A comparative analysis of lethal and non-lethal fish sampling techniques

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Abstract

FERGUSON, RACHEL. A comparative analysis of lethal and non-lethal fish sampling techniques. (Under the direction of Dr. Tamara Pandolfo.)

Muscle tissue samples from fish have long been used to analyze the presence of contaminants in fish populations. However, muscle tissue collection is a lethal sampling technique. The use of lethal methods has caused concerns over collecting samples from endangered species as well as having a negative impact on ecosystems. The purpose of this literature review was to summarize the current knowledge of non-lethal fish sampling methods and to determine how they compare to the traditional lethal methods of sampling. Most studies used fin clips as the non-lethal sampling method, however, other studies utilized mucus swabs, scales, and blood samples. The use of non-lethal methods has proven to be a useful alternative to lethal methods, and results of published studies indicate that non-lethal and lethal sampling methods are generally comparable. Fin clipping is the most preferable non-lethal method due to minimal harm to the organism and the regeneration of clipped fins. More research needs to be done in order to provide further information on the efficacy legitimacy of non-lethal sampling techniques.

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1.0 Introduction

Bioaccumulation can be defined as the uptake, storage, and accumulation of contaminants in organisms (Streit, 1998). Bioaccumulative contaminants, such as polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), organochlorines, and mercury, are known to accumulate in organisms and persist in the environment. Aquatic ecosystems are exposed to these contaminants through human activities such as urbanization, industrialization and agricultural activities (Bashir et al., 2020). Bioaccumulative contaminants are then deposited in fatty tissues and accumulate in aquatic organisms. In fish, there are two routes of contaminant uptake: aqueous uptake through gills and dietary update by ingestion (Streit, 1998). Biomagnification occurs when contaminants are passed up the food chain to higher trophic levels (Alexander, 1999). As contaminants, such as PCBs, pass up the food chain, the concentration increases in each organism (Figure 1).

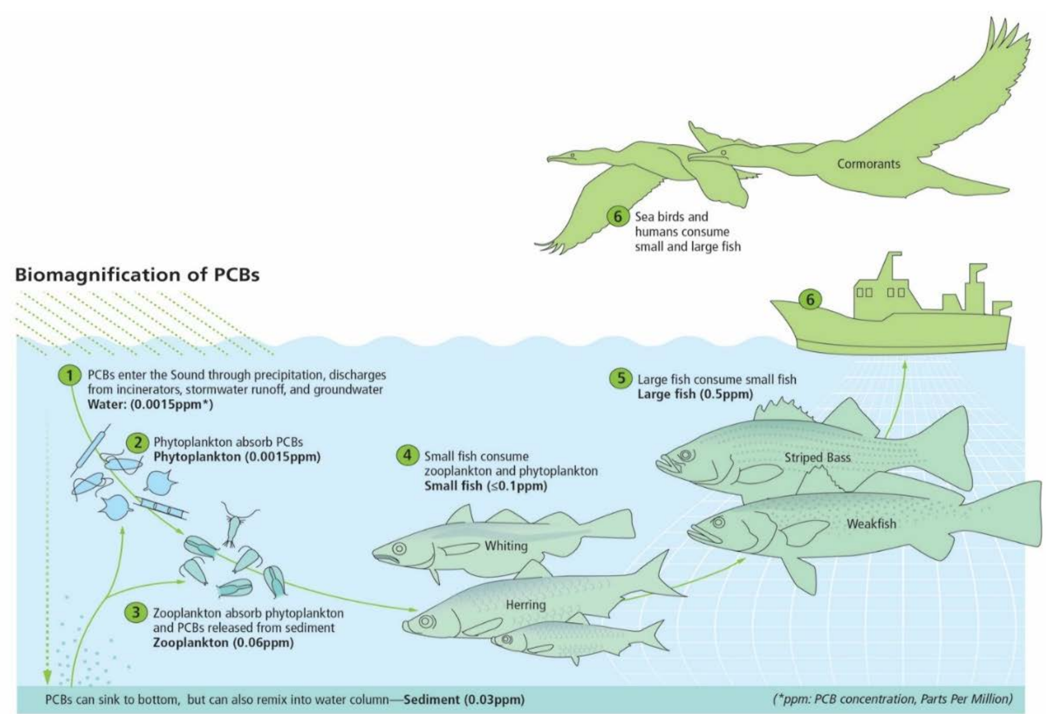

Figure 1. Biomagnification of PCBs. (Long Island Sound Study, 2010)

