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Acoustic Conditioning to Reduce the Impact of Escapement in Atlantic Salmon (Salmo Salar) Net Pen Aquaculture

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ACOUSTIC CONDITIONING TO REDUCE THE IMPACT OF ESCAPEMENT IN ATLANTIC SALMON (*SALMO SALAR*) NET PEN AQUACULTURE

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Abstract

Net pen culture is the most common method used to raise salmon, however these farms are typically located in areas through which wild salmon inhabit or migrate and escapes may influence wild stocks. Commercial salmon farms can potentially reduce both economic losses to the producer and impacts on wild stocks by recapturing escapees. Acoustic signaling has been used to concentrate fish species for feeding or harvest and to deter fish from specific areas. The present study investigated the approach of conditioning salmon to associate a tone with feeding to entice return to a specific location allowing recapture. The ability of juvenile and sub-adult Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* to detect various frequencies within their hearing range (below 380 Hz) was assessed. Subsequently, the fish were conditioned to associate a 250 Hz pure tone with feeding. Juvenile and sub-adult fish readily conditioned to acoustic signals (87 % in salmon and 97 % in trout) over a period of 4 – 7 days. Following initial conditioning the fish retained the training, regardless of the degree of reinforcement (exposure to a single tone every one, two or four weeks) for a 6 month period without a significant decrease (88 % in salmon and 97 % in trout). No significant differences were observed in either species in response to signal frequency (89 % in salmon and 96 % in trout) or intensity (91 % in salmon and 96 % in trout). Preliminary release and recapture trials conducted in Narragansett Bay to determine the feasibility of recovering fish did not result in return of fish.

Key Words: Atlantic salmon, rainbow trout, *Salmo salar*, *Oncorhynchus mykiss*, salmonids, acoustics, conditioning, recapture

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Preface

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Introduction

Commercial aquaculture of Atlantic salmon (*Salmo salar*; ATS) has increased markedly to meet a rapidly expanding demand. In 2004, worldwide annual production of farmed salmon reached 1.5 million metric tons (FAO, 2004). Currently, net pen culture is the most cost-effective method to raise salmon with some 94 percent of all adult ATS produced within commercial aquaculture facilities (Milewski, 2001). Unfortunately, escapement of salmon from net pens does occur due to husbandry practices and catastrophic events. Because many farms are located in areas through which wild salmon inhabit or migrate, the potential for escaped domestic salmon to impact wild stocks is high.

In the U.S., much attention has been focused on restoration of native ATS in Maine where eight rivers contain distinct populations listed as endangered (Federal Register, 2000). Recently, the Draft Atlantic Recovery Plan released by the National Marine Fisheries Service has proposed strategies for restoring these populations of salmon. In addition to reducing acid rain, restoring habitat, limiting water withdrawals and reducing predation, this plan seeks to minimize the mixing of wild and farmed fish. In Maine, the ATS aquaculture industry has over 750 net pens rearing approximately 10 million salmon in waters inhabited by endangered native stocks (NMFS, 2004).

Escapement can occur via storms, poor husbandry practices, theft, boat collisions, equipment failures, predator damage and vandalism. Published accounts likely provide a conservative estimation of the impact as not all escape events are reported and accurate quantification of escaped individuals is difficult. In Maine in 2000, a storm caused a cage to collapse releasing 100,000 sub-adult salmon averaging 2.3 kg (Whoriskey, 2001) and in Scotland some 600,000 salmon were reported to have escaped in February 2005 (IntraFish, 2005). Worldwide variations in legislation, enforcement and monetary penalties can deter farmers from reporting an event. Although precautions are taken to prevent escapes, no fail-safe strategies for net pens have been developed. Structural reinforcement of net pens might reduce losses from storm events but increases capital costs markedly and does not address escapes related to other factors such as handling errors. In the absence of 100 percent retention of cultured salmon, alternatives are needed to reduce potential environmental impacts.

The focus of the current study was to investigate the approach of reducing the impact of domestic salmon on wild stocks by conditioning salmonids to associate a particular acoustic tone with a reinforcing stimulus (feeding). Fish trained to respond to specific tones could be enticed back to the farm or a specific location allowing recapture. Acoustic signaling has been successful in concentrating fish for feeding or harvesting and in deterring a variety of species from specific areas including Atlantic cod (*Gadus morhua*; Ings and Schneider, 1997), Atlantic salmon (*Salmo salar*; Knudsen et al., 1994), tilapia, blue acara and petenia (*Oreochromis* *mossambicus, Aequidens pulcher* and *Caquetaia kraussii*; Levin and Levin, 1994), red sea bream (*Pagrus major*; Tateda et al., 1985), thick-lipped mullet and common carp (*Creimugil labrosus* and *Cyprinus carpio*; Wright and Eastcott, 1982) and rainbow trout (*Oncorhynchus mykiss*; Abbott, 1972). The timely retrieval of escaped salmonids could considerably reduce the potential effect on wild stocks, as well as the financial loss to the industry.

Materials and Methods

Environmental Parameters

ATS fry were obtained from North Attleboro National Fish Hatchery (North Attleboro, MA) and cultured in the University of Rhode Island Aquaculture Center at East Farm in Kingston, RI. Eyed rainbow trout (*Oncorhynchus mykiss*; RBT) eggs were obtained from Trout Lodge in Sumner, WA and also grown at East Farm.

Fish were maintained under a 12L:12D light regime and tanks were supplied with single pass freshwater ranging seasonally from 6 to 16 °C. The tanks were aerated using 15 cm diffusers to maintain levels of DO in the 6 to 10 mg/l range. Tanks were siphoned to remove solids as needed and scrubbed on a weekly basis.

Salmon and trout from 15 to 25 cm in length were selected from the general population and transferred to four 3.7 × 1.2 × 0.3 m freshwater raceways (1150 l) at East Farm. Forty fish were held in each of four raceways. A 25-cm water column was maintained in the raceways with a flow rate of 20 lpm. New groups of salmon and trout from 20 to 40 cm were transferred to one 3.7-m and two 1.5-m diameter cylindrical tanks receiving single pass seawater in the Blount Aquaculture Research Laboratory (BARL) at the Narragansett Bay Campus, Narragansett, RI. Seawater was filtered (20 μm) and heated or chilled to maintain a temperature range from 10 to 20 °C. During tone conditioning experiments groups of forty fish were transferred and held in the 3.7-m tank. Additional fish were held in the 1.5-m cylindrical tanks. During the pen acclimation experiment a group of ten salmon was transferred into the 3.7-m tank. Excess fish were transferred to one of the-1.5 m tanks. A water column of 68 cm was maintained in the 3.7-m (7200-l) tank and 76 cm in the 1.5-m (2000-l) tanks with a flow rate of 50 lpm in the 3.7-m tanks and 30 lpm in the 1.5-m tanks. A set of 0.9-m tall airlift pumps (consisting of 7.6-cm diameter PVC, each with a 90° elbow attached attached 66 cm from the bottom of the tank and a tee attached to the top) were used to circulate the water clockwise in the 3.7-m tank. Fish were added to the tanks to replace any mortalities.

Sound Generation

The acoustic signal was generated on a Dell Inspiron 1000 notebook computer (PC) using GoldWave Digital Audio Editor software (GW; GoldWave Inc., St. John's Newfoundland, Canada). Pure sine waves were programmed using the formula $\sin(2 * \pi * f * t)$ where f = frequency and t = time. A 20-cm underwater speaker (AQ339 Clark Synthesis, Littleton, CO) was centrally suspended (at 2.5 cm in raceways and at 24 cm in the 3.7 m tank) in the water column at a constant location and received signals from the 8-Ω output of a 75-watt amplifier (Peavey IPA 1502, Lubell Labs, Columbus, OH). The speaker was rated with lower and upper frequency limits of 10 Hz and 800 Hz. The front dial of the amplifier was set at 5 to provide a gain of up to 43 decibels depending on frequency and all decibels are reported in micropascals (μPa). The PC volume control remained constant at a level of 50 percent. Intensity was digitally altered by varying the amplitude (from 1 to 10) on GW. Duration of tones was regulated by pre-programming wave files set for specific time periods on GW. To avoid variation in sound pressure thresholds, acoustic signals were generated using this standardized method throughout the trials.

Determination of Signal Level (SL)

Acoustic signals were analyzed using an ITC 6083 hydrophone (International Transducer Corporation, Santa Barbara, CA) and quantified by determining the root mean square (rms) voltage (V_{rms}) from a selected section of the received signal. The source level (SL) in dB was determined using the following formulas:

(1) $(V_{rms}) = 0.707 * \text{peak}$

(2) $SPL = | M_x | - G + 20 * \log_{10} (V_{rms})$

SPL is sound pressure level, $| M_x |$ is hydrophone sensitivity and G is amplifier gain

(3) $TL = 20 * \log_{10} (r)$

TL is transmission loss where r is the range in meters between the hydrophone and source (i.e., 1).

(4) $SL = SPL - TL$

Calibration of the hydrophone was done by the University of New Hampshire Center for Ocean Engineering (UNH/COE).

Acoustic Conditioning

All fish were trained to feed at a constant location using floating feeding rings. During raceway trials 26.7 cm^2 square feeding rings were used and for the 3.7-m tank and Narragansett Bay trials the rings were 56 cm in diameter. Each tone was played for one minute, separated by five minute intervals and repeated in triplicate. Acoustic conditioning regimens were conducted at different times to avoid conditioning to time of day. To reduce the potential for fish to entrain on visual cues, movement of personnel in the vicinity of the tanks also occurred during non-feeding periods. Groups of 40 fish were transferred to raceways or cylindrical tanks and acclimated to the size, shape and color of the underwater speaker by placing a dummy speaker (yellow plastic circular sprinkler, a yellow plastic paint lid and three black wire wraps) into the tank prior to and during conditioning. A pure 250-Hz sine wave signal was generated, amplified and broadcast into the tank. Following acclimation to the yellow dummy speaker the tone was played daily and feed was presented for the duration of the tone (3 replicates) as a reinforcing stimulus. Upon demonstration of a positive response (by active swimming to the feeding ring in anticipation of feed) to the stimulus (tone) without a food reward, the fish were considered conditioned.

Retention of Conditioning

A second variable of conditioning investigated was the length of time fish retained training. Groups of 40 fish were entrained to the 250-Hz tone for 14d and then re-exposed without feed reinforcement for various intervals. Fish were exposed to a single acoustic signal at intervals of one, two or four weeks and the total number of fish responding (active swimming to feeding ring to the tone without a food reward was recorded. One group each of salmon and trout was employed for each regime. Trials lasted until the fish ceased responding or six months, whichever came first.

Light Conditioning

A 26,000 lumen underwater quartz halogen lamp (DropLite 1000 Watt, DeepSea Power and Light, Inc., San Diego, CA) was used to supplement the tone conditioning regime. The light served as a visual cue for orientation as sound localization in salmonids and other hearing generalists is not completely understood (Popper, 1998). In tank trials the light was partially shaded using an opaque PVC sheath which was removed for release trials in Narragansett Bay where both water turbidity and distance potential were greater. Fish were exposed to the light for the 1 minute duration of the tone during the standard 14-d conditioning period. Again, attraction (active swimming to feeding ring in anticipation of feed) to stimuli (tone and light) without a food reward was considered a conditioned response.

Acoustic Stimuli Discrimination

Once conditioned, the specificity of response to various frequencies was investigated. Two approaches (positive and negative reinforcement) were assessed to determine the ability of the fish to discriminate between tones. Groups of 40 fish were conditioned for 14 d to a specific tone and then observed for their response to a different frequency in the absence of a reward. Tones were played in triplicate and separated by five minute time intervals.

In the positive reinforcement trials fish were conditioned for 14 d at 250 Hz and then observed for a response to 100 Hz in the absence of feed. The number of fish responding during each tone was recorded. Data were collected daily for the duration of each experiment.

In the negative reinforcement trial fish were exposed to a 100-Hz tone in the absence of feed for a 14-day conditioning period. Feeding took place at a separate time point with no tone presentation. Subsequently, fish were exposed to a 300-Hz tone during feeding for a period of 5 days. Following conditioning the response to 100-Hz versus 300-Hz tones (3 replicates of each tone) was assessed.

Additionally, the responses to two different decibel levels (146 and 127 dB) were investigated using two groups (one salmon and one trout) of 40 fish. The fish were conditioned to the 250-Hz tone (3 replicates) at 146 dB for 14 days with feed and then exposed to the 250-Hz tone (3 replicates) at 127 dB.

Response in Tanks

The ability to attract salmon back to a small pen within the 3.7-m diameter tank was examined. Initially, 10 salmon was enclosed inside a 77-cm^3 mesh pen. Approximately 9 cm of the pen was above the water surface with four 15 × 78 cm sections of fine rigid mesh extending longitudinally, 66.0 cm to 81.3 cm from the bottom of the tank to retain floating feed (Fig. 1). Fish were conditioned to feed within the closed pen to the 250 Hz tone for 14 d and the number of fish feeding during the tone was recorded. After 14 d of feeding within the pen, a side panel was removed to allow movement in and out of the pen. Movement into the pen during the tone playback was used as an indicator that the fish were conditioned. Fish were observed using an underwater camera system (Aqua-Vu ZT-50, Nature Vision, Inc., Brainerd, MN).

Narragansett Bay Return

In subsequent investigations the pen was suspended from the Environmental Protection Agency Laboratory (EPA) dock on Narragansett Bay using a 6-m length of nylon rope. Fish were transferred into two 18.9-l buckets and anesthetized with a solution of tricaine methane sulfonate (75 mg/L; Argent Chemical Laboratories; Redmond, WA) at the Blount Laboratory and driven to the EPA dock. In the first three releases groups of 10 previously conditioned fish were placed inside the pen and allowed to acclimate for five days. Release number four did not utilize the pen; rather, conditioned fish were released directly into the open water. During feeding the pen was raised to allow the top surface of the pen to be exposed. The underwater speaker was suspended into the water column at a 48 cm depth. The acoustic conditioning tone and light were presented during feeding. After five days of active and repeated feeding with the tone the fish were released during incoming or slack tide. A feeding ring identical to the one used in tank studies was floated next to the pen and secured with monofilament to provide a visual cue. The number of fish returning or remaining in the designated feeding area immediately following the release was recorded. Residency of the fish in the area was observed using the underwater camera if permitted by turbidity conditions. Video output was recorded to the PC using a video capture system (VideOh! DVD AVC-2210, Adaptec, Inc., Milpitas, CA) and supporting software (Sonic MyDVD, Sonic Solutions, Novato, CA). The tone was presented daily until no fish returned.

Statistical Analysis

Initially, salmon and trout data were analyzed using X^2 (chi-squared) to test for independence with two degrees of freedom. Three groups each of salmon and trout were compared among three conditioning and three extinction regimes. Data for conditioning and extinction within the raceways were subjected to one-way analysis of variance (ANOVA). Tukey's post hoc test was performed to determine where differences occurred. Salmon and trout conditioning data within the single 3.7-m tank were analyzed separately using independent t-tests. Discrimination and return-potential data were also analyzed using independent t-tests. All data analysis was conducted on the program SPSS (LEAD Technologies, Charlotte, NC). Statistical significance was at the level of $P < 0.05$. Values reported in text and figures are expressed as mean percentage ± SEM unless otherwise noted. Transformations were not required because the n remained constant giving equal strength to the percentage data.

Hz (87 ± 1) tones in the absence of feed. Trout also responded equally to the 300-Hz (94 ± 2) and 100-Hz (93 ± 1) tones under negative reinforcement (Fig. 7). Exposure to varying dB levels indicated that the salmon were unable to distinguish over a range of 11 dB. The percent response for salmon and trout to decibel levels of 147 and 126 was 90 ± 1 and 92 ± 1 and 96 ± 1 and 97 ± 1, respectively (Fig. 8).

Response in Tanks

In the 3.7-m tank salmon were enclosed within the mesh pen and successfully conditioned to the tone. Upon release however, a limited number of fish returned (< 50 %). A second feeding ring was deployed in the tank adjacent to the pen to assess whether those fish refusing to swim into the pen (confined space) for feeding might return to an open structure. Feeding from either the ring or within the pen was considered a conditioned response. The response of 10 salmon held within the pen during conditioning was 76 ± 3 and did not differ from the response of fish that returned to the pen or feeding ring following release (77 ± 3, N = 3 replicates; Fig. 9). The average return response over a 15-d period upon release of a group of 40 fish was 76 ± 1 for salmon and 97 ± 0 for trout (N = 3 replicates) (Fig. 10).

Release

A series of four releases were conducted where groups of salmon or trout were transferred into a mesh pen suspended into Narragansett Bay (Table 5). The recapture of fish upon release was not attempted. The first release was conducted at higher than ideal water temperatures (18 °C) with 7 mortalities occurring prior to the release. The remaining 15 fish failed to return within 48 hours. The fish from release trial two suffered four mortalities thought to be cause by increased swell activity. In the third trial no mortalites occurred. Fish were observed to respond (active feeding) during transmission of the acoustic signal, although at a distance of 30 m from the feeding ring. The acoustic tone was played for a period of two hours and then discontinued when no further feeding activity was observed. The following day (24 h later) no fish were witnessed responding during the 40 minute observation and 35-40 back-to-back 1 minute duration acoustic signal broadcasts. Release four was conducted without acclimation time in the pen. Five trout were directly released into Narragansett Bay and no fish responded positively to the tones within the first two hours of release or after 24 h.

Discussion

Salmonids are hearing generalists or non-specialists capable of detecting sound but not with the acuity of species equipped with accessory hearing structures, such as goldfish. Both salmon and trout were rapidly conditioned to aggregate at a specific location in response to a 250-Hz tone of 134 dB associated with feeding. The associative learning of trout was consistently higher than salmon. Trout behavior was more aggressive and typically the fish completed feeding before the tone ended (1 minute). In contrast, salmon were less aggressive and required extra time to complete feeding on the ration. Salmon behavior was also altered by human presence and it became necessary to use opaque "hides" to keep salmon from being "spooked" during raceways trials. While feeding rings were utilized to control the movement of feed, aggressive behavior often caused displacement of feed outside the rings.

The findings of the current work are in agreement with previous research investigating the use of sound to control fish behavior. RBT have been conditioned to respond to a 150-Hz tone by swimming to a specific pond site in anticipation of feed (Abbott, 1972). The same fish responded equally when exposed to a 300-Hz tone but not a 600-Hz tone. The thick-lipped mullet (*Crenimugil labrosus*) and common carp (*Cyprinus carpio*) were successfully conditioned together to associate an acoustic signal of 150 Hz with food (Wright, 1982). In research to determine the ideal conditioning stage of red sea bream (*Pagrus major*) differences in responses were observed among size classes with dominant fish interfering with the movement and feeding success of smaller individuals (Tateda, 1985).

Audiograms demonstrate the diverse range of hearing thresholds observed among fishes ranging from infrasonic (< 35 Hz) to sonic (35 - 20,000 Hz) to ultrasonic (> 20,000 Hz) (Popper, 1998). Ultrasound (10 Hz in a pulse pattern) has been found to deter ATS smolts from hydroelectric power plant intake turbine pumps whereas 150 Hz had no repelling effect (the intake pumps likely masked the tone) (Knudsen, 1994). However, ATS breached acoustic barriers of 16 Hz when enticed by feed (Bullen, 2003).

In the present report neither ATS nor RBT demonstrated sensitivity to variations in tone frequency and intensity under positive or negative reinforcement within their hearing range. Certain tones produced by the sound generation system in the tanks and raceways were too weak or distorted and had to be eliminated from trials (including 50 Hz and 200 Hz). The usable range of signal intensity also was limited by the equipment from 109 to 150 dB (Baldwin, 2005). To realize the utility of acoustic conditioning it is necessary to understand how fish respond to sound and design protocols consistent with behavior. While not all frequencies within the range of hearing were examined in these trials, tone presentation of any frequency from 50 Hz to 400 Hz elicited a positive response after successful conditioning in both salmon and trout. Cardiac conditioning methods determined that ATS have a shift in threshold around frequencies within their hearing range due to masking by ambient noise (Hawkins, 1978). In contrast, cod are capable of discriminating between tones differing by as little as 36 Hz and also can detect direction within 10 to 20° (Schuijf, 1980).

Impressively, ATS and RBT were able to retain conditioning for up to seven months which is of potential utility to aquaculture operations. The lack of significant changes in response over a seven month period for all three conditioning retention regimes suggests a farmer could expose fish to tones less frequently with equal results. The domesticated nature of the trout likely explains the greater response when compared to feral salmon. Over seven months, there was no decline in response, which could have been a result of an all-or-none reaction or due to visual stimuli; neither of which could be eliminated from the aquaculture setting. Red sea bream were conditioned to a 200-Hz tone (20 - 30 dB) after two weeks in tanks but required two months to condition in field training to the identical acoustic tone. Upon release of 10,000 individuals, some 1,494 fish were recalled after a period of three months (Fujiya, 1980).

Salmon and trout both successfully responded to the secondary stimulus of light presented in the 3.7-m tank by swimming toward the light to obtain feed. In the present study the initial response of fish to the underwater light was a startle reaction, but eventually they did condition to the light in conjunction with the tone. Previous studies successfully conditioned salmon to be attracted to light for video observation (Lines, 1997) and to move through a tunnel by following light with reluctance to voluntarily swim towards darker water (Lekang, 1995). The lumen rating for the underwater light was much greater than in other studies attempting to attract or condition fish.

During the release into Narragansett Bay, an increase in activity was observed during acclimation of fish enclosed within the suspended pen in response to the tone prior to release. Release days were selected based on time of low tide and wind and weather forecasts (preference for calm water conditions and clear skies). The first release was conducted early in the summer with waters approaching the maximum tolerable temperature for salmon and resulted in mortalities during acclimation.

Upon release the fish moved out of view and due to high turbidity were not visible. During the second release trial, storms with high winds and swell activity resulted in mortalities during the acclimation periods preceding release (Table 5). Current speeds in Narragansett Bay can exceed 77 cm/s (Spaulding, 1990) and considering the previous exposure of fish to tank flow rates, the strong tidal movement likely caused stress and mortalities. During the third release on December 2, 2005, the water was dead calm (as even small ripple waves distorted the water surface enough to make observations of fish difficult). Fish were observed repeatedly breaching the water to obtain pellets during tone transmission although not adjacent to the feeding ring or pen. Fish were observed in two locations: near the shore in a cove or in the opposite direction toward deeper water. The tide/current altered directions during the tone application and transported feed to these two different areas. After the first hour of release the feeding stopped. Typically RBT will feed continuously within tanks but the larger than normal pellets used for easier visualization may have caused rapid satiation.

Controllable parameters (equipment and settings which had 70 to 90 % positive responses) were duplicated during the release experiments in Narragansett Bay. Temperature, pressure, salinity, air bubbles, organisms and other particles could all contribute to the attenuation of acoustics (Richards, 1998). Differences in tank and Narragansett Bay bottom composition were unavoidable and could have influenced tone propagation (Lurton, 2002). While sound travels in water at 1500 m/s the issues of absorption, reverberation and directional confusion arise as low frequency sounds propagate poorly at shallow depths because the wavelength is larger than the water depth. Therefore in shallow habitats fish likely detect low frequency sound only within extremely close range (Rogers, 1988). The 250-Hz tone utilized in the present study produces a 6-m wave and the depth of water at the release site at low tide was less than 6 m. To further complicate reception, underwater ambient noise levels are high arriving from both biological and mechanical sources and often mask tones (i.e. fish, outboard motors, pumps, or wave action). Boat engines produce 100- to 300-Hz tones and can have decibel levels from 142 to 176 (Urick, 1975). Ambient noise within aquaculture systems constructed of fiberglass, concrete and earthen ponds can produce decibel levels from 90 to 130 at frequencies less than 400 Hz (Bart, 2001).

Unfortunately, tracking of fish was outside the scope of the present project. The ability to track fish using acoustic tags such as HTI 795s micro acoustic tags and the HTI 291 acoustic tag tracking system (Hydroacoustic Technology, Inc., Seattle, WA, USA) would have provided valuable information on movement of the fish following release for up to 28 days. The vast space and diurnal tide cycle at the release site may have stimulated the fish to disperse in an effort to locate better conditions. Additionally, noise induced stress (100 to 10,000 Hz at levels of 110 to 170 dB) has been shown to instinctively trigger fish to disperse into a larger space and away from a tone in both hearing specialists and generalists (Smith, 2004).

Stimulation of the lateral line by nearfield (0.95-m) waves cannot be ruled out in the present study and may have been the primary stimuli in tanks. The lateral line detects particle motion produced by frequencies ranging from less than 1 Hz to several hundred hertz (Enger, 1993). Nearfield waves can attenuate rapidly affecting the distance in which the fish can detect the signal. It can also be argued that fish responding successfully within tanks are familiar with surroundings and part of their learned response includes repeated maneuvering. Once placed into unfamiliar territory (Narragansett Bay) the fish are at a disadvantage. Upon release, they are exposed to a new environment in which directional confusion may arise. However, in a previous study over half of the steelhead trout transplanted 1000 m from a commercial aquaculture site in Bay d'Espoir, Newfoundland returned within a few days without prior knowledge of the surroundings suggesting the feasibility of recapture. The fish were not acoustically conditioned and ability of the trout to locate the site was attributed to olfaction (Bridger, 2001). Fish were observed remaining in the area and even returned into pens (Bridger, 2002). Another study in the Bay of Fundy (subject to 300 cm/s tidal exchange) found that ATS released in large numbers did not remain in groups and routes taken were highly variable which would make recapture difficult (Whoriskey, 2004). In Newfoundland, salmonids remained near cages within the first few days of release and then moved into shallower water < 3 m (Brothers, 1999).

Although unlikely, the salmon and trout may have been non-responsive due to hair cell damage from long term exposure to tones at high intensities. Hearing generalists tend to exhibit less vulnerability to noise-induced hearing loss. Neither the bluegill sunfish (*Lepomis machrochirus*) nor tilapia (*Oreochromis niloticus*) exhibited significant threshold shifts when exposed to intense sounds (Scholik, 2002; Smith 2004). While effects of excess noise on fish are poorly understood, several hearing specialists including the goldfish (*Carassius auratus*), the zebrafish (*Danio rerio*) and the fathead minnow (*Pimephales promelas*) have had hearing damage or threshold shifts in response to anthropogenic sounds (Smith, 2004; Scholik, 2002). After exposure to intense tones for a prolonged period of time (within their hearing range) a temporary threshold shift occurred. Recovery to the previous threshold was reached within a matter of days from long term exposure in the hearing specialists.

Sustainable aquaculture is a balance between social, economic and environmental factors (Bridger, 2005). Among other challenges, Atlantic salmon producers must reduce the impact of escapees on wild stocks to achieve desired sustainability of commercial salmon culture and wild salmon populations. While the decrease in wild populations of most stocks of salmon cannot be definitively attributed to escapement from aquaculture facilities, counts of wild fish have declined while the number of escapees has increased (by percentage of total production the numbers of escapees have actually decreased (Bridger, 2002)). Suggested improvements including additional structural engineering to reinforce net pens, eliminating farms within range of natal rivers and monitoring systems to observe escapes are expensive and not fool-proof. Escapes also occur without warning due to husbandry errors and rogue storms to further complicate the issue. Escapees have been found in the immediate vicinity of, as well as great distances from farm sites making recovery more difficult (Whoriskey, 2004). Therefore, acoustic recall of fish concentrating individuals to one area would help alleviate impact potential.

Conclusions

The results of the present study indicate that ATS and RBT can be conditioned to associate a 250 Hz tone with feeding at a set location. Retention of conditioning continued for a period of seven months under each of the three exposure regimes. Neither ATS nor RBT exhibited the ability to discriminate between variation in tones by frequency and intensity. Both were reluctant to swim into a confined area suggesting trapping challenges.

Table 1. The percentage of (a) salmon and (b) trout in triplicate raceways responding to a 250-Hz conditioning tone on days 1 to 3 and 12 to 14 over a 14-day conditioning period. Positive responses were indicated by active swimming during tone transmission to feeding rings in the absence of a reward (feed). The overall average response from day 1 to day 14 was 79.9 % in salmon (increasing from 0 to 88.3) and 93.4 % in trout (increasing from 0 to 98.3).

(a)

	Day								
Tank	**0**	**1**	**2**	**3**	**Mean**	**12**	**13**	**14**	**Mean**
1	0	75.8	62.5	74.2	70.8	89.2	89.2	90.8	89.7
2	0	56.7	79.2	73.3	69.7	75.0	83.3	75.8	78.0
3	0	55.8	62.5	67.5	61.9	92.5	92.5	93.3	92.8
Mean					**67.5** ± 4.9				**86.8** ± 4.5

(b)

	Day								
Tank	**0**	**1**	**2**	**3**	**Mean**	**12**	**13**	**14**	**Mean**
1	0	85.0	89.2	88.3	87.5	93.3	95.8	98.3	95.8
2	0	80.8	80.8	94.2	85.3	93.3	99.2	98.3	96.9
3	0	76.7	78.3	75.8	76.9	97.5	97.5	96.7	97.2
Mean					**83.2** ± 3.2				**96.7** ± 0.4

Table 2. The percentage of (a) salmon and (b) trout in triplicate repetitions held within a single 3.7-m diameter tank responding to a 250-Hz conditioning tone on days 1 to 3 and 12 to 14 over a 14-day conditioning period. Positive responses were indicated by active swimming during tone transmission to feeding rings in the absence of a reward (feed). The overall mean response from day 1 to day 14 was 76.5 % (increasing from 0 to 84.2) in salmon and 92.6 % (increasing from 0 to 97.7) in trout.

(a)

	Day								
Rep	**0**	**1**	**2**	**3**	**Mean**	**12**	**13**	**14**	**Mean**
1	0	47.5	60.0	70.0	59.2	77.5	75.0	75.0	75.8
2	0	75.0	62.5	67.5	68.3	87.5	85.0	77.5	83.3
3	0	85.0	62.5	75.0	74.2	87.5	75.0	87.5	83.3
Mean					**67.2** ± 4.4				**80.8** ± 2.5

(b)

	Day								
Rep	**0**	**1**	**2**	**3**	**Mean**	**12**	**13**	**14**	**Mean**
1	0	77.5	87.5	87.5	84.2	95.0	90.0	95.0	93.3
2	0	87.5	93.0	90.0	90.2	95.0	98.0	98.0	97.0
3	0	95.0	93.0	98.0	95.3	95.0	98.0	100.0	97.7
Mean					**89.9** ± 3.2				**96.0** ± 1.4

Table 3. The response of (a) salmon and (b) trout acoustically conditioned for 14 days and subsequently exposed over a seven-month period to one of three regimes consisting of a single exposure to acoustic signal at intervals of one, two or four weeks without positive reinforcement. Values are the mean percentage of 40 fish responding to the signal at monthly interval

(a)

	Month						
	1	**2**	**3**	**4**	**5**	**6**	**7**
S1	91.7	90.8	88.3	86.7	91.7	87.5	90.8
S2	84.2	90.0	84.2	85.0	90.0	86.7	90.0
S4	87.5	87.5	89.2	88.3	90.8	86.7	89.2
Mean	**87.8**	**89.4**	**87.2**	**86.7**	**90.8**	**86.9**	**90.0**
SEM	± 2.2	± 1.0	± 1.5	± 1.0	± 0.5	± 0.3	± 0.5

(b)

	Month						
	1	**2**	**3**	**4**	**5**	**6**	**7**
T1	94.7	98.3	92.5	99.2	96.7	97.5	93.3
T2	97.5	95.8	98.3	96.7	95.8	98.3	96.7
T4	95.0	99.2	97.5	97.7	99.2	98.3	97.5
Mean	**95.7**	**97.8**	**96.1**	**97.8**	**97.2**	**98.1**	**95.8**
SEM	± 0.9	± 1.0	± 1.8	± 0.7	± 1.0	± 0.3	± 1.3

Table 4. Parameters utilized to examine discriminatory capabilities (frequency in hertz (Hz) and intensity in decibels (dB)) in salmon and trout within their hearing range. Reinforcement parameters are given in (a) and mean percent responses to each parameters for each species shown in (b). Each species was conditioned with three replicates by initial tone presented for 14d followed by the secondary tone at day 15.

(a)

Reinforcement Parameters		
Regime	**Initial Tone**	**Secondary Tone**
(+)	**250 Hz, 135 dB**	**100 Hz, 135 dB**
(-)	**100 Hz, 135 dB**	**300 Hz, 135 dB**
(dB Δ)	**250 Hz, 147 dB**	**250 Hz, 126 dB**

(b)

Positive Reinforcement (+)	**Salmon**	**Trout**
250 Hz	**89.8** ± 0.6	**98.1** ± 0.4
100 Hz	**89.3** ± 0.7	**97.3** ± 0.6
Negative Reinforcement (-)		
300 Hz	**89.1** ± 1.0	**93.8** ± 1.5
100 Hz	**87.3** ± 1.0	**93.2** ± 1.3
Sound Pressure Level Change (dB Δ)		
147 dB	**90.0** ± 0.8	**95.7** ± 0.6
126 dB	**92.1** ± 0.8	**96.6** ± 0.5

Table 5. Conditions and results for four separate releases of salmon or trout.

Release Date	Temp	Turbidity	Current	Morts	Fish	Returns
7/15/2005	18°C	High	Med	7	15 Salmon	No
11/23/2005	10.5°C	Med	Low	4	6 Trout	No
12/2/2005	10°C	Low	Low	0	9 Trout	?
01/24/2006	5.8°C	Low	Med	0	5 Trout	No

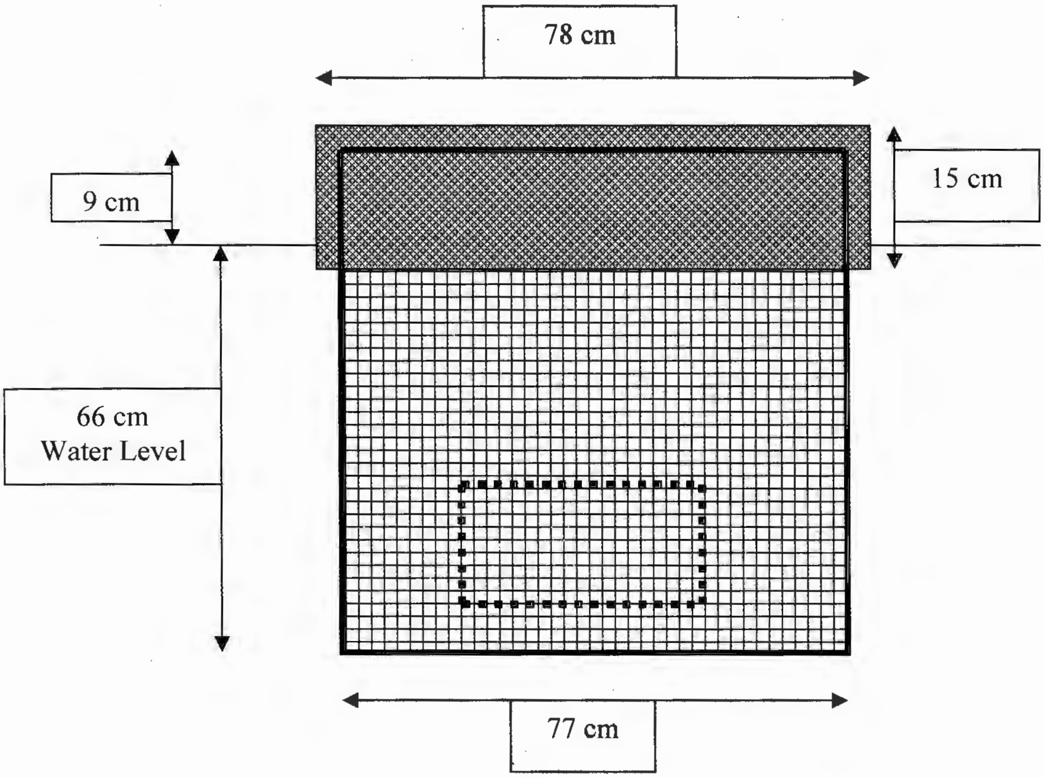


Figure 1. Schematic of 77-cm^3 mesh pen used to hold fish during tank trials and acclimation periods in Narragansett Bay. Rigid mesh lined the top of pen 66 cm from the tank bottom to retain floating feed overlapping with the water level of 68 cm in tank trials. Schematic is not to scale.

(a)

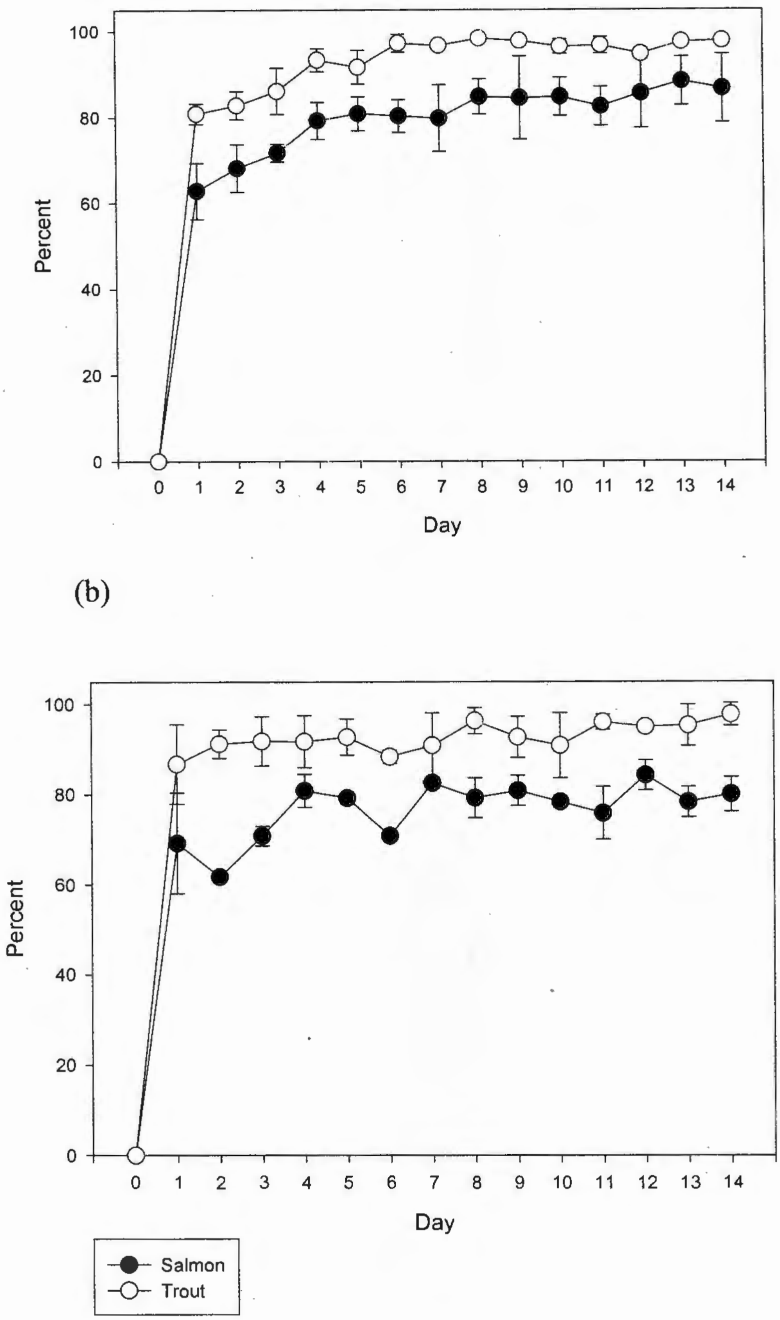
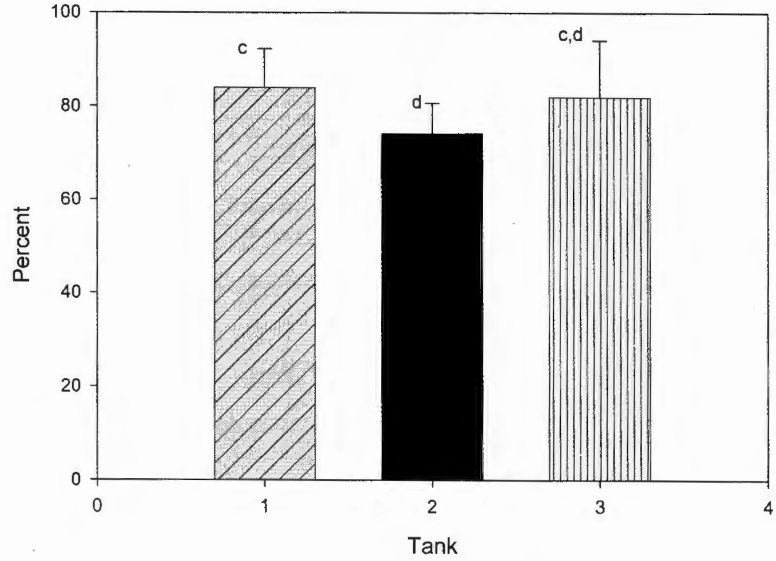


Figure 2. Percent of salmon and trout responding to a 250-Hz tone over a 14-d period in (a) freshwater raceways (triplicate repetitions in triplicate tanks) and (b) saltwater cylindrical tank (triplicate repetitions). Positive responses were indicated by active swimming during tone transmission to feeding rings in the absence of a reward (feed). N = 40. Values are expressed as percent mean ± SEM.

(a)



(b)

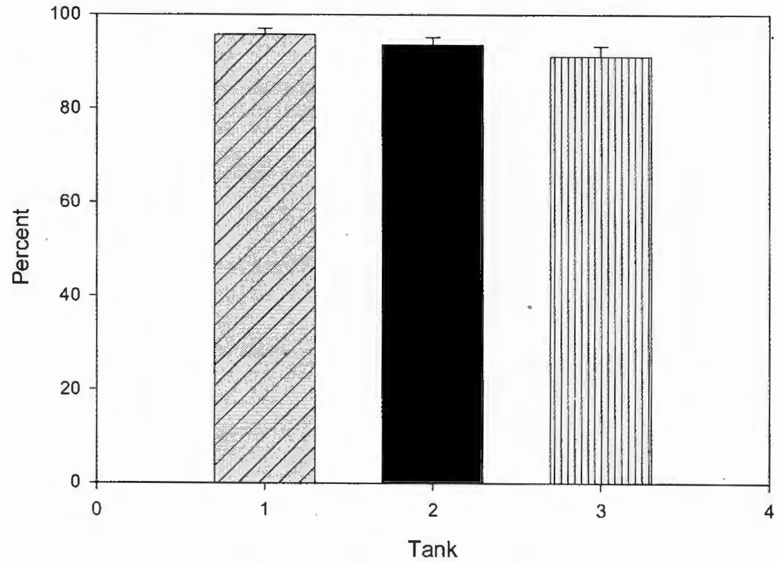


Figure 3. Average conditioning response in triplicate raceway tanks for (a) salmon and (b) trout over a 14-d conditioning period. Values are expressed as percent mean ± SEM. Significant differences in response are denoted by differing superscripts. No significant differences were found in trout.

(a)

(b)

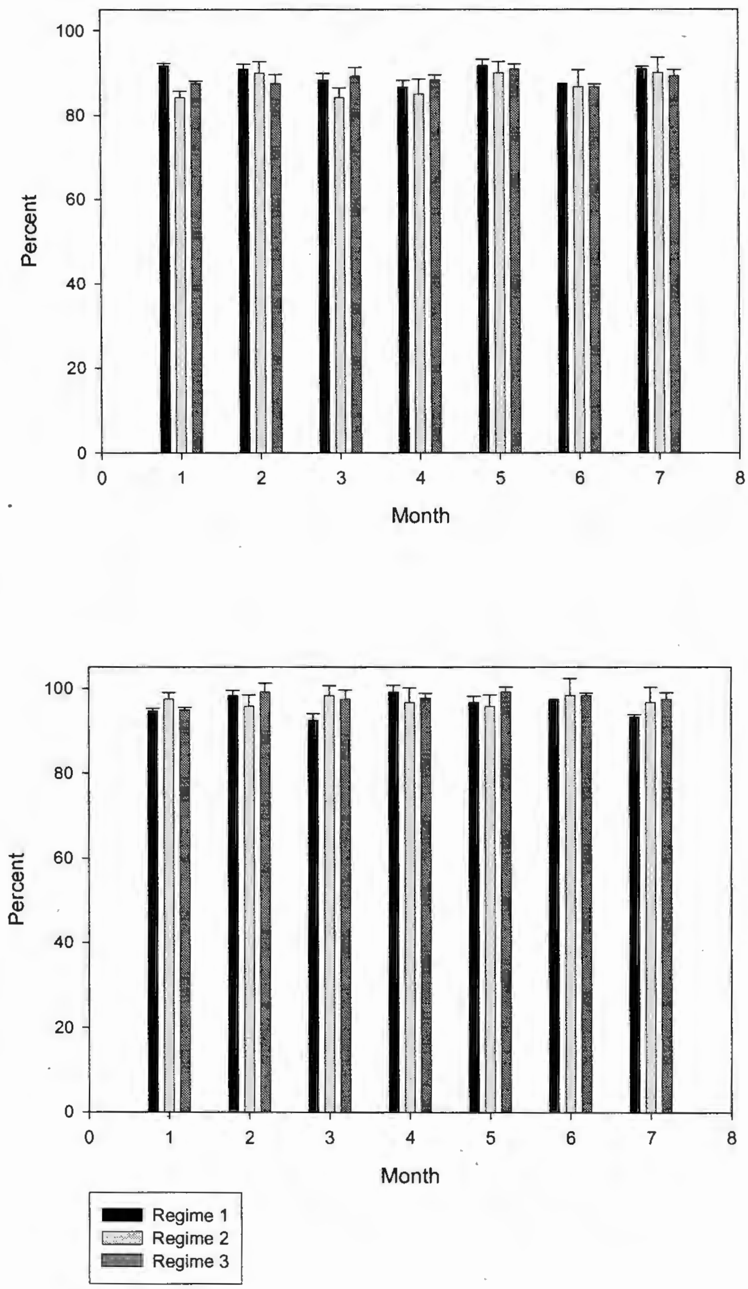


Figure 4. Retention response to conditioning in (a) salmon and (b) trout under three tone regimes (1 = 1 tone play per week, 2 = 1 tone play every 2 weeks and 3 = 1 tone play every 4 weeks) over a seven-month period. Values are expressed as percentage of positive responses (triplicate replications) ± SEM. There were no significant differences among regimes for each species. N = 40.

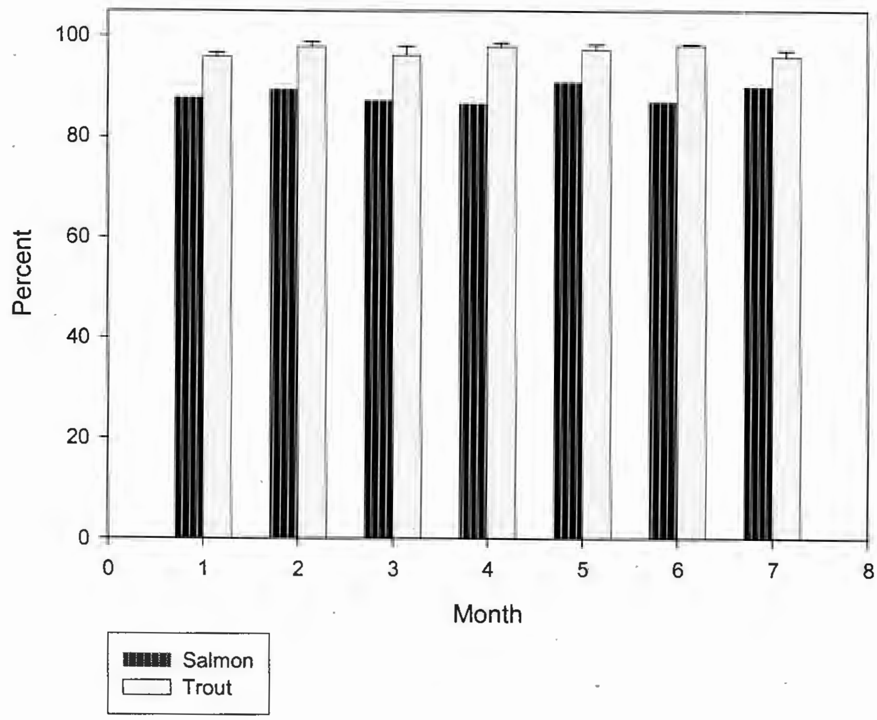
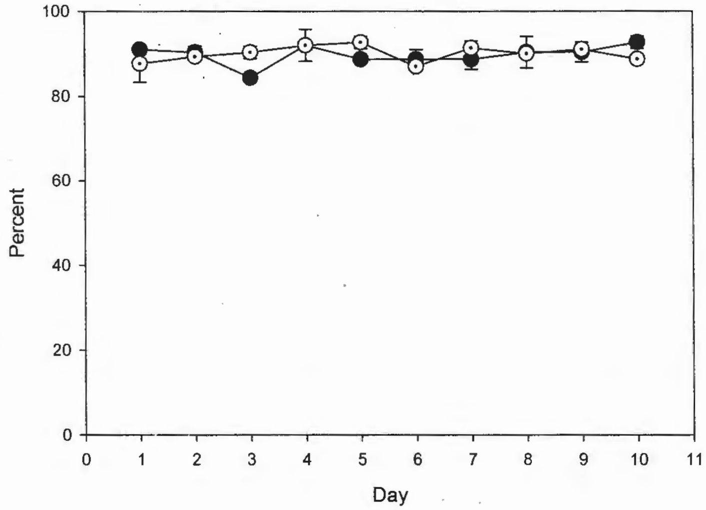


Figure 5. Retention means to conditioning for all three regimes responses (1 tone play per week, 1 tone play every 2 weeks and 1 tone play every 4 weeks) in salmon and trout during seven-month trial. Trout significantly outperformed salmon at each time point at the 250-Hz tone. Values are expressed in mean percent response for triplicate raceways ± SEM. N = 40.

(a)



(b)

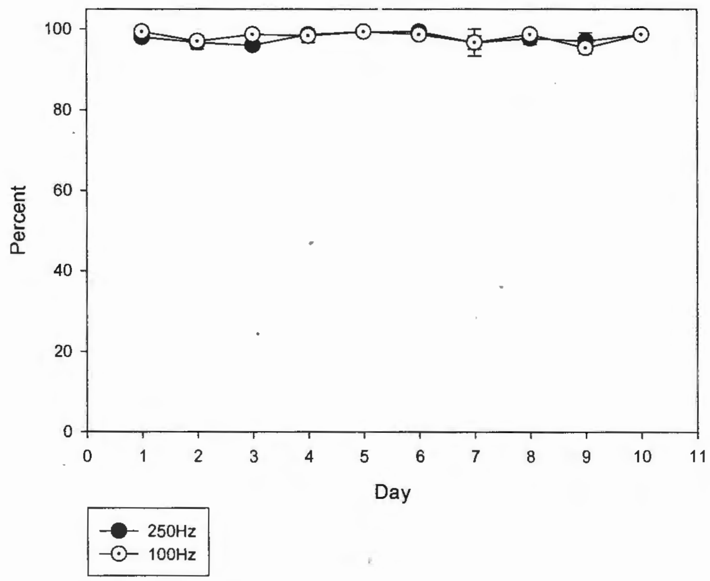


Figure 6. Percentage response to positive reinforcement in (a) salmon and (b) trout using 250 Hz initially as the conditioning tone and then replacing that tone with 100 Hz in the absence of feed to determine effect (N = 40). Values are expressed in mean percent response to two different tones in each species ± SEM. No significant differences were found between the tones in either species.

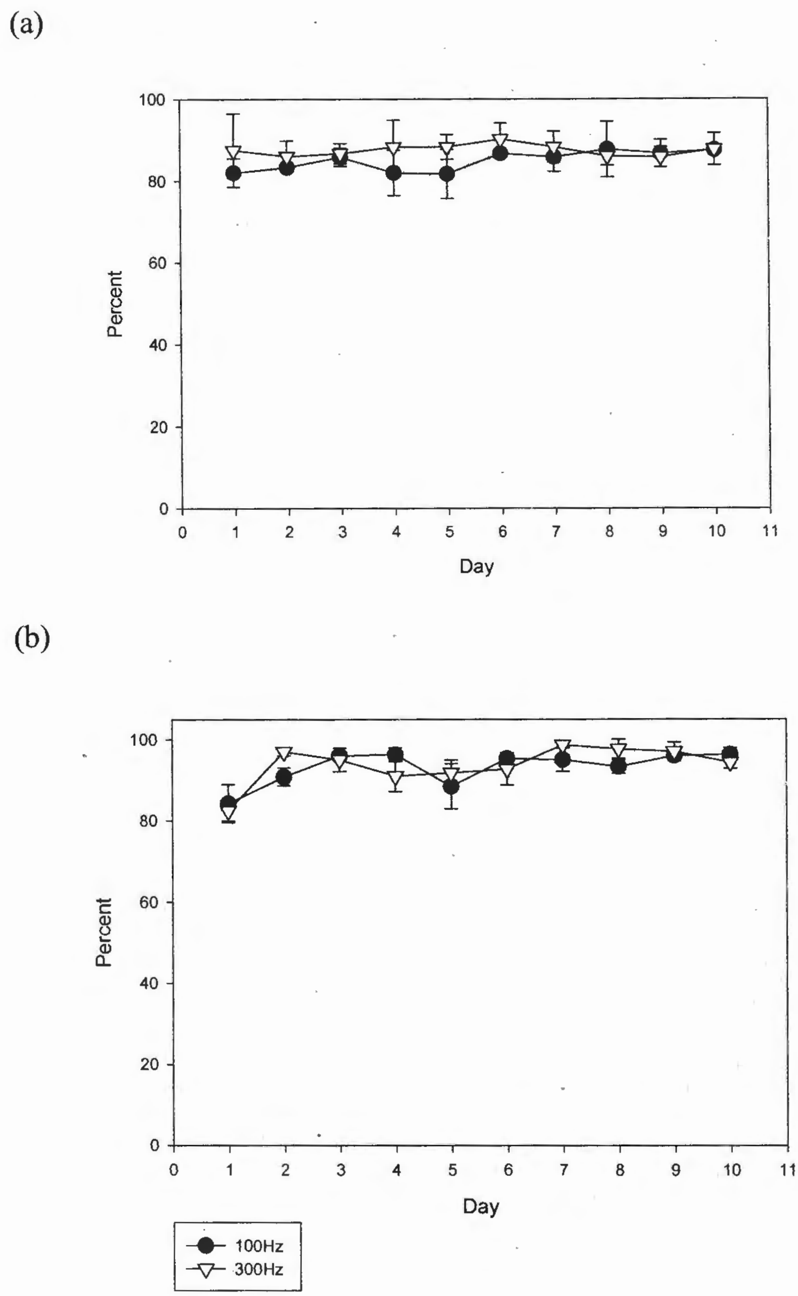
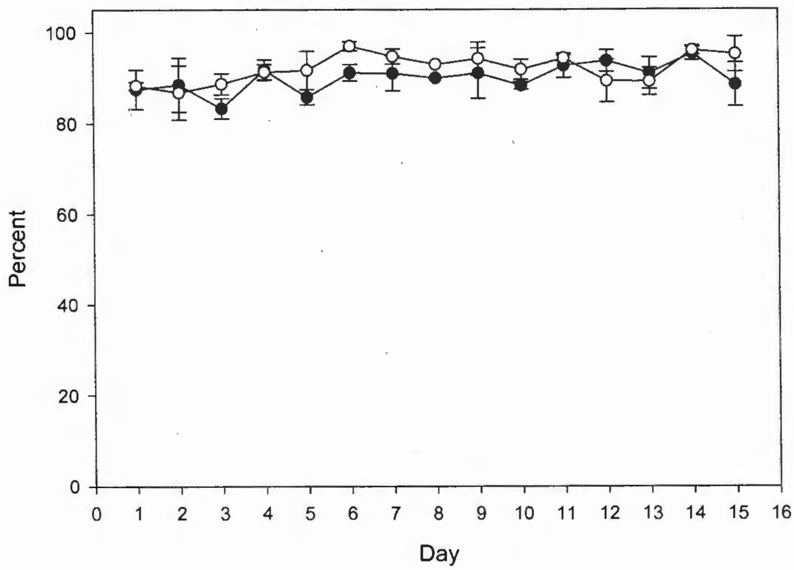


Figure 7. Percentage response to negative reinforcement in (a) salmon and (b) trout using 100 Hz initially as the conditioning tone and then replacing with 300 Hz in the absence of feed to determine effect (N = 40). Values are expressed in mean percent response to two different tones (triplicate replications) in each species ± SEM. No significant differences were found between the tones in either species.

(a)



(b)

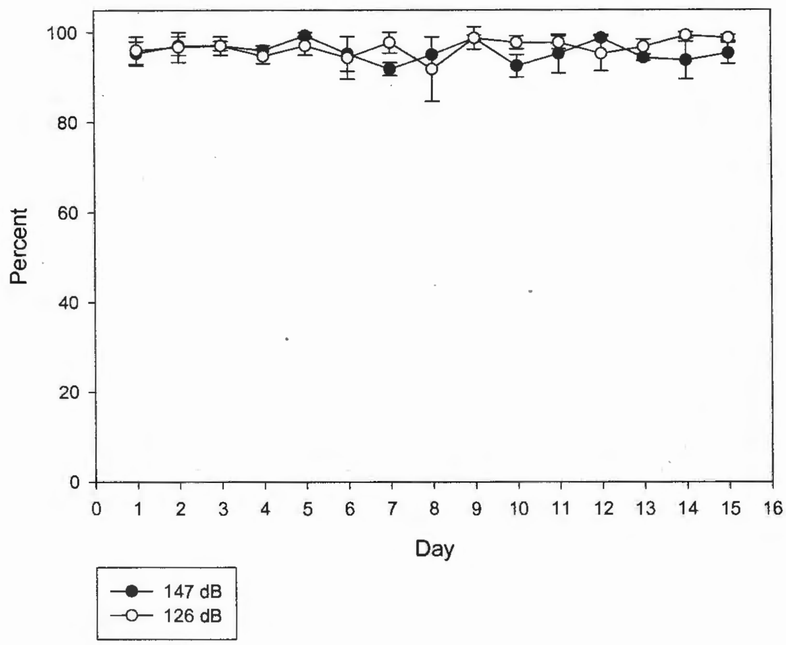


Figure 8. The percent response to intensity level change in (a) salmon and (b) trout using a 250-Hz tone at 147 decibels (dB) initially as conditioning tone and then replacing it with a 126-dB tone in the absence of feed to determine effect in raceways (N = 40). Values are expressed in mean percent response to two different decibel levels in each species ± SEM. No significant differences were found between the two different tones in either species.

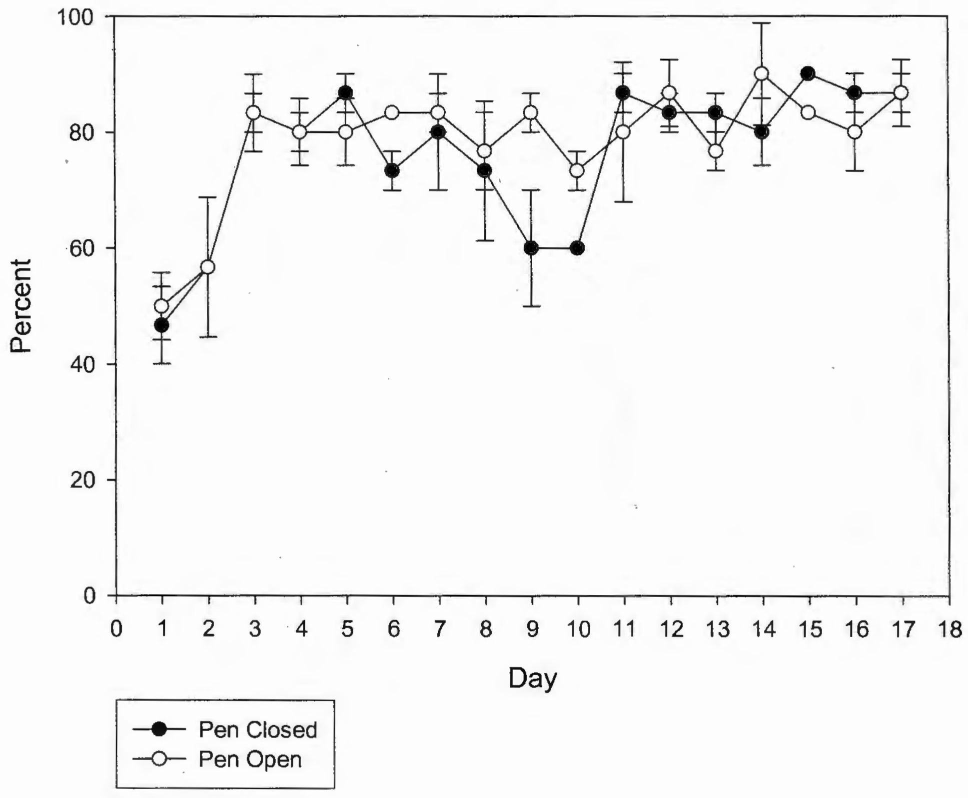


Figure 9. Percent response (active swimming to feeding ring) of conditioned salmon held within a pen compared to salmon after release from pen (N = 10). Values are expressed in mean percent response (triplicate replications in the cylindrical tank) ± SEM. No significant differences were found between two groups.

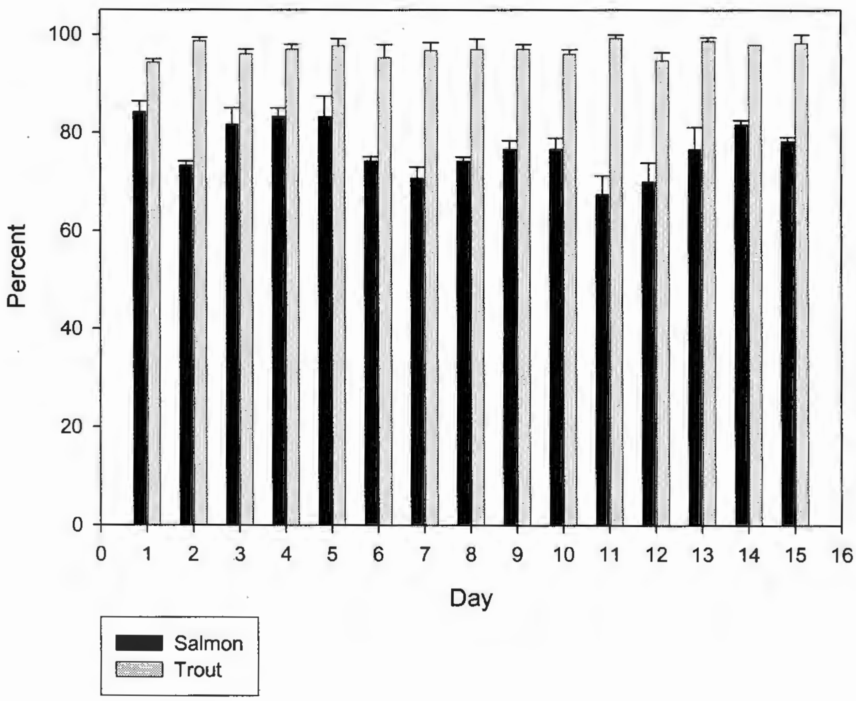


Figure 10. Comparison of the percent response of salmon and trout within the 3.7-m diameter tank over a 15-day period following conditioning (triplicate replications). A feeding ring was floating adjacent to the pen. Values are expressed in mean percent response to tones in each species for trials run on successive days from 1-15 ± SEM. N = 40.

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Appendix. Literature Review

Characteristics and Life Cycle

Atlantic salmon (ATS), *Salmo salar*, and rainbow trout (RBT), *Oncorhynchus mykiss*, both have fusiform body shapes with soft fin rays and adipose fins. They also have paired pelvic and pectoral fins and single dorsal, anal and caudal fins (Pennell, 1996). The ATS have silver dorsal (with black spots), silver side and white ventral coloration (Shearer, 1992). RBT have metallic blue coloration dorsally with black spots and are silver on sides. Being coldwater ectotherms, ATS prefer a temperature range from 10 to 17 °C while RBT can tolerate a wider range of temperatures up to 26.5 °C. ATS can grow to 27 kg and 122 cm and live up to 12 years. RBT can grow to 20 kg and 122 cm and live up to 8 years. ATS and RBT have both anadromous and freshwater forms. Anadromous RBT are referred to as steelhead trout (STT) and have a maximum temperature threshold of 23.9 °C (Pennell, 1996).

Olfactory and visual cues are utilized by salmonids to return to their natal river to spawn. Imprinting is done with a high degree of fidelity which in turn creates reproductively separate stocks or populations. The life cycle entails upstream migration to the natal freshwater for spawning and placement of eggs into redds for fertilization. Eyed eggs then hatch into alevins and emerge as fry. The next life stage is the parr phase which can extend from one to six years followed by the parr-smolt transformation to smolts or smoltification (biochemical, physical, morphological and behavioral changes) which enables seawater survival (Pennell, 1996). Smolts migrate to seawater at lengths ≥ 15 cm and spend one to four years before returning to spawning grounds as mature adults.

ATS and RBT both belong to the order Salmoniformes and the family Salmonidae. The historic distribution of wild ATS ranged from the Connecticut River to northern Canada across the Atlantic to Greenland, Iceland and northern Europe. RBT ranged from Mexico to the Bering Sea in Alaska and from eastern Asia to the Continental Divide (Laird, 1988). Although introductions have increased the distribution ranges of both species, major rivers have lost stocks due to industrial pollution and installments of weirs, locks and dams (Laird, 1988).

Culture

ATS and RBT are the most widely cultured of all salmonids and have been selectively bred since the 1830's with increased success (Pennell, 1996). The acceptable culture conditions are pH levels ranging from 6 to 8.8 and dissolved oxygen (DO) for salmon above 5ppm. RBT are capable of tolerating lower DO levels (Shearer, 1992). Un-ionized TAN levels exceeding 0.025 mg/l can be problematic (Laird, 1988). Salmonids are carnivores requiring a high percentage of protein in their diet. Rearing units can vary from ocean net pens to indoor recirculating systems depending upon stage of development and the location of the farm.

Salmonids are economically significant fishes in North America and create a challenge in terms of management (Lynch, 2002). With the growth of the industry and a flood of product, market prices have decreased forcing farmers to increase production efficiency. This has included attempting to increase profit margins by minimizing escapes. Escapees initially were not seen as a threat to wild populations because it was believed that without the natal river they would be lost at sea (Volpe, 2001). Intensive culture in both the hatchery and grow-out stages at locations adjacent to wild salmonid populations has led to farmed and wild ATS breeding proving prior assumptions incorrect. In some cases, salmonids have returned to aquaculture sites as opposed to swimming up wild salmon runs. In a release study, the return of transplanted fish indicated a homing response that could be attributed to an olfactory response to excess feed as ATS have been artificially imprinted to chemical additives (Bridger, 2001).

While ATS are capable of repeated spawns in the wild, only a small percentage actually does. In culture of ATS this allows for multiple spawning of broodstock (Laird, 1988). Broodstock are held at low densities and decreased temperatures. Eggs and milt are extracted and combined externally for fertilization. Eggs are then rinsed and placed into tray incubators. Upon emergence, the fry are reared in tanks until they reach the smolt stage and can be transferred to net pens for grow-out. Typically, the production of smolts takes over one year under traditional methods. However, manipulation of photoperiod and temperature have enabled zero age smolts and shortened overall production time (Pennell, 1996). Knowledge of degree days and environmental parameters along with selective breeding has maximized potential growth. The growth of salmonids can be projected by multiplying degree days (degrees above 0°C) by the constant species specific growth rate (with site specific restrictions).

Behavioral differences are apparent between domesticated and feral stocks of salmonids. Behavior of escapees can be influenced by genetic characteristics and effect predator avoidance, aggression and habitat selection (Bridger, 2002). Escapees may not have the knowledge required to successfully time spawning or migration, crucial to progeny output. Escapees may become more aggressive and display less schooling behavior to decrease competition (Bridger, 2002). A successful strategy for survival has been hypothesized where steelhead trout escapees move between aquaculture sites in the summer and spillways from hatcheries in the winter increasing the probability of recapture (Bridger, 2002). Foraging is poorly developed in hatchery fishes and starvation would lead to mortality (Brown, 2003).

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