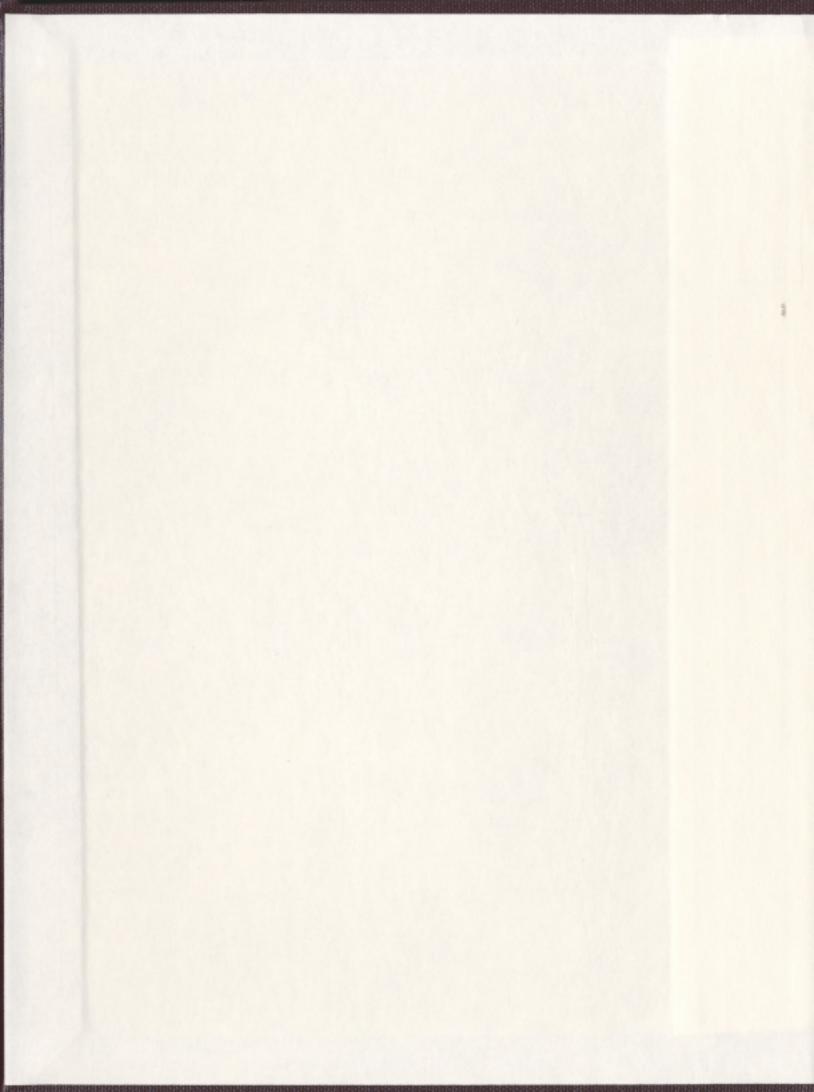
## MOVEMENT AND MITIGATION OF DOMESTIC TRIPLOID STEELHEAD TROUT (Oncorhynchus mykiss) ESCAPED FROM AQUACULTURE GROW-OUT CAGES

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#### MOVEMENT AND MITIGATION OF DOMESTIC TRIPLOID STEELHEAD TROUT (Oncorhynchus mykiss) ESCAPED FROM AQUACULTURE GROW-OUT CAGES

by

<sup>©</sup>Christopher J. Bridger

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School of Graduate Studies

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#### Abstract

Cultured fish may occur in the wild from intentional release for restocking and sea ranching purposes or aquaculture escapees from ocean grow-out facilities. Aquaculture facilities lose some individuals during the production cycle, especially when sea cages are used. In addition to economic loss incurred to the fish farmer from escapement, potential disease, ecological and genetic interactions between escapees and wild conspecifics are of concern. In this study I monitored escapee movement, by tracking transmitter-implanted domestic female triploid steelhead trout in the wild using sophisticated biotelemetry fixed data-logging and manual tracking techniques, in Bay d'Espoir, Newfoundland, Canada. Of the 68 triploid steelhead released on-site, in July 1998, 51 (75%) remained within a 500 m radius of the summer grow-out site 32 days after release. Similar to on-site released triploid steelhead, 17 of 66 (26%) triploid steelhead released approximately 1000 m outside of the summer grow-out site returned to the site within 4 hours of release. Subsequent tracking found that an additional 26 triploid steelhead had returned to the summer grow-out two days after release, bringing the total number of off-site released triploid steelhead return to 65%. Triploid steelhead trout released during the winter displayed lower fidelity than those released in summer.

Dispersing triploid steelhead during summer were detected in the vicinity of other salmonid aquaculture sites throughout the bay. Summer and winter released triploid steelhead both displayed a directed movement upstream towards the hydroelectric spillway – also the location of the local salmonid hatchery. Results suggest escaped triploid steelhead trout may survive in the wild – moving between summer grow-out sites and the hydroelectric spillway, while feeding on excess farm feed during the summer season. A biotelemetry methodology was

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developed, and also described herein, to monitor and optimize potential recapture traps for the salmonid aquaculture industry. Recapturing escapees aggregating near aquaculture sites may help mitigate negative implications through removal of escapees from the wild.

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This research was directed by a committee comprised of Scott McKinley (Waterloo Biotelemetry Institute), Dave Scruton (Department of Fisheries and Oceans), Nigel Allen (Marine Institute of Memorial University of Newfoundland), Gary Hoskins (Conne River Aquaculture; CRA), Steve Moyse (Newfoundland Salmonid Growers Association; NSGA), and Keith Stoodley and George Niezgoda (Lotek Marine Technologies Inc.; LMTI). Throughout the field portion of the research, I am forever indebted to Dr. Rick Booth for teaching me the intricacies of telemetry research, and for a continued friendship that goes well beyond our desire for field research. Several additional people were instrumental throughout the field component of this research, namely, Todd Lindstrom and others from LMTI for all technical aspects of the study; Everest Hinks and the crew of CRA for providing boat support, fuel and field assistance; Dr. Michael Tlusty, Chris Diamond and Randy Mercer (NSGA) for field assistance as required; and Valerie Kempers (Applied Biometrics Inc.) for telemetry assistance. Otolith analysis was completed following a move to the Gulf Coast Research Lab in Ocean Springs, MS. Here, Dr. Bruce Comyns and Glenn Zapfe showed me the techniques and potential of counting otolith rings as a means to determine fish age and growth. The tracking portion of this research was funded through the Ocean Technology Fund and the Canadian Centre for Fisheries Innovation.

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#### 1.0. Introduction

In this introduction I first briefly summarize salmonid aquaculture with regard to the process of producing fish for the market starting with the egg stage. I then discuss the problem of fish escaping from cage sites, including a discussion of potential interactions as it relates to disease/parasite transmission, ecological and genetic interactions. I then discuss the situation in Bay d'Espoir, my study site, with regard to steelhead trout (*Oncorhynchus mykiss*), the species I focussed on for my research. The use of telemetry is discussed next and finally I present my research objectives.

#### 1.1. Salmonid Aquaculture

Successful grow-out of salmonids occurred in Norway in the 1960s with rainbow trout, although successful incubation and various stages of grow-out in salmonids were documented earlier than this date (Saunders, 1995). Following fertilization of female ova (egg) by male milt (sperm), eggs are incubated until hatching in various types of trays, raceways and troughs with constant water flow. Dead eggs are frequently removed to prevent fungal infection (especially *Saprolegnia*) to all eggs.

Trays may be removed subsequent to eggs hatching to maintain yolk-sac fry, or alevins, in a raceway system. Upon yolk-sac exhaustion (approximately 4-6 weeks after hatching), salmonid fry are immediately fed a commercial artificial "crumb" feed of size 0. As fry grow, larger crumb size is given until eventually small pellets are offered to the

fingerlings, or parr. Fingerlings are typically size-class graded throughout early rearing to separate larger dominant and smaller subordinate individuals, thereby reducing hierarchical systems within the growing environment, allowing optimal feed size usage, preventing cannibalism, and maintaining individuals with comparable growth traits as a cohort. Eventually, anadromous fingerlings, such as Atlantic salmon (*Salmo salar*) and steelhead trout (*Oncorhynchus mykiss*), smoltify and undergo major physiological changes in preparation for the migration from fresh to salt water (Willoughby, 1999).

Once smolts reach the desirable minimum size for cage grow-out, they are transferred from early rearing land-based tanks to grow-out cages in an estuary. Smolts are initially located in the low salinity portions of the estuary and gradually move further out to sea and full salinity to complete the grow-out cycle (comparable to a natural lifecycle). Salmonids typically reach market size after completion of a 2-2.5 year grow-out cycle.

Within the aquaculture industry, broodstock are selected which produce offspring with economically desirable grow-out traits such as rapid growth rate, delayed age of maturation, resistance to disease, and high survival at the densities experienced in captivity (Friars *et al.*, 1996; Gjøen and Bentsen, 1997). In addition, strains and species of aquaculture fish can be exotic to the grow-out region. Due to these industry practices, concerns exist regarding the impacts exotic strains and species may have on wild fish

populations (including salmonid conspecifics) should escapement from grow-out cages occur.

#### 1.2. Occurrence of Aquaculture Escapees in the Wild

Cultured fish may occur in the wild from intentional release for restocking and sea ranching purposes or aquaculture escapees from open-ocean grow-out facilities. It is likely that aquaculture facilities lose some individuals during the production cycle, especially when sea cages are used. Aquaculture escapement may be classified as either chronic or acute. Chronic leakage is the result of slow, continuous loss of salmonids to the wild. Such loss results from improper farm practices, small holes in the containment netting caused by general wear and tear or localized, small-scale predation attempts. Large, rapid loss of fish due to storm damage or predation from seals or tuna is acute escapement. Acute escapement may result in the loss of one to several cages of fish in a relatively short period of time and therefore may be economically devastating to an aquaculture venture.

Throughout the world, fisheries biologists have documented strains of salmonids of aquaculture origin in the wild. Escapees can be differentiated from wild conspecifics either by external appearance or the presence of synthetic flesh colorants from salmonid farm feed (Lura and Sægrov, 1991a; Hiilivirta *et al.*, 1998). Marine wild fisheries in Norway reported 29.1% of the salmon caught to be of reared origin with 5.8% of the

catch of domestic strains caught in the freshwater fisheries in 1989 (Lund *et al.*, 1991). Likewise, the proportion of farmed salmon, potentially from the Norwegian aquaculture industry, ranged from 25 to 48% in the Faroes fishery (Hansen *et al.*, 1993). Similarly within Scotland, the frequency of farmed salmon escapees in the fisheries was 22% in 1990 (Webb and Youngson, 1992), and in 1989 an Icelandic salmon river had 30.1% reared salmon escapees in the catch, almost all of which were sexually mature (Gudjonsson, 1991).

This trend is no different in Canadian waters. Carr *et al.* (1997) reported up to 55% of the salmon redds sampled in the Magaguadavic River, within the Bay of Fundy, New Brunswick, originated from cultured escapees. Within British Columbia, Atlantic salmon escapees were first documented in 1987. From 1988 to 1995, 97, 799 escapees were reported from 18 incidents. These salmon have been discovered in fresh water with 188 being caught or sighted in fresh water systems from 1990 to 1995. Most of the Atlantic salmon recovered in fresh water displayed gonadal development and external coloration associated with sexual maturation (McKinnell *et al.*, 1997). However, up to 1997, there were no reports of Atlantic salmon spawning within British Columbia (McKinnell and Thomas, 1997). No estimates of cultured salmonids in the wild have been documented in Bay d'Espoir, Newfoundland. However, examples of escapement exist with 4000 market size steelhead trout, a non-indigenous cultured salmonid species, escaping during one storm in 1996 (Anonymous, 1999c).

With such high global incidence of salmonid escapees, many governments and industries have adopted regulations and a code of conduct to mitigate potential interactions between escaped salmonids and wild conspecifics. In Norway, salmon farming is prohibited near important salmon rivers. As well, authorities have introduced strict regulations for the transport and trade of live fish (Bergan *et al.*, 1991). Recently, European salmon producers have met to discuss standards and systems to prevent escapement and a new cage inspection and approval system will soon be implemented in Norway (Northern Aquaculture, May 1999). In Newfoundland, the Newfoundland Salmonid Growers Association has also adopted a local code of practice which, amongst numerous other issues, deals with escaped fish elimination and removal from the wild (Anonymous, 1999a).

#### 1.3. Potential Interactions of Aquaculture Escapees with Wild Stocks

International symposia have been held to discuss concerns of possible interactions between escapees and wild conspecifics (Hansen *et al.*, 1991; Hutchinson, 1997). Wild stocks of salmonid populations are believed to be adapted to their home stream conditions, existing in reproductively discrete populations (brook charr, *Salvelinus fontinalis*, Angers *et al.*, 1995; Atlantic salmon, Verspoor, 1997; coho salmon, *Oncorhynchus kisutch*, Small *et al.*, 1998; chinook salmon, *Oncorhynchus tshawytscha*, Nelson *et al.*, 2001). Potential interactions between domestic salmonids escaping or

introduced to the wild (also through stock enhancment or sea ranching) and native populations may be detrimental to the stock through genetic dilution and/or competition. Disease, ecological and genetic interactions are frequently cited as the potential interactions of greatest concern.

#### 1.3.1. Disease Interactions.

One frequent opposition to fish farming is that the opportunity for disease infection and transfer is increased in an aquaculture setting compared to a wild stock. Rearing conditions could raise the likelihood for opportunistic diseases and/or parasites owing to increased stocking density and associated stress. However, the same disease/parasite agents are present within wild salmonid stocks, as well as within other marine fish species (Saunders, 1991; Kent *et al.*, 1998). Apparent increases in diseases and parasites in fish farms may be misleading owing to rigorous observation and documentation in aquaculture that are absent for wild fisheries and stock surveys (McVicar, 1997).