PCBs belong to a class of contaminants known as persistent organic pollutants (POPs). POPs are toxic organic chemicals that remain intact for long periods of time and bioaccumulate in fatty tissues due to their high lipid solubility (Stockholm Convention, 2019). There are 12 key POPs which were produced after World War II to be used in agriculture (i.e. pesticides, insecticides), disease control, and manufacturing (EPA, 2009). Although many POPs have been banned, they persist in the environment and are highly toxic. Some of the most common POPs include PCBs, DDT, PBDEs, and organochlorines. These compounds are associated with long-term toxicity resulting in impaired immune systems, endocrine systems, growth, nervous systems and reproduction, cancer, allergies, and altering the hormonal systems in organisms such as fish (Stockholm Convention, 2019; Assuncao et al., 2020). For example, PBDEs are brominated flame retardants that can result in endocrine disruptions and neuro-behavioral defects in fish (Zhang et al., 2019).

Mercury is a heavy metal that, like POPs, persists in aquatic environments and bioaccumulates in tissue (Krabbenhoft et al., 2018). Mercury undergoes a series of chemical transformations in the atmosphere, land and water, resulting in the presence of several forms of mercury in the environment. Methylmercury is formed when microscopic organisms combine mercury with carbon converting it from an inorganic to organic form. This form of mercury is highly toxic, bioaccumulative, and biomagnifies as it travels up the food chain (DEC, 2020). Methylmercury affects the central nervous system and can potentially cause irreversible damage to the brain (Krabbenhoft et al., 2018).

Biomagnification of contaminants in fish can be harmful not only to the fish itself, but also potentially to humans. In many coastal towns throughout the world, fish are an essential source of protein. Consuming fish from contaminated waters puts humans at risk of exposure to bioaccumulative contaminants (Ding et al., 2019). In one of the worst known cases of methylmercury poisoning, hundreds of people died in Minamata Bay, Japan in 1956 due to the consumption of contaminated fish (Krabbenhoft et al., 2018).

Because of the potential for harmful effects on human health and the environment, it is important for researchers to collect and analyze samples from aquatic organisms in waters polluted by bioaccumulative contaminants. Traditionally, monitoring for these contaminants requires lethal harvesting of fish to obtain fillet samples. There are concerns with lethal sampling among those who consider the methods cruel, as well as concerns regarding the potential for negative impacts on ecosystems (Rolfhus et al., 2008). Non-lethal sampling techniques are a potential alternative option; however, these techniques need to be assessed to verify that sampling results are comparable to that of lethal methods.

A 2006 study in the Indian River Lagoon, Florida compared the use of lethal and non-lethal sampling methods to assess the concentration of organic contaminants in hardhead catfish (Heltsley et al., 2006). The Indian River Lagoon is a narrow estuarine lagoon extending from Ponce de Leon Inlet south to Jupiter Inlet between Cocoa Beach and Melbourne Beach in Florida (Figure 2). The Lagoon has suffered for decades due to human-induced impacts such as accelerated population growth, urbanization, habit alteration, pollution, and toxic spills (Adams et al., 2019). Organochlorines have been found in the Indian River Lagoon due to the use of insecticides in the Florida Crop and Citrus Program in the 1970s (Wang, 1983). The Indian River Lagoon has also been contaminated with pesticides containing PCBs as well as brominated flame retardants such as PBDEs. Because these contaminants are persistent and degrade at a slow rate, residue concentrations can still be found in the Lagoon.

