WRAC Project Termination Report
Part I: Summary

PROJECT TITLE:	Physiological Changes Associated with Live Haul: Maintaining Healthy Fish
REPORT GIVEN IN YEAR	2010
PROJECT WORK PERIOD:	9/01/06 – 8/31/09; no-cost extension approved through 8/31/10
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REASON for TERMINATION:	Project completed

PROJECT OBJECTIVES:
The overall objective of this program was to improve the fish health and survival of transported fish. Sub-objectives for tilapia and rainbow trout included:

1. Documentation of current holding and hauling protocols and transport systems for long-haul conditions.
2. Evaluate the impact of hauling conditions on fish appearance, tissue quality, and overall customer acceptance.
3. Develop simple computer models to predict water quality as a function of time, density, and other important operational parameters.
4. Construct experimental transport systems to simulate long-haul conditions. Develop "standardized" hauling conditions and compare with observed data.
5. Evaluate the impacts of chemical addition, temperature modification on fish quality and post-haul mortalities using an experimental transport system.
6. Develop a mortality model to allow prediction of post-haul mortality based on the hauling conditions and quality of fish at the end of haul.

7. Develop hauling criteria and protocols. Make recommendations for modification of existing transport systems to industrial cooperators.
8. Develop outreach products that can be used by live-haulers to make informed decisions. This will consist of WRAC Extension Publications and a workshop.

PRINCIPAL ACCOMPLISHMENTS - TILAPIA:

(#1) Documentation of current holding and hauling protocols and transport systems for long-haul conditions.

Water quality information was collected in rearing raceways, hauling systems, and in retail holding systems for tilapia. Additional information was collected on the design and operation of hauling systems and retail holding systems.

Water quality at tilapia farms is typical of low temperature geothermal waters in Idaho. DO concentrations in the hauling tanks were in excess of 19 mg/L at the end of the haul. Over the duration of the haul, the pH dropped by 1.0 - 1.7 units. Carbon dioxide concentrations ranged from 40 to 80 mg/L. TAN concentrations ranged 17-29 mg/L but the un-ionized ammonia concentration ranged from 20 -70 µg/L.

There was a wide variation in the water quality parameters at the retail stores. The pH varied from 4.2 to 7.4 and the TAN ranged from 0.0 to 65 mg/L. Based on published environmental requirements, the most important parameters in retail holding systems are temperature, pH, and un-ionized ammonia. Dissolved oxygen and carbon dioxide were not limiting in retail holding.

(#2) Evaluate the impact of hauling conditions on fish appearance, tissue quality, and overall customer acceptance.

Physical Damage. A fluorescein dye method was used to assess physical injury incurred by tilapia during transport. Fluorescein analysis revealed puncture wounds and abrasions in all tilapia sampled directly from the raceway, before crowding. However, these fish were apparently crowded and moved the day before sampling. Fish sampled after crowding and loading into the truck showed a similar amount of puncture wounds, as well as, many more abrasions to the cranial and ventral sides of the body and eye injury. After arrival at Richmond, BC, tilapia sampled directly from the truck had similar bodily injury as pre-loading with more abrasions to the caudal and pectoral fins noted. Tilapia 24 h and 48 h post-holding at the retailers did not look much different after fluorescein exposure than the fish sampled at unloading. However, at this point, lesions and hemorrhaging on skin were apparent to the naked eye.

Comparison of (1) knotted net or (2) a smooth, large-mesh rubber net showed greater bodily injury from the use of knotted nets. Injury to the eye area was more common in the fish sampled in the knotted nets. Because of the serious observed damage from conventional loading techniques, an alternative loading system was evaluated. Tilapia (*Oreochromis mossambicus*) were sampled from Pacific Aqua Farms (Niland, CA) where they utilize a fish pump. Very little injury was found throughout our sampling at any stage of the loading process at this farm.

Gill histology. After an experimental haul from one farm in Idaho, high post-haul mortality occurred. Histological analysis of the gill tissue from these fish indicated some abnormalities such as fusion of the gill lamellae and hyperplasia of the respiratory epithelium; which likely resulted in severely restricted intake of oxygen.

