A fish sampling protocol for the Musquash Estuary Marine Protected Area

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ABSTRACT

In 2006, the Musquash Estuary was designated as a Marine Protected Area (MPA). To support the monitoring of the MPA, a fish sampling protocol is proposed in this document. The main objectives of this protocol are to: 1) Understand the nekton community within nearshore habitats of Musquash MPA by characterizing baseline spatial and temporal variability and environmental drivers; 2) Assess the health of the estuary across multiple habitats for long-term evaluation of potential human sources of contamination; and 3) Compare Musquash fish community to reference locations outside of the MPA. Two sampling gears (beach seine and fyke net) are proposed for the collection of nekton data at four Musquash Estuary nearshore habitat sites (Black Beach, Five Fathom Hole, Hepburn’s Basin and Gooseberry Cove) and three reference sites (Saints Rest Marsh, Dipper Harbour and Chance Harbour). The protocol also recommends potential locations for salt marsh habitat sampling by fyke net. Sampling considerations such as timing of sampling and specific site accessibility are discussed. Data to be collected on fish communities includes: species richness, abundance, and lengths. Atlantic silversides are also proposed for collection for use as a sentinel species to assess potential impacts of contamination. Data to be collected on free-swimming invertebrates includes species richness and abundance, which will provide complementary data on the community utilizing nearshore habitats. This information will provide insight into the annual variability associated with the Musquash Estuary MPA fish community.

RÉSUMÉ

En 2006, l'estuaire de la Musquash a été désigné zone de protection marine (ZPM). Un protocole d'échantillonnage pour le poisson a été mis en place dans le cadre du suivi de la ZPM et est décrit dans le présent document. Le protocole a trois principaux objectifs : 1) comprendre l'activité du necton au sein des habitats littoraux de la ZPM de l'estuaire de la Musquash en caractérisant la variabilité temporelle et spatiale normale ainsi que les facteurs environnementaux; 2) mesurer la santé de l'estuaire dans de multiples habitats pour évaluer à long terme les sources de contamination humaines; et 3) comparer l'activité des poissons de la Musquash et des sites de référence à l'extérieur de la ZPM. On propose de réaliser la collecte des données sur le necton avec deux des engins d'échantillonnage (senne de plage et verveux), dans quatre endroits d'habitats littoraux de l'estuaire de la Musquash (la plage Black Beach, le trou Five Fathom Hole, l'anse Hepburn Basin et l'anse Gooseberry Cove), ainsi que dans trois sites de référence (le marais Saints Rest Marsh, le havre Dipper Harbour et le havre Chance Harbour). Le protocole recommande aussi des emplacements d'habitat de marais salé pour l'échantillonnage par verveux. Sont également discutés certains éléments à prendre en compte tels que le moment de l'échantillonnage et l'accessibilité des sites. Voici certaines données à recueillir sur les communautés de poissons : richesse, abondance et longueur des espèces. On propose également de faire des échantillonnages pour de la capucette, laquelle constituerait une espèce sentinelle pour évaluer les incidences potentielles de la contamination. Des données doivent être recueillies sur les invertébrés nageant librement, entre autres la richesse et l'abondance des espèces. Elles fourniront des données complémentaires sur la faune vivant dans les habitats littoraux. Ces renseignements donneront un aperçu de la variabilité annuelle associée à la communauté de poissons dans la ZPM de l'estuaire de la Musquash.

1.0 OVERVIEW OF FISH SAMPLING PROTOCOLS

1.1 Long-term Monitoring

Monitoring programs vary in intensity based on objectives. Biological data collection can range from low intensity collection such as public observations or landings from commercial vessels to more intense forms including rigorous systematic sampling designs involving multiple sampling techniques with members of the scientific community and/or trained community groups (Singh et al. 2000, Honey et al. 2004, Thériault et al. 2006, Weldon et al. 2007, Higdon and Paulic 2013). Data collected from sampling protocols also vary in terms of answering questions at the histological, individual, population or community level (Doyle et al. 2011).

Long-term sampling protocols are usually established in areas that are in need of conservation, considered to be ecologically sensitive areas at high risk of being altered, or in need of restoration (Raposa et al. 2003, De Mutsert and Cowan 2012). Monitoring can coincide with restoration efforts or occur in areas that will experience a major alteration (addition of a dam, dredging, or impact of point and non-point pollution sources; De Mutsert and Cowan 2012). Long-term studies of fish assemblages in ecologically and commercially important areas are essential in order to establish natural variability. Without understanding the natural shifts in fish communities both spatially and temporally, it becomes difficult to distinguish between natural/annual shifts based on physical cues (e.g., temperature, salinity, and lunar cycles) from overexploitation or other anthropogenic disturbances (Macdonald et al. 1984, Ayvazian et al. 1992, Lazzarri et al. 1999). For example, data collected from a five-year study (inshore and offshore sampling) in the Passamaquoddy Bay area (Macdonald et al. 1984) revealed that fish assemblages migrated inshore during summer months (June – October) and then offshore during winter months with returns beginning in the early spring. These changes in assemblages were largely influenced by small fluctuations in temperature. The study from Macdonald et al. (1984) emphasized the need for monitoring fish in inshore habitats, particularly estuaries, as being important for commercially important species. Understanding fish movements at a local scale allowed for more accurate estimations of fish abundances. The authors from this article suggest that important inshore recruiting cohorts were highly correlated with successful offshore fishing the following year.

1.2 Estuarine Monitoring

Estuarine systems are sensitive areas in need of conservation and preservation (Able and Fahay 1998, Elliot and Hemingway 2002). They are considered the most productive environments alongside tropical rainforests (Blaber et al. 2000). Because of this, they are ecologically important areas for fish and crustaceans, acting as nursery areas-supporting large numbers of juveniles, providing feeding grounds, migration routes for anadromous species, as well as overwintering sites (Macdonald et al. 1984, Able and Fahay 1998, Elliot and Hemingway 2002). It has been found that a continued decline in estuarine habitats is highly correlated with declines in abundance of early life stages of fishes (Able and Fahay 1998). However, estuaries are part of coastal areas, which suffer the most from anthropogenic disturbances (e.g., filling, channelization, industrial outputs; Able and Fahay 1998, Elliot and Hemingway 2002). Therefore, monitoring these locations is important for the assessment of ecosystem health and the maintenance of their productive capacity (Ayvazian et al. 1992).

1.3 Use of Fish as Monitoring Tools

Fish species that are considered permanent residents in estuaries are rare (Able and Fahay 1998). Estuarine waters have been found to carry only 10% of the species found in adjacent waters (Able and Fahay 1998). Fishes must be highly tolerant of abrupt changes in temperature, salinity, oxygen, turbidity and strong seasonal changes for them to be able to cope with environmental changes associated with estuaries (Able and Fahay 1998). Adult stages of most marine species are typically absent from estuaries while larval and juvenile stages of these species are more tolerant of the environmental variability. Productivity in estuaries is high, as large biomasses of a small number of species tend to dominate the ichthyofauna. Thus, collecting fish within estuarine environments typically focuses on smaller bodied species or larvae/juveniles, as these fish typically are the most tolerant. Sampling methods typically used in estuarine systems depend on the study objectives, but largely focus on the nearshore area (<5m depth) and include: seines, trap nets, and trawls, which usually target fish <200mm in length (Methven and Schneider 1998). Estimates on productivity and habitat use can be made by monitoring the nearshore community, thus providing insight into the health of the estuary.

The collection of fish within estuaries is important to assess across multiple habitats of varying salinity, substrate type and vegetation differences. Fish characterization of an area often involves both the biological and environmental collection of parameters. Biological parameters that are widely used include:

- Richness, for identifying invasive, indicator, commercially important species or species at risk (Macdonald et al. 1984, Methven and Schneider 1998, Raposa et al. 2003)
- Abundance, for estimates of Catch Per Unit Effort (CPUE) and biomass (Gibson et al. 1996)
- Individual lengths, for age estimations (Able and Fahay 1998, Methven and Schneider 1998).

Monitoring programs have been found to be more successful when they combine physical, chemical and biological data (Carlisle et al. 2002). Monitoring factor interactions allows for the potential to detect disturbances that may be causing habitat degradation or changes in species compositions (i.e., reduction of indicator, rare, commercially important species or increasing numbers of invasive species; Raposa et al. 2003). Environmental characteristics taken along with fish samples often included: temperature, salinity, dissolved oxygen, turbidity, and sediment samples. Other characteristics such as fish weight or sex are sometimes assessed but are difficult to establish in the field, requiring fish to be euthanized and brought back to the laboratory (Macdonald et al. 1984, Gibson et al. 1996, Honey et al. 2004). An alternative to this issue is through the collection of a sentinel species, which can be used to characterize sex ratios, weights, somatic indices and condition factors (Doyle et al. 2011).