The Indian River Lagoon is an ideal sampling location to compare lethal and non-lethal methods due to its history of contamination. Validation trials to confirm the utility of non-lethal sampling methods are necessary (Leis et al., 2018) However, in 2006 there were not many other studies that utilized non-lethal sampling methods. The objective of this project was to perform a literature review on articles that have been published since the 2006 Indian River Lagoon study comparing the use of lethal and non-lethal sampling methods to assess contaminant concentrations in aquatic organisms. Ultimately, this review will help to verify whether non-lethal methods are a feasible alternative to traditional methods.

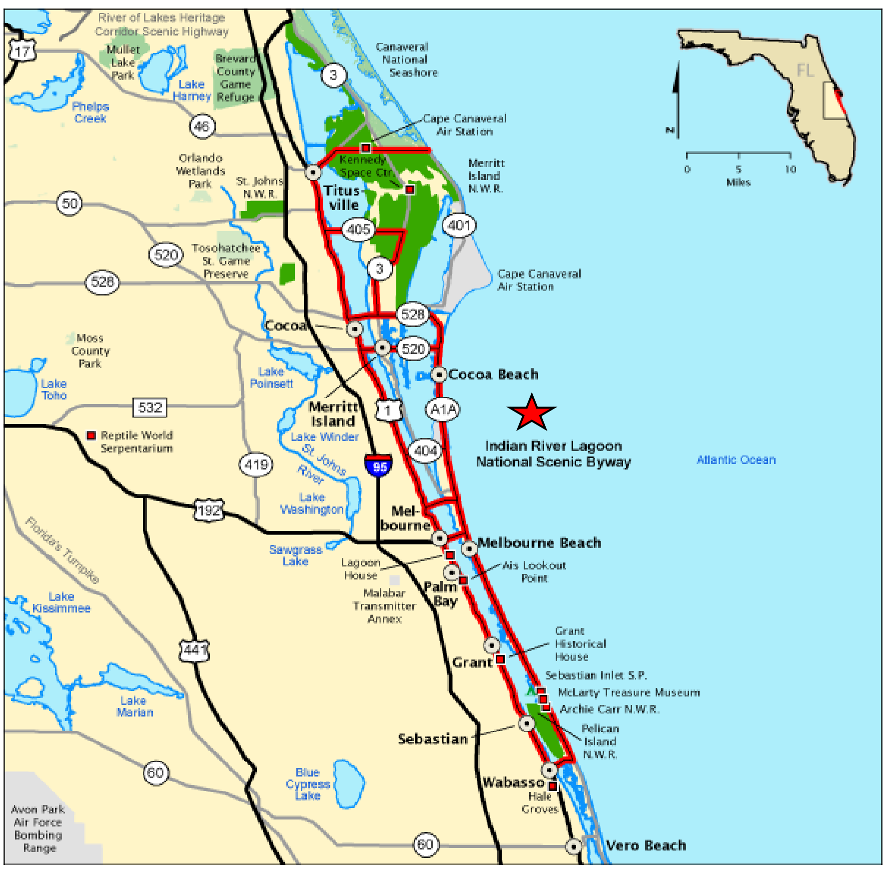

Figure 2. Map of Indian River Lagoon, Florida. (US DOT, 2020)

2.0 Methods

In order to identify relevant research that has been conducted after the Heltsley et al. (2006) Indian River Lagoon study comparing lethal and non-lethal sampling methods on fish, a literature search was completed using the ScienceDirect, Web of Science, and PubMed databases. For the initial search, the terms "fish", "marine or saltwater", "non-lethal sampling" and "biomonitoring" were searched in each database for articles published between 2006 and 2019. From the extensive list of over 1,000 scientific journal articles, abstracts were reviewed to identify studies that compared non-lethal and lethal sampling methods on fish. A total of 10 studies met this criteria. The 10 articles as well as the Heltsey, et al. (2006) article were then reviewed in depth. Each study was assessed based on test organism, study area, contaminants of concern, lethal and non-lethal sampling methods, analytical methods, and results (Tables 1-4). Results from non-lethal and lethal sampling methods were compared and assessed for differences based on researcher's original analyses (Table 4).