Infectious agents may be spread from wild stocks to cultured fish (and vice versa) through horizontal (direct contact either via the water or escapees interacting with wild conspecifics) or vertical (from infected wild broodstock or successful spawning of infected escapees) transmission. Both horizontally and vertically transmitted diseases require extensive contact for infection. Therefore, it is possible disease and parasite

agents could be more likely transmitted through stock enhancement programs that promote extensive contact and spawning between hatchery-derived and wild fish to increase depleted wild populations. For example, spread of the monogenean parasite *Gyrodactylus salaris* has been correlated with the introduction of infected hatchery salmon to Norwegian rivers through stock enhancement programs (Johnsen and Jensen, 1986). Fish in numerous nearby rivers that have never been stocked, but are in close proximity, also have the parasite. These fish likely originate from stocked parr migrating to neighboring streams (Johnsen and Jensen, 1986; Lund and Heggberget, 1992). Although stock enhancement programs are considered the primary cause of *G. salaris* in the wild, infected transport tanks, birds and escapees may also be additional sources of infection.

Some diseases of wild stocks—such as enteric redmouth disease (*Yersinia ruckeri*)—have been attributed to transmission of the pathogen from infected salmonid farms. However, no major outbreaks or clinical signs of the disease have been observed in wild fish (Håstein and Lindstad, 1991) that are likely serving as carriers of the pathogens. It may therefore be possible that the greatest risk of disease transfer exists from wild carriers to aquaculture stocks (Saunders, 1991). If suboptimal conditions exist for the cultured stock, the same opportunistic bacteria, not causing clinical signs of infection in wild fish, may result in a disease outbreak for cultured fish. To prevent opportunistic disease outbreaks, it is imperative that good husbandry practices are

followed and optimal growing conditions maintained in culture environments. Following best management practices will decrease the opportunity for pathogen transmission and disease outbreak in aquaculture, which, in turn, would mitigate the opportunity for disease transmission to wild fish should escapement occur.

#### 1.3.2. Ecological Interactions.

Age, season, location of escapement, and juvenile experience influence movement patterns and upstream migration of escapees. Sutterlin *et al.* (1982) documented successful return of mature salmon to the smolt area of release after 13-25 months at sea. Decreased return can be expected for salmon escaping during winter. Up to 60% straying by salmon smolts released during the winter has been observed (Hansen and Jonsson, 1991). Rate of straying increases with distance between salmonid home-river location and escapement site (Heggberget *et al.*, 1991). Increased straying will result in escaped adults entering rivers at random to spawn (Hansen *et al.*, 1987). Haphazard river entry is observed when escaped fish have no previous river experience which results in a lack of discernible sign-posts (cues that allow return to a specific site; Hasler, 1966) to follow during migration. Reduced homing precision to a river also exists for escapee steelhead trout adults (Jonsson *et al.*, 1993).

Juvenile river experience will impact escapee upstream migration behavior. Atlantic salmon will return to the river they left as juveniles, regardless of their genetic

origin (Hansen and Jonsson, 1994). Hatchery smolts released in the mouth of a river (but without river experience) will return to that river as mature adults at the same time as wild conspecifics. However, hatchery adults enter the river later in the season illustrating the influence of river experience on behavior (Jonsson *et al.*, 1990, 1991, 1994). Escaped farm salmon having no river experience will also delay river ascent compared to wild conspecifics (Eriksson and Eriksson, 1991; Gudjonsson, 1991; Lund *et al.*, 1991; Heggberget *et al.*, 1993). In contrast, hatchery smolts released within a river enter the river at the same time as wild conspecifics (Jonsson *et al.*, 1994). These results illustrate the importance of river experience—and aquaculture practices—on timing of river ascent. Salmonid aquaculture industries typically raise early life stages either in tank facilities or ponds/lakes and transport juveniles to an estuary without allowing river experience. Escapees would be expected to return to this estuary, assuming they are raised in the vicinity of the estuary of juvenile origin, and enter rivers at a later time than wild conspecifics.

Once in a river, escapee and wild salmon have been observed together in spawning areas (Økland *et al.*, 1995; Heggberget *et al.*, 1996; Thorstad *et al.*, 1998). Escapees of both sexes may spawn (apparently in equal numbers) with wild conspecifics (Lura and Sægrov, 1991b; Webb *et al.*, 1991). However, escapees tend to spawn later in the season (Webb *et al.*, 1991). Having spawned later, escapees may dig redds in areas being used by wild salmon, thus destroying the redds of wild salmon and decreasing their

fitness (Webb *et al.*, 1991). This would also indicate that escapee females with no river experience are able to locate suitable spawning areas and successfully dig redds.

Youngson *et al.* (1993) indicated that Atlantic salmon escapees tended to hybridize with brown trout (*Salmo trutta*) more frequently than wild salmon. This may be attributed to overlapping habitat with brown trout and/or abnormal sexual behavior of escapee female salmon which limits spawning opportunities with wild salmon. Lura and Sægrov (1993) observed escaped Atlantic salmon spawning earlier than native wild salmon in the River Vosso. Earlier escaped Atlantic salmon spawning time overlapped with brown trout spawning. Escaped Atlantic salmon reproductive behavior could result in sterile progeny, negatively affecting the brown trout population. Additionally, earlier spawning will extend the growing season of hybrid (Atlantic salmon x brown trout) alevins. Assuming hybrids overlap juvenile habitat with wild Atlantic salmon, longer growing seasons will result in larger hybrid juveniles compared to wild salmon, and give a competitive and territorial advantage to hybrid progeny (Berejikian *et al.*, 1996).

Although successful spawning has been documented, escapee salmon are reproductively inferior to wild conspecifics. After entering a river to spawn, escapees remain in the river for a shorter period of time, with a notable percentage of both males and females leaving unspawned (Jonsson *et al.*, 1990, 1991; Økland *et al.*, 1995). Escapees in Norway move up- and down-stream more often; are observed to be further up

the river; and are injured more frequently during spawning than wild individuals (Jonsson et al., 1990, 1991; Heggberget et al., 1996; Thorstad et al., 1998). Increased movement and upstream distribution suggests escapees cannot locate "their" spawning area owing to the absence of juvenile river experience. Escapees tend to spawn in the lower reaches of northern Scottish rivers (Webb et al., 1991, 1993). Webb et al. (1991) noted that later escapee river entry compromised further river ascent owing to decreased water levels later in the season. Inferior escape female spawning results from reduced spawning behavior, construction of fewer redds, and retention of more eggs than wild females (Fleming et al., 1996). Decreased fecundity and spawning behavior will result in less reproductive output from female escapees. Escapee males also display inappropriate courting behavior resulting in decreased success in entering redds to fertilize the eggs (Fleming et al., 1996). In North America, hatchery steelhead have reduced reproductive success relative to wild conspecifics (Chilcote et al., 1986; Leider et al., 1990). Predation and competition decrease the survivorship of offspring from hatchery steelhead. Owing to high mortality, hatchery steelhead contribution to the total population decreases from 85-87% at the egg stage to 42% at the adult stage (Leider et al., 1990).

#### 1.3.3. Genetic Interactions.

Genetic interactions may involve competition and inbreeding that lead to decreased natural population size thereby resulting in increased inbreeding within the population. Competition for limited space and food could remove wild conspecifics and

change the allelic frequency of natural populations (Krueger and May, 1991; Utter, 1998). Escapees spawn in nature and hybridization has been observed between wild and cultured salmon creating another genetic threat to wild stocks. This is critical to local salmonid populations that home to a specific river system. Altered genetic composition may alter homing ability and affect progeny survivorship, rendering wild populations unable to locate suitable spawning areas.

Samples taken from the Glenarm River in Northern Ireland indicate the genetic composition of wild salmon became more like that of escaped salmon following a sea cage escape. In addition, heterozygosity of the wild population had been reduced. The large degree of allelic frequency shift indicates either the ratio of escaped to wild spawners must have been quite high or the escaped spawning population was predominately males that spawned with multiple wild females (Crozier, 1993). Mitochondrial DNA and minisatellite markers indicate escaped juveniles complete their life cycle, return to the area of escapement and breed with wild salmon (Clifford *et al.*, 1998). Hybrids of farmed and wild Atlantic salmon display intermediate performance on survival, growth, migration and parr maturity. Also, hybrids tend to show reduced genetic variation that will be detrimental to wild populations in the long term (McGinnity *et al.*, 1997).

#### 1.4. Steelhead Trout (Oncorhynchus mykiss Walbaum)

Although the names steelhead and rainbow trout (previously *Salmo gairdneri* Richardson) have been used interchangeably, these terms represent two different life strategies of the same species. Rainbow trout inhabit fresh water throughout its life cycle within streams, rivers and lakes. Anadromous members of the species, using fresh water for their early life prior to migrating to sea for growth and returning to fresh water to spawn, are steelhead trout. *O. mykiss* are native to coastal and inland waters of British Columbia. Rainbow trout typically spawn during spring, from late March to mid-May. This is in contrast to the native Newfoundland salmonids that spawn in the fall. Following spawning, fry emerge from mid-June to mid-August. Rainbow trout remain in the stream system throughout its life while steelhead trout migrate to sea after 2 years, returning to spawn at age 3-5 (Scott and Scott, 1988).

While in fresh water, young steelhead feed on aquatic insect and crustacean larvae. With increasing size, they may consume smaller fish. Salmon eggs, mostly coho, are the most prominent food item for rainbow trout during the winter season (Idyll, 1942). Consumption of salmon eggs may cause concern regarding steelhead escapees in Newfoundland. In addition, juvenile Atlantic salmon and rainbow trout, living in the same fresh water system, may be direct competitors for food and space with heightened competition due to the territorial nature of both species (Chadwick and Bruce, 1981).

Newfoundland rainbow trout were first introduced to Long Pond, near St. John's, in 1887 (Chadwick and Bruce, 1981). Additional introductions in the early 1900's occurred in ponds along the railway route to enhance the local recreational fishery. More recent introductions have been documented in ponds on the Avalon Peninsula, Notre Dame Bay region and near Corner Brook. Few of these populations have been observed to be diadromous. However, occurrence and extension of the range of steelhead populations in Newfoundland suggest introduced populations may now be successfully reproducing and expanding in Newfoundland (Chadwick and Bruce, 1981).

Bay d'Espoir, on the south coast of Newfoundland currently is considered the only region in the province to have the necessary bio-physical attributes for open-ocean cage aquaculture for salmonid species. Newfoundland salmonid aquaculture began in 1985 with Atlantic salmon. Steelhead trout was first grown in Bay d'Espoir in 1988 and until 2000 only female triploid steelhead were permitted within the region. Four companies operate seven active steelhead trout sites within Bay d'Espoir.

Prior to 2000, the industry raised triploid steelhead with high mortality (20-25%), poor food conversion ratios (2.5:1 by the second year), high disease incidence, and low market weight. Such industry characteristics do not easily allow for an economically competitive industry in the global market place. The Bay d'Espoir situation is considered an exception to other steelhead growing regions with comparable environmental

conditions but raising diploid steelhead. Due to local investor concerns regarding the aquaculture performance of triploid salmonids, the provincial Department of Fisheries and Aquaculture requested the use of diploid females for sea cage culture in the region. After much discussion and risk assessment, the Bay d'Espoir industry was granted permission in 2000 to import all-female diploid steelhead eggs. The proposal to import diploid steelhead trout requested commercial grow-out trials in Bay d'Espoir, and pending evaluation of the requested strain, potential commercial-scale aquaculture of diploid steelhead. Use of all-female populations eliminates the risk of escapees to establish self-sustaining wild populations. Additionally, the proposal outlined several methods to minimize escapement from cages, including equipment checks, improvement of husbandry practices, and an increase in predator control, to further eliminate adverse interactions with native wild salmonid stocks.

#### 1.5. Telemetry

Telemetry is the process of conveying information from one location to another. Transmitted information may include environmental data from remote locations or in the case of fisheries and wildlife biotelemetry, information is transmitted from the animal, through the use of an appropriate transmitter, via a specific carrier frequency, to a collection site (Lotek Engineering Inc., 1994). Telemetry systems vary immensely in the equipment used and complexity depending on the environment and scope of the research. Typically, either a radio or acoustic transmitter is attached to the study animal, which

transmits an appropriate radio or acoustic signal that is received by an antenna or hydrophone and tracked manually or from a fixed data-logging station. Transmitted data vary according to the transmitter capabilities but typically include an identification code, which may be accompanied by some other measured parameter of the environment or physiology of the experimental animal. Advantages of telemetry over conventional methods (i.e., direct observation and catch-and-release programs) to collect biological fisheries data include the ability to monitor fish behavior continuously over large spatial areas and temporal periods following a single intrusive event for transmitter attachment.

Underwater biotelemetry has proven to be a powerful technique for studying the behavior and movement of aquatic animals since its introduction with acoustic transmissions (Trefethen, 1956) and first application (Johnson, 1960). It was apparent early in underwater telemetry development that acoustic and radio transmissions were suited for different environments. Acoustic transmissions offer greater range per unit power in conditions where the water has high conductivity and depth, which cause unacceptable radio signal attenuation (Stasko and Pincock, 1977). In contrast, radio frequency (RF) transmissions offer a greater range per unit power in fresh, shallow water. As well, RF is less affected by turbulent water and debris (Schieffer and Power, 1972; Ziebell, 1973) and may be detected through ice (Lonsdale and Baxter, 1968). Owing to biocompatibility issues of transmitter size and weight associated with double-tagging experimental fish, most studies have been limited to either fresh water (Winter *et al.*,

1973; McCleave *et al.*, 1978; Eiler, 1995), employing RF, or estuarine and salt water environments (Stasko, 1975; Fried *et al.*, 1978; Tytler *et al.*, 1978; Moore *et al.*, 1990, 1992, 1995; Greenstreet, 1992; Lacroix and McCurdy, 1996), utilizing acoustic telemetry.