Incorporating both fish and crustaceans into long-term estuarine monitoring programs is common. Thériault et al. (2006) used nekton collected under the Community Aquatic Monitoring Program (CAMP) to assess the impacts of a seafood processing plant on overall estuarine health. A Before-After-Control-impact (BACI) study by the Louisiana Department of Fisheries and Wildlife used nekton community characteristics collected from 1986 to 2007 in a similar way as the CAMP protocol. Data collected was found to be successful in monitoring the effects of freshwater discharge into the estuary on commercially important species (De Mutsert and Cowan 2012). These programs established a once to twice monthly sampling regime and found that sampling nekton alongside other physical characteristics provided data that could be used as an indicator for monitoring estuarine health. Additional measures, such as collaboration with other established monitoring programs, as well as the use of sentinel species have been found to complement these protocols to give a more holistic picture of estuarine health.

Biological sampling in estuaries have been shown to include a unique set of challenges. Successful monitoring programs and studies of these habitats have demonstrated strong links between fish (or nekton) characteristics and physical factors influencing the environment. Piecing together the dynamics of the fish community as it pertains to local climate, tidal dynamics, habitats within the estuary, and potential anthropogenic threats requires a multi-level approach of monitoring. Collecting information on richness, abundance and size alongside abiotic factors such as time of year, temperature, salinity, dissolved oxygen, turbidity and habitat type (salinity gradient, substrate type), provides information on the community and how it may be utilizing its environment. Combining this information with additional sampling gears (differing mesh size, active vs passive gear types) as well as with the use of sentinel species enables researchers to obtain a holistic representation of how fish (or nekton) are utilizing the estuary. Previous monitoring techniques have also been shown to be successful when they involve members of the community since they offer a sense of stewardship over these ecologically sensitive areas.

2.0 MUSQUASH ESTUARY MONITORING PLAN

2.1 Monitoring Musquash Estuary

It has been estimated that approximately 85% of the salt marsh habitats within the Bay of Fundy have been either significantly altered or destroyed (Harvey et al. 1998, Singh et al. 2000). Musquash Estuary remains a valuable system as it's considered to be the last of the remaining ecologically intact estuaries in the Bay of Fundy (Singh et al. 2000). As a result of its designation as a Marine Protected Area (MPA) in 2006, Musquash has been protected from a wide variety of activities. The establishment of management zones allows for restrictive human use of different habitats based on sensitivity (Singh et al. 2000, Cooper et al. 2014, Greenlaw et al. 2014; Figure 1). Because of its size and importance to wildlife, the protection and monitoring of Musquash Estuary is a priority for conserving its valuable habitats, high biodiversity, productivity and influence on the surrounding environment (Blaber et al. 2000, Singh et al. 2000).

The biological monitoring framework of the Musquash MPA requires that protocols allow for the ability to identify changes in ecological characteristics, and monitor current activities and perceived threats (Singh et al. 2000, DFO 2011). Conservation objectives, as stated in the CSAS monitoring framework (DFO 2013), aim to achieve an ecosystem-level monitoring framework to "ensure that there is no unacceptable reduction or human-caused modification in productivity, biodiversity or habitat". Twelve indicators are listed to monitor the conservation objectives associated with monitoring the MPA. The conservation objectives and associated indicators (bold letters associated with indicators established in the Musquash Monitoring Framework; DFO 2013) include: Productivity, Biodiversity and Habitat.

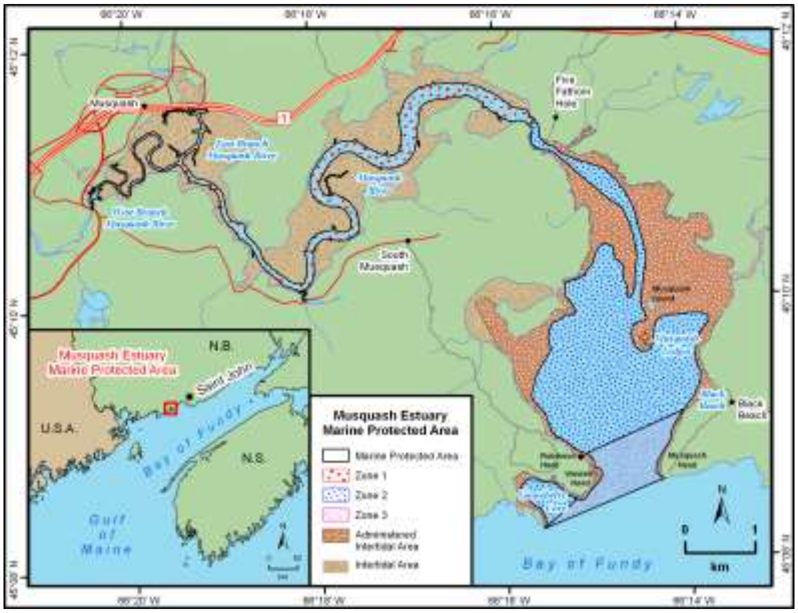

Figure 1: Musquash Estuary Marine Protected Area (MPA) and Administered Intertidal Area (AIA).

<u>Productivity</u>

This protocol will provide data regarding the total biomass and spatial distribution of nekton species (both fishes and free-swimming invertebrates) representing different trophic levels (**P1**). Fish abundance (biomass) and length data (to estimate life stage) will be collected using beach seine and fyke net samples from zone 2 (Figure 1), and fyke net samples from zone 1. Standardized sampling with these two gears at specific locations will provide information on CPUE measures that can be compared annually and spatially. This monitoring protocol recommends an intensive baseline-sampling period of 3-5 years, which will include sampling at least once monthly from May to October. After a baseline is established, sampling may only focus on key indicator species or sampling periods as established in the baseline. Sites recommended in the monitoring protocol within these two zones consider habitats (established in Greenlaw et al. 2014), the naturally occurring salinity gradient within the estuary as well as differing substrate types which may provide specific habitat use for fish species (for example, important nursery sites).

<u>Biodiversity</u>

Indicators of biodiversity addressed in this monitoring protocol will include data collected on nekton species richness. Frequency of sampling (at least once monthly) will enable the characterization of species utilizing different habitats within the estuary, including rare (**B1**) or species at risk (**B2**). The monitoring plan identifies external standardized sampling sites adjacent to Musquash Estuary that can be used as a reference to the species found within the MPA. From data collected during baseline sampling, as recommended in this monitoring protocol, species richness can be assessed spatially and annually for natural fluctuations in nekton diversity followed by long-term assessments to monitor change.

Habitat

During standardized sampling as recommended in this monitoring protocol, measurements associated with habitat will be collected alongside biological data. These contribute to the characterization of different habitats within the estuary (**H5**) through salinity, temperature, dissolved oxygen and turbidity metrics. Indicators of contaminant concentrations through the use of a sentinel species (Atlantic silverside, *Menidia menidia*, as mentioned in section 7.2) will also aid in monitoring chronic impacts of potential contamination (**H7**) on nearshore fish within the estuary.

The long-term monitoring of proposed future sampling in this document focuses on fish communities with additional invertebrate sampling, as indicators of conservation objectives identified in the monitoring framework. This monitoring protocol aims to further establish a fish community baseline and provide guidance for long-term monitoring. The objectives of this protocol are to:

i) Understand the fish community within multiple habitats of Musquash MPA by characterizing baseline spatial and temporal variability and environmental drivers by collecting information on:
    a. Abundances and lengths of individuals (productivity),
    b. Species richness (biodiversity)
    c. Environmental characteristics (habitat): Salinity, temperature, dissolved oxygen
ii) Assess the health of the estuary across multiple habitats for long-term assessments of potential human sources of contamination through:
    a. Use of Atlantic silverside as a sentinel species
    b. Weight, sex, age, Liver Somatic Index (LSI), Gonadosomatic Index (GSI) and condition factor (K) of individuals
iii) Compare Musquash fish community to reference locations outside of the MPA.
    a. Objectives i and ii are applicable to reference locations outside of the MPA.

A baseline for characterization of Musquash Estuary nearshore fish community has already begun with sampling events conducted by Arens (2007) and Ipsen (2013). These data allowed insight into the natural variability and habitat of the Musquash fish community. Arens (2007) identified sampling methodology issues such as tidal and diel restrictions, as well as the variability associated with spatial and temporal factors within a small geographic scale. The author also conducted a spatial comparison across multiple sites, including two of which were also sampled by Ipsen (2013). Strong temporal shifts due to seasonal changes (temperature) were apparent. Spatial differences associated with salinity and habitat characteristics were also observed with regards to fish utilizing areas as nursery sites.