Secondary and tertiary searches were also completed to gather supplementary information on the Indian River Lagoon and biomagnification of contaminants using the terms "Indian River Lagoon", "fish", "contaminants", and "biomagnification". From this search, an additional 6 articles were selected.

3.0 Results

Non-lethal and lethal sampling method studies have occurred in several locations around the world. Of the eleven studies, eight studies took place in the United States (Table 1). The remaining three studies took place in Canada, England, and Australia. The articles were published in a wide range of publications related to the environment and/or fish (Table 1).

Environmental Science and Technology and *Archives of Environmental Contamination and Toxicology* published two each of the eleven total articles.

Table 1. Studies selected for the literature review of non-lethal and lethal fish sampling techniques described by author(s), date published, publication, and study location.

Author(s)	**Date Published**	**Journal**	**Study Location**
Assuncao et al.	2020	Marine Pollution Bulletin	Northeast, England
Heltsley et al.	2005	Environmental and Science Technology	Moore & Lee County, North Carolina, USA
Heltsley et al.	2006	Presented to the American Chemical Society	Indian River Lagoon, Florida, USA
Henderson et al.	2016	Fish Physiology and Biochemistry	Queensland, Australia
Kambeitz et al.	2019	Integrated Environmental Assessment and Management	Saskatchewan, Canada
Kelly et al.	2006	North American Journal of Fisheries Management	Massachusetts, USA New Brunswick, Canada
Leis et al.	2018	Journal of Aquatic Animal Health	Wisconsin, USA
Piraino et al.	2013	Archives of Environmental Contamination and Toxicology	Rhode Island, USA
Rolfhus et al.	2008	Environmental Science and Technology	Minnesota, USA Wisconsin, USA
Sanderson et al.	2009	Transactions of the American Fisheries Society	Idaho, USA
Schmitt et al.	2006	Archives of Environmental Contamination and Toxicology	Missouri, USA

3.1 Analysis of Study Endpoints

Out of the eleven studies, three (including the original Heltsley, et al. (2006) Indian River Lagoon study) analyzed fish for organic contaminants, three analyzed total mercury, and two analyzed stable isotopes (Table 2). The three remaining studies analyzed for Largemouth Bass virus, effect endpoints (i.e. survival, growth, reproduction, and energy storage), and sampling mortality.

Table 2. Sampling endpoints for each of the studies analyzed in this literature review of non-lethal and lethal fish sampling techniques.

Study Reference	**Endpoint Analyzed**
Assuncao et al., 2020	Polybrominated diphenylethers (PBDEs), polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (p,p'-DDE), hexachlorobenzene (HCB)
Heltsley et al., 2005	PCBs, organochlorine pesticides (OCPs)
Heltsley et al., 2006	PCBs, OCPs, brominated flame retardants
Henderson et al., 2016	Sampling Mortality
Kambeitz et al., 2019	Effect Endpoints (Survival, Reproduction, Growth, Energy Storage)
Kelly et al., 2006	Stable isotopes (δ15N, δ13C)
Leis et al., 2018	Largemouth Bass Virus (LMBV)
Piraino et al., 2013	Total Mercury
Rolfhus et al., 2008	Total Mercury
Sanderson et al., 2009	Stable isotopes (δ15N, δ13C)
Schmitt et al., 2006	Total Mercury

3.2 Organisms and Sampling Methods

Each of the eleven studies sampled different species of fish (Table 3). Seven out of the eleven studies sampled saltwater fish and the remaining four studies collected freshwater species. Seven out of the eleven studies used fin clips as the non-lethal method of collection on the fish sampled. Four of these studies took samples from the adipose fin and three took samples from the caudal fin (Figure 3). One study (Rolfus et al., 2008) sampled several different species of fish using samples from several different fins including pelvic, caudal, dorsal, adipose, and anal fins. The other non-lethal collection methods included mucus swabs (Leis et al., 2018), blood samples (Schmitt et al., 2006), muscle biopsy punch (Schmitt et al., 2006; Henderson et al., 2016), and scales (Kelly et al., 2006; Piraino et al., 2013; Henderson et al., 2016).