Biotelemetry probably has its greatest merit in aquaculture research to determine the three-dimensional position of cultured fish within grow-out cages, thereby facilitating evaluation of position relative to ambient environmental conditions and culture practices. In the past, positioning studies have required direct observation (Sutterlin et al., 1979), underwater camera systems (Kadri et al., 1991; Furevik et al. 1993), or acoustic monitoring (Juell et al., 1994). Although each of these techniques has merit, they monitor the cage of fish as a unit and frequently have incomplete data sets with regards to a 24-hour diel cycle. Lotek Marine Technologies Inc. in association with researchers from the Marine Institute and Conne River Aquaculture (unpublished data) employed an advanced telemetry system to monitor four individually tagged steelhead trout in a commercially stocked aquaculture cage with approximately twenty thousand individuals. The study was a proof-of-concept for the use of telemetry within the high stocking densities of aquaculture cages. Fish tracks were obtained throughout a 24-hour cycle for the duration of the experiment. In addition, data allowed calculation of cage volume utilization by individual fish and swimming speed within different areas of the cage. This technology will be invaluable for future research to determine environmental effects on

cultured fish, optimal cage design, feeding schedules, and fish social interactions within an aquaculture cage.

It has long been the desire of fisheries biologists to measure the activity, and subsequently stress, of fish in their natural environment. In addition, activity data would allow aquaculturists to determine the stress levels displayed by cultured fish associated with the culture environment and practices. Many researchers have collected data regarding activity and metabolic rates of fish in laboratory surroundings, or in the field at discrete intervals of time. However, lab and field studies have frequently required indirect measures of fish activity or have experimental fish tethered to monitoring equipment to attain data (Priede and Tytler, 1977). Tethering fish may limit the movement and activity of experimental fish, thereby affecting metabolism. This constraint makes it difficult to extrapolate results to the natural environment where additional complexity is expected between the environment, activity and metabolism.

The well being of cultured fish affects growth rate, health status and survival. Stress to cultured fish may be induced by environmental factors, culture practices or both (Barton and Iwama, 1991; Hedrick, 1998). Probably the most noteworthy use for EMG/ECG telemetry in aquaculture would be the determination and extent of potential stressors to cultured fish. Monitoring behavioral characteristics and stress response associated with aquaculture farming practices and confinement with telemetered ECG is

relatively novel. Bjordal et al. (1988) demonstrated feeding, delousing, removal of dead fish, net changing and sampling procedures all increased the heart rate of farmed Atlantic salmon using acoustic telemetry. Recently, Atlantic halibut (*Hippoglossus hippoglosus*) has received substantial attention as an aquaculture candidate species. Rabben and Furevik (1993) tested the effectiveness of ECG telemetry to monitor halibut stress in lowdensity conditions. They reported a 24-hour cycle in pulse rate with a peak at midday and minimal value around 2300 hours. A preliminary investigation of farming practices was conducted with no effect on heart rate with the tank being lit by a flashlight beam, formalin treatment, or presence of people in the tank. However, an increase was observed when a nearby door was opened allowing daylight to enter the room and during competitive feeding. Bradycardia, beat skipping, was observed in the presence of larger halibut. Armstrong et al. (1989) reported bradycardia in pike as a car approached the pike location. This may have consequences for farmed fish with numerous boat approaches to aquaculture cages throughout a 24-hour cycle. Stress responses and longterm effects in Atlantic salmon associated with approaching boats have not been documented.

Tracking and spatial positioning telemetry systems outside of the cage may be employed to monitor the movements and behavior of escapees and determine potential interactions with wild conspecifics. Although the effects of domestic fish on wild stocks has been the focus of much debate (Hansen *et al.*, 1991; Hutchinson, 1997), few studies

have utilized telemetry to monitor potential interactions. To date, all telemetry studies have employed RF to monitor the movement of salmonid escapees within river systems (Heggberget *et al.*, 1993, 1996; Økland *et al.*, 1995; Thorstad *et al.*, 1998). Much more escapee telemetry research is required to determine the effects of escapees on the environment and the feasibility of recapture to return escapees to grow-out cages for further growth, thereby reducing financial losses to the aquaculture industry. In addition, predator movement studies may be conducted to determine the likelihood and potential mitigative procedures against predatory attacks on aquaculture cages, and therefore the stock. Finally, studies may be performed to determine the extent of aquaculture cages acting as artificial reefs to wild fish stocks and home range of artificial reef fish.

#### 1.5.1. Remote, Combined Acoustic/Radio Biotelemetry System.

The research reported in this thesis employed biotelemetry to monitor the movement and behavior of aquaculture steelhead trout in the wild, as they moved throughout Bay d'Espoir, Newfoundland. Several technical challenges had to be overcome to optimize telemetry for this study. First, diadromous fish utilize water with varying salinity throughout their life cycle. Second, commercial power supplies are often lacking for remote, fixed data-logging stations which may also be inaccessible for data downloading and receiver configuration and interrogation for parts of the study season. Finally, extreme reaches of water may create problems for conventional tethered

hydrophone systems. Each challenge was separately addressed and integrated to develop the remote, combined acoustic/radio biotelemetry system described.

Gradients in the aquatic environment associated with depth and conductivity, requiring both acoustic and RF transmissions to optimize a study, may be considered bimodal in nature. Owing to biocompatibility issues, such as weight and size restrictions of the transmitter, it is not feasible to double tag most animals with separate acoustic and radio transmitters. A combined acoustic and radio transmitter (CART) was subsequently developed to operate in a bimodal environment and overcome these limitations (Potter, 1988; Solomon and Potter, 1988). Initial CART transmitters were limited by power and battery life. Further, they were designed to transmit in acoustic mode for a predetermined number of days, according to the expected fish movement, after which the transmitter switched to radio transmission until the transmitter battery was exhausted (Solomon and Potter, 1988). This configuration limited the capability of a study; particulary when animal movement patterns were not predictable. To overcome this limitation, a new CART transmitter, which switches between transmission modes based on the ambient environmental conductivity, was developed (Niezgoda *et al.*, 1998).

In the past, data retrieval from fixed data-logging stations required a hard-wired connection to link the receiver to a host computer. Hard-wired connections become a concern when fixed stations are in remote locations that are costly to access repeatedly or

are periodically inaccessible during the study period. Remote access to fixed telemetry stations has employed geostationary operational environmental satellite (GOES) communication (Eiler, 1995). Collected data were transmitted every 3 hours to a satellite-linked receiving station and accessed daily via telephone modem by the researcher. This system, however, lacks a two-way link to allow receiver operational parameter adjustments such as frequency tables, antenna gain, and scan time to optimize the system in dynamic ambient conditions.

In my study, a remote, combined acoustic/radio, biotelemetry data-logging system was used as a means to monitor fish movement and behavior. It is based upon a field proven radio receiver with combined acoustic and radio transmitters (CART) and augmented with remote data links (Lotek Marine Technologies Inc., St. John's, Newfoundland) (Figure 1.1).

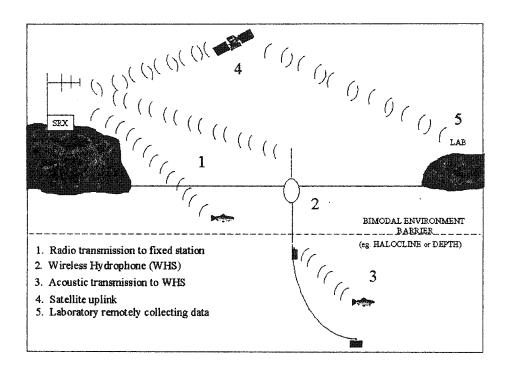


Figure 1.1. Components of the remote, combined acoustic/radio biotelemetry system.

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#### 1.6. Research Objectives

Owing to the characteristics of the Bay d'Espoir steelhead trout industry and the economic concerns to the aquaculturist associated with escapees, there is considerable incentive to determine escapee steelhead trout movement and behavior. Steelhead trout are not indigenous to the Atlantic region and may create problems for native fish populations should introduction (i.e. escapement) occur. Further, to develop optimal recapture strategies to remove escapees from the wild, it is necessary to determine the movement and behavior of steelhead escapees in the natural environment. This study attempts to address escapee movement by tracking transmitter-implanted domestic female triploid steelhead in the wild. Results will assist in determining the feasibility of developing a recapture strategy for the Bay d'Espoir triploid steelhead aquaculture industry. Specific objectives of my study are to:

1/ determine the degree of seasonal site fidelity to the aquaculture site after escapement and return following off-site release of triploid steelhead trout;

2/ determine the degree of dispersion from aquaculture sites and the patterns and cues of dispersal from aquaculture grow-out sites;

3/ suggest a possible life-history strategy for steelhead escapees that supports long-term survival in the wild;

4/ outline potential negative impacts of observed escapee behavior to the environment, public at large, and the aquaculture industry; and,

5/ determine the appropriateness of using telemetry to assist in developing optimal recapture strategies for removal of escapees from the wild.

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# 2.0. Steelhead Fidelity and Dispersion

## 2.1. Materials and Methods

# 2.1.1. Study Area.

Bay d'Espoir, on the south coast of Newfoundland, Canada (Figure 2.1), is the largest salmonid aquaculture region in the province with summer grow-out sites

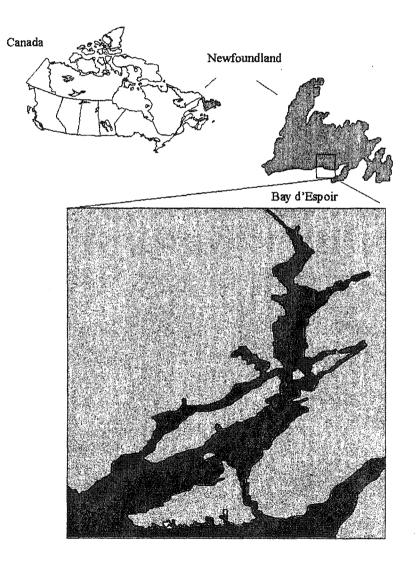


Figure 2.1. Location of the study site in Bay d'Espoir, Newfoundland, Canada.

throughout the bay and the largest overwintering site within Roti Bay (Figure 2.2). Like salmonid aquaculture industries elsewhere, summer grow-out sites require adequate water depth and flow to allow dispersion

of wastes and replenishment of oxygen while providing sufficient shelter from extreme wind and waves. Winter sites may have shallower depth and less water flow owing to reduced feed input and faeces production during the colder winter season. However, overwintering sites must have shelter from moving ice and minimum water temperature

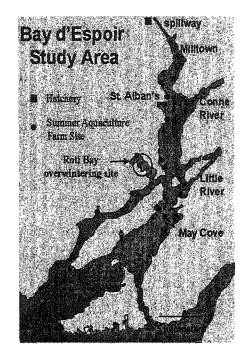


Figure 2.2. Bay d'Espoir salmonid aquaculture industry.

greater than -0.7°C, to avoid superchilling and winter fish kills. Movement of cages between summer grow-out and overwintering sites allows for at least a 4 month fallowing interval each year. A site may be licensed as both a summer grow-out and overwintering site in Bay d'Espoir only if there is sufficient space to separate summer and winter cage positions by 400 m within the site boundaries (Anonymous, 1999b). Most cages are towed to the Roti Bay overwintering site for the winter season. Here, winter ice conditions are less threatening to cage structures and nets and farms are more protected from winter storms. This farming strategy minimizes the likelihood of acute fish escapement during harsh winter conditions.

Bay d'Espoir water temperatures vary between 18-21°C in summer and reach subzero Celcius thermal minima in winter. Some areas south of May Cove (Figure 2.2) can experience winter water temperatures below the lower lethal temperature of -0.7°C (Anonymous, 1999b). The bay is considered very dynamic with regards to its halo/thermocline that may vary in depth on an hourly basis (Newfoundland Salmonid Growers Association, personal communication). Water flow from both tides (inward and outward) and hydroelectric discharge from the local spillway (outward), water depth (ranging from 6-250 m), remoteness of much of the bay, inaccessibility during the winter season and vastness of some stretches of water to monitor were all challenges to overcome for this telemetry project.

### 2.1.2. Experimental Animals.

Conne River Aquaculture (CRA), owned and operated by the Council of the Conne River Micmacs, provided steelhead trout for the study. All steelhead were triploid females from the 1996 year class. Sizes ranged from 1.5-2.0 kg in weight. In total, 150 triploid steelhead trout were surgically implanted with a combined acoustic/radio transmitter and released from the May Cove summer grow-out site. An additional 90 triploid steelhead were implanted and released from the CRA Roti Bay overwintering site.

### 2.1.3. Combined Acoustic/Radio Transmitter (CART).

CART transmitters are cylindrical, measuring a minimum of 14 mm diameter by 53 mm length and 12.2 g fresh water weight with a variable external antenna length. Each CART transmitter broadcasts a unique identification code at a study specific repetition rate. Acoustic frequencies are quartz crystal derived for 65.535 Khz and 76.8 Khz and transmitted omnidirectionally with a source level of 154 and 156 dB re 1 uPa @ 1 m, respectively. Radio frequencies are also crystal controlled and factory programmed to suit local regulations.

Both static and dynamic CART transmitters were used for the study. Static CART alternates its mode of transmission between acoustic and radio irrespective of the ambient conductivity. As an acoustic signal requires much more power to transmit compared to RF transmissions, it is considered inefficient to be transmitting in the least optimal mode for the ambient environment. Dynamic CART measures the ambient electrical water conductivity to choose the optimal mode of transmission thereby maximizing detection range and increasing transmitter longevity (Niezgoda *et al.*, 1998).