Objectives in Ipsen (2013) were to begin the characterization of nearshore fish communities as part of a baseline within zone 2 of the MPA (Figure 1). In addition, sampling was to incorporate two reference sites, Dipper Harbour and Saints Rest Marsh, to serve as comparative sites outside of the MPA. Species richness, abundances, seasonal and temporal trends were consistent with other studies in similar areas. This study was comparable to Arens (2007) and attempted to characterize fish parameters within different nearshore habitats within the MPA along the natural salinity gradient and included: an intertidal sand and gravel beach, intertidal flat and brackish/cobble substrate (Black Beach, Hepburn’s Basin, Five Fathom Hole, respectively). This study found that temporal shifts in the community were more dominant than spatial differences, while each site displayed unique aspects of the community.

Information gathered from both Arens (2007) and Ipsen (2013) can be used as a starting point for a sampling baseline. This baseline can thus be developed alongside a long-term sampling regime. A long-term monitoring program within Musquash will allow a further understanding of fish habitat utilization together with physical, chemical, and biological factors that may be influencing temporal and spatial changes. The current protocol uses techniques from previous sampling methods (Arens 2007 and Ipsen 2013) in order for future sampling to be comparable. In addition, recommendations are given associated with: increasing community data by sampling both fish and other free swimming invertebrates (nekton); additional environmental parameters such as dissolved oxygen and turbidity; the use of a sentinel fish species; as well as additional reference sites to allow comparisons of the Musquash community.

As with the majority of estuarine systems, these environments are associated with extremely high environmental variability, exhibiting large daily changes in chemical and biological structure due to high fluctuations in salinity, water levels from tidal and other hydrodynamic processes (Macdonald et al. 1984, Ayvazian et al. 1992). Therefore, a long-term approach to establishing a baseline is important to assess the natural variability occurring within the area. This is necessary in order to identify existing or potential problems associated with human activities and enable mitigation measures.

2.2 Overview of Protocol

In order to begin sampling within Musquash Estuary MPA, proper permits need to be obtained from the Department of Fisheries and Oceans Canada (DFO). Proponents of sampling need to submit 1) an Application Form for Activity within the Musquash Estuary Marine Protected Area (MPA) and Administered Intertidal Area (AIA), at least 60 days prior to sampling, and 2) an Application for Licence Issued Pursuant to Section 52 of the Fishery (General) Regulations, at least 30 working days prior to the proposed date of activity.

Three main techniques are recommended for sampling the nekton community (free swimming invertebrates and fish) within Musquash Estuary: 1) nearshore sampling, conducted with a beach seine and fyke net (section 4.1, 4.2 and 7.1); 2) salt marsh sampling completed with a fyke net (section 4.2 and 7.1); and 3) the collection of Atlantic silversides (*Menidia menidia*) using the beach seine, for use as a sentinel species (section 7.2).

3.0 PERSONNEL AND TRAINING

It is recommended that a minimum of two people sample for security reasons during each sampling event. However, if resources permit, having three or four people is more favourable, particularly during fyke net setup (establishing pole locations) or during peak season when abundances of fish are high. At least one person of the group should have a biology background (minimum Bachelor’s degree) and be trained (by personnel experienced with local species) to be able to efficiently identify species of both fish and invertebrates. Considerations for a workshop provided by DFO may be useful, such as for the CAMP program (Weldon et al. 2007). This person should also have experience with the sampling methods and be able to make decisions in the field regarding sampling gear placement. Other members of the group do not need extensive training (community group members or interns) but it would be beneficial if they were familiar with, or able to learn, sampling methods (beach seining), species identification techniques, and fish measurements.

4.0 SAMPLING GEARS

Sampling gears used for estuarine fish collection, for the most part, use seines (Ayvazian et al. 1992), fyke nets (Honey et al. 2004) as well as throw traps (Raposa et al. 2003) for sampling the nearshore area (water depths <2m). Honey et al. (2004) evaluated the use of a seine in nearshore environments for long-term monitoring in which fry, juveniles, yearlings and year 2+ fish of different species were caught. The seines ability to be used relatively quickly in habitats ranging from beaches to vegetated areas was emphasized as an important attribute. However it has been noted that seines have low collection efficiency (38%; De Mutsert and Cowan 2012). Therefore, the seine should be used in conjunction with other sampling gears, as long as both gears are found to be complementary. In Honey et al. (2004), the seine was often used with other gears such as trawls and fyke nets to characterize fish assemblages. Specific to the nearshore area, fyke nets are typically used to capture fish during spawning migrations and tagging studies. Dual gears (fyke net and beach seine) were also utilized in Musquash (Ipsen 2013). At Five Fathom Hole anadromous species such as smelt and American eel were often caught in the channel with the fyke net, while smaller bodied fish and juveniles were captured in the beach seine. This method also enabled data collection pertaining to the succession of cohorts within the estuary. For example, *Pollachius virens* (pollock) were detected in the spring and early summer in the beach seine followed by catches solely in the fyke net in later summer months.

It is therefore recommended that only two gear types be used for the collection of fish within Musquash Estuary and reference sites; beach seine and fyke net. Both of these gears are easy to manage, perform minimal harm to the sampled fish, and are non-destructive to the habitat in which they are sampling. Both gear types are relatively lightweight (when dry) and can be easily carried for shore access or placed in a small boat (canoe) to be transported to sites (Gibson et al. 1996). However, sampling gears will get significantly heavier to carry following a sampling event, and might require two people to transport back to the truck. For a list of other equipment recommended for sampling see Appendix A.

4.1 Beach Seine Sampling Methods

A beach seine (Figure 2) is an active sampling gear that is commonly used in sampling shallow water (<1.5 m). A beach seine of 9 x 1.5 m (9 mm stretch mesh) with a central collection bag, is recommended for use to be comparable with previous studies (Arens 2007, Ipsen 2013).

It is recommended that sampling with a beach seine should take place at, or within a maximum of 2 hours after a low, slack tide. Sampling within an hour of low tide has been found to yield the greatest numbers of individuals and highest species richness compared to other tidal stages (Gibson et al. 1996, Arens 2007). Sampling conducted two hours after low tide may render access (or departure) from site difficult. Beach seining requires two people to operate. Sampling involves the seine to be towed so the net is perpendicular to the shore at a depth comfortable for the outermost person (~1 – 1.5m) and about knee to waist height for the person closest to the shore.

Seining should be performed for 3 minutes at a slow walking pace. At three minutes the person closest to the shore will slow their pace, while the person furthest from the shore will begin to curve toward the shore until both people are towing so the net is parallel to the shore. The net is hauled to the shore, making sure the bag remains centered and the lead line remains on the bottom. Once the bag end of the seine is out of the water the contents can be emptied into a 20 L bucket with an aerator. This is considered a single tow. It is important that the lead line remains against the substrate for the duration of the tow. It is helpful to hold the poles at a slight angle so that the bottoms of the pole are angled in towards the net. Sampling with the seine should be conducted during the day.

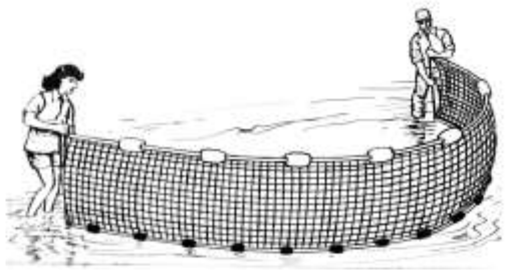

Figure 2: Beach seine sampling gear. From: https://en.wikipedia.org/wiki/Beach_seine#

4.2 Fyke Net Sampling Methods

A fyke net (Figure 3) is a passive sampling gear. Recommended fyke nets should be 3.7 m long and consist of four hoops (mesh size 38.1 mm stretch mesh in the wings and body, and 22.2 mm stretch mesh in cod end) and have two 3 m long wings attached to the opening of the net. Wings taper from 100 cm deep (where they are secured by metal rods driven into the sediment) to 70 cm at the opening of the net (Ipsen 2013).

The fyke net will take 2-3 people to initially set up, establishing secure pole locations, and two people to hook up the net and retrieve the following day. This will involve carrying poles (3 rebar poles: at least 8 ft long) and a post driver to each location. It is recommended that the poles be installed at the sampling location either permanently or for the duration of the sampling season. ***Important:*** Poles and fyke nets should be labeled according to requirements under the Fishery General Regulations, SOR-93-53. These include:

- The name of *one* person responsible for the gear securely affixed to a tag, float or buoy
- The name be legible and readily visible at all times without having to manipulate (raising the gear or removing ice and snow)
- The name be in solid, black capital letters in Roman characters with no ornamentation; at least 75 mm in height; and in a contrasting colour compared to the background
- The tag, float or buoy should be affixed to each end of the gear

Additionally the tag, float or buoy should include the MPA approval number and Section 52 license number.