Seven out of the eleven lethal methods used in the studies were muscle tissue fillets. One study used kidney and spleen tissue incisions as the lethal method (Leis et al., 2018). Two other studies did not use lethal sampling methods. One of the studies used past literature to compare non-lethal sampling results (Assuncao et al., 2020). The other study's objective focused on the suitability of non-lethal methods by studying the mortality levels of fish that are sampled non-lethally via biopsy punch (Henderson et al., 2016).

It should be noted that one of the eleven studies does not mention the specific use of lethal or non-lethal methods. For this study, an Environmental Effects Monitoring (EEM) program for Canadian Metal and Diamond Mining was evaluated to determine the number of fish sacrificed through lethal methods. A focus was put on the total number of fish sacrificed and lethal and non-lethal endpoints rather than specific methods used (Kambeitz et al., 2018).

Table 3. Organism sampled, non-lethal technique, and lethal method used for each of the studies chosen for the literature review.

Study Reference	Organism	Non-Lethal Method	Lethal Method
Assuncao et al., 2020	Atlantic salmon, Sea trout	Adipose fin clip	No lethal methods used
Heltsley et al., 2005	Flathead catfish	Adipose fin clip	Axial muscle fillet-right dorsal region
Heltsley et al., 2006	Hardhead catfish	Adipose fin clip	Axial muscle fillet-right dorsal region
Henderson et al., 2016	Russell's snapper, Grass emperor	Scales, Muscle biopsy punch	No lethal methods used
Kambeitz et al., 2019	Spottail shiner, Lake chub	Not specified	Not specified
Kelly et al., 2006	Sunfish, Slimy sculpin	Scales-above and below lateral line	Muscle tissue-under left dorsal fin; Fin tissue-caudal fin
Leis et al., 2018	Largemouth bass	Mucus swabs-dorsolateral region	Kidney and spleen tissue incisions
Piraino et al., 2013	Black sea bass, Bluefish, Striped bass, Summer flounder, Tautog	Caudal fin clip, Scales	Axial muscle tissue-dorsal region above the operculum
Rolfhus et al., 2008	Northern pike, Walleye, Arctic grayling, Winter flounder	Fin clips-pelvic, caudal, dorsal, adipose, anal	Skinless fillets (axial muscle)
Sanderson et al., 2009	Chinook salmon, Rainbow trout	Caudal fin clip	Dorsal muscle plug
Schmitt et al., 2006	Smallmouth bass	Blood samples-caudal venipuncture, biopsy needle and punch-beneath dorsal fin	Fillet samples

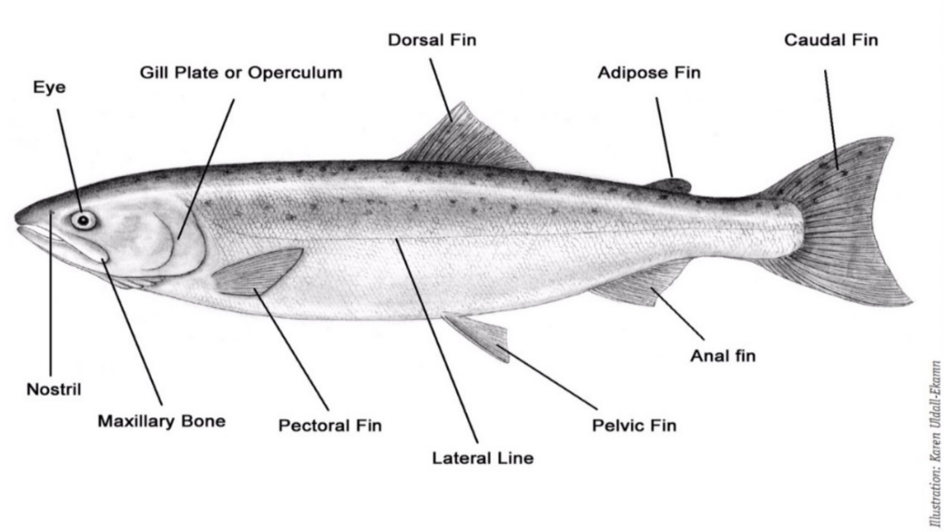

Figure 3. External anatomy of a fish (The Reel Angle, 2017)

