska there are five subareas which are divided into depths <100 m, 100-199 m and >200 m and for each core species there are size groups that are sampled.

There are two parts of the stomach sampling protocol:

1. Stomach content analyses at sea – this is time consuming and usually they can do 10 stomachs per haul before the next haul. Stomach content analyses must wait for other biological sampling or data collection to finish first. When the catch is on the sorting table, first the length and weight are recorded, then special projects with additional sampling requirements such as otoliths take their samples, then the stomachs are sampled.

2. Stomach collection – for each haul there are 20 stomachs per haul that are preserved for later laboratory analyses

They have tried to do some analysis with microscopes at sea but they have not been successful, even when at anchor, due to the motion of the vessel.

There are three analysts working on stomach content analysis in their lab. They use the On Screen Lab Form for electronic data entry as the stomachs are being analyzed. For each prey there is a list of what needs to be recorded since it might vary by prey item, e.g. prey length, contents and weight, and data are recorded for individual fish. All prey fish are weighed and measured. For crabs they measure the length (king crab) and width (other commercially important crab).

The group has very good online resources. The diet data are in an Oracle database and are freely available using the Diet Analysis Tool (https://access.afsc.noaa.gov/REEM/WebDietData/DietTableIntro.php) with %Weight, %#, or %FO data for each haul or for each region; count data not always available (e.g. when there are hundreds of copepods).

For taxonomic identification they have developed the online Stomach Examiners Tool (SET) which helps analysts identify species based on different body parts, e.g. some vertebrae, gill rakers, setae can help distinguish species. It is a web tool that maybe publically available at https://access.afsc.noaa.gov/REEM/SET/Index.php. Workshop participants suggested making the tool available offline so analysts could use it at sea.

The REEM Stomach Content Analysis Procedures Manual is available at: https://www.afsc.noaa.gov/refm/reem/manuals/labmanual.pdf

2.2. DFO Pacific Region Stomach Content Analyses Programs

2.2.1. Strait of Georgia Juvenile Salmon Surveys

Chrys Neville, (DFO, Pacific Biological Station, Nanaimo, BC) presented stomach content procedures employed on the DFO juvenile salmon surveys in the Strait of Georgia. The standardized trawl surveys are conducted twice annually with 80-100 sets per survey. Some samples are also collected by purse seine. The focus is juvenile salmon however all pelagic fish species caught are identified and enumerated.

Over 90% of the stomachs are analyzed at-sea and from 400-700 samples are processed per day. The rest are frozen or preserved in ethanol for analysis back in the lab. The prey in juvenile salmon stomachs are identified to help examine diet overlap between species, fish health, condition and changes in diet with environmental conditions. When time permits they also examine the stomachs of salmon predators.

Stomach content analysis has been done by the same analyst (private contractor) since 1997 with help from a survey staff team of three other people who process, collect other samples (e.g. tissue for DNA, otoliths, etc.) and record data. The stomach content analyst determines sex of the fish and assesses the gut fullness based on the size of the stomach and stomach lining thinness. The stomach is removed from the just posterior to the gill arch but the intestine is not included. The stomach is cut open with scissors and contents are then scraped into a petri dish. The stomach content volume is visually estimated using experience, comparable volume blocks or graduated containers. The digestion state is recorded in 10% increments, from fresh (0%) to fully digested (100%). Water is added to the stomach sample and the species are identified with a hand lens. The percentage species composition is recorded. Any group with a volume of less than 0.1cc is considered a trace amount and is not included in the subsequent analysis. The fish remains that cannot be identified are sometimes brought back to the lab for identification. Other specimens are also preserved for more detailed analysis in the lab.

The long-term stomach content analyst for this survey is now retiring from going to sea so DFO Science leads of these surveys will need to modify how they conduct stomach content analyses. Plans include creating a photo catalogue to assist current and new sea-going staff with identification in stomach content enumeration. Also, perhaps fewer samples will be collected and there will be increased collaborations with other programs who are examining diets. One suggestion to improve at-sea identification was to take a picture of the stomach contents and have them enlarged on a screen.

It was noted by a participant that consistencies between the survey protocols used on the DFO juvenile salmon survey, the Canadian hake survey, DFO historic groundfish bottom trawl surveys are in part due to retired staff involvement in early groundfish surveys. The general procedure, that was established in groundfish surveys and subsequently applied to other surveys, was to measure the volume of the bolus, separate the contents out by prey groups and estimate prey group volumes and sometimes measuring prey fish lengths.

2.2.2. Joint US-Canada Pacific Hake Surveys

Alicia Billings from the Northwest Fisheries Science Center, Newport, OR, presented stomach content analyses conducted for the Canada/US Pacific Hake surveys. The survey is an acoustic-trawl survey with midwater trawl hauls used to validate acoustic backscatter. The survey supports stock assessment analyses. On the US portion of the survey, stomachs are collected at-sea for later lab analysis. Individual stomachs are labeled with barcodes, put in cloth bags and preserved in 10% formalin. If samples are going to be stored for a long time they are transferred to 70% ethanol once back in the lab. Fish are randomly sampled until a target number of fish is reached per tow. Regurgitated and everted stomachs are noted but not included in target number. The stomachs are opened and the degree of stomach fullness is estimated. The bolus is removed, placed on a tray and blotted. The digestion state and total bolas weight (g) are recorded, and contents are separated into the lowest taxon possible. The weight, volume and count are recorded for each taxon with subsamples taken when necessary.

On the Canadian portion of the survey diet analyses are done at sea. The three most dominant prey species (by volume) in the diet are recorded for up to 50 individual Pacific hake per trawl haul. The stomach contents are extracted and taxon identified to the lowest possible level. Empty and everted stomachs are recorded and included in the n=50 target. The prey volume is estimated by arranging prey groups into piles 1 cm wide x 1 cm high. The prey digestion states are also estimated in 25% increments from fresh to fully digested.

Alicia discussed the back log of samples and that there may be quite a bit of old diet data that need to be located. They are working on getting the data into a database and methods to compare the US and Canadian datasets. The US surveys are conducted by volunteers so they don't have the capacity to do stomach content analysis at sea. They are investigating analyzing stomach samples stratified by length.

2.2.3. West Coast Vancouver Island Pelagic Ecosystem Surveys

Linnea Flostrand (DFO, Pacific Biological Station, Nanaimo, BC) described stomach content analysis on the west coast Vancouver Island pelagic ecosystem surveys. There have been several programs collecting stomach content data on the west coast, differing in objectives and sampling design but surveys have been associated with monitoring ecological trends with Pacific Herring and/or Sardine stocks. The survey programs include:

- The WCVI Pelagic Trawl Survey – summer surface tows using transect or stratified random designs, day fishing 1997-2005 and night fishing 2005-2015 (2005 both night and day fishing).
- La Perouse Acoustic Trawl Survey – summers 2013-2014, parallel transects with 6 nm spacing

On the earlier WCVI pelagic sardine surveys, the at-sea protocol (described in McFarlane et al. 2010) was to collect 10 – 20 sardine stomachs per set and preserve them in 3.7% buffered formalin (10 to 20 pooled per jar per set). In the lab an analyst estimated the total volume (cm^3), percent stomach fullness, percent digestion and separated out the prey contents to the lowest taxonomic group. There were 14 major groups described by frequency of occurrence and percent volume . A modified index of relative importance and the Morisita-Horn index of overlap were also used for analyses.

During a study examining herring and sardine interactions in 2012, stomachs were collected, placed in individually labeled mesh bags, then preserved in 3.7% formalin for later lab analysis by the same stomach content analyst (private contractor) used for juvenile salmon surveys. In the lab the weight of the whole stomach and empty stomach was recorded, and the % fullness estimated (based on distention). The total volume of prey was estimated by comparing groups of prey to known volumes of wheat germ. The phytoplankton and zooplankton were separated using a sieve. The presence of phytoplankton taxa were recorded in subsamples. The total volume of zooplankton was estimated (phytoplankton volume was the difference between total and zooplankton volume estimates). Large prey were counted and identified. Numerous prey (>200) were subsampled. Analyses included comparing diets using a variety of approaches such as ANOVA and ANOSIM.

Since 2010, these survey programs have put more effort into consistent protocols and multi-species sampling. Since 2013, data have been entered electronically in the field. A lot of effort has gone into a database but there there is a lack of support for database management.

Currently, work is underway to link the diet data to the DFO Institute of Ocean Sciences zooplankton database.

2.2.4. Genetic Techniques Applied in Pacific Region

Angela Schulze (DFO, Pacific Biological Station, Nanaimo, BC) describe genetic techniques for species identification being used in DFO's Molecular Genetics Laboratory. Environmental DNA (eDNA) is a new technique that can describe the species composition in the environment without first targeting the specimen. eDNA metabarcoding can provide information on mixed samples with high taxonomic resolution and sample processing is relatively fast. With eDNA metabarcoding, Next Generation Sequencing is used to process 40 million reads at a time and multiple reads/sample, whereas with traditional DNA barcoding there is only one sequence read/sample and up to 96 samples/run.

Angela described the key considerations for applying metabarcoding such as the sampling design (e.g. number of biological replicates), experimental design (e.g. genetic markers are appropriate), bioinformatics (e.g. using validated algorithms), data transferability and comparability (e.g. is there a reference DNA database), and interpretation of the data (e.g. are there data available for validation).

The eDNA techniques were recently used to assess biodiversity on the Canada C3 study. Three primer sets were used on water samples from 100 locations in Canada's three oceans. They also intend to analyze zooplankton, marine invertebrates and bacteria samples.

They have also used DNA metabarcoding on marine mammal scat analysis to assess pinniped predation on salmon. Scat samples were collected and separated into hard parts for microscope analysis and soft parts for DNA analysis. In the study, 1400 seal scats were amplified with 3 primer sets and they identified 230 species. Relative correction factors were applied to examine the proportions of species. They identified 255 fish species and 5 cephalopods. Over 75% of the population diet was hake and herring, but there was a mean of 1.8 species per scat. Some terrestrial taxa also showed up in the diets.

Angela concluded with some references to other recent studies where DNA metabarcoding has been used on stomach contents including on lionfish in Puerto Rico (Harms-Tuohy et al. 2016) and on Antarctic Toothfish (Yoon et al. 2016).

One participant asked about the differences between the different metabarcoding in the eDNA study and the seal study. Angela noted that they get the same results but use different methods. In response to another question about identifying salmon stocks from eDNA, Angela said that is the goal but there are still major advances that need to be made before it's achievable.

2.3. Stomach Content Analyses Programs in Other DFO Regions

2.3.1. DFO Newfoundland/Labrador Region

Mariano Koen-Alonso is a Research Scientist from DFO's Northwest Atlantic Fisheries Centre in St. John's, NF, and was invited to describe how stomach content analyses are integrated into the Region's survey and research programs. Groundfish stock assessment bottom trawl surveys have been the major focus for stomach sampling, however, the stomach content collection has been done under B-based funding which has resulted in gaps in the time series. With DFO's Ecosystem Research Initiative in 2008, the stomach content analyses program was

revamped and the goal of sampling is now to provide diet composition of the main prey species across large spatial scales.

There are three main types of stomach contents sampling in the region:

i) Stomach contents proper – full stomach analysis on 9-10 groundfish species and 3 or more forage species

ii) Called stomachs –sampler calls out main prey item in stomachs of core groundfishes while collecting other biological data. These data give the frequency of dominant prey.

iii) Stable isotopes – this activity is still being developed and is a complementary component

There is a fall and spring survey with different sampling for groundfish and forage fish. Groundfish are sampled on every set and selected by size classes. They aim for about 6-12 fish per species per set for 30-50 fish minimum per region. The sample size is determined by cumulative trophic diversity plots. Individual groundfish are measured and weighed if possible, then their stomachs are frozen and taken back to the lab for processing. In the lab, the stomach is weighed and individual prey are identified, weighed and measured.

Forage species are also sampled on every set but only to a maximum to 25 fish per set. One set per strata group is selected for detailed analysis. Individual fish are frozen and sent to the lab for processing. Stomach contents are analyzed under a microscope. For a subset of fish the fullness index, total stomach weigh and % of main prey is recorded. For a smaller subset full stomach content analysis, including weights of all prey) is done. The processing is done by two technicians.

Mariano described some of the program's resources and requirements and concluded with some advice for stomach content programs. A key message was that a stomach content program must be part of a larger program that has clear ecosystem objectives. Other advice included:

- Define the spatial scale you want to characterize and sample accordingly
- Use simple but quantitative metrics (e.g. digestion code)
- Spread sampling out in space and time
- The combined use of full stomach content analysis and called stomachs is useful
- Focus on main prey for energy transfer
- Be adaptive. Keep the stable older techniques in addition to exploring new techniques.