Fyke nets situated in a channel should be placed so that the opening of the net will face upstream, catching fish on the ebb tide. Similarly, fyke nets located on a beach will face the shore, catching fish on the ebb tide. Poles for fyke nets located on beach sampling locations should be placed in a location either deep enough, or off to the side, as to not interfere with beach seining tows. The fyke net will be secured onto the poles at low tide, and retrieved 24 hours later, sampling fish through two tidal cycles.

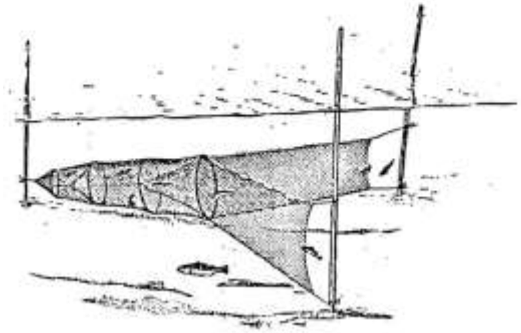

Figure 3: Fyke net sampling gear and poles. From FAO 2001.

<u>4.2.1 Tidal Considerations</u>

Bay of Fundy tides provide challenges for sampling with passive gears. Tidal height should be a consideration while establishing fyke net locations. The following points should be kept in mind while establishing fyke net locations.

- It is important to monitor the fyke net at low tide, when first established at a new sampling location.
- If nets are set at low tide during a spring tide, they may be difficult, or impossible, to access during neap tidal cycles.
- Oppositely, if net locations are established at low tide, during neap tidal events, then fyke net sampling during different tidal stages may result in the net becoming completely exposed at low tide. This will result in high mortalities of fish during low tide.
- It is recommended that nets be installed at low tide during the transition period between spring and neap tides. This will allow for greater flexibility in sampling schedule, so as not to be restricted to extreme tidal events.
- Take note of the low water level at the time of sampling (often provided on tidal websites of the area; Appendix B). This will provide insight into the location's ideal time to sample, as well as providing consistency.
- When the net is retrieved, 24 hours after setup, specimens will be placed in a bucket with an aerator for further processing to occur (see section 7 for details and Appendix A, for equipment).

5.0 FREQUENCY OF SAMPLING

Sampling frequency in estuarine systems varies throughout the literature, and is heavily depended on resource availability and study objectives. In most cases where the characterization of fish communities were performed for the establishment of a baseline, sampling frequency included daily (Honey et al. 2004), twice-monthly (Honey et al. 2004, Arens 2007, De Mutsert and Cowan 2012, Ipsen 2013), and monthly (Thériault et al. 2006, Weldon et al. 2007, De Mutsert and Cowan 2012) sampling regimes in order to capture temporal variability.

Increasing sampling frequency is favourable when establishing a baseline as it reduces the variability between samples (Raposa et al. 2003) and reduces the occurrence of type II statistical errors. This reduction in variability was noticed in Ipsen (2013) where sampling at Black Beach, twice a month, allowed for the quantification of 20% more species and 40% more individuals when compared to once monthly sampling events. High variability in fish communities in temperate estuaries is a common issue. Not only do fish communities differ spatially and temporally due to factors such as dynamic seasonal temperatures or site characteristics (e.g., turbidity, substrate heterogeneity; Able and Fahay 1998, Arens 2007, Ipsen 2013) but also have been known to change following tidal, diel, and lunar cycles (Gibson et al. 1996, Arens 2007). Efforts to reduce this variability include utilizing a standardized group of differing sampling gears to correct for sampling bias (Honey et al. 2004), maintaining consistency in tidal height and time of day (Gibson et al. 1996, Thériault et al. 2006, Weldon et al. 2007, Arens 2007), achieving consistent sampling frequency and duration over multiple years (Thériault et al. 2006 and Weldon et al. 2007), as well as having accurate identification of specimens (Thériault et al. 2006).

In order to establish a thorough baseline for Musquash Estuary nearshore fish community, it is recommended that an intensive sampling period be completed. This will enable an understanding of the natural variability occurring within the estuary. Baseline sampling should be pursued for at least a three to five year period, followed by ongoing monitoring periods that are established by what intensive baseline data reveals. Data collected during baseline collection can also be compared to Arens (2007) and Ipsen (2013). Previous studies characterizing fish or nekton communities within estuaries have at least two years (Ayvazian et al. 1992, Able et al. 2002, Clark et al. 2009, Courrat et al. 2009, Dolbeth et al. 2010); five years (Lazarri et al. 1999, Selleslagh and Amara 2008); or to up to over a decade of continuous intensive monitoring (De Mutsert and Cowan 2012).

Sampling should take place at intervals of at least once monthly from May to the end of October, and be repeated annually, at all locations. Sampling more frequently (twice a month instead of once monthly) would be beneficial if resources permit. However, due to sampling limitations enforced by the tides, sampling once monthly is currently recommended for this sampling protocol. By sampling twice a month, an estimate of spatial and temporal patterns of dominant species, and rare or transient species, can be more easily identified. Sampling only once a month may miss rare and transient species, as seen in Ipsen (2013). By sampling once a month, temporal and spatial trends of dominant species may be characterized. Where resources are limited, uniquely sampling during summer months (July, August, and September) will provide an estimate of the spatial distribution of dominant species utilizing nearshore habitats during peak season. If sampling only during one season is all that is feasible for a sampling, it is first recommended to sample all three months, twice monthly. Quantitative data on species richness and abundance can be pooled and compared among sites (n=3, averaged two sampling periods per month), reducing sample variability when compared to a single monthly sampling design (n=3).

6.0 SAMPLING LOCATIONS

The following map (Figure 4) shows the location of Musquash Estuary relative to three other estuaries proposed for sampling as control sites including Saints Rest Marsh (to the east) as well as Chance Harbour and Dipper Harbour (to the west).

Figure 4: Google Earth image showing estuaries to be sampled for proposed long-term monitoring (DigitalGlobe 2015a)

6.1 Sampling within Musquash Estuary MPA

Musquash Estuary fish sampling can be divided into two separate main habitats of focus; 1) the nearshore habitat and 2) the salt marsh habitat consisting of salt marsh channels. Sampling these two main habitats will most likely co-occur during sampling events. It is recommended that the nearshore sampling use both a beach seine and fyke net, whereas salt marsh channels use a fyke net. Further considerations regarding the two main habitats (nearshore and salt marsh) can be seen in section 6.1.3.

Habitats not included in this protocol
Habitats not included in this protocol are those that may be considered for future sampling but are not recommended as part of a long-term protocol at this time. These habitats include the subtidal main channel or bay and salt marsh pannes. In the case of the subtidal main channel or bay, sampling restrictions associated with the use of gears that may alter the habitat, such as trawls (DFO 2011) make sampling the subtidal portions of Musquash not applicable at this time. However, sampling this habitat may provide insight into larger species of fish utilizing the MPA and should be included in future considerations depending on gear type and study design.

The fish community within salt marsh pannes has not been previously documented. Access to these locations is time consuming. Previous sampling attempts within salt marsh pannes at Hepburn's Basin, with minnow traps showed both low catch numbers and low species richness (Ipsen, personal observation). Future considerations for this habitat are encouraged but are difficult to justify within a long-term monitoring plan due to the effort (man hours vs outcome) associated with these locations. Therefore, this sampling protocol focuses on salt marsh channels as sampled in Gratto (1986) and Ipsen (2013).

6.1.1 Nearshore Beach Seining and Fyke Net Locations

The nearshore habitat consists of four sampling sites in zone 2 of the MPA (Figure 5). These include: Five Fathom Hole, Black Beach, Hepburn's Basin and Gooseberry Cove. These recommended sites are chosen based on previous sampling events using similar gears that can be incorporated into baseline information (Arens 2007, Ipsen 2013) or have been known to be accessible (Matt Abbott, Conservation Council of New Brunswick; pers. comm.).

Although, these sites are classified as "nearshore", each of them uniquely represents their own habitat type. These four locations represent the natural salinity gradient from Five Fathom Hole, the most brackish site, to Gooseberry Cove, the outermost region of the MPA (Figure 5). Similarly, substrate types differ among nearshore sampling sites, encompassing rocky cobble substrates (Five Fathom Hole/ Gooseberry Cove), an intertidal sand and gravel beach (Black Beach) and an intertidal flat (Hepburn's Basin) (Greenlaw et al. 2014). These characteristics, both salinity gradient and substrate type, have been shown to represent differing fish community characteristics, affecting the occurrence of demersal and pelagic species or presence of juveniles, within Musquash (Ipsen 2013) and within estuaries in general (Gibson et al. 1996, Albaret et al. 2004). It is recommended that all four of these sites be assessed annually for the establishment of a nearshore baseline and continue to be sampled for long-term monitoring.

Figure 5: Musquash Estuary proposed sampling sites (DigitalGlobe 2015b). Four sites are recommended for beach seine and fyke net sampling in zone 2 of the MPA.