3.3 Study Objectives

The objectives of all eleven studies focused on the utility of non-lethal sampling methods. All eleven studies stressed the importance of validating the use of non-lethal sampling methods to confirm their effectiveness. Nine of the eleven studies directly compare non-lethal and lethal sampling methods for determining contaminant levels, stable isotopes, or LMBV. One study's focus is on an EEM program in Canadian metal and diamond mines. The EEM program does not provide non-lethal critical effect sizes for effect endpoints which limits their utility for comparison with lethal endpoints (Kambeitz et al., 2019). Another study focused on the stress levels and levels of mortality in non-lethally biopsied fish (Henderson et al., 2016). Instead of comparing the results of contaminant levels from non-lethal and lethal methods, this study's objective is to determine if non-lethal biopsy punches are suitable for field use (Henderson et al., 2016).

3.4 Non-Lethal vs. Lethal Results

Although the eleven studies all had similar objectives, methodology and sample analysis in each study varied (Table 4). Some studies compared the concentration of contaminants in the fish samples collected using different techniques (Schmitt et al., 2006; Piraino et al., 2013). Other studies were more general in describing the correlation between non-lethal and lethal sampling method results. For example, Rolfhus et al. (2008) described the total amount of mercury in the non-lethal samples as being “less than” total mercury in lethal samples. A few studies focused on statistical correlation and linear relationships to determine the strength of relationships between non-lethal and lethal methods (Heltsley et al., 2005; 2006).

The remaining six studies determined that non-lethal and lethal methods are comparable based on the assessment of the authors. The Kambeitz et al. (2019) study, which found that non-lethal and lethal methods were not comparable, was not based on sampling but on a technical guidance document from Environmental Canada which provided non-lethal study design recommendations. The authors of this study found that the design of the EEM program needed to be greatly improved as the recommendations for non-lethal surveys are not as extensive as for lethal surveys. In order for this to be accomplished, more research needs to be conducted using non-lethal sampling techniques. Two studies do not compare the two methods. For one, only non-lethal methods were used and the results were compared to literature of past lethal method studies (Assuncao et al., 2020). The Assuncao et al. (2020) study found that further non-lethal studies should source more tissue from the fish in order to improve detection limits for analysis. The other study did not focus on analytical results from contaminants in fish, but on the survival rate and stress levels on non-lethally biopsied fish

(Henderson et al., 2016). For this study, all of the biopsied fish survived and the clipped fins fully regenerated.

Table 4. Non-lethal and lethal results for each of the studies chosen for the literature review.

Study Reference	**Non-Lethal Results**	**Lethal Results**	**Main Findings of Study**	**Is the non-lethal technique validated by the authors?**
Assuncao et al., 2020	Higher concentrations of organics in sea trout samples than salmon samples	N/A	Larger tissue samples should be taken in order to improve detection limits and analyze lower concentrations	Yes
Heltsley et al., 2005	Organic concentrations in adipose fin are highly correlated with muscle fillets Linear relation R^2 = 0.87		Adipose fin of certain fishes may be used to accurately estimate tissue concentrations	Yes
Heltsley et al., 2006	Organohalogen concentrations in adipose fin are significantly correlated to those found in muscle fillets Pearson correlation coefficients p <0.0001		Adipose fin removal is a promising non-lethal technique to assess contaminant concentrations	Yes
Henderson et al., 2016	Removal of muscle tissue-no effect on survival Gill beat rate-no significant differences in biopsied vs non-biopsied p = 0.004	N/A	There were no instances of mortality or significant stress to the biopsied fish. This method was found to be a suitable non-lethal technique	Yes
Kambeitz et al., 2019	No critical effect sizes	Critical effect sizes known	There needs to be more guidance on non-lethal methods provided by the EEM program	No (Not enough non-lethal literature guidance to be significant)