# 2.1.4. Surgical Procedure.

Transmitters were surgically implanted into each triploid steelhead on floating platforms near the rearing cages (Figure 2.3). Individual triploid steelhead were removed from the cage and bathed in an anaesthetic solution of clove oil (60 ml  $L^{-1}$ ) until the

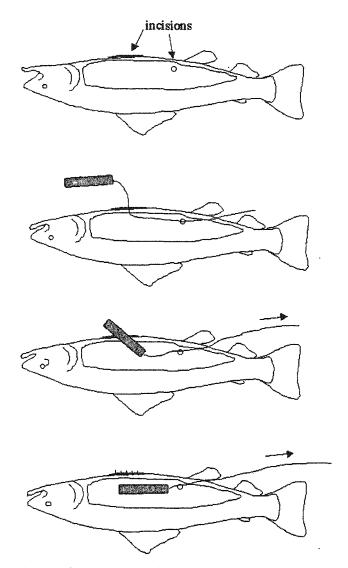


Figure 2.3. Surgical technique to implant CART transmitters into the body cavity of steelhead trout.

opercula rate slowed and became irregular (Anderson *et al.*, 1997). Anesthetized triploid steelhead were removed from the clove oil bath and placed dorsal side down on a V-shaped surgical table. Throughout the procedure, the gills were irrigated with a dilute solution of clove oil (20 ml L<sup>-1</sup>). An incision, approximately 20-30 mm long, was made on the ventral surface anterior to the pelvic girdle for transmitter implantation into the body cavity. The antenna exited near the anal fin through a small hole pierced in the flesh with a 16-gauge needle. For dynamic CART, the conductivity probe exited the body through a larger hole pierced in the flesh, again near the anal fin but to the opposite side of the antenna. The large incision was closed with 3-4 independent sutures (2/0 Ethicon silk). No sutures were required for the smaller incisions. Triploid steelhead recovered in a holding cage for a minimum of 24 hours prior to release to facilitate physiological recovery from the anaesthetic.

## 2.1.5. Release Procedure.

Two sets of 'escapee' scenarios were conducted to test seasonal movements of escaped triploid steelhead (Figure 2.4). The first set occurred in the summer of 1998 from the CRA grow-out site in May Cove. The initial release occurred on July 12, 1998 involving 68 triploid steelhead trout. Release was accomplished by simply dropping the cage netting on one side of the cage frame, sinking the mesh and allowing tagged triploid steelhead to swim from the cage. This was meant to mimic a hole that would have been torn in the net resulting in an acute loss of fish. A second release occurred on August 10,

1998 with 66 triploid steelhead trout. This release was performed after the cage was towed approximately 1000 m from the May Cove grow-out site. Tagged triploid steelhead were released in a similar manner as the first release. Following release, the net was replaced in the cage and promptly returned to the cage flotilla in May Cove. This release mimicked a cage towing disaster resulting in an acute fish loss unknown to the operator. This scenario is relevant to the Bay d'Espoir salmonid aquaculture industry due to the practice of cage towing twice a year between summer grow-out sites and overwintering sites throughout the Roti Bay

bay.

A second set of releases occurred at the CRA overwintering site within Roti Bay in December (Figure 2.4). Here, three individual releases of 30 tagged triploid steelhead were performed. An on-site release was performed in a similar fashion to the summer on-site release. It may be argued that the presence of the towed cage may have acted as

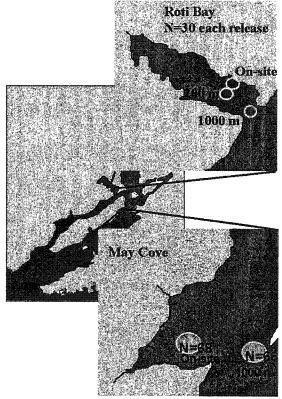


Figure 2.4. Triploid steelhead release sites for summer (May Cove) and winter (Roti Bay) escapee scenarios.

a cue to return to the grow-out site in the first off-site release. To eliminate this bias, two off-site winter releases were performed without the presence of a cage. Tagged triploid steelhead were held in 100 L tubs in a boat and transported 200 m and 1000 m from the overwintering site for manual release from the side of the boat.

#### 2.1.6. Remote Data-logging Stations.

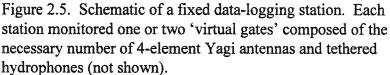
Nine remote data-logging stations were fixed and entirely self-sustaining. A platform was built to keep the system off the ground and hold all components. An insulated and watertight enclosure, constructed to house the electronic components, was bolted to the platform. The system included a Lotek SRX\_400 radio receiver, ASP\_8 (antenna switching peripheral), UUC (ultrasonic upconverter), photocontroller and battery supply, and satellite data transceiver. A drainpipe was fitted in the top of the enclosure to act as a

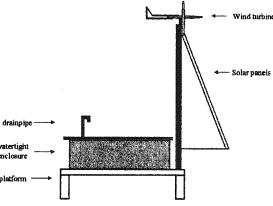
vent for the batteries and allow passage of external wires into the enclosure, while preventing

reaction waterlight enclosure → platform → Figure 2.5. Schematic of a fi

entering (Figure 2.5). Each data-logging

precipitation from





station was equipped with the appropriate number of tethered hydrophones and fourelement Yagi antennas to create 'virtual gates' throughout the bay. 'Virtual gates' essentially recorded the passing of transmitter-implanted fish from a passive perspective.

Remote stations, lacking a public power source, have to generate enough power from the environment to be self-sustaining for an indefinite period of time. A hybrid system of wind turbines and photovoltaic cells was installed on each platform (Figure 2.5). This configuration maintained a 400 amp-hour battery and was designed to withstand 10 days of autonomy (i.e. no wind or sun). Each system was effectively maintenance free requiring only annual inspection.

The SRX data-logger, with a memory capacity of up to 35,000 records, is designed to cease data collection after the memory is filled. Fixed data-logging stations were strategically located for escapee monitoring, sometimes in areas that were difficult to access either some or most of the year, thereby limiting traditional data retrieval methods. A necessity existed for a remote data link to these stations for data downloading and initialization of the SRX data memory. Within the current study, a satellite based data link was established utilizing the MSAT communication system owing to a lack of other potential data links (e.g. telephone, cellular, local radio data network (i.e. VHF or UHF)). Transmitted data included the date and time of transmitter detection, channel, code, antenna, power level, number of events and hourly battery status

indication. A two-way link was necessary between the fixed receiver and a local PC to allow remote interrogation and configuration.

## 2.1.7. Monitoring Procedure.

Nine fixed CART data-logging stations (previous section) were strategically placed throughout Bay d'Espoir in the spring of 1998 (Figure 2.6). All stations utilized an appropriate number of 4 element Yagi antenna and tethered hydrophones to create

'virtual gates' throughout Bay d'Espoir that essentially recorded the passing of transmitter implanted triploid steelhead trout.

To augment fixed station data, an intensive manual tracking effort was developed and implemented. Manual tracking was necessary to allow precise positioning of triploid steelhead between virtual gates. In addition, to gain information on directed triploid steelhead movements, manual tracking allowed

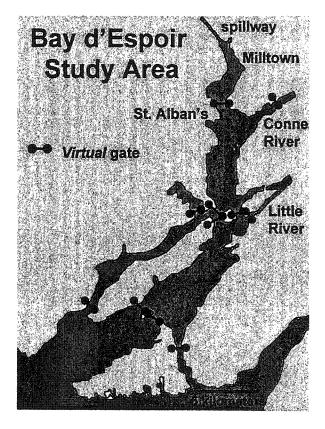


Figure 2.6. 'Virtual gate' locations throughout Bay d'Espoir to monitor triploid steelhead movements.

relocation of triploid steelhead over time. Manual tracking began at the site of release.

To allow consistent data collection, manual tracking was performed at fixed locations within May Cove and Roti Bay. Site fidelity was defined as the identification of triploid steelhead within 500 m of the farm site after release. This distance is consistent with Norwegian regulations, the only regulation of its kind, governing domestic salmonid escapement and subsequent recapture within 500 m of the cage site (Anonymous, 1997). In addition, this radius represents the expected range per unit power of the transmitter from the receiving hydrophone.

Owing to the large study area, manual tracking efforts away from the release sites were focused on areas with known fish locations. Transects were created approximately 1 km apart throughout the bay. Continuous radio tracking was permitted regardless of boat motion, with periodic stops to allow acoustic tracking with a hydrophone. Transects were completed monthly, time and weather permitting.

In addition to determining site fidelity, it would have been desirable to determine potential home range of triploid steelhead within May Cove in relation to release cage and feed storage barge location. This would require use of received power levels to determine the relative distance of a transmitter to a 270° baffled hydrophone in each of the possible 90° of monitoring. Successful crude positioning would also require constant gain of the receiver throughout manual tracking. However, with a high concentration of transmitters in a relatively small area, gain adjustment was necessary to allow

identification of all implanted triploid steelhead within May Cove, which was the primary objective. In addition, it may be expected that escaped fish will follow the feed barge around May Cove during feeding times to achieve the desired level of satiation on any farm feed passing through the aquaculture nets. Therefore, to allow appropriate home range determination, tracking must correspond with either feeding or non-feeding events throughout the study. However, this was not the case as both feeding and manual tracking times varied with each tracking effort of May Cove.

## 2.1.8. Stomach Content Analysis.

In addition to monitoring triploid steelhead movements in the wild, it was necessary to determine the feeding habits of escapees to further substantiate interpretation of a life history strategy. Ten triploid steelhead were angled from each of two cohorts for stomach content analysis. The first cohort was angled in the summer near aquaculture cages while the second was angled during winter at the entrance to the hydroelectric spillway. Following capture, triploid steelhead had their stomachs removed and preserved in 10% formalin solution for later laboratory analysis to determine stomach contents for seasonal feeding habits.

# 2.1.9. Otolith Analysis.

Scales may be analyzed to determine the life history of most teleost species. However, in the case of anadromous salmonids, scale margin resorption during spawning

migrations and regeneration of lost scales have been documented, both of which will result in inaccurate interpretation of age and life history of the individual. Otoliths are calcium carbonate deposits in the inner ear of teleost fish. The exact function(s) of otoliths are not entirely known but have been speculated to serve as piezoelectric bodies for depth and/or sound recognition in teleost fish (Degens *et al.*, 1969). Frequently, otoliths may be used for life history studies, with the one drawback that its use requires death to the individual thereby allowing for one-time sampling. In addition, resorption of the otolith is also possible depending on stressors, associated with spawning, metamorphosis and migration, that the fish has experienced (McKern *et al.*, 1974).

Pannella (1971) first correlated the sub-annual otolith increments in some coldtemperate fish species to the days in a year, followed by several other researchers with numerous other species (Struhsaker and Uchiyama, 1976, *Stolephorus purpureus*; Tanaka *et al.*, 1981, *Tilapia nilotica*). Daily increments in accretion were assumed to be related to the internal circadian rhythms of the fish. Similar daily ring patterns have been documented in numerous Pacific salmonids (Wilson and Larkin, 1980, *Oncorhynchus nerka*; Volk *et al.*, 1984, *O. keta*; Neilson and Geen, 1982, *O. tshawytscha*). However, numerous environmental parameters have been documented to influence the number of otolith growth bands per day and band width. Three pairs of otoliths are found in teleost fish – the sagitta, asteriscus and lapillus – with the sagitta, usually the largest pair, used most often in ageing studies (McKern *et al.*, 1974).

To verify escapee ability to survive in the wild, sagittal otoliths from harvested (summer 2000) and escaped triploid steelhead angled near the mouth of the hydroelectric spillway (winter 1999) were analyzed and compared. Harvested steelhead, of known age, were analyzed to determine the expected otolith increment formation for those individuals raised entirely in captivity. Otoliths from escaped steelhead were compared to otoliths from harvested steelhead to determine fish survival following escapement (i.e. assuming harvested steelhead age range 2-3 years, any escapee otolith determined to be of greater age could indicate escapee survival in the wild).

Prior to placing sagittal otoliths in molds, rubber mold bottoms were covered with a hardener-resin mixture and dried at 50°C for a minimum of 12 hours. Sagittal otoliths were placed, sulcus acusticus side up, in the mold and completely encased in the hardener-resin mixture. This mixture composed of Amine Encapsulating Hardener (Hardner HT 8700) and Epoxy Encapsulating Resin (Araldite CY 8702) in a 1:5 g ratio. Following encapsulation, the mold was placed in an oven, at 50°C, for 5 minutes to allow clearing. Otoliths were checked to ensure proper placement within the mold and returned to the oven for 24 hours.

Encapsulated otoliths were sectioned in the sagittal plane as close to the primordia as possible on one side, and excess mold removed from the opposite side. Sectioned

otoliths were subsequently mounted on slide glass, with "Crystalbond", a thermosetting plastic resin, with the primordia end nearest to the slide. Thin sections were required for light microsopy inspection of the otolith. Excess mold and otolith sections were sanded with 120c and 1500c silicone carbide waterproof sandpaper, followed by polishing with Buehler Micropolish until most of the surface scratches were removed and to allow light penetration for optimal viewing. Prepared otolith sections were viewed and analyzed at 125X magnification. Distances between the primordia and dark bands and otolith edge were measured, using an optical micrometer, near the sulcus in a line perpendicular to the bands.

### 2.2. Results

### 2.2.1. Site Fidelity.

Of the 68 triploid steelhead released on-site, in July, 51 (75%) remained within a 500 m radius of May Cove 32 days after release (Figure 2.7A). Triploid steelhead fidelity to the May Cove site declined with time. September manual tracking totals within May Cove are not complete representations owing to rough water conditions making acoustic tracking with a hydrophone difficult. Degree of fidelity rapidly declined in late-September (77 days after release) following the appearance of several tuna in the vicinity of the sea cages. Implanted triploid steelhead were not subsequently located elsewhere throughout the bay and were therefore assumed to have been prey to the tuna outside of the grow-out cages. Predation prematurely terminated manual tracking efforts.

Similar to on-site released triploid steelhead, 17 of 66 (26%) triploid steelhead released approximately 1000 m (August) outside of May Cove returned to the grow-out site within 4 hours of release. Subsequent tracking found that an additional 26 triploid steelhead had returned to the May Cove grow-out site 2 days after release, bringing the total number of off-site released triploid steelhead returning to the grow-out site to 65% (Figure 2.7A). Again predation is thought to have eliminated these fish. This prematurely terminated manual tracking efforts.

