

## Effects of commercial live transportation and preslaughter handling of Atlantic salmon on blood constituents<sup>#</sup>

Efecto del transporte comercial y manejo *ante mortem* sobre constituyentes sanguíneos de salmones del Atlántico

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### RESUMEN

Se evaluaron los efectos de la cosecha con transporte en wellboat y el manejo *ante mortem* sobre los constituyentes sanguíneos relacionados al estrés en salmones (*Salmo salar*). Diez peces fueron muestreados en cada una de las seis etapas denominadas: centro; después de la carga; después del transporte; después de la descarga; después del reposo y después del bombeo hacia la planta procesadora. Se determinaron las concentraciones sanguíneas de cortisol, glucosa, lactato, sodio, cloruro y osmolalidad. En cada etapa, las concentraciones de los constituyentes sanguíneos analizados cambiaron de acuerdo con la severidad del tratamiento aplicado. Los resultados de este estudio indican que una de las etapas más estresantes fue el bombeo desde las jaulas de reposo hacia la planta de proceso. La concentración de todas las variables estudiadas disminuyó después de 24 horas en las jaulas de reposo, y todas, a excepción de la glucosa, aumentaron después de que los peces fueran bombeados a través del salmoducto desde las jaulas de reposo a la planta de proceso. Esto confirma que este último