Black Beach

With a main road leading to the beach (Figure 6), this site is the easiest site to access and sample. It is also the most public location of the sampling locations. To prepare sampling equipment and drive from Saint John to Black Beach sampling can be completed within 2-3 hours depending on fish abundances.

Figure 6: Black Beach sampling site (DigitalGlobe 2015c). Straight line indicates path of multiple tows at approximate low tide mark and the x marks where nearshore fyke net sampling could take place.

Five Fathom Hole

Five Fathom Hole is accessed from the Five Fathom Hole wharf (Figure 7). It is necessary to carry gear to the sampling location (~550m: 10-15 minutes). To access the beach seine location, Ferguson Creek will need to be crossed. At low tide, this creek is very shallow with gravel patches, which makes crossing easy. It is important that the sampling team have proper fitting wader boots at this site, as the terrain in some spots is deep mud where someone can easily get stuck. Life jackets are also recommended as the beach where seining takes place drops off quickly. Sampling Five Fathom Hole takes about 2-4 hours. *Time saving tip:* At Five Fathom Hole, the fyke net was stored in the woods, hidden in the trees so it did not need to be carried each time.

Figure 7: Five Fathom Hole beach seining site (marked with a straight line) and fyke net location in the channel (marked with an x) (DigitalGlobe 2015d)

Hepburn's Basin

Hepburn's Basin is the most time-consuming site to access (Figure 8). To get to the area to seine and where the fyke net is placed, is approximately 1.5 km of walking with sampling gear (~15-20 minutes). Leaving from Saint John, it takes approximately 4-6 hours to sample at this location with the beach seine and 4-5 hours to collect the fyke. It is important that researchers wear proper fitting waders/boots for sampling this location, as it is necessary to cross the intertidal flat to seine and set up the fyke net. *Time saving tip:* Similar to Five Fathom Hole, the fyke net was stored hidden in the woods, so it did not need to be carried to the site for each sampling event.

Figure 8: Hepburn's Basin seining site (marked with a straight line) and fyke net locations (nearshore and in the channel; marked with an x) (DigitalGlobe 2015e). For channel sampling see salt marsh sampling (Section 6.1.2).

Gooseberry Cove

Gooseberry Cove beach (Figure 9) is accessible by vehicle. Accessing this site will take a longer commute. Estimated time for commute from Saint John and sampling at this location is 3-4 hours with beach seine, 2-3 hours to collect fyke net.

Figure 9: Gooseberry Cove seining site (marked with a straight line) and potential fyke net location (marked with an x) (DigitalGlobe 2015f).

6.1.2 Salt Marsh Fyke Netting Locations

The salt marsh system in Musquash Estuary is considered very valuable for productivity parameters in the Bay of Fundy (Singh et al. 2000). The salt marsh system is located along the channel in zone 1 as well as an area by Hebpurn's Basin in zone 2 (Singh et al. 2000) (Figure 1, 8 and 10). Accessing salt marsh habitats within Musquash Estuary is challenging. There have been very few studies characterizing fauna in this system. Gratto (1986) identified species within a salt marsh channel at Hepburn's Basin, and Ipsen (2013) identified species utilizing a salt marsh channel, at Five Fathom Hole within Ferguson Creek. Based on the value placed on the productivity of the salt marsh system in Musquash Estuary, characterizing the fish community within this habitat as part of the long-term monitoring program, will provide important information of species utilizing this habitat type.

Musquash Estuary salt marsh habitats provide unique challenges. Gears used for sampling salt marsh habitats vary depending on accessibility and resources. Methods used to collect fish in these habitats include: throw traps, lift nets, minnow traps, breder traps, fyke nets, towed nets/seines and trawls (Carlisle et al. 2002, Raposa et al. 2003, Ipsen 2013). It is recommended to use a passive gear such as a fyke net to sample tidal creeks and channels. Small traps, such as minnow traps, may become easily buried in mud or swept away due to the dynamic force of the tide. The salt marsh habitat of Musquash consists of narrow channels and is usually surrounded by steep banks and mud, making access and the use of active sampling gears such as beach seining difficult. Fyke net sampling in this habitat should be carried out in a similar fashion as nearshore habitats in that the net should be placed facing upstream to catch fish on the ebb tide (Section 3.2).

Suggested salt marsh sampling sites within the MPA are located both in the main channel (zone 1; Figure 10) as well as in the salt marsh channel located at Hepburn's Basin (zone 2; Figure 8 and 10). Approximately 7 channels could be recommended for sampling (Figure 10). However, most of these, except two (accessible by road, or by foot at Hepburn's Basin), would only be accessible by canoe. These sampling locations were chosen based on the width of the channel compared to Ferguson Creek at Five Fathom Hole. Alternative locations may need to be considered based on site accessibility and resources at the time of choosing sites and sampling.

Figure 10: Potential salt marsh sampling locations for fyke net (marked with an x) (DigitalGlobe 2015g). Sampling location in orange signifies a known road access site. All other locations may need to be accessed by canoe (zone 1) or by foot (zone 2). Alternative sampling locations to those portrayed here may be considered depending on ease of access to the channel or other habitat limitations.

6.1.3 MPA Sampling Considerations

Timing of sampling events

Depending on the site and season (differing abundances of fish), varying times may be required to successfully sample a location. It is difficult to access multiple sites during the same day without being rushed, particularly for seining, unless fish abundances are low and quickly processed. For example, it may be possible to seine Black Beach, at low slack tide, set up the fyke net, and make it over to Five Fathom Hole within an hour of low slack tide, seine, and set up the fyke net before water becomes too high. Similarly, it may be possible to retrieve multiple fyke nets on the same day for nearshore and salt marsh sampling events, but this will depend on the ability of the group leader to identify species, as well as monitor tidal restrictions. For the purposes of this proposal, it is recommended that sites be sampled on as few consecutive days as possible, due to diurnal differences that may affect species compositions (Gibson et al. 1996). However, differing days may be more realistic for first time sampling at a site. The possibility of sampling multiple sites will be at the discretion, and comfort level of the lead member of the group. For an example of a sampling period and tidal restrictions, see Appendix B and Appendix C.

Site accessibility
Musquash Estuary is a challenging environment to access. Sampling locations range from fairly easy to access to more time consuming. For example Black Beach is the fastest site to sample because it is a relatively short commute from Saint John, provides the ability to drive right to the sampling location, and allows for sampling with few obstacles (in contrast, sand and gravel beach compared to intertidal flats or rocky cobble). Sampling Hepburn's Basin consists of a longer commute (45 minutes – 1 hour), and a hike down a trail and across an intertidal flat (10-15 minutes).

Although all sites are recommended for sampling annually, limitations arise which may prevent access to one or more sites. Black Beach is an ideal site to continue monitoring as it has two previous sampling events (Arens 2007 and Ipsen 2013) and is a very quick site to sample. However, Black Beach was found to have reduced species richness, with decreased numbers of rare species and juveniles, compared to other sites within the estuary (Ipsen 2013). This site is also more public which may provide problems with gear tampering (fyke net) or disturbances (people swimming). Choosing which sites will be sampled over others will be at the discretion of the group leader, the resources available (time and people) and the time of year.

Other considerations for sampling sites within Musquash may be the use of a small vessel to access sites instead of by road. This may be a possibility for reducing time between sampling events. Accessing salt marsh channels in zone 1 will require a non-motorized vessel as per the Musquash Estuary Marine Protected Area Regulations (SOR/2006-354). A small-motorized boat may work between some sites in zone 2 (Five Fathom Hole and Black Beach) but difficulties may arise while attempting to access other sites, particularly Hepburn's Basin, from the water. Hepburn's Basin is an intertidal flat and has a low gradient of shallow water, making access with a boat possibly problematic around low tide.

6.2 Reference Sampling Locations Outside the MPA

In areas where fish are highly migratory, Higdon et al. (2013) suggest that sampling in a larger geographic area (outside the MPA) was an effective way of characterizing the fish community that may be utilizing a protected area. Fish community comparisons for long-term monitoring could include Saints Rest Marsh (Doyle et al. 2011, Arens 2007, Ipsen 2013), Chance Harbour (Arens 2007), and Dipper Harbour (Arens 2007, Ipsen 2013). These sites have already been established as locations that are accessible and have been previously sampled for nearshore fish communities. They are also estuaries that bracket Musquash (Figure 4), enabling assessment of spatial trends associated with potential salinity gradients or anthropogenic disturbances stemming from point source pollution sites within close proximity (Singh et al. 2000, Doyle et al. 2011). Salt marsh habitats located at Saints Rest Marsh and Dipper Harbour have also been known to be accessible through extensive studies involving salt marsh vegetation (Dipper Harbour) and field trips by the University of New Brunswick ecology class (Saints Rest Marsh).