Triploid steelhead released in Roti Bay displayed different results. On-site released triploid steelhead in the winter displayed lower fidelity than those released in summer. Return of released triploid steelhead in the absence of a cage resulted in less site fidelity. Of 30 triploid steelhead released 200 m from the CRA overwintering site only 5 (16%) returned to the site within 2 days of the release. This percentage declined over time with 4 (13%) triploid steelhead remaining on-site 6 days post-release and finally 3 (10%) remaining 16 days after release. Similar results were documented for triploid steelhead released 1000 m from the CRA site. Of 30 triploid steelhead released, 2 (6%) displayed site fidelity to the CRA site within 4 days post-release. Returning steelhead remained in the vicinity of the cages for the remaining 16 days of monitoring (Figure 2.7B). Manual tracking of winter-released steelhead was terminated with ice formation that made it impossible to utilize a hydrophone or visit the overwintering site (to prevent fish disturbance and potentially lead to winter fish kills).

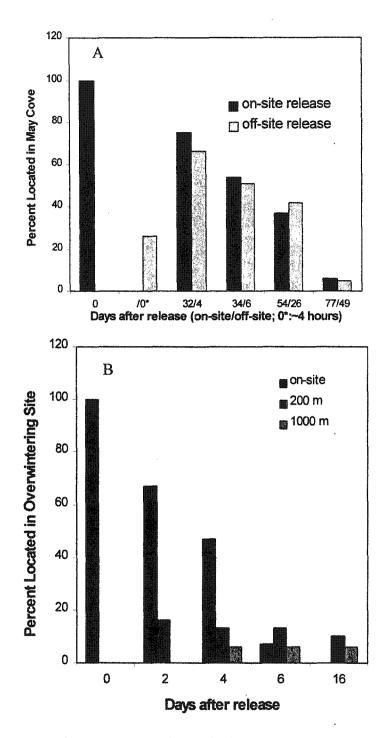


Figure 2.7. Site fidelity (percent located within 500 m from cage system) observed for steelhead trout released on-site and off site (1000 m) in May Cove during the summer grow-out season (A) and Roti Bay on-site and 200 and 1000 m off site during the overwintering season (B). Numbers represent % of escapees remaining within 500 m of the summer (A) and winter (B) aquaculture sites.

#### 2.2.2. Stomach Content Analysis.

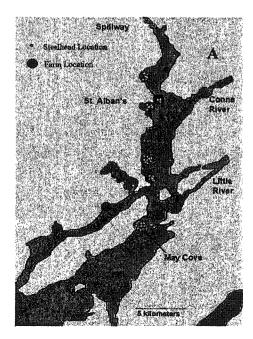
No similarities exist between stomach contents of triploid steelhead angled during the summer and winter seasons. All summer angled triploid steelhead had full stomachs consisting mostly of excess farm fish feed falling through the cages during feeding (Table 2.1). In addition, some mussel spat was identified that may inadvertently be ingested during feeding on excess feed near the cage netting. Winter triploid steelhead stomachs contained rocks, some quite large, and bottom debris. Only one stomach contained wild prey, a small portion of trichopteran insect larvae, thus it can be assumed these fish are not successfully feeding on wild food items during winter. Three individuals contained large *Panulus* shrimp that are used by local anglers as bait.

Table 2.1. Stomach contents of triploid steelhead captured during the summer and winter seasons (F = full stomach, E = empty stomach).

		Summer	Winter			
Fish	Fullness	Contents	Fullness	Contents		
1	F	Fish feed	E			
2	F	Fish feed + mussel spat	F	Rocks + branches + bait		
3	F	Fish feed	1/4	Rocks + bait		
4	F	Fish feed	1⁄4	Rocks + branches +		
				trichopteran larvae		
5	F	Fish feed + mussel spat	1/4	Rocks + branches		
6	F	Fish feed	E			
7	F	Fish feed	E			
8	F	Fish feed	< 1/4	Rocks		
9	F	Fish feed	F	Rocks + branches + bait		
10	F	Fish feed + mussel spat	1/4	Rocks + branches		

## 2.2.3. Dispersion.

Figure 2.8 illustrates the last recorded manual tracking location for triploid steelhead released during summer (A) and winter (B). Many implanted triploid steelhead had limited summer dispersal prior to presumed tuna predation (with large presence of triploid steelhead in May Cove). With dispersion, many triploid steelhead were detected in the vicinity of other salmonid aquaculture sites throughout the bay, with the hydroelectric spillway as the final destination for upstream migration (Figure 2.8A). One triploid steelhead from the summer release was located within the Conne River estuary. Winter released triploid steelhead also exhibited a directed movement towards the hydroelectric spillway but without stops along the way in the absence of other aquaculture sites throughout the bay during winter (i.e. summer grow-out sites are abandoned in winter with cages towed to overwintering sites) (Figure 2.8B).



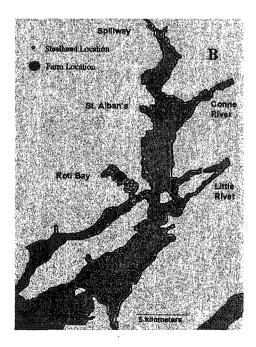


Figure 2.8. Dispersion of implanted triploid steelhead trout illustrated through last recorded location from the summer (A) and winter (B) release sites.

## 2.2.4. Environmental Parameters.

Environmental data were collected from various sources and analyzed for monthly differences that might serve as a dispersion cue for upstream migration of triploid steelhead from May Cove (Figures 2.9-2.15). Meteorological data were acquired from an Environment Canada weather station and included maximum, minimum and average daily air temperature, total daily precipitation, and average daily cloud opacity for the months of July and August. All parameters are plotted below (Figures 2.9-2.11) and in each case, no monthly statistical differences were found (Kruskal-Wallace p>0.05).

Salinity, temperature and dissolved oxygen at 1, 3 and 5 m depth is routinely collected by CRA site workers within May Cove and was acquired from the fish farmers for this analysis. Hydrological data showed a high contrast between July and August. However, owing to the highly dynamic aquatic environment of Bay d'Espoir, high variability is also evident within each month. Salinity data revealed a contrast in daily salinity at all depths at the time of data collection (Figure 2.12). Temperature data also showed some variability, although an overall warming trend is evident (Figure 2.13). Likewise, dissolved oxygen had some variability within each month but a decreasing trend at all depths is evident (Figure 2.14). Caution should be used in interpreting the hydrological data presented as different site workers would take the measures and data collection discontinues after August 6, prior to termination of triploid steelhead tracking date of August 12.

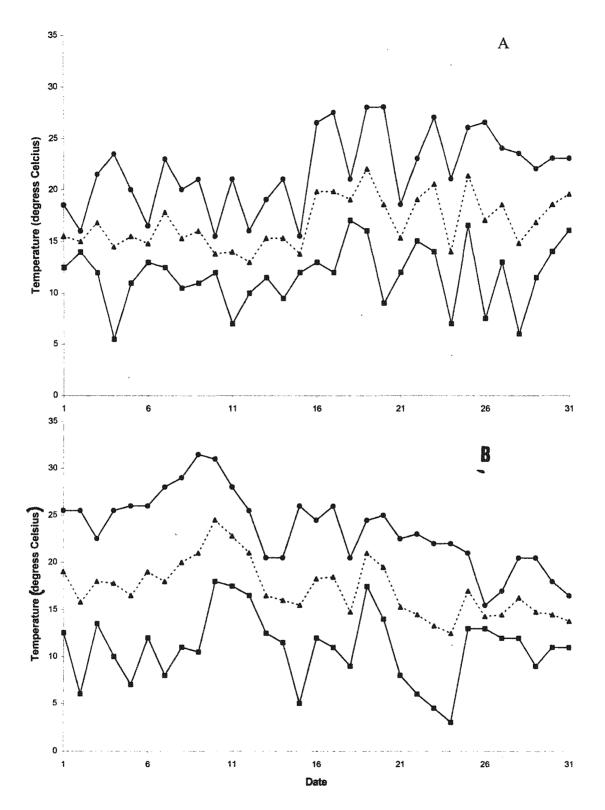
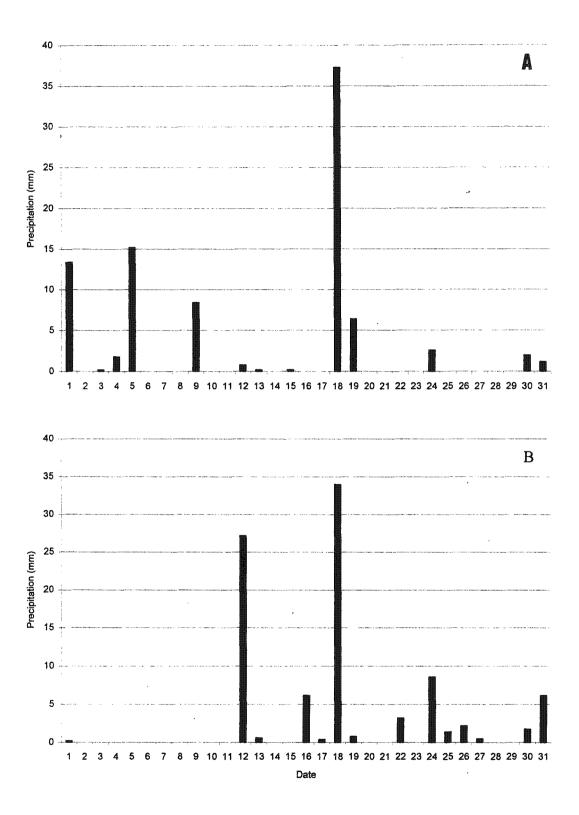


Figure 2.9. Maximum (circles), average (triangles), and minimum (squares) daily air temperature for July (A) and August (B) 1998.



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Figure 2.10. Total daily precipitation during July (A) and August (B) 1998.

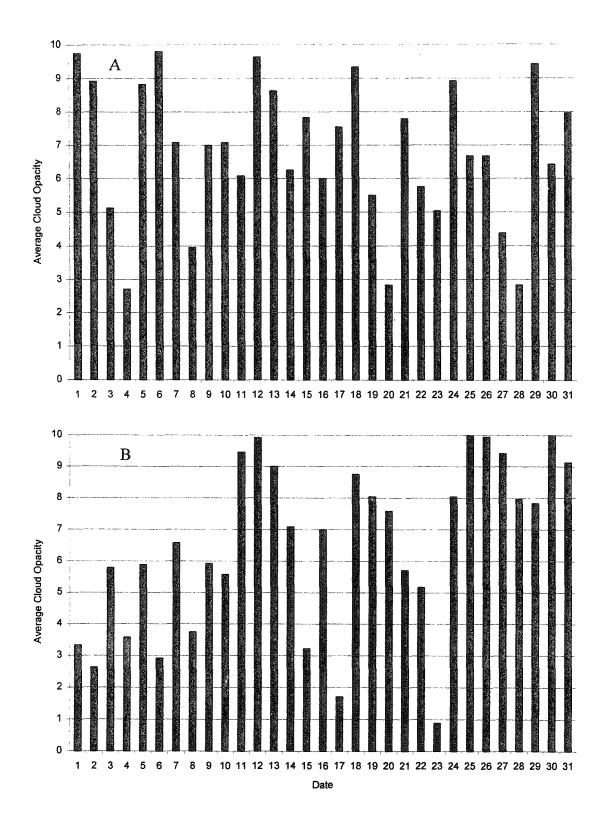


Figure 2.11. Daily average cloud opacity for July (A) and August (B) 1998.

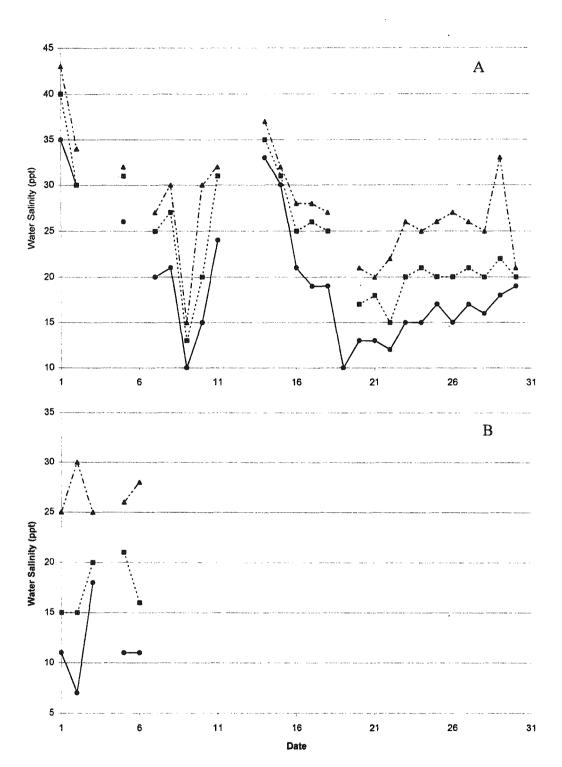
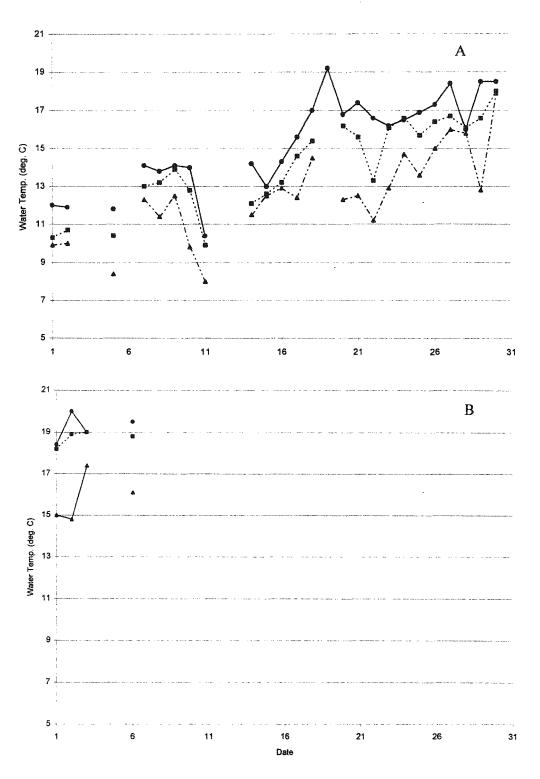


Figure 2.12. Water salinity at 1 (circles), 3 (squares) and 5 (triangles) m depth in May Cove for July (A) and August (B) 1998.



