Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety

14 May 2008

Transportation of fish within a closed system

Contents

Summary

There are no exact statistics for transportation of farmed fish, but fish are at least transported twice during their lifetime; once from hatchery to growing sites and secondly to slaughter stations. Thus, the production data of farmed fish gives an indication of the amount of fish being transported. In 2007, Norway produced 750.000 tons Atlantic salmon, 80.000 tons rainbow trout and 12.000 tons Atlantic cod (Directorate of Fisheries 2007). The number of transported fish is increasing mainly due to increased production and to centralisation of slaughter houses. In Norway, well boats are the most common way to transport fish, but trucks are also used for transportation of fingerlings and smolt. Transportation of live fish by air is less common for economical reasons.

Recently, regulations and restrictions due to occurrence of infectious diseases have to some extent required that more fish have to be transported in closed systems. Regulations concerning transportation of aquatic animals are presently being evaluated by the Norwegian Food Safety Authority.

The Norwegian Food Safety Authority asked the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) to conduct a risk assessment on the transportation of fish in a closed system and have put 5 specific questions to the committee. The questions to be answered were:

1) Which circumstance or circumstances might put the fishes´ welfare at risk? If several such circumstances exist, do these evolve independently of one another or do they influence one another in any manner?

2) Do problems occur after a specific period of time in the transport container, or will the time lapse before the onset of problems be dependent on various specific factors?

3) Is it possible to prevent the occurrence of circumstances that compromise the welfare of the fish? If so, outline the necessary or relevant measures applicable.

4) Does the reduction in water temperature in itself have a negative welfare implication? Could the reduction in water temperature result in the reactions of the fish becoming difficult or impossible to observe, and thus make the interpretation of their welfare status more difficult?

5) Can the water temperature be reduced in such a manner that the welfare of the fish is not put at risk?

VKM appointed an *ad hoc* group consisting of both VKM members (Panel 8, Panel on Animal Health and Welfare) and external experts to answer the request from the Norwegian Food Safety Authority. The report from the *ad hoc* group has been evaluated and approved by Panel 8 of VKM.

The Panel on Animal Health and Welfare is of the opinion that transportation of life fish will always imply some kind of risk. The fitness of the fish will have great impact on the risks associated with transportation. Sick or immunocompromized fish will be extremely vulnerable and should not be transported. Care should be taken to minimize handling stress prior to and during transportation.

Open system transportation is generally considered to be less risky than transportation in closed systems. However, the risks connected with closed systems can to a greater extend be subject to calculations and modelling than risks in opens systems. This is because the open system is exposed to ambient environment and its natural fluctuations in temperature and changes in water chemistry.

Scientific data on large scale closed systems are few, and risk modelling depend to a large extend on empiric data from the industry and experiments conducted in laboratory facilities.

Transportation in closed systems requires water treatment, as well as systems for observation of fish and monitoring of water quality. Consequently, well trained crew and skills to handle emergency situations are needed.

The metabolism of the fish will influence the water chemistry and thus the welfare of the fish during transportation. With the exception of very young fish (larvae), all fish should be starved at least for a period of three to five days before as well as under transportation.

It is difficult to give exact upper safe limits for water chemistry parameters, since many of the parameters interact in a complicated manner. However, some figures derived from industry experience exist and they are included in this report. These values should be used with care and considered as guidelines.

Oxygen, carbon dioxide and TAN represent limiting factors during closed transports. While elevated carbon dioxide often is a first limiting factor in the transport water, it can be degassed by increased dimensions of water treatment system. There is, however, a risk that the gained improvement in water quality is used for optimising transport biomass, and thereby risks for elevated TAN and pH, and eventually toxic un-ionised ammonia. In closed transportations of salmon fingerlings and smolt, the concentration of CO_2 should be below 20 mg/L. At transportation of fish for slaughter with chilled water, the light sedation due to accumulation of CO_2 is considered beneficial. Thus, a higher concentration of CO_2 up to 30 mg could be accepted. This will depend on biomass, temperature and duration of transportation.

It is not possible to give an exact limit for the maximum transportation time in a closed system. This will depend on fish species, density, and temperature and water treatment in the transport unit. Transportation in closed systems should be as short as possible, both in distance and time, while extended transportations should be performed with reduced biomasses. By adding seawater to reach a salinity of 1 ‰, the buffer capacity in fresh water will increase and the risks associated with accumulation of CO_2 and mixing of different water qualities will to some extent be reduced. Necessarily treatment of the seawater must be applied.

Accumulation of CO_2 and TAN is faster in warmer water in the autumn when 0+ smolts are transported. The risk for the fish welfare problems is thus probably higher for transportation of 0+ smolts than in 1+ smolts due to weather and climatic conditions. Field data suggest that the toxicity of nitrogenous wastes and CO_2 can be reduced to a certain degree by keeping oxygen concentration around 100 %. The risk can be avoided by proper planning.

A lowering of the water temperature during transportation can reduce the impact of several factors on fish physiology and welfare. The temperature reduction must be carried out with great care and is recommended not to exceed 1, 5 °C/hr. For Atlantic salmon and rainbow trout, the transportation temperature should not aim to be below 6 °C.

Most of the information used in the assessment is either based on small scale laboratory studies or on industry derived experience. There is a clear need for more scientific based knowledge on most of the aspects related to transportation of live fish in closed systems.

1. Key words

Transport of fish, animal welfare, water quality, water chemistry parameters, well boats,

2. Contributors

Persons working for VKM, either as appointed members of the Committee or as *ad hoc*-experts, do this by virtue of their scientific expertise, not as representatives for his/her employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed an *ad hoc* group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority.

The members of the *ad hoc* group are:

VKM members

Brit Hjeltnes (Chair), Panel on Animal Health and Welfare

Rune Waagbø, Panel on Animal Health and Welfare

External experts:

Bengt Finstad, Norwegian Institute for Nature Research, NINA

Bjørn Olav Rosseland, Norwegian University of Life Sciences

Trond Rosten, Norwegian Institute for Water Research (NIVA)

Sigurd Stefansson, University of Bergen

3. Assessed by

The report from the *ad hoc* group has been evaluated and approved by Panel on Animal Health and Welfare (Panel 8) of VKM.

Panel on Animal Health and Welfare:
Wenche Farstad (chair), Bjarne O. Braastad, Knut E. Bøe, Arne Flåøyen, Brit Hjeltnes, Kristian Hoel, Tore Håstein, Espen Rimstad, Rune Waagbø and Olav Østerås

Scientific Coordinators from the Secretariat: Ingfrid Slaatto Næss

4. Acknowledgements

5. Terms of reference

The Norwegian Food Safety Authority requests that the following aspects be assessed in connection with transports operating with closed systems encompassing also those transports that occur with a reduction in water temperature and those that do not: There is a need to differentiate between transports that make use of fresh water and those that use sea water and also where it is deemed necessary, between transports in well boats and lorries. (It is possible for well boats to open their valves during transport. The resultant change in salinity needs only to occur in small pockets of transport water and such varying salinity is considered unfortunate from a fish welfare point of view.)

1) Which circumstance or circumstances might put the fishes´ welfare at risk? If several such circumstances exist, do these evolve independently of one another or do they influence one another in any manner?

2) Do problems occur after a specific period of time in the transport container, or will the time lapse before the onset of problems be dependent on various specific factors?

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5) Can the water temperature be reduced in such a manner that the welfare of the fish is not put at risk?

This commission is limited to Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic cod (*Gadus morhua*). Where knowledge exists concerning the species Atlantic halibut, (*Hippoglossus hippoglossus*), turbot (*Scophthalmus maximus*) and wolf fish (*Anarhichas minor*) it would be useful if this knowledge also is evaluated. The Norwegian Food Safety Authority expects that most of the scientific data concern salmon and rainbow trout, but it would be advantageous to know the risk attached to lorry transportations of turbot fry from Spain to Norway of long duration (3 days with chilled water temperatures).

6. Interpretation of the mandate

This assessment focuses on the transportation of Atlantic salmon, rainbow trout and Atlantic cod. Only limited information on other species is included. Transportation assessed is movements of live fish from hatcheries to nursery or on growing sites and for slaughter. Transportation of eggs is not

considered and minor transportation of fish within the hatchery is not assessed. Furthermore, traditional transport of fish for cultivation purposes carried out in sealed plastic bags with O2 and transportation of ornamental fish is not assessed. The main focus has been on Atlantic salmon since this is the most important cultured species in Norway. Transportation by air is not assessed in depth since there is limited information available. Definitions of welfare often have either a feeling-, nature- or function based approach (FSBI 2000, Fraser 2004, Duncan 2004). Agreement on how to correctly and best define welfare has shown to be difficult (Wolfrom T 2004). The function-based approach analyses the animals coping with the environment, or, as Broom (1986) quotes, "the welfare of an animal is its state as regards its attempts to cope with its environment". This definition is based on the assumption that the animal tries to maintain homeostasis, equilibrium, in its physiological system. It is mainly this approach that has been used in this report.

7. Background

There are no exact statistics for transportation of fish, but fish are at least transported twice during their lifetime; once from hatchery to growing sites and secondly to slaughter houses. Thus, the production data of farmed fish gives an indication of the amount of fish being transported. In 2007, Norway produced 750.000 tons Atlantic salmon, 80.000 tons rainbow trout and 12.000 tons Atlantic cod (Directorate of Fisheries 2007). The number of fish transported is increasing mainly due to increased production and to centralisation of slaughter stations. In Norway, well boats are the most common way to transport fish, but trucks are also used for transportation of fingerlings and smolt. Transportation of live fish by air is less common for economical reasons. Recently, regulations and restrictions due to occurrence of infectious diseases have to some extent required that more fish have to be transported in closed systems. Regulations for transportation of aquatic animals are presently being evaluated by the Norwegian Food Safety Authority.

The Norwegian Food Safety Authority asked the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) to conduct a risk assessment on the transportation of fish in a closed system. VKM appointed an *ad hoc* group consisting of both VKM members (Panel 8, Panel on Animal Health and Welfare) and external experts to answer the request from the Norwegian Food Safety Authority. The report from the *ad hoc* group has been evaluated and approved by Panel 8 of VKM.

All transportation of fish will to some extent induce stress, related to factors like changes in water chemistry, pH, temperature, foaming, loading and unloading, transportation time and biomass.

Boats and trucks used for transportation of live fish normally carry containers designed to keep the fish alive. In boats the live fish container is called a well, and this well is connected to a system of pipes and pumps for recirculation of transport water. While recirculation systems are common in well boats, these are also found in modern trucks. In the regulation (Mattilsynet 2007), the well, pumps and pipes are defined as the transport system, which also includes water oxygenation equipment. The regulation includes specific rules for approval, washing and disinfection of transport systems.

Both trucks and well boats will normally supply extra oxygen to the fish during loading, transport and unloading. Several systems for oxygen supply are used. Degassers for water carbon dioxide (CO_2) and nitrogen (N_2) are not standard equipment in all vessels, but are common on vessels specialized on closed transport. Ozone gas produced by a generator on-vessel seems to be the preferred solution for disinfection of large scale modern well boats. This is due to economical and efficiency considerations. A few well boats are equipped with refrigerated seawater (RSW) cooling system for the transport water. Furthermore, some boats are equipped with specially designed water and oxygenation distribution system in the well, to ensure a more optimized current, but the most common flow through systems moves water from front to back of the boat through valves. The water exchange in most well boats can be controlled by the opening of the valves, the speed of the vessel and/or by using large circulation pumps. In trucks, water exchange must be carried out by water tapping and refilling.

Cooling of the transport water is in most cases applied commercially to reduce the metabolism of the fish and thereby delay the deterioration of the transport water in closed systems.

7.1 The live fish transport industry in Norway

An official list of approved vessels for live fish transport is presented by the Norwegian Food Safety Authority (http://mattilsynet.no/fisk/godkjente_produkter_virksomheter). The list shows that there is 89 approved vessels for live fish transport at the moment.

7.1.1 The truck fleet

There is no segregation of trucks and boats form the list of approved vessels, but based upon comments by Paul Nergaard from Norwegian Food Safety Authority (pers. comm) there are 7 trucks approved for live fish transport in Norway. A common size of the fish containers on a truck is 1 -5 m^3. There are often several fish containers and a total transport volume on a truck with trailer is often 20 – 30 m^3. No detailed information about on the water treatment, surveillance technology and competence levels on these trucks have been available.

7.1.2 The well boat fleet

The Norwegian well boat fleet is estimated to 136 boats of very different age, equipment, size and well volume. At present, it seems that 82 of these boats are approved for live fish transport. The first Norwegian well boats were originally fishing vessels used for live transport of saithe (*Pollachius virens*) and cod. In the 70'ties, well boats came into common use in transportation of farmed salmonids. Table 1 shows the numbers and age in the Norwegian fleet up to year 2010. A large proportion of the fleet was built in the years between 1995 and 2000. Approximately ten vessels of large size (499 – 2043 m^3 volume of fish well) have been built during the later years, and 5 more are contracted up to year 2010. (Bjørn Atle Krohn Johansen, Shipping publications, pers. comm.). Included these new boats, another 20.500 m^3 capacity is added to the Norwegian fleet since 2002.

The technical standard of the vessels may contribute to the risk associated with the transport of fish in general and specifically for transport on closed system.

Table 1. The composition of the Norwegian well boat fleet in 2008 (Shipping publications)

Building year	No of boats	% of fleet
2010	1	0,7
2009	2	1,5
2008	2	1,5
2007	3	2,2
2006	3	2,2
2005	0	0
2004	1	0,7
2003	4	2,9
2002	4	2,9
2001	8	5,9
1995-2000	34	25
1990-1995	6	4,4
1970-1990	18	13,2
1950-1970	37	27,2
1900-1950	12	8,8
1800-	2	1,5
Total	**136**	**100**

Table 2. The size distribution of the well boat fleet (Shipping publications)

Capacity (m^3)	No boats	% of fleet	Total capacity	% of capacity
1300 -2250	2	1,6	4284	12,5
1000 - 1300	7	5,4	8106	23,6
500-1000	7	5,4	4889	14,2
400-500	6	4,7	2 740	8,0
300-400	15	11,6	5 020	14,6
200-300	19	14,7	4 247	12,4
100-200	29	22,5	4 252	12,4
50-100	8	6,2	571	1,7
<50	7	5,4	263	0,8
No info	29	22,5	0	0,0
Total	**136**	**100**	**34 372**	**100**

7.2 The welfare issue

7.2.1 Fish welfare during transport

Since the Brambell Committee Report postulated the "five freedoms" in 1965, animal welfare has been a subject of intense public and scientific debate. Attitudes towards animal welfare are rapidly changing. In particular, the welfare of fish is increasingly brought to the forefront of public concern (Pottinger, 1995; Rose, 2002; Braithwaite and Huntingford, 2004; Chandroo et al., 2004; Sneddon, 2004; Ashly 2007; Branson 2008). Some authors have concentrated on identifying conditions that must be fulfilled if an animal's welfare is to be considered acceptable. Even still under debate suggested welfare criteria for fish are freedom from severe (long lasting) stress, suffering and pain. However, Dawkins (2004) summarizes different aspects of welfare by introducing the view that one should only ask two questions in order to describe the welfare of an animal; (1) "is the animal healthy?"and (2) "does the animal get what it wants?". While suffering and pain is not as easy to deal with in fish, stress responses are much better documented in farmed fish species. Rose published his overview article (Rose, 2002), and concluded that fish cannot experience pain and suffering. According to Rose, fish do not have the brain structures necessary to perceive the emotional side of pain. He suggested that the nociceptive activity in the nervous system observed in fish exposed to a potential noxious stimuli, are reflex responses. During the last years though, researchers have now shown that fish do have a nociceptive system such as nociceptors and nerve fibres (Sneddon, 2002, 2003b; Braithwaite and Huntingford, 2004), and that change of behaviour in trout is actually a consequence of noxious stimulation (Sneddon, 2003a; Sneddon et al., 2003; Sneddon, 2004). Stress responses in fish (Donaldson, 1981; Mazeaud and Mazeaud, 1981) including farmed salmon (Pickering, 1992; Pottinger et al., 1992; Fløysand, 1993; Olsen, 1993; Knudsen, 1994; Sverdrup et al., 1994) are well established and primary, secondary and tertiary stress responses occur during a farmed fish's lifetime (Sverdrup, 1994). Physical disturbances during the transport (Sigholt et al., 1995; Erikson et al., 1997; Jittinandana et al., 2005) and slaughter of fish (Azam et al., 1989; Faergemand et al., 1995; Roth, 1997; Robb et al., 2000; Van de Vis et al., 2003) are unavoidable in aquaculture and have potential to induce stress responses that affect meat quality. In general, stressed fish undergo accelerated, post-mortem metabolism and develop softer muscle texture in a manner similar to that off mammals (Jerrett et al., 1996; Jerrett and Holland, 1998; Sigholt et al., 1997). Reducing *ante mortem* stress is important in assuring high quality fillets from cultured fish, and limiting *ante mortem* struggle is a key component for optimizing slaughter quality as it is in pigs and cattle. On this basis there is reason to believe that there is a good correlation between fish welfare in transport and unloading, stunning and slaughter quality.

There is a need for close attention to the welfare issues for transport in closed systems. Some aspects of particular interest is: fish species, size, life stage and fitness of the fish, handling, water quality, time and temperature, weather conditions, equipment, bio security, human factors (knowledge, experience and training,). The scientific knowledge is limited, even though there is already an established industrial protocol for closed salmon transport approved by Freedom Food in vessels operating in Scotland. There are several years' of practical experience with closed transport in Scotland. Due to this, an operational approach to fish welfare is relevant in this case. A way to group and organize operational welfare indicators is presented in chapter 7.3.

7.2.2 Fish welfare, transport stress and harvest quality

There is a link between pre-rigor time and meat quality (Erikson et al 1998), and a correlation between pre-rigor time and measures of fish welfare (Rosten et al 2004). Dependent on the pre-killing stress response in the salmon, the duration of the pre-rigor period can vary from one to 36 hours. A short-pre rigor time is normally a sign of elevated anaerobic muscle activity prior to slaughter, and this can be regarded as a compromised welfare. Rigor can be measured by dip tail analysis or by assessing the rigor index (Oka et al., 1990). Pre-rigor time up to 18 h (see figure 1) has been reported from field studies on closed transport of adult Atlantic salmon (Rosten et al unpublished data). This indicates that closed and cooled transportation may show welfare advantages compared to open transport, but this is depended upon the whole harvesting and slaughtering process. The advantage of closed transport

might be associated with the sedative effect of mild CO_2 exposure, given sufficient oxygen and slow cooling of the water (see figure 5). The results correspond with practical experiences from Scotland, where fish transported in a closed system with cooling are calmer (pre- stunned) before stunning and bleeding (see also 7.7.1.)
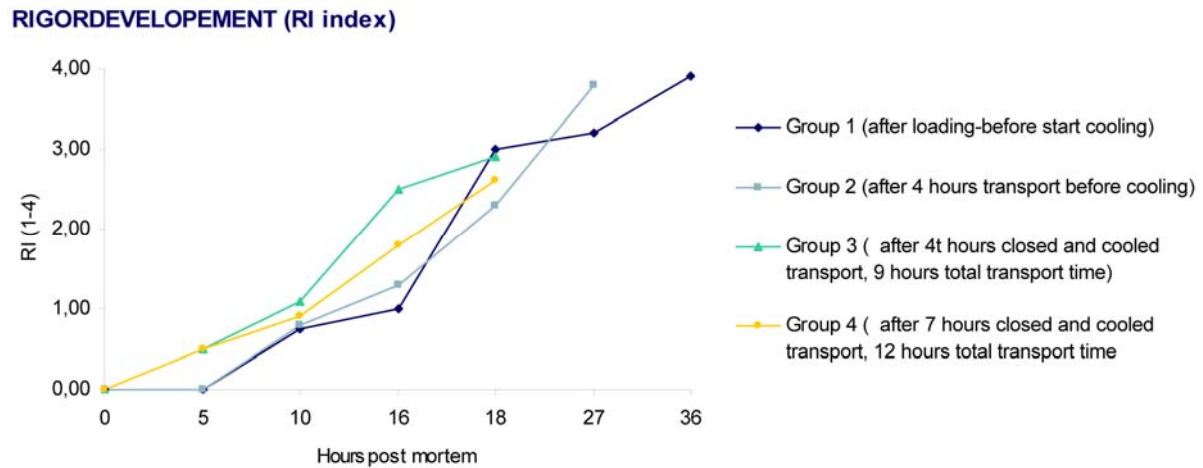

Figure 1. Rigor mortis development from killing (RI=0) until maximum rigor (RI=4) for six adult salmon from loading phase (blue line), after 4h transport before closing valves (light blue line), after 9h transport (4h closed and cooled water) (green), and after 12h transport (7h closed and cooled water) yellow (Rosten et al, unpublished data).

7.3 Operational welfare indicators in question for closed transport

As described in the report on welfare in aquatic animal production by Rosten et al (2007a), the basis for establishing operational welfare indicators (OWIs) is that the indicator must be scientifically sound and validated. However, when scientific data are missing or are scarce, empirical data may be used, although cautiously. When such data obtained through professional experience indicate that a certain factor does have a positive effect on the welfare of the individual animal or group of animals, it is reasonable to try to use this information to create a favourable environment for these animals. Welfare is normally considered individually, but the report suggests that an OWI in aquatic husbandry must also be applicable to a group of fish. Likewise it is suggested to divide the OWIs in two categories;

I.   *Direct* conditions (fish physiology, behaviour and health) that can be observed or recorded in fish individuals or group

II.   *Indirect* (operational or resource based) environmentally defined conditions (physical and chemical)

In Appendix X the OWIs of relevance for closed transport are listed in a more detailed form.

7.4 The stress concept

Stress is defined as a condition in which the dynamic equilibrium of animal organisms called homeostasis is threatened or disturbed as a result of actions of intrinsic or extrinsic stimuli, commonly defined as stressors (Selye 1950, 1973; Schreck 1982; Wendelaar Bonga 1997; Iwama et al., 1997; Portz et al. 2006). Acute stressors produce effects that threaten or disturb the homeostatic equilibrium, and they elicit a coordinated set of behavioural and physiological responses thought to be

compensatory and/or adaptive, enabling the animal to overcome the threat. If an animal is experiencing intense chronic stress, the stress response may loose its adaptive value and become dysfunctional, which may result in inhibition of growth, reproductive failure, and reduced resistance to pathogens. Responses to both acute and chronic stress typically involve all levels of animal organization and are collectively called the integrated stress response (Wendelaar Bonga 1997; Barton 2002; Iversen et al. 2004). Closed transport includes many potential stressors that elicit short or long tem physiological responses, many of which are specified as OWIs in Appendix I.

The general pattern of the stress response tends to be similar whether the challenge has resulted from fish cultural procedures (netting, transportation, disease treatments), water chemistry changes (turbidity, pH, temperature), or changes in behaviour (fright, dominance hierarchies). A convenient paradigm for the stress response is to think of it as occurring in three stages as described in Wedemeyer (1996) and Iwama et al. (2006) This include an initial alarm reaction (primary response) characterised by activated pituitary-interrenal axis with release of catecholamine and corticosteroid hormones. Approximate resting and stressed plasma levels for salmonids are <3 and 20-70 nM for adrenaline and 10 and 150-500 nM for cortisol, respectively (Iwama et al. 2006). The secondary response, or stage of resistance, includes successful compensation and acclimation, often with energy loss and growth retardation. If the stressful challenge has exceeded acclimation tolerance limits, the fish reach the final stage of exhaustion (tertiary response), with maladaptation, immune deprivation, and secondary diseases involving the whole fish population (Iwama et al. 2006).

7.4.1 Stress during transport

Handling
Transportation and handling procedures consists of several potential stressors, such as capture, on-loading, transport, unloading, temperature differences, water quality changes and stocking (Iversen et al. 1998, 2003, 2005; Finstad et al. 2003; Portz et al. 2006; Ashley 2007). Handling and transport have been shown to initiate a severe stress response in salmonids (*Salmo* spp and *Oncorhynchus* spp) (Specker and Schreck 1980; Barton and Iwama 1991). Among others, stress related cortisol releases in fish may suppress immunological capacity (Ellis 1981; Schreck et al. 1993; Einarsdóttir et al. 2000), affect seawater tolerance (Iversen et al. 1998; Sandodden et al. 2001), growth (Beitinger 1990; Bernier and Peter 2001) and survival (Barton and Iwama 1991; Wendelaar Bonga 1997).

Crowding
There is a difference between loading and density (Portz et al. 2006). While loading is defined as the weight of fish per unit of flow (kg/l/min), density refers to weight of fish per unit space (kg/m^3). Confinement may describe the entire volume of a small tank (Fevolden et al. 2003) or to a restricted net volume within a larger tank (Ruane et al. 1999). When the system is static the fish have a decreased volume for water exchange, potentially affecting water quality and related stress responses. This type of stress is often associated with high stocking densities leading to crowding stress. Short term crowding stress occurs commonly in aquaculture practices; possess characteristics of acute as well as chronic stress with long-term compromised immune systems resulting in disease or death (Portz et al. 2006). Therefore, optimal densities at loading and in transport tanks should always be taken care of regardless of profitability or convenience (Ellis et al., 2002; Portz et al., 2006).

Temperature
Fish are poikilotherms and an increase in ambient temperature will increase their metabolic rate. Thermal stress occurs when the water temperature exceeds the optimal temperature range, with energy demanding stress responses, and potential decrease in individual survivorship (Elliott 1981; Portz et al. 2006). Most fish can gradually acclimate to normal temperature changes but rapid changes in temperature, as may happen under fish loading and transportation, may result in thermal stresses or lethal conditions (Portz et al., 2006). Current knowledge indicate that 6 ºC is a lower limit for targeted water cooling (Sigholt and Finstad 1990), while experiences show that salmon can acclimate to 4,5 ºC given sufficient time to acclimate (Handeland et al., 2000).
The ion- and osmoregulatory functions may be depressed due to thermal stress (Finstad et al., 1988; Houston and Schrapp 1994). Reduced feed intakes and growth, reduced swimming behaviour, sudden or erratic movements with possible collision with the tank wall or other fishes, increased regurgitation, defecation and gill ventilation are among thermal stress related behaviour (Elliott 1981; Kieffer 2000; Portz et al., 2006). For a more general review on behavioural and physiological effects of water temperature in fish, see Wedemeyer (1996).

Recommendation for short-term holding of fish according to the recent review article by Portz et al. (2006), states that the temperature should be similar to the original source, to avoid thermal shock when the fish are transferred to the holding system (Wedemeyer 1996). For some fish, transportation can be less stressful if it takes place during cooler months or in chilled water (Carmichael et al. 1984; Erickson et al. 1997). Lower water temperatures typically decreases metabolic processes (e.g. oxygen consumption rate, NH$_3$ excretion rates, activity levels), while water oxygen solubility increases. If fishes are to be transported at a lower temperature, the holding water should be slowly cooled (≤1 ºC/day) to the desired temperature before transportation (Wedemeyer 1997). Upon arrival to the new destination, the temperature difference between the transportation water and the new holding tank should be minimized. Large deviations in temperatures may hide stress related behaviour responses in fish. In this area there is a need for further knowledge on acceptable lower range temperature

tolerances for commercial species such as salmonids and cod, especially regarding use of low temperature as a stress alleviating factor during transport.

Water quality

A recently published book summarises natural and seasonal variability in freshwater quality in Norway, water quality criteria for salmonids in relation to life history stage, methodology for water treatment, the situation in Norwegian fish farms from raw water to tank water, examples of adverse situations leading to negative fish welfare or mortality with suggested counteracts, as well as experiences from transport of live fish in Norwegian waters (Bjerknes et al. 2007). According to Portz et al. (2006), however, there are many water quality information sources for long term and intensive culture of fishes (Pickering 1981; Adams 2002), but sparse information related to short term holding of fish in confinement. Temperature, dissolved oxygen, ammonia, nitrite, nitrate, salinity, pH, carbon dioxide, alkalinity and hardness in relation to aluminium and iron species are the most common water quality parameters affecting physiological stress (Stefansson et al., 2007). In Portz et al. (2006), water quality variables for different fish species and general water quality recommendations for holding of fishes (modified from Timmons et al. 2002) are given. For salmonids during the last weeks of smoltification, even short episodes of adverse water quality (<3 days of increased aluminium (Al) exposure) could seriously reduce the seawater tolerance (Kroglund et al. 2007) and increase susceptibility for sea lice infestation (Finstad et al. 2007), thereby increasing the vulnerability to transport stress. The effects are clearly linked to an accumulation of Al on gill tissue, documented by reduced Na-K-ATPase activity, increased plasma glucose and reduced plasma chloride with increased gill-Al (Kroglund et al. 2007, Fig 1.) Increased susceptibility to sea lice infestation has also been observed after exposure to 150 % oxygen supersaturation for a short period (< 2 weeks) before seawater transfer (Finstad et al, unpublished data).
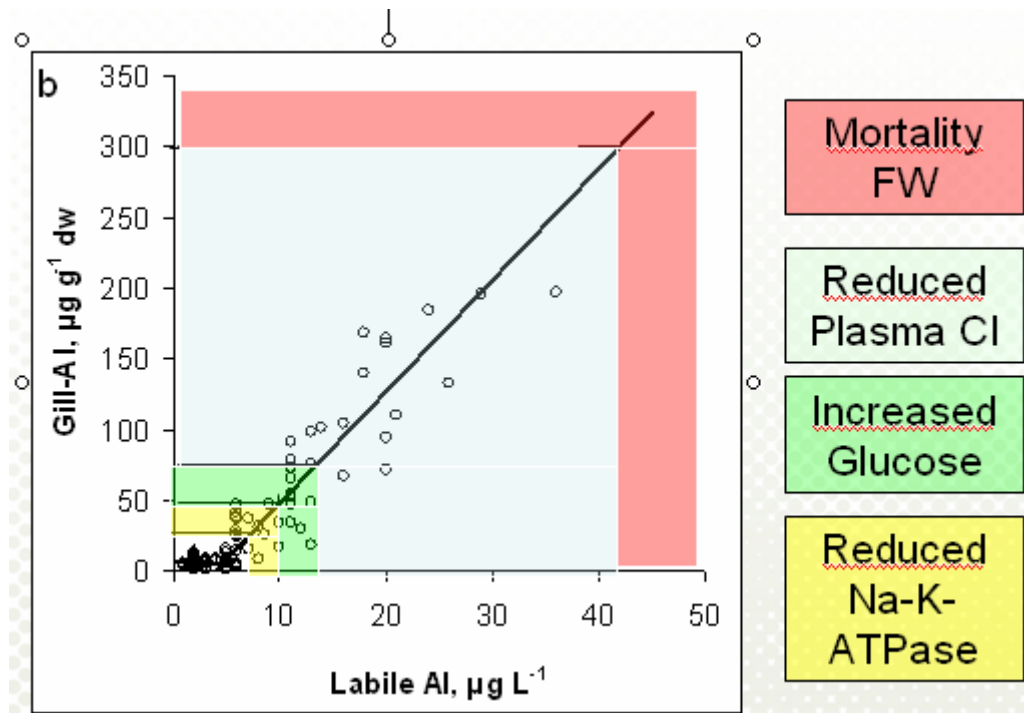
Figure 2. The concentration of inorganic labile aluminium (LAl) in water and the concentration of Al in gills of Atlantic salmon smolts. The figure shows levels of Al in water and gill leading to physiological reactions prior to seaward migration. A gill concentration > 25 µg Al/g gill dry weight (dw) reduces Na-K-ATPase activity while >300 µg Al/g gill dw results in mortality in freshwater. A gill concentration of >60 µg Al/g gill dw at seaward migration

have resulted in a reduction of return to the home river by 50%. (Data from Kroglund et al. 2007, figure modified by Rosseland 2007).
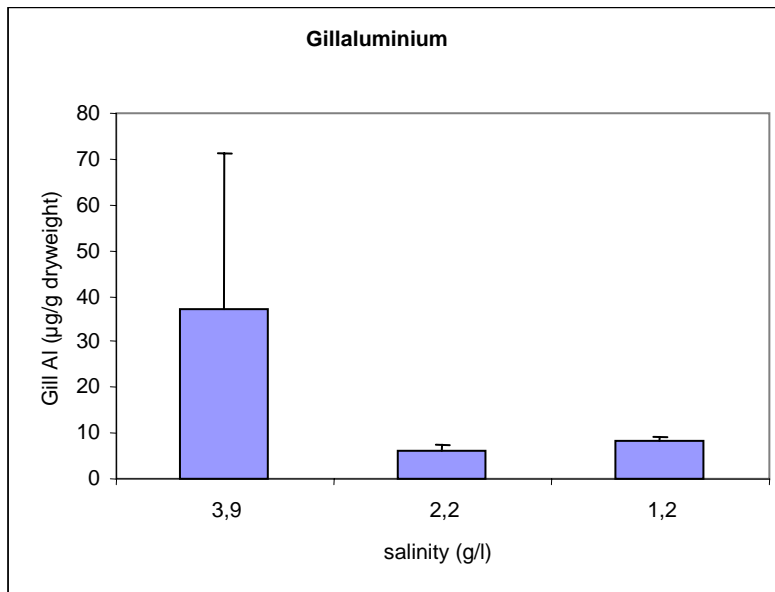

Figure 3. Increased gill-Al accumulation can be related to acidic raw water, or an effect of humic where organic bound Al is mixed with seawater between 1 -15 ppt, creating an estuarine mixing zone (Rosseland et al. 1998, Staurnes et al., 1998; from Rosseland et al., 2007)

Transport of fish together with humic freshwater from the smolt farm and into a well boat half filled with seawater, can create estuarine mixing zones within the well boat during loading with gill Al-accumulation (Fig. 2). In such cases, the freshwater from the farm tank should be separated from the fish before entering the transport well, loading the fish directly into seawater.

The physiological benefits provided by either the simple or complex mineral salt formulations in transport water are probably mainly due to the protection they afford against life-threatening blood electrolyte losses and ionoregulatory dysfunction that occur when the diuresis stimulated by handling and crowding stress is prolonged (Mazik et al. 1996; Southgate 2008). Survival rates of transported fish can be increased by adding NaCl at 0.5-0.8 % (5-8 g/L) to the transport water (Wedemeyer 1996a; Southgate 2008) and it is also showed that survival is even better if the fish can be allowed to recover in salt-enriched water after release from the transport tank (Mazik et al. 1991). Further, according to Wedemeyer (1996b) more complex mineral formulations have also been developed that are particularly useful in mitigation stress and reducing the mortality of fish transported in water of low total hardness.

With respect to water quality, it is essential to keep an optimal water quality in transport tanks during the whole transport to reduce the stress response, thereby optimizing the wellbeing of the fish and increasing survival and growth after release (Rosten et al., 2006).

7.4.2 Cortisol levels during closed transport

Several conditions in the well will change during a transport with closed system (see figure 1). Water quality, water current and sounds in the boat will change due to the use of pumps and water treatment equipment. It is inevitable that the fish notices these changes and field documentations show that plasma cortisol may increase moderately (see figure 4 and 5). Hauling, grading and loading and

unloading are stressors and the transport period itself *may* be a recovery adaptation phase when handled properly.
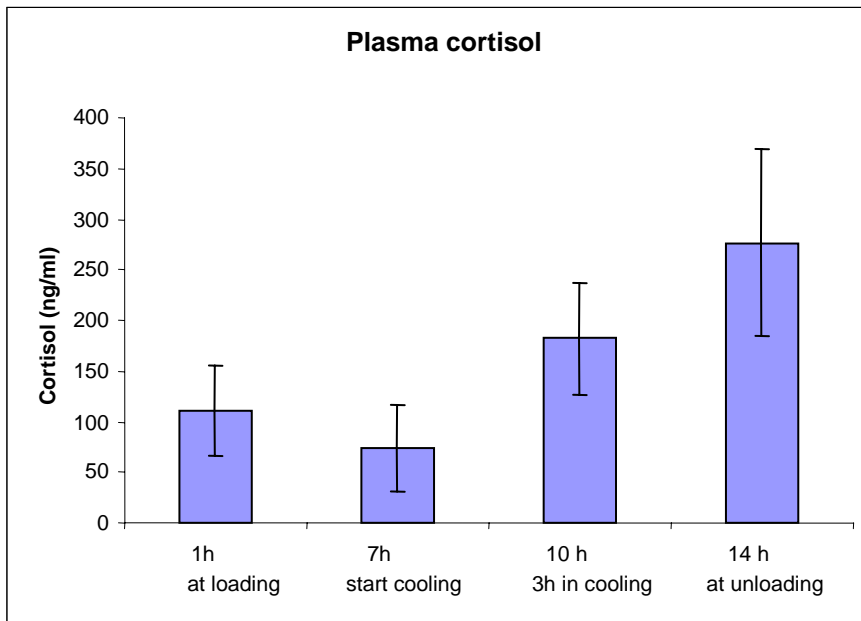
Plasma cortisol

Figure 4. Changes in plasma cortisol before and during a 7 hours closed transport of adult salmon with average body weight of 4, 1 kg at a fish density of 118 kg/m^3 (Rosten et al., unpublished data).

For Atlantic salmon parr, Rosten et al., (unpublished data) found that the plasma cortisol during the transport operation changed depending on the length of starvation prior to transport in a closed system. Highest plasma cortisol concentrations were seen after loading and unloading of the salmon parr (see Figure 4).
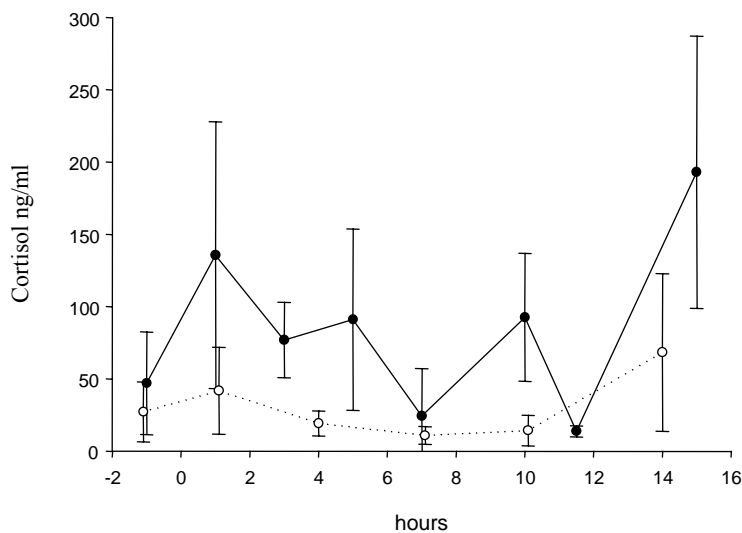
Figure 5. The cortisol response (ng/ml) in Atlantic salmon parr pre (-1h), during (1-12 h) and after a 14 and 15 hours closed transport in freshwater (oxygen 10-12 mg L^{-1}, temperature 9-10 ^{o}C, conductivity 1200 mikroS m$^{-1)}$. The filled circles are fish starved 48 hours prior to transport, open

circles are fish starved 144 hours prior to transport. Fish density was 11, 5 kg/m^3 in first transport (filled circles) and 12, 8 kg/m^3 in the second transport. CO_2 levels where kept within 2-8 mg L^{-1}. TAN levels reached 450 – 750 ug L^{-1} during the closed transport. Degassers were used as water treatment (unpublished data from Rosten et al).

7.5 Critical water quality parameters at high fish density and low specific waterflow

7.5.1 Oxygen (O$_2$)

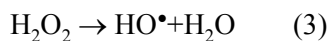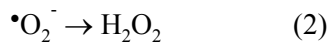Intensive smolt production generally involves high biomass, low specific water consumption and the use of oxygen supplementation to ensure enough O_2 in the tanks. Oxygen is often supplied by super-saturating the inlet water and/or tank with O_2, exposing the fish chronically or acutely to high O_2 levels. By adding pure oxygen to super-saturation, water consumption can be reduced to a minimum. However, this strategy can create serious problems. Firstly, these systems must be backed up by advanced security and control systems, as failure in oxygen supply quickly will cause anoxia and suffocation. On the other hand, there are serious concerns related to toxic effects of oxygen itself.

Oxygenation using pure O_2 gas will create a toxic O_2 environment for the fish, which has nothing to do with gas bubble disease or high gas pressure in itself. Rather, the toxic effects are related to the formation of the free oxygen radical superoxide (O_2^-).

$$O_2 \rightarrow {}^{\bullet}O_2^- \qquad (1)$$
$${}^{\bullet}O_2^- \rightarrow H_2O_2 \qquad (2)$$
$$H_2O_2 \rightarrow HO^{\bullet} + H_2O \qquad (3)$$
$$HO^{\bullet} \rightarrow H_2O \qquad (4)$$

Under normal conditions this is a radical which is formed as part of the aerobic respiration chain. The mechanism of action for O_2^- in water will be partly through direct effects on membrane transport proteins, partly through changes in protein synthesis and partly through oxidation of membrane lipids (Rohn et al. 1996). To counteract such consequences, animals have evolved defence systems which include e.g., the antioxidants vitamin A, C and E, and Glutathione. Recent data show that the partial pressure (PaO$_2$) in arterial blood of many fish species contain oxygen levels corresponding to no more than 30 % saturation (Massabuau 2001), most likely because the formation of free radicals increases dramatically above this level. Atlantic salmon, however, is not able to reduce PaO$_2$ to less than 50% of saturation (60-70 % in mean), and at oxygen pressures far above normal, e.g., in case of super-saturation, oxygen partial pressure in blood increases, increasing the formation of free radicals (Kristensen et al. unpublished). Hydrogen peroxide (H_2O_2) may also negatively influence production of erythropoetin (EPO), ie., reduce production of haemoglobin and erythrocytes. At transfer to an environment containing less O_2 (e.g., seawater) this may cause further problems. Recent findings of the low deregulation capability therefore suggest that Atlantic salmon may be equally or more vulnerable to the formation of free radicals caused by hyperoxia compared to other species, unless the salmon has more efficient detoxification systems than other fish species.

Ongoing research is likely to resolve these questions in more detail, but we have enough evidence to warn against to high and long lasting super saturation of oxygen during closed transport.

7.5.2 Carbon dioxide (CO_2)

Studies have shown that background levels in water sources for more than 100 smolt sites range from 1-2, 5 mg CO_2/L. During dry periods, however, CO_2 rich groundwater may dominate in a water source, and at low temperatures this may cause CO_2 super-saturation which can not easily be removed using traditional aeration methods. CO_2 from the water will come in addition to CO_2 produced by the fish in the rearing unit. Free CO_2 may therefore become a problem in land-based production using addition of oxygen and in closed transport of fish. In situations with sufficient water exchange and without addition of oxygen, O_2 becomes limiting long before CO_2 even approaches critical levels. In intensive production with addition of oxygen and reduced specific water consumption, CO_2 from metabolism may accumulate in the water. CO_2 reacts with water to form H_2CO_3 which dissociates to H^+ and HCO_3^{-} causing a reduction in pH. CO_2 concentration may therefore have both direct and indirect effects on the physiology of fish; it interacts with the important bicarbonate (HCO_3^{-}) buffer system and may affect blood pH, acid-base balance and so also hydro-mineral balance (Fivelstad et al 2003). Recent studies have further pointed to negative effects of high CO_2 levels on bone mineralisation (Fivelstad et al 2003). Elevated levels of CO_2 in intensive culture (30 - 40 mg CO_2/L) are a consequence of limited water supply, high fish density and extensive use of oxygen supplied at super-saturation. As outlined above, CO_2 reduces pH and thereby affects any substances which show different forms depending on pH. Accordingly, a reduction in pH caused by elevated levels of CO_2 may re-mobilise metal ions, e.g., Al (Fivelstad et al 2003). The consequences of a CO_2 dependent reduction in pH would be the same as discussed above. High CO_2 can further cause deposition of calcium carbonate in kidney tissue, known as nephrocalcinosis. This condition is characterised by visible, white, cheese-like, calcium rich depositions in the kidney tissue. Nephrocalcinosis has been observed at concentrations as low as 10 mg CO_2/L following long-term exposure. Increased capacity for binding metabolic CO_2 can be achieved by increasing bicarbonate contents of the water using addition of seawater or liming (Fivelstad et al., 1999, Liltved et al., 2007), a reduction from 2-4 mg CO_2 has been observed when small doses of seawater have been added to smolt rearing tanks (Åtland et al., 2007, Rosten et al., 2007c).

7.5.3 TAN – Total Ammonia Nitrogen ($NH_4^+ + NH_3$)

Ammonia is the most important waste product from the metabolism of proteins in fish. It is determined as Total Ammonia Nitrogen (TAN expressed as N mg/L) and expresses the sum of ionised NH_4^+ and unionised (gas) NH_3. The distribution of the two forms is highly dependant on pH, temperature and salinity (see figure 6). NH3 is the most toxic form, among others, due to its high membrane permeability, and its toxicity increases with reduced temperature. In flowthrough systems, concentrations of TAN are low (Rosten et al., 2007c, Åtland et al., 2007) but can be significant in recirculation and closed transport systems. Concentrations of 25 μg to 300 μg NH_3/L have been reported to cause mortality in salmonid fishes, and 10 μg NH_3/L to cause negative gill interaction. In freshwater hatcheries, Canadian authorities recommend < 10 μgNH_3/L (SECL 1983).

Based on the work by Knoph (1996), a conservative critical limit for Atlantic salmon in Norway has been set to 3–5 μg NH_3-N/L, dependant on temperature (Rosseland 1999). The literature does not give an absolute limit, but Rosten et al. (2004) has suggested < 2 μgNH_3/L as optimal and > 25 μgNH_3/L as not acceptable. No data on the sensitive smolt stage exist. Effects on plasma cortisone, plasma catecholamine, respiration, osmoregulation, circulation, haematology, and histology of gills, kidney and liver have been observed (Smart 1978, U.S. EPA, 1985 and 1989, Alabaster and Lloyd 1982). It is, however, important to stress that unionised ammonia never exist alone but will act synergistic or additive to other contaminants.

In seawater, the permeability of the gill membrane increases by a factor of 10. With the high pH and increased transformation of NH_4^+ to NH_3 (Figure 6) may explain the increased toxicity of NH_3 in seawater (Girard og Payan, 1980). US-EPA has set a maximum 1 Hr exposure pr. every 3rd year of

wild fish to 5000 μg TAN/L at pH 8.0, and ca. 2500 μg TAN/L at pH 8.5 (EPA 1998). Experiences from Norway indicate that a freshwater transport should not exceed 5000 μg TAN/L at CO_2 > 45 mg/L and < 70% O_2 saturation (Rosten 2000), supporting the US-EPA recommendations. Toxicity of ammonia increases at hypoxic conditions (Alabaster and Lloyd 1982). From the Norwegian WQ-project, the level of TAN in freshwater hatcheries using flow through systems, is between 400 -500 ugTAN/L, with less than 2, 5 ugNH3/L (Rosten et al. 2007c, Åtland et al. 2007).
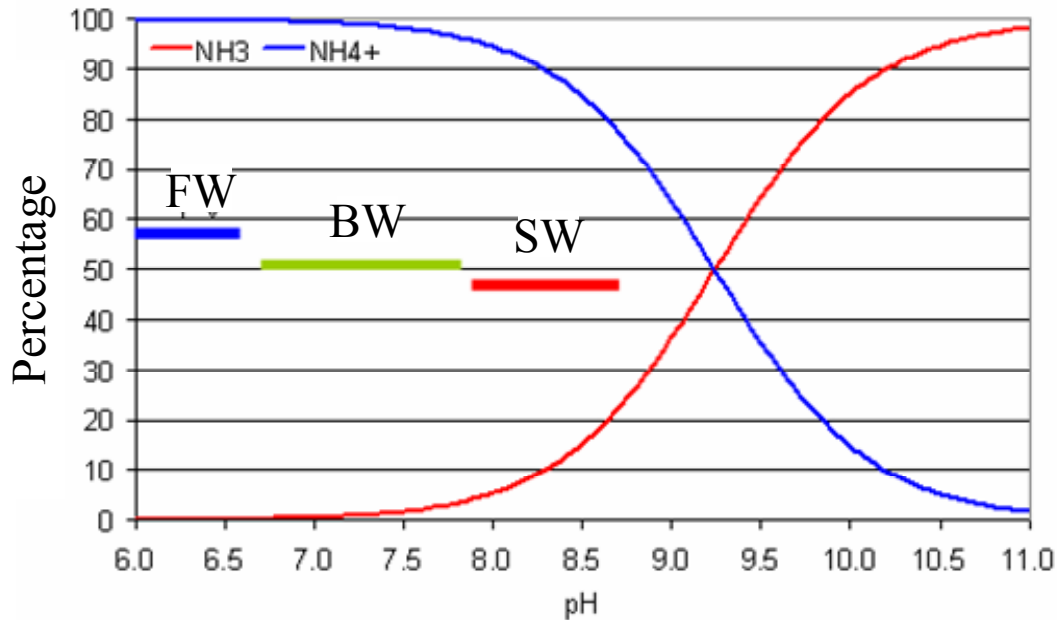

Figure 6. Percentage of TAN being in form of NH_4^+ and NH_3 as a function of pH. Normal pH of freshwater (FV), brackish water (BV) and seawater (SV) are indicated (Stefansson et al., 2007)

7.5.4 The creation of foam in marine transport water

By introducing air, oxygen or ozone bubbles into a transport water (only seawater) containing amounts of organic material, foam will form (Rosten et al., pers comm). This has both advantages and disadvantages. The foam might be a way to clean the transport water even though it is a by-product of degassing or aeration and should be removed. Left unremoved, the foam might represent a problem by making it difficult to observe the fish. Likewise, the foam might presumingly (not validated) in some cases, contain fish pathogens. These may enter the environment during transport, if not removed or treated.

The process is also know as protein skimming, and is held as an important aspect of keeping a healthy marine aquarium system by ability to remove DOC's (dissolved organic compounds) active protein skimming can be used as water treatment. Foam is created in the borderline between air and seawater. Tri dimensional foam is created on the sea surface when air bubbles that are trapped under water rises to the surface. At the start, the foam is created from the sea surface micro layer and the surface material of the bubbles. The residential time on the surface is depended upon the stability of the bubble and the presence of small particles (dissolved or in a solid state), Aveyard and Clint, (1996), Peltzer and Griffin, (1988). The creation of foam in seawater is known to be influenced by water with high productivity (Vogt, 1982), reduced surface conductivity due to presence of surface active compounds (Aveyard and Clint, 1996, Peltzer and Griffin, 1988), electrolytes (Weissenborn and Pugh,

1996), humic acids (Oppo et al., 1999), organic films (Slauenwhite and Johnson, 1996), algae (Lancelot et al., 1987, Magnusson et al.., 1988).

7.6 Fish metabolism and the impact on water quality in a closed transport system

The metabolism in fish can be expressed by oxygen consumption. In aquaculture one often expresses oxygen consumption as specific oxygen consumption (MO_2) in mg O_2 kg fish^{-1} min^{-1}. In a fish tank this can be estimated by the following simple equation;

(1) $MO_2 = (DO_{in} - DO_{out})$ x Q/ B (after Forsberg 1997)

Where DO_{in} and DO_{out} are the dissolved oxygen concentration of the outlet and the inlet water in mg L^{-1}, and Q is the water flow in litre min^{-1}, and B is the biomass of fish in kg. Oxygen consumption is influenced by many factors, such as temperature and fish size (Brett and Glass 1973, Fivelstad and Smith 1991, Forsberg 1994), feed ration (Brett and Groves 1979, Forsberg 1997), feed composition (Roberts 1990), swimming speed and salinity (Rao 1971, Forsberg 1994), stress levels (Smart 1981, Barton and Schreck 1987) and photoperiod (Whitney and Saunders 1973). It is also shown that metabolite production, as carbon dioxide and nitrogen, increases proportionally with increasing feed intakes (Kaushik 1980, Beamish Bæverfjord and Krogdahl (1996) and Thomas 1984, Forsberg 1997).

In a closed transport system the accumulation of total ammonia-nitrogen (TAN) and carbon dioxide (CO_2) from fish metabolism is relevant. In a fish tank the excretion of these metabolites can be simply be expressed as;

(2) $SX = (X_{out} - X_{in})$ x Q/B (after Forsberg 1997)

SX is the excretion of either TAN or CO_2, and X_{out} and X_{in} are the outlet and the inlet concentration (mg L^{-1}) of each metabolite. An ammonia quotient AQ and a respiration quotient (RQ) can be calculated as mol TAN / mol O_2^{-1} and mol CO_2 / mol O_2^{-1} and be presented as;

(3) $AQ = (32/14)$ x TAN / MCO_2 (after Forsberg 1997)
(4) $RQ = (32/44)$ x MCO_2 /MO_2 (after Forsberg 1997)

The fractions are used to convert oxygen consumption rates and metabolites excretion from mg to mole. From these (1-4) equations we can understand the simple practical principle that increasing the biomass (B) and limit the water exchange (Q) the concentrations (SX) and CO_2 and TAN in the transport water will increase. The rate is influenced by all the factors mentioned above, and concentration in water by the time and volume available for accumulation. A field study of closed transport of harvestable salmon carried out by Rosten et al., (unpublished data) confirms this (see figure 6 and 7).
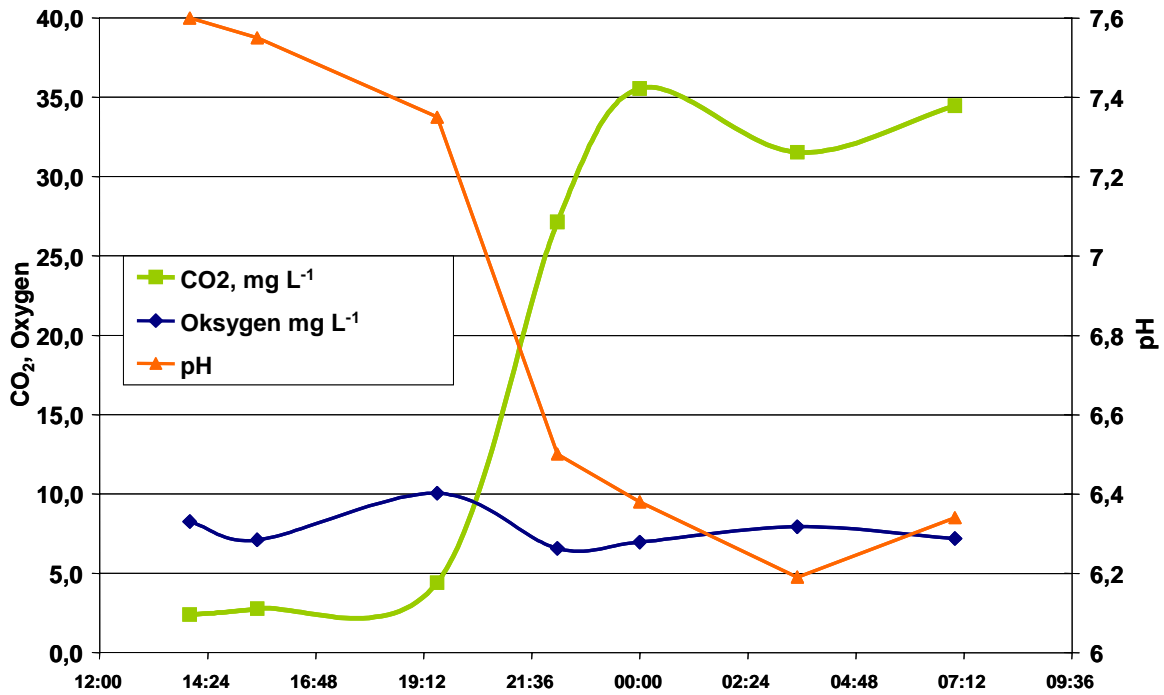
Figure 6. Figure 6 describes the levels of CO_2, H^+ (pH) and oxygen concentration in the well water during a 18h transport of salmon average weight 4, 1 kg, 118 kg /m^3. Time 14:24 (2:24 pm) to 19:12 (7:12 pm) is open system. Time 19:12 to 07:12 (07.12 am) is closed system. The flattening in CO_2 and pH from time 00:00 to 07:12 is due to the use of degassing equipment onboard the vessel. The relatively stable oxygen concentration is due to feedback controlled adding of extra oxygen to the well.
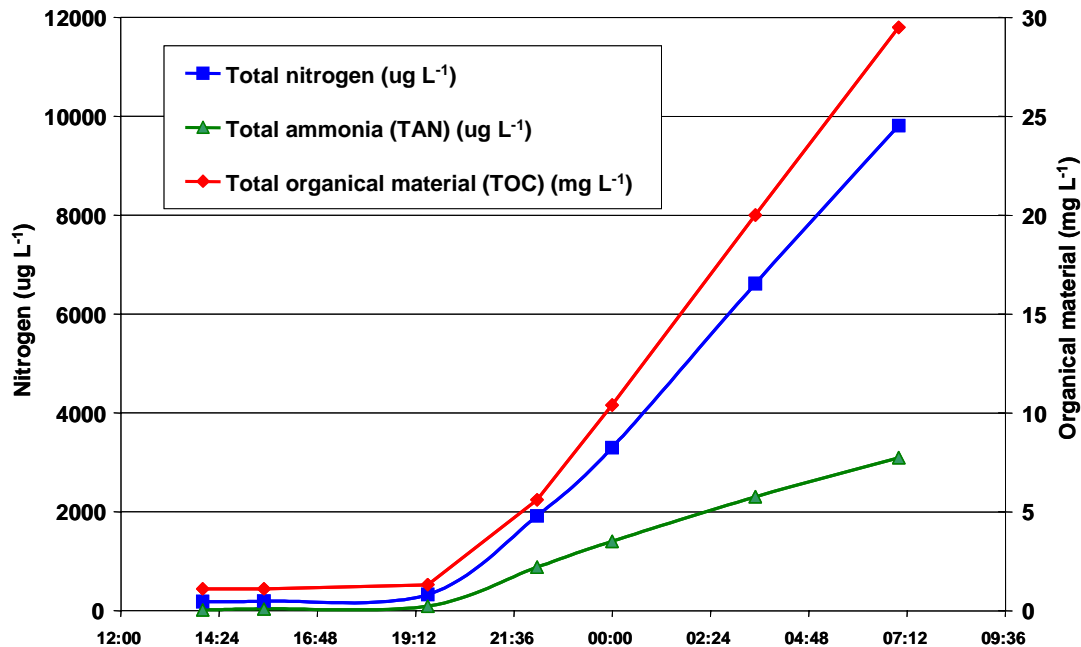
Figure 7. Figure 7 describes the levels of TAN, Total-nitrogen (Tot-N) and total organic material (TOC) concentration in the well water during a18h transport of Atlantic salmon average weight 4, 1 kg, 118 kg / m^3. Time 14:24 (2:24 pm) to 19:12 (7:12 pm) is open system. Time 19:12 to 07:12 (07.12 am) is closed system. The increase in TAN, Tot-N and TOC from 19:02 to 07:12 is due to no water exchange during the closed period. It is likely to assume that the accumulation rate is influenced by the

common known factors that influences fish metabolism. No water treatment was applied to these parameters.

NIVA has recently carried out some calculation relevant for water quality estimation in fish transport for large salmon. A model published by Grøttum and Sigholt (1998) was chosen. The model for oxygen consumption was based on swim respirometer experiments on starved Atlantic salmon (size range: 1, 1-2 kg, temperature 5-15 °C). The estimated 4 variable model relates oxygen consumption (VO2, mg kg^{-1} h^{-1}) to body weight (BW, kg), temperature (°C) and swimming speed (U, body lengths sec^{-1}). The equation is given without (1) and with (2) uncertainty estimates (±SE).

$$VO2 = 61, 6 * BW^{-0,33} * 1,03^T * 1,79^U \qquad (1)$$
$$VO2 = 61,6\ (\pm6,6)*BW^{-0,33(\pm0,11)} * 1,03\ (\pm0,10)^T * 1,79\ (\pm 0,10)^U \qquad (2)$$

The incorporated parameters in the model are considered to constitute the core parameters needed to estimate oxygen consumption. Relatively large uncertainty estimates is inherent in such experiments, as individual variation in oxygen consumption and other parameters is relatively large. The obtained mass exponent for correction for body size corresponds to the theoretical scaling factor for allometric effects of changes in surface area: body volume ratio (0, 67). The factor for temperature effects on metabolic rate, commonly denoted as Q_{10} was 1, 34, which is a fairly low estimate, possibly underestimating the temperature effect on metabolic rate (i.e. overestimating metabolism at low temperatures while underestimating metabolism at high temperatures). The incorporation of swimming speed, in body lengths pr second takes fish size into account. Figure 8 illustrates the fact that smaller fish have higher metabolism and contributes more with metabolites to the transport water, than larger fish. Figure 9 illustrates the fact that increasing the swimming speed will result in higher metabolism and higher contribution of metabolites to the transport water. Both calculations were done using fishsize 9-14 kg, and a temperature range 4 to 12 ºC. Swimming speed was estimated to 0, 5 bl/sec.
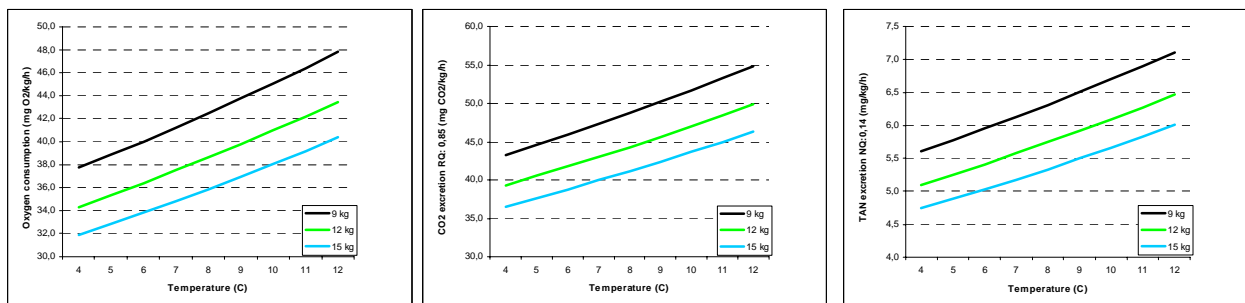

Figure 8. Effects of fish size on oxygen consumption, CO_2 excretion and TAN excretion in the temperature range 4-12 ºC. Data from: the model (1).
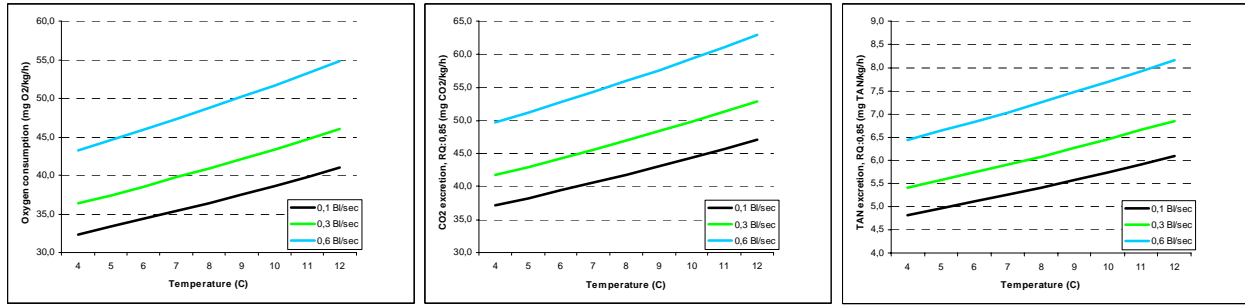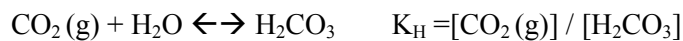
Figure 9. Effects of swimming speed (U, bl sec^{-1}) on oxygen consumption, CO2 excretion and TAN excretion in the temperature range 4-12 ºC. Data from: the model (2).

By studying of Forsberg (1997), we find specific oxygen consumption rates in 2 kg Atlantic salmon kept at 8,5 ºC ranging form 0,95-0,99 mg kg^{-1} min^{-1} in starved fish, to 1,91 – 2,06 mg kg^{-1} min^{-1} in fish fed with 0,6- 0,75 % bodyweight day[-1]. Likewise we see that the TAN excretion rates ranged from 11,8-12,8 μg N kg^{-1} min in starved fish, to 76,4 – 81,7 μg N kg^{-1} min^{-1} in fish fed with 0,59- 0,62% bodyweight day[-1]. The production of CO_2 was estimated to be 0,85 – 0,86 mg kg^{-1} min^{-1} in starved fish, and 2,17 – 2,12 mg kg^{-1} min^{-1} in fish fed with 0,59- 0,62 % bodyweight day[-1]. From this experiment, we can clearly see the impact of starvation prior to the transport on the water quality in the closed transport system. We can also see that the CO_2 excretion is approximately in a 1:1 relationship with the specific oxygen consumption in starved fish, and 10 % increase in CO_2 excretion in fed fish. Likewise it can be estimated that TAN excretion is in 1: 100 relationship the specific oxygen consumption in starved fish, compared to an approximated 1:10 relationship in fed fish.

7.6.1 The buffering capacity of water

Blancheton et al., (2007) has described that the carbonate system is responsible for determining the pH of most natural waters. It consist of the dissolved forms of carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-) and the carbonate ion (CO_3) Free CO_2 , or total dissolved CO_2 in the water is normally considered to be same as the $[H_2CO_3]$ since only a small fraction of the CO_2 is hydrated to H_2CO_3.   In a farming or a transport system for fish, the equilibrium reactions and the corresponding constants can be expressed as;

$CO_2 (g) + H_2O \leftrightarrow H_2CO_3$ $K_H = [CO_2 (g)] / [H_2CO_3]$

$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ $K_{a,1} = [H^+] [HCO_3^-] / [H_2CO_3]$

$HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$ $K_{a,2} = [H^+] [CO_3^-] / [HCO_3^-]$

Where K_H is Henry's law constant and the two equilibrium constant (K) are function of temperature and salinity (Stumm et al 1996). From the equations we see that accumulating CO_2 in the water will result in increased $[H^+]$, which will result in a drop in pH. The drop in pH is depended upon the buffering capacity of the water, which could be expressed as alkalinity. Alkalinity (ALK) is the capacity of water to neutralize acid, that is $[H^+]$, to H_2CO_3 and is defined by Doe (1994) as;

$ALK = [HCO_3^-] + 2 [CO_3^-] + [B(OH)_4^-] + [NH_3] + [SiO(OH)_3^-] + [HPO_4^{2-}] + 2[PO_4^{2-}] - [H_3PO_4] + [OH^-] - [H^+]$

In most aquaculture relevant applications, the alkalinity expression is simplified to include only carbonate, hydrogen and hydroxyl terms;

$ALK = [HCO_3^-] + 2 [CO_3^-] + [OH^-] - [H^+]$.

The buffering capacity might also be expressed by Acid Neutralizing Capasity (ANC) defined by Reuss and Johnsson (1986). The buffering capacity judget by ANC, is generally better the more ions there is present in the water. By adding lime or seawater one will gain more buffer capacity both by increasing the $[HCO_3^-]$ and ions (Liltvedt et al., 2007). By this reasons, seawater is more robust towards pH drop caused by accumulation of CO_2 than low ionic freshwater.

7.6.2 Maximum levels of metabolites in transport water and transport length

The major water quality effects experienced by fish during a closed transport are: low dissolved oxygen (O_2) levels due to consumption, accumulation of carbon dioxide (CO_2), depression of pH due to CO_2-accumulation and increased total ammonia nitrogen (TAN) levels (Paterson et al., 2003, Erikson et al., 1997, Rosten et al., 2007a). In fish transport operations the dissolved CO_2 is not recommended to increase above 20-30 mg L^{-1}, to prevent blood CO_2 concentrations from rising (hypercapnia) and the resulting Bohr-effect from impairing adequate oxygen transport to the tissues. The oxygen carrying capacity of fish Hb will also decrease if the blood pH declines (Root effect). In hypercapnia, the Root effect will compromise oxygen transport to the tissues only if the blood CO_2 increase is large enough to cause metabolic acidosis. Increased blood lactic acid concentrations (hyperlacticemia) due to excitement and struggling, can overwhelm the blood buffering capacity causing acidosis and reduced oxygen transport to the tissues (Wedemeyer 1996). This can cause rapid and strong rigor, and reduced flesh quality in salmon (Erikson et al., 1997, 1998) Because of this, closed transport is an operation where fish welfare might be challenged if not planned and carried out properly.

In a closed transport of harvestable fish, one may take advantage of the possibility to cool the water temperature, establish a controlled environment of O_2, and let CO_2 levels naturally accumulate to a certain level. The latter may be called "self-inductive sedation", where increased CO_2 and reduced temperature seem to make the fish calmer and more sedate (Ronja Commander; Rosten et al; pers comm.; see also 7.7.2.). On the other hand, accumulation of TAN and CO_2 represent potential stressors during closed transport, and the crew needs to have a tight control and the knowledge and measures to prevent undesirable and life threatening conditions for the fish.

Most well boat transports are typically carried out with flow trough system (open valves). The advantage of an open transport strategy compared to a closed is lower risks for accumulation of harmful metabolites (Rosten et al., 2005a). The disadvantage is the lack of possibility to lower the core temperature of the fish prior to slaughter. There is also no possibility to benefit from self-inductive sedation by moderately elevated CO_2.

During closed transport it is important that the water CO_2 levels do not reach toxic levels. In the case of European sea bass (*Dicentrarchus labrax*), the LC_{50} levels of CO_2 were about 100 mg L^{-1} (Grøttum and Sigholt 1996). From practical experience with salmon growers in closed seawater transport, a maximum allowed CO_2 concentration in the well is set to 50 mg L^{-1} (Håvard Bjørndal pers. comm.). In a closed well boat transport system with Atlantic salmon smolt a CO_2 level of 44 mg L^{-1} were reached after 2 hours transport (Rosten et al., 2005a).

Oxygen is very important in closed transport system. With no extra oxygen supply the fish will start to die within a very short time. On the other hand, transport of salmon under hyperoxic conditions causes abnormal behaviour and osmoregulatory disturbances (Erikson et al., 1998 and Chapter 7.5.1). Gill damage and osmoregulatory disturbances due to oxidative cell damage is shown during hyperoxia for Atlantic smolt (Brauner et al., 2000) and might be an explanation for the observed mortalities often seen 12-48 hours after transport and transfer to seawater (Rosten et al., 2007). However, low oxygen levels are known to increase the toxicity of ammonia (Merkins and Downings, 1957, Alabaster et al., 1982, Thurston et al., 1981). When extra oxygen is supplied, this might be a possible explanation why fish very seldom dies during a closed transport on trucks, even with high fish loads and presumingly high total ammonia concentrations. A few such cases are known to the *ad hoc* group (Rosten pers. comm.).

Un-ionized ammonia (NH_3) causes problems in a closed transport (Figure 6), but since the accumulation of CO_2 results in a decrease in pH, the ammonia will be in its more bio-tolerable ionic form (NH_4+). Practical experience with well boats shows that mortality can occur when opening the valves for water-exchange, since this operation leads to a rapid increase in pH and chemical equilibrium driven towards increased concentration of toxic un-ionized ammonia.

There is limited information about safe levels of water quality parameters in closed transport. There seems to be a relationship between the CO_2 concentration in water and the dissolved oxygen (DO) levels necessary to provide sufficient oxygen to the tissues. Basu (1959) showed that the DO levels had to increased from 6 mg L^{-1} when pratical no CO_2 was present, to more that 11 mg L^{-1} when CO_2 levels reached 30 mg L^{-1}. Due to this oxygen levels must be kept above 80% saturation during closed transport (Wedemeyer 1996). The role of NH^{4+} is uncertain since most studies have been done on un un-ionized ammonia (NH_3). A maximum 4 hours safe exposure level of 100 ug L^{-1} is recommended for salmonids på the US Environmental Protection Agency 1986. In Norwegian context this is regarded as high (Rosseland pers.med). Cod fry is reported to be relatively tolerant to ammonia. No growth limitation was observed for cod fry (3-4g) during 96 days exposure for 60 μg/l NH_3 (Foss et al., 2004). However, it is suspected that NH_4^+ might be more permeable to the gill membrane in seawater than in freshwater and thus might have more influence on the toxicity in sweater (Girard and Payan, 1980).

A seldom case study of a transport with fatal outcome is described by Rosten et al (2007). The case described a 30 hours closed transport with smolt (97g, 36 kg/m3) in freshwater, water temperature 4-6 ºC, pH 5, 8 – 6, 4. Fish started to get problems after 15 hours, when TAN levels reached 5, 5 mg L^{-1}. At that time un-ionised ammonia levels had reached 0, 5 ug L^{-1}, CO_2 concentration reached 40 mg L^{-1}, oxygen levels dropped to 68 % saturation, and TOC increased by 7 mg L^{-1}. The mortality was assessed to be caused by a combination of high levels of metabolites, to low level of oxygen under such conditions. Using these critical water quality concentrations, it is possible to calculate the maximum load and advisable transport length in closed system for Atlantic salmon fry and smolts. A three step risk level for metabolites in transport water was suggested by Rosten et al., (2007), with maximum upper levels of 60 mg L^{-1} for CO_2, 5 mg L^{-1} for TAN and 0,5 ug L^{-1} for NH_3-N (see table 3). This model has not yet been verified, so it should not be used as guidelines. The uncertainty is especially linked to the assumed fish metabolism under such conditions. However, this model clearly illustrates the complexity in predicting safe transport length and transport loads.

Table 3. Water quality criteria suggested to calculate the maximum transport length in closed transport.

Parameter	Risk level (RL)1	Risk level (RL) 2	Risk level (RL) 3
CO_2 mg L^{-1}	20	40	60
TAN μg L^{-1}	3000	4000	5000
NH_3 μg L^{-1}	0,5	1	2

Table 4. Maximum recommended transport time in closed system with freshwater (pH 6.0; alkalinity 0.04 mmol L^{-1}) for 1-2 g salmon fry with three different fish loads, three different risk levels (RL), three different temperatures and three different specific oxygen consumption. The model is highly dependent on pH, temperature and buffer-capacity (alkalinity).

Temperature and oxygen consumption rate	25 kg/m³ Maximum time in closed system (hours)	50 kg/m³ Maximum time in closed system (hours)	75 kg/m³ Maximum time in closed system (hours)
2 °C ($5 \text{ mg } O_2 \text{ kg}^{-1} \text{ min}^{-1}$)	RL 1. 3,7 RL 2. 8,0 RL 3. 10,0	RL 1 1,7 RL 2. 4,0 RL 3. 5,0	RL 1 1,1 RL 2. 3,0 RL 3. 3,0
10 °C ($14 \text{ mg } O_2 \text{ kg}^{-1} \text{ min}^{-1}$)	RL 1. 1,0 RL 2. 3,0 RL 3. 3,0	RL 1. 0,4 RL 2. 1,3 RL 3. 1,7	RL 1. 0,3 RL 2. 0,9 RL 3. 0,8
15 °C ($19 \text{ mg } O_2 \text{ kg}^{-1} \text{ min}^{-1}$)	RL 1. 0,4 RL 2. 1,0 RL 3. 2,0	RL 1. 0,1 RL 2. 0,7 RL 3. 1,2	RL 1. 0,1 RL 2. 0,4 RL 3. 0,6

Table 5. Maximum recommended transport time in closed system with freshwater (pH 6, 0, Alkalinity 0,04 mmol L^{-1}) for 70 g salmon smolt with three different fish loads, three different risk levels (RL), three different temperatures and three different specific oxygen consumption.

Temperature and oxygen consumption rate	25 kg/m³ Maximum time in closed system (hours)	50 kg/m³ Maximum time in closed system (hours)	75 kg/m³ Maximum time in closed system (hours)
2 °C ($2 \text{ mg } O_2 \text{ kg}^{-1} \text{ min}^{-1}$)	RL 1. 5,5 RL 2. 16,0 RL 3. 25,0	RL 1 2,7 RL 2. 8,0 RL 3. 12,0	RL 1 1,7 RL 2. 5,0 RL 3. 8,0
10 °C ($5 \text{ mg } O_2 \text{ kg}^{-1} \text{ min}^{-1}$)	RL 1. 2,6 RL 2. 7,0 RL 3. 10	RL 1. 1,2 RL 2. 3,0 RL 3. 5,0	RL 1. 0,8 RL 2. 2,1 RL 3. 3,0
15 °C ($8 \text{ mg } O_2 \text{ kg}^{-1} \text{ min}^{-1}$)	RL 1. 1,7 RL 2. 3,8 RL 3. 6,0	RL 1. 0,6 RL 2. 1,6 RL 3. 2,8	RL 1. 0,2 RL 2. 0,9 RL 3. 1,8

7.6.3 Monitoring of water quality during closed transports

Monitoring of a closed live fish transport is critical since rapid changes in water quality may occur under transport conditions. Currently, systems for monitoring and controlling water quality in well-boats and trucks vary. A full covering system for control and management of water quality parameters is not constructed. The most important parameters to monitor in a closed system are dissolved O_2, CO_2, total ammonia, total organic carbon and pH together with temperature and conductivity (salinity). During transport in closed wells the increased content of mucus and debris from the fish is a challenge for automatic on-line sensors to give reliable data, and self cleaning sensors is highly recommended. In addition will the content of algae and special potential harmful algal be of importance to monitor in the incoming water (from outside the boat). Presently sensors like temperature and conductivity should work satisfactory if the recommended maintenance is followed, but more difficult will be sensors based on membrane technology as e.g. O_2 or electrodes (pH). Sensor technology based on fibre optics is

currently available for O_2, and may be tested as an alternative to current standards. Measurement of CO_2 is very often based on alkalinity titration and pH measurements, or basic titration. These methods are based on assumptions of equilibrium in the bicarbonate system and that the bicarbonate system is the dominant buffering system. Both assumptions may be incorrect in high-density transport and comparison with a chemical based CO_2 determination in the laboratory has shown unreliable data by such analysis (Kristensen and Rosseland 2005). New prototype sensors based on direct measurement of the CO_2 partial pressure is developed and promising results are achieved.

Direct measurement of the toxic un-ionized ammonia (NH_3) is presently not possible with any sensor, but is based on pH measurements and chemical determination of ammonium. New and improved technology is urgently needed to give better estimates of this toxic substance.

7.6.4 System for monitoring of water quality in closed transport

A water quality monitoring systems with sensors for O_2, CO_2, pH, salinity and temperature is already implemented on some well boats specialized for closed transport. The water monitoring system consists of sensors mounted in-line with the water flow either pumped from outside the ship or from the wells on-board. The signal from the sensor are recorded using software available from the instrument supplier and they are presented on-board the ship for the operator or transferred via cables to monitors on the bridge.

Figure 10. Water sampling from units leading water from two fish wells and outside water into a central monitoration and sampling unit onboard a well boat designed for closed transport. Photo: Trond Rosten

7.6.5 Water treatment

It is critical to treat the water during a closed transport. It is also important to gain optimal water quality before closing the well. Equipment for treating water may include oxygenation, water cooling, degassing of CO_2 and protein skimming. It is not common to use any equipment to treat unionized ammonia. Keeping sufficient low pH is a way to avoid ammonia toxicity. To our knowledge, water treatment equipment is taken into use in both well boats and trucks design for closed transports (see figure 11 and 12). It is not common to use any equipment to treat unionized ammonia, but keeping sufficient low pH is a way to avoid ammonia toxicity. The use of Zeolitt is potential method to clean fresh water for ammonium–N. This method is not commonly used in Norway, but a prototype for trucks is developed in Chile (Parada 2008). We do not have any more information upon this method.

Figure 11. Water treatment unit onboard one well boat designed for closed transport. Photo: Kai Sørensen (NIVA).

Figure 12. Water treatment unit (degasser) on one well boat designed for closed transport. Photo: Kai Sørensen (NIVA)

7.7 Starvation before transportation

The rationale for introducing a period of starvation before transportation is to secure acceptable welfare during and after the transport. Experiences show that starvation reduces metabolism prior to and during the transport phase, it reduces negative impacts from uneaten feed, fish metabolism and

faeces on water quality; it reduces population hierarchy, fight and stress, as well as improves performance after transfer.

In the wild, many fish species may survive in the absence of food for periods of several months, like for example migrating sexually mature salmonids and over-wintering carps. Thus, in-house transport procedures of fish normally involve withdrawal of feed for an appropriate period (from two days to one week depending on fish size) prior to and under transport, mainly to provide safe environment and minimise stress during, under and after transport. An acceptable transport results is essential both for on-growers (quality for farming) and slaughter fish (sustain market quality). For harvest fish, starvation is also essential to reduce contamination by intestinal content during slaughter and to improve fillet quality (Einen et al., 1998).

There seem to be differences in fasting metabolism between fish species, developmental stages, and relative to time and rearing temperatures (Petri 2003). Juveniles, with rapid growth and development, and less stored energy are more sensitive to fasting. Metabolic rate increases with temperature, and induces relative changes in nutrients (glucose, lipids and amino acids) released for catabolism.

Starvation reduces growth rate, metabolism and hence respiration rate in fish. To survive adverse periods like starvation, energy saving strategies is induced to maintain the supply of energy substrates to life bearing tissues such as the brain and neural tissue. The strategy of conserving body substrate levels with reduced energy expenditure during food deprivation, while maintaining an efficient assimilation strategy when food is available; makes the fish able to survive adverse conditions for much longer periods than mammals. A delayed adaptation of metabolic rate to starvation is, however, observed which means that a starvation should be initiated for an appropriate period before transportation (Petri 2003).

Observed changes during short-term starvation include:
o The intestinal mucosa changed towards a typical "resting appearance" in Atlantic salmon postsmolts (500 g) fasting through three weeks compared to fed fish (Bæverfjord and Krogdahl 1996).
o Higher relative losses in body mass was observed initially (during 2-7 days) compared to later losses in carp, rainbow trout and Sibirian sturgeon during 28 days fasting, indicating emptying of the gastrointestinal tract and adaptation to lower metabolic rates (Petri 2003)
o Body mass loss during starvation is higher at elevated temperatures indicating elevated catabolism and energy expenditure (Petri 2003)
o Body lipid, protein and glycogen are degraded and used for energy during starvation; the relative contribution differs between species and rearing conditions (Petri 2003)
o Energy saving strategy involves endocrine changes, such as circulating growth hormone and thyroid hormone and hepatic level growth hormone receptor (GHR) observed already 2 days after fasting (Deng et al 2004)
o Short- or long-term fasting and re-feeding regimes do not seem to be associated with nutritional stress and reduced fish welfare (Pottinger et al 2003).

The fish should be starved some days prior to transportation to reduce general metabolism and to temporary adapt their behaviour to a no feed regimes. Partly or restricted feeding will cause competition, aggressive behaviour and increased risk for transportation injuries. Stress and increased stocking densities during transport are likely to reduce feed intake (Ellis et al 2002), which further supports fasting as a welfare measure. During transport, general metabolism may be reduced both due to starvation and hypoxia (Zhou et al., 2001), and reduced water temperature.

Besides a maintained oxygen level, threshold tolerance limits for long term negative effects of ambient unionized ammonia is one of the most critical water quality parameters for optimal performance in intensive rearing facilities (see 7.6). Since ammonia excretion is directly related to protein intake and time after feeding (Handy and Poxton, 1993), starvation prior to transport are beneficial to the water quality.

It is important to know species-specific tolerance limits for water quality parameters, even though these may prove to be too simplistic due to interactions with situation dependent parameters (temperature, oxygen, carbon dioxide, pH, salinity, nitrite, and suspended solids). One should consider welfare implications of transporting fish between waters of different qualities, since the fish may need time to adapt to changes in water chemistry (HSA 2007).

7.8 Sedation

7.8.1 The use of other sedatives during transport

Anaesthetics are useful to reduce activity and to minimise stress in fish as described by Iversen et al., (2003). Anaesthetic agents added to the water at low doses may also be used to sedate fish prior to transport. This reduces metabolic rate and hence oxygen demand, reduce general activity, increase ease of handling, and mitigate the stress response (Cooke et al. 2004). The ideal level of sedation for fish transport is referred to as a deep sedation, including loss of reactivity to external stimuli, and decrease in metabolic rate but with maintenance of homeostasis (McFarland 1959). This level is consistent with anaesthesia stage two, as described by Summerfelt and Smith (1990). If heavily sedated, the fish will loose equilibrium, cease swimming and die from suffocation if they all are settled to the tank bottom. The choice of anaesthetics generally depends on several considerations: 1) Safety for the user 2) Availability; 3) Cost-effectiveness; 4) Ease of use; and 5) Nature of the study (Cho and Heath 2000). Marking and Mayer (1985) produced a list of characteristics of an ideal anaesthetic. In addition to these characteristics, a particular anaesthetic should have a stress-reducing capacity, with blocking of the hypothalamus-pituitary- interrenal (HPI) axis and render the fish unable to respond to additional stressors (Olsen et al., 1995; Keene et al., 1998).

Metomidate (d1-1-(1-phenylethyl)-5-(metoxycarbonyl) imidazole hydrochloride) is a rapid acting water-soluble non-barbiturate hypnotic in several species (Mattson and Riple 1989; Masse et al. 1995). Metomidate has also been shown to block cortisol synthesis and prevent handling-related glucose increase (Thomas and Robertson 1991; Nilssen et al. 1996). In Norway it has been used in research but it has never been introduced to or approved by the commercial aquaculture industry, partly due to the absence of analgesic properties (Horsberg and Samuelsen 1999).

Benzoak[®] (now labeled Benzoak[®] VET, Euro-Pharma AS) contains the active substance benzocaine (ethyl-4-aminobenzoate) at a concentration level of 20% (200 g L^{-1}). Benzocaine is a local anaesthetic agent closely related to the widely used anaesthetic metacaine (MS-222) (Mattson and Riple 1989; Burka et al. 1997; Ross and Ross 1999). However, it is 250 times less soluble than metacaine in water, and therefore any solutions containing benzocaine have to be made in ethanol, acetone or propylene glycol (Burka et al. 1997; Ross and Ross 1999). Benzocaine has a good margin of safety, although this appears to be reduced at higher temperatures. Water hardness or pH do not affect its efficacy. As with metacaine, benzocaine is fat-soluble and recovery times can be prolonged in older or sexually mature animals (Ross and Ross 1999). Generally the tissue levels of the drug fall to undetectable levels within approximately 24 h (Allen 1988), but the withdrawal time for use in fish for food in USA (Ross and Ross, 1999) and Norway is 21 days (Horsberg and Samuelsen 1999).

Clove oil is derived from the stem, leaves and buds of the *Eugenia caryophyllata* tree, and it is used throughout the world for applications ranging from food flavouring to local anaesthesia in dentistry profession (Anderson et al. 1997). Eugenol (4-allyl-2-methoxyphenol), the active substance makes up 90-95 % of the clove oil (Briozzo et al. 1989), and as a food additive is classified by the FDA to be a substance that is generally regarded as safe (GRAS) (Anderson et al. 1997). Due to these positive features reported on eugenol (clove oil) as anaesthetic on aquatic animals, a new anaesthetic compound for fish was developed in New Zealand, called Aqui-S™ (Ross and Ross 1999). Aqui-S™ is reported to contain 50 % (540 g L^{-1}) iso-eugenol (2-methoxy-4-propenylphenol) and 50 % polysorbate 80. These materials are classified as GRAS by the FDA (Ross and Ross 1999). The eugenol based

anaesthetics (clove oil and Aqui-S™) show promise as effective anaesthetics for Atlantic salmon based on good efficiency at low dosage and stress-reducing capabilities (Tort et al., 2002; Iversen et al., 2003). Similar effects have been shown on largemouth bass (*Micropterus salmoides)* (Cooke et al., 2004) and on channel catfish (*Ictalurus punctatus)*, (Small 2004). Eugenol based anaesthetics are also easily and inexpensively obtained, and are organic substances safe for the environment and user.

Anaesthetics for use in commercial fish transports in Norway (aquaculture and compensatory releases) have not been performed yet. This method has yet to be licensed both in Norway and in the rest of Europe and more studies have to be performed if this method is to be implemented in commercial fish transports. In view of the positive research results using anaesthesia, this may however be an alternative strategy for increasing fish welfare during transport leading to better survival and growth of fish at the releasing site.

7.8.2 Self inductance CO_2 –sedation during closed transport

It is shown that fish will be anesthetized under extremely high CO_2 levels ($>>100$ mg L^{-1}) (Yoshikawa et al., 1994; Bernier and Randall, 1998). Robb et al., (2000) found that there was significant different in time for onset of brain failure between fish stunned by percussive blow or spike, and those stunned by CO_2. Hower it is also found that oxygen levels prior and during CO_2 stunning can modulate adult Atlantic salmon's stress response to CO_2 stunning and prolong the short pre-rigor time associated with pure CO_2 stunning (Erikson et al 1998). The levels during closed transport studied in Scotland by Rosten et al were very moderate compared to those previously used in CO_2 stunning tanks. The temperature in the transport water may play a major role, since it is affecting oxygen uptake. The lowering of temperature and the moderate increase in concentrations of CO_2 might induce a lower affinity of oxygen in the fish blood, thus stimulating the release of oxygen to the tissues and reducing the gill respiration frequency (root off effect, see Chapter 7.6.1), but this might also impair the uptake of oxygen through the gills. One can speculate if the procedure of lowering the water temperature is counteracting the negative effect of CO_2 on the oxygen uptake. Anyhow, during such transport conditions it can be vital to prevent hypoxia. Figure 14 shows that the partial pressure of oxygen and CO_2 in the blood is increasing during the period with closed transport with water temperatures lowered from 12 to 6 ^{o}C. This mechanism must be studied more carefully to understand the sedative responses in fish during a controlled closed and cooled transport.

In the full scale pilot study of water quality and fish physiology during two commercial transports of adult salmon using closed well boat system, by lowering the water temperatures and controlling accumulation of CO_2 and oxygen levels, it was reported that the fish seemed to develop a sedative status, and that the pre-*rigor mortis* time was significantly prolonged compared to normal pre-rigor time experienced in Norwegian harvesting plants (Rosten et al., unpublished data). A study of the breathing movements indicated that there might be a correlation between CO_2 content in the well water and fish getting into a pre- anaesthesia phase (see Figure 13).
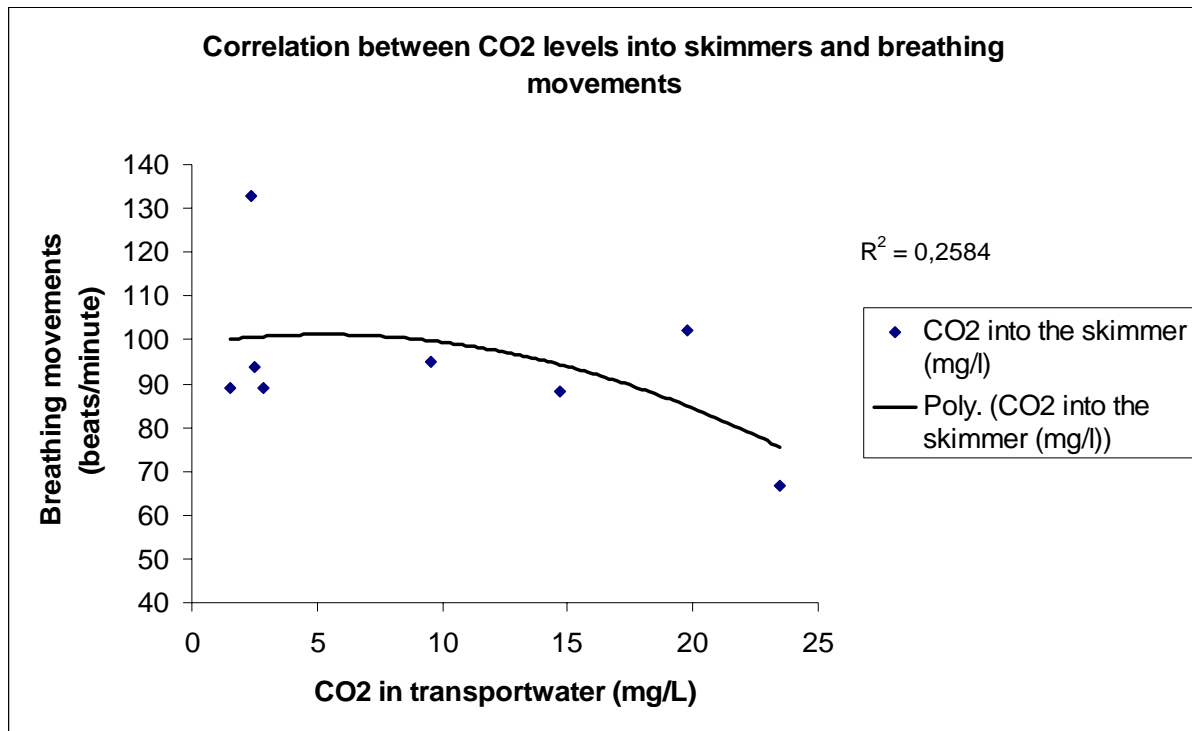
Figure 13. The potential relationship between carbon dioxide concentration in the well water and recorded breathing movements (respiration frequency) in closed transport. After Rosten et al., (unpublished data).

O₂ partial pressure in fish blood

CO₂ partialpressure in fish blood
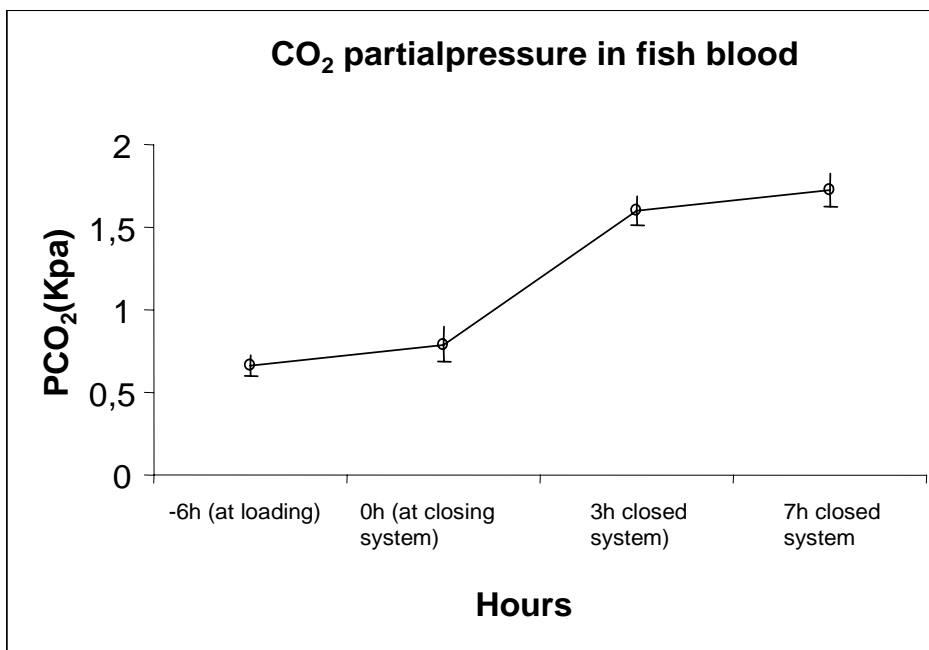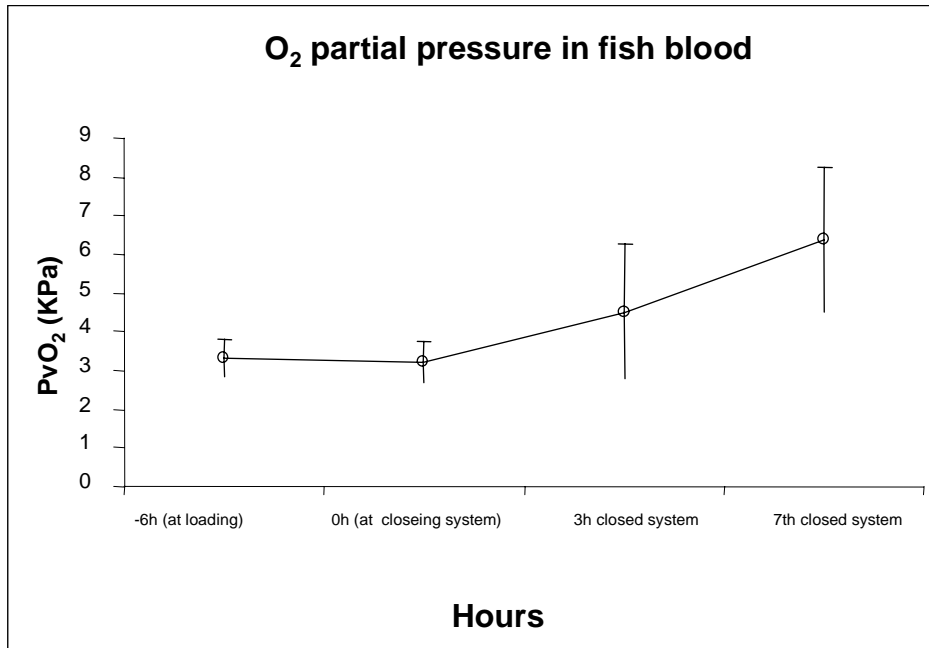
Figure 14. The figure on top show oxygen levels in adult Atlantic salmon blood (kPa) and to the bottom shows CO_2 levels in blood (kPa), sampled with heart puncture pre, and during a transport (7 hour) with closed system. Rosten et al. (unpublished data).

7.9 Remarks on critical operations in closed fish transports

A way to reduce the accumulation of metabolites is to try to reduce the metabolism of the fish. This can be done by lowering swimming speed, lowering the temperature, and using degassing equipment. It is important to monitor water quality in the well and combine this with observations of the fish. Underwater cameras in several places in the well are recommendable for control purposes. One should

avoid a too large and rapid temperature drop (max 10 ^{o}C with max 1.5 ^{o}C change hour^{-1}). The temperature in the well-water should not be below 4 ^{o}C (Håvard Bjørndal, pers.comm).

7.9.1 The importance of information and experience

It is vital to any transporter to know the condition of the fish to be transported; are they healthy, harmed or by any way compromised or stressed. The fish should be fit for transport, and if not judged so, the transport should be cancelled. In this context, it makes sense to consider the condition of the fish group and not single fishes. An experienced transporter will adjust transport conditions based on his opinion about fish health and robustness. For stressed fish, actions might be to reduce loading and increase oxygen supply. In well boats it is common to talk about rule of thumb. This vague wording often covers years of experience in observing fish during transport, while the importance of this must not be overruled. There is no special education for transporting fish so the competence is in most cases handed over from an experienced skipper to a new recruit. However, when it comes to transporting fish in closed system in well boats, the experience is weak or lacking. This might represent both a practical and a psychological barrier to overcome for the industry. Boats and crew fit for closed transport are available, but constitute a minor part compared to the large number of boats and crews rigged for open transport.

7.9.2 Preparation for transport

To be able to carry out a successful transport with live fish, it is important to ensure sufficient preparation before the transport is carried out. The importance of food restriction prior to the transport is stressed by many transporters, since this procedure seems to improve both robustness of the fish and the visual appearance of the transport water due to a down regulation of metabolism and less faeces in the gut. Faeces in the transport water will cause more organic carbon and higher turbidity in the transport water. To the human eye such water will seem more polluted. Higher levels of organic material are also causing the creation of skim when air, oxygen or ozone is mixed into the water. In closed transport systems with seawater, the problem with skim during summertime (naturally occurring organic load in the seawater) and with higher fish densities or insufficient feed restriction period, must be solved technically. An assessment carried out by the committee concludes that such technology is available.

Planning the truck or sailing route is of high importance since this might affect the time necessary to transport with closed system, the transport length, the risk for intermixing with water from other fish farm and the risk for rough sea, algae blooms etc. Dialogue with fish health authorities is of highest importance. The time schedules for transportation of live fish, and especially transportation of smolts, can be very tight. Well boats and trucks are normally booked well in advance. This gives limited possibilities for postponing transports due to unfavourable environmental conditions.

Pre-transport stress is problematic. It is well known both scientifically and practically that fish tend to get stressed when they are crowded together in a dip- or brail net. Crowding and netting fish is also often associated with hypoxia, and these two stressors create a massive stress response in the fish (see Chapter 7.4.) These operations must therefore be carried out with gently hands, clear minds and the necessary time. Transporters often reports problems with too much stress in fish tanks or brail nets prior to transport. This lowers the robustness of the fish and affects the fitness for transport. If the transport is to be carried out with closed valves it event more important that the pre-transport stress is kept as low as possible. The way to regulate and minimize this is to stimulate good workmanship and understanding by the fish technicians that carries out operation. In addition, time spend in a brail net is very important, and in this context the loading equipment plays a significant role.

7.9.3 Loading

Up to the late 90'ties a large dip net was used to load the fish into the boat. In the 70'ties and 80'ties it was not common to use plastic lining on the dip net, so the operation was "dry" and very stressful. The plastic linings was introduced in the mid 80'ties in well boats, and was a major improvement in the loading technology. Since it was discovered early that the loading stress could affect the quality of the transport (and the wellbeing of the fish), there was a drive for innovation of new loading technologies. Since the density and the total cargo originally was estimated by counting dip nets, there was also a drive for establishing technology that could automatically calculate the load from counting the number of fish and analyzing average weight. In the late 90'ties a new system based upon online video analyses of live fish passing a weighing – counting unit on its way into the boat, was designed. When this unit was combined with the idea of using the possibility of siphoning the fish from the brail-net onto the boat, great progress was made in minimizing the loading stress. The next generation of low stress loading equipment was the idea to establish a sub atmospheric (-0, 2 ATM) pressure inside the well so that the fish was sucked into the boat through the large siphon tube passing the weighing and counting equipment. The advantages of the siphoning and sub atmospheric pressure system were that these systems were much faster than loading by a dip net. The time spent in a brail-net could therefore be dramatically reduced. The accuracy of the current biomass estimation equipment is held to be within 1-2 %. This was almost a revolution compared to conditions in the mid 90'ties where a 10 % accuracy was more common. Today, all new boats tend to have such equipment, however, an unknown number of operating vessels do not have this technology.

Loading fish directly into a closed system has recently been shown to be risky, due to physical conditions in the well water, particularly the risk for a total gas-pressure above 100 % combined with the super saturation with nitrogen gas, which might cause problems for the fish (Rosten et al. 2007b). The cause of the super saturation is probably that air bubbles are trapped in the pipes and well and are put under pressure by circulation pumps. This can be counteracted by degassing the transport water 1.5 -2 hours prior to loading the fish into the closed system, a procedure which physically homogenizes the air in the water.

There is no doubt that the initial loading stress is significant. Using modern loading equipment it is probably correct to assume that the stress is originating from crowding and brail-netting before transport. Establishing an initial recovery window post loading may improve welfare of the fish. Experience and operational procedures for transporting large salmon in closed, temperature dropping systems have counted in a period of recovery before the system is closed. This might be of vital importance. Recommendations for fish loads in closed systems using two temperature regimes have been obtained from the company *Sølvtrans AS* and are presented in Figure 15.
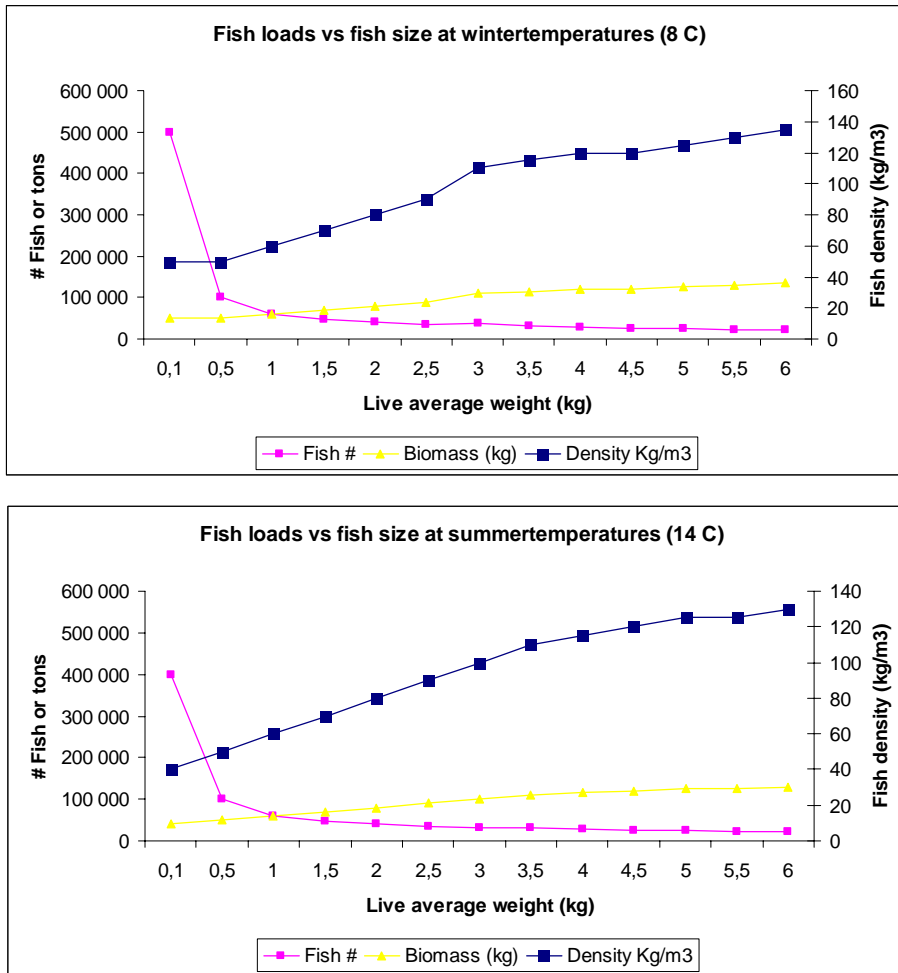
Figure 15. An example of practical criteria used to decide fish load on high (14 °C) and low (8 °C) temperatures (summer and winter conditions). We can se that the higher metabolism of small fish is causing a reduction in fish load. Likewise there is a reduction in fish loads under summer temperatures. (Data from Sølvtrans AS, Håvard Bjørndal)

7.9.4 Unloading

Arriving and the unloading phase is another critical moment in closed transport. It is important to avoid quick pH rise which can cause rapid transformation from ammonium (NH_4) to ammonia (NH_3) leading to ammonia toxicity. Unloading the fish from a well boat using vacuum pumps, without intake of fresh seawater with higher pH is almost inevitable. Opening the valves may thus increase acute risk for ammonia toxicity. Due to this problem boats with moving bulkhead are the ones mostly suited for unloading fish from a closed system. There are indications that fish may be mechanically wounded in the transporting process. (Aunsmo et al., 2008).

7.9.5 Emergency situations

During a closed transport the transporters should plan for emergency situations. Examples of such situations and actions are listed in table 7.

Table 7. Emergency situations and counteractions that should be planned for in a closed transport

Emergency situation	Probability to occur (*p*) [1(low)-6 (high)]	Damage (*d*) 1(low)-6 (high)]	Risk (*r*) *r=p*d*	Action
Failure of oxygen supply	2	6	12	Backup system
Failure of degassing of CO_2	2	6	12	Backup system
Wrong calibration of water sensors	3	5	12	Training, procedures and inter calibration
Rapid pH rise in TAN rich water	3	6	18	Avoid intake of water with higher pH after a long period of closed system or change water with a same or lower pH
Delayed and prolonged transport (truck)	3	3	9	Planning travelling route, bring enough oxygen supply
Delayed and prolonged transport (boat)	2	3	6	Planning travelling route bring enough oxygen supply
Fish starved to little prior to transport	5	4	20	Open system, change water if possible
Fish not fit for transport	3	6	18	Abort mission
Weak fish die during transport and disturbs circulation	1	5	5	Remove dead fish if possible. Use extra oxygen supply.
Failure in loading and un-loading equipment	1	6	6	Maintenance
Weather conditions	3	3	9	Planning

7.9.6 Additional comments for operations in transporting smolt and fry

The same issues as described for transport of large salmon apply for transport of salmon smolts in open and closed systems. In addition, special concerns during the transport of smolts are addressed below. We have distinguished between the challenges of three different transport situations:

1. Open transport
2. Closed transport
3. Alternating open and closed transport

It is important to note that existing transport regulations may limit the possibilities of carrying out the recommendations mentioned in this report. The points put forward below are discussed only in the context of water quality and fish welfare during transportation in seawater.

Smolt status, the importance of seawater tolerance

The first basic requirement for a meaningful discussion of smolt transport is the smolt status of the fish; specifically to what extent the juvenile salmon have developed seawater tolerance. Methods are available for the assessment of smolt status, e.g., seawater challenge tests and the determination of gill Na^+,K^+-ATPase activity, and the discussion below builds on the assumption that the fish are fully smoltified. If this is not the case, the fish should not be transported.

1. Open transport

Challenges related to water quality during open transport are primarily the quality of the water which is brought into the well, as well as the ratio between fish biomass and flow through capacity in the well. This is normally not a problem in a modern well-boat. Flow through rate is typically 3-4 times what is normally found in land-based rearing facilities. In most cases the flow through rate is sufficient to provide oxygen and remove metabolic wastes. Based on experience, 35 – 50 kg /m³ are considered safe densities when transport is mainly carried out with open valves. Carbon dioxide and ammonia will normally not accumulate to dangerous levels under the conditions described above (Rosten et al., 2004). One important consideration is the risk of exposing the fish to chemicals from ships and industry when transporting with open valves. Such exposure is often the cause when acute mortalities

are experienced during open transport. In order to determine the cause of mortality, water samples must be secured quickly, and fish must be taken for autopsy. Similarly, fish transported in open systems run a risk for of becoming infected if the well boat moves through an area with populations of infected fish.

Water quality during open transport, mixing zone chemistry
When two volumes of water with different qualities (pH, ionic strength, metals, salinity and content of organic substances) are mixed, unstable water chemistry is formed from the time of mixing, persisting for some time, depending on temperature. The classic situation is when acid Al-rich fresh water is limed or mixed with NaOH or seawater. The resulting increase in pH causes changes in Al species, from low molecular weight which aggregate to form larger molecules, so called polymers. During this polymerisation, Al becomes increasingly bio-available to the fish gills, before reverting to harmless Al-polymers or colloids after a period of time (minutes – hours depending on temperature, calcium levels and pH; Rosseland et al., 1992; Kroglund et al., 2001).

Series of experiments and the VK studies of KPMG, NIVA and UMB have further documented that a similar phenomenon may occur when small volumes of seawater are added to brown, fresh water, rich in humic acids or water with high clay content (which is harmless as untreated fresh water) to final salinities between 1 – 15 ‰ (Bjerknes et al., 2003; Rosseland et al., 1998, 2002; Strandring et al., 2003; Staurnes et al., 1998). This may happen during loading of smolts, as fresh water rich in humic acids is mixed with seawater in the well, or when the well-boat passes through the estuary of acid or humic rich rivers in narrow fjords. In order to avoid these situations, it is important to separate fresh water during loading of the well-boat so it is not mixed with seawater in the well, and to plan the route through the fjords to avoid passing through estuaries with unstable brackish water.

During transport with open valves through brackish water where metal-rich freshwater meets seawater (known as estuarine mixing zones) smolts may be exposed to aluminium deposition on the gills. Al deposition on gills have been described in relation to mortality of farmed salmon in open cages in fjords with large supply of acid Al-rich fresh water. Mortality is often associated with short-term episodes during winter, when mild weather, snow-melt and rainfall cause heavy run-off and a marked reduction in salinity (from > 20 ‰ to < 10 ‰) and temperatures (from 7 to 4 °C in surface water). The low-salinity surface water may form a layer 5-6 m deep with high concentrations of aluminium (> 100 ug/l). Situations have been described with increase in gill aluminium in farmed salmon from < 10 ug/g under normal conditions to > 200 ug/g during fresh water influence. One of the main reasons for the toxic conditions in the range 1 – 15 ‰ is the increase in ionic strength following seawater mixing mobilises Al bound to humic acids or colloids from the freshwater source (Teien, 2005; Teien et al., 2006). 0+ smolts which are transported in open systems during autumn may be exposed to toxic mixing zones if transported through fjords.

2. Challenges during closed transport
Water quality during closed transport is primarily determined by the fish metabolism. Metabolism increases with increasing temperature, stress level, reduction in fish size, increasing swimming speed/activity and increasing digestion. We are primarily concerned with carbon dioxide, CO_2, nitrogenous wastes (NH_4^+ and NH_3, below referred to as TAN), and total organic carbon, TOC.

Temperature during transport is probably the most critical factor for the accumulation of metabolites in transport water. Temperature in the sea is normally highest during late summer and autumn, so transport of 0+ smolts in August – September normally coincides with high temperatures. The fish metabolism will be high, and consequently there will be smaller margins during transport under such conditions. For 0+ smolts which are transported later in the autumn and winter, temperature will be lower and challenges related to metabolism will be reduced.

Production of carbon dioxide is related to oxygen consumption in an approximate 1:1 ratio, while TAN is closer to a 10:1 ratio. Consequently, CO_2 accumulates much faster than TAN levels. CO_2

accumulation in the water causes pH to drop, more so in a freshwater transport than in a seawater transport, caused by the higher buffering capacity of seawater.

For smolt transport in closed systems, field data suggest that CO_2 concentrations should be kept below 45 mg/l. Studies of juveniles in land-based tanks have revealed physiological responses at levels as low as 10 mg/l. However, studies also suggest that high CO_2 levels may be tolerated when water is slightly supersaturated with oxygen. The most common problems from high CO_2 are changes in blood chemistry (increase in pCO_2, $pHCO_3^-$, and reduced plasma chloride levels and reduced blood pH, Rosten et al., 2005). Both NH_4^+ and NH_3 are toxic to fish in a dose dependent manner. NH_3 (ammonia) is considered the most toxic component (Knoph, 1995) and is most studied. Experience from practical smolt transport suggests that TAN levels should not exceed 5 mg/l. Studies during transport of 0+ smolts in closed wells suggest that TAN levels of approximately 2 mg/l are achieved at a fish density in the range 25-40 kg/m^3 at a water temperature in the range 10-13 °C. Toxicity of ammonia compounds increases at oxygen concentrations below 70 % saturation and in combination with CO_2 above 45 mg/l (Rosten, 2002; Rosten et al., 2002).

3. Challenges during alternating open and closed transport, supply of new seawater
The challenges described for open and closed transport also apply in general for transport which alternates between open and closed system. The most critical factor is the duration of the period with closed valves. Experience suggests that problems occur when opening the valves to supply new seawater after a period of closed transport. This phenomenon is poorly studied; however, theoretical calculations suggest the cause being a sudden change in the ammonia – ammonium equilibrium caused by rapid increase in ionic strength and pH. Replacement of water in a well does not take place without the formation of temporary and unpredictable mixing zones between pockets of old and new water.

During closed transport in seawater, ammonia, NH_3, always represents the most critical factor for mortality and reduced welfare. The most important precaution is to keep pH below 6, 5 which will drive the equilibrium towards ammonium, maintaining a low ammonia concentration. This situation will normally occur after 1-2 hours of closed transport in seawater, caused by the gradual accumulation of CO_2 (Rosten et al., 2005). Opening the valves and taking in new seawater with pH 8, 2 represents a critical situation with potentially acute ammonia toxicity caused by the pH induced drive of the NH_4^+ - NH_3 equilibrium towards NH_3. This phenomenon is, however, poorly studied scientifically, and protocols which may prevent this phenomenon are not established, neither scientifically or in practical terms. As a consequence, unloading of smolts must take place without the addition of new water if the transport has been closed. For a well-boat, this means that water from the unloading must be returned to the well.

7.10 Transport of Marine fish

Knowledge on transportation, stress and related welfare considerations in marine fish is scarce. The information in the following chapter is therefore fragmentary relative to the previous chapters on salmonids, and is mostly based upon industrial experiences and in house guidelines.

7.10.1 Atlantic cod

Atlantic cod is regarded as a very promising species in coldwater fish farming, and more attention has been directed toward its culture which has a 20 year history in Norway (King and Berlinsky 2006) and is underway in Iceland, Scotland and Canada (Despatie et al. 2001). However, the effect of transport stress on fish is mainly reported on salmonid species such as Atlantic salmon (Portz et al. 2006). For Atlantic cod there are few studies on the stress response. However, a recent study has shown that Atlantic cod are very sensitive to acute increases in water temperature (Perez-Casanova et al. 2008). Resting levels for cortisol in cod have previously been reported by Staurnes et al., (1994) and the 24

hours recovery time for cortisol levels in cod has previously been reported by King et al. (2006) and King and Berlinsky (2006). Also, the effect of acclimation temperature on the acute stress response in juvenile cod showed that cod responded to a common, acute stressor in a manner similar to other teleosts and it was shown that plasma cortisol returned to baseline levels slower at lower than at higher temperatures (King et al., 2006). In a recent study by Dwyer and Iversen (in prep.), Bodø University College, a comparison of the primary and secondary stress response in Atlantic cod and Atlantic salmon during crowding has been performed. Atlantic cod subjected to multiple crowding stresses exhibited a delayed primary stress response compared to Atlantic salmon. While plasma cortisol in cod returned to pre-stress levels 24 hours after crowding, salmon showed no such recovery even one week after crowding, indicating a more severe stress response in salmon compared to cod. Plasma osmolality, magnesium, and glucose increased in response to crowding in salmon, indicating a possible disturbance in hydromineral and metabolic balance. For cod, no consistent changes in secondary stress responses were observed, with the exception of an increase in magnesium during the recovery period. The results indicate that salmon exhibited a greater primary and secondary stress response than cod. Whether the difference detected in primary stress response in this experiment indicates a delayed response in cod compared to other teleosts, or is simply a result of environmental or experimental factors, warrants further investigation. In adult cod, a delay in the secondary stress response after physical handling was also observed as a consequence of feed composition, Cod fed elevated dietary carbohydrates showed sustained post stress hyperglycaemia as compared to cod fed a non-carbohydrate diet (Hemre et al., 1991). This calls for attention with respect to feed and feeding also for cod prior to transport.

The effect of Aqui-S^{TM} sedation (4 mg/l) on the primary and secondary stress responses in salmon and cod, relative to two controls, was also evaluated during crowding Dwyer and Iversen (in prep.). While crowding provoked the primary stress response in the Aqui-S and no-sedation groups, metomidate was effective in blocking cortisol release in Atlantic salmon. In cod, crowding caused significant increases in the primary stress response in all treatment groups compared to resting concentrations. The stressor had no real effect on the secondary stress responses in salmon or cod, with the exception of a possible influence on plasma osmolality in salmon and plasma chloride and glucose in cod. As a sedative, Aqui-S^{TM} was ineffective in reducing the primary stress response in both species during crowding. Although metomidate was effective in alleviating the primary stress response in salmon, the anaesthetic did not have this effect on cod. Higher dosages of Aqui-S^{TM} (and metomidate) may be needed to reduce or alleviate the primary stress response in Atlantic cod. Since no known published investigations have been done on the efficacy of Aqui-S^{TM} as a stress alleviator in cod, further investigation is needed to determine optimal dosages to alleviate the stress response in this species during transportation.

Cod fry is reported to be relatively tolerant to ammonia. No growth limitation where observed for cod fry (3-4 g) during 96 days exposure for 60µg/l NH_3 (Foss et al., 2004).

7.10.2 Turbot and halibut

There is very limited published information on transport of turbot and Atlantic halibut. A recent report by Rosten et al (2007) covered aspects of welfare in these species during the production cycle, including comments on transportation. Halibut larval and juvenile stages are often farmed on same sites, while juveniles (30-350 g) are transported by truck, boat and plane (Iceland) to sea sites or marine tank facilities. Until now, slaughter fish are killed and bled at site prior to transport to packing and distribution facilities for markets, and well boats are rarely used. Compared to other farmed species the total production of halibut in Norway is approx 1200 metric tons per year (2006), thus the need for transportation has until now been limited to 60-380 000 juveniles (2003-2006) (Directorate of Fisheries 2007). Future transport of live halibut for slaughter will, however, require development of suitable hauling, transfer and transport equipment.

Commercial production of turbot in Norway is estimated to be within 2300 metric ton/y in 2006 (Fiskeridirektoratet 2007); with production of both juveniles and market fish by use of industrial heated water. Today the industry includes importation of juveniles (around 10 gram) from hatcheries to on-growing sites, with three days truck transports from Spain to Norway. In such closed transportation, water is supplied with oxygen and is ventilated, and the fish is cooled to approx 10 °C. Like halibut, market fish is slaughtered at site and then transported to packing and distribution facilities. Transport of juveniles between 1-3 g is commonly carried out by air transport where weight limitations apply.

In all aquaculture, it is in the interest of all parties to transport the fish as carefully as possible, with fish welfare in focus (HSA 2007). However, published best practices are not available. Experiences are drawn and practices adjusted according to the running situation and this do not always allow control of all transport parameters. An example of practical transportation guide for flatfishes, extracted from personal information from Dr. J. Stoss (leading competence at Stolt Seafarm AS, Øye, Norway) is presented in Appendix II. The given welfare indicators are in line with those discussed by Rosten et al. (2007).

Transport of marine species has to be differentiated into (1) Short term transport (< 24 hrs; available oxygen) or (2) Long time transport (> 24 hrs; water pH regulation and CO_2 cleaning necessary, in addition to oxygen supply). Further, it is important to differentiate the physiological status of the juveniles, ranging from newly metamorphosed juveniles to completely bottom staying fish. Younger fish seem to cope less well with low water temperatures during transport, due to physiological disturbances and osmoregulation problems (Staurnes 2001).

Indicators of final outcome represent good and objective measures of a correct and safe transport with respect to welfare and health:
1. A correct transport should not result in increased mortality compared to normal farming (< 0.1%).
2. The fish should be interested in feed in few hrs after entering the receiving unit, however dependent on temperature and body size. Fish (juveniles until 100 g) that do not feed within 24 hrs represent an unacceptable transport. Larger fish like brood fish or market sized fish will however be stressed by any transport and will typically not eat in several days.
3. Traumatic damages related to high densities should be avoided; typical injuries include physical eye damages from collisions, fin and tail bleeding, haemorrhages at the blind side, high mucus losses etc.

8. Assessment

8.1 Question no 1

"Which circumstance or circumstances might put the fishes´ welfare at risk? If several such circumstances exist, do these evolve independently of one another or do they influence one another in any manner?"

The fitness of the fish will influence the welfare of the fish during and after transportation. There is a significant risk of compromising the welfare if the fish is not in a physiological equilibrium when being transported. Diseased fish will be especially vulnerable to transportation. The severity will depend on the nature and stage of the disease. Ideally, from a welfare point of view, such fish should not be transported.

Loading and unloading of the fish are stressful processes that may even cause physical damage to the fish. Special care should be taken when fish is transferred from one water temperature to another. A rapid drop in temperature is generally considered to create more stress than a temperature increase. The loading and unloading stress must be compensated by adding sufficient oxygen to the transport water to avoid hypoxia and metabolic stress due to hyperlacticemia.

In a closed transportation system the aquatic environment within the container will deteriorate due to the accumulation of metabolites and to oxygen consumption. The concentration of carbon dioxide, total ammonia nitrogen (TAN), particles and total organic carbon will increase with time and the pH of water and oxygen concentrations will decrease. If left uncorrected, these alterations will, depending upon concentrations and duration, result in a deleterious aquatic environment, which eventually may be lethal for the fish. This can occur within a relatively short time period (20 to 30 minutes) if replacement oxygen is not provided.

Since seawater has a far higher buffer capacity than fresh water; transportation in fresh water will normally be associated with greater risks. This is due to chemical relationships involving both CO_2 and TAN.

As it is the metabolism of the fish that results in the alterations in the chemistry of the transport water, these changes will be influenced by various attributes of the transported fish, including species, size, life-stage, activity, feed intake and stress levels. Conditions associated with a risk of reduced welfare are therefore biomass and transport duration, in addition to those factors that may result in alterations in the chemical equilibrium of the water. The water parameters that, both individually and synergistically have the greatest risk associated with reduced welfare, are concentrations of oxygen, carbon dioxide, and ammonia. Important chemical interactions between these three water parameters are related changes a) in CO_2, pH and NH_3; b) CO_2 and O_2 levels; and c) O_2 and NH_3 levels.

Finally, fitness of the fish, water chemistry and temperature will all interact in a complicated manner with influence on coping and welfare.

Emergency situations which include the risk scenarios given above may also arise from breakdown of equipment, technical failure, bad weather conditions, road conditions and other unforeseen external and human factors.

8.2 Question no 2

"Do problems occur after a specific period of time in the transport container, or will the time lapse before the onset of problems be dependent on various specific factors?"

The biomass, size of the fish, time of pre transport starvation as well as temperature and general water quality (fresh- and seawater) are all important factors for the time lapse before water quality problems occur.

With respect to oxygen concentration, problems will occur within minutes should the holding water not be compensated for by provisional oxygen.

Total ammonia nitrogen (TAN) accumulates en route in the transport vessel or unit. In association with water changes in transit or with discharge, there is an increased risk of ammonia poisoning as a result of a rapid increase in water pH. The push of equilibrium towards production of toxic un-ionised ammonia can occur in a matter of minutes.

Water carbon dioxide production will lower the pH and thereby reduce the formation of ammonia from the increasing TAN, but it will on the other hand be stressful acting negatively on the acid-base balance in the fish. Carbon dioxide increase in water will rapidly cause an increase in carbon dioxide partial pressure in the fish blood that affects the oxygen binding to haemoglobin. This leads to increased requirement for available oxygen either by lowering the water temperature, increased oxygen supply, or a combination of both. The accumulation of CO_2 in the blood (hypercapnia) is compensated physiologically by increase in plasma bicarbonate. The rapidness and the magnitude of the CO_2 accumulation are important, since the fish has a limited possibility for adaptation. In such cases it is important to avoid extra acid base stress that might arise from hypoxia and struggling, causing pH drop in muscle and blood due to hyperlacticemia. There is reason to believe that there is an interaction between CO_2 levels in the water and the toxicity of ammonia, especially at simultaneously low dissolved oxygen levels in the transport water.

8.3 Question no 3

"Is it possible to prevent the occurrence of circumstances that compromise the welfare of the fish? If so, outline the necessary or relevant measures applicable".

It is entirely or partially possible to avoid those conditions which result in reduced welfare, depending upon the individual transport circumstances. Hypoxia can be prevented by adding extra oxygen to the transport water. One may avoid hyperoxia by using feedback controlled oxygenation systems. Hypercapnia may be counteracted by using water treatment, like degassing. The size of the degassing pump must be adapted to the CO_2 production rate of the biomass in the transport and the degassing efficiency. The risk for total gas pressure above 100 % can be counteracted by running the degassing system prior to loading the fish into a closed system. This procedure will ensure that any compressed air from filling the well or starting the circulation system will evacuate and water will adjust towards equilibrium. Ammonia poisoning can be avoided by keeping the pH in a safe area, suggested to be in lower limit range 6.2 – 6, 5, and avoid rapid pH rise due to intake of new water volumes with higher pH. Un-loading of fish is a typical risk operation which can cause such pH rise, but this can be counteracted by using technical solutions like moving bulkhead and the return of transportwater to the well. Models for transport lengths and loads based on critical water chemistry parameters can be developed but are not commonly in use in the transport business today.

Excessive formation of foam due to oxygenating and degassing of transport water, especially in seawater during summer season, is a known problem during closed transport. This can be partly counteracted by using different kind of skimmers. The process might on the other hand be a way to treat the water in a closed system.

High metabolism will result in reduced water quality. Metabolism can be lowered by introducing an adequate period (3-5 days) of starving the fish prior to transportation, transport the fish at a lowered temperature, and reducing the stress and swimming activity.

In order to minimise the risks, the fish needs to be disease free and in good physiological condition, Atlantic salmon smolts needs to be completely smoltified before transportation. Care should be taken during loading and unloading to reduce stress and physical damage. If the freshwater at the smolt farm contain particles, humus or freshwater with high aluminium content, the well boat should not mix seawater and freshwater from tanks. A fish/water separation should be used at the boat level.

If flooding from neighbouring rivers creates a brackish environment when loading smolts from a smolt farm, the well boat should not have open valves during loading but use tanks filled with full salinity.

If the well boat has used closed transportation, fresh seawater should not be mixed into the wells during unloading at the netpen rearing site to avoid ammonia formation at pH increase. The risk is modulated by the accumulated TAN levels (biomass and transport length)

Since the exposure is derived from concentration multiplied with time, the overall risk associated with exposure to unfavourable water qualities in a closed transportation can in general be reduced by decreasing the transportation time and reducing the fish density.

The concentrations of oxygen, nitrogen and carbon dioxide should be carefully monitored during the transportation. Monitoring equipment should be properly maintained and calibrated prior to transportation. It is important that crew on the well boat and the driver on the transporting trucks are well trained in transportation routines and emergency plans. A closed transportation system is more vulnerable to technical failures and requires more skill to operate and to handle in emergency situations according to action plans.

Anaesthetics for use in commercial fish transports in Norway (aquaculture and compensatory releases) have not been performed yet. This method has yet to be licensed both in Norway and in the rest of Europe and more studies have to be performed if this method is to be implemented in commercial fish transports. In view of the positive research results using anaesthesia, this may, however, be an alternative strategy for increasing fish welfare during transport leading to better survival and growth of fish at the releasing site.

8.4 Question no 4

 "Does the reduction in water temperature in itself have a negative welfare implication? Could the reduction in water temperature result in the reactions of the fish becoming difficult or impossible to observe, and thus make the interpretation of their welfare status more difficult?"

Large and rapid changes in water temperature will affect water chemistry, as well as the physiology and the behaviour of the fish, and will thereby have the potential to influence fish welfare.

Gradual reduction in water temperature prior to or during transportation will however contribute to reduced risk for welfare problems by decreasing the metabolism and stress responses in the fish, and by increasing the oxygen saturation level in the transport water. These factors will all to some extent reduce the impact on water quality during transportation.

However, too rapid temperature reduction will result in thermal stress, which can be lethal. This might happen when the fish are transferred from one water temperature to another during loading or unloading. It is therefore recommended that the temperature is gradually reduced, either in the rearing facility or within the transport container.

Because fish are less active at low temperatures, the temperature reduction makes it more difficult to interpret behaviour associated with compromised welfare induced by osmotic disturbances, handling injuries, changes in water quality or too rapid cooling.

Indication from studies of closed transport in Scotland points in the direction that a combination of mild CO_2 accumulation, mild lowering of transport water temperature and sufficient oxygen supply generates a slower and less responsive fish, with high slaughtering quality (long pre-rigor time). Longer rigor time is normally regarded as positive for fish welfare.

8.5 Question no 5

"Can the water temperature be reduced in such a manner that the welfare of the fish is not put at risk?"

Gradual cooling will reduce the risk of thermal stress and decreased welfare. However, there is limited knowledge on clearly-defined optimal rates for temperature reduction for different species and for different transport conditions. If welfare is measured by death-rate, slaughter quality, oxygen saturation of the blood, ion regulation and blood glucose concentration, field data show that that cooling of salmon at the rate of 1.5 °C per hour after a restitution period is of minimal risk and constitute a rule of thumb. However, if other welfare indicators are used (e.g. behaviour and cortisol concentrations), it is more difficult to provide a defined level at which welfare risk is compromised. Current knowledge indicate that 6 °C is a lower limits for targeted water cooling, while experiences show that salmon can acclimate to 4,5 °C given sufficient time to acclimate.

9. Conclusions

Transportation of life fish will always imply some kind of risk. The fitness of the fish will have great impact on the risks associated with transportation. Sick or immunocompromized fish will be extremely vulnerable and should not be transported. Care should be taken to minimize handling stress prior to and during transportation.

Open system transportation is generally considered to be less risky than transportation in closed systems. However, the risks connected with closed systems can to a greater extend be subject to calculations and modelling than risks in opens systems. This is because the open system is exposed to ambient environment and its natural fluctuations in temperature and changes in water chemistry. Scientific data on large scale closed systems are few, and risk modelling depend to a large extend on empiric data from the industry and experiments conducted in laboratory facilities.

Transportation in closed systems requires water treatment, as well as systems for observation of fish and monitoring of water quality. Consequently, well trained crew and skills to handle emergency situations are needed.

The metabolism of the fish will influence the water chemistry and thus the welfare of the fish during transportation. With the exception of very young fish (larvae), all fish should be starved at least for a period of three to five days before as well as under transportation.

It is difficult to give exact upper safe limits for water chemistry parameters, since many of the parameters interact in a complicated manner. However, some figures derived from industry experience exist and they are included in this report. These values should be used with care and considered as guidelines.

Oxygen, carbondioxide and TAN represent limiting factors during closed transports. While elevated carbon dioxide often is a first limiting factor in the transport water, it can be degassed by increased dimensions of water treatment system. There is, however, a risk that the gained improvement in water quality is used for optimising transport biomass, and thereby risks for elevated TAN and pH, and eventually toxic un-ionised ammonia. In closed transportations of salmon fingerlings and smolt, the concentration of CO_2 should be below 20 mg/L. At transportation of fish for slaughter with chilled water, the light sedation due to accumulation of CO_2 is considered beneficial. Thus, a higher concentration of CO_2 up to 30 mg could be accepted. This will depend on biomass, temperature and duration of transportation.

It is not possible to give an exact limit for the maximum transportation time in a closed system. This will depend on fish species, density, and temperature and water treatment in the transport unit. Transportation in closed systems should be as short as possible, both in distance and time, while extended transportations should be performed with reduced biomasses. By adding seawater to reach a salinity of 1 ‰, the buffer capacity in fresh water will increase and the risks associated with accumulation of CO_2 and mixing of different water qualities will to some extent be reduced. Necessarily treatment of the seawater must be applied.

Accumulation of CO_2 and TAN is faster in warmer water in the autumn when 0+ smolts are transported. The risk for the fish welfare problems is thus probably higher for transportation of 0+ smolts than in 1+ smolts due to weather and climatic conditions. Field data suggest that the toxicity of nitrogenous wastes and CO_2 can be reduced to a certain degree by keeping oxygen concentration around 100 %. The risk can be be avoided by proper planning.

A lowering of the water temperature during transportation can reduce the impact of several factors on fish physiology and welfare. The temperature reduction must be carried out with great care and is recommended not to exceed 1, 5 °C/hr. For Atlantic salmon and rainbow trout, the transportation temperature should not aim to be below 6 °C.

9.1 Future needs

An increase in transportation of life fish in closed system will require an upgrading of the present well boat fleet and transportation trucks with respect to systems for water treatment and monitoring, quality control and education of the crew.

9.2 Knowledge needs

Most of the information used in the assessment is either based on small scale laboratory studies or on industry derived experience. There is a clear need for more scientific based knowledge on most of the aspects related to transportation of live fish in closed systems.

Use of sedatives like clove oil, may have an overall beneficial impact on the welfare of fish during transportation. More scientific data is needed before firm conclusion can be reached.

The effects of a combination of low CO_2-level, slight reduction of temperature and sufficient oxygen levels have to be studied more in detail to explain the responses (physiology, behaviour and flesh quality) of the fish during a controlled closed and cooled transport.

- Further physiological testing of fish response during transport (helicopter, truck and well-boat) and a special attention should be given to marine species such as Atlantic cod, turbot etc. where there exists little knowledge of the fish response during transport
- Test the use of anaesthetics (e.g. clove oil), salt addition etc. as stress reducers during transport in small and big scale experiments

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11. Appendix I

11.1.1 OWI - Physiology

1. Nourishment
   1.1. Deprivation from food before transport reduces risk of mortality during transport and handling.
2. Changes in respiration
   2.1. Increase in respiration is linked to stress or adverse water quality; decrease in respiration is linked to water quality in transport water. Changes in respiration are also linked to the current / swimming speed in the transport container.
   2.2. Experience shows that observation of respiration rate is possible by using underwater videocameras (see figure X)
3. Ability to maintain osmoregulation
   3.1. Fish are tolerant to high or low salinity within limited borders depends upon live history stage and or species. The water quality before, under and after transport is relevant.
   3.2. In a closed transport, there might be limited possibility to change salinity
4. Changes in body color
   4.1. From practical farming and live fish transport, colour changes of the fish might indicate stress or mal-adjustments.

11.1.2 OWI - Behaviour

5. Changes in feeding response and appetite
   5.1. It is normally taken as a good sign that transported fish regain their appetite and feeding behaviour shortly after a transport.
6. Changes in swimming pattern
   6.1. This is by operators normally taken as an indicator of the condition of the fish during transport, e.g.: the ability to maintain balance and position in the transport container.
   6.2. Might be observed by underwater video cameras.
7. Agressivity and/or cannibalism
   7.1. To our knowledge, although not reported in transport, occurrence of aggressive behaviour or cannibalism might be and indicator of stress after transport in combination with deprivation of food

11.1.3 OWI - Health

8. Mortality
   8.1. This is the classical and most used parameter to evaluate the fitness of a transport
9. Deformities
   9.1. Not in question as far as we know, but fish with deformities might be less fit for transport.
10. Gill tissue changes
    10.1. Unspecific gill disease with inflammation, excess mucus secretion, necrosis or apoptosis might occurred as a reaction to adverse water quality during transport, caused either by hyper oxygenation, high concentration of NH_3 or estuarine mixing zone water (Al depositions)
11. Wounds
    11.1. Wounds can occur as a consequence infectious disease, mucus and skin injures during loading, transport and unloading. Design and use of equipment, fish density and rough weather are the most likely causes of skin injuries and must be taken into account during evaluation of the suitability of the transport vessel.
    11.2. It is important to avoid wounds both in open and closed transport, since wounds might affect the fitness of the fish before transport
12. Infectious diseases

12.1.   The outbreak of an infectious disease will strongly reduce the fitness of the fish. The severity depends on the nature of the fish.

12.2.   Stress during loading and transport might suppress the immunosystem, leading to increasing risk for outbreak of disease.

12.3.   Closed transport system is often used when the fish is sick, with the aim of preventing spread of fish disease. Since a fish disease will affect the fitness of the fish, special attention should be drawn to this practice.

12.4.   The use of a closed transport system might in one side lower the risk for spreading fish diseases (in the case where the fish cargo is infectious). On the other side, there is also a risk for contamination from infectious agens still in transport system (due to insufficient or faulty wash and disinfection from previous cargo(s).

13. Parasites

13.1.   The spreading of *Gyrodactylus salaris* to the Norwegian River Skibotn was probably water exchange from a truck carrying trout.

13.2.   High infection rate of sea lice shortly after transport to sea might indicate a reduced immunity caused by multiple stressors like exposure to adverse water quality conditions prior to and/or during transport and the stress from transport itself.

11.1.4 OWI - Operation

14. Protection against predators

14.1.   Compared to towing of open nets with tuna fish, with the risk of shark attacks, will a transport in side a well boat or truck offer protection against predators. Besides this, this is not in question.

15. Anaesthetization and slaughtering

15.1.   In closed systems with large salmon, there is established a practice of letting the fish get sedated during transport due to mild self-induced hypercapnia, combined with gently lowering the water temperature and sufficient oxygen supply. Experience and field studies from Scottland confirm that this practise produce fish that is calm before killing. See chapter 7.7.1.

15.2.   Mild anaesthetization of the fish with a cortisol blocker added to the water is not common practise, but might lower the metabolism and / or lower the stress response associated with handling and transport.

16. Design of equipment

16.1.   This is very important in the context of offering condition suitable for fish welfare. Experience and field studies (Rosten et al, 2006) show that oxygen supply, carbon dioxide degassers are necessary to carry out a closed transport. In addition, an ability to control water temperature and unload fish with high water stand in the well (that is: moving bulkhead).

12. Appendix II

An in-house ethical guide for transport of flat fishes derived from the industry (Dr. J. Stoss, pers. comm..) include following considerations:

1. *Sorting;* Sorting (grading) by size should be done about a week prior to transport to avoid aggression during pre-transport starvation.
2. *Vaccination or prophylactic treatments* against ectoparasites should be completed in due time before transport to prevent development transport injuries or wounds, and outbreak of diseases after transport.
3. *Juvenile fish should be kept starving* for several days prior to transportation. Ten gram juveniles should be fasted for 2-4 days; shorter period at elevated temperatures >15 °C, and longer at lower temperatures. While the gastrointestinal tract should be completely emptied, care should be taken to prevent degradation of somatic tissues. The aim with starvation is to ensure acceptable water quality during the transport - prevent excess faeces and reduce the requirement for oxygen.
4. *Temperature reduction.* The temperature should be gradually reduced under the starvation period and kept low during transportation. This can be problematic, especially during long term transport. Transports can still be done without temperature control but one needs to consider possible temperature effects which might develop during transport (depending on duration of transport and ambient temperatures). To prevent problems, fish densities during transports can be reduced. In certain instances (long term transport over 1-3 days and rather high or low ambient temperatures) transports have to be postponed. It is difficult and probably not convenient to recommend optimal transport temperatures, since this depends on ambient rearing temperature. Atlantic halibut juveniles reared at 15 °C, can be transported successfully at 8 °C, while 2 °C represents thermal stress (Staurnes 2001). The main point is to reduce the temperature gradually to a temperature relative to the ambient.
5. *Temperature increase.* At receiving site, the fish will be transferred to water with elevated temperatures than in the transport. This is considered unproblematic as long as the temperature is kept below the normal temperature range of the species.
6. *Temperature fluctuations.* Expected temperature under long distance transportation may vary with the weather situation, and should be carefully evaluated and planned to prevent mortalities. Small water volumes and thin cage insulation may allow the water to reach critical temperatures depending on ambient temperature in the area receiving the fish; for example transportation in summer to China (> 30 °C) with extended transport periods (20-30 hrs) after landing. Likewise, open transports on truck or boat to and from South Europe, may experience high temperature fluctuations. It is therefore of great importance to plan the transport and include temperature control.
7. Optimal *water oxygen level* should be secured under the transport (150 % is acceptable), while super saturation (>200 %) should be avoided. The ventilation frequency is regulated by water oxygen saturation and physiological status. Accordingly, increased ventilation is observed at declining water oxygen and increased metabolism, stress, gill diseases etc.
8. *Water buffering/CO_2 removal* should be considered in all long transports (Grøttum et al 1997). High carbondioxide values cause hypercapnia, suffering and losses. Seawater pH should not decline to below 7. In closed transport units (for example plastic bags with oxygen atmosphere, a buffer should be added (for ex Tris buffer)**.** In open units, like large tanks on trucks or boat should be equipped with ventilation, which effectively removes CO_2; here no extra buffer is needed.

Transportation without water. It is possible to transport turbot (fish without scales; i.e. not halibut) in a moisture environment without water, however with temperature control. This is normally used in long distance air transport where the transport weight is of major importance. While this is acknowledged as an acceptable way of transportation, there is no described procedure for such transport available.