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Oxygen consumption of snow-trout (Schizothorax plagiostomus) and Red Zebra (Metriaclima estherae) during simulated transport in a closed system

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ABSTRACT

In the struggle for poverty alleviation in developing countries, the use of aquaculture could contribute to establish increased economic income and provide food security. The transportation of fish from brood stock hatchery to buyer is a severe element with the risk of threatening fish health and survival. For fish and seller/buyer's best interest, solutions to improve fish health should be a priority. In rural areas, this is further complicated by low availability to advanced transport systems.

In this study, simulated transport of the Himalayan snow-trout carp and the Lake Malawian Red Zebra cichlid in closed containers and the effect of fish strain, stocking density, air phase and the use of an anesthetic (MS-222) on oxygen consumption and possible prolonging of transport time was investigated. The impact of a sedative treatment on blood chemistry was further documented. Experiments on simulated transport of snow-trout fingerlings showed a strain related stocking density dependent increase in weight specific oxygen consumption. Improving oxygen availability by substituting 2/5ths of the transport container volume with an air phase resulted in increased oxygen consumption and reduced possible transport time compared to when performing transport with totally water filled container. Exposing fish to a sedative treatment during transport had the ability to reduce oxygen consumption and therefore increase possible transport time. However, subjecting fish to a sedative MS-222 treatment resulted in changes in plasma sodium ion, glucose and lactate concentrations, indicating possible hypoxic conditions to the fish.

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INTRODUCTION

World poverty and food security

The World Bank Reports has estimated that almost one billion people will be living under extreme poverty (< 1.25 USD a day) in 2015. The countries classified as low- or middle-income (sub-Saharan Africa and Southern Asia) account for nearly 40 per cent each, with a concurrent underweight prevalence of 21% in sub-Saharan Africa and 31 % in Southern Asia (*2*).

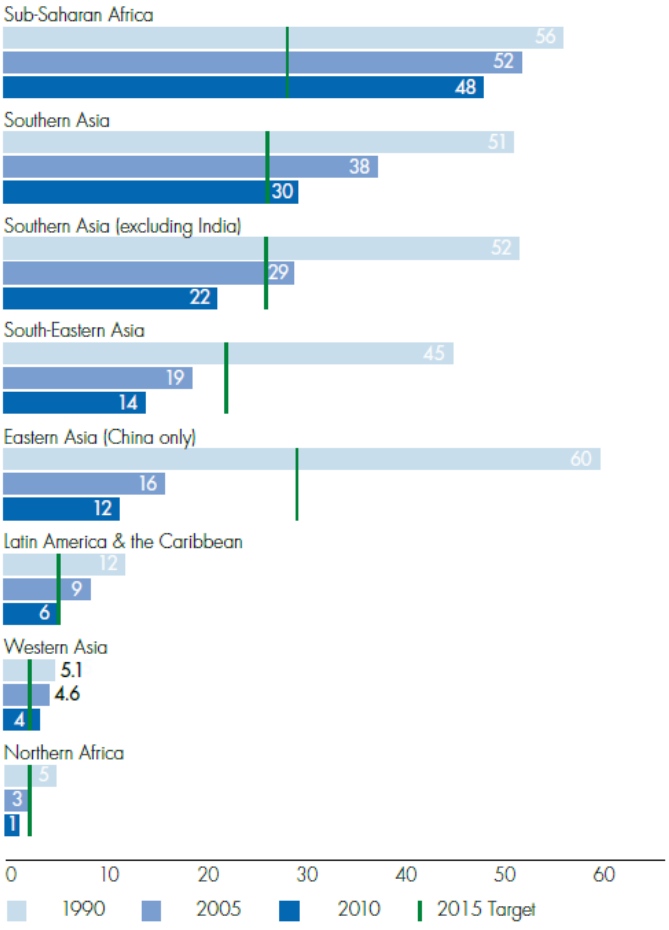


Figure 1: Proportion of people living on less than 1,25 $ a day over last three decades. Modified from UN 2013 (*2*).

During the last three decades, aquaculture has experienced a twelve time increase in food fish production, making it one of the fastest growing food industries. Fish is considered an important source of nutritious food and animal protein (*3*). 40 per cent of world aquaculture production is derived through rural small-scale fish farming, where it in these areas is a major contributor to poverty reduction (3). Still, fish food consumption remains too low in many areas of sub-Saharan Africa and Southern Asia, where they are failing to benefit from contributions that fisheries and aquaculture are increasingly providing elsewhere in the world, in terms of sustainable food security and income (*4*).

The high prevalence of poverty within the rural areas is accompanied by their remote location and poor road connections, contributing to further detachment from the rest of the world, making potential export/trade difficult and time consuming (*5*). Extending private small-scale fish farming could lead to poverty alleviation in these areas. To make this possible, the fish has to be transported from brood- and live-stock locations to its designated production sites.

Among the countries located in the sub-Saharan Africa and Southern Asia are the two countries Malawi and Nepal. According to the Human Development Report from 2013, both countries range within the thirty countries in terms of lowest human development, and is ranked 220 and 207th in terms of gross domestic product (4). They both hold great water resources, with Malawi holding the world's 9th largest lake, Lake Malawi, and Nepal with its numerous river systems. Conditions like these provides the basis necessary to establish fish farming for further poverty alleviation and food security establishment.

The transport of live fish

Live fish trade requires transportation of fish from seller to buyer. Type of transportation varies, depending on purpose of the fish destination (slaughter, livestock, ornamental use etc.) and must also be fitted to the technology available. Industrialized countries most often transport live fish by truck or well-boats, while more primitive methods such as transportation of fish in polyethylene bags are applied in less developed countries.

The transportation can be performed in two systems; open or closed. An open system is a water container in which necessary additives required for fish survival are supplied from the outside. A closed system is a sealed non-outside supplied container (*6*).

The transportation procedures of live fish may cause stress exposures (*7-10*). In closed systems, oxygen availability may be a limiting factor (*11*), while accumulation of metabolic end products (carbon dioxide and ammonia) may deteriorate water quality further (*12-14*). An ideal saturation of oxygen in the transport water throughout procedure is 100% (*15*), while CO_2 levels is recommended to be kept below 30 – 40 mg L^{-1} (*16*). CO_2 excreted in the water container reacts with H_2O forming HCO_3^- and H^+, resulting in lowering of water pH. As pH declines, the proportion of carbonate species shift from bicarbonate to carbon dioxide (see *figure 2*). In open transport systems, the limiting factor of oxygen and carbon dioxide can be met by the application of pure oxygen and aeration (*17*), leaving ammonia toxicity as limiting factor. Mean acute ammonia toxicity for fish is reached at concentrations surpassing to 2.8 mg L^{-1} (*18*).

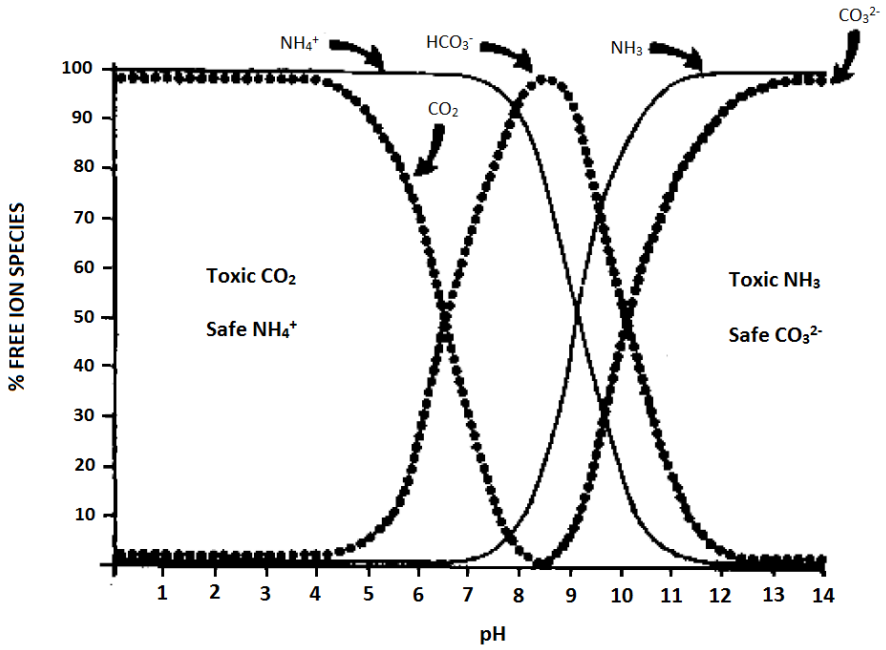


Figure 2: Proportions of carbon dioxide species and ammonia in water as a function of pH. Modified from Berka 1986 (*11*).

Fish, being poikilothermic, has biochemical reaction rates depending on ambient temperature. The rate with which oxygen is consumed, therefore increases with ambient temperature (*19*). Furthermore, oxygen solubility in water is inversely correlated with temperature (*20*), causing further challenge for transportation of live fish in closed systems.

The respiratory medium for most fish is water, and its level of dissolved oxygen poses the most critical and limiting variable factor (*1*).Thus, oxygen availability is the primary limiting factor for fish survival during transport in closed systems. As fish consumes oxygen, oxygen availability is reduced causing environmental hypoxia to the fish, finally to a saturation where fish is no longer capable of maintaining an adequate supply of oxygen to meet its metabolic demands (*21*), a point termed the critical saturation (S_{crit}). Such anoxic circumstances may cause suffocation of the fish. Additionally, the progression of an hypercapnic environment, due to the accumulation of excreted carbon dioxide, could severely disrupt gas transfer across the gills (*22*).

Fish O_2-respiration

The demand for oxygen in animals arises from the dual functions of maintaining homeostasis performing work (*23*). The gills are the main site of gas exchange in fishes. The counter current blood flow in the gill lamella facilitates the exchange of gasses between blood and water (*24*). The exchange of O_2 is due to diffusion, following the partial pressure gradient down the lamellar epithelium. Maintenance of O_2-partial pressure gradient between water and blood is facilitated through gill ventilation, continuously replacing the respiratory medium in contact with the respiratory surface, and lamellar perfusion providing O_2-depleted blood to the site of gas exchange (*25*).

Oxygen is transported throughout the cardiovascular system in two forms, as plasma dissolved oxygen or as bound to erythrocyte hemoglobin (*1*). Due to low solubility of oxygen in fish plasma the main proportion (95 %) of blood oxygen is hemoglobin-bound (*26, 27*). As a result, the O_2 carrying-capacity of the blood is hemoglobin dependent. By regulating the amount of erythrocytes present in the blood plasma, the fish can meet its metabolic demand if increased. The spleen is considered main reservoir for erythrocytes. The recruitment of erythrocytes is generated through contraction of smooth muscle associated with the spleen (*28-30*), facilitated through direct innervation of the sympathetic nervous system or by circulatory catecholamines (*30*).

Hemoglobin-oxygen (Hb-O_2) affinity is regulated through several allosteric modulators, assisting on- and offloading of oxygen. Lowering of pH causes reduced Hb-O_2 affinity, resulting in decrease of % Hb-O_2 binding at a given oxygen partial pressure (Bohr effect), whereas a decrease in the metabolic precursors ATP and GTP, causes a higher affinity to O_2. Further, a reduction in blood pH can also cause a decrease in the maximal binding capacity of hemoglobin to O_2 (Root effect) (*26*).

Shortage of oxygen, known as hypoxia is divided into categories of environmental hypoxia and functional hypoxia. Environmental hypoxia is defined as the partial pressure of water oxygen where fish physiological function is first compromised, at which fish compensates by increasing gill ventilation and perfusion, blood delivery to tissues and recruitment of stored erythrocytes. Functional hypoxia occurs during exercise, acidosis and may also be due to increased lamellar epithelial thickness (*31*).

Fish stress

Fish are exposed to stressors both free living and within captivity (*32*). Stress is described as a state when the threatened homeostasis of the fish requires a complex suite of physiologic and behavioral adaptive responses to re-establish a normal state (*33, 34*). If the stressor persists and fish is unable to recover homeostasis, the stressor is considered maladaptive. This state will be detrimental to fish's health and well-being (*35*).

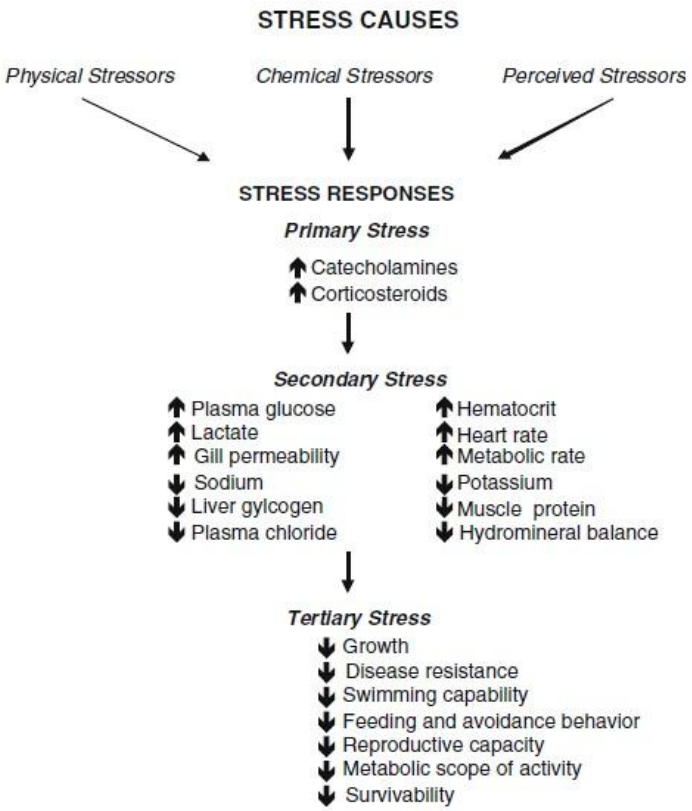


Figure 3: Possible stressors and physiological effects of the primary, secondary and tertiary stress responses. From Portz et al. 2006 (*1*).

A stress response is categorized into three stages, first described by Selye as the alarm reaction and the following stages of resistance and exhaustion (*36*). This was later termed the primary, secondary and tertiary response (*37, 38*). The primary response to stressor exposures is characterized by an increase in stress related blood hormones. This involves up regulation of the hypothalamic-sympathetic-cromaffin - and hypothalamic-pituitary-interrenal axis activity, resulting in increased blood catecholamines (CAs), adrenocorticotrophic hormone and cortisol. To assist the fish with mobilizing fuels for increased energy demand, physiological and behavioral changes occurs, a following state, triggered upon release of the primary response hormones can be categorized as the second stress response (*1*). Hyperglycemia, occurring from CAs and cortisol stimulated glycogenolysis (*39*) to satisfy the increased metabolic demand, is one of the main parameters used to measure stress. Sustained hyperglycemia, after the effects of initial catecholamine, is maintained through the stimulatory effect of cortisol (*35*). Generally, secondary stress responses occur within a few minutes to an hour after stress exposure, usually persisting for longer time periods (*1*). Further, CAs stimulate to increased ventilation rate, gill lamellar recruitment and cardiac output for the purpose of increasing oxygen availability to the body cells. Increasing blood volumes moving through an increased surface of gill epithelium may cause loss of ions in

freshwater fishes (*1*). Increasing circulating catecholamines also have the ability to increase cell metabolism by stimulating to increased glycolysis resulting in elevated use of oxygen (*40*).

Chronic stress exposure will be detrimental causing osmoregulatory dysfunction, decreased body immune-competence and body growth with mortality following (*41*). At this point, the stress response can be categorized as a tertiary response, often relating to whole animal response.

Anesthesia

The term "welfare" refers to the state of an individual in relation to its environment (*42*). Recently, fish welfare has received more attention and is today a topic of great interest (*43, 44*). Maintaining fish welfare is of producer's best interest, not only for public perception, marketing and product acceptance, but also for fish production itself, affecting efficiency, quality and quantity. Little is known about the feelings of an animal, but behavioral and physiological responses are measurable (*42*). Ongoing scientific debate argues whether fish is neural capable for awareness, fear and pain (*45*). Experiments done by Sneddon on rainbow trout injected with bee venom in the lip where nociceptors are located, showed increased gill ventilation, extended achievement of normal feeding and abnormal behavior, concluding ability of sensing pain (*46*). However, the lack of neocortex in the fish brain is argued by some to conclude the opposite, being that this part of the brain provides the basis for pain and fear consciousness (*47, 48*). The recent increasing extent of scientific activity concerning fish welfare predicts an even greater interest of this topic in the future (*49*).

Anesthetic agents are applied to fish to induce loss of sensation and immobilization, by depressing central and peripheral nervous systems (*50*), causing reduced voluntary movement and reduced sensory perception (*51, 52*). Anesthesia is applied either by physical (electric tension or refrigeration) or chemical (agents), latter being most widely used (*53*) . Anesthetic agents have shown to reduce physiological parameters related to stress and to reduce mortality upon stress exposure (*50, 54*). The agent is subjected by exposing the fish to water applied with the agent, where its absorbed through the gills and skin, entering the circulatory system further passing the blood-brain barrier, causing an effect on the central nervous system (*8, 55*). Fish show varying behavioral traits upon exposure to anesthesia, depending on species, agent and concentrations used (*56*). Several stages from anesthesia have been characterized.