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Figure 2.13. Water temperature at 1 (circles), 3 (squares) and 5 (triangles) m in May Cove for July (A) and August (B) 1998.

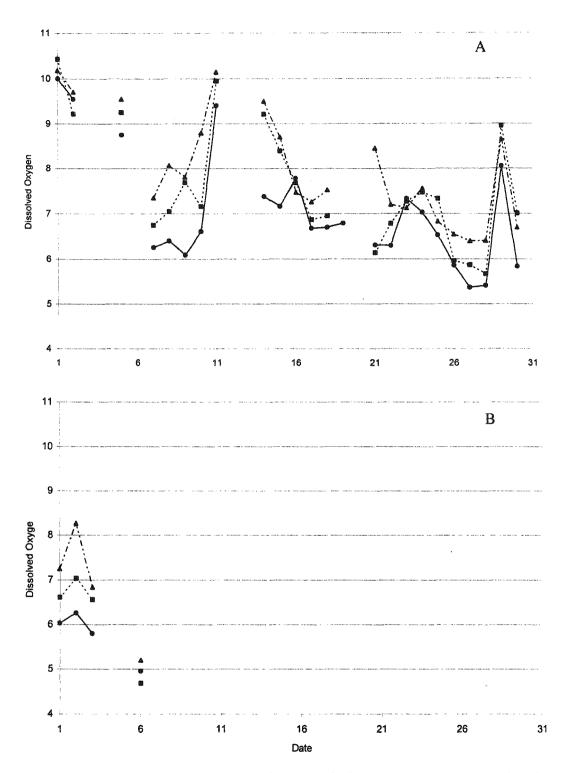


Figure 2.14. Dissolved oxygen (mg/L) at 1 (circles), 3 (squares) and 5 (triangles) m depth in May Cove in July (A) and August (B) 1998.

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Spillway outflow was acquired from Newfoundland Hydro turbine use data. A significant difference existed between July and August outflow, with July having more outflow throughout the month than August. However, from Figure 2.15, it is apparent that outflow in mid-August, the time of triploid steelhead dispersion and directed movement toward the spillway, was within the range experienced throughout July.

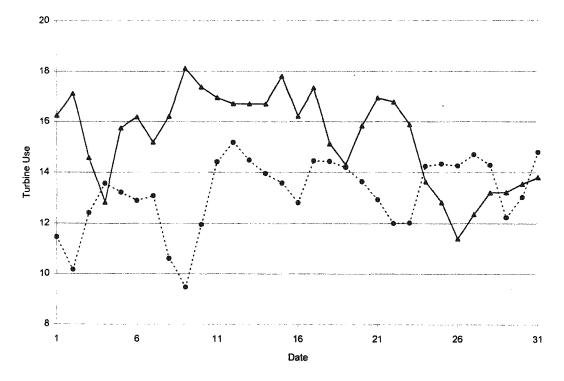


Figure 2.15. Newfoundland Hydro spillway outflow (MCM) for July (triangles) and August (circles) 1998 within Bay d'Espoir

### 2.2.5. Otolith Analysis.

Several otolith bands could be observed in prepared otolith sections (Table 2.2). A discrete transition from opaque inner to translucent outer portions of each otolith could be viewed at a fairly consistent location from the primordia. Two outer bands could also regularly be observed in otoliths of approximately the same size, prior to reaching the otolith section edge. A prominent change from an inner optically dense area to a more translucent outer portion could potentially correspond to the early freshwater stage and move to the estuarine/marine stage of grow-out (Volk *et al.*, 1995).

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- Sar Toylatan			••••••••••••••••••••••••••••••••••••••	tolith Checks fo	r Harvested Stee	elhead Trout			
#1C	0 <sup>a</sup>	6	11	21.5	275	35.5°	45 <sup>d</sup>	61.5°	67 <sup>f</sup>
#8C	0	7	17	27.5	35	39.5	49.5	61	68
#13C	0				30	36	45	58	66
#9C	0		20	25	31	37	45	60	66
	<u> </u>		Otolith Check	ts for Escaped Si	eelhead Trout A	Ingled near the S	piliway		
#6W	0	4		24		38	49		
#8W	0		17	27	35		53		
#2W	0				30	44	52		
#10W	0	7	19.5	27	33	38	46	60	70
#16W	0	11.5	18	27	37	42	51	56	67.5
#11W	0		18	25.5	35	42	56.5		

Table 2.2. Measured distances (125X) of otolith ring increments and otolith edge from the primordia for steelhead trout (*Oncorhynchus mykiss*) taken during harvest and angled in the wild following escapement from grow-out cages.

a) 0 values represent the primordia with measurements taken from this location out to the otolith edge.

b) Bold values represented the drastic change from opaque to translucent areas of the otolith. This area would possibly

represent movement from freshwater to sea and smoltification stress.

c) Minor check occurs small distance from migration change.

d) Major band potentially representing the first annuli check.

e) Major band representing second annuli and therefore aging the fish to two years.

f) Otolith edge is the last value to be listed for each analyzed otolith.

N.B. In all cases, checks of comparable distance have been placed in the same column, but all checks were not necessarily observed in each fish analyzed.

## 2.3. Discussion

The data suggest a high degree of fidelity by released triploid steelhead trout to a commercial aquaculture summer grow-out site. Attraction to sea cages is in accordance with previous studies documenting high concentrations of wild fish in the vicinity of aquaculture cages (Collins, 1971; Loyacano and Smith, 1976; Carss, 1990). Three possible explanations may be given for steelhead homing to previously experienced aquaculture grow-out sites. First, an attraction to the sea cage structure and its associated aquatic community for increased shelter. Attraction of fish to a sea cage structure may be comparable to a 'shoaling' effect found at artificial reefs, presumably due to the benefits from the associated aquatic community and increased shelter (Spanier et al., 1985; Workman et al., 1985; Hair et al., 1994). Shoaling may account for the high degree of fidelity displayed by on-site released triploid steelhead. Triploid steelhead returning to the May Cove site from the off-site release within 4 hours post-release may also provide support to the notion of fish affinity to the cage structure being towed back to May Cove. Fish loss during towing is possible as unsuitable weather conditions sometimes occur before towing operations can be completed. Attraction of fish to the towed cage may facilitate substantial recapture of escapees at this time. In contrast, 4 days following the towed release, 43 (65%) fish were found in May Cove of which 26 (60%) were additional fish to those within May Cove 4 hours post-release. Additional triploid steelhead were 'finding' their way to the May Cove site and not following the towed cage structure. Additional triploid steelhead provide the most compelling evidence to indicate fidelity, or

homing, to the site as they received no obvious stimulus to return to May Cove (i.e. towed cage). Fidelity implies some level of orientation exhibited by triploid steelhead based on cues or imprinting established while growing within the May Cove aquaculture grow-out site.

A second explanation for site fidelity may be a schooling effect created through domestication, attracting escapees to other fish present at the grow-out site. Domestic triploid steelhead are selected for fast growth in high stocking densities. Schooling behavior to other individuals, inside or outside the cages, may be anticipated following escapement (Ruzzante, 1994 for review). Schooling in culture conditions may also create a site-specific fidelity of escapees to other individuals of that species. For this to be the case, escaped triploid steelhead will be attracted only to cages holding steelhead trout regardless of the site of rearing. However, dispersing triploid steelhead displayed an attraction to other aquaculture sites holding other species of salmonids within the Bay d'Espoir region as they migrated upstream. Owing to the apparent attraction to sites other than just the site of origin, it may be concluded that triploid steelhead are displaying 'aquaculture site fidelity' and **not** 'site-specific' or 'species-specific fidelity'. Attraction to other fish may still exist, however, this will not account for dispersal of triploid steelhead from the May Cove aquaculture cages over time, despite the constant high concentration of fish, both inside and outside of the cages, remaining in the May Cove grow-out site area.

A final explanation that may better explain 'aquaculture site fidelity' is an olfactory response to the odor of aquaculture fish feed in the vicinity of **all** aquaculture sites throughout the bay. An olfactory response may also be responsible for the triploid steelhead following the towed cage back to May Cove owing to the feed odor present on the towed netting. An olfactory response will also explain the high 'aquaculture site fidelity' throughout Bay d'Espoir as triploid steelhead move upstream. An olfactory response is substantiated by the stomach content analysis data of triploid steelhead angled in the summer near grow-out cages. From the data, it is clear escapees feed primarily on excess fish feed falling through the sea cages. Aquaculture sites may provide increased feeding opportunity near the sea cages with excess feed falling through the cages during feeding. Again with this explanation, the question arises of why triploid steelhead disperse from an aquaculture site during the summer grow-out season when both feed levels and excess feed are plentiful?

A change in local environmental conditions may serve as a cue for upstream migration and account for the seemingly unnecessary dispersal of triploid steelhead from May Cove (e.g. river flow (Stabell, 1984; Smith *et al.*, 1994)). However, from the environmental data, meteorological, hydrological and hydroelectric spillage conditions, in July and August, provide no evidence for an environmental cue for dispersion. No significant difference was found in the July and August meteorological data of mean air

temperature, total daily precipitation, and mean total daily opacity. Although hydrological parameters (water salinity, temperature, and dissolved oxygen) at three depths (1, 3 and 5m) were significantly different between July and August, care should be expressed in interpreting hydrological data with regards to triploid steelhead dispersion. August data collection ceased prior to the off-site release and subsequent return of triploid steelhead to May Cove. Therefore, with returning escapees to May Cove during fluctuating and significantly different hydrological conditions, little significance of hydrological data on dispersion is expected at this time (i.e. off-site released triploid steelhead returned to May Cove and therefore would not explain dispersion from the grow-out site). In addition, large hydrological fluctuations within each month are anticipated within the bay owing to local wind and tidal conditions at the time of data collection.

A more likely explanation for seemingly unnecessary dispersal may be predator density dependent. Escaped triploid steelhead have been raised on fish feed since first feeding as parr and therefore are initially attracted to feed odor in the water near the May Cove grow-out site. Within an aquaculture cage, the nearest-neighbor distance (NND) is very small with high stocking densities. However, small NNDs are allowable within the cage environment with increased feeding opportunities and an ample supply of feed provided to the fish (typically fed *ad labitum*), thereby decreasing competition with negligible risk of cannibalism. For escaped triploid steelhead outside the cages, the same

odor of farm feed is present in the water passing through the cages but owing to attempts by the fish farmer to minimize feed wastage, escapees will receive less feed outside the cages. Low levels of accessible feed outside the cages will adequately sustain only a small 'predator' population. Transmitter-implanted triploid steelhead are also presumably competing with wild fish populations and other escapees attracted to the cages for the same food source. Eventual dispersal is reasonable to assume to increase the NND and decrease feeding competition. Following the odor of fish feed in the water will result in a directed upstream movement in Bay d'Espoir. Odor induced dispersion likely accounts for the high degree of attraction displayed by triploid steelhead to other aquaculture sites throughout the bay.

Rapid upstream movement was further corroborated during autumn 1999 when Department of Fisheries and Oceans (DFO) personnel responded to an acute escapement from a site in the outer portion of Bay d'Espoir. Satisfactory recapture was accomplished only in the mid-portion of the bay, despite high feeding levels ongoing at the site of escapement (DFO personnel, personal communication). This movement, and subsequent recapture, was similar to observed results in my study, indicating upstream movement of escapees directed towards the odor-laden water, possibly to increase the NND outside of the cage, and allow effective feeding by escapees. DFO observations suggest a complex relationship between feeding opportunities, predator density in the vicinity of the sea

cages, and time of year influencing local environmental conditions with respect to movement patterns of escaped triploid steelhead in the wild.

Triploid steelhead trout released in Roti Bay during winter demonstrated a lower degree of site fidelity when released on-site and decreased return to the CRA overwintering site when released off-site. Decreased feeding opportunity is presented at the overwintering sites and therefore decreased fidelity corroborates the notion of a response to feed odor, or in this case lack of feed odor.

Probably the most interesting biological result of my study was the directed movement of escaped triploid steelhead to the hydroelectric spillway and not to any of the local salmonid rivers. This is of particular importance since transmitter-implanted triploid steelhead passed the mouth of Conne River, a natural salmonid river, to gain access to the hydroelectric spillway. My study therefore differs from other escapee studies (Jonsson *et al.*, 1990, 1991, 1994; Eriksson and Eriksson, 1991; Gudjonsson, 1991; Lund *et al.*, 1991; Heggberget *et al.*, 1993; Økland *et al.*, 1995; Heggberget *et al.*, 1996; Thorstad *et al.*, 1998) reporting escapees entering natural salmonid river systems. However, in Newfoundland the hydroelectric spillway is also the discharge location of the hatchery effluent for the Bay d'Espoir salmonid aquaculture industry. Webb *et al.* (1991) reported a similar behavior by escaped adult Atlantic salmon. Many domestic salmon were observed in the River Polla where movement was directed to the discharge

water of a salmonid hatchery. Some escapees were captured in the hatchery during times when the hatchery outflow pipes were submerged. This may suggest the escaped triploid steelhead are homing to their 'natal stream' (i.e. industry hatchery effluent).