Saints Rest Marsh
Also known as Irving Nature Park or Manawagonish Creek (Figure 11), this site has been previously sampled for the nearshore fish community by Ipsen (2013), and has been accessed by the University of New Brunswick ecology field class for sampling of the salt marsh. It is in close proximity to Musquash Estuary, is not protected and is in close proximity to Saint John Harbour. It has also been studied for potential contamination impacts in Doyle et al. (2011). At lower tidal

heights the beach turns more into an intertidal flat, similar to Hepburn's Basin, and can be difficult to walk on, particularly to place the fyke net.

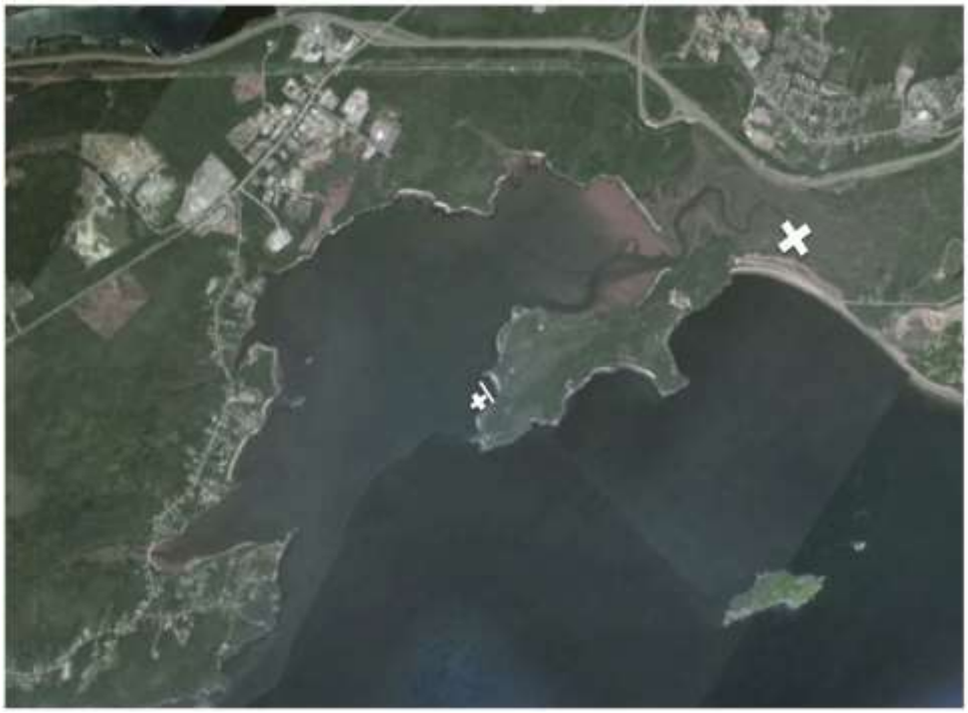

Figure 11: Saints Rest Marsh seining site (marked with a straight line) and fyke net locations (nearshore and in the channel; marked with an x) (DigitalGlobe 2015h).

Chance Harbour

Chance Harbour (Figure 12), was previously studied in a spatial comparison of fish community by Arens (2007). This site has easy beach access and a salt marsh that appears to be accessible by the road.

Figure 12: Chance Harbour potential seining site (marked with a straight line) and fyke net locations (nearshore and in the channel; marked with an x) (DigitalGlobe 2015i).

Dipper Harbour
Located the furthest from Musquash (Figure 13), Dipper Harbour was sampled as part of Ipsen (2013) for the nearshore fish community. Dipper Harbour's beach is easily accessible by a road. However, permission from landowners is needed. Substrate at Dipper was similar to Black Beach. Dipper Harbour's salt marsh is easily accessible and salt marsh monitoring from vegetation studies has been previously conducted by Chmura et al. (1997).

Figure 13: Dipper Harbour seining site (marked with a straight line) and fyke net locations (nearshore and in the channel; marked with an x) (DigitalGlobe 2015j).

7.0 NEKTON COMMUNITY SAMPLING

7.1 Community Assessments

Both invertebrates and fish species will be counted and identified to species. Fish lengths will be measured (Fork length (FL)) to estimate age. In instances where high abundances of a single species of fish are caught, only 30 individuals are measured to decrease likelihood of mortality. A list of potential fish species either previously collected within Musquash Estuary or in surrounding habitats can be seen in Appendix D.

Data
Data will be obtained for fyke net and beach seine collections separately. Due to differing mesh sizes this information should also be analyzed separately. The data collected from the fish sampling protocol should include: number of fish caught, species identification, and individual lengths to estimate the life stage of the fish. For a template of metrics sampled see Appendix E. These data were simple to transfer into an excel file to perform filtering, pivot tables and analyses. An example of these data can be seen in Table 1.

Table 1: Example of raw data collected within Musquash Estuary at sites Black Beach (BB) and Five Fathom Hole (FFH) during either the first two week period of the month (E- Early) or the last two week period (L- Late).

Day	Month	Year	Location	Gear	Tow	Species	Length
L	10	2009	BB	Seine	2	Winter Flounder	53
L	10	2009	BB	Seine	2	Winter Flounder	48
L	10	2009	BB	Seine	2	Winter Flounder	37
L	10	2009	FFH	Seine	1	Atlantic silverside	43
L	10	2009	FFH	Seine	1	Atlantic silverside	65
L	10	2009	FFH	Seine	1	Atlantic silverside	43
L	10	2009	FFH	Seine	1	Atlantic silverside	43
L	10	2009	FFH	Seine	1	Atlantic silverside	61
E	11	2009	BB	Seine	1	Atlantic silverside	64
E	11	2009	BB	Seine	1	Atlantic silverside	60
E	11	2009	BB	Seine	1	Atlantic silverside	85
E	11	2009	BB	Seine	1	Atlantic silverside	66
E	11	2009	BB	Seine	1	Atlantic silverside	43
E	11	2009	BB	Seine	1	Atlantic silverside	62
E	11	2009	BB	Seine	1	Atlantic silverside	66

These data can be analyzed using univariate analyses (ANOVAs for factors site and season, as well as their interactions), non-parametric analysis on fish sizes, and multivariate analysis (PERMANOVA's, nMDS plots and SIMPER comparisons for community data in PRIMER; see Ipsen 2013).

7.1.2 Large Abundances of Nekton

During warmer seasons there may be large numbers of fish and invertebrates (counts easily determined as greater than 300). In such cases, deciding to estimate the total number of individuals of these species is best, to avoid mortality from overcrowding in the bucket. This instance is likely to take place for sticklebacks (in the spring) as well as Atlantic silversides and sand shrimp. Methods used by Weldon et al. (2007) suggest that between 2 to 5 subsamples (depending on time and number of persons sampling) of individuals is taken using square, 20-cm, aquarium dip nets. Individuals in the subsample are enumerated, and the remainder of the individuals are emptied out of the bucket into the dip net and released, taking note of the counts of dip nets that are filled. It is important to ensure that only the high abundant species is present in dip net and all other, less common, species are accounted for and processed in the proper way. It is also important to do this quickly, particularly for oxygen sensitive species such as Atlantic silversides.

7.2 Contaminantion Indicator through Sentinel Species Collections

Nearshore environments are subjected to various sources of contamination. Although established as an MPA, Musquash Estuary, located within 20 km of Saint John Harbour, has the potential to be impacted by contamination affecting the harbour. Although the evaluation of sediment contamination sources in Musquash is underway, there is currently no biological monitoring for chronic effects of potential contamination on fish. The most popular sentinel species used for characterization of anthropogenic disturbances in marsh ecosystems are the mummichog, rock gunnel and tomcod. Although present, these fish were not ubiquitously found in large abundances within Musquash Estuary. In the case of Atlantic tomcod, high catches were limited to fyke nets (Ipsen, 2013). Based on data of fish abundances within Musquash Estuary (Arens 2007, Ipsen 2013), the only other species that would match the criteria for a sentinel species would be the Atlantic silverside. Doyle et al. (2011) used the Atlantic silverside as a sentinel species to characterize contamination gradients in Saint John Harbour. It is therefore recommended that a baseline for fish health for Atlantic silversides become established as a way to assess potential impacts of contamination occurring within the estuary.