Table 1: Stages of anesthesia and fish behavioral characteristics. (Øistein Preus Hveding 2008, modified from McFarland 1959, Schoettger et al. 1967 and Burka et al. 1997 (*57-59*).

Stage	Description	Behavior
0	Normal	Active swimming; reactive to external stimuli; equilibrium, opercular rate and muscle tone normal
1	Light sedation	Reduced swimming activity; slight loss of reactivity to external visual and contractile stimuli
2	Light narcosis	Equilibrium loss with efforts to right; increased respiratory rate
3a	Deep narcosis	Total loss of equilibrium; decreased muscle tone and respiratory rate; some reactivity to stimuli
3b	Surgical anesthesia	Total loss of reactivity and muscle tone; very low respiratory rat, depressed heart rate
4	Medullary collapse	Respiration ceases; cardiac arrest; death normally follows

The use of fish anesthetic agents is frequent within aquaculture and research procedures, because of their mobility- and stress-reducing abilities. For procedures such as handling and transportation, anesthesia- and sedation-achieving concentrations are used, respectively. Fish is subjected to deeper stages of anesthesia when performing blood sampling and surgery (*53*).

Exposing the fish to an anesthetic agent, can itself be disadvantageous, being that the chemical restraint itself can be negative to the fish, causing physiological disturbances similar seen in a stress-response. Still, sedation and anesthesia produce a lower stress response compared to when performing non-anesthetic applied handling and transport, in terms of elevations in primary and secondary stress responses (*60-62*). When transporting fish, maintaining water quality is of uttermost importance (*6*). A decreased demand of oxygen, due to sedation induced lowering of metabolism may result in slower depletion of total oxygen and excretion of metabolic wastes, such as carbon dioxide and ammonia into the water (*61, 63, 64*).

Since its introduction in the 1960s, tricaine methane-sulfonate (MS-222) has been one of the most used anesthetic worldwide (*65*). It is applied to reduce stress during research and aquaculture procedures such as handling, transport, blood sampling, surgery (*50*). MS-222 is supplied as 100 % pure drug for the purpose of direct application in water, with the solubility of 11 %. Due to high lipid solubility, MS-222 is considered a suitable anesthetic for both fresh- and saltwater fish. The level of anesthesia or sedation varies depending on exposure time and concentration of the drug. Environmental factors such as temperature, pH, oxygen content, hardness and salinity, as well as

biological factors i.e. species, sex, age, weight, size, lipid content and biomass density are known to affect drug efficacy (*65*).

Absorbed through the gills and skin of the fish, MS-222 enters the blood and is further distributed throughout the fish body (*66*), where it suppresses nerve membrane excitability by inhibiting entrance of Na^+ into the nerve (*67*), reducing voluntary movement and sensory perception of the fish (*51, 68*), as well as blocking brain activity and sound sensitive neurons (*51, 69, 70*). Due to its acidity, application of MS-222 in un-buffered water can alter water pH considerably (*71*). Exposure to MS-222 itself has shown to elevate physiological stress parameters (*50, 72*). However, acute immobilizing concentrations of MS-222 prior to handling and loading has shown to reduce physiological stress responses during and after live fish transport (*10*). Thus, the importance of establishing suitable anesthetic concentrations and treatment methods that will not act as a stressor has been elucidated (*65*).

The purpose of this study

Stress exposure during loading and transport due to handling and deteriorating water quality have detrimental effect on fish, and may lead to mortality. The use of an anesthetic could reduce both oxygen depletion and release of fish metabolic wastes and therefor prolong possible time of transport. This would benefit rural areas characterized by its poor technology and/or time consuming transport. Accordingly, by monitoring oxygen consumption within a closed system, the aims of the present work were to study snow-trout and Red Zebra and answer the following:

1. Is weight specific oxygen consumption affected by stocking density?
2. Can sedation reduce oxygen consumption and thereby prolong transport time?
3. Does the sedatory chemical have negative impact on the fish blood chemistry?

MATERIALS & METHODS

Study site and object

The study was divided in two parts, first one in Nepal, where simulated transport of live snow-trout (*Schizothorax plagiostomus*) and the possible prolonging effect of MS-222 on transport time, by its efficacy on oxygen consumption, was investigated. When returning to Norway, a comparative study on the metabolic and blood response to varying sedative treatments on Red Zebra (*Metriaclima estherae*) was conducted.

Substudy 1 – Snow-trout

The snow-trout, also known by the Nepalese as the Buche Asla, was the first object of this study. Experimental fish consisted of 170 Melamchi (2.4 ± 1 g) and 150 Trishuli (3.3 ± 1.5 g) fingerlings. Melamchi fish had been bred at the station from a brood stock originating from the Melamchi River. Trishuli fish had been collected the same season from Trishuli River. Breeds were kept separate in cylindrical concrete tanks, until last days of experimental period when conducting high stocking density studies. An acclimation tank and a recovery tank were each used for 24 hour per group, prior and after experiment, respectively, to ensure that intestines were emptied and prevent any repetitive use of fish from day to day. Tanks, two meters in diameter, were all identical in shape and size. Small rocks were applied inside the tanks to provide fish shelter. Tanks were continuously supplied with fresh water, originating from an up-hill creek. Tank water temperature varied between 12 and 15.5 °C as a result of changing ambient temperature. Water level was kept at 30 cm. Fish were daily fed dry egg powder from local supplier. Experiments were performed at the Nepal Agricultural Research Council (NARC) Fisheries Research Division, Godawari during March – April 2013.

Substudy 2 – Red Zebra

The Lake Malawian Red Zebra cichlid was chosen as the second study object. Fish was supplied from a hobby aquarist located in Levanger, first batch in May 2009 and a second in June 2013. Fish were stored in numbers up to 8 animals per 180 L glass aquariums, kept in a climate room holding 21°C at NTNU Gløshaugen, Trondheim. The aquariums were equipped with chopped gutter tubes, sand grains and water pumps. Water was collected from 250 L water filled stocking barrels, added 5 grams of salt (Felleskjøpet). Photo period was set at 07 – 19:00 hours, resulting in a 12L : 12D light regime. Fish used in the experiment consisted of both juvenile and adult specimens, weighing from

7.6 to 36.6 gram. Fish were fed daily with commercial flake food (TetraPro Algae™) between 12 – 14:00 hours. Experiments were performed during May – July, 2013.

Anesthesia

The anesthetic used in the experiments was MS-222 (tricaine methane-sulphonate 100 %). Concentrations used in the experiments are expressed in miligrams per liter (mg L^{-1}). To induce anesthesia and sedation, concentration determination done by Øystein Preus Hveding (*73*) and Stine Ims (*74*) was used. Throughout all sedative experiments, fish status were checked regularly to ensure that stage 2 of anesthesia (table 1) was not entered.

Respirometry

Cylindrical plexiglas tubes by volumes of 50, 12 and 3.7 L were used as respiratory chambers. Plexiglas discs were applied as bottom and top lids, sealing off the chamber. A small fine netted chamber was attached onto the bottom lid, holding a magnetic stone. Removable top lid was supplied with customized edges, providing static positioning. Two holes with transecting air tube nipples were applied through the top lid, from where water sampling or refilling was performed. To ensure total sealing and prevention of gas diffusion across air phase (outside) and water phase (inside), vaseline was applied between lid and cylinder tube. A hole was made through the wall of the chamber, in order to insert a nipple keeping the cable from an optical oxygen electrode (inside) connected to an oxygen meter (YSI ODO™) on the outside. A magnetic stirrer was located below the chamber, providing a uniform environment in the water masses, by rotating the magnetic stone inside the chamber. Walls of the respiratory chambers was either covered with black plastic bags (Nepal) or applied two layers of black paint (Norway), to reduce potential stress caused by external movement in proximity of the chamber.

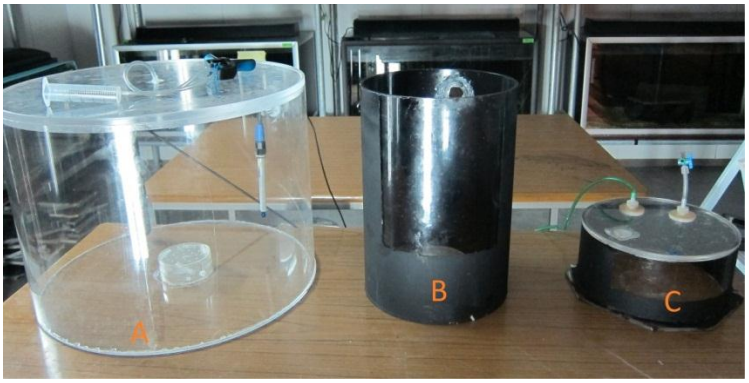


Figure 4a: The three sizes of respiratory chambers used in the experiments with A) snow-trout (50 L), B) group of Red Zebra (12 L) and C) single individuals of Red Zebra (3.7 L). During snow-trout experiments, the chamber wall was covered with black folded plastic bags in similar manner as of chamber B and C.

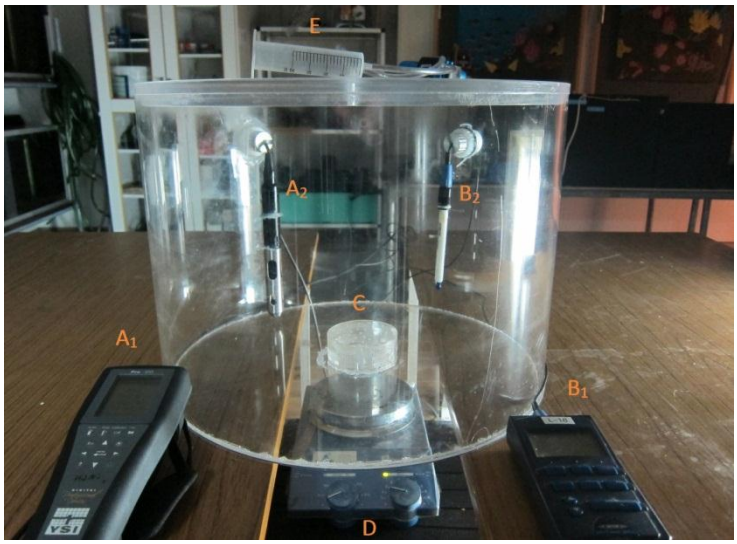


Figure 4b: The respiratory chamber applied for simulated transport of snow-trout. The 50 L cylindrical plexiglas chamber had A_1) a handheld oxygen meter and B_1) a handheld pH meter connected to an inside A_2) optical oxygen probe and B_2) a pH probe, respectively. Inside connected to the bottom lid was C) a fine netted chamber holding a magnetic stone and contact with D) an outside magnetic stirrer. E) A refill hose was connected through the removable top lid

Acrifix® 192 was applied to connect bottom lid to cylinder and the fine meshed netting to the walls of the chamber of the magnet stone. The chamber, housing the magnetic stone, was connected to the bottom lid by standard aquarium silicone. After gluing process, water and water pump was applied to the chamber and held over-night, circulating. Water change was performed three times, once before and after over-night circulation, before subjecting fish to the chamber.

Experimental procedure

When studying oxygen consumption on snow-trout, experiments containing only groups of fish were carried out. It was also planned to perform experiments on individual fish, however lacking fulfillment of bilateral working contracts from NARC with respect to fish availability and working location prohibited the execution of such measurements. In order ensure documentation of aspects reflecting real life conditions during transport of live fish, measurements on group fish oxygen consumption was a priority.

Experiment 1a – Oxygen consumption of snow-trout at different stocking densities in a closed system

One day prior to experiment, fish were transferred from outside stocking tank to a neighbor tank, for a following 24 hour feed deprivation. Further, fish were transferred to an inside rectangular glass aquarium, containing fish shelter and 40 L water from feed deprivation- tank. Additional water from same tank was added to a 50 L cylindrical respiratory chamber. Fish were kept in the aquarium for 60 minutes. During this time, both waters of aquarium and respiratory chamber were supplied with aeration to provide equal oxygen saturations. Side walls of aquarium and respiratory chamber were covered with folded black plastic bags to ensure a non-stressful setting by eliminating fish's detection of human movements close to the aquarium. Additional non-see-trough top cover was applied to aquarium, preventing fish from jumping out.

Fish were netted and transferred to the respiratory chamber at fixed time intervals, until the oxygen saturation level was down to 3 mg L^{-1} (DO_2). Water samples were collected at start and every 2 ½ h during experiment for later ammonium analysis (Analysesenteret, Trondheim). After experiments, fish was transferred in batches (≈20 piece) to an aquarium containing anesthetic dose of MS-222 (50 mg L^{-1}). At loss of equilibrium fish were netted and individual weight was recorded (ScalTech SPB 42), for further transfer to a barrel containing untreated water for recovery.

Table 2: Weight and stocking density for groups of snow-trout subjected to 50 and 30 L water. Mass and temperature values are expressed in mean ± SD.

Origin	N	Volume (L)	Mass (g)	Tot weight (kg)	Density (kg L^{-1})	Start DO_2 (mg L^{-1})	Temp. (°C)	pH interval
Trishuli	50	30	3.0 ± 1.00	0.16	0.0054	8.13	13.3 ± 0.8	7.91 – 7.56
"	50	50	2.9 ± 1.00	0.15	0.0030	8.4	14.9 ± 0.6	7.96 – 7.53
"	100	30	3.2 ± 1.09	0.31	0.0105	8.09	13.8 ± 0.1	7.96 – 7.59
"	100	50	3.2 ± 1.13	0.30	0.0062	8.31	15.9 ± 0.2	8.04 – 7.57
"	150	30	3.2 ± 1.31	0.48	0.0158	8.3	14.4 ± 0.1	8.00 – 7.59
"	150	50	3.3 ± 1.50	0.50	0.0104	8.38	12.1 ± 0.2	---
Melamchi	50	30	2.5 ± 0.89	0.13	0.0043	8.26	13.9 ± 0.3	7.96 – 7.57
"	50	50	2.4 ± 0.74	0.12	0.0026	8.2	14.2 ± 0.8	8.00 – 7.52
"	100	30	2.3 ± 0.90	0.24	0.0080	8.13	14.8 ± 0.0	8.05 – 7.63
"	100	50	2.3 ± 0.90	0.24	0.0050	8.62	12.6 ± 0.4	8.03 – 7.57
"	150	30	2.4 ± 0.93	0.36	0.0120	8.21	15.2 ± 0.2	8.05 – 7.61
"	150	50	2.4 ± 0.93	0.36	0.0075	8.57	13.8 ± 0.3	7.99 – 7.57
T & M	300	50	2.9 ± 1.49	0.85	0.0180	8.31	14.6 ± 0.1	7.96 – 7.59

Experiment 1b – Oxygen consumption of snow-trout when subjected to MS-222 sedative treatments

Procedure for following experiment was performed similar to that described above in *Experiment 1a.* Fish were transferred into the respiratory chamber containing 0, 15 or 25 mg L^{-1} [MS-222]. The sedative solutions were achieved by adding 0.75 and 1.25 grams of MS-222 directly into 50 liters of water.

Table 3: Groups snow-trout exposed to concentrations of MS-222. To perform experiment with 300 snow-trout, both Melamchi and Trishuli fish was used. Mass values are mean ± SD.

N	Sedative treatment	Origin	[MS-222] Treatment	Mass (g)	Tot weight (g)	Density (kg L^{-1})	Temp. (°C)
150	High	Melamchi	15 mg L^{-1}	2.5 ± 0.83	374.2	0.007484	15.5 ± 0.1
150	Heavy	Melamchi	25 mg L^{-1}	2.5 ± 0.83	374.2	0.007484	15.1 ± 0.1
300	Zero	M & T	0	2.9 ± 1.50	849.2	0.016984	14.6 ± 0.1
300	High	M & T	15 mg L^{-1}	2.9 ± 1.50	849.2	0.016984	15.4 ± 0.1
300	Heavy	M & T	25 mg L^{-1}	2.9 ± 1.50	839.2	0.016784	15.1 ± 0.2

Experiment 1c - Oxygen consumption of snow-trout when subjected to MS-222 sedative treatments, following pre-anesthesia

Procedure for following experiment was performed similar to that described in *Experiment 1a*, until netting and transferring fish into the respiratory chamber. After the 60 minute rest in the 40 L aquarium, fish was anesthetized in a 50 mg L^{-1} solution, by adding 2 gram MS-222 dissolved in 100 mL water into the aquarium through a hose. Water aeration provided sufficient mixing of the anesthetic solution to the water. When all fish lost equilibrium (within 5 minutes), fish were netted and transferred to the respiratory chamber containing 5, 10 or 15 mg L^{-1} MS-222. Desired concentrations were achieved by adding 3 L of 50, 100 and 150 mg L^{-1} MS-222 stock solution to the respiratory chamber, holding 27 L pure water. During all three experiments, the respiratory chamber was holding 3:2 water to atmosphere.

Table 4: Snow-trout exposed to different MS-222 treatments. Weight was measured by bulk measurements.

N	Sedative treatment	Origin	MS-222 Pre-anesthesia (mg L^{-1})	MS-222 Sedation (mg L^{-1})	Total weight (g)	Density (kg L^{-1})	pH interval	Temp. (°C)
65	Low	Trishuli	50	5	194.6	0.0065	7.91 – 7.68	15.2 ± 0.2
65	Medium	Trishuli	50	10	0190	0.0060	7.92 – 7.52	16.0 ± 0.0
65	High	Trishuli	50	15	197	0.0066	7.79 – 7.62	16.8 ± 0.2

Temperature between the experiments differed, as seen in table above. To compare rates of oxygen consumption of the three group, MO_2 values were temperature corrected to 16 °C with a $Q_{10} = 2$.