2.3.2. DFO Maritimes Region

Adam Cook was invited to describe the Food Habits Program from DFO's Maritimes Region; he was unable to attend the meeting, but prepared notes and a presentation which was given by Jackie King. There have been a number of sampling programs with variations in space, time and objectives since 1998, but sampling has been more consistent since 2007. The focus has been to develop ecosystem models. They conduct stomach sampling during depth stratified random bottom trawl surveys on DFO research vessels (RV surveys) as well as on specific projects such as the lobster bottom trawl survey and MPA survey (snow crab survey that does some sampling in MPAs). The RV surveys originally sampled 40-50 species, but that was too

difficult and now there is only diet sampling on 20-25 species. The species list is on a 2-year cycle which is more cost effective, and samples are length stratified within a species and set.

Stomach content analysis on the RV surveys is done both at-sea and in the lab, but they prefer at-sea analysis because specimens are fresh. The other programs send frozen samples back to the lab where stomachs are examined individually. Taxonomic resolution is dependent on prey species, level of digestion and available time.

The Maritimes Region uses an Oracle database and an at-sea entry system with multiple checks as data are recorded. They use different types of analyses depending on the questions being asked. Species accumulation curves are used to determine sampling adequacy and Adam referred to Warren et al. (1994) for sampling design considerations. Data are being used mainly for stock assessment (for predation rates), and also for ecosystem modeling and some Marine Protected Area planning.

2.3.3. DFO Quebec Region

Denis Chabot from the Maurice Lamontagne Institute in Mont-Joli, QC, was invited to summarize stomach collection protocols in DFO's Quebec Region. Stomachs are sampled annually in August on multi-species ecosystem surveys. The surveys are stratified based on depth, and were originally designed for Atlantic cod, but stomach data are also used to describe the diets of Greenland halibut, Atlantic halibut, redfish and other species periodically for special projects. The time series started in 1993 but there are some gaps.

Originally, a range of sizes classes were sampled on all sets, but now only half of the sets and only two size classes are sampled to save time. It is faster but less effective to cover the range of sizes and also, large fish are under-sampled. Denis suggested that it would be much better to have three or more size classes. Whole stomachs are collected at-sea, frozen and analyzed back in the lab. However, small fish (typically < 15 cm) are frozen whole. Increasing the size of fish that are brought back whole is considered as one way on increasing the number of species studied during a given survey, and this will likely be tried in 2018.

Denis described the lab protocols as follows:

1. Whole stomach weighed (allows them to estimate predator length and help with data checking)
2. Stomach content bolus weighed
3. Prey are separated into taxa and the digestion stage is noted (There are three digestion categories: whole, near-whole and partial. There used to be more but it took too much time to record)
4. A photo is taken (this helps with identification checks)
5. Prey with usable length are measured
6. Prey weighed by taxa & digestion stage
    - In recent years prey are counted as well
    - Subsampling for small prey
7. Data entry in Excel
8. Data imported into R

Planktivores aren't studied as much, but when they are, the protocols are different. Large prey are counted and weighed as for other predators. Copepods are weighed together (Observed Copepod Mass, OCM) and then counted by species and ontogenic stage. A database of 'typical masses' of an undigested specimen for each taxa and ontogenic stage is used to estimate copepod mass by species and stage, i.e. number times typical mass. If the sum of all copepod masses (SM) is greater than OCM, then a correction factor is calculated (CF=OCM/SM) which represents the difference in typical masses of an undigested specimen and the partially copepod mass in the specimen's stomach. If copepods are abundant, a subsample is counted.

The advantages of the Gulf Region program are that they get very reliable and detailed taxonomic data, they can study predator-prey relationships based on size, and that they can look at ontogenic, temporal and spatial changes in diet because they have large samples sizes for the main predators. Disadvantages of the approach are that it is labour intensive, it takes a long time to process the samples, and they do not do as many species as they would like because they are limited by the number of people that they can send to sea. Denis noted that they may try the call method described by Mariano to collect data on more species.

In response to a question about using a stomach fullness index for redfish, Denis commented that fullness indices don't work for some species because they regurgitate frequently and it cannot be assumed that the full stomach content is available for analysis. This underestimates stomach fullness and makes it unreliable.

Another question was asked about other types of analyses they are using. Denis noted that they are looking at genetic barcoding in collaboration with universities. They have also considered doing fatty acids and stable isotopes for specific projects.

2.3.4. DFO Gulf Region

Hugues Benoit also from the Maurice Lamontagne Institute gave a brief summary of marine fish stomach content analyses in the Gulf Region. Prior to 2004, efforts varied over space and time and sampling objectives changed. In 2004 to 2006, they conducted intensive sampling with complete species coverage (e.g. five individuals of each species in each set), and from 2005 to 2013 they conducted sporadic seasonal sampling for cod. They have tried stomach analysis at-sea but in recent years it has been too difficult with increasingly smaller fish, at-sea capacity and lack of funding. The objective of the stomach content analysis program is to obtain diet estimates that are spatially representative for the range of predator sizes.

The typical protocol since the late 1980s has been to take individual prey weights (blotted wet weight), identify prey at the 'highest' taxonomic resolution possible, record the digestion state (4-level), and measure prey lengths if possible.

They have a unique program for cod condition monitoring which includes diet or at least total stomach weights. Data are available across seasons and are used in bioenergetics models to estimate consumption.

Hugues also described some work using stable isotopes to examine Atlantic salmon feeding habits and return rates to streams (linked to survival rates). They examine stable isotopes in the outer growth ring of scales from one season and apply a Bayesian Isotope Mixing Model to describe the components in the diet.

A participant asked if the index of herring from cod stomachs correlates with herring survey indices to which Hugues replied that there is a broad scale signal but that the data are too sparse.

2.4. University of British Columbia Stomach Content Protocol

Evgeny Pakhomov from the University of British Columbia (UBC) was invited to describe the stomach content analysis protocols used by UBC and the Hakai Institute. All of their sampling is opportunistic, often on DFO surveys, and is project-based. As a result processing is done in a laboratory. They have comprehensive data but no central database. Long-term monitoring is difficult in universities when you have students for only one or two years. Their diet data are used mainly for publications and more recently ecosystem modeling, and have been collected on the following projects:

- Rivers Inlet Ecosystem Study: 2008-2012, seining, summer season
- Pacific herring feeding ecology, Central BC, 2007-2015
- Eelgrass Study: feeding ecology of pacific salmon juveniles
- Southern SoG: feeding ecology of dominant forage fish species
- Discovery Islands – Johnston Strait interface
- Sockeye juveniles (Samantha James): 2016-2016
- Pink & chum (Vanessa Fladmark): 2016-2017
- High seas feeding ecology of forage fish and juvenile salmon

They also collect stable isotopes and scales, though not to the same extent. For example, of 30 stomachs they might collect 10 stable isotope samples, depending on funding.

The protocol for analyzing stomach contents varies with preservation method, for example formaldehyde preserved whole specimens vs. fresh or frozen whole specimens:

- Specimen is thawed (frozen specimens only)
- Specimen is weighed and measured (total, standard and fork length)
- Belly is cut open with scissors and the stomach is removed, blotted dry and weighed
- Stomach is opened and emptied into a petri dish
- Gut fullness is estimated
- The empty stomach is blotted and weighed
- Water is added to the petri dish to suspend and separate contents
- Contents are identified to the lowest taxonomic grouping possible using a microscope with an ocular micrometer
- Prey are counted or if there is a large number a randomized subsample is taken
- Prey are weighed or if there is a large number only a random subset of 10 individuals is weighed (fresh or frozen specimens only)
- In each prey group, prey items are separated by digestion state using one of three (or four) indices

For stomachs that are preserved in 95% ethanol the protocol is:

- Remove stomach from ethanol, blot, fill vial with water and let stomach soak for 30 minutes to re-hydrate
- Remove stomach from water, cut from top of esophagus to posterior sphincter and estimate fullness visually
- Remove stomach contents, blot away excess moisture and weigh
- Place under microscope with water and separate items into different taxonomic groups, each with four digestive states
- Count and weigh each group (blotting before weighing) and take max and min lengths

- Transfer contents to 20ml vial of 95% ethanol for long term storage.

Stomach juices are accounted for by drying everything. If the stomach is very small, they visually estimate the volume then convert it into dry weight. Evgeny also described an express method which is similar except that they just identify the main groups and they don't do counts of prey.

Evgeny recommended always measuring fullness in percent and to express it as a proportion of the body weight. Their method is very thorough and everything possible is measured, but sometimes it takes two days to do one stomach.

There were several questions about the methods and some discussion about preservation methods. Formalin affects weight and you have to use a conversion factor or the dry weight will be underestimated by up to 30%. The Hakai Institute preserves in 95% ethanol for genetic ID, but the samples are re-hydrated making it difficult to estimate fullness.

3. LAB DEMONSTRATIONS

In the morning on the second day, several demonstrations were given on protocols and tools that were described on the first day. Microscopes and other lap equipment were set up so participants could try out the different protocols (Figure 2). One microscope was connected to the projector so the parts of the procedure could be shown on a projector screen.

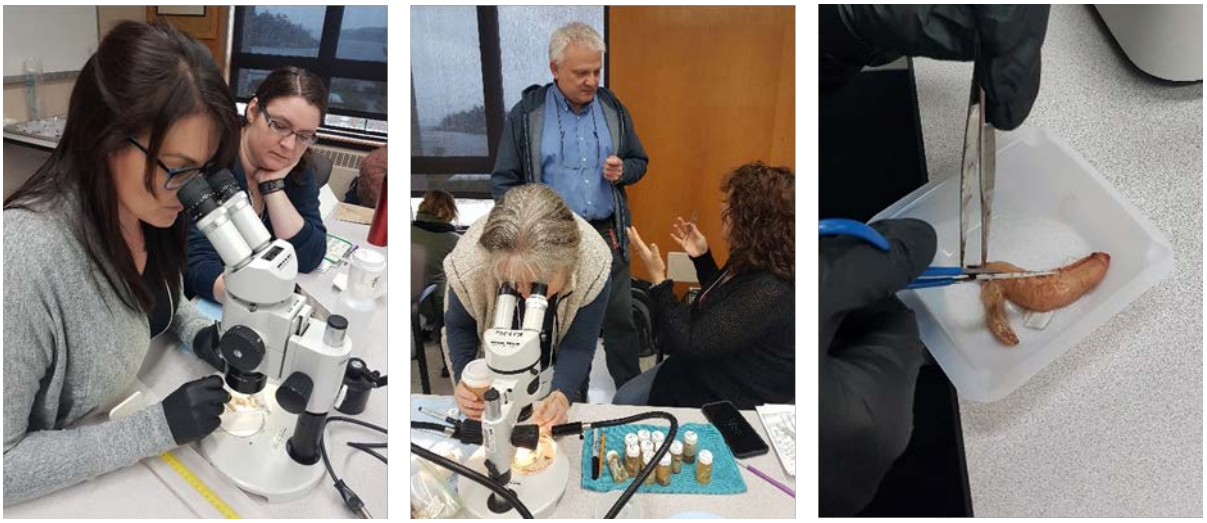

Figure 2. Participants work through various lab protocols for stomach content analysis.

3.1. UBC protocol

Evgeny Pakhomov and Vanessa Fladmark (UBC, Vancouver, BC) demonstrated the UBC/Hakai protocol (Figure 3) following the methodology outline on Day 1 and reported in Appendix C. Vanessa separated out different life stages and found one parasitic worm. For parasites, they make a note of them, measure and weigh them, but do not include in the volume.

For large numbers of prey, a subset is selected in order to capture a range of sizes. This might include laying each prey item out and visually sampling a range of sizes, or if there were a very large number (e.g. >100) record the smallest and largest individuals and then take a random selection of 10. Vanessa noted that the copepod species cannot usually be determined because they're digested quickly.

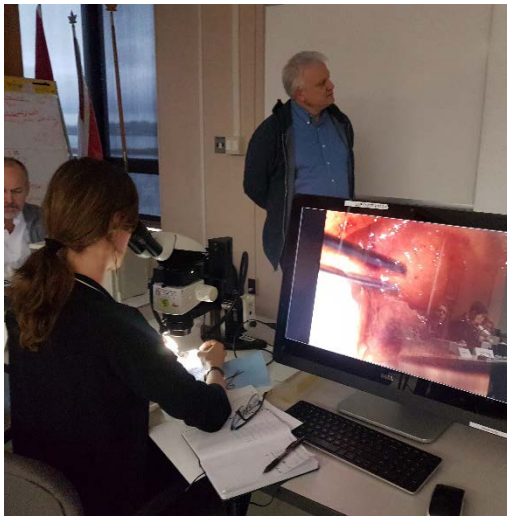

Figure 3. Vanessa Fladmark cutting open a fish stomach under a microscope.