Menidia menidia or Atlantic silverside (Figure 14) is a common species resident to coastal habitats, marshes, and intertidal creeks from southern Gulf of Saint Lawrence to northern Florida (Scott and Scott 1988, Colette and Klein-MacPhee 2002, Able and Fahay 1998). Atlantic silversides can be found in these nearshore habitats throughout the year (Able and Fahay 1998). Silversides start spawning at sizes as small as 42 mm total length (Able and Fahay 1998). The spawning season varies depending on latitude but typically starts in spring (May-June) and continues to late summer (July-August) (Able and Fahay 1998). Because they are smaller bodied, these fish are successfully caught in the nearshore environment with a beach seine. In Ipsen (2013), data collections of Atlantic silversides showed that CPUE was the highest in later summer months (August – November). Abundances decreased (1-30 individuals) between December and May. Hepburn's Basin nearshore sampling showed a spike in abundance in June, followed by the other nearshore sampling sites. However, silverside catches have the potential to be highly variable: absent or in small numbers (<50) during one sampling event followed by numerous individuals (>500) the following event (Ipsen 2013). Silverside collections within Musquash Estuary align with other observations in the area. Future collections and sampling of Atlantic silverside for use as a sentinel species are recommended based on Doyle et al. (2011).

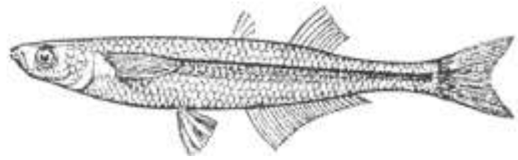

Figure 14: Atlantic silverside, *Menidia menidia*. From: http://www.glf.dfo-mpo.gc.ca/Gulf/FAM/IMFP/2000-2004-Silversides-PEI

Atlantic silversides meet many criteria for the use as a sentinel species. Doyle et al. (2011) found that while inshore, Atlantic silversides are locally resident. However, they tend to leave inshore areas during late spring to spawn, and again in late autumn for winter. Considerations for sample size and lab techniques are suggested. However these methods are recommended based on information collected by a single study using Atlantic silversides collected from Saint John Harbour (Doyle et al. 2011). As more information is collected on Atlantic silversides found within Musquash Estuary these recommendations may change. As per the Environmental Effects Monitoring Program guidelines in Canada, it is recommended to sample spawning fish just prior to spawning when gonads are developed and at similar growth stages. For Atlantic silverside, sampling should occur in late May or June, prior to the full moon. However, it is also recommended that silversides be taken upon their return in late summer as well, when abundances are higher, to estimate growth (Thériault et al. 2006, Doyle et al. 2011).

During these two sampling periods, it is recommended that at least 60 - 100 adult silversides (to try to capture at least 20 of each sex) of lengths approximately >45mm FL are kept while completing nearshore fish community sampling (Section 6.1). Fish brought back to the lab will be placed in a cooler full of ice and anesthetized with tricaine methanesulfonate (Western Chemical Inc., Ferndale, Wash., U.S.A.; 160 mg/L for approximately 2 to 5 minutes). Fish will be weighed and measured to determine the total FL (± 0.1 cm) and the total body weight (± 0.01 g).

After the severance of the spinal cord, the fish will be dissected to determine the gonad and liver weights to the nearest 0.001 g and the removal of scales or otoliths for the determination of age. This information will be used to calculate Gonadosomatic Indices (GSI), Liver Somatic Indices (LSI), and condition factor (K). Comparisons of these factors can then be made between Musquash Estuary sites and reference sites outside of the MPA. Additionally, a section of the liver should be taken after weight, and immediately placed in a cryovial to be stored at -80°C for the analysis biomarkers indicating contaminant exposure (Thériault et al. 2006, Doyle et al. 2011).

8.0 SUMMARY

Sampling Musquash Estuary and its reference sites consists of using two sampling gears (beach seine and fyke net) for the collection of nekton data. Data collected on fish communities includes: species richness, abundance, lengths as well as Atlantic silversides for use as a sentinel species. Data collected on invertebrates includes species richness and abundance, which will provide complimentary data on the community that is utilizing nearshore habitats. In order to establish a baseline at recommended sites it is necessary to conduct an intensive sampling period over the course of 3-5 years depending on resources. Sampling frequency is recommended to occur at least once monthly between May and October. This information will provide insight into the annual variability associated with the fish community. Based on data collected during the intensive baseline, further decisions can be made as to the most effective way to sample Musquash Estuary and its reference sites.

Musquash Estuary is a dynamic system and considerations will need to be made as to the best sampling regime for each site. It is important to maintain a level of consistency (tidal height, time of day, sampling methods) as fish communities are highly influenced by physical factors in these environments. Care must be taken with passive gears as to provide sufficient depth at low tide to reduce fish mortalities. An estimated timing for sampling for each site can be seen in the Appendix C as a starting point for establishing a sampling regime.

This protocol does not cover all habitats found in Musquash Estuary. The main channel/bay as well as salt marsh pannes may be considered for future sampling. These systems may provide insight into other fish communities utilizing the Estuary.

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Appendix A: Equipment checklist for field

- Beach seine
- 3-6 Fyke net (s)
- Other field equipment
    - A truck
    - Well fitting chest waders
    - GPS
    - Post pounder (first trip only to set poles for fyke net)
    - Weatherproof (Rite in Rain) notepad
    - Pencil
    - YSI with probes for
        - Temperature
        - Salinity
        - Dissolved oxygen
    - Secchi disk
    - Buckets
        - Three for beach seining events
        - Two for fyke net sampling
    - Dip nets
    - Portable, battery powered oxygen stone and bubbler
    - Fish measuring board
    - Containers for unknown species
    - Containers or bags, cooler and ice for sentinels

- General equipment
    - Camera
    - Lifejackets
    - First aid kits for field location and or vehicle
    - Cell phone
    - Rain gear, polarized glasses
    - Sunscreen, hat, bug spray
    - Extra batteries for sonde

Appendix B: Sample tidal sampling

Consistency was maintained in Ipsen (2013) by sampling in the morning and within tidal ranges around 1 to 1.8 m (when possible). In Table 2, an example of sampling periods is shown. This example runs from April 5th to April 12th. Sampling in later months will allow more flexibility due to the increased daylight period. An example of how sampling may occur would include going to sites to set up the fyke net prior to low tide events (but not necessarily in the dark). The sites that are reached at low tide would be seined along with the fyke net being set up (within a maximum of 2 hours of low tide). As the morning progresses, it would be possible to continue to set up fyke nets at other sites as the tide rises (as long as the low water mark is known). This is important so fish don't become exposed during the next low tide period, and ensures that the net can be adequately retrieved 24 hours later while not jeopardizing the safety of the sampling crew.

Table 2: Tide table corresponding with Figure 15 (below). Table shows example time frame of when sampling could occur (shaded low tide rows). Sampling times over several days, and seasons, may change with changing low tide time and light availability (sunrise).

Date	Time	Tidal height (m)	Phase
Sunday 5 April	0:57	7.44	High tide
	6:58		Sunrise
	7:12	0.9	Low tide
	7:32		Moonset
	13:17	7.41	High tide
	19:33	1.11	Low tide
	19:57		Sunset
	21:16		Moonrise
Monday 6 April	1:34	7.48	High tide
	6:56		Sunrise
	7:52	0.93	Low tide
	8:03		Moonset
	13:56	7.41	High tide
	19:58		Sunset
	20:12	1.22	Low tide
	22:16		Moonrise
Tuesday 7 April	2:12	7.48	High tide
	6:54		Sunrise
	8:32	1.01	Low tide
	8:38		Moonset
	14:36	7.37	High tide
	19:59		Sunset
	20:52	1.37	Low tide
	23:16		Moonrise
Wednesday 8 April	2:53	7.45	High tide
	6:52		Sunrise
	9:15	1.12	Low tide
	9:18		Moonset
	15:18	7.3	High tide
	20:01		Sunset
	21:34	1.54	Low tide

Date	Time	Tidal height (m)	Phase
Thursday 9 April	0:15		Moonrise
	3:36	7.41	High tide
	6:50		Sunrise
	9:59	1.24	Low tide
	10:03		Moonset
	16:04	7.23	High tide
	20:02		Sunset
	22:19	1.68	Low tide
Friday 10 April	1:10		Moonrise
	4:25	7.36	High tide
	6:49		Sunrise
	10:48	1.33	Low tide
	10:55		Moonset
	16:55	7.16	High tide
	20:03		Sunset
	23:11	1.78	Low tide
Saturday 11 April	2:02		Moonrise
	5:18	7.34	High tide
	6:47		Sunrise
	11:43	1.36	Low tide
	11:53		Moonset
	17:50	7.13	High tide
	20:04		Sunset
Sunday 12 April	0:09	1.76	Low tide
	0:45		Last
	2:49		Moonrise
	6:16	7.37	High tide
	6:45		Sunrise
	12:42	1.29	Low tide
	12:56		Moonset
	18:50	7.18	High tide
	20:06		Sunset