To assess the potential impact of gill damage on transport mortality, gill samples were collected from five tilapia farms in Idaho. No differences were observed between the different farms and overall all the gills appeared healthy. Of the 20 fish sampled, only three had signs of hyperplasia of the respiratory epithelium and the three fish were each from a different farm. All 20 fish showed signs of mild chronic inflammation in the gills. However, this level of inflammation can be considered background inflammation that is not indicative of diseased fish. Additional sampling and research will be needed to document the development and impacts of gill damages on transported tilapia.

(#3) Develop simple computer models to predict water.

A simulation program was developed to improve our understanding of the water quality changes that occur during hauling. This program estimates oxygen consumption, carbon dioxide excretion, and ammonia excretion as a function of temperature and activity. The program does predict the rapid decrease in pH at the start of hauling, followed by an increase in pH over the haul, as was observed in the experimental hauls from Idaho. The accuracy of this model strongly depends on the values of metabolic production factors used and the specific geometry of the hauling tanks and LOX distribution system.

(#4) Construct experimental transport systems to simulate long-haul conditions.

Because of the difficulty of riding with commercial drivers and the distances involved, it was necessary to build a number of experimental systems for hauling work: portable hauling system and prototype transport system. Two systems were developed to evaluate the use of video systems in determining fish distribution and behavior within hauling tanks. The video systems work well for small fish at low densities, but are unable to determine fish distribution at production hauling densities because of overlapping fish and increased turbidity.

(#5) Evaluate the impacts of chemical addition, temperature modification on fish quality and post-haul mortalities using an experimental transport system.

Tilapia are typically hauled in a simple salt (sodium chloride) solution of approximately 2-5 g/kg, depending on the individual hauler. An "isotonic" hauling solution was developed using an EXCEL spreadsheet. Two trials were conducted to compare pretreatment with 30 ppt Instant Ocean for 1 minute before loading into transport tanks. In the first haul, the pre-haul salt treatment significantly reduced post-haul mortality. In the second haul from a different farm, there was no beneficial effect of the salt dips, but many of the fish arrived in a moribund condition. The overall pre-haul quality of the fish used for this experiment might have played a role in driving the mortality observed, and thus been a factor in affecting our ability to discern treatment effects.

(#6) Develop a mortality model and hauling constant.

Based on published literature, a "safe" hauling density depends on length (or weight) of the fish, duration of the haul, and temperature:

$$\text{Density(g/kg)} = K_{\text{species}}\left[\frac{\text{Fish Length}^{a}}{\text{Time}^{b} \times \text{Temperature}^{c}}\right] \qquad (1)$$

Higher densities can be used for shorter times, lower temperatures, and larger fish. For this equation to be dimensionally homogeneous, the right side of the equation must have the same dimensions. The Buckingham π theorem provides a method for computing these dimensionless parameters (a, b, & c) from the given variables. Work is ongoing to define the dimensionless groups that are important in hauling. When this is complete, experimental verification of the hauling constant approach will be conducted with tilapia.

(#7) Develop hauling criteria and protocols.

Formal recommendation for hauling criteria and hauling protocols will be developed in the outreach products and will include recommendations for the following major items:

- Operation of pure oxygen system,
- Transition from the distribution tanks to the retail tanks,
- Crowding and netting techniques,
- Operation of retail holding systems
- Pre-haul fasting,
- Pre-haul salt treatment, and

- The ability of the fish to cope with haul-related stressors.

(#8) Develop outreach products.

The following outreach products will be prepared:

1. A workshops in Idaho to present project results to commercial haulers,
2. Two WRAC Extension Publications, and
3. User-friendly spreadsheet models (fish stress and mortality) will be available for downloading from the WRAC web site.

PRINCIPAL ACCOMPLISHMENTS – RAINBOW TROUT:

This section presents work that was done with rainbow trout. Objectives 3, 4, 6, & 8 for tilapia apply to both tilapia and rainbow trout.

(#2) Evaluate the impact of hauling conditions on fish appearance, tissue quality, and overall customer acceptance.

Increased Density. Trout of mixed diploid/triploid stock from the Oregon Hatchery Research Center (OHRC) in Alsea, OR were hauled to Oregon State University Fish Performance and Genetics Laboratory (OSU) in Corvallis, OR at varying densities: 1x (1lb/gal), 2x (2 lb/gal), and 3x (3lb/gal). The fish were catchable-sized rainbow loaded onto the truck following typical raceway crowding, netting, weighting in a basket and stocking into respective tanks. The haul from OHRC to OSU was approximately 1 hour, and we then left the fish in the haul tanks for another 16.5 hours. Two receiving water quality conditions were tested:

"Good"	water temperature = 13.5°C and DO = 9.5 mg/L
"Bad"	water temperature = 20°C and DO = 6.0 mg/L

No mortality was observed in the 1X or 3x treatment, immediately following the haul. All fish from the 3x treatment placed in the "Good" holding condition survived, however, mortality was observed in the 3x treatment placed in the "Bad" holding condition. This suggests that 3x (3lb/gal) is an achievable hauling density and survival is dependent on the receiving conditions. Additionally, we are interested in determining whether additives to the haul medium can further increase the densities at which fish can be transported.

Bodily Injury During Crowding and Loading. Bodily injury was assessed using the fluorescein technique. Overall, very little bodily injury was detected. The only real noteworthy injury was in three out of the five fish sampled from the 3x haul treatment that had eye injury. No fish transported at 1x and only one from the 2x treatment had eye injury. This increased eye injury may be a consequence of the increased density in the 3x treatment.

(#7) Developed hauling criteria and protocols.

Formal recommendation for hauling criteria and hauling protocols were developed in the outreach products and included recommendations in the following major items:

- Rainbow trout can be transported at 2 and 3 lb/gallon with reasonable mortality,
- Loading protocols and diffuser layout may be more important at higher density,
- Little bodily injury was detected with the fluorescein technique,
- Eye injury may be more prevalent at 3 lb/gallon and require smoother interior surfaces, and
- Chemical additions may be useful at higher densities.

Other Accomplishments:

The characteristics of oxygen flow through ceramic fine bubble diffusers depends strongly on the type of diffuser and prior history of its use. This observation was examined and the results disseminated.

Basically, the mass transfer rates and gas flow rates are much lower when the diffuser is saturated with water before the gas flow starts. This means that this type of fine bubble diffusers performance can change drastically depending upon whether the gas flow was temporarily interrupted such as by changing gas supplies or other actions or by wetting the diffusers before turning on the gas flow.

IMPACTS:

Relevance: In certain markets, live fish can be sold for substantially higher prices than fresh dressed fish. A significant live-haul industry has developed in the U.S. and fish are commonly hauled 1,500-2,000 miles (25-30 hours) to market. Increase feed and transportation costs have reduced profits in this industry. Because of the economic importance of the live fish market, improved systems and protocols are needed to allow this industry to expand and prosper. One major constraint to improving overall fish health in transport systems is that very little information has been published on the chemical and physical conditions in transport systems during long-distance transport and this limited data may not be representative of current commercial systems.

Response: Water quality data was collected from the farm, hauling tanks, and retail stores. Physical damage to the fish from crowding and loading was documented using a fluorescein dye technique. The characteristics of oxygen flow through ceramic plate and carbon diffusers were studied. This research was directed toward hauler and retail store owners. Based on this work, changes in the following tasks or processes were recommended to minimize physical and physiological damage to transported fish:

- The use of knotted nets should be avoided.
- The use of a fish pump may result in less physical damage to tilapia than conventional loading techniques.
- In retail holding systems, potential water quality problems include suboptimal water temperature, high un-ionized ammonia concentrations, and high gas supersaturation levels.
- The transfer of fish from the distribution tanks to retail holding is a very stressful event that may result in serious physiological and endocrinological impacts.
- Rainbow trout can be transported at 2 and 3 lb/gallon with reasonable mortality.
- The characteristics of oxygen flow through fine bubble diffusers depends strongly on the type of diffuser and prior history of its use.

Results: The impact of this project will be increased survival and product quality of transported fish as a result of adopting the recommended protocols and utilization of the models. A survey comparing pre and post project live-hauling success among the targeted audience using the recommended protocols should provide the necessary information to evaluate project impact. The survey may either be conducted by phone or mail, and will take place one to two years after project completion to allow for protocol adoption.

While the main impacts of this work will occur after the outreach workshops and publications have been completed, the following changes have occurred:

- Following a presentation of our results to participating haulers in Idaho, an open dialog between haulers was established. After this meeting, one hauler reduced his hauling density.
- Based on documentation of net damage, another hauler replaced all their harvest basket netting material to softer knotless mesh to reduce physical damage.

Collaborators: Significant roles were played by fish farmers, distributors, and manufacturers: Leo Ray, Fish Breeders of Idaho; Mark Lupher, Epicenter Enterprises; John Lambregts, Falls Services; Brian Tadlock, Pristine Springs; Bob Williams; Blundell Seafoods Ltd.; Jim Parson, Troutlodge; Ken Beer, The Fisheries; Mark Francis, Aquaneering, Inc.

Contact: John Colt, 2725 Montlake Blvd. East, Seattle, WA 98112, john.colt@noaa.gov; Carl Schreck, Oregon State University, Department of Fish and Wildlife, 104 Nash Hall, Corvallis, OR 97331, carl.schreck@oregonstate.edu; Gary Fornshell, University of Idaho Extension, Twin Falls County, 246 3rd Avenue East, Twin Falls, ID 83301, gafornsh@uidaho.edu.

RECOMMENDED FOLLOW-UP ACTIVITIES:

The impact of hauling on tilapia was found to depend strongly on the origin of the fish. Additional work is needed to define the nutritional, physiological, and disease status of commercially reared tilapia and the impacts of these parameters on survival and product quality of transported fish.

Rainbow trout can be transported at 2-3 times normal density with acceptable mortality. The use of chemical additives to increase hauling density needs to be documented. This might include: buffers, ammonia lock, and anaesthetics.

SUPPORT:

YEAR	WRAC-USDA Funding	OTHER SUPPORT					Total Support
		University	Industry	Other Federal	Other	Total	
2006	$89,456	$3,891[a]		$37,500[b]		$41,391	$130,847
2007	$81,856	$3,500[a]		$30,000[b]		$33,500	$115,356
2008	$86,299	$4,000[a]		$20,000[b]		$24,000	$110,299
2009	$96,789	$4,000[a]		$30,000[b]		$34,000	$130,789
2010	$0			$35,000[b]		$35,000	$35,000
TOTAL	$352,100	15,391		117,500		$132,891	$487,291

[a]Salary for Carl Schreck, OSU/USGS; [b]Salary for John Colt and Eric Kroeger, NOAA

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

Publications in Print and Manuscripts:

National Marine Fisheries Service, Oregon State University, University of Idaho. 2006. Working Paper No. 1: Environmental Requirements of Nile Tilapia (*Oreochromis niloticus*).

National Marine Fisheries Service, Oregon State University, University of Idaho. 2006. Working Paper No. 2: Water Quality in Farm, Hauling, and Retail Holding Systems.

Colt, J. 2008. Water quality model for long-haul transport of fish. Version 1.0.3, Absoft FORTRAN 99, 21 pages.

Colt, J. 2009. Draft recommendations for the computation of hauling mixtures, Excel spreadsheet, 2 pages.

Colt, J. Schuur, A., Cryer, E. Miles, T. 2009. Modeling of multiple stocks and programs for master planning and feasibility studies. Aquacultural Engineering. 41, 176-187.

Colt, J., Watten, B., Rust, M. 2009. Modeling carbon dioxide, pH, and un-ionized ammonia relationships in serial reuse systems. Aquacultural Engineering 40, 28-44.

Colt, J. 2010. Computation of Dissolved Gas Concentration in Water as Functions of Temperature, Salinity and Pressure, Second Edition, Draft, 325 pages.

Colt, J., Kroeger, E., Rust, M.2010. Characteristics of oxygen flow through fine bubble diffusers used in aquaculture hauling applications. Aquacultural Engineering. In press, 9 pages, doi:10.1016/j.aquaeng.2010.06.001.

Colt, J., Watten, B., Pfeiffer, T. 2010. Carbon Dioxide Stripping in Aquaculture –Terminology, Reporting, and Modeling. Accepted for publication, September 6, 2010, Aquacultural Engineering.

Colt, J., Momoda, T., Chitwood, R., Fornshell, G., Schreck, C. 2010. Water quality in tilapia transport: from the farm to the retail store. Internal NOAA review, To be submitted to Aquaculture, 44 pages.

Colt, J. Impact of alkalinity and respiratory carbon dioxide on the pH, un-ionized ammonia, and free carbon dioxide in fish transport systems. In Preparation.

Momoda, T. Chitwood, R., Colt, J., Schreck, C.B. Effects of Stress and Injury Associated with Transporting Live Tilapia. In Preparation.

Papers presented:

Chitwood, R., Feist, G., Momoda T.S., Schreck, C.B and Colt, J. 2007. Stress effects of transporting tilapia to the live fish market and recommendations to enhance health and survival. World Aquaculture Society. San Antonio, Texas.

Chitwood, R., Momoda T.S., Feist, G., Colt, J., and Schreck, C.B. Mortality and effects associated with stress and handling in transporting live tilapia. 2008. Idaho Aquaculture Association. Twin Falls, Idaho, June 21, 2008.

Colt, J., Chitwood, R., Momoda, T., Feist, G., Schreck, C. 2008. Water quality in retail tilapia holding systems. Aquaculture American 2008, Orlando, Florida, February 9-12, 2008.

Colt, J., Rust, M. 2008. Modeling of water quality in warmwater transport systems. Aquaculture American 2008, Orlando, Florida, February 9-12, 2008.

Momoda, T., Chitwood, R., Feist, G., Colt, J, and Schreck, C. 2008. Stress and injury associated with transporting tilapia to the live fish market affects pathology-related survival. Aquaculture American 2008, Orlando, Florida, February 9-12, 2008.

Schreck, C., Momoda, T., Chitwood, R., Feist, G., M. Kent, R. Holt, and Colt, J. 2008. "Effects of injury and stress associated with transport of live tilapia: Do they inoculate each other with pathogens?" International Congress on the Biology of Fish. Portland, Oregon, July 2008.

Colt, J., Watten, B., Rust, M. 2009. Impact of attached algae, suspended bacteria, and re-aeration on oxygen and carbon dioxide balances in series raceways. Aquaculture America 2009, Seattle, Washington, February 15-18, 2009.

Colt, J, Kroeger, E., Rust, M. 2010. Impacts of alkalinity and carbon removal on the mortality and product quality of tilapia. Annual meeting, World Aquaculture Society, San Diego, California, March 1-5, 2010.

Colt, J. Ray, L. 2010. Hauling channel catfish fingerling from Arkansas to Idaho: an adventure with Leo Ray. Annual meeting, World Aquaculture Society, San Diego, California, March 1-5, 2010.

Kroeger, E., Colt, J, Rust, M. 2010. Fine bubble diffusers: impacts of pressure, water, and handling on mass flowrates and transfer efficiency. Annual meeting, World Aquaculture Society, San Diego, California, March 1-5, 2010.

Schreck, C., Momoda, T. 2010 Effects of stress and injury associated with transporting live tilapia. Annual meeting, World Aquaculture Society, San Diego, California, March 1-5, 2010.

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SUBMITTED BY: **9/10/10**

Title: (Work Group Chair or PI) Date

Dallas Weaver

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APPROVED: Date 9/14/10