It was noted that recording the weight of each prey group by digestion state was time consuming and probably more detail than most projects require. Evgeny noted that this level of detail was useful if you wished to identify ration size, but noted that overall it wasn't necessary and if you have lots of stomachs, then weighing the prey group and estimating proportion of digestion state is sufficient—they have done both approaches and are within 10% difference in weight. The participants asked if there were

discrepancies between the sum of individual weights and the total bolus weight initially recorded. Evgeny noted that yes they are often different, but if you are using the data to assess the diet overall, the discrepancy is not an issue. Strait of Georgia Juvenile Salmon Survey Protocol

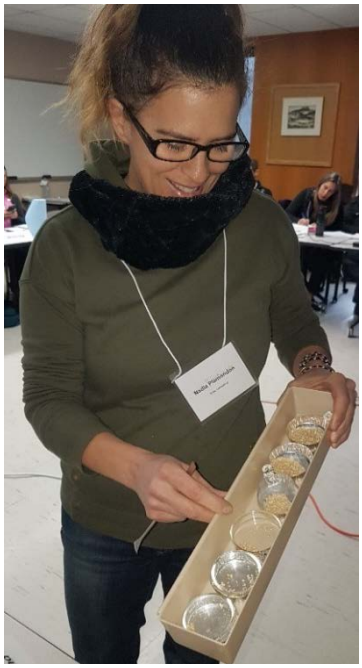

Figure 4. Nadia Plamondon shows the containers of wheat germ with a known volume to help estimate volume.

Chrys Neville and Nadia Plamondon (Zotec Consulting Ltd., Nanaimo, BC) demonstrated the procedure used at-sea on Strait of Georgia juvenile salmon surveys. When removing the stomach contents, the intestine is not included. Initially the stomach fullness is estimated. It was noted that they never have 100% fullness; a fullness of 90% would be bursting, but this is not typical. Usually they get 70-80% as the maximum stomach fullness observed. In the field they use blobs of latex to help estimate stomach fullness, in addition to noting the thickness of the stomach lining.

They have dishes of measured volumes of wheat germ to assist in visually estimating the total volume of the bolus in cm^3 (Figure 4). Digestion is estimated as a percentage from 0 (fresh) to 100% (fully digested) in increments of 10%, and the number is based on the average for all the stomach contents. Usually at 60% digested stomach contents can't be identified to species, and in those cases the sample is preserved and brought back to the lab for identification. Nadia noted that the volume tends to be the hardest thing to estimate. Prey item volumes are estimated by proportion of that group to the total volume of the bolus. Currently, stomach content weights cannot be measured in the in the field (lack of high accuracy field scales) so they do volume in both the field and the lab. The program has a collaborative project that is comparing stomach content volume and weights so that the differences can be resolved.

Participants discussed the subjectivity of the stomach fullness estimate. Nadia noted that this technique does not really work if there is a fish which can really distend the stomach lining. One comment was that the subjectivity doesn't matter as much if you have a long-term analyst but it becomes more a problem if you're comparing datasets from different analysts. The program's long-term analyst is retiring from going to sea. They are going to continue to do stomachs at sea and bring some back for validation. Chyrs noted that they don't use the fullness estimate for anything at this point, but they do use the proportion of stomachs that are empty. For example, the proportion of empty stomachs in juvenile sockeye salmon was high in 2007 which may have been related to the low sockeye return in 2009. In 2015 stomachs were full so they are looking at ways to use these data.

3.2. Taxonomic Identification resources

Mei-sun Yang demonstrated the AFSC's Stomach Examiners Tool (SET) available online at:

https://access.afsc.noaa.gov/REEM/SET/.

SET is a catalogue of photos and tips to help identify prey species. For example, it could be used to distinguish walleye pollock, capelin and sculpin using photos and descriptions of gill arches, or walleye pollock from Pacific herring based on vertebrae. They focused on main prey

items so some species (e.g. squid) aren't included. Mei-Sun noted that SET is used quite often by analysts in the lab and that it currently is not available offline (for at-sea work), but this is possible. Participants noted this would be helpful for at-sea identifications.

The Food Web Dynamics Program (FWDP) at the Northeast Fisheries Science Center provides their training materials online and includes guides for prey identification:

https://www.nefsc.noaa.gov/femad/pbb/fwdp/training/.

Denis Chabot presented an excellent set of common invertebrate species identification guides developed by Claude Nozères (Institut Maurice-Lamontagne, Mont-Joli, QC) in DFO Quebec Region. Claude has developed a series of posters to help identify common invertebrates that are now widely used in the Quebec region. The posters are accessible online at the following site:

www.researchgate.net/publication/312193447_Mini-posters_of_macroinvertebrates_in_captures_of_the_NGSL_surveys

3.3. Other tools

Participants brought other tools used for stomach content analysis. Jackie King and Tyler Zubkowski (Pacific Biological Station, Nanaimo, BC) developed a volume measuring trench. The tool is made of plexiglass with a ruler (mm) embedded on one side of a trench that is 1 cm wide and 1 cm deep (Figure 5). Prey items are placed in the trench, and packed such that they fill the trench evenly and do not extend past 1 cm high. Once packed, the volume (cm^3) is measured as the length along the ruler. For example, if the prey item once evenly packed in the trench extends along the ruler to 1.6 cm, then the total volume of that prey item is 1 cm x 1 cm x 1.6 cm or 1.6 cm^3. Small fish prey can be chopped up to facilitate packing the trench evenly. The tool can be dipped in a bucket of water between samples to clean it out, but the trench does have one end open so that contents can be rinsed out for disposal or into a ziploc bag for preservation.

Brian Smith showed the 'wind chimes' used for estimating stomach volume (cm^3) by the FWDP, NEFSC; this is a series of wooden dowels of varying diameters marked with volume in cm^3 (Figure 5). A dowel is selected that best matches the diameter of the bolus and the volume is estimated from the cm^3 markings.

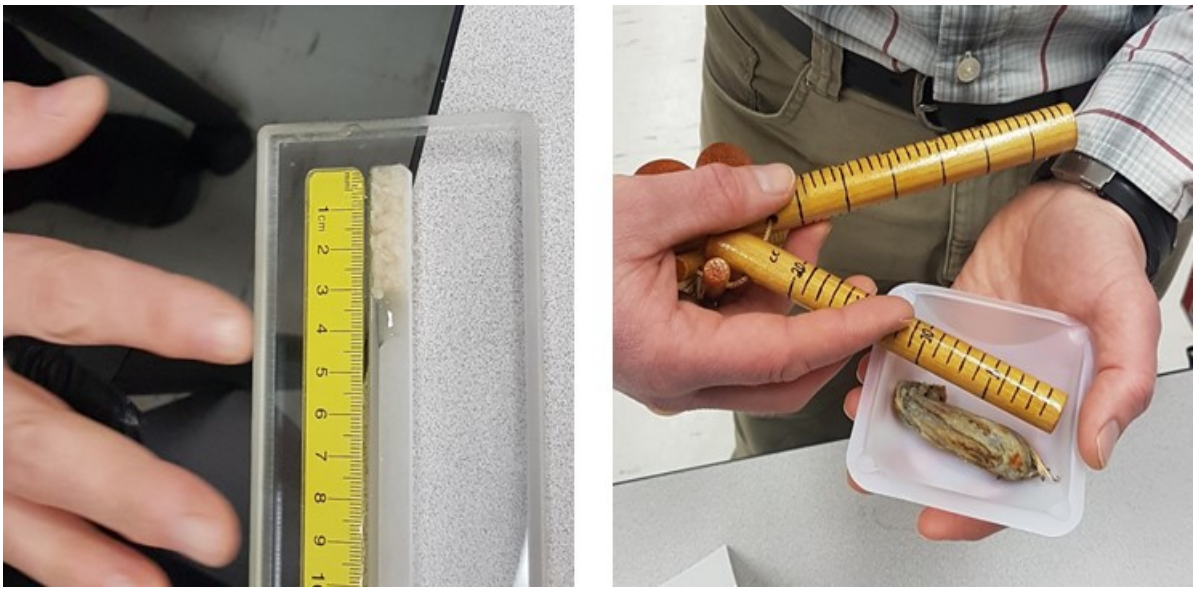

Figure 5. Tools used to estimate prey volume at-sea: left: the King-Zubkowski volume measuring trench used by pelagic ecosystem surveys at the Pacific Biological Station; right: the Food Web Dynamics Program (FWDP) at the Northeast Fisheries Science Center uses 'wind chimes' for prey volume estimation.

4. PACIFIC REGION NEEDS FOR DIET DATA

4.1. Ecosystem modelling requirements

Caihong Fu (DFO, Pacific Biological Station, Nanaimo, BC) described the diet data needs for the OSMOSE (Object-oriented Simulator of Marine ecOSystEms) individual-based model. The diet data are used to:

i) Provide information for selecting modelled species and their trophic interactions
ii) Determine predator-prey size ratio as model input
iii) Provide the basis for constructing a diet suitability matrix
iv) Calibrate the ecosystem model and validate the diet matrix

The model includes 10 to 15 key species. Other species are background species and included as biomass aggregates. Diet data help with this selection. One example was from a simulated study of predation pressure on Pacific herring (Fu et al. 2017).

One question was asked about how the maximum ingestion rate estimates are determined. Caihong said they come from literature and other models such as ECOSIM. Size ratios can be drawn from available data in the Pacific Region.

OSMOSE includes 85% of the biomass and commercial catch of fish. The model doesn't deal with different depth distributions of fish; however, there is a modified version that includes a diet suitability matrix where depth can be included. For example, if spiny dogfish are smaller than 60 cm they eat prey near the surface of the water column instead of bottom-oriented prey, such as shrimp.

4.2. Marine spatial planning requirements

Stephanie Archer (DFO, Pacific Biological Station, Nanaimo, BC) was asked to discuss applications of diet data in the Marine Spatial Ecology and Analysis Section (MSEAS). MSEAS works on identifying important areas and MPAs and also provides advice related to monitoring, risk and vulnerability in these areas. One example of their work is using a tropic approach to developing ecosystem indicators for sponge reefs in Hecate Strait. Other components of their work deal with estimating ecosystem function and identifying critical species interactions. Fish can be used as samplers to identify productivity hot spots and range shifts. Diet data could help identify risks to ecosystems, such as accumulation of microplastics in filter feeders and impacts of anomalous events in MPAs (e.g., like the pyrosome bloom in 2017). The group is still figuring out what kind of diet data they need, but diet data and prey preferences need to be spatially referenced. Prey composition could inform biodiversity, and to build energy flux networks. Measures of stomach fullness or proportions of empty stomachs may also be useful.

4.3. Alternative diet analyses and bioenergetics linkages

Strahan Tucker (DFO, Pacific Biological Station, Nanaimo, BC) gave a presentation about using fatty acids (FA) and stable isotopes as alternate or complementary means of diet estimation. There are 35 core fatty acids that tend to be stable after digestion and can be used to make quantitative estimates of diet composition with a measurement of error. They use a mixture model to estimate the combination of prey that creates the signature in the predator. An example of use was given in Budge et al. (2004) where estimates in fish were within 10% of the actual values. Strahan also referenced several recent publications with new developments and applications (Bromaghin et al. 2017a, b and Bromaghin 2017). There was some discussion about how to tell the difference between omnivores eating omnivores.

Next Strahan described the use of stable isotopes to describe trophic position (nitrogen) and carbon source (e.g. terrestrial/aquatic; pelagic/benthic; nearshore/offshore). Differences are transferred throughout the food webs and they provide a 2-dimensional snapshot of diet. Advantages to biochemical approaches to diet estimation include that they are space/time integrated and that each individual is an independent sample.

Finally Strahan discussed transposing taxonomic descriptions of diet into other ecologically relevant currencies most notably an energy context; important for understanding energy content of consumers and prey can be measured directly through bomb calorimetry or the biochemical approaches previously described are already relevant indexes. Fatty acid analysis provides an estimate of total lipid content or individual FAs can be grouped to understand proportions of essential FAs. From stable isotope analysis, the ratio of total carbon to nitrogen provides a good surrogate of total lipid content. Moreover, the SI signature itself is already an integrated, standardized index of diet readily amenable to contrast and dietary niche concepts.

For FA of fish, samples come from the belly flap or muscle and are ideally kept at -80°C but it's not as much of an issue for larger fish. For both approaches, different tissues have different turn over times so multiple tissues could be measured to look at diets over different time periods. Costs are $15 to 22 / sample for stable isotopes and $ 100 to 120 for fatty acids (compared to stomach contents which average $21/ stomach).

5. DISCUSSION

5.1. At-sea vs. laboratory sampling

The Co-Chairs reviewed a table comparing at-sea and laboratory stomach content analysis to help establish some recommended protocols (Appendix D Table 1). Participants agreed that a major advantage of processing samples at-sea is that it's more cost effective. It eliminates the time spent preserving samples and potential sample labelling errors when fish are processed for later lab analysis. However, some platforms do not have the space or time to analyze samples at sea (e.g. DFO Newfoundland/Labrador surveys). Also ship time is expensive so it depends on how the analysis fits in with other work. Again it was noted that the study by Link et al. (2008) illustrated that on average, stomach content analyses at sea adds an additional 10 minutes in the processing and sampling of research tows.

It was noted that analyzing small body predators at sea can be challenging, however it was pointed out that small or difficult samples can always be brought back to the lab. At sea analysis will generally have lower taxonomic resolution and may be more prone to misidentification. However, there are not always resources for lab analysis, so even if the at-sea data are lower in taxonomic resolution, at least they are collected. Doing analysis at sea allows the researcher to see what's happening in real-time and potentially adapt sampling.