However, triploid steelhead in the Bay d'Espoir salmonid aquaculture industry would have no imprinted sign-posts for the spillway until nearly to the St. Alban's area, as required by the sequential learning hypothesis. Within the industry, smolts are transported from the hatchery and placed in the bay at a wharf several kilometers from the hatchery, and at a comparable distance from the Conne River estuary. From here, the practice at the time of this study was to tow the cages from low salinity to high salinity water for the grow-out phase with first year overwintering in Roti Bay. Following their second summer, the cages were towed back to St. Alban's, near the processing plant. Therefore, some other mechanism must account for triploid steelhead swimming past the Conne River estuary and migrating to the hatchery water. With the seemingly high affinity of escaped triploid steelhead to fish feed odor, it is probable that triploid steelhead are homing to the hatchery outflow and its associated feed odor. Attraction to the hatchery effluent would also explain directed movement of winter escapees to the hydroelectric spillway in the absence of other cages and being triploids therefore presumably not displaying a spawning response. In addition, upstream movement to the spillway may not be a genetic response to pheromones as suggested by the pheromone hypothesis for salmonid upstream migration. Instead, in the case of escapees, attraction

may simply be from odors associated with fish farming and the feed that the escapees have been raised on. Cooper and Scholz (1976) demonstrated that steelhead trout imprint their home stream odor as juveniles and use this to return to the natal river to spawn. Other artificial chemical imprinting of salmonids has been documented for Atlantic salmon imprinted to morpholine (Sutterlin et al., 1982). Atlantic salmon imprinted for as little as 5 days to low concentrations of morpholine homed to a marine site with morpholine added to the water. From this, it is evident that long periods of farm feed odor imprinting of aquaculture fish may contribute to the attraction of triploid steelhead to the spillway water. Some concern may exist for the Bay d'Espoir salmonid industry if the hatchery were to be moved to a location outside of the bay and the effects this may have on escapee movement. However, from my data, it seems reasonable to suggest adding the appropriate odor to the spillway water during autumn may attract escapees to the spillway and prevent entry of escaped fish to local river systems, regardless of hatchery presence. Sutterlin et al. (1982) also demonstrated the ability of Atlantic salmon to be artificially imprinted to return to a marine site even though these salmon originated from a nearby hatchery. Salmon reared in a distant hatchery and released at a marine site 12 km from their parent stream returned to the imprinted marine site after 1 or 2 years at sea. It appears very important to time imprinting with smoltification to be most effective. Of course, without further experimentation focusing on the attraction of escaped triploid steelhead trout to the spillway, one cannot entirely rule out the possibility that these fish are influenced by the direction of the hydro discharge current, also being stronger than the

natural salmonid rivers, followed by an attraction to a freshwater thermal regime (or avoidance of the marine thermal regime).

Although the majority of released triploid steelhead moved upstream to the hydroelectric spillway, one tagged individual was tracked to the Conne River estuary. Dempson *et al.* (1996) also notes that escaped rainbow trout have been captured in the Conne River and Dempson *et al.* (2001) summarizes occurrence of farmed salmon identified in Conne River from 1993 to 2000. In my study, this individual, from the summer releases, equates to a 1% straying rate of escaped triploid steelhead to other river systems. This percentage is below the estimated 4% straying rate of wild salmonids by Stabell (1984), potentially to allow some gene flow between river system populations. This lower rate of straying may be attributed to the strong homing response to fish feed odor and a potential lack of desire to spawn by triploid females in the wild. It is worthy to note, however, that this escapee was consistently present in the same location of the Conne River estuary. Therefore, it is also likely that the signal may in fact have been from a discarded transmitter removed from an angled experimental escapee and thrown in the estuary.

With a high degree of fidelity to the cages evident during the summer grow-out season and directed movement to the hydroelectric spillway during the overwintering season, the question of whether escapees can survive in the wild remains. Stomach

content analysis data certainly provide some evidence to suggest escapees may survive following escapement. Escapees feed heavily on excess farm feed during the summer grow-out season followed by a period of relatively unsuccessful feeding during the overwintering season. However, assuming escapees are adequately feeding during the summer, a duration of non-feeding may be sustained on supplies of fatty tissue during winter, and would be comparable to wild salmonid spawning behavior. Triploid steelhead sampled in the late winter provided visual evidence for this survival strategy with sufficient adipose tissue remaining within the body. In fact, this strategy is not unlike that experienced by triploid steelhead retained within the cage environment. Aquaculture fish in Bay d'Espoir are fed to satiation during the summer grow-out season to maximize growth, followed by an overwintering maintenance ration to sustain activity and metabolism. This feeding cycle is continued until the fish are harvested possibly 2  $\frac{1}{2}$ -3 years after hatching. In addition to collected stomach content data, anecdotal evidence also exists of triploid steelhead being caught in the local recreational fishery at sizes much larger than the desired harvestable size in aquaculture. It is therefore possible that angled fish had escaped some time previously and survived in the wild using a similar strategy as described above.

To verify the proposed life history strategy of escapees to ensure survival, otoliths were collected from harvested and angled triploid steelhead and compared. From the otolith analysis, escapees were likely angled shortly after escapement as all were younger

than the harvested individuals examined. Additional otolith analysis should be performed to verify survival of escapees in the wild. Documentation of steelhead otolith growth under aquaculture practices and in the wild is recommended to assist future interpretation of otolith increment deposition.

Otolith research with steelhead trout is somewhat limited. However, much research has been performed to describe otolith increment formation and factors affecting deposition in the Pacific salmonid genus *Oncorhynchus*. A major setback to interpreting otoliths from aquaculture fish is the lack of published information characterizing otolith growth in domestic environments and correlated with the stresses associated with aquaculture operations. McKern *et al.* (1974) characterized recognizable bands on otoliths taken from steelhead trout. They were able to age individuals according to the number of freshwater and ocean annuli and recognize bands associated with nuclear and migration checks associated with smoltification and movement to the ocean.

For individuals raised in the confines of a stable hatchery environment, no seasonal variation should be observed in otolith growth provided that constant quantities of feed are offered to the fish (McKern *et al.* 1974). Within the hatchery environment, all parameters (including water temperature, photoperiod, feeding frequency, stocking density and water flow) affecting fish growth are held to an optimal level to allow maximal growth and attainment of market size in the shortest period of time. Within the

ideal hatchery situation, daily growth increments of fish should be consistent resulting in a fixed width for daily otolith increments. Upon reaching an appropriate size for transfer to grow-out cages, these fish are held initially in low salinity water through smoltification. Fully smoltified salmon are moved to water of higher salinity. Stress associated with smoltification and corresponding lack of feeding at this time may result in the presence of a check on the otolith.

In addition, transfer from the hatchery environment to the open ocean environment could be represented by a change in width increment measured in daily otolith rings. Width change results from transfer from the constant hatchery environment to the dynamic open ocean environment with its fluctuating temperature, salinity, photoperiod, cloud cover and operations. As previously discussed, all of these parameters, either individually or together, may act as a zeitgeber for otolith ring formation or affect ring width increment. Volk *et al.* (1995) discerned the entry of pink salmon smolt to the marine environment by a distinct change in otolith appearance. Smolt freshwater residence period corresponded to an optically dense region close to the primordium and contrasted to increments that are more translucent and with wider increments, corresponding to marine residence. Decreased growth may be expected just prior to seaward migration due to intense and stressful smoltification processes, accompanied by major physiological and environmental change. Such growth deterrents might be offset once in the marine environment when normal feeding levels resume.

Neilson et al. (1985) also differentiated the freshwater and estuarine growth in juvenile chinook salmon (O. tshawytscha) by a 25% increase in increment width following entry to sea. Although an increment increase could not be determined, all samples from harvested and escaped steelhead trout in the present study had a similar prominent change from an inner optically dense area, potentially corresponding to the freshwater stage, to a more translucent outer portion, corresponding to the estuarine/marine stage of grow-out (measures in **bold** print in Table 2.2). Location of this transition within the otolith was relatively the same for all harvested individuals and some escapees. In my study, the remaining escaped individuals displayed this transition zone at a much earlier time. An earlier ring deposited that represents the freshwater to seawater transition could identify those individuals that escaped during the transfer to the cage from the live haul truck or shortly after being placed in cages in the freshwater portion of the estuary. Individuals remaining in the cage would be moved out to sea according to the industry requirements but those escaping early in the grow-out cycle could move to sea on their own accord and therefore display the transition at a earlier age. Interpretation of an earlier time of escape also implies young escapees have survived and thrived in the wild.

Neilson and Geen (1982) distinguished distinct event checks within the regular otolith increment pattern. Checks could be correlated with major stressful events experienced by chinook salmon including hatching, transport stress and abrupt temperature change. However, unlike individuals in my study, Neilson and Geen (1982)

monitored ambient environmental conditions and hatchery procedures to correlate checks with date of occurrence. As eluded to earlier, daily growth increments in otoliths taken from salmonids in sea cages should be constant during the grow-out cycle assuming aquaculture operations and growth are constant. Such principles assume that enough feed is provided to the fish to allow uniform growth throughout the stock. However, it is often apparent that within each cage, a hierarchy is established that results in dominant and subordinant individuals. This, in turn, may affect feeding frequency and quantity of individual fish thereby affecting growth. Finally, within aquaculture operations, there are numerous occasions when normal behavior is disrupted and stress levels increase (ie. grading, net changing, sampling). It is not known how long after these operations before feeding and growth are returned to pre-disruption levels. Event checks would have benefited this study and potentially allowed determination of escapement time. However, in the absence of comprehensive data monitoring the experiences of sampled individuals, interpretation of otolith bands and correlation to migration, transport, storm events or even annual increment checks is not possible. Checks observed in the opaque inner portion of the otoliths could correspond to numerous grading events in the hatchery, vaccination of all parr prior to transfer to sea, and initial placement in pond cages prior to transport to the sea in a live haul truck. All of these operations would create elevated levels of stress and may result in a stress check on the otolith at that time.

Many of the cited researchers noted that employing otolith increment production to age Pacific salmonids in the wild is extremely difficult owing to numerous and intricate factors affecting growth. This difficulty applies to aquaculture fish as well. Although culture feeding regimes should allow maximal growth, ambient environmental conditions and numerous stressors associated with culture may influence fish growth and subsequent otolith increment periodicity and width. To ensure appropriate interpretation of otolith characteristics of escapees, it is highly recommended to thoroughly document the 'aquaculture life history' experienced by captive fish, paying particularly close attention to the date and frequency of farm stressors. This will allow optimal interpretation of otoliths and assist in identifying increment checks associated with farm handling and environmental conditions. Without such information for aquaculture individuals, it is difficult to interpret escapee otolith patterns to determine the life history strategy and success of escapees in the wild.

#### **3.0. Implications of Fidelity and Solutions**

Several negative implications are evident from 'aquaculture site fidelity' by escaped triploid steelhead. These may include the presence of drug residues in fish feeding outside the cages, increased risks of escaped salmonids acting as disease and parasite vectors to wild stocks, and increased attraction of predators to the site for escapees. Recapturing escapees aggregating near aquaculture sites may alleviate negative implications.

Owing to the economic loss associated with escapement and the above possible negative implications, it should be the goal for all aquaculturists to eliminate fish loss from grow-out cages. However, elimination may be an unrealistic goal owing to the harsh and sometimes unpredictable environment of open ocean aquaculture. A more appropriate solution may be to recapture escapees and return them to the cages for further growth.

Efficient development of suitable recapture strategies for any aquaculture industry should follow three logical phases. Movement and behavior of escapees must be determined to ensure effective recapture is feasible. As discussed throughout this text, this phase has been completed for triploid steelhead trout in Bay d'Espoir, NF. The evidence provides a reasonable expectation that escaped triploid steelhead aggregate in predictable patterns to allow effective recapture. Next, an effective method to monitor potential recapture trap configurations must be developed, preferably from a passive

perspective so as not to disturb escapee behavior in relation to the recapture strategy. Finally, potential recapture traps would need to be tested to develop optimal recapture strategies for the industry.

This chapter will focus on the use of telemetry to monitor potential recapture strategies for the salmonid aquaculture industry. Telemetry will allow ease of monitoring, from a passive perspective, of fish in potential recapture traps and help to optimize appropriate trap development. The telemetry system described was field tested with triploid steelhead in the Conne River Aquaculture overwintering site in Roti Bay (Figure 2.2) in December 1998.

## 3.1. Methods and System Calibration.

A recapture trap was deployed within the site to determine the feasibility of employing telemetry to monitor its fishing success. This trap had a similar configuration to a traditional Newfoundland caplin trap. The trap itself was affixed to the plastic floatation collar of a fish culture cage structure with a 15-m leader oriented towards shore. Two hydrophones were used to monitor the trap for nearby acoustic signals from transmitter implanted triploid steelhead (Figure 3.1). An omnidirectional hydrophone (B) was placed in the center of the trap and configured to monitor only within the cage. Hydrophone B would give a presence/absence recording for triploid steelhead within the trap. Another hydrophone (A) was configured to monitor the leader. Hydrophone A was placed at the near-shore end of the leader and fitted with a 270° baffle, with the open 90° pointing along the leader towards the trap entrance. Both hydrophones had cables running towards shore where the receiver and necessary electronic components were situated.

A Lotek SRX\_400 receiver was used to monitor both hydrophones. This radio receiver cannot process received acoustic frequencies. Therefore, each hydrophone was connected to an individual Lotek Ultrasonic Upconverter (UUC) which converts the electrical signal from the hydrophone to a SRX compatible radio frequency for the receiver to process. The SRX received, processed and logged identification codes of transmissions within the range of each of the hydrophones.

The most important aspect of this methodology involves calibration of each of the hydrophones to allow appropriate data collection. For trap calibration, a single transmitter was placed in the water at several points around the trap with the SRX monitoring the Hydrophone B. Hydrophone gain was set so the hydrophone could not receive transmissions from outside the netting. This was to ensure Hydrophone B would not receive signals from an external transmitter that would be interpreted as a fish within the trap (Figure 3.1).

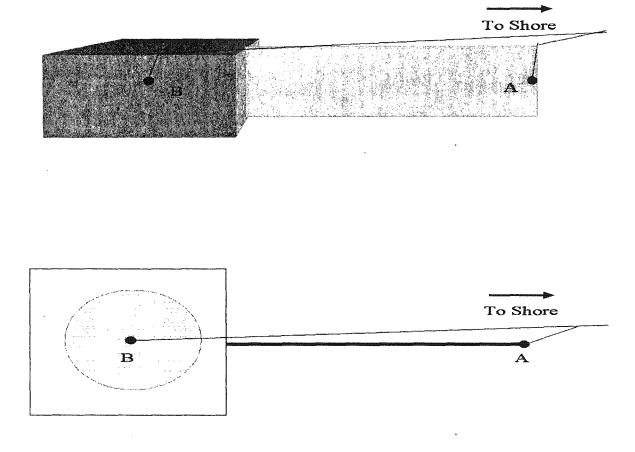


Figure 3.1. Recapture trap with monitoring hydrophone locations; (upper) Side view; (lower) Top view with trap hydrophone coverage area (stippled area).