Kelly et al., 2006	Strong correlations between sunfish and slimy sculpin *p* <0.001		Values in scale and muscle tissues of sunfish and in fin and muscle tissue of slimy sculpin were strongly correlated. The values of scale and fin tissues can be substituted for muscle tissue values.	Yes
Leis et al., 2018	28 of 60 mucus samples positive for LMBV ~46% samples detect LMBV	18 of 60 tissue samples positive for LMBV ~30% samples detect LMBV	Non-lethal techniques showed promise as alternative to traditional counterparts because they consistently detected LMBV. Mucus swabs are a more sensitive method than that of tissue.	Yes
Piraino et al., 2013	Scales mean coefficient of determination R^2 = 0.53 Fins mean coefficient of determination R^2 = 0.71		Scale analysis was deemed ineffective as there was a high level of uncertainty within the regression models. Fin clips are better predictors of muscle Hg	Yes-fins No-scales
Rolfhus et al., 2008	Mean concentrations of Hg in fins ranged from 2.7 to 8.9% of those in fillets Coefficient of determination r^2 = 0.52		The analysis of fin clips could be a useful screening tool for assessing Hg contamination	Yes
Sanderson et al., 2009	The relationship between fin and muscle tissue were consistent across years and streams δ15N P < 0.001 δ13C P < 0.001		Fin clipping is a promising non-lethal technique. Fin clips is not a viable option for	Yes

		smaller fish as too much caudal fin is needed for analysis	
Schmitt et al., 2006	Blood and fillet Hg concentrations were linear and statistically significant $p < 0.01$	Any of the non-lethal techniques are satisfactory. Both blood and plug samples were easier to perform than needle	Yes

3.5 Non-Lethal Concerns

Each of the studies addressed potential issues when using non-lethal sampling methods on fish. For one study that used blood samples as one of the non-lethal methods, it is determined that using single-use disposable needles and syringes generates solid waste (Schmitt et al., 2006). Although this method does produce accurate results, blood sampling may not be the most viable non-lethal method. Another study suggested that fin clipping is practical for large fish (>65 mm) because less than one-third of the fin is needed for samples (Sanderson et al., 2009). For smaller fish, a full fin would be needed for acceptable samples thus affecting the ability of a fish to swim. This presents a major limitation for non-lethal sampling in smaller fish. Many studies mentioned concerns that non-lethal sampling methods may still harm fish that are being sampled. One study was solely dedicated to investigating mortality rates and stress levels in non-lethally biopsied fish (Henderson et al., 2016). This study determined that there were no significant differences between gill beat rates of biopsied fish and non-biopsied fish in the study. All of the biopsied fish also survived the non-lethal sampling.

4.0 Discussion

Lethal sampling techniques have long been used by researchers to analyze contaminant levels in fish; however, the use of non-lethal methods allows for the potential to sample fish humanely and to sample the same fish over time. The objective of this literature review was to summarize the current knowledge of non-lethal sampling methods on fish and how they compare to the lethal alternative. Seven of the eleven studies used fin clips as the non-lethal sampling method. Fin clipping is the most appropriate non-lethal technique due to minimal harm to the organism and because partially clipped fins can regenerate (Rolfhus et al., 2008). The study detecting LMBV in Largemouth Bass used mucus swabs as the non-lethal method. This method was chosen because infected fish are known to shed LMBV in their mucus (Leis et al., 2018). Thus, the use of mucus swabs may not be appropriate when detecting contaminants in fish populations. One study used blood sampling with a needle and syringe as one of the non-lethal methods. This technique is moderately difficult to perform and did not produce values directly comparable to muscle concentrations (Schmitt et al., 2006). Three of the eleven studies removed scales as the non-lethal method. Scales have proven to be variable and less reliable than fin clips. One study's results showed that the high level of uncertainty within the regression models indicated that scales were an ineffective tool for estimating mercury concentrations (Piraino et al., 2013). Scales also are mostly bone and can contain large amount of inorganic carbonate that would need to be removed prior to analysis, thus adding another step to the procedure (Sanderson et al., 2009). All of the studies that used lethal sampling methods included some type of muscle tissue fillet. Muscle tissue fillets are the most commonly used lethal sampling method in fish populations and there has been extensive research on the results of these methods.