Chemicals can be an issue for samples that are brought back to the lab. Safety and disposal are both issues. On the hake surveys, they are required to have someone that is specially trained in working with chemicals on board. Freezing is an alternative to preserving the specimens in chemicals.

Several participants agreed that the type of sampling will depend on the objectives and breadth of the research questions. A good option might be to include both at-sea and laboratory analysis in the protocols. In addition, taking pictures of the samples at-sea can help with verification.

5.2. Discussion on diet metrics

The group developed a table of pros and cons on the various metrics for stomach content analysis (Appendix D Table 2). Generally:

<u>Counts</u>

- Counts do not give enough information because of the different energy contents of different individuals, but they can be useful for prey preference and selectivity.
- Counts can be a useful measurement, but weight provides a measure of energy transfer.
- Digestion rates of prey varies, possibly skewing what is observed in the stomach.
- Secondary counts (from prey inside of prey) can also be a problem.

<u>Weights/volume</u>

- For small fish measuring weights at sea is not always practical, but volume can be estimated. Some analysts find that weight is quicker. In the Quebec Region, analysts at sea can measure to 0.1 g on a motion-compensated scale which works for larger prey.
- A solution is to come up with a cross reference between volume and weight.
- Volume can be estimated using a visual method, a volumetric sampler or wind chimes. Volume estimates can be quick but measurements can take longer.

- Some studies suggest that weights are better; they are more objective and can be reconstructed but it is a problem for meal size determinations.
- Blotting small prey can be a problem. Suggested solutions included:
    - moving the prey that is less digested to the side and weigh the liquid
    - wet the cloth and ring it out so that prey don't get stuck to the cloth when they are blotted

Lengths

- Predator:prey size ratio is important for modeling trophic interactions.
- Measuring zooplankton lengths allow you to understand prey selectivity; for herring or other prey, this can help identify size/ages classes.

Digestion state

- When prey are highly digested, the diet data are less reliable
- The general preference is to have fewer categories (e.g. three categories used by NEFSC and the Quebec Region).
- This is one of the least crucial metrics and is subjective.
- There must be an unidentifiable prey category.
- Can be useful for identifying peak feeding times, or identifying feeding chronology.
- It depends on fish and temperature.
- One suggestion was to exclude it for fieldwork, but in the lab do it because there is more time.

Stomach fullness

- It's a subjective index if estimated visually.
- It could be based on content weight as a % of body weight, in which case it is not subjective.
- Time consuming, except if estimated from other metrics taken during stomach content analysis.
- It's useful for recording the extremes, i.e. very full or empty; however empty stomachs are always recorded).
- It could be expressed as three categories.
- Some groups do it because it's always been done, but it's not used.
- It is related to depth and species because of regurgitation.
- Some groups also use it to classify between everted, regurgitated and empty, but it was noted that those observations should be recorded in a dedicated field in the database.

Other comments

Mariano Koen-Alonso suggested called stomachs (as used in Newfoundland/Labrador Region) is a good option for establishing dominant prey and participants spent some time discussing the method. It helps optimize time, and other information can be obtained from subsampling. Calling more than one prey item per stomach is also an option. However, Link et al. (2008) showed that there is a marginal investment in time for weighing stomach contents. However, it was noted that calling is even faster because the way fish are cut open for calling is different. Mariano noted that their protocols are different depending on the species and priorities. For groundfish they measure length, weight and diet (i.e. stomachs preserved for laboratory analyses), but only fish that are selected for maturity are called stomachs.

5.3. Methods for statistical techniques

At the end of the second day statistical methods for summarizing and analyzing stomach content data were reviewed and discussed, with a focus on methods summarized in Chipps and Garvey (2006) (Appendix D Table 3). For an examination of predator impact on prey, measures such as frequency of occurrence (FO) and percent composition by number or weight can be used. Relative prey importance can be assessed using the mean percent number/ weight, stomach fullness, or indices of prey importance such as the Index of Relative Importance (IRI) or the Prey Specific IRI. Other indices are available to examine prey selectivity, diet overlap and energy flow. Energy density estimates are becoming more important in the Pacific region and are a quantitative way to get at the nutritional value of prey.

Participant comments on metrics included:

- Observed weights, numbers, size classes and partial fullness indices are better indices than FO.
- Fish that have spawned may weigh less therefore, calculating stomach fullness as a percent of body weight may be misleading. Length cubed could be used as an alternative to weight of predators that have spawned.
- When calculating indices, prey weights should be examined in individual fish and then averaged over all fish at a station.
- Bootstrapping is a good method for getting variance estimates.
- It is difficult to estimate the variance for IRI and often the IRI has to be deconstructed to interpret and understand it. A better alternative to the index of prey importance is a 3-dimensional representation (i.e. percent weight, percent occurrence, percent number).
- The Maritime Region uses a gastric evacuation model to correct the relative abundance in the stomach, because of varying prey digestion rates.
- Energy flow indices describe the contribution by energy, but the predator can't always use all the calories.
- The energetic value of prey is affected by the proportion of indigestible parts, which varies with species.

6. RECOMMENDED PROTOCOLS

Several discussion periods were set aside to allow for group development of recommended protocols for at-sea and laboratory stomach content analyses. An initial point of discussion was the selection of species to sample for stomach contents. It was agreed that the intent of broad-scale diet studies is to elucidate the predator-prey interactions underlying the ecosystem structure, then sampled species should be ecologically relevant, and not only commercially important. Participants suggested sampling 'species of interest' and functional groups' rather than commercially important species. The disadvantage of this approach may be the selection of species that are rare which may be difficult to sample consistently over the long-term. It was stressed that the species list needs to be adaptive to accommodate changes.

Another consideration for discussion was the number of specimens to sample per species, which depends on the objective of the research questions but agreement on some generalities

was reached. First that stomach content data should be collected for individual fish, i.e. not pooled across fish. Since most surveys employ a random stratified design, discussion focused on the number of samples to analyze per tow per strata, so inferences could be made for each stratum. One approach would be to collect a few samples per tow, and collect samples across multiple tows in a strata to avoid pseudoreplication. The Pacific Region analyst on juvenile salmon surveys indicated that about 5 stomachs per species per tow may adequately represent the diet composition in that tow. That might be applicable where species examined are roughly the same size, but in other instances it is preferable to collect length-stratified diet data. In Newfoundland/Labrador Region, 6 samples per species (aside from redfish) per area is a target number, with 2 'small', 2 'medium' and 2 'large' fish selected. The NEFSC collects length-stratified diet data; they do not have a target number but rather attempt to sample one fish per 5 cm interval across the species' length distribution for each stratum. There are systematic ways to select sample size such as cumulative prey curves or power analyses. It is important to remember that sample size determination based on archived data must match the taxonomic resolution that will be employed in the field or laboratory. Stomachs from small predators (e.g. 60 grams) should be preserved and sent back to the lab; it is more reliable to identify prey with a microscope and easier to weigh prey items in a lab setting.

In some of the protocols, the bolus is weighed as well as prey groups. Total bolus weight is one way to account for digested material. Some groups don't weigh the bolus, but use the cumulative weight of prey groups, eliminating one step in the analysis, and making it easier to record diet data.

It was noted that some programs in the Pacific Region are reluctant to initiate at-sea diet data collection because it would add to sample processing time, and, if prey weights are needed, would require new fine-scale motion-compensating scales. While motion-compensating scales with an accuracy of 0.01-0.05 g are available, they are expensive and currently not owned by programs in the Pacific Region. Also, some programs do not have the staff capacity or funds to conduct stomach analyses in the laboratory. The group agreed that at a minimum, quantitative volume by prey group determined with the NEFSC 'wind chime' or with the King-Zubkowski volume sampler (Figure 5) would be a suitable starting at-sea protocol; particularly if it was seen as a pilot protocol to which adjustments could be made. While it was discussed twice previously, it was again noted that the study by Link et al. (2008) illustrated that stomach content analyses at sea adds on average an additional 10 minutes in the processing and sampling of research tows. It might be a misperception by Pacific Region staff that additional sampling time is a constraint to initiating at-sea diet data collection.

Regarding electronic data recording, the DFO groundfish group has developed their own system that includes diet data collection options that could be further improved. The US Hake surveys are exploring the option of using GitHub so data are open source. The Atlantic region uses an MS Access system in the field which is linked to Oracle. Other groups use Python but have encountered issues using it on a tablet.

It is difficult to identify net feeding, but if it's suspected, the NEFSC protocol is to discard the fish. Participants agreed that suspected net feeding, regurgitated and everted samples should be recorded. Fish that have regurgitated or everted stomachs are not preserved for laboratory analyses. Earlier discussion noted that preservation of stomachs affects prey weights; different preservation methods have varying impacts on prey weights. Most participants recommended freezing as the best method to minimize effects on prey weights and to eliminate the use, transport, and disposal of chemicals; however, lack of freezer space on ships is sometimes an obstacle.

There are a wide range of digestion codes used in the Pacific Region and elsewhere, and most participants agreed that assignment of a percentage of fully digested, particularly at increments of 10%, are subjective and difficult to standardize between recorders. Participants could not identify instances where digestion state was used in analyses or reported in scientific publications. For the most part, it serves as a useful filter in the database for selection of records. The group agreed that the digestion codes of NESFC were preferred (Fresh, Partial, Well) and should be used, with the intent that state of digestion would be used as a data filter for reliability of prey identification or possible net feeding.

Prey weights are needed for ecosystem modeling, and while difficult to measure accurately at sea they can be done in the lab. It was noted that at sea sampling takes minimal extra time per tow. Laboratory stomach content analyses will require additional staff or funding resources for most survey programs.

If prey length measurements are required, the DFO's Institute of Ocean Science zooplankton lab standards should be used. However, it was noted that if the prey species and life stage are identified, then average length and dry weight estimates are already available.

There is no universal taxonomic resolution to implement, since the resolution will depend on the project but it is strongly encouraged that prey are identified to the highest taxonomic resolution that the recorder feels confident making.

6.1. At-sea protocol recommendations

1. Data should be collected for individual fish (i.e., not pooled), along with other fish morphometric and biological data*.
2. Select species to sample based on those that are representative of functional groups in the ecosystem and species of interest for directed studies. The list of species should be adaptive and could change over time.
3. Number of specimens by tow and area: determine the minimum sample size required based on objectives and species in identified areas, strata, or area of interest (e.g., using cumulative curves). Sample size will vary by species. Consider the survey design when determining sample size – it's better to get a small number from each tow in multiple tows per area; distribute the effort across areas. Ensure the sample size matches the intended taxonomic resolution of diet data and analyses that will be done. Identify a standard sample size for unknown areas/species (e.g., 5 samples per size stratum and species). Identify a maximum sample size.
4. Predator size stratification: ensure diet data are collected across predator size strata; e.g., 2 fish per 3 size classes.
5. If possible, it may be advantageous to use electronic data acquisition software to ensure data are available faster; this can be Quality Assured and Quality Controlled easily, imported directly into a database, and can help samplers identify when to collect a stomach.
6. Remove stomach from the anterior end of the oesophagus to the pyloric sphincter; for small predators, preserve for lab analysis**.
7. Identify and record empty stomachs
8. Identify, record and reject for further sampling, everted, regurgitated stomachs, or in-net feeding (look in mouth)

9. Remove bolus from pyloric and cardiac parts of the stomach to petri dish and sort prey taxa
10. Identify prey to lowest taxonomic level that sampler is comfortable with using naked eye or hand lens (if more detailed level needed, preserve for later lab analyses)
11. Quantify prey categories using one of the following methods:
    - Weigh prey categories (possibly modify data acquisition software to collect cumulative weights for taxonomic categories) OR
    - Collect quantified volume estimates of prey categories.
12. Identify digestion state (Fresh, Partial, Well) for each prey category.

*special projects and protocols to collect tissues for fatty acid and stable isotopes and DNA, as required.

**label fish for lab analysis; ensure labels do not get lost; barcoding may work

6.2. Laboratory protocol recommendations

1. Stomachs or fish to be preserved at sea should be frozen (alternatives such as formalin, as needed with consideration for transport, spill prevention and disposal costs). Samples that are sent to the lab should meet sample size, predator, predator size, and area sampling requirements and should exclude fish stomachs that were everted, regurgitated, or indicated as in-net feeding.
2. Data should be collected for individual fish (associated with morphometric and other biological data)
3. If possible, it may be advantageous to use electronic data acquisition software to ensure data are available faster; this can be Quality Assured and Quality Controlled easily, imported directly into a database.
4. Remove stomach from anterior end of the oesophagus to the pyloric sphincter.
5. Identify and record empty stomachs.
6. For whole fish, identify, record, and reject for further sampling regurgitated or everted stomachs that were missed in the field.
7. Record stomach content weight (~0.001g):
    - Blot dry
    - Weigh full stomach
    - Remove bolus from the pyloric and cardiac portions of the stomach and put into petri dish
    - Weigh empty stomach
8. Identify prey to lowest taxonomic level possible (higher resolution than field protocol; level of taxonomic identification will be project-dependent; e.g., fish and euphausiids identified to species; life history stage may or may not be needed [e.g., crabs megalops vs zoeae]);
9. Identify digestion state (Fresh, Partial, Well) for each prey category
10. Weigh each taxonomic category (~0.001 g)
11. If prey are in good condition, measure prey (or subsample of prey); length measurements to be standardized (i.e., standard lengths of fish, copepod length

measurement standards vary; refer to DFO's IOS zooplankton lab for standards); length and weights can be reconstructed from historic data (e.g., zooplankton database)

6.3. Additional protocols (project-specific):

- Count prey items (depends on goal); weigh or reconstruct weight
- Lengths of prey items (depends on goal; needed for ecosystem modeling, such as OSMOSE)
- Photos of prey items could be taken for archive or for double-checking identifications, volumes, etc; the constraint of collecting photos is that they have to be filed, catalogued and stored; also need to set up a photo station to get quality photos

7. CAPACITY BUILDING RECOMMENDATIONS

After reviewing the protocols, participants discussed options for capacity building. Co-op programs and graduate students provide a collaborative opportunity, but they are often around for only a few months and graduate projects often take years to complete. There is an excellent opportunity for capacity building with First Nations and citizen science programs, and partnerships could be made with NGOs. For some techniques, only limited training is needed. The Quebec Region uses Co-op students, but it takes more than a week to get them trained. Other options are contractors, but they tend to be expensive and their rates are going up. Ideally, DFO's Pacific Region would develop a stomach content analysis program with dedicated staff similar to the existing programs at US NMFS science centers. This program would align with DFO's mandate for ecosystem-based fisheries management and support ecosystem science research programs. Valuable initial steps include conducting training workshops and developing manuals and guides. Pictures of prey categories are great training tools (e.g. posters that Quebec Region has developed).

8. STANDARDIZATION PROJECTS

There are several standardizations projects that should be undertaken in order to address calibration between historical measurements and the recommended quantitative approaches outlined above, and to calibrate between at-sea and laboratory analyses:

1. Qualitative stomach fullness estimates vs. stomach content weight (as percent body weight) for calibration of archived stomach fullness data;
2. Stomach content volume to weight conversion for calibration between the two measures;
3. Qualitative volume estimates vs. quantitative volume estimates for calibration of archived volume estimates based on qualitative approaches.
4. Corrections for preserved prey (frozen, ethanol, formaldehyde) vs. fresh prey to obtain conversion factors.

9. ACKNOWLEDGEMENTS

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APPENDIX A. MEETING AGENDA

Pacific Region Science Workshop on Stomach Content Analyses

February 27-March 1, 2018

AGENDA

Seminar Room, Pacific Biological Station

Nanaimo, BC

Objectives:

6. Review historic and current approaches for stomach content analyses conducted by DFO Pacific and other Regions
7. Review programs from other jurisdictions and from academia that have extensive, ongoing stomach content analyses projects.
8. Identify advantages and short-falls of various approaches for stomach content analyses.
9. Identify current and future needs for diet data of Pacific Region ecosystem, food-web, and predator-prey dynamics research.
10. Recommend protocols in stomach content analyses to meet those needs.

DAY 1: Tuesday, February 27th			
Time	**Agenda Items**		**Speakers**
9:00	*Introduction* • overview and objectives of the workshop (attached is a modified version of the funding proposal)		Jackie King Jennifer Boldt
9:10	*Literature Review Part I* • methodologies from the literature		Jackie King
9:25	*Programs from other jurisdictions* *30 minutes each* • Food Web Dynamics Program (Northeast Fisheries Science Center) • Trophic Interactions Laboratory (Alaska Fisheries Science Center)		Brian Smith Mei-Sun Yang
10:25	**Health Break**		**20 min**
10:45	*DFO current and historic approaches for stomach content analyses 20 minutes each* • Pacific Region o juvenile salmon surveys o joint Canada-US hake surveys o pelagic ecosystem surveys o genetic approaches		Chrys Neville Alicia Billings Linnea Flostrand Angela Schulze
12:00	**Lunch (not provided)—the cafeteria has sandwiches, soup, salads and a hot lunch special (<$12), cash only (Canadian)**		

DAY 1: Tuesday, February 27th			
1:00	*DFO current and historic approaches for stomach content analyses continued* *20 minutes each* • Gulf Region • Quebec Region • Newfoundland/Labrador Region • Maritimes Region		Hugues Benoit Denis Chabot Mariano Koen-Alonso Jackie King
14:20	**Health Break**		**20 min**
14:40	*Discussion of Day 1 – sampling methods* • commonalities/differences • other programs and methods that exist but not represented? • pros and cons of each method/protocol o do we agree with authors' assertions (table in literature review)		All
16:00	**End for the day**		
18:00	**Event dinner (not provided) – Coach & Horses Pub, 321 Selby Street**		

DAY 2: Wednesday, February 28th			
Time	**Agenda Items**		**Speakers**
9:00	*Programs at universities and student project opportunities* • Institute for the Oceans and Fisheries (UBC)		Evgeny Pakhomov
9:20	*Laboratory Demonstration and Show & Tell* • UBC/Hakai Institute • DFO juvenile salmon surveys • Trophic Interactions Laboratory (AFSC) • Food Web Dynamics Program (NEFSC)		Evgeny Pakhomov Nadia Plamondon Mei-Sun Yang Brian Smith
10:40	**Health Break**		**20 min**
11:00	*Laboratory Demonstration and Show & Tell* • group look at samples – try out protocols • if you have any specialized gear that your program has developed or used, please bring for our Show & Tell		All
12:00	**Lunch (not provided)—the cafeteria has sandwiches, soup, salads and a hot lunch special (<$12), cash only (Canadian)**		
1:00	*Pacific Region needs for diet data* • ecosystem modelling requirements • marine spatial planning requirements • alternative diet analyses & bioenergetics linkages		Caihong Fu Stephanie Archer Strahan Tucker
2:00	Literature Review Part II • diet indices • statistical approaches		Jackie King Jennifer Boldt
2:15	*Discussion of Day 2*		All

DAY 2: Wednesday, February 28th			
	• identify the current and future needs for diet data that can realistically be supported • sources of funding • student opportunities – pros and cons for DFO • pros and cons of statistical approaches o do we agree with authors' assertions (table in literature review)		
15:00	**Health Break**		
	Discussion of Day 2 continued • how are data linked within survey databases? • how are data linked to zooplankton/other data?		All
16:00	**End for the day**		

DAY 3: Thursday, March 1st			
Time	**Agenda Items**		**Speakers**
9:00	*Workshop Recommendations* • at sea protocols • laboratory protocols • analytical approaches • capacity building • diet protocol standardization		All
10:15	**Health Break**		
	Workshop Recommendations continued		All
12:00	**End for the day**		

APPENDIX B. LIST OF PARTICIPANTS

Name	Affiliation
Stephanie Archer	DFO-Pacific Region
Sonia Batten	Sir Alister Hardy Foundation for Ocean Science
Hugues Benoit	DFO-Quebec Region
Alicia Billings	NMFS, Northwest Fisheries Science Center
Jennifer Boldt	DFO-Pacific Region
Manon Cassista-Da Ros	DFO-Maritimes Region
Kristina Castle	DFO-Pacific Region
Denis Chabot	DFO-Quebec Region
Adam Cook*	DFO-Maritimes Region
Lindsay Dealy	DFO-Pacific Region
Hilari Dennis-Bohm	DFO-Pacific Region
Vanessa Fladmark	UBC
Linnea Flostrand	DFO-Pacific Region
Caihong Fu	DFO-Pacific Region
Moira Galbraith	DFO-Pacific Region
Vanessa Hodes	DFO-Pacific Region
Jackie King	DFO-Pacific Region
Stephanie King	Sea This Consulting
Mariano Koen-Alonso	DFO-Newfoundland Region
John Morris	DFO-Pacific Region
Chrys Neville	DFO-Pacific Region
Evgeny Pakhomov	UBC
Nadia Plamondon	Zotec Consulting
Cliff Robinson	DFO-Pacific Region
Angela Schulze	DFO-Pacific Region
Brian Smith	NMFS, Northeast Fisheries Science Center
Chelsea Stanley	DFO-Pacific Region
Strahan Tucker	DFO-Pacific Region
Malcolm Wyeth	DFO-Pacific Region
Mei-sun Yang	NMFS, Alaska Fisheries Science Center
Tyler Zubkowski	DFO-Pacific Region

* unable to attend

APPENDIX C. A REVIEW OF THE LITERATURE ON METHODS FOR ASSESSING FISH DIET COMPOSITION

C1. INTRODUCTION

Diet composition is a fundamental part of understanding trophic dynamics and ecological function. There are a number of methods used for data collection and statistical analysis, and several reviews are dedicated to describing and comparing the available techniques (Hyslop 1980, Cortés 1997, Chipps and Garvey 2006, Baker et al. 2014). Visual stomach content analysis is the most common method for estimating diet composition, but other technologies, such as fatty acids, stable isotopes and DNA analysis, have become more widely used in recent years (Brodeur et al. 2017). Methods should be chosen based on the research application, and often studies will incorporate more than one method to address the research question (Link and Almeida 2000, Chipps and Garvey 2006, Litz et al. 2017).

There is great deal of scientific literature on methods for determining diet composition. However, there is a lack of agreement on best practices or standard protocols for many of the methods (Cortés 1997, Chipps and Garvey 2006), and furthermore, methodological approaches are often summarized without adequate detail about the data or its precision (Cortés 1997, Ferry and Cailliet, Gregor 2014, Buckland et al. 2017). Field sampling methods and survey design (e.g., time of day, sample size, etc.) will affect the results, but those topics were considered to be beyond the scope of this review.

This literature review describes the main methods for assessing diet composition in fish and provides examples of applications of these methods. The main methods for quantifying diet are summarized, with an emphasis on visual enumeration supported by examples of documented protocols. Methods for statistical analysis of stomach contents are also described. Methods related to collecting stomach contents from live fish (e.g. gastric lavage) were not considered.

C2. METHODS FOR DETERMINING DIET

2.1. Visual enumeration of stomach contents

Stomach content analysis is the visual inspection of prey in a predator's stomach in which prey are generally identified to the lowest possible taxonomic level (Link and Almeida 2000, Simth and Link 2010, REEM 2015, Litz et al. 2017, etc.). The taxonomic resolution can vary depending on the research question (Chipps and Garvey 2006). Stomach content analysis can be done at sea (e.g. Link and Almeida 2000, Sweeting and Beamish 2009) or later in a lab if samples are preserved in formalin or frozen (e.g. Landingham et al. 1997, Laurinollli et al. 2004, REEM 2015, Livingston et al. 2017). The Northeast Fisheries Science Center protocol historically processed stomach contents in a lab, but switched to at-sea processing to reduce

costs and to obtain better results by processing fresh stomach contents (Link and Almeida 2000, Smith and Link 2010). Shipboard personnel are trained at workshops and have identification aids on board. Likewise, portions of samples from the annual Alaska Fisheries Science Center (AFSC) bottom-trawl program are analyzed at sea (Livingston et al. 2017). The results from stomachs analyzed at-sea and in the lab are generally comparable, however there may be better detection of soft bodied prey in the at-sea samples. One disadvantage to analyzing samples at sea is that the samples sizes are limited by the time available to personnel between hauls; when the prey diversity is high and when stomachs are full, stomach analysis takes longer (Livingston et al. 2017).

Generally, all individual prey items are identified and quantified by:

a) weight,
b) volume,
c) counts,
d) points or
e) some combination of methods.

Prey identification is one of the most challenging aspects of stomach content analyses and is influenced by a number of factors including digestion rates, time since consumption, species characteristics (Simenstad and Cailliet 2017). In addition to prey species identification, attributes such as stomach fullness, state of digestion and prey length are often recorded (REEM 2015).

The samples can be weighed wet, dried, or both (Landingham et al. 1997), although dry weight estimates tend to be more time consuming (Hyslop 1980). For example, stomach contents of Arctic groundfish caught between the 1960s and 70s in DFO research surveys used the dry weight and counts of stomach contents (Atkinson et al. 1991). An alternative to weight is volumetric analysis, which may give the most representative determination of bulk, and can be measured using displacement, settling, or indirect methods (Hyslop 1980).

2.1.1. Documented protocols for visual enumeration of stomach content

Methods employed vary between laboratories and by application. Here we review several published protocols for longer-term programs. The NOAA North Pacific observer program has a manual for fish observers to reference while onboard commercial fishing vessels which includes the detailed protocols for collecting and analyzing stomach contents (Alaska Fisheries Science Center, 2016; section 17). The data generated from this program begins in 1981, and once data are checked for quality, they are made publically available online. Lab procedures used by Alaska Fisheries Science Center (AFSC) Trophic Interactions Lab are described in the Resource Ecology and Ecosystem Modeling Stomach Content Analysis Procedures Manual (REEM 2015). The Northeast Fisheries Science Center (NEFSC) provides an online overview of their protocols which includes weighing and counting prey (https://www.nefsc.noaa.gov/femad/pbb/fwdp/training/). From DFO's Maritimes Region, stomach contents data, including counts and weights, from over 100,000 stomachs collected between 1958 and 2002 were compiled for the Diet Composition and Consumption Estimation Project (Laurinollli et al. 2004). That report summarizes the sampling methods from the various sampling programs. In DFO's Pacific Region, a number of studies using stomach content analysis (e.g. Beamish et al. 2010) point to the methods described in Sweeting and Beamish (2009). Other examples of studies using stomach content analyses in the Pacific Region include a report on the biology of Pacific Sardines using volumetric methods (McFarlane et al.

2010), comparison of feeding habits of the five species of juvenile salmon (Brodeur et al. 2007) and diet analyses on the Strait of Georgia synoptic bottom trawl survey (King et al. 2013). The fish stomach content analysis protocol used by UBC's Institute of the Oceans and Fisheries, and developed jointly with the Hakai Institute, is to record the counts and weights of prey in the stomach of previously frozen fish (Bresch et al., unpublished manual). Methods similar to this protocol were applied in various studies at UBC (Ajmani 2011, Podeswa and Pakhomov 2015) and the University of Victoria (Collicutt 2016).

At sea protocols

A) Stomach collection

AFSC protocol: Fish are randomly selected for sex/length samples, then stomachs are collected from these fish for defined size strata. Fish are taken from hauls sampled for species composition and only fish that do not show signs of net feeding or regurgitation are collected. Empty stomachs are collected unless collected immediately following a fish that was discarded because of regurgitation. In this case, the empty stomach is discarded as well. Stomach samples are prepared using the following procedures:

- Cut through the skin and open the body cavity
- Excise the stomach by cutting just anterior to the pyloric caeca and posterior to the gill chamber (different method for flatfish stomachs)
- Place the stomach in a specimen bag with a label
- Place the bag in a 5-gallon bucket of 70% formalin to be analyzed later in a lab
- Collect up to 80 stomachs per bucket or until the bucket is full

DFO Maritimes Region protocol: At sea, stomach samples were prepared as follows:

- Stomach fullness was estimated
- Contents in the oesophagus were pushed back into the stomach
- Stomachs, including the oesophagus down to the pyloic caeca, were removed from the specimen and placed in a Whirl-Pak bag or 8-lb poly bag
- Stomach wall was cut open in the bag and a salt brine added to the bag
- Samples were frozen

DFO Pacific Region protocols: Published protocol (McFarlane et al. 2010) for the west coast Pacific sardine survey (1997-2008) outline that stomachs were collected from each set and pooled by set.

Prior to 2010:

- stomachs were excised
- about 10 to 20 were pooled into one jar and
- preserved in 3.7% buffered formalin

After 2010:

- Each individual stomach was placed in an individually labelled bag
- 10 bags were put in a jar with 3.7% buffered formalin

B) Stomach content enumeration

AFSC protocols: Livingston et al. (2017) note that a portion of stomachs are also analyzed at sea by AFSC. The **Qualitative Method** is followed at sea:

- Prey species are identified to the lowest taxonomic level and recorded by National Oceanographic Data Center code
- Prey categories are weighed to the nearest 0.001g
- One of four procedures is used to sort and quantify prey:
    1. For a small bolus or large, undigested prey (most common) – contents are separated into taxonomic categories, each group is enumerated then blotted and weighed to the nearest 0.001 g.
    2. For a large bolus with undigested prey and one abundant prey group – less numerous prey are sorted then subtracted from the total weight. The major prey group is enumerated by dividing the prey taxon's total weight by the average weight of an individual prey.
    3. For a large bolus with undigested prey and more than one abundant prey group - less numerous prey are sorted out. The remaining prey are rinsed and placed in quartering dish and prey are weighed and enumerated from one quadrant. The remaining group weight is determined by subtraction and the enumerated using the method in procedure 2.
    4. For prey that is more digested – an attempt is made to determine the weight of the specific prey taxa by taking a sub-sample, sorting taxa using one of the procedures above and categorizing the remaining weight with the major prey taxa.

NEFSC protocol: The Food Web Dynamics Program has analyzed all stomachs at sea since 1985. The most recent protocols published (Link and Almeida 2000, Smith and Link 2010) are:

- The stomach is cut open and emptied onto a measuring board or sorting tray
- Total bolus volume is estimated to the nearest 0.1cm^3 using a volumetric gauge
- Prey are identified to the lowest taxon practical and sorted into prey groups
- The proportion of total volume and average digestion for the group is estimated
- Larval fish are preserved for laboratory examination
- When feasible, important species (e.g. fishes, crabs, and squids) are counted and measured for length to the nearest mm. Count can also be estimated. If there are more than 10 individuals, the length is measured for only a random subsample of 10 individuals.

DFO Pacific Region protocols: Protocols vary by survey.

The Strait of Georgia juvenile salmon surveys (Sweeting and Beamish, 2009):

- opening the stomach from the cardiac to pyloric constrictions and removal of the contents to a Petri dish.
- a visual estimate of fullness (%)
- prey volume (cc) estimated
- total stomach volumes estimated to be less than 0.1 cc are considered empty

The Strait of Georgia synoptic bottom trawl survey (King et al. 2013):

- stomach sampling is done for the first tow of the day, the tow hauled aboard immediately after lunch, and the final tow of the day
- stomach content information is collected from as many species as possible in the selected tow, starting with the most abundant species in the tow, and working through the catch to the least abundant species as time permits

- an overall target for survey is at least 200 stomach specimens per species for species captured in at least 10 tows.
- stomach contents are identified to the lowest taxonomic level possible
- prey volume estimated to the nearest cubic centimetre (cc)
- unidentifiable remains (categorized as digested matter) also expressed estimated to nearest cubic centimetre (cc)
- the prey digestion state was estimated as 1="fresh," 2="half digested," 3="three-quarters digested," or 4="fully digested."

The West Coast of Vancouver Island La Perouse acoustic-trawl survey and the Night Pelagic Ecosystem Survey:

- The stomachs of the first 10-20 measured fish of each species in each trawl haul are examined, including empty stomachs.
- The volume (cc) of each prey item, identified to the lowest possible taxon and life history stage using a magnifying glass or naked eye, is recorded (e.g., copepods, euphausiids, crab megalops, Pacific herring, unidentifiable prey).
- The prey digestion state was visually estimated as 1="fresh," 2="half digested," 3="three-quarters digested," or 4="fully digested."
- Records of diet composition accompany individual fish weight and length measurements.
- For some years with special diet analysis projects, individual stomachs were placed in individually labeled bags and preserved in 3.7% buffered formalin for later laboratory analyses using microscopes (i.e., prey were identified to finer taxonomic and life history stage categories and, in some cases, prey were measured).

Laboratory Protocols

AFSC protocols: Lab procedures used by AFSC's Trophic Interactions Lab are described in the Resource Ecology and Ecosystem Modeling Stomach Content Analysis Procedures Manual (REEM 2015) and are summarized here:

- The cloth bag containing the samples are removed from the formalin bucket
- The stomach, specimen label and any loose prey items are removed from the cloth bag with forceps and placed on toweling in a dissection tray
- Cloth bags are washed for future use
- Data are recorded electronically in an On-Screen Lab Form on a lab PC
- For each stomach the haul number, specimen number, predator length (cm), sex, maturity, stomach fullness, total stomach content weight (g) and intestine content weight is recorded
- The stomach weight is obtained by:
    - Removing the intestines below the pyloric caeca (the esophagus is included with the stomach)
    - Making a longitudinal incision to the stomach
    - Blotting the food bolus with paper toweling to remove moisture
    - Removing non-prey items such as parasites, stomach lining and rocks
    - Weigh the bolus to the nearest 0.01g on an electronic balance
- The stomach fullness is described categorically and based on the degree of distention and the weight of the bolus relative to the size of the fish

- For each prey item the digestion state, life history stage, and parts codes is recorded
    - Digestion state categories are 1 - stomach empty; 2 - traces of prey items; 3 - < 50% intact; 4 - 50-75% intact; 5 - 75-100% intact 6 – no digestion

Prey contents are analyzed using the **Qualitative** (as above in at-sea section) or **Quantitative** Methods depending on the analyst and what data already exists for the predator. The **Quantitative** Method is used if more detailed information is required on a species, for special studies, or if there is a new analyst working on the data. The method is as follows:

- Prey species are identified to the lowest taxonomic level and recorded by National Oceanographic Data Center code
- Prey categories are weighed to the nearest 0.001g and an estimate is made for each category's percentage of the total stomach content volume
- Commercially important species are sorted, weighed and counted precisely
- Other prey are enumerated when their numbers are reasonable (e.g. <100)
- One of three procedures is used to sort and quantify prey:
    1. Most common – Prey are sorted and commercially important species are counted and weighed. Other species assigned percentages based on their estimated volume.
    2. When the stomach is mostly commercially important species – Remove other prey from the sample and weigh, then determine the weight of the remaining commercially important species.
    3. When the sample has few large prey and/or commercially important species – Weigh the large prey individually, the small prey together and add the individual weights to get a total stomach weight, then sort and enumerate. Individual % volumes can then be estimated after the % of the larger prey have been calculated.

- The count is the minimum number that can be proven (e.g. if there are 10 euphausiid eyeballs, the count is 5)
- Commercially important species are measured for length if possible
- Intestine weights are measured in some cases
- Suspected net-fed prey fish or bait fish can be separated from the sample
- A final check of the predator data is made when all of the stomachs in a bucket have been analyzed.

The primary difference between the **Qualitative** and **Quantitative** Methods is the level of sorting, enumerating and weighing of prey other than fish and crab found in a stomach. The **Quantitative** Method obtains an exact count and weight (0.001 g) for every prey group. The **Qualitative** Method relies on visual estimates by experienced personnel of the prey composition of the remaining weight after the fish and crabs have been weighed and counted. For the **Qualitative** Method, prey counts of the remaining prey groups may be estimated but are usually left as null (Livingston et al. 2017).

DFO Maritimes Region protocol: As per Laurinollli et al. (2004) :

- Stomachs were thawed and strained
- Stomach and contents were emptied onto a plastic tray and weighed (to 0.01g)
- The empty stomach was weighed separately

Stomachs were analyzed in the lab by two technicians working as a pair. Each prey item was identified, weighed, measured for length and the quality described using digrestion codes:

1 – Good condition

2 – Partly digested
3 – Well digested
4 – Unidentifiable

DFO Pacific Region protocols:

The west coast Pacific sardine survey (McFarlane et al. 2010):

- contents of the cardiac stomach region were extracted with curved end forceps into a petri dish.
- total volume of stomach contents visually estimated in cubic centimetres (cc) using a syringe marked at every 0.1 cc
- proportion of a full stomach was expressed as a percentage;
- where 0% denoted an empty stomach, and 100% signified a completely full stomach.
- degree of stomach contents digestion also expressed as a percentage, where 0% denoted fresh contents and 100% indicates completely digested contents.
- contents examined under dissecting microscope
- prey items identified to lowest taxonomic group possible, then collated to a major prey group
- contribution of each major group expressed as percent of the total stomach volume
- unidentifiable remains (categorized as digested matter) also expressed as percent total stomach volume

Protocol for special diet analysis projects for the Night Pelagic Ecosystem survey:

- The full stomach is weighed in grams.
- Percent fullness is estimated based on known size of a “full” sardine stomach (i.e., fullness and how distended the stomach is).
- Contents from the stomach (oesophagus to pyloric sphincter), are extracted and placed into a petri dish
- Weight of the empty stomach recorded in grams
- Total volume of prey is recorded by comparing to known volumes (i.e., using pre-measured volumes of rice as a comparison tool)
- For phytoplankton consumed,
    - Contents are poured over a 90 um seive, allowing phytoplankton to pass through to a beaker
    - Remaining zooplankton prey are tranferred to a petri dish.
    - Volume difference between zooplankton prey and total prey volume is recorded as phytoplankton volume.
    - To identify phytoplankton, a slide is prepared with a subsample of the phytoplankton. Only presence/absence of phytoplankton species were recorded.
- For zooplankoton prey:
    - Estimate total volume (cc)
    - Remove all euphausiids and large prey items from the sample and enumerate and identify to species and life history stage.
    - For numerous (>200 count) other prey, split and subsample using a Folsom splitter
    - Under a dissecting microscope, identify and enumerate to the lowest taxonomic group possible

- Volume (CC) of each general prey category is estimated (e.g., copepods, crab, etc)

UBC/Hakai protocol: The UBC/Hakai diet protocol for analyzing stomach contents in a lab is described in (Bresch et al., unpublished manual) and summarized here as follows:

- Specimen is thawed, weighed and measured (total, standard and fork length)
- Belly is cut open with scissors and the stomach is removed, blotted dry and weighed
- Stomach is opened and emptied into a petri dish
- Gut fullness is estimated
- The empty stomach is blotted and weighed
- Water is added to the petri dish to suspend and separate contents
- Contents are identified to the lowest taxonomic grouping possible using a microscope with an ocular micrometer
- Prey are counted or if there is a large number a randomized subsamples is taken
- Prey are weighed or if there is a large number only a random subset of 10 individuals is weighed
- In each prey group, prey items are separated by digestion state using one of three indices
    1. Midoli + Nikita

        1 - body intact, easily identifiable “fresh” food item

        2 - may be missing some parts but the body is still in good condition

        3 - chunks, or parts of bodies that are still identifiable to a major group

        4 - mushy, have lost their shape but can still tell e.g. whether it was a copepod

        5 - contents are like soup, mostly liquid and unidentifiable
    2. From Evgeny

        1 - intact prey, did not lose coloration

        2 - prey lost coloration/transparency, may have lost some appendages

        3 - prey in various stages of digestion, from lost appendages to quite digested but still identifiable to genus or order

        4. - highly digested prey
    3. The AFSC codes:

        1 - stomach empty;

        2 - traces of prey items;

        3 - < 50% intact; 4 - 50-75% intact;

        5 - 75-100% intact 6 – no digestion
- Each digestion subgroup is blotted then weighed on an analytical balance

2.2. Fatty acid analysis

Fatty acids (FA) can be used as biological markers to study trophic interactions by comparing the FA signatures found in predators with those found in their prey. After consumption, FA in the prey generally remain intact, even after digestion, and are deposited in the predator's tissue which can then be analyzed to quantify the predator's diet (Budge and Iverson 2003). Samples can come from tissue or whole individuals and analysis is done in a lab. One of the standard methods for extracting the FA containing lipids from animal tissue is the Folch method (Folch et al. 1957; and described in Budge and Iverson, 2003; Iverson et al., 2002, etc.). Following extraction, analysis is performed by gas chromatography using methods such as those described in (Litz et al. 2017). The FA are expressed as a weight percent of total FA, using a nomenclature that describes the carbon chain length, the number of double bonds and the double bond location (Iverson et al. 2002).

In a review of FA trophic markers in the marine environment, Dalsgaard et al. (2003) questions the use of FA trophic markers as a quantitative tool because of issues around dynamics of FA in the marine environment. However, they note that FA are still useful for assessing trophic interactions, that can compliment methods such as stomach content analysis by providing information on orgins of lipid reserves over time.

In order to use FA analysis effectively, the FA patterns and their variations must be first understood in the prey species assemblage (Iverson et al. 2002). Studies, such as the one by (Litz et al. 2017), use biomarkers established in the literature that indicate water types, plankton ratios and piscivory.

The metabolism of FA in fish is linked to sex, age, size and sexual maturity, as well as to environmental factors such as temperature and food availability (see review by Dalsgaard et al., 2003). In a study in Prince William Sound, (Iverson et al. 2002) determined the FA signatures from 22 fish and invertebrate species and used discriminant and classification and regression tree analyses to distinguish species with up to 95% accuracy. They demonstrated that while the signature of a species can vary with location, lifecycle, fish size and seasonal or interannual shifts in diet, the data show that the between species variation in signature is often greater than the within species variation. Similar results were found in a study on Canada's east coast, where the 16 most numerous species (n>18) were classified with 98% accuracy (Budge et al. 2002). For species with similar feeding habits that are more difficult to distinguish from each other, FA analysis can be used with other types of stomach content analysis (Iverson et al. 2002).

The majority of research on using FA for fish diet composition has focused on qualitative markers of trophic interactions in high-latitude, shelf seas (Dalsgaard et al. 2003, Litz et al. 2017), and in lower trophic level species or marine mammals (references in Dalsgaard et al. 2003). In a study in the Beaufort Sea, DFO scientists collaborated with several other organizations to compare trophic niches of three species of cod using FA and stable isotope analysis (Brewster et al. 2017).

2.3. Stable isotope analysis

Stable isotope analysis gives information on the energy flow in the food system and can be used to describe the diet of a predator over weeks or months (Litz et al. 2017). The isotopes commonly used in the marine environment are those of carbon (δ13C) and nitrogen (δ15N). The signature of δ13C is similar between predators and their prey and can be related to sources of primary production (Chipps and Garvey 2006). δ15N varies with nutrient sources and between trophic levels, and can be used to describe energy pathways (Litz et al. 2017).

Isotope analysis is done on samples taken from the tissue of fish, usually around the dorsal fin, or on whole samples of smaller fish and invertebrates (Chipps and Garvey 2006). Samples are collected at sea and frozen until analysis. If the sample is a whole fish, the stomach contents should be evacuated so the sample is not contaminated. After samples are dried and ground up, they are analyzed in a mass spectrometer. One study reported that the cost to analyze one stable isotope sample is about half the cost to analyze one stomach's content (Vinson and Budy 2011).

The data can be used to calculate trophic position which can be used to assess feeding patterns in a population including omnivorous feeding behaviour (Chipps and Garvey 2006). For example, a study from the coast of Oregon identified the timing of piscivory in juvenile Chinook salmon by the increase in δ13C and δ15N, along with fatty acid biomarkers (Litz et al. 2017). The study also acknowledged other studies in which δ13C is depleted offshore and enriched onshore, or where δ13C is associated with lower sea surface temperatures (see references within). Because of these confounding factors, the authors emphasized that combining the isotope data with fatty acid analysis can aid in the interpretation of results. Another series of studies out of UBC have used stable isotopes to assess Sockeye Salmon declines on the BC central coast (Ajmani 2011, Doson Coll 2015). A University of Victoria and DFO study on the feeding ecology of Chinook Salmon used δ13C to link juvenile salmon survival to large-scale climate variability (Hertz et al. 2016).

2.4. DNA-based diet determination

DNA-based approaches are being used more frequently for species identification in stomach contents as techniques develop and become more widely available (Brodeur et al. 2017). Much of the interest stems from the potential to increase the identification rate of stomach contents (Paquin et al. 2014), especially for larval fish, soft body prey or taxa with morphological similarities often not detected or distinguished by visual methods. DNA barcoding can be used to identify stomach contents that are highly digested and degraded. For example, a study comparing methods for describing fish diets in Lake Erie demonstrated that DNA can achieve better taxonomic resolution compared to visual analysis of stomach contents (Carreon-Martinez et al. 2011).

Analysis can be done on small samples, in which species can be identified by diagnostic PCR or DNA-barcoding (Pompanon et al. 2012). DNA barcode sequences from cytochromes commonly used for species ID are available in public databases such as the Barcode of Life Database (http://www.barcodeoflife.org/; (Paquin et al. 2014). In a review of molecular analysis of predation, King et al. (2008) describe the common techniques and issues with sampling and laboratory procedures. They note that this technology is early in development and a wide range of techniques should still be explored. Next generation sequencing is one technique making the technology more powerful by allowing more efficient and precise diet characterizations (Pompanon et al. 2012).

DNA based diet estimation methods are employed by the Marine Mammal Research Unit at UBC (e.g. Thomas et al. 2014) and in collaboration with other organizations including DFO (Tollit et al. 2009). One study out of the University of Washington used DNA to identify highly digested stomach contents in Chinook Salmon (Buser et al. 2009).

C3. STATISTICAL ANALYSES OF STOMACH CONTENTS

There are a number of ways stomach content data can be summarized and analyzed. In this section, the main statistical methods are described along with their advantages and disadvantages. In the widely cited Hyslop review (1980), one of the main conclusions is that investigators should employ both a measure of the amount of the stomach contents (e.g. numerical methods) and a measure of the bulk of the stomach contents (e.g. weight/volume). Cortés (1997) notes that many studies qualitatively describe variations in diet in terms of biological and environmental conditions but do not provide statistical support. In the review, he suggests improvements to a number of methods and a method for testing significance of interaction terms (i.e. season, size, etc.). The analysis chosen will depend on the research question. For example, if there is interest in describing the impact of predators on prey, it may be more suitable to use the % weight or volume for the whole sample (Chipps and Garvey 2006).

3.1. Frequency of occurrence (using presence/absence data)

The frequency of occurrence (FO) is the number of predator stomachs containing the prey species out of the total number of predator stomachs examined (Link and Almeida 2000). FO provides a simple and fast method describing the diet of a population (Hyslop 1980). It is widely used in the literature, but it is also criticized for its crudeness. For example, the species database FishBase (http://www.fishbase.org), suggests that FO is not a good indicator of how much the prey item contributes to the diet because it doesn't account for differences in prey size. (http://www.fishbase.org/manual/fishbasethe_diet_table.htm). On the other hand, Baker et al. (2014) suggests the method is robust enough for most applications, and is simple and less costly than more detailed analyses. With a large enough sample size, FO provides similar results of prey importance and diet composition when compared to bulk methods (Baker et al. 2014, Buckland et al. 2017).

A variation of FO is the dominance method which is the proportion of predator stomachs dominated by the bulk of a certain prey species (Ahlbeck et al. 2012). However the method still does not represent the actual quantity of prey in a stomach, and there are multiple criteria for assessing dominance which limits the comparability between studies (Hyslop 1980).

Both the Food Web Dynamics Program at the Northeast Fisheries Science Center (NFSC) and studies out of the Alaska Fisheries Science Center report a number of statistical estimators including FO (Link and Almeida 2000, Livingston et al. 2017). It is used by DFO Pacific Region survey programs.

3.2. Bulk methods (mass or volume)

Bulk stomach content analyses methods measure the contribution of a prey species by weight or volume to the stomach and give the most detail about a predator's stomach contents. The measurement is usually expressed as a percentage of total stomach weight or volume, and can be calculated in a number of ways. For example, Ahlbeck et al. (2012) describe three methods for determining the diet composition: i) take the percent mass of the prey found in each stomach, then average for all predators in the sample; ii) pool all stomach contents before calculating the %mass; and iii) pool contents but divide the prey mass by the mass of the predator before pooling. The main methods used by the NEFSC Food Web Dynamics Program are the simple unweighted percent mass and weighted percent mass (by number of individuals at length per tow and by total number of individuals per tow; similar to Link and Almeida 2000; Latour et al. 2008). On the DFO Strait of Georgia salmon surveys, the diet composition is expressed by percent volume for each prey group (Sweeting and Beamish 2009).

In one evaluation based on modeled results, the bulk methods were found to describe the diet most accurately (Ahlbeck et al. 2012). However, (Baker et al. 2014) take issue with these methods because it's not possible to accurately separate out different prey types because of digestion, and also because the composition may be related to a variety of unquantifiable factors that prevent an accurate representations of the actual composition of the prey consumed (e.g. the type of prey, time in the stomach, the feeding mode of the predator, etc.). Attempts to minimize these issues include reconstructing to their original size (Hyslop 1980, Baker et al. 2014). Hard structures in the stomach contents can be used to back-calculate for a total prey weight, but there must be a known relationship between the dimension of the hard structure to the whole body weight (Chipps and Garvey 2006). These reconstructions can be timing consuming, do not account for rapidly digested prey items, and they assume that the prey was consumed whole (Baker et al. 2014).

3.3. Numeric counts method (using count data)

This method describes the diet by the number of prey in each prey category out of the total number of prey (Hyslop 1980). It can be calculated in various ways to accommodate the mass of the fish or to reflect the different amounts of prey in each fish (Ahlbeck et al. 2012). It is relatively fast and simple to apply, and can be used to describe individual feeding behaviour. Numeric counts are most suitable to use in situations when the prey species are a similar size, given that the method tends to overestimates small prey taken in large numbers (Hyslop 1980). It will also overestimate prey that have a long passage time, and is confounded by prey that has been broken up.

The AFSC REEM Laboratory uses detailed counts for stomachs of species that in special studies or that have relatively little information, or for commercially important prey species. In a study on salmonids, counts were expressed as the percent composition (Vinson and Budy 2011).

3.4. Points methods and stomach fullness indices

The points method is a system where points are awarded for the estimated prey contribution to the total prey mass or volume (Ahlbeck et al. 2012) and is often used as an index for stomach fullness (Hyslop 1980). The method is easy and can be performed quickly, but is subjective and there are a number of ways it can be applied therefore making it difficult to compare between studies (Hyslop 1980).

Chipps and Garvey (2006) suggest a more objective approach referred to as the mean stomach fullness index (MSF) whereby the observed prey volume is compared to the estimated stomach capacity. They note that the MSF data can be analysed by a number of statistical procedures and is useful for providing information on the energetic contribution of different prey types.

For the AFSC bottom trawl surveys, stomach fullness is qualitatively estimated, but for the whole stomach and not individual prey contributions (Livingston et al. 2017). Others make the estimate based on volume (Link and Almeida 2000, Smith and Link 2010) or mean stomach weight as a percent of the total body weight (Litz et al. 2017).

3.5. Indices of prey importance

The term 'importance' is used loosely in the literature, but the extensively cited paper by (Hyslop 1980) defines it as simply the prey amount and bulk in the diet. There are a couple of indices for prey *importance* (Cortés 1997), the most commonly used is the:

Index of Relative Importance (IRI)

$$IRI = \%FO \times (\%W + \%N)$$

where,

%FO is percent frequency occurrence

%W is percent weight, determined from mass method

%N is proportion of numeric counts, determined from count data.

A variation on the index is to divide by the sum of all products (Ahlbeck et al. 2012), which makes it more comparable among food types (Cortés 1997). Simenstad and Cailliet (2017) and references therein, suggested that the IRI should be replaced by the prey-specific index of relative importance (PSIRI) which is calculated using the prey specific %W and %N. King and Beamish (2002) used a modified index of Relative Importance (RI) based on their available data, with %FO as in IRI, but using %C (prey proportion of individual stomach contents) and %V (percentage ratio of prey volume across samples to stomach content volume across samples) instead of %W and %N.

Kaeriyama et al. (2000) used a number of methods to assess the feeding ecology of Pink and Sockeye Salmon in the Gulf of Alaska and felt that the modified IRI was the best method for evaluating stomach contents. Sometimes the IRI is calculated from dry weight (Landingham et al., 1997). Proponents for the use of these indices suggest that by combining the different measurements the bias from the individual methods is reduced and that dietary importance is more accurately portrayed (Cortés 1997, Liao et al. 2001). On the other hand, some argue that

there is no additional information added when using these indices (Chipps and Garvey 2006), or that these methods add more sources of error Hyslop (1980).

3.6. Indices for diet similarities

Overlap indices are used to measure the overlap of resources between species and may suggest competition or an abundance of prey (Chipps and Garvey 2006). A number of indices are reviewed in (Cortés 1997) including:

Morisita's Index of Similarity between sample *j* and *k*

$$C_{\lambda}=\frac{2\sum X_{ij}X_{ik}}{(\lambda_1+\lambda_2)N_jN_k}$$

where,

X_{ij}, X_{ik} = number of individuals of species *I* in sample *j* and sample *k*

$N_j = \Sigma\, X_{ij}$ = total number of individuals in sample *j*

$N_k = \Sigma\, X_{ik}$ = total number of individuals in sample *k*

$$\lambda_1 = \frac{\sum\left[X_{ij}\left(X_{ij}-1\right)\right]}{N_j\left(N_j-1\right)}$$

$$\lambda_2 = \frac{\sum[X_{ik}(X_{ik}-1)]}{N_k(N_k-1)}$$

the **Simplified Morisita index**

$$C_H = \frac{2\sum X_{ij}X_{ik}}{\left[\left(\sum X_{ij}^2/N_j^2\right)+\left(\sum X_{ik}^2/N_k^2\right)\right]N_jN_k}$$

and **Horn's index of Similarity** for samples *j* and *k*

$$R_0 = \frac{\sum\left[\left(X_{ij}+X_{ik}\right)log\left(X_{ij}X_{ik}\right)\right]-\sum\left(X_{ij}logX_{ij}\right)-\sum(X_{ik}logX_{ik})}{\left[\left(N_j+N_k\right)log\left(N_j+N_k\right)\right]-\left(N_jlogN_j\right)-(N_klogN_k)}$$

Morisita's index is recommended for use when prey numeric counts are available, and Horn's index is recommended when proportions or biomass estimates are available.

In addition to calculating the IRI , Landingham et al. (1997) also report the simplified Morisita's index to assess diet overlap in juvenile salmon.

3.7. Other methods (be reviewed and revised at the Workshop during Discussion periods)

Multivariate analysis, such as principal components analysis or cluster analysis, are useful in cases where there are a large number of prey and to assess the structure of the stomach contents of a set of fish (Crespin de Billy et al. 2000). As an alternative to describing data in a tabular form, when there are two or more variables the data can be presented graphically and used to describe population-level data such as feeding strategies, relative prey importance and diet variably (Cortés 1997, Chipps and Garvey 2006).

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APPENDIX D: TABLES COMPARING METHODS

Appendix D Table 1. Pros, cons, and solutions of stomach content analyses at-sea and in the laboratory.

	Pros	Cons	Solutions
At-sea	• Fresh specimens • Digestion state • Data are quickly available • Best when all samplers collect data • Process more samples • Collect diet data (staff/cost-effective) • Opportunity to do adaptive sampling	• Staff not necessarily experts or consistent across surveys • Lower taxonomic resolution • Misidentification • Small-bodied predators difficult to analyze • Dependent on number of samplers and platform (affects sample size) • Chemicals (storage and disposal and spills) • Transfer to the lab is another chance to introduce error	• Manuals (online and printed) • training • Workshops • Lab validation (send to lab) • Photo (file storage is a con) • Can combine different levels of sampling intensity (field and lab; e.g., 'called' vs full stomach analysis)
Lab	• more likely to have dedicated staff – better consistency • more predators • Reduces challenges at sea • Higher taxonomic resolution • higher weight precision • Broader range of questions can be addressed	• Preservation impacts digested state • Shrinkage/expansion • Takes longer • Funding dependent • Staffing/expertise dependent • Time to preserve samples • Fish identification often lost • Chemicals (storage and disposal and spills)	• Rehydrate ethanol-preserved stomachs • Conversion factor for alcohol and formaldehyde-preserved stomachs • Photo (file storage is a con) • Freezing reduces the use of chemicals

Appendix D Table 2. Pros, cons, and solutions of the different metrics of stomach content analyses.

Metric	Pros	Cons	Solution
Individual counts	• Can rely on presence only of partial prey • Prey preference studies • Can reconstruct weights (numbers x known weights; structure size related to animal size)	• Difficult when many prey present • Time consuming • Not representative of energy consumed • Biased by undigested prey • May see prey of prey (secondary prey; contamination)	
Volume	• Quicker than counts (volume estimates quicker than measurements) • Can be related to weight • Easier for smaller prey (especially at sea) • Reflective of energy consumed	• Consistency across samplers is difficult • Need to convert to weight?	• Use quantitative approach (various tools) • Create volume:weight relationship
Weight (wet or dry)	• Fastest (depends on situation) • Quantitative • Objective • Can be reconstructed • describe true diets (energy consumed) most accurately (Ahlbeck et al. 2012)	• Influenced by water • Overestimate small prey • Underestimate digested prey • Difficult to get precise weights at sea sometimes • Dry weights take more time	• Denis to tell Malcolm the best scales to use at sea • When weighing prey, also weigh liquid • Use damp cloth to blot dry prey before weighing • Dry:wet weight ratio • See Jason Link's ppt regarding amount of time added for doing diet analyses
'Called' stomachs (i.e. identify dominant prey only)	• Fast • Presence • Low cost/time • Easier to process fish	• Not full analysis • Not quantitative • Some methods: Only major prey item (or 2) • Lower taxonomic resolution	• In lab, use probability of prey in stomach and mean weights of stomach, etc from other detailed studies • Could call all prey items (not just dominant prey) • Use in combination with a higher level diet analysis on subsamples or designated stations

Metric	Pros	Cons	Solution
Prey length	• Predator-prey size ratios for ecosystem modeling • Predation vs. fishing mortality • Prey size-selection • Identify year classes of prey fish	• Subsampling needed if many prey • Time consuming	
Digestion state (categorical)	• Enables identification of meal size • Enables interpretation of diel feeding chronology, timing • Indicates reliability of content identification • Used for quality control	• Subjective • More time needed • One of the least crucial metrics • Time consuming/distraction to data collection	• Captured in 'unidentified prey' and weights • Simplify to 3 categories to reduce time spent deciding
Stomach fullness (categorical)	• Identify empty vs everted stomachs • Field indication of high feeding areas	• Subjective, varies among samplers • Influenced by water • Least crucial metric • Time consuming/distraction to data collection	• Content weight (volume) as a percent of body weight • Empty, regurgitated, everted stomachs should be recorded in a separate field (i.e., not in a stomach fullness field) • Stop using this

Appendix D Table 3. Summary of different statistical analyses for stomach contents.

Statistical Analysis	Description	Data used	Needs/assumptions	References
t-test		any; often composition data are arcsine square root transformed	normal distribution; large sample size	Chipps and Garvey 2006
ANOVA	Analysis of variance. Post-hoc: e.g., Fisher; Tukey; Scheffe	e.g., stomach weight as %body weight; often composition data are arcsine square root transformed	normal distribution; large sample size; individual fish stomachs collected from independent experimental units.	Brodeur et al. 2007; Chipps and Garvey 2006
Kruskal-Wallis	nonparametric ANOVA. Post-hoc e.g., Dunn	e.g., average % number prey		
Repeated measures ANOVA	Repeated measures analysis of variance to deal with confounding problems of spatial or temporal autocorrelation; randomized-complete-block repeated-measures ANOVA; or a split-plot ANOVA. Post-hoc: e.g., Bonferroni	any; repeated measures for same individual or site	randomized-complete-block approach difficult to employ, requires sphericity of variance–covariance matrix of within-subject factor (variance of difference between any two levels of within-subject factors must be constant; Mauchly's test of sphericity)	Chipps and Garvey 2006
ANCOVA	Analysis of covariance; to look for diel changes in diet	content weight, fish weight	stomach content weight regressed against fish weight (covariate). If linear (can be transformed), compare slopes; if slopes parallel, compare intercepts. Significant differences among intercepts indicate a diel pattern	Chipps and Garvey 2006
ARIMA	Autoregressive integrated moving average (ARIMA) models used to identify nonrandom patterns through time	any	assumes observations available in discrete, evenly spaced intervals; requires >50 dates	Chipps and Garvey 2006
MANOVA	parametric multivariate analysis of variance; test statistics e.g., Wilks' lambda, Hotelling-Lawley trace, Pillai's trace, Roy's largest root criterion	prey weights or volumes (not proportions), with factors (e.g., locations) as treatments	assume multivariate normality and similar variance–covariance structure among samples; sample size has to be large relative to the number of variables	Chips et al. 2007; Anderson and Walsh 2013

Statistical Analysis	Description	Data used	Needs/assumptions	References
nonparametric MANOVA	non-parametric randomized one-way multivariate analysis of variance to test for differences in diet between two samples; Hotelling's T2 for testing difference between means; F-statistic used to test for treatment effect among three or more factors. Post-hoc: univariate t-tests to detect differences owing to prey types	prey proportions, with factors (e.g., locations) as treatments	used if assumptions of MANOVA are not met	Cortes et al. 1997; Chipps and Garvey 2006
2DKS	two dimensional Kolmogorov–Smirnov test		two or more bivariate plots of spatial distributions of prey occurrence in diets of individual fish may be compared; also can be used to determine if spatial distributions within single plots differ significantly from randomly generated ones.	Chipps and Garvey 2006; William and Teukolsky 1988
Canonical discriminant analysis	detects individual prey items responsible for differences among factors	prey weights or volumes, with factors (e.g., locations) as treatments	variables should have an approximate multivariate normal distribution within each class, with a common covariance matrix	Cortes et al. 1997; Manly 1994
Multiway contingency table	Multiway contingency table analysis based on log-linear models; contingency table, number of prey categories by number of predator categories	prey numbers	large samples sizes are needed so that less than 20% of the cells have an expected frequency less than five	Cortes et al. 1997; Chips and Garvey 2006
Mantel test	randomization test to look at autocorrelated spatial patterns in diet data	Spatial variation among individuals compared to relative proportion of a specified diet item in stomachs	compare to distance matrices	Mantel 1967; Chipps and Garvey 2006

Statistical Analysis	Description	Data used	Needs/assumptions	References
ANOSIM	Analysis of similarities to test for differences in community composition among factors (e.g., years); uses ranked distance or dissimilarity. Post-hoc SIMPER (similarity percentages procedure)	e.g., %prey composition, weight, …	uses ranks of Bray-Curtis similarities (resemblence matrix) calculated from fourth-root transformed data to compare sites or standardized to compare species; distribution free	software PRIMER (Plymouth Routines in Multivariate Ecological Research); Clarke and Green 1988; Clarke and Warwick 2001; Clarke 1993
PERMANOVA	permutational multivariate analysis of variance; uses distance or dissimilarity; evaluates differences in location and spread simultaneously	e.g., %prey composition, weight, …	uses Bray-Curtis similarities (resemblence matrix) calculated from fourth-root transformed data (to compare sites) or standardized to compare species; distribution free	Anderson 2001; Anderson and Walsh 2013
NMDS	Nonmetric mutidimensional scaling analysis to visually assess prey species; descriptive	e.g., %prey composition		software PRIMER (Plymouth Routines in Multivariate Ecological Research); Clarke and Green 1988; Clarke and Warwick 2001; Clarke 1994
PCA	Principal components analysis with Euclidean bi-plots of PCA scores	e.g., proportions, %GII (i.e., (%N+%W)/2)	1. log-ratio analysis (%PCA) on logarithms of percentages, no zeros in data. 2. correspondence analysis; zeros in data	Bizzarro et al. 2007; Chipps and Garvey 2006
Cluster analysis	To compare composition; descriptive	e.g., overlap indices; Morisita's index	distribution free	Bizzarro et al. 2007