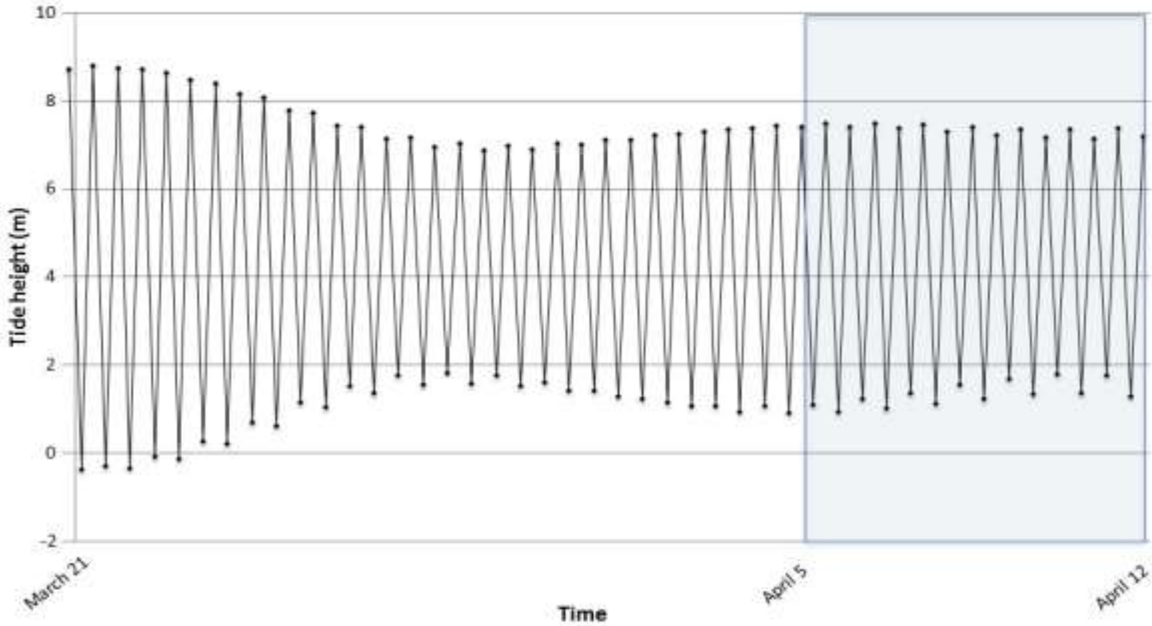

Figure 15: Example of high and low tidal cycles showing both spring and neap tides for Lorneville, New Brunswick. Shaded area represents an ideal sampling cycle (tidal height, time of day). Example sampling period shown in Table 2 (above). Tide information taken from: http://www.tide-forecast.com/locations/Lorneville-New-Brunswick/tides/latest

Appendix C: Summary table of sampling sites and times

Table 3: Summary table of sites, habitats and gears with estimates of sampling time, including the commute from the city of Saint John, and number of personnel needed. Fyke net time for sampling on Day 1, estimated at 10 minutes, is under the assumption that fyke net setup is done alongside beach seining at that site. Time for sampling estimates marked with a * are based on fyke net sampling setup in the salt marsh channel within close proximity to nearshore habitats and could be combined within the same trip. Shaded areas represent sampling within salt marsh channel.

Area	Zone	Habitat	Site	Map	Gear	Minimum number of personnel		Time for sampling (hours)		Data collection
						set up	sampling	Day 1	Day 2	
MPA	Zone 2	*Nearshore*	*Five Fathom Hole*	*7*	Seine		2	2-4		Nekton/sentinel
					Fyke net	3	2	10min	2-3	Nekton
			Black Beach	*6*	Seine		2	2-3		Nekton/sentinel
					Fyke net	2	2	10min	2-3	Nekton
			Hepburn's Basin	*8*	Seine		2	4-6		Nekton/sentinel
					Fyke net	3	2	10min	4-5	Nekton
			Gooseberry Cove	*9*	Seine		2	3-5		Nekton/sentinel
					Fyke net	2	2	10min	4-5	Nekton
		Salt marsh	*Hepburn's Basin*	*8*	Fyke net	3	2	1*	1-2*	Nekton
	Zone 1	*Salt marsh*	*Channel*	*10*	Fyke net	3	2	2-3	2-4	Nekton
Outside MPA	Dipper Harbour	*Nearshore*		13	Seine		2	3-5		Nekton/sentinel
					Fyke net	2		10min		
		Salt marsh	*Channel*	13	Fyke net	3	2	1*	1-2*	Nekton
	Chance Harbour	*Nearshore*		12	Seine		2	2-4		Nekton/sentinel
					Fyke net	2	2	10min		Nekton
		Salt marsh	*Channel*	12	Fyke net	3	2	1*	1-2*	Nekton
	Saints Rest Marsh	*Nearshore*		11	Seine		2	3-4		Nekton/sentinel
					Fyke net	3	2	10min	2-4	Nekton
		Salt marsh	*Channel*	11	Fyke net	3	2	1*	1-2*	Nekton

Appendix D: Species list

Table 4: Species list of fish caught within and around Musquash MPA.

Class/Family	Species name	Common name	References
Ammodytidae	*Ammodytes americanus*	American sand lance	Arens 2007
Ammodytidae	*Ammodytes sp.*	sand lance	Ipsen 2013
Anguilidae	*Anguilla rostrata*	American eel	Ipsen 2013, Arens 2007, Gratto 1986
Atherinopsidae	*Menidia menidia*	Atlantic silverside	Ipsen 2013, Arens 2007, Gratto 1986
Clupeidae	*Clupea harengus*	Atlantic herring	Ipsen 2013, Arens 2007, Gratto 1986
Clupeidae	*Alosa aestivalis*	blueback herring	Arens 2007, Groto 1986
Clupeidae	*Alosa pseudoharengus*	alewife	Ipsen 2013, Arens 2007, Gratto 1986
Clupeidae	*Alosa sapidissima*	shad	Groto 1986
Cottidae	*Myoxocephalus scorpius*	shorthorn sculpin	Ipsen 2013, Arens 2007, Gratto 1986, Stevens 1997
Cottidae	*Myoxocephalus aenaeus*	grubby	Arens 2007
Cottidae	*Myoxocephalus octodecemspinosus*	longhorn sculpin	Arens 2007
Cottidae	*Hemitripterus americanus*	sea raven	Ipsen 2013, Arens 2007, Gratto 1986
Cyclopteridae	*Cyclopterus lumpus*	lumpfish	Ipsen 2013, Arens 2007, Gratto 1986
Fundulidae	*Fundulus heteroclitus*	mummichog	Ipsen 2013, Gratto 1986, Stevens 1997
Fundulidae	*Fundulus diaphanus*	banded killifish	Gratto 1986
Gadidae	*Microgadus tomcod*	Altantic tomcod	Ipsen 2013, Arens 2007, Gratto 1986
Gadidae	*Gadus morhua*	Atlantic cod	Ipsen 2013, Arens 2007
Gadidae	*Urophycis tenuis*	white hake	Ipsen 2013, Arens 2007
Gadidae	*Pollachius virens*	pollock	Ipsen 2013, Arens 2007, Gratto 1986, Stevens 1997
Gadidae	*Urophycis chuss*	squirrel hake	Gratto 1986
Gasterosteidae	*Apeltes quadracus*	fourspine stickleback	Arens 2007
Gasterosteidae	*Pungitius pungitius*	ninespine stickleback	Ipsen 2013, Gratto 1986
Gasterosteidae	*Gasterosteus aculeatus*	threespine stickleback	Ipsen 2013, Arens 2007, Gratto 1986,
Gasterosteidae	*Gasterosteus wheatlandi*	blackspotted stickleback	Ipsen 2013, Arens 2007
Osmeridae	*Osmerus mordax*	rainbow smelt	Ipsen 2013, Arens 2007, Gratto 1986
Pholidae	*Pholis gunnellus*	rock gunnel	Ipsen 2013, Arens 2007, Gratto 1986
Pleuronectidae	*Limanda ferruginea*	yellowtail flounder	Gratto 1986
Pleuronectidae	*Pseudopleuronectes americanus*	winter flounder	Ipsen 2013, Arens 2007, Gratto 1986
Pleuronectidae	*Liopsetta putnami*	smooth flounder	Ipsen 2013, Gratto 1986
Salmonidae	*Salvelinus fontinalis*	brook trout	Gratto 1986
Scophthlmus	*Scophthalmus aquosus*	windowpane flounder	Arens 2007
Zoarcidae	*Zoarces americanus*	ocean pout	Gratto 1986

Appendix E: Nekton Sampling Sheet Template

Sampling Team:____

Date:__________________________________Site Name:__________________________

Latitude:_______________________________Longitude:__________________________

Time:__________________________________Tidal height:_________________________

Gear:__________________________________ Photographs taken:___________________

Weather:____

Comments:____

____

Environmental parameters

Temperature (°□):________Salinity (PPT):_______DO (mg/L):______________

pH: ______Turbidity:______

Species collected

Gear	Tow	Species	Length mm (FL)	Sample preserved and labeled?	Sentinel species collected?	Notes