Of the seven studies that used fin clipping as the non-lethal method, there were several different fins used. The caudal fin was the most commonly used fin among the eleven studies due to its large size. For one study, the caudal fin was chosen due to its quick regeneration time of 6 weeks and its large biomass which could yield replicate samples (Sanderson et al., 2009). Caudal fin collection is also noninvasive and relatively easy (Piraino et al., 2013). The adipose fin was chosen to analyze the presence of PCBs and OCPs in one study since it does not provide a vital physiological function in the fish and is easily accessible and removed. However, the adipose fin has high lipid content compared to muscle tissue, and normalization of contaminant concentrations to lipid content was required for analysis (Heltsley et al., 2005).

4.1 Limitations

There were several limitations from the literature review that are worth noting. One of the biggest limitations was that these studies were not designed to be compared with one another. Although the main objective of each study was to compare non-lethal and lethal sampling methods, the variables analyzed varied greatly. Only six of the eleven studies monitored for specific contaminants. The remaining studies analyzed isotopes, LMBV, mortality from non-lethal methods, and effect endpoints.

There were also limitations regarding the contaminants analyzed. For example, one study that analyzed the presence of mercury in fins found that there was a significant difference in mercury between specific portions of pelvic fins (i.e. proximal vs. distal). Methylmercury binds preferentially to thiol-containing amino acids which may lead to unequal distribution between skin, cartilage, bone, and muscle (Rolfhus et al., 2008). However, standardized sampling protocol for taking fin clips could reduce this from occurring.

Another limitation from the studies was the use of the adipose fin as a sampling point. The adipose fin is only found on 20% of ray-finned species such as salmon, catfish, and trout (Aiello et al., 2016). While clipping this fin proved to be a comparable non-lethal alternative, there are limitations to the type of fish that can be sampled. However, several of these families of fish include those that are important to commercial and sport fish species that are consumed by humans (Heltsley et al., 2005). As mentioned above, the adipose fin is also lipid-laden which is a limitation when comparing contaminant results to that of muscle tissue. It was also discussed that non-lethal sampling methods are only ideal for larger fish (>65 mm) due to the size of fin clip that is needed for analysis (Sanderson et al., 2009).

5.0 Conclusion

Non-lethal sampling methods are an effective alternative to lethal sampling methods on fish. The utilization of non-lethal sampling techniques can prevent mortality of the fish and potential impacts on the ecosystems that they inhabit. Non-lethal sampling is perhaps most beneficial for the sampling of valuable wild fish populations or threatened and endangered species. Non-lethal methods may also be preferable if a large number of predatory fishes is needed for sampling as this removal can modify food web structures and lead to overpopulation of non-predatory organisms. (Rolfhus et al., 2008).

This literature review indicated that fin clipping is the most preferable non-lethal method. Further research should be conducted using these methods to verify that non-lethal methods provide results as accurate as the lethal alternatives, because there are still a limited amount of studies which directly compare both methods.

Further, studies comparing the usage of caudal fins and adipose fins would be beneficial. Although the adipose fin is found in limited species, it does not have a vital function, unlike the caudal fin which could impact fish survival. However, the adipose fin is known to have high lipid content which could skew analytical results of contaminants when comparing muscle tissue as the lethal alternative. More studies comparing both fin clips to muscle tissue would provide further information on the most reliable non-lethal techniques for sampling in fish.

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