It was necessary to calibrate Hydrophone A so that movements of transmitter implanted triploid steelhead could be determined as the fish moved along and responded to the leader. Gain for Hydrophone A was set initially to monitor the length of the leader only. Received signal power levels from transmitters will be greatest when the transmitter is closest to the receiving hydrophone. From this assumption and Hydrophone A location, highest power levels would have been recorded nearest to shore with a steady decrease in signal strength anticipated as triploid steelhead moved away from Hydrophone A towards the trap entrance, with the lowest signal power levels received when the fish was at the trap entrance. With the gain set properly, transmitted signals would not be received by Hydrophone A as triploid steelhead entered the trap. Owing to environmental variability (depth, conductivity and temperature) and fish/transmitter orientation effects on signal strength, received power levels will not be constant for each point along the leader but would remain fairly consistent within a predictable range. Signal strength range will have a probability density function (p.d.f.) approximating a normal distribution and symmetric standard deviation with the upper tail missing (Figure 3.2). A lower tailed p.d.f. occurs because most power levels from a given point are centered around the mean (i.e. most likely signal strength for the ambient environment and distance from the hydrophone) with some power levels diminishing slightly along the propagation path (giving lower tail values). Power level values above the mean are optimal for that distance if the system was monitored in a stable

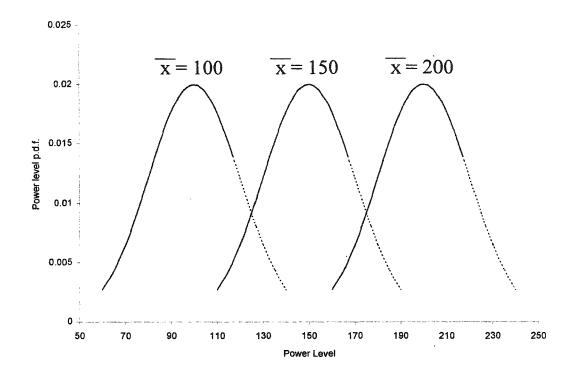


Figure 3.2. Theoretical probability density function (p.d.f.) of power levels from each point of leader calibration. Dotted lines complete each normal p.d.f..

environment. With ample calibration time and infinite calibration stations along the leader, a continuum of power levels would be achieved for the entire leader length. The mean for each calibration station would correspond to a received power level to track fish movement along the leader with a high degree of certainty for predicting the movement pattern.

However, this degree of calibration requires substantial effort and is unfeasible for a field study. To decrease calibration effort, the leader may be calibrated at a minimum of three points along the leader--nearest to Hydrophone A, middle and entrance to the trap (Figure 3.3). Again, a single transmitter was used for calibration by placing the transmitter at mid-leader depth at each calibration station. The transmitter was tied to the head rope of the leader and monitored by the SRX for a minimum of 5 minutes. With a 10 second acoustic transmission repetition rate, the transmitter transmits 30 signals during a 5 minute minimum time span. To optimize monitoring of triploid steelhead movement in relation to the leader, calibration should also be performed approximately 1 m from each station on both sides of the leader, again at mid-depth. Depending on the exact distance of additional calibration points from Hydrophone A, received power levels away from the leader may be more similar to the next furthest calibration station from Hydrophone A along the leader (Figure 3.3). Owing to the nature of this relationship, received power levels associated with a single calibration point on the leader may be placed confidently anywhere within the corresponding shaded area of Figure 3.3. However, by plotting subsequent power levels, the most probable fish track with regards to the leader and trap may be determined.

With ample calibration, all possible power levels will be recorded from at least one of the calibration stations along the leader. In this manner, all power levels from fish tracking may be placed at one of the calibration stations with a degree of certainty defined by the calculated p.d.f.. Calibration plots may have a degree of power level overlap between nearby calibration stations. However, the calculated p.d.f. would determine

from which calibration station signals were more likely to originate from with fish location placed at the calibration point with the greatest p.d.f. value. In circumstances where p.d.f. plots from nearby calibration stations cross, fish position may be placed between calibration stations. Although placing fish between calibration stations may not occur often, a more complete picture of fish movement over time could be achieved. Fish positioning may be superimposed on the trap and leader to determine probable fish tracks (Figure 3.4).

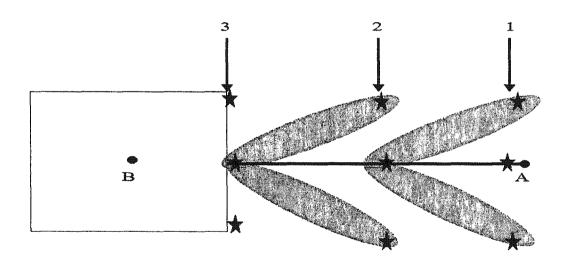


Figure 3.3. Trap calibration illustrating zones of similar power levels received by the receiver (gray area). A  $\bigstar$  represents exact points of leader calibration. Numbers represent calibration regions along the leader.

Triploid steelhead movement data in the vicinity of the trap and leader were collected for 4 days. With the absence of reverse winkers at the trap entrance, fish were allowed to swim freely in and out of the trap. Therefore, there was no need to remove captured fish from the trap during the study. Data were downloaded to a laptop using the Lotek Wildlife Host program and copied to Microsoft Office Excel and Minitab for Windows version 9.2 for analysis and plotting.

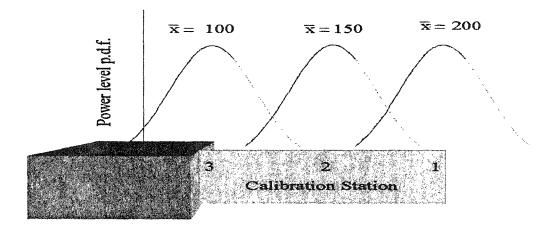


Figure 3.4. Probability density function superimposed on the recapture trap to determine fish movement along the leader.

# 3.2. Calibration Results and Triploid Steelhead Movements.

Field calibration along the leader (Figure 3.5) was similar to the theoretical calibration plot previously discussed (Figure 3.2). Owing to different standard deviations of each data set, normal distributions of the data had slightly different shapes. More

overlap was evident between the middle calibration station and the stations at both ends of the leader. In addition, the mean value (130.21) of the furthest calibration station from Hydrophone A was much higher than the expected mean (100). The other two calibration stations displayed means close to that which was anticipated according to the theoretical p.d.f. (Figure 3.2).

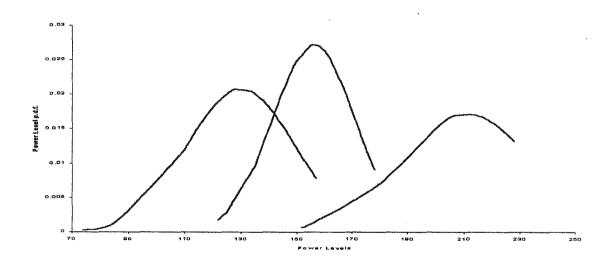


Figure 3.5. Probability density function of field collected power levels during leader calibration.

By using the p.d.f. plot attained from calibration data (Figure 3.5), triploid steelhead movement could be plotted and superimposed on the leader (Figure 3.6). Figure 3.6 illustrates some examples of triploid steelhead movement as determined from received power levels and compared with the calibrated p.d.f. shown in Figure 3.5. Illustrated are three movement scenarios with respect to the trap and leader. The fish with tag 99 was first received with a power level of 219. From this, it may be assumed that the triploid steelhead approached Hydrophone A from its baffled side. Following leader encounter, the triploid steelhead turned and swam away from the leader. The fish with tag 91 displayed contrasting behavior swimming along the length of the leader. However, owing to the trap design, it appears that the triploid steelhead followed the leader to the trap entrance, rounded the end of the leader at the trap entrance, and followed the leader again but on the other side, without ever entering the trap. The steelhead with tag 98 also followed the leader but this time was contained by the trap for a period to complete several trips around the inside of the trap. Again owing to the trap design, the triploid steelhead eventually found its way out of the trap and swam away.

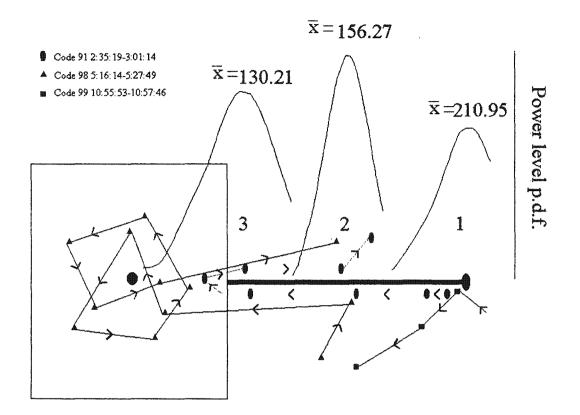


Figure 3.6. Triploid steelhead trout movement with respect to the recapture trap leader as determined from received power levels and leader superimposed probability density function.

## 3.3. System Considerations.

Previous methods employed to monitor the effectiveness of potential recapture traps have involved closing the trap and hauling it at set intervals to determine fishing success. This approach is labor intensive and does not provide a detailed description of fish behavior with respect to the trap and leader. Hauling of traps determines only the quantity of fish captured at discrete time intervals. Trap hauling at set intervals, may not determine optimal fishing duration as fish may be retained only for the period of time within these intervals (i.e. the trap may be hauled every 6 hours but the optimal fishing period may be 4 hours, therefore less fish will be apparent at the time of trap inspection). In addition, fish behavior will be disrupted by periodic trap inspection possibly biasing collected results. Use of telemetry as described can monitor the trap passively thereby eliminating problems of fish disturbance, allow complete observation of fish movement in the vicinity of the trap, and determine optimal fishing duration and when to haul the trap.

Using telemetry, I successfully monitored the movement of triploid steelhead in the vicinity of a potential recapture trap and leader. For simplicity, received power levels for only a few fish were plotted along the leader (and not considered to be away from the leader). However, for trap design purposes, a researcher should consider a received power level to originate from anywhere within the corresponding area described in Figure 3.3. Careful plotting of subsequent power levels will provide an accurate description of fish movement. It should be noted that the trap, lacking necessary reverse winkers for successful recapture, was not designed to capture fish but rather to field test telemetry for a recapture application. For this reason, fish were not retained for long periods of time following trap entry but free to swim within the trap and exit at will.

Future users of telemetry for recapture purposes should be aware of several critical considerations for optimal data collection and analysis. First, leader calibration allowing meaningful data analysis is of utmost importance. Leader calibration should be performed with every trap set-up to ensure collected data correspond to expected power levels. In addition, calibration may be performed using as many stations along and away from the leader, with the longest period of time considered feasible by the researcher. In the present study, the leader was calibrated at three stations for a minimum of five minutes at each station, which should be considered a minimum. Second, owing to the intrinsic environmental (depth, temperature and conductivity) and transmitter orientation effects on received signal strength, it should be expected that calibration will produce the most consistent values only in extremely stable environments. In Bay d'Espoir, both water conductivity and temperature are changing periodically possibly affecting received signal strengths, and resulting in a high degree of power level variation from monitored transmitters. Next, longer leaders may be more practical for telemetry use as this would result in less overlap of power levels between each calibration station. This is evident using a leader approximately 15-m long that demonstrated much overlap between middle calibration values and both ends of the leader observed (Figure 3.5). Finally, basic telemetry will provide only horizontal data along the leader. To acquire more complete data sets of fish movement, transmitters capable of measuring fish depth should be used to further augment collected horizontal data.

#### 3.4. Allowing Escapees to Remain in the Wild

Fish from aquaculture cages are appearing in the wild. Although recapturing escapees to remove them from the wild would mitigate potential interactions with wild fish stocks, several advantages to leaving escapees in the wild may also be argued. From my data, escapees left in the wild would remove excess feed falling through the cages thereby decreasing nutrient loading resultant from aquaculture practices. Escapees may provide additional economic gains associated with a farm lease site if fish farmers are allowed to charge recreational fishers for access to the site and angled escapees (comparable to fish out ponds). Finally, development of recapture technologies will require increased legislation on such topics as how long after escapement does the farm own the fish, for what duration should a farm be liable for the escapees, how far from the farm site should recapture be allowed, who owns recaptured escapees, and what are the implications of inadvertently capturing wild conspecifics? Increased legislation also will increase the liability and complexity to an already financially-challenged aquaculture industry.

### 4.0 Future Considerations

This research may be considered a model escapee tracking study with a primary objective to determine triploid steelhead movement within Bay d'Espoir, NF. Promising results have been collected from this initial study that help to address some of the environmental concerns associated with aquaculture. Implementation of recapture strategies may increase economic feasibility to aquaculture ventures. However, this study was conducted only on a single year-class of domestic triploid steelhead trout. Future escapee research is recommended to use similar telemetry methodologies while focusing on the following areas:

A. Other salmonid species/strains/size classes.

i. Domestic salmonid species and strains may behave differently when released in the wild.

ii. Diploid salmonids may become reproductively mature and therefore behave more aggressively with wild stock interactions during spawning season.

iii. Previous results have demonstrated directed movements to the hatchery water. However, in many instances hatcheries may be far from grow-out sites and the movement and behavior of these escapees may be different.

iv. Escapee imprinting and homing may be orchestrated through the addition of specific chemo-stimulants to hatchery water (i.e. imprinting to fish feed odor).

B. Capture wild/domestic salmonids during upstream migration to determine micro-scale interactions in the wild.

Now that some data have been collected for escaped triploid steelhead, it is evident that escapees may be capable of surviving on their own in the wild. Data are necessary from otoliths obtained from triploid steelhead captured in the wild to verify survival of escapees. In addition, many aspects relating domestication effects on salmonid behavior have to be studied to determine the full implications of escapees on the environment.

Finally, for a successful recapture strategy, a next step would be to determine optimal leader length, depth, twine color, and fishing duration to recapture escaped steelhead trout in the wild. Recapture research also should be carefully monitored to fine tune development of a model recapture strategy that is easily transferable to salmonid aquaculture industries globally.

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