Experiment 2a – Oxygen consumption of Red Zebra when subjected to MS-222 sedative treatments, following pre-anesthesia

One day prior to experiment, fish were transferred from stocking aquarium to a neighbor non fed acclimation aquarium, containing fish shelter (chopped gutter pipes) and 40 L water. An additional 0.5 L from the fishes tank of origin was added to provide any prior accustomed odorants. Water was under continuous aeration. Aquariums were painted black on three of its sides, leaving one see-through side exposed to an area with no human traffic occurring. A black plate was used as lid, covering 5/6th of the top opening.

After 24 hours of acclimation, fish were netted and transferred into a respiratory chamber where the drop in dissolved oxygen (DO_2) was logged by an oxygen meter (YSI ODO). Single fish experiments were carried out in a 3.7 L chamber, while group (N=6) experiments were assigned to a 12 L chamber. Water used in the respiratory chamber was collected from same stocking barrel as fish were accustomed to 24 h prior to experiment. Experiments included pre-anesthesia prior to transfer and a sedative concentration in the respiratory chamber. Pre- anesthesia was induced by subjecting fish into a black bucket containing an anesthetic water bath. Water samples were collected before and after experiments, further frozen immediately for later pH measuring.

Table 5: Red Zebra exposed MS-222 treatments, as single or in groups of six fish. Values are expressed in mg L^{-1}.

Groups	Pre-anesthesia	Sedation
Control	0	0
No sedation	150	0
Low sedation	150	10
Medium sedation	150	30
High sedation	150	50

After transferring fish into the respiratory chamber, a lid applied with a smooth layer of vaseline under its edge, was put on top. Additional water was applied through a transectional lid hose until the chamber was completely filled with water.

Table 6: Single individuals and groups of Red Zebra exposed to different sedative treatments of MS-222. Individual weights of group experiments and single fish density are in mean ± SD.

	Treatment [MS-222]	N	Mass (g)	Tot weight (g)	Density (kg L^{-1})	pH interval	Temp. (°C)
Single	0 -- 0	7	18.0 ± 3.9	-	-	7.82 – 7.80	20.4
«	150 -- 0	6	19.5 ± 6.5	-	-	7.69 – 7.68	20.9
«	150 -- 10	5	27.3 ± 6.5	-	-	8.03 – 7.74	21.4
«	150 -- 30	6	22.8 ± 7.9	-	-	7.73 – 7.43	20.8
«	150 -- 50	5	18.8 ± 2.0	-	-	7.48 – 7.31	21.4
Group	0 -- 0	6	10.9 ± 1.7	65.4	0.0055	7.73 – 7.34	21.4
«	150 -- 0	6	9.9 ± 1.9	59.4	0.0050	8.05 – 7.68	20.8
«	150 -- 10	6	11.2 ± 1.7	67.2	0.0056	7.81 – 7.92	20.8
«	150 -- 30	6	9.0 ± 1.9	54.2	0.0045	7.83 – 7.47	20.2
«	150 -- 50	6	15.6 ± 3.7	93.6	0.0078	7.53 – 7.30	21.2

Experiment 2b – Blood chemistry of Red Zebra when subjected to a MS-222 sedative treatment, following pre-anesthesia

Groups of Red Zebra (N = 6) were exposed to untreated and treated simulated transport for 2 ½ hour after 24 h in a non fed acclimation aquarium. At the end of transport, blood from caudal vein was sampled by use of 1 mL syringes (Omnifix®-F) after a quick blow to the head. Blood chemistry analyses of sodium, glucose, lactate and hemoglobin were performed by use of i-STAT™ and EC8+ cartridges. Top lid was re-applied between single fish blood sampling to prevent diffusion of gases in or out of the tank. Dissolved water oxygen was monitored to ensure that these were kept stabile throughout the sampling procedure. In order to compare values, blood samples were collected from fish exposed to 24 hour acclimation and 2 ½ hour untreated simulated transport.

Table 7: Exposed and non-exposed MS-222 groups (N=6) of Red Zebra. Control group experienced no handling or transferring to respiratory chamber. Individual weight values are mean ± SD.

N	Sedative treatment	MS-222 Pre-anesthesia (mg L^{-1})	MS-222 sedation (mg L^{-1})	Mass (g)	Total weight (g)	Density (kg L^{-1})	pH interval	Temp. (°C)
6	Control	-	-	20.5 ± 5.9	-	-	-	21.0
6	Zero	0	0	10.9 ± 1.3	63.5	0.0055	7.75 – 7.63	21.0
6	Medium	150	30	9.0 ± 1.9	54.2	0.0045	7.83 – 7.47	20.2

Analytic procedure

Estimating transport time of different stocking densities of snow-trout

Experiment duration was defined by the time for oxygen saturation to reach 3 mg L^{-1} (DO_2). Start saturation varied between experiments (table 2), resulting in different total oxygen availability. To compare groups, the difference from start to end oxygen saturation of the group starting off at the lowest saturation (100 T 30 L ; 8.09 mg L^{-1}), was set as new DO_2 interval (5.09 mg L^{-1}). The time for each group to reduce oxygen saturation equal to this interval was further used for estimating transport time.

Slope analysis

Values of oxygen saturation were exported from oxygen measuring software (YSI Data Manger) into a statistics program (SigmaPlot). Saturation values (milligrams of oxygen per liter) and time (minutes) were plotted at Y- and X-axis, respectively. Shape of curve, illustrating post-handling oxygen consumption was further divided into three phases. Phases were as follows

A: Initial phase

B: Transitional phase

C: "Resting" phase

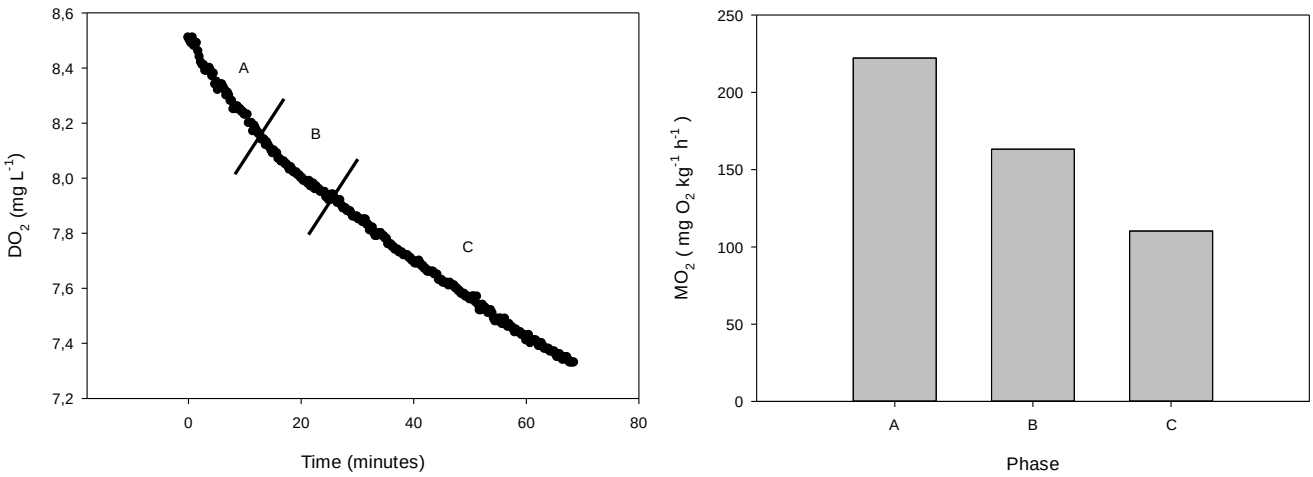


Figure 5: Dissolved water oxygen during simulated transport time (left) and the rate of oxygen consumption at the different phases (right).

Slope to the left illustrates a typical reduction of dissolved oxygen (DO_2) seen after handling and transferring fish. Starting off as an initial steep fall in DO_2 (initial phase), the rate of oxygen depletion declines after a 15 – 20 minute period (transitional phase). Within 30 – 40 minutes the regression slope decreases less than earlier, representing a state where the fish starts settling down from the earlier stress exposure ("resting" phase). Oxygen consumptions were calculated from stable C-phase periods in order to ensure that estimates reflected a period of where fish showed constant consumptions for longest period.

Estimating C-phase oxygen consumption

In closed respiratory chambers, the rate of oxygen consumption (MO_2) during stabile consumption phases is estimated by multiplying ΔDO_2 from a ten minute interval by the water volume of the respiratory chamber, further dividing this by Δt and body mass, yielding the rate of oxygen consumption per unit weight (O_2 fish weight^{-1} time^{-1}) (*75*). To ensure rate consumption estimates that reflected the phase, all DO_2 values from a minimum of ten minutes within this period was used when calculating MO_2, using linear regression (SigmaPlot for Microsoft Windows 12.5 Systat Software Inc.). All C-phase MO_2 estimates were retrieved from oxygen saturations above 4 mg L^{-1}.

Estimating oxygen consumption in a sedative solution

Trends in oxygen consumptions varied during fish respirometry in different sedative treatments of MS-222. When studying simulated transport of snow-trout in Nepal, all trends of oxygen depletions in a sedative solution illustrates the sum of oxygen consumption of the n individuals inside the closed respiratory chamber. Individual fluctuations in O_2 consumption are therefore "covered" by the consumption of the other n-1 fish present in the chamber. However, studying oxygen depletion from single Red Zebra individuals, clearly illustrated cross-individual differences in MO_2 seen with two overall trends; a) smooth reduction in dissolved oxygen or b) repetitive bursts of increased depletion of dissolved oxygen (see figure below). Using SigmaPlot 12.5, linear regression of the representative overall reduction trend of the phase was found. If plot showed all through stabile reductions, then rate of consumption could be estimated easily.

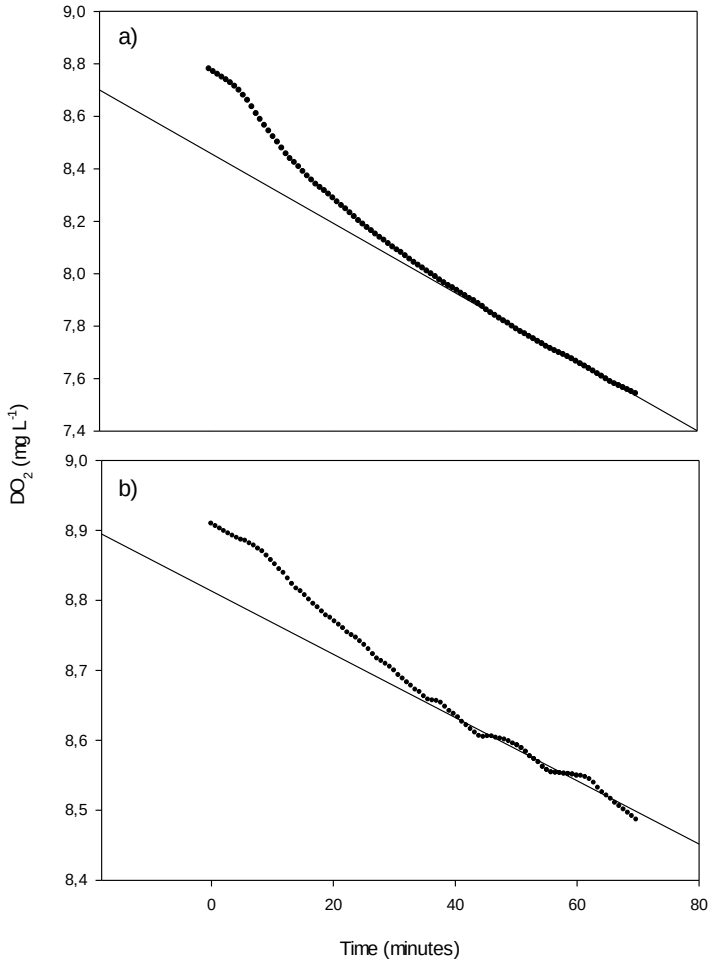


Figure 6: Dissolved oxygen of the respiratory water in a closed chamber applied single Red Zebra individuals. Both (a) stabile and (b) repetitive burst oxygen uptakes were observed.

Estimations of C-phase oxygen consumption on Red Zebra were performed at fairly similar time interval to ensure equal recovery time after handling stressor. During this period dissolved oxygen levels were all above 7 mg L^{-1}, with a few exceptions.

Estimating initial "A" phase oxygen consumption in a sedative solution

During sedative experiments of Red Zebra, the efficacy of the anesthetic MS-222 on reducing oxygen consumption during the initial "A" phase" was also included.

Ammonia estimations

Proportion of un-ionized ammonia was estimated using temperature, pH and ammonia pKa (*76*) in Microsoft Windows Excel spread sheet (Trond Rosten, SINTEF).

Graphic and statistics

Graphic illustrations in this study was performed in SigmaPlot 12.5 (Systat Software Inc.) for Microsoft Windows. Graphics illustrating oxygen consumption at sedative treatments following pre-anesthesia have X-axis (A – B) values representing anesthetic MS-222 concentration prior to netting (A), and sedative treatment in the respiratory chamber (B). Values are expressed in mg L^{-1}.

Data failing normality, not meeting assumptions for parametric statistical tests, were log 10 transformed for later Tukey HSD in IBM® SPSS® Statistics 21.

Experiments of this study were performed as a pilot study of the greater Sustainable Poverty Reduction in Nepal (SPRN) program. Due to inadequate material availability when studying the transport of live snow-trout in Nepal, alternative solutions to experiment conduction was done. To obtain measurements of value, experiments had to be planned and performed on the basis of the resources available (fish and stocking tanks). It was decided to sacrifice the opportunity of performing statistical analysis in order to obtain qualitative information regarding the possible effects of strain, stocking density, tank water:air relationship and anesthetic treatment.

RESULTS

Exp. 1a – Oxygen consumption of snow-trout at different stocking densities in a closed system

Average constant oxygen consumption varied from a low 108.6 mg O_2 kg^{-1} h^{-1} to a high 262.8 mg O_2 kg^{-1} h^{-1} depending on transport system, density and snow-trout strain. Snow-trout fingerlings of Trishuli strain showed a lower rate of oxygen consumption throughout all experiments, when compared to the Melamchi fingerling strain. Both breeds showed higher consumption rates when transport was performed at higher densities. When increasing stocking density from low to high, Melamchi strain showed a 65 % higher MO_2 increase than the Trishuli strain. Increasing stocking density from low to high in a transport system containing atmosphere resulted in higher changes in MO_2 compared to stocking density increases in transport systems with no atmosphere, by about 350 and 200 % for Trishuli and Melamchi strain, respectively. When Melamchi and Trishuli strain were combined, fingerlings showed a consumption rate of only 168.6 mg O_2 kg^{-1} h^{-1}.

A tendency of a non-linear MO_2 development with increasing stocking densities was seen for almost all groups, with the exception of highest density of Melamchi fingerlings in a container holding 5:0 water to atmosphere.

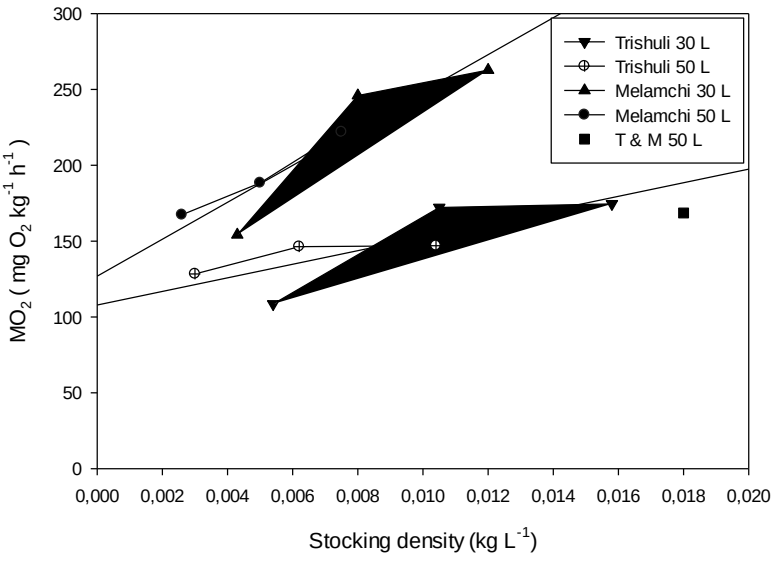


Figure 7: Weight specific oxygen consumption for Trishuli and Melamchi snow-trout fingerlings kept at different stocking densities. O_2 measurements were done using a closed transport tank holding 5:0 or 3:2 parts water:atmosphere. During experiments temperature and pH varied between 12.1 – 15.9 °C and 7.52 - 8.05, respectively.

When allowing a drop of 5 mg L^{-1} in dissolved oxygen the corresponding estimated possible transport time for the different combinations of snow-trout strain, tank water volume and density is shown in figure 8. If pairwise compared, almost identical transport time is shown for both fingerling breeds, despite higher stocking densities of Trishuli fingerlings.

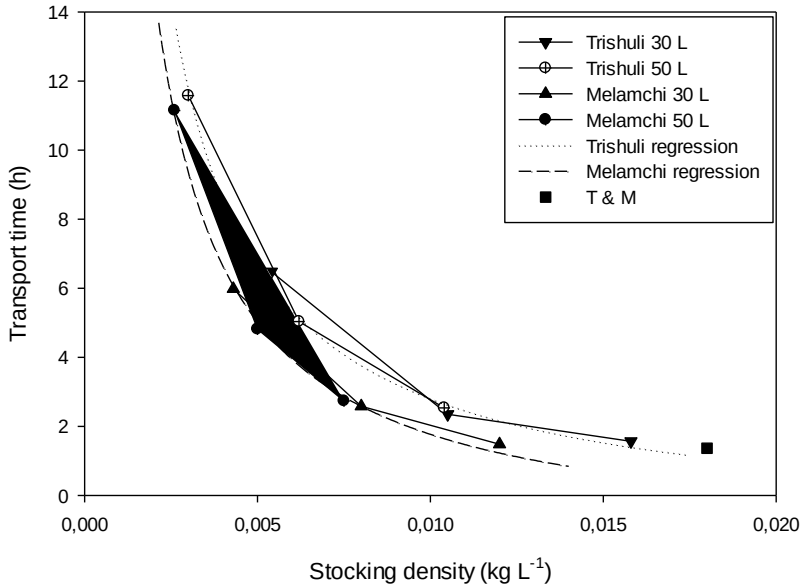


Figure 8: Time of transport for Trishuli and Melamchi snow-trout fingerlings kept at different stocking densities. O_2 measurements were done using a closed transport tank holding 5:0 or 3:2 parts water:atmosphere. During experiments temperature and pH varied between 12.1 – 15.9 °C and 7.52 - 8.05, respectively.

From the different stocking density transport time of each fingerling strain, two functions were estimated, yielding;

Trishuli: $$(kg\ L^{-1}) = -1.0143 + \frac{0.0381}{kg\ L^{-1}} \quad (1)$$

Melamchi: $$(kg\ L^{-1}) = -1.4822 + \frac{0.0325}{kg\ L^{-1}} \quad (2)$$

for $0.0026 \leq kg\ L^{-1} \leq 0.016$

where,

$$kg\ L^{-1} = \begin{cases} \dfrac{\left(\dfrac{n \times \overline{m}}{1000}\right)}{L} \\ \overline{m} = 3.5\ (Trishuli)\ or\ 2.9\ (Melamchi) \\ L = \ 5{:}0\ or\ 3{:}2\ water\ to\ air \end{cases},$$

with total hours of transport time, t, as a function of stocking density (kg L^{-1}), derived by multiplying number of fish (n) by mean body weight (in grams), further dividing this weight (in kilos) by volume of water in the sealed container, holding either 5:0 or 3:2 water to atmosphere.

For practical use, transporter would more likely be interested in estimating maximum stocking density possible for a given hours of transport, obtained by rearranging the function 1 and 2;

Trishuli: $$kg\ L^{-1}(t) = \frac{0{,}0381}{1.0143+t} \quad (3)$$

Melamchi: $$kg\ L^{-1}(t) = \frac{0{,}0325}{1.4822+t} \quad (4)$$

Two graphs were plotted from function 3 and 4 illustrating the possible stocking density as a function of desired transport time of Melamchi and Trishuli fingerlings;

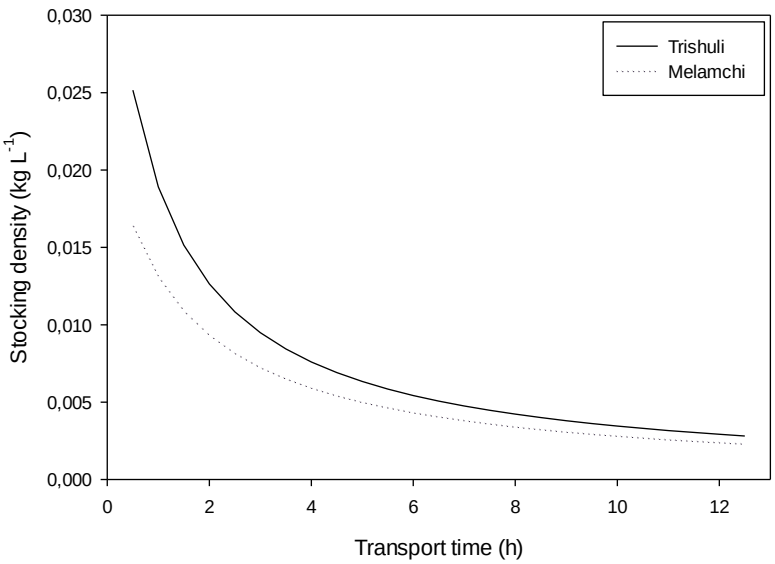


Figure 9: Maximum stocking density of the two snow-trout fingerling strains Melamchi (BW 2.4 g) and Trishuli (BW 3.3 g) as a function of desired hours of transport time at a temperature of ≈ 14 °C.

Water quality changes during transport of snow-trout

Changes in water pH and ammonia content were measured during the simulated transport of snow-trout.

pH changes

Total pH intervals of the individual transport experiments are shown in *table 2*. Progression of pH reduction is illustrated in Appendix A. Water pH changed from 8 to 7.5 during transport when water O_2 saturation dropped from 8 to 3 mg L^{-1}. Simulated transport performed with 5:0 parts water:atmosphere showed greater pH reductions when compared to 3:2 of same fish quantity. In all experiments, trend of pH reduction throughout simulated transport was characterized by inverse proportionality. When comparing log DO_2 and pH, a non-linear relationship is seen.

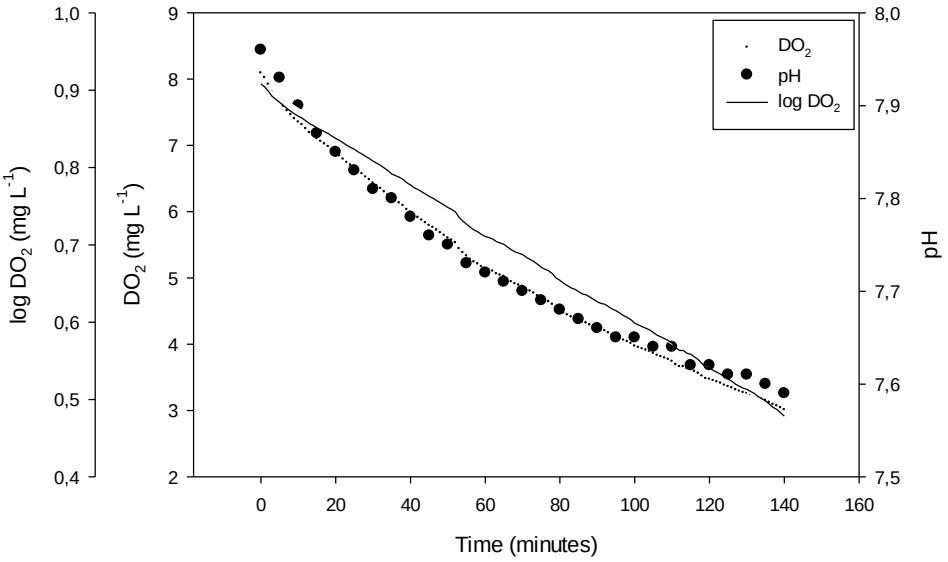


Figure 10: Changes in pH and dissolved oxygen during simulated transport of 100 Trishuli snow-trout (0.0105 kg L^{-1}) in a closed (3:2, water:atmosphere) tank. T = 13.8 ± 0.1°C

Ammonia accumulation

An increase of ammonia concentration from 0.00048 to 0.0015 mg NH_3 L^{-1} was seen during 1.7 hours for simulated transport of the highest density. At lowest density of snow-trout ammonia concentrations in the water increased from 0.00022 to 0.00087 during first 10 hours of transport.

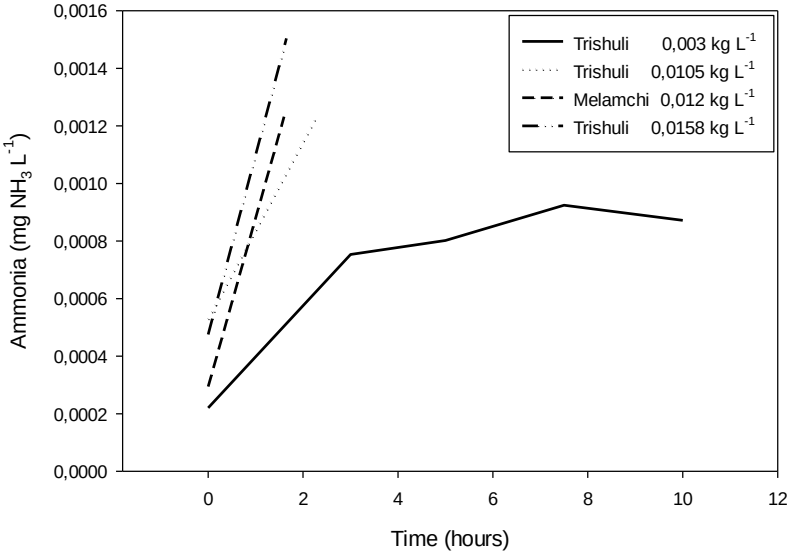


Figure 11: Changes in un-ionized ammonia during simulated transport of Trishuli and Melamchi snow-trout fingerlings during a period with DO_2 drop from 8 to 3 mg L^{-1}.

Exp. 1b – Oxygen consumption of snow-trout when subjected to MS-222 sedative treatments

The purpose of this experiment was to study the effect of increasing sedative concentrations of MS-222 on oxygen consumption of snow-trout kept at different stocking densities.

C-phase oxygen consumption rates

Snow-trout fingerlings stocked at different densities showed different changes in oxygen consumption between exposures to two MS-222 sedative concentrations during simulated transport. Greatest change in MO_2 was seen during low stocking density (Melamchi) transport with a 25 mg L^{-1} sedative concentration, lowering the rate of consumption of controls from 222 to 110.4 mg O_2 kg^{-1} h^{-1}. This reduction was only slightly greater when compared to 15 mg L^{-1} MS-222 exposure, which resulted in a MO_2 of 128.4 mg O_2 kg^{-1} h^{-1}. During high stocking density transport of snow-trout fingerlings, any sedative concentration exposure during transport resulted in only minor changes in rates of consumption, with a reduction of 18 % when exposed to the highest sedative concentration, compared to control. A higher sedative concentration was necessary to achieve any MO_2 reduction by MS-222 sedation during transport of highest density.

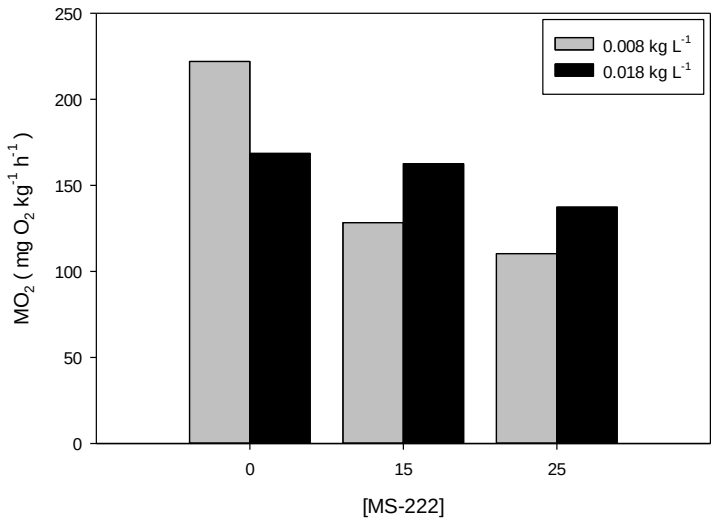


Figure 12: C-phase rate of oxygen consumption per unit weight (Y-axis) of snow-trout at 0.008 (Melamchi) and 0.018 kg L^{-1} (both strains) exposed to sedative concentrations of MS-222 (X-axis). T = 15.2 ± 0.3

Transport time

Low stocking density transport of snow-trout subjected to sedative concentrations of MS-222 resulted in a marked prolonging of total transport time from 2 ¾ (control) to 4 ¾ - 5 hours. However, total transport time of low stocking density was not very affected when increasing the sedative concentration from 15 to 25 mg L^{-1}. When exposed to similar concentrations, snow-trout transported in a high stocking density showed only minor prolonging of total transport time.

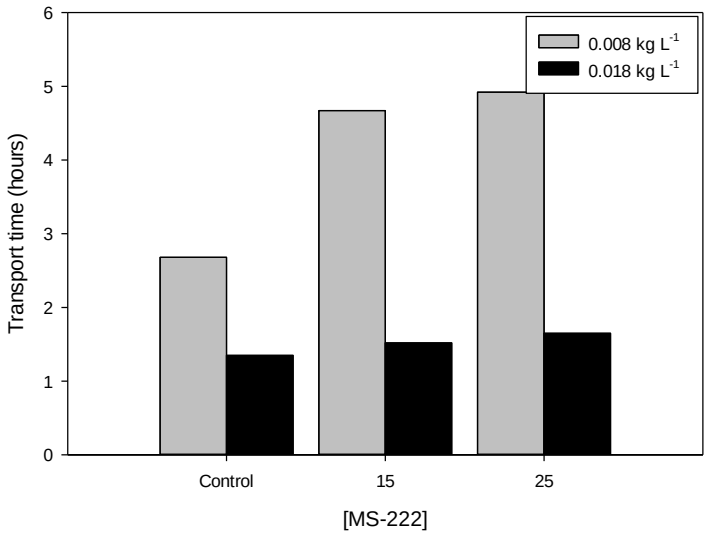


Figure 13: Total transport time of two stocking densities of snow-trout exposed to different sedative concentrations to water oxygen saturation reached a drop by 5.04 units (mg L^{-1}). T = 15.2 ± 0.3.

Exp. 1c - Oxygen consumption of snow-trout when subjected to MS-222 sedative treatments, following pre-anesthesia

The purpose of this experiment was to determine the effect of increasing sedative concentration on oxygen consumption of snow-trout.

C-Phase oxygen consumption

Fish exposed to varying sedative concentrations following a pre-anesthetic treatment of MS-222 showed slight differences in oxygen consumption. Snow-trout exposed to lowest sedative concentration showed highest rates of oxygen consumption. MO_2 was reduced from 135 mg O_2 kg^{-1} h^{-1} when subjected to a 5 mg L^{-1} sedative treatment to 110 – 120 mg O_2 kg^{-1} h^{-1} when subjected in 10 and 15 mg L^{-1} MS-222.

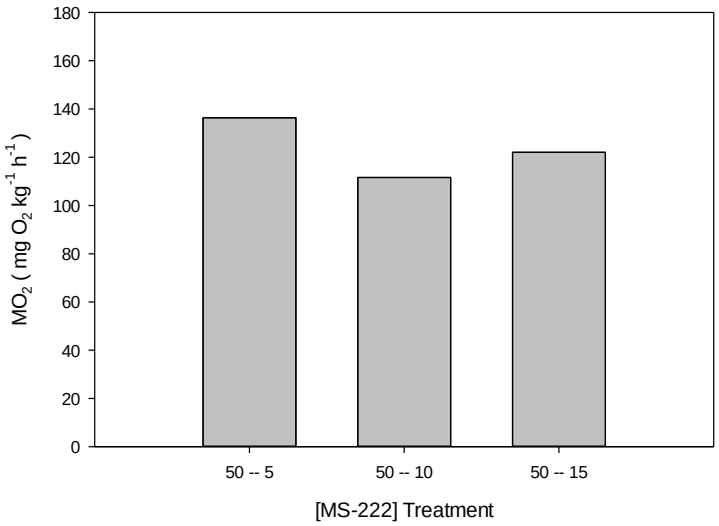


Figure 14: Rate of oxygen consumption per unit weight (Y-axis) of snow-trout (Trishuli) exposed to sedative concentrations of MS-222 (X-axis) during simulated transport at 0.0065 kg L^{-1}. T = 16.0 ± 0.6 °C and pH = 7.8 ± 0.1. Rates was temperature corrected to 16 °C for comparison, with Q_{10} = 2.

Total transport time

Subjecting snow-trout at a similar stocking density to simulated transport of different sedative concentrations resulted all in approximately 3 hours for the oxygen saturation in the container to reach a 2.94 unit reduction. A slightly prolonged time of possible transport time was seen when subjecting snow-trout to 10 and 15 mg L-1 MS-222.

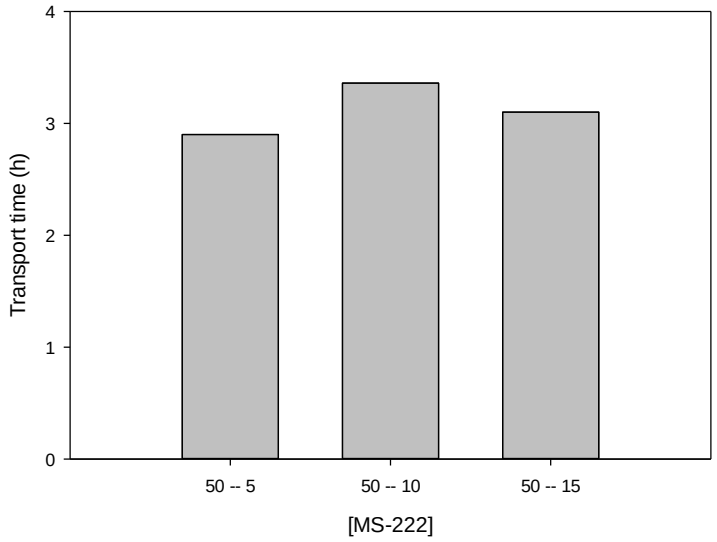


Figure 15: Time from subjecting Trishuli snow-trout (0.0065 kg L^{-1}) to water oxygen saturation reached a drop of 2.94 units (mg L^{-1}). T = 16.0 ± 0.6 °C and pH = 7.8 ± 0.1

Exp. 2a – Oxygen consumption of Red Zebra when subjected to MS-222 sedative treatments, following pre-anesthesia

The purpose of this experiment was to study the reduction efficacy of different sedative treatments of MS-222 on oxygen consumption on Red Zebras. The experiment was conducted on single and group individuals.

During initial A phase both individual and group showed variations of oxygen consumption depending on sedative treatment following pre-anesthesia. Untreated control of both individual and group expressed highest MO_2 of 200 and 325 mg O_2 kg^{-1} h^{-1}, compared to fish exposed to an anesthetic treatment which showed MO_2 rates of about 150 mg L^{-1}. Even though not significant different, fish subjected in 0 and 10 mg L^{-1} MS-222 following pre-anesthesia showed slightly increased MO_2 compared to fish subjected in 30 and 50 mg L^{-1}. Greatest difference was seen comparing untreated control to fish exposed to 30 and 50 mg L^{-1}, showing significant difference ($P < 0.05$ Tukey HSD).

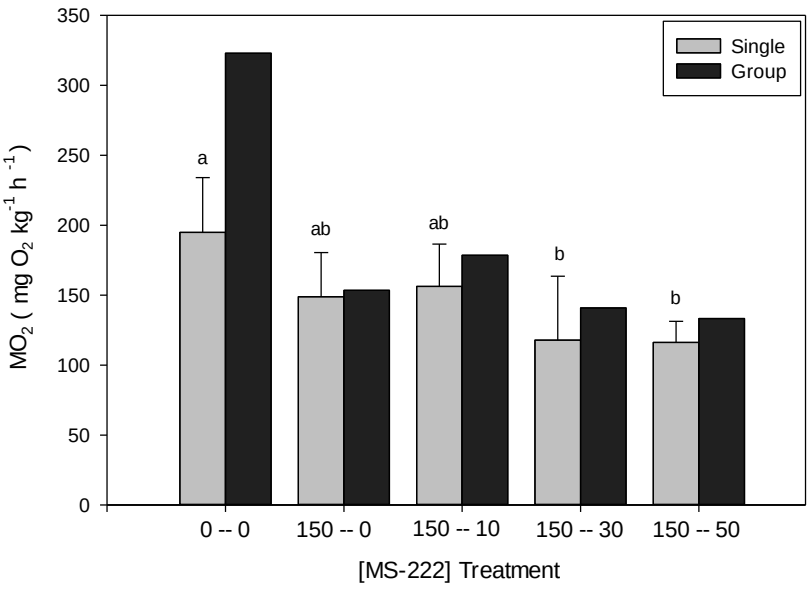


Figure 16: Rate of oxygen consumption per unit weight (Y-axis) of single and groups of Red Zebra exposed to treatments of MS-222 (X-axis) during A phase. T = 20.2 – 21.4 °C and pH = 7.31 - 8.05. Values are mean ± SD. Bars with different letters are significantly different ($P < 0.05$ Tukey HSD).

C phase MO_2 of single Red Zebra exposed to different anesthetic treatments showed treatment dependent variations, significant different by $P < 0.05$ (ANOVA). During C phase, highest rates of oxygen consumption of individuals (130 mg O_2 kg^{-1} h^{-1}) and group of fish (130 mg O_2 kg^{-1} h^{-1}) were at exposure to 0 mg L^{-1} following pre-anesthesia. Further, exposure to 10 mg L^{-1} resulted in higher consumption rates of when compared to fish exposed higher sedative concentrations, with single Red Zebra subjected to 30 mg L^{-1} showing significantly lower MO_2 compared to fish exposed to pre-anesthetic treatment only.

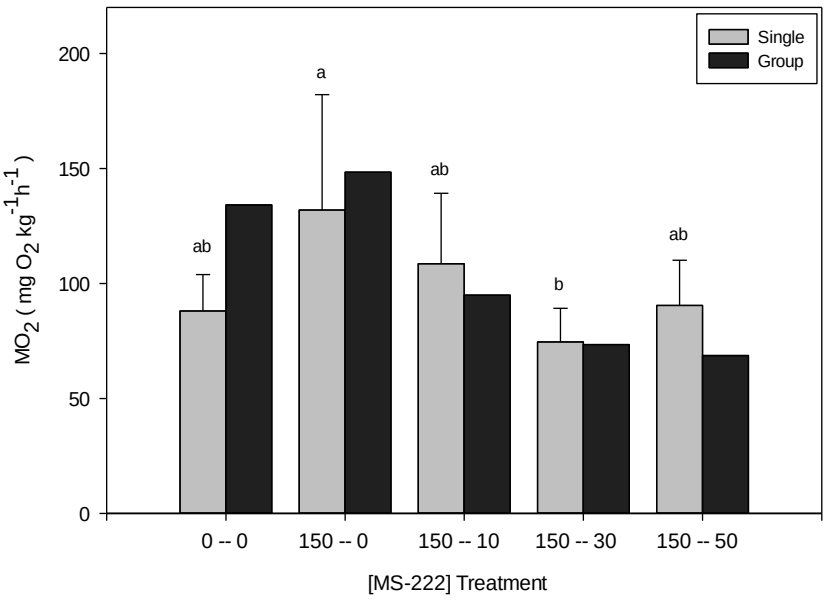


Figure 17: Rate of oxygen consumption per unit weight (Y-axis) during C-phase on single and groups of Red Zebra exposed to different sedative treatments of MS-222 (X-axis). T = 20,2 – 21,4 °C, pH = 7.31 - 8.05. Values are mean ± SD. Bars with different letters are significantly different ($P < 0.05$ Tukey HSD).

Total oxygen consumed after 60 minutes of simulated transport of Red Zebra varied with type of treatment. Untreated group transport (control) showed highest rates of 240 mg O_2 kg^{-1}. Individual fish showed significant different TOC_{60} between single fish MS-222 treatments ($P < 0.05$ ANOVA), with TOC of 110 – 120 mg O_2 kg^{-1} when exposed to 30 and 50 mg L^{-1}. Group consumption is comparable to single fish within anesthetic treatment exposures.

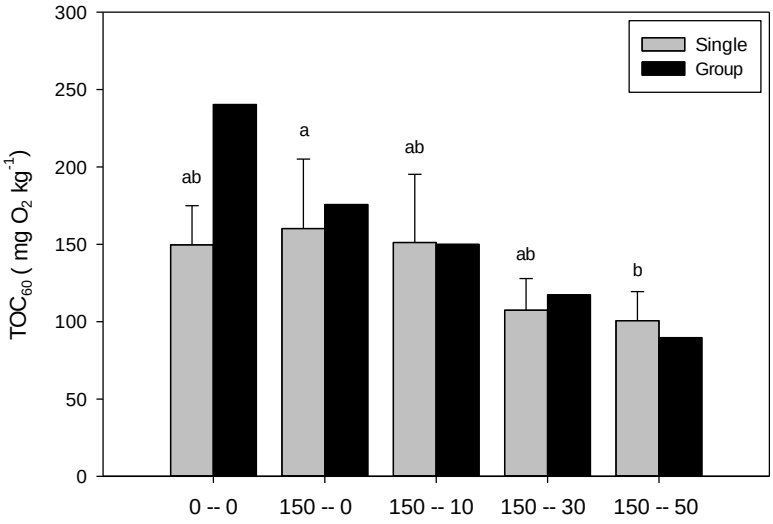


Figure 18: Total oxygen consumption after 60 minutes on single individuals and groups of Red Zebra exposed to different sedative treatments of MS-222, including control. T = 20,2 – 21,4 °C, pH = 7.31 - 8.05.Values are mean ± SD. Bars with different letters are significantly different (Tukey HSD, $p < 0.05$).

When exposing groups of Red Zebra (N=6) to a 2 ½ hour simulated transport, total oxygen consumption (TOC) decreased with increasing sedative MS-222 concentrations. During transport in a non-sedative concentration following pre-anesthesia fish showed a TOC of 420 mg O_2 kg^{-1}. When a sedative concentration of 10 mg L^{-1} was added to the transport container, TOC was markedly reduced to 300 mg O_2 kg^{-1}. A further increased MS-222 sedative concentration of 30 mg L^{-1} resulted in a decrease in TOC of 245 mg O_2 kg^{-1}. During exposure to 50 mg L^{-1} MS-222 fish did not show equilibrium recovery from pre-anesthesia. Experiment was therefore terminated after 60 minutes of simulated transport.

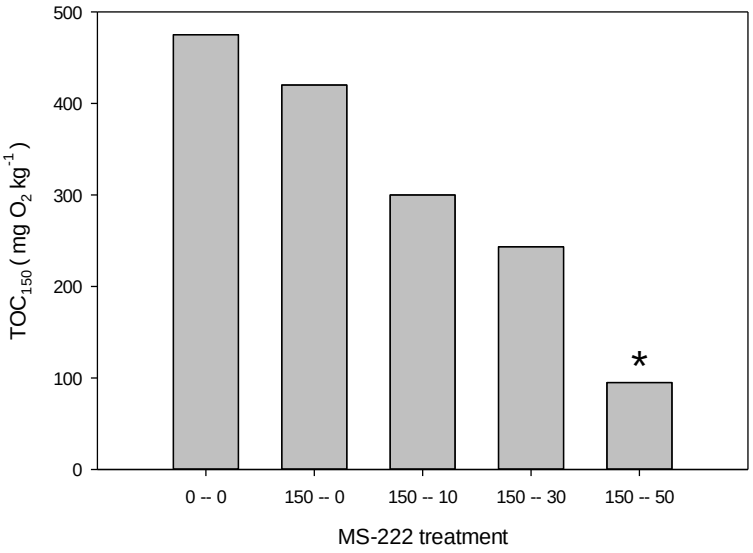


Figure 19: Total oxygen consumption of groups of Red Zebra (N=6) exposed to different MS-222 sedative concentration. Mean temperature ranged between 20.2 to 21.5 °C, while pH was 7.30 to 8.05. Experiment involving exposure to 50 mg L^{-1} was terminated after 60 minutes.

Exp. 2b – Blood chemistry of Red Zebra when subjected to a sedative MS-222 treatment, following pre-anesthesia

The purpose of this experiment was to determine effects of MS-222 on blood chemistry after a 2 ½ h exposure to a sedative solution, following a pre-anesthetic treatment. A 2 ½ hour exposure to 30 mg L^{-1} following pre-anesthesia resulted in significant lower levels of hemoglobin ($P < 0.05$ Tukey HSD), from 8 to 5.5 g 100mL. Untreated fish subjected to simulated transport had Hb levels between these. Fish exposed to unsedated and sedated simulated transport showed elevated blood lactate concentration from 0.6 mmol L^{-1} (control) to approximately 1 mmol L^{-1}. Still, no significant difference was seen. Oxygen saturation in the respiratory water at sampling time (2 ½ h) for group exposed to untreated (0 – 0) and treated (150 – 30) simulated transport was 6.3 and 7.5 mg L^{-1}, respectively.

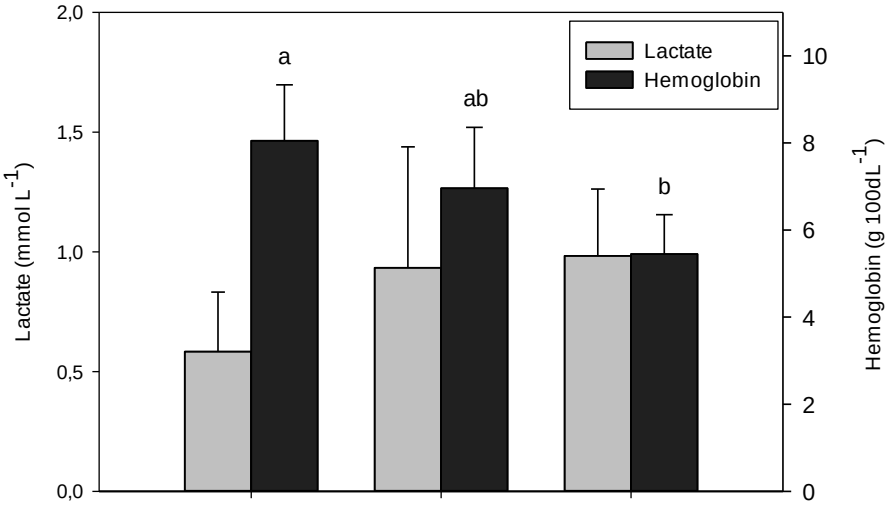


Figure 20: Hemoglobin and lactate (mean ± SD) of MS-222 treated and un-treated Red Zebra. Values are mean ± SD. Bars with different letters are significantly different (Tukey HSD, $p < 0.05$). Lactate values showed no significant difference. Temperature ranges between 20 to 21 °C.

Blood glucose was significantly higher at 2 ½ hour for both groups subjected to simulated transport when compared to controls ($P < 0.05$ ANOVA). Sodium concentrations was markedly lower at 2 ½ hour of sedative simulated transport, slightly insignificant from controls ($p = 0.054$ Tukey HSD).

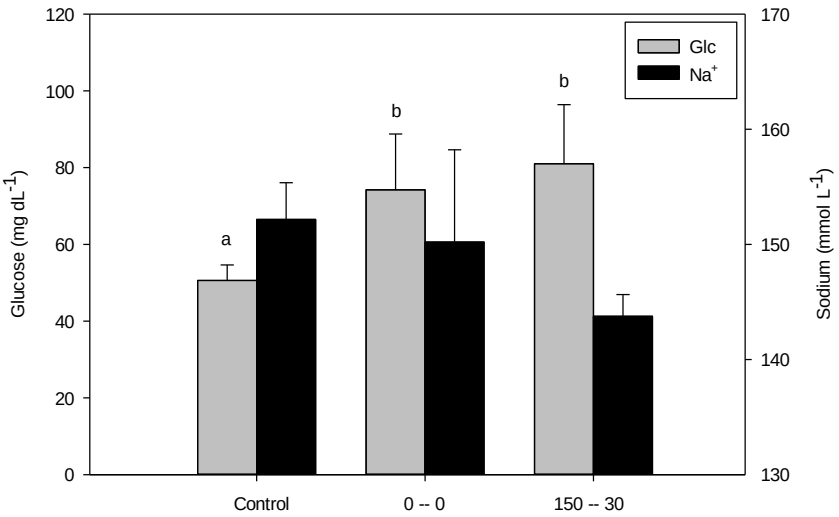


Figure 21: Change in blood sodium and glucose concentration of Red Zebra exposed to unsedated (0 – 0) and sedated simulated transport following pre-anesthesia (150 – 30), and control. Values are mean ± SD. Bars with different letters are significantly different ($P < 0.05$ Tukey HSD).

Video monitoring of fish exposed to simulated transport without any anesthetic treatment (0 – 0) showed higher swimming activity with fish distributed throughout entire water column, compared to a steady bottom positioning observed in fish transported in 30 mg L^{-1} MS-222.

DISCUSSION

Introduction of fish farming to the poor people of developing countries may significantly contribute to poverty alleviation. Constituting a major role in aquaculture, the transportation of fish must be performed in such a way as to ensure fish health and survival. Limited technical logistics and poor transportation infrastructure, demands the application of simple methods when performing live fish transportation in remote rural areas. Application of sealed plastic bags for back packing is a well know used system. Resulting mortality does, however, document the need for improvement of these crude transport forms. Thus, knowledge on fish oxygen consumption and the possible effects of variable factors involved in transport procedure (water volume, stocking density, fish size and stress) is essential for further improvement of fish transport.

In this study, the effect of fish stocking density on rate of oxygen depletion in a closed system was examined. Fish stress is often accompanied when performing fish netting, crowding and transport. Application of an anesthetic (MS-222) was accordingly investigated for its possible improvement on total transport duration through sedation of fish activity and oxygen consumption.

Due to inadequate material availability when studying the transport of live snow-trout in Nepal, it was decided to change study procedure in such a manner that more qualitative data were obtained, this at the expense of planned repetitive measurements and statistical analysis**.**

Two relevant transport scenarios (within developing countries) were chosen for testing fish use of oxygen during closed transport. These included back-packing of snow-trout fingerlings in hilly area of Nepal, and small container transport of ornamental fish within Malawi.

SNOW-TROUT (SCHIZOTHORAX PLAGIOSTOMUS)

Carrying weight is of considerable importance when performing live fish transport by a back-pack system in the hilly Himalayan areas. After interviewing professional (sherpa) carriers, it was recommended to develop a system load of 50 – 60 kg. Accordingly, a closed tank system containing 50 L of water was studied.

Oxygen consumption in a closed system

Weight specific oxygen consumption of snow-trout fingerlings documented an overall increased oxygen consumption at higher stocking densities. Trishuli fingerlings showed an increase from only 130 to 175 mg O_2 kg^{-1} h^{-1} when fish stocking density was increased from a minimum of 0.003 kg L^{-1} to maximum of 0.0158 kg L^{-1}, indicating that fingerlings of the Trishuli strain were only little affected by crowding. Their average rate of oxygen consumption are close to what is reported for other carp fingerlings at similar temperatures, being approximately 150 mg O_2 kg^{-1} h^{-1} (*77*).

When increasing the stocking density of Melamchi fingerlings, rates of oxygen consumption showed much higher increase than compared to the Trishuli strain, changing from 170 mg O_2 kg^{-1} h^{-1} at low density (0.0026 kg L^{-1}) to 260 mg O_2 kg^{-1} h^{-1} at the higher density (0.012 kg L^{-1}). This approximately 100 % higher increase in oxygen consumption from lowest to highest stocking density of Melamchi fingerlings, compared to Trishuli strain raises interest. Possible explanations for this could be related strain specific higher activity or increased stress as a result of strain specific aggressive behavior. Trishuli-Melamchi differences are not likely due to differences in domestication since the Trishuli fingerlings were collected wild same season from the Trishuli River.

A possible way to examine whether or not the increased MO_2 seen at higher stocking densities of Melamchi fingerlings is caused by aggressive behavior would be to “dilute” these fishes with those from another non-aggressive strain. Accordingly, when combining Trishuli and Melamchi fingerlings, giving a 50 % increase in stocking density (0.018 kg L^{-1}), the rate of oxygen consumption was only 170 mg O_2 kg^{-1} h^{-1}, some 90 - 100 mg O_2 kg^{-1} h^{-1} lower than for the Melamchi strain alone. It is therefore likely that the increased oxygen consumption seen in the Melamchi strain at higher stocking densities is due to increased dominant behavior of this strain, compared to Trishuli strain.

As fish increases in weight its weight specific standard metabolism decreases (*78*). Further, when studying the relationship of body weight to oxygen consumption ($VO_2 = aBW^b$) in pre-smolt Atlantic salmon, Cook et al. reported a scaling exponent (*b*) of 0.85 following a 24 hour feed deprivation (*79*). Further, when comparing 69 teleost species of post-larval stage, Clarke et al. (1999) derived an

exponent of 0.79 (*80*). However, this does not account for the high difference in MO_2 seen between Melamchi (mean BW of 2.4) and Trishuli (mean BW of 3.3). The role of body weight as an explanation to the higher Melamchi oxygen consumption can therefore be rejected.

O_2 availability improvements

When performing live fish transport in closed systems, adjustments should be tested for the purpose of increasing O_2 availability or reducing O_2 consumption. Possible ways could be the use of a gas phase of air or pure oxygen, or adding sedatives to the water (*15, 81*). Availability of pure oxygen in rural areas is low. Therefore, in this study, the effects of 3:2 parts water:air and the possible impacts of the anesthetic MS-222 on total transport time was investigated.

Air phase impact

Performing simulated transport of snow-trout fingerlings with a 20 L atmosphere in the closed transport container did not contribute to any prolongation of transport time. On the contrary, estimates of oxygen consumption showed higher values compared to transport performed with 5:0 parts water:air, indicating negligible diffusion of oxygen from the atmosphere into the water. Thus, by adding an air phase, thereby increasing tanks holding of total oxygen due to higher oxygen availability in air, actually only worsen the scenario. This is believed to be because of the low solubility of oxygen in water. Furthermore, during real conditions of live fish transport, a container holding an atmospheric phase results in water movements when carried. This could potentially act as a stressor resulting in fish seasickness with mortality following (pers. com. Nilssen).

Sedatory impact

In this study the positive effects of different anesthetic treatments to reduce rate of oxygen consumption, thereby prolonging transport time and maintaining water oxygen saturations above critical levels during transport was documented.

The use of MS-222 sedative concentrations during transport of snow-trout fingerlings resulted in slight decreases in rate of oxygen consumption when exposed to concentrations from 5 to 15 mg L^{-1}, following pre-anesthesia. Thus, the transport time, defined by a drop in 2.94 units of dissolved oxygen (DO_2), was not markedly affected. If oxygen consumption continued at similar rates as shown in figure 14, the time for DO_2 to reach 3 mg L^{-1} would be 5 ½ hours. This is a slight increase compared to transport time of Trishuli fingerlings at similar density (100 T 50 L ; 5 h).

Fish stocking density has been shown to affect anesthetic efficacy. When studying MS-222 resistance of equilibrium loss on three freshwater species Sylvester et al. (*82*) found higher drug resistance with

an increase of 2 – 3 time stocking density. During simulated transport of snow-trout an increased transport time of 20 % was seen at highest stocking density of 0.018 kg L^{-1} when subjected to the highest sedative concentration (25 mg L^{-1}). The effect of MS-222 on oxygen consumption at this fish density was minor compared to the 84 % increased transport time witnessed at lowest density (0.008 kg L^{-1}) at similar concentration. The small effect of these concentrations on reducing oxygen consumption at highest stocking density is believed to result from a increased rate of which MS-222 is metabolized at higher biomass present in the container. Thus, it is obvious that stocking density do affect the efficacy of MS-222 on reducing oxygen consumption on snow-trout fingerlings.

The additional lowering on rates of oxygen consumption and prolonging of transport time seen when comparing 15 and 25 mg L^{-1} MS-222 at lowest stocking density is minor. Dosage dependent stress responses to MS-222 exposure have been documented (*10, 83*). Thus, it is suggested that when applying a sedative for prolonging transport time of snow-trout fingerlings at 0.008 kg L^{-1} a sedative concentration of 15 mg L^{-1} should be used.

Impact from water quality

When performing live fish transport in closed systems water quality may deteriorate due to accumulation of carbon dioxide (causing lowering in pH) and ammonia (*12-14*). When performing snow-trout transport, water pH and ammonia was therefore measured.

Water pH and carbon dioxide

As the fish use oxygen it releases carbon dioxide (in a relationship of 1:1) in the water. CO_2 then reacts with water producing H^+-ions, resulting in a lowering of pH. According to the equilibrium of carbonate species in water, the proportion of carbonate species shifts from bicarbonate to carbon dioxide when pH decreases. Thus, lowering of pH would require higher additional amounts of CO_2 added to the water.

When performing simulated transport of snow-trout in the closed container pH dropped from 8 to 7.5. Slope representing pH showed a non-linear trend indicating the likelihood of water buffering capacity, when compared to log DO_2. It is reasonable to assume that this minor environmental change in pH would not compromise the blood buffering capacity of the carp during short lasting transportations (*84, 85*).

However, if CO_2 is allowed to reach high levels, a resulting hypercapnia has shown to impair oxygen consumption when exposing fish to acute hypoxia (*86*). The actual concentrations of water CO_2 in this study are unknown. In the future, the possible effects on oxygen consumption from higher CO_2

accumulation rates, caused when performing high stocking density transport in a closed system, should be investigated.

Water carbon dioxide solubility is high, being 26 times greater than oxygen at 25°C. In natural conditions, the low atmospheric partial pressure of CO_2 (0.04 %) mediates the diffusion of elevated CO_2 from the water into the atmosphere. When comparing pH interval of simulated transport with 5:0 and 3:2 parts of water:air at similar number of fish in the container (table 2), lower pH was reached in all simulated transports performed with 5:0 water to air, indicating the possibility of some CO_2 diffusion from water to air. If fish were to be under some kind of respiratory restriction due to hypercapnia, the application of an air phase, facilitating CO_2 diffusion from water to air, could have the possibility to alleviate fish from this. However, at a given stocking density the presence of an air phase did not alter transport time (figure 8). This would indicate that 1) if there actually was hypercapnic CO_2 levels present in the water, a 20 L air phase would not contribute to any respiratory improvement to the fish, or 2) that the levels of CO_2 present in the container holding 5:0 parts water:air did not pose any restrictions on fish respiratory functions and that the adding of an air phase would only reduce transport time by removing oxygen availability to the fish.

The application of a CO_2 absorbent (i.e. carbonate ceramics) should be included in future studies on transport of snow-trout in closed systems to investigate the effect of elevated water CO_2 on fish oxygen consumption.

Water ammonia

The toxic component ammonia is the un-ionized ammonium NH_3. Due to its low gill permeability, water NH_4 increases poses minor harm to fish health. The pK for NH_4^+ / NH_3 is 9.5, thus the proportion of toxic NH_3 increases with pH in which can have detrimental effects if high concentrations are reached, having the potential of causing neural necrosis in the brain (*18*). Acute toxic levels of ammonia in freshwater fish is 2.8 mg NH_3 L^{-1} (*18*). A 24 hour LC50 of NH_3 on Atlantic salmon smolts has been documented to 0.15 mg NH_3 L^{-1} at oxygen levels equal to air saturation, but decrease to 0.09 mg NH_3 L^{-1} when lowering oxygen saturation to DO_2 = 3.5 mg L^{-1} (*87*). However, due to CO_2 accumulation during transport in closed systems water pH increase is prohibited (*15*).

During simulated transport of snow-trout in a closed container, water ammonia concentrations reached $15x10^{-4}$ mg NH_3 L^{-1} when transported at highest stocking density of 0.016 kg L^{-1}. At lowest stocking density (0.003 kg L^{-1}), ammonia concentration at end of transport was $8.7x10^{-4}$ mg NH_3 L^{-1}. At other densities, ammonia concentrations fell within this range. Due to that snow-trout is transported in a closed container, the accumulation of carbon dioxide caused lowering in pH. The

higher proportion of ammonium is favored with decreases in pH, causing low levels of ammonia. It is therefore concluded that increases in ammonia concentrations during closed transport of snow-trout are negligible in terms of toxicity to the fish.

Possible time of transport

Total transport time, defined by the time for dissolved oxygen in the closed container to drop 5 units, decreased with increasing stocking density, with both fingerling strains showing similar reduction trend. When performing simulated transport of Trishuli fingerlings at stocking densities of 0.003 to 0.016 kg L^{-1}, transport time decreased from 11 ½ to 1 ½ h, whereas increasing the stocking density of Melamchi fingerlings from 0.0026 to 0.012 kg L^{-1} resulted in reduced total transport time from 11 to 1 ½ h. Because of the increased aggressive behavior, the higher MO_2 of the Melamchi fingerlings resulted in decreased transport time compared to the Trishuli strain at a given stocking density. Thus, despite higher stocking density obtained for the Trishuli fingerlings, both strains showed fairly similar total transport time. As a result, when transporting snow-trout fingerlings, higher biomass can be transported for equal duration when transporting the Trishuli strain compared to Melamchi fingerling transportation.

Two functions were derived from transport time of the two strains different stocking densities, estimated in order to predict possible transport time at any given strain stocking density. For practical applicability, functions were rearranged in order to estimate maximum stocking density at approximately 14 °C for any desired transport time.

RED ZEBRA (METRIACLIMA ESTHERAE)

The strict ranking system within a Red Zebra cichlid group may release aggressive behavior and increase oxygen expenditure if new individuals are added. Accordingly, this study tried to eliminate or limit such behavior as all experiments were performed with individuals pre-habituated to each other.

Oxygen consumption in a closed system

During the initial A phase of closed simulated transport, the average single Red Zebra oxygen consumption was 200 mg O2 kg^{-1} h^{-1}. As these individuals progressed into steady C phase, average MO_2 was down to 80 mg O2 kg^{-1} h^{-1}, indicating that these single individuals had settled down. The comparable results for simulated group transports were 325 and 135 mg O2 kg^{-1} h^{-1}, respectively. Thus, after initially displaying high activity the individual group members also settled down. Still, the average weight specific oxygen use was almost 70 % higher for group than for single transport. This is consistent to video monitoring showing bursts of increased swimming activity of the group. The rates discussed are reflected in the total oxygen consumption (TOC) seen for single individual and group after the first 60 minutes of closed simulated transport, being 150 and 240 mg O_2 kg^{-1} h^{-1}, respectively.

O_2 availability improvement

Sedatory impacts on Red Zebra oxygen consumption

During A phase, pre-anesthetic treatment of single individuals for a following exposure to a non-sedative concentration (0 mg L^{-1}) during transport resulted in oxygen consumption of 150 mg O_2 kg^{-1} h^{-1}. This compares to what seen during 10 mg L^{-1} exposure. However, sedative concentrations of 30 and 50 mg L^{-1} resulted in slightly lower MO_2 of 120 mg O_2 kg^{-1} h^{-1}. As pre-anesthetized single individuals progressed into C phase fish exposed 0 mg L^{-1} MS-222 showed MO_2 of 130 mg O_2 kg^{-1} h^{-1}. When a sedative concentration of 10, 30 or 50 mg L^{-1} was added to transport container, single fish consumed oxygen at rates of 110, 75 and 90 mg O_2 kg^{-1} h^{-1}, respectively. A pre-anesthetic treatment of a following transport in 0 and 30 mgL-1 resulted in significant lower ($P < 0.05$ Tukey HSD) MO_2 for sedated fish.

During A phase groups exposed to an anesthetic treatment showed, all though slightly higher, rates of oxygen consumption comparable to what seen during single fish transport. However, as group individuals progressed into C phase, the group exposed to 0 mg L-1 MS-222 showed MO_2 of 150 mg O_2 kg^{-1} h^{-1}. Apart from control, this was the only group that showed consumption of oxygen higher

than its respective single fish treatment during C phase. At higher sedative concentrations all groups showed MO_2 equal or lower to the single fish exposed to same concentrations.

As a result, after 60 minutes of single Red Zebra transport, total oxygen consumed at 0 and 10 mg L^{-1} MS-222 was 150 – 160 mg O_2 kg^{-1}, compared to during 30 and 50 mg L-1 exposure showing oxygen consumption of 100 – 110 mg O_2 kg^{-1}.However, during group transport TOC reduced linearly with increasing sedative concentration from 0 to 50 mg L^{-1} by 175 to 90 mg O_2 kg^{-1}.

Further, during a 2 ½ hour simulated transport of groups of Red Zebra, the total oxygen consumed was 420 mg O_2 kg^{-1} at transport in a non-sedative concentration. This was reduced to 300 mg O_2 kg^{-1} when adding 10 mg L-1 MS-222 to the transport water. A further reduction to 245 mg O_2 kg^{-1} was witnessed upon exposure to 30 mg L^{-1}. When compared to the untreated transport control group, showing a total consumption of 475 mg O_2 kg^{-1}, all MS-222 treated groups showed a lower consumption. The 50 % reduction of total oxygen consumed seen between the untreated control group and transport in a 30 mg L^{-1} MS-222 clearly illustrates the efficacy of MS-222 to reduce oxygen consumption during transport of Red Zebra.

Hence the aggressive behavior common for the cichlid fish, it is likely that the increasing sedative concentration during transport may cause less interaction between individuals within the group, as a result of lower swimming activity. Thus, high difference in MO_2 and TOC_{60} seen between single and group transport of control fish is greatly reduced when adding a sedative concentration to the transport container. This is consistent to what seen during video monitoring at exposure to a non and 30 mg L^{-1} sedative concentration following pre-anesthesia. It is concluded that MS-222 is considered to be efficient in reducing increased oxygen consumption during group transportation by reducing swimming activity in Red Zebra.

There is sparse knowledge of MS-222 effect on fish oxygen consumption. However, many authors have studied fish blood responses during or after MS-222 exposure.

Sedatory impacts on blood chemistry

Hematological response

Assessing hematological measurements would provide indications of fish oxygenation status by its primary role in oxygen transport. A significant decrease in hemoglobin concentration from 8 to 5.5 g $100ml^{-1}$ was seen in Red Zebra after 2 ½ hour exposure to a sedative concentration of 30 mg L^{-1} MS-222. On the contrary, after a two hour simulated transport of gilthead sea bream in a MS-222 sedative solution of 25 mg L^{-1}, Gonzales et al. (1995) found elevated hemoglobin values of 6 g $100ml^{-1}$ significantly higher than controls of 4.75 g $100ml^{-1}$, seen despite that oxygen levels were kept equal to air saturation (*83*). The increases was argued to result from decreased gill ventilation causing functional hypoxia to the fish (*88, 89*). Further, studies on MS-222 anesthesia on fish has shown to decrease arterial oxygen saturation (*90*), thus the present finding on Red Zebras decreased Hb levels upon MS-222 exposure seems illogical.

Higher Hb values measured in control groups could be a result of beta-adrenergic stimulated recruitment of erythrocytes, as an effect of sampling procedures. Thus, the efficacy of MS-222 to reduce a stress response upon acute stressor exposure, arising during blood sampling procedure, would be illustrated through these findings. Therefore, the possible explanation to lower levels of Hb on sedated fish could relate to MS-222 inhibition of sympathetic nervous stimulatory affecting erythrocyte recruitment, otherwise rapidly increased when exposed to handling, as seen in untreated fish.

In order to compensate for hypoxic conditions, teleost fish regulate intracellular erythrocyte pH through beta-adrenergic stimulation of Na^+/H^+ antiporter, increasing cell volume and intracellular pH, resulting in a Bohr shift increasing Hb-O_2 affinity (*91*). The neural pathway activating catecholamine secretion is believed to be controlled by blood oxygenation status (*30*). Catecholamine secretion in teleost fish has shown to increase when fish experience a decline in blood oxygen to about 50 – 60 % saturation (*92-94*). The reduction of mobility and sensory perception by MS-222 exposure is documented as a result of its binding to specific sites in voltage-gated Na^+-channels blocking Na^+ flow currents (*95*). Speculation rises whether or not MS-222 affects the current of Na^+ through the Na^+/H^+ antiporter or the catecholamic stimulated recruitment of erythrocytes from the spleen. If so, MS-222 would have the potential of affecting hematological compensatory adjestments upon hypoxia. Before recommending the use of MS-222 sedation in order to prolong transport time, one has to verify its possible impact of oxygen content in the blood returning from the gills.

Lactate

Oxygen delivery to the tissue insufficient of meeting metabolically demands from carbohydrate metabolism would be indicated by increases in plasma lactate levels. Plasma lactate is affected by fish capture for following transport and transport itself and has shown continue to increase following exercise (*96*). Even though insignificant from controls, a rise in mean plasma lactate of 60 – 70 % for both transport group compared to non transport controls indicate that this was the case. Thus, it is indicated that both transport with and without the use of an anesthetic causes metabolic demands that succeed O_2 delivery capacity of the fish during some stage during transport.

Sodium

Sodium ion concentration was measured due to its osmoregulatory role and indicator of osmolytical status in fishes. At stressor exposure, higher secretion of circulating levels of catecholamines results in increased perfusion of gill lamella, thus increasing the amount of blood in contact with the thin respiratory surface of the gills. This is often mediated by the demand of increased blood oxygenation (*41*). Due to the respiratory compromise, freshwater fish therefore experience increased passive ion loss and increased water influx during stressor exposure. When performing MS-222 treated simulated transport, sodium concentrations was lower (slightly insignificant by $p = 0.063$), when compared to fish not exposed to transport. Sedated fish had mean sodium concentration also lower than fish exposed to untreated transport. This indicates that MS-222 produce a lowering of blood sodium by increasing electrolyte loss or increasing water diffusion into the blood.

Glucose

Compensating the loss of electrolytes during sustained stress, has shown to be mediated through the release of plasma cortisol. Cortisol plays an osmoregulatory role, stimulating increased activity of ion-transporting enzyme Na+/K+-ATPase. When performing 2 ½ hour transport of Red Drum in a 25 mg L^{-1} MS-222 sedative solution, following a pre-anesthetic treatment of 80 mg L^{-1}, Robertson et al. (1987) documented elevated blood glucose concentrations in both exposure and control group at end of transport, with slightly higher levels in sedative exposed fish (*10*). This compares to what is seen after a 2 ½ hour transport of Red Zebra. Further, after 2 hours of simulated transport of gilthead sea bream, Gonzales et al. (1995) found MS-222 concentration dependent increases in cortisol and glucose (*83*). Authors suggested the possible role of hypoxia-induced increased cortisol and glucose, indicated by elevated hemoglobin and hematocrit values. This further contributes to the suggestion that MS-222 acts as an asphyxiant on fish resulting in a stress response.

Mean concentration values of both transport groups are significantly higher than controls. These findings may therefore suggest that sedation can induce hypoxic stimulatory release of cortisol with accompanying increase of glucose, despite that water oxygen levels were close to air saturation.

CONCLUSIONS

1a. Simulated snow-trout fingerling transport demonstrated a strain related stocking density dependent increase in weight specific oxygen consumption. This dependency was probably due to difference in the aggressive behavior between strains.

1b. Simulated Red Zebra group transport demonstrated higher weight specific oxygen consumption as compared to single fish. Video monitoring verified increased swimming activity of fish when stocked in group.

2. Snow-trout and Red Zebra oxygen consumption of a can be reduced if sedated during transport, which can prolong possible transport time.

3. Sedating Red Zebra during simulated transport caused changes in plasma sodium ion, glucose and lactate concentrations. This may imply circulatory changes indicating hypoxic conditions and should therefore be investigated before sedation of such fish during transport is recommended.

PERSPECTIVES

Future experiments of oxygen consumption during real back-pack transportation are necessary to determine the effect of transport on oxygen consumption under real conditions, and further how the use of MS-222 could contribute to decrease elevations in oxygen consumption, at concentrations similar as to what used in this study.

Further, during future studies on snow-trout transport in closed systems the use of a CO_2 absorbent should be included. This would document any effect from increased progression of CO_2 accumulation on fish oxygen consumption, present at higher stocking density transport.

The strain dependent increase in MO_2 seen during simulated transport of snow-trout fingerlings should be further investigated. This could be performed by comparing single individual snow-trout MO_2 of both strains with MO_2 during simulated transport of the two strains and a 50/50 mixing of the strains, all at similar stocking density.

Furthermore, in order to relate the oxygen consumption at different stocking densities and sedative treatments to that of an unstressed individual, oxygen consumption of fish not exposed to handling or transfer should be obtained. This would provide a comparable basis necessary to determine the effect of a stressor on post-stress oxygen consumption.

REFERENCES

1. D. E. Portz, C. M. Woodley, J. J. Cech, Stress-associated impacts of short-term holding on fishes. *Rev Fish Biol Fisher* **16**, 125 (May, 2006).
2. UN, "The Millennium Goals Report 2013" (United Nations, New York, 2013).
3. F. F. a. A. Department, F. I. Division, Ed. (FAO, Rome, 2000).
4. F. F. a. A. Department, The State of World Fisheries and Aquaculture 2012. (2012).
5. P. Starkey, *Improving rural mobility : options for developing motorized and nonmotorized transport in rural areas*. World Bank technical paper, (World Bank, Washington, D.C., 2002), pp. vi, 64 p.
6. FAO, R. Berka, Ed. (1986), pp. 79.
7. K. B. Davis, N. C. Parker, Plasma Corticosteroid Stress Response of 14 Species of Warmwater Fish to Transportation. *T Am Fish Soc* **115**, 495 (May, 1986).
8. L. G. Ross, B. Ross, Anaesthetics & Sedative Techniques for aquatic animals (2.ed). *Blackwell Science Ltd., Oxford*, 157 (1999).
9. C. R. Weirich, J. R. Tomasso, Confinement-Induced and Transport-Induced Stress on Red Drum Juveniles - Effect of Salinity. *Prog Fish Cult* **53**, 146 (Jul, 1991).
10. L. Robertson, P. Thomas, C. R. Arnold, Plasma-Cortisol and Secondary Stress Responses of Cultured Red Drum (Sciaenops-Ocellatus) to Several Transportation Procedures. *Aquaculture* **68**, 115 (Feb 1, 1988).
11. R. Berka, in *EIFAC technical papers 48*. (FAO, Rome, Italy, 1986).
12. J. A. Grottum, M. Staurnes, T. Sigholt, Effect of oxygenation, aeration and pH control on water quality and survival of turbot, Scophthalmus maximus (L), kept at high densities during transport. *Aquac Res* **28**, 159 (Feb, 1997).
13. U. Erikson, T. Sigholt, A. Seland, Handling stress and water quality during live transportation and slaughter of Atlantic salmon (Salmo salar). *Aquaculture* **149**, 243 (Mar 31, 1997).
14. L. C. Gomes, J. I. Golombieski, A. R. Chippari-Gomes, B. Baldisserotto, Effect of salt in the water of transport on survival and Na^+ and K^+ body levels in fingerlings of silver catfish Rhamdia quelen (Pimelodidae). *Journal of Applied Aquaculture* **9**, 1 (1999).
15. T. S. Harmon, Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Rev Aquacult* **1**, 58 (Mar, 2009).
16. G. A. Wedemeyer. (Chapman and Hall, New York, 1996).
17. G. J. Carmichael, R. M. Jones, J. C. Morrow, Comparative Efficacy of Oxygen Diffusers in a Fish-Hauling Tank. *Prog Fish Cult* **54**, 35 (Jan, 1992).
18. D. J. Randall, T. K. N. Tsui, Ammonia toxicity in fish. *Mar Pollut Bull* **45**, 17 (2002).
19. M. Maricondi-Massari, A. L. Kalinin, M. L. Glass, F. T. Rantin, The effects of temperature on oxygen uptake, gill ventilation and ECG waveforms in the Nile tilapia, Oreochromis niloticus. *J Therm Biol* **23**, 283 (Oct, 1998).
20. J. Colt, Computation of dissolved gas concentrations in water as functions of temperature, salinity, and pressure. . *American Fisheries Society* **Special Publication No. 14, Bethesda, MD.**, (1984).
21. M. Jobling. (Chapman & Hall, London, 1994).
22. D. Randall, C. Daxboeck, Oxygen and Carbon-Dioxide Transfer across Fish Gills. *Fish Physiol* **10**, 263 (1984).
23. J. R. Brett, Metabolic Demand for Oxygen in Fish, Particularly Salmonids, and a Comparison with Other Vertebrates. *Resp Physiol* **14**, 151 (1972).

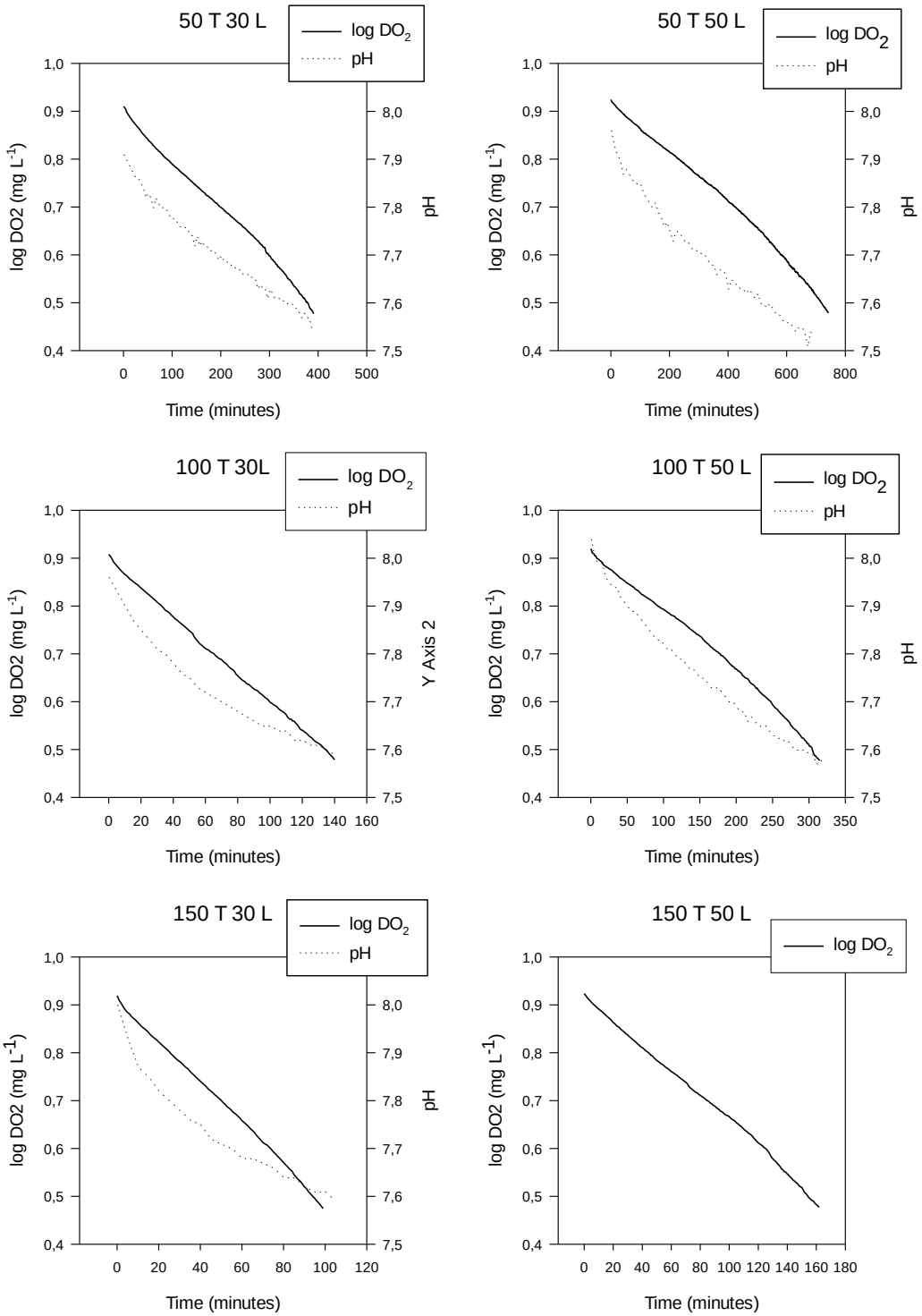
24. D. H. Evans, P. M. Piermarini, K. P. Choe, The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiol Rev* **85**, 97 (Jan, 2005).
25. M. N. Fernandes, *Fish respiration and environment*. (Science Publishers, Enfield, NH, 2007), pp. xvi, 392 p.
26. D. H. Evans, *The Physiology of fishes*. CRC marine science series (CRC Press, Boca Raton, 1993), pp. 592 p.
27. R. G. Boutilier, T. A. Heming, G. K. Iwama, Appendix - Physicochemical Parameters for Use in Fish Respiratory Physiology. *Fish Physiol* **10**, 403 (1984).
28. K. Yamamoto, Y. Itazawa, H. Kobayashi, Direct Observation of Fish Spleen by an Abdominal Window Method and Its Application to Exercised and Hypoxic Yellowtail. *Jpn J Ichthyol* **31**, 427 (1985).
29. R. M. G. Wells, R. E. Weber, The spleen in hypoxic and exercised rainbow trout. *J Exp Biol* **150**, (1990).
30. S. F. Perry, R. Kinkead, P. Gallaugher, D. J. Randall, Evidence That Hypoxemia Promotes Catecholamine Release during Hypercapnic Acidosis in Rainbow-Trout (Salmo-Gairdneri). *Resp Physiol* **77**, 351 (Sep, 1989).
31. J. G. Richards, A. P. Farrell, C. J. Brauner, *Hypoxia*. A. P. Farrell, C. J. Brauner, Eds., Fish Physiol (Academic Press, 2009), vol. 27.
32. G. K. Iwama, Stress in fish. *Stress of Life* **851**, 304 (1998).
33. G. P. Chrousos, P. W. Gold, The Concepts of Stress and Stress System Disorders - Overview of Physical and Behavioral Homeostasis. *Jama-J Am Med Assoc* **267**, 1244 (Mar 4, 1992).
34. Chrousos, Stressors, Stress, and Neuroendocrine Integration of the Adaptive Response. *Annals of the New York Academy of Sciences* **851**, 355 (1998).
35. B. a. Iwama, Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Rev. of Fish Deseases*, 26 (1991).
36. H. Selye, Stress and the General Adaptation Syndrome. *Brit Med J* **1**, 1383 (1950).
37. G. A. Wedemeyer, B. A. Barton, D. J. McLeay, *Stress and acclimation*. C. B. Schreck, P. B. Moyle, Eds., Methods for Fish Biology (American Fisheries Society, Bethesda, MD, 1990).
38. T. G. Pottinger, P. H. M. Balm, A. D. Pickering, Sexual Maturity Modifies the Responsiveness of the Pituitary-Interrenal Axis to Stress in Male Rainbow-Trout. *Gen Comp Endocr* **98**, 311 (Jun, 1995).
39. M. M. Mazeaud, F. Mazeaud, in *Stress and fish,* A. D. Pickering, Ed. (Academic Press, New York, 1981), pp. 49 - 75.
40. A. L. Lehninger, D. L. Nelson, M. M. Cox, *Lehninger principles of biochemistry*. (W.H. Freeman, New York, ed. 5th, 2008).
41. S. E. W. Bonga, The stress response in fish. *Physiol Rev* **77**, 591 (Jul, 1997).
42. D. M. Broom, Animal-Welfare - Concepts and Measurement. *J Anim Sci* **69**, 4167 (Oct, 1991).
43. P. J. Ashley, Fish welfare: Current issues in aquaculture. *Appl Anim Behav Sci* **104**, 199 (May, 2007).
44. F. A. Huntingford *et al.*, Current issues in fish welfare. *J Fish Biol* **68**, 332 (Feb, 2006).
45. F. S. Conte, Stress and the welfare of cultured fish. *Appl Anim Behav Sci* **86**, 205 (Jun, 2004).
46. L. U. Sneddon, V. A. Braithwaite, M. J. Gentle, Novel object test: Examining nociception and fear in the rainbow trout. *J Pain* **4**, 431 (Oct, 2003).
47. G. K. Iwama, The welfare of fish. *Dis Aquat Organ* **75**, 155 (May 4, 2007).
48. J. D. Rose, The Neurobehavioral nature of fishes and the question of awareness and pain. *Rev Fish Sci* **10**, 1 (2002).
49. A. B. Lawrence, in *Fish Welfare,* E. Branson, Ed. (John Wiley & Sons, 2008).
50. G. N. Wagner, T. D. Singer, R. S. McKinley, The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (Oncorhynchus mykiss Walbaum). *Aquac Res* **34**, 1139 (Nov 4, 2003).

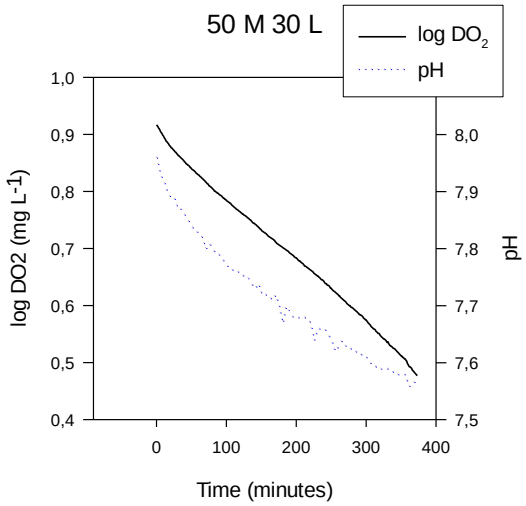
51. M. Spath, W. Schweickert, Effect of Metacaine (Ms-222) on Activity of Efferent and Afferent Nerves in Teleost Lateral-Line System. *N-S Arch Pharmacol* **297**, 9 (1977).
52. D. E. W. Arnolds *et al.*, Physiological effects of tricaine on the supramedullary/dorsal neurons of the cunner, Tautogolabrus adspersus. *Biol Bull* **203**, 188 (Oct, 2002).
53. T. Brattelid, *Compendium in Labotory Animal Science for Fish Researchers*. T. Brattelid, A. J. Smith, Eds., (Norwegian School of Veterinary Science & Norecopa, 2011).
54. K. M. Carter, C. M. Woodley, R. S. Brown, A review of tricaine methanesulfonate for anesthesia of fish. *Rev Fish Biol Fisher* **21**, 51 (Mar, 2011).
55. E. A. B. Brown, E. G. Trams, E. Pratt, J. E. Franklin, Contributions to Pharmacology of Quinaldine (Uptake and Distribution in Shark and Comparative Studies). *Comp Biochem Physiol* **42**, 223 (1972).
56. D. L. Neiffer, M. A. Stamper, Fish Sedation, Anesthesia, Analgesia, and Euthanasia: Considerations, Methods, and Types of Drugs. *Ilar J* **50**, 343 (2009).
57. W. N. McFarland, in *Publications of the Institute of Marine Science* (University of Texas, 1959), vol. 6, pp. 431 - 440.
58. R. A. Schoettger, A. M. Julin, "Efficacy of MS-222 as an anesthetic on four salmonids" (Washington D.C., 1967).
59. J. F. Burka *et al.*, Drugs in salmonid aquaculture - A review. *J Vet Pharmacol Ther* **20**, 333 (Oct, 1997).
60. K. Bressler, B. Ron, Effect of anesthetics on stress and the innate immune system of gilthead seabream (Sparus aurata). *Isr J Aquacult-Bamid* **56**, 5 (Mar, 2004).
61. T. C. Crosby, J. E. Hill, C. A. Watson, R. P. E. Yanong, R. Strange, Effects of tricaine methanesulfonate, hypno, metomidate, quinaldine, and salt on plasma cortisol levels following acute stress in threespot gourami Trichogaster trichopterus. *J Aquat Anim Health* **18**, 58 (Mar, 2006).
62. B. C. Small, N. Chatakondi, Routine measures of stress are reduced in mature channel catfish during and after AQUI-S anesthesia and recovery. *N Am J Aquacult* **67**, 72 (Jan, 2005).
63. S. J. Cooke, C. D. Suski, K. G. Ostrand, B. L. Tufts, D. H. Wahl, Behavioral and physiological assessment of low concentrations of clove oil anaesthetic for handling and transporting largemouth bass (Micropterus salmoides). *Aquaculture* **239**, 509 (Sep 30, 2004).
64. P. Hoskonen, J. Pirhonen, The effect of clove oil sedation on oxygen consumption of six temperate-zone fish species. *Aquac Res* **35**, 1002 (Aug 20, 2004).
65. N. T. Popovic *et al.*, Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *J Appl Ichthyol* **28**, 553 (Aug, 2012).
66. J. B. Hunn, J. L. Allen, Movement of Drugs across Gills of Fishes. *Annu Rev Pharmacol* **14**, 47 (1974).
67. F. J. Carmichael, in *Principles of medical pharmacology,* H. Kalant, W. H. E. Roschlau, E. M. Sellers, Eds. (Department of Pharmacology, University of Toronto, Toronto ,Ontario, 1985), vol. 4th edn, pp. 265 - 289.
68. L. U. Sneddon, Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, Oncorhynchus mykiss. *Neurosci Lett* **319**, 167 (Feb 22, 2002).
69. H. Hensel, B. Bromm, K. Nier, Effect of Ethyl Meta-Aminobenzoate (Ms-222) on Ampullae of Lorenzini and Lateral-Line Organs. *Experientia* **31**, 958 (1975).
70. S. C. Kestin, S. B. Wotton, N. G. Gregory, Effect of Slaughter by Removal from Water on Visual Evoked Activity in the Brain and Reflex Movement of Rainbow-Trout (Oncorhynchus-Mykiss). *Vet Rec* **128**, 443 (May 11, 1991).
71. L. A. Brown, in *Fish Medicine,* M. K. Stoskopf, Ed. (W.B. Saunders Company, Philadelphia, Pennsylvania, 1993), pp. 79 - 90.
72. K. B. Davis, N. C. Parker, M. A. Suttle, Plasma Corticosteroids and Chlorides in Striped Bass Exposed to Tricaine Methanesulfonate, Quinaldine, Etomidate, and Salt. *Prog Fish Cult* **44**, 205 (1982).

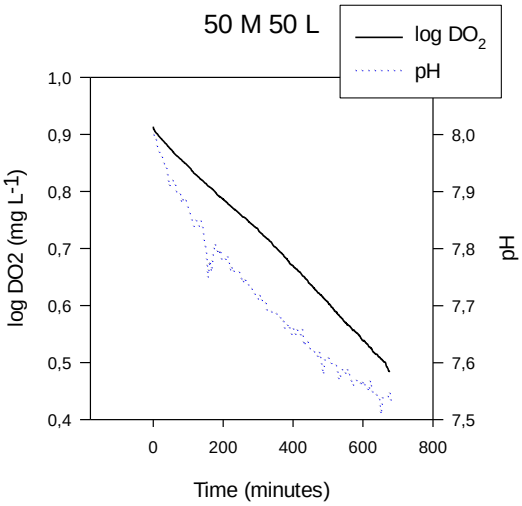
73. Ø. P. Hveding, NTNU, Norwegian University of Science and Technology (2009).
74. S. Ims, NTNU, Norwegian University of Scienve and Technology (2011).
75. J. F. Steffensen, Some Errors in Respirometry of Aquatic Breathers - How to Avoid and Correct for Them. *Fish Physiol Biochem* **6**, 49 (Jan, 1989).
76. K. Emerson, R. C. Russo, R. E. Lund, R. V. Thurston, Aqueous Ammonia Equilibrium Calculations - Effect of Ph and Temperature. *J Fish Res Board Can* **32**, 2379 (1975).
77. A. B. Tabinda *et al.*, Rate of Oxygen Cosumption in Fingerlings of Major Carps at Different Temperatures. *Pakistan Journal of Biological Sciences* **6**, 1535 (2003).
78. J. R. Brett, Environmental factors and growth. *Fish Physiol* **8**, 599 (1979).
79. J. T. Cook, M. A. McNiven, A. M. Sutterlin, Metabolic rate of pre-smelt growth-enhanced transgenic Atlantic salmon (Salmo salar). *Aquaculture* **188**, 33 (Aug 1, 2000).
80. A. Clarke, N. M. Johnston, Scaling of metabolic rate with body mass and temperature in teleost fish. *J Anim Ecol* **68**, 893 (Sep, 1999).
81. D. R. T. Sampson, D. J. Macintosh, Transportation of Live Carp Fry in Sealed Polythene Bags. *Aquaculture* **54**, 123 (May 15, 1986).
82. J. R. Sylvester, L. E. Holland, Influence of Temperature, Water Hardness, and Stocking Density on Ms-222 Response in 3 Species of Fish. *Prog Fish Cult* **44**, 138 (1982).
83. A. Molinero, J. Gonzalez, Comparative Effects of Ms-222 and 2-Phenoxyethanol on Gilthead Sea Bream (Sparus-Aurata L) during Confinement. *Comp Biochem Phys A* **111**, 405 (Jul, 1995).
84. J. N. Cameron, Regulation of Blood-Ph in Teleost Fish. *Resp Physiol* **33**, 129 (1978).
85. D. J. Randall, N. Heisler, F. Drees, Ventilatory Response to Hypercapnia in Larger Spotted Dogfish Scyliorhinus-Stellaris. *American Journal of Physiology* **230**, 590 (1976).
86. A. P. CruzNeto, J. F. Steffensen, The effects of acute hypoxia and hypercapnia on oxygen consumption of the freshwater European eel. *J Fish Biol* **50**, 759 (Apr, 1997).
87. J. S. Alabaster, D. G. Shurben, G. Knowles, Effect of Dissolved-Oxygen and Salinity on the Toxicity of Ammonia to Smolts of Salmon, Salmo-Salar L. *J Fish Biol* **15**, 705 (1979).
88. G. D. Campbell, D. H. Davis, Effect of ethyl m-Aminobenzoate (MS 222) on the elasmobranch electrocardigraph. *Nature* **198**, (1963).
89. D. J. Randall, L. S. Smith, V. R. Brett, Dorsal aortic blood pressures recorded from the rainbow trout (*Salmo gairdneri)*. *Canadian Journal of Zoology* **43**, 863 (1965).
90. A. Soivio, K. Nyholm, M. Huhti, Effects of anaesthesia with MS 222, neutralized MS 222 and benzocaine on the blood constituents of rainbow trout, *Salmo gairdneri*. *J Fish Biol* **10**, 91 (1977).
91. F. B. Jensen, Red blood cell pH, the Bohr effect, and other oxygenation-linked phenomena in blood O-2 and CO2 transport. *Acta Physiol Scand* **182**, 215 (Nov, 2004).
92. S. F. Perry, S. G. Reid, Relationships between blood oxygen content and catecholamine levels during hypoxia in rainbow trout and Amercan eel. *American Journal of Physiology* **263**, 240 (1992).
93. S. F. Perry, S. G. Reid, The Effects of Acclimation Temperature on the Dynamics of Catecholamine Release during Acute-Hypoxia in the Rainbow-Trout Oncorhynchus-Mykiss. *J Exp Biol* **186**, 289 (Jan, 1994).
94. S. Thomas, S. F. Perry, Control and Consequences of Adrenergic Activation of Red-Blood-Cell Na+/H+ Exchange on Blood-Oxygen and Carbon-Dioxide Transport in Fish. *J Exp Zool* **263**, 160 (Aug 15, 1992).
95. C. Nau, G. K. Wang, Interactions of local anesthetics with voltage-gated Na+ channels. *J Membrane Biol* **201**, 1 (Sep 1, 2004).
96. E. C. Black, I. Barrett, Increase in levels of lactic acid in the blood of cutlthroat and steelhead trout following handling and live transportation. *Canadian Fish Cultivist* **20**, (1957).

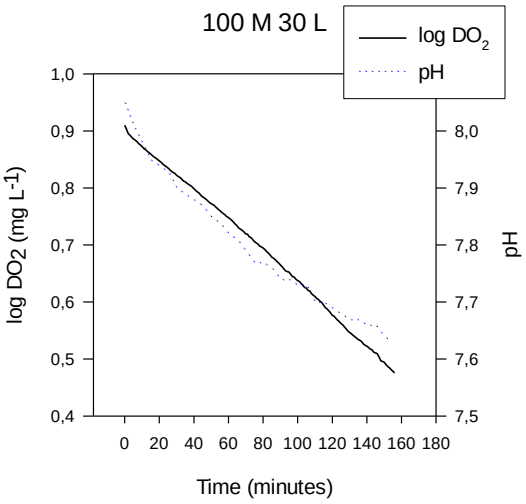
APPENDIX

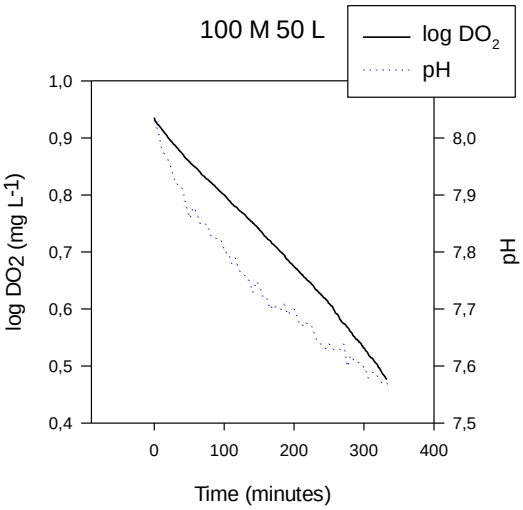
DO_2 and pH during transport of snow-trout

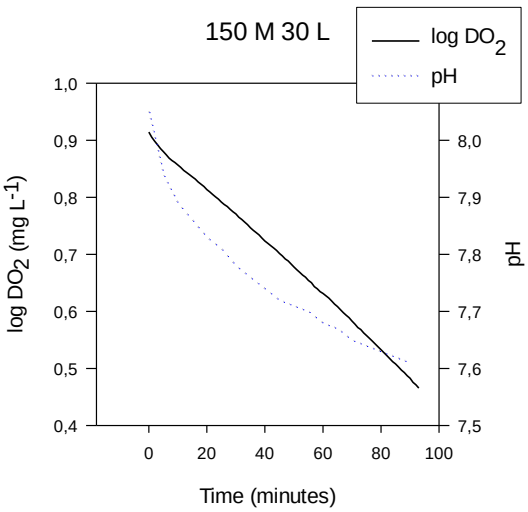


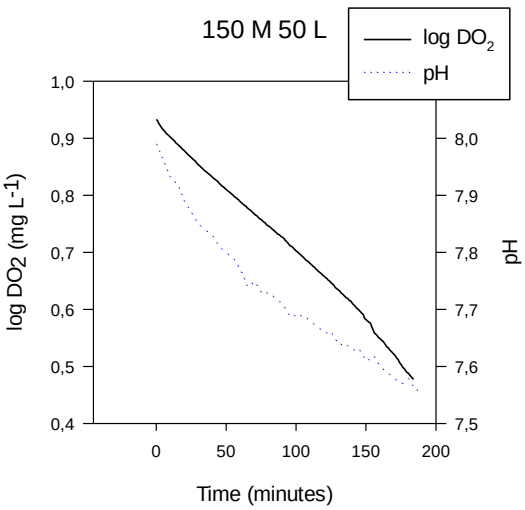

50 M 30 L
log DO2
pH
log DO2 (mg L-1)
pH
Time (minutes)


50 M 50 L
log DO2
pH
log DO2 (mg L-1)
pH
Time (minutes)


100 M 30 L
log DO2
pH
log DO2 (mg L-1)
pH
Time (minutes)

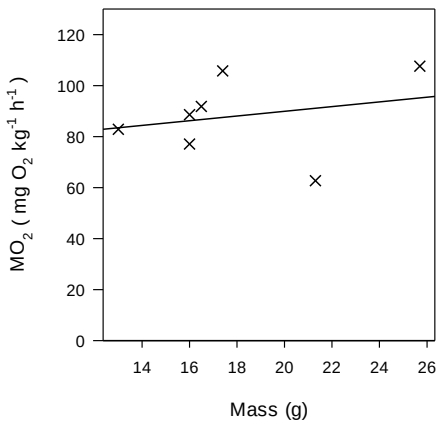

100 M 50 L
log DO2
pH
log DO2 (mg L-1)
pH
Time (minutes)


150 M 30 L
log DO2
pH
log DO2 (mg L-1)
pH
Time (minutes)

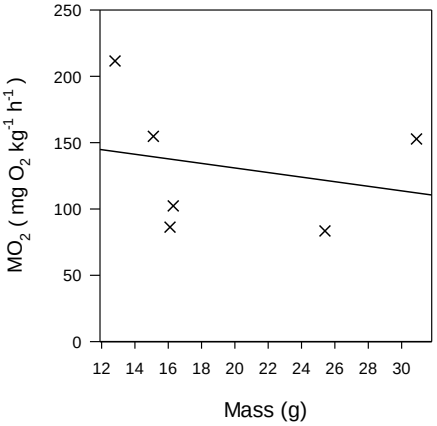

150 M 50 L
log DO2
pH
log DO2 (mg L-1)
pH
Time (minutes)

Red Zebra rate of oxygen consumption at MS-2222 sedative treatments

0 -- 0



150 -- 0



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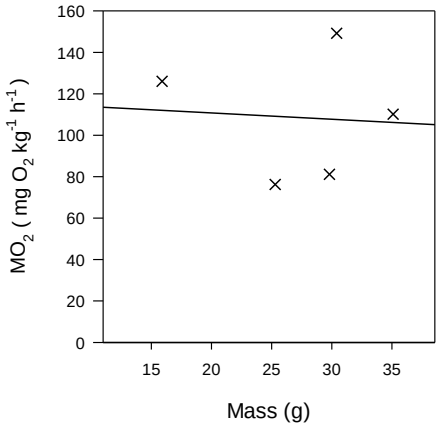

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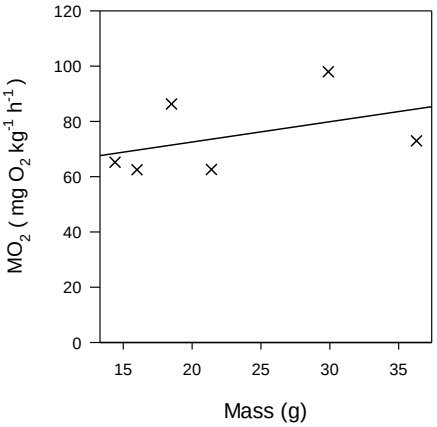

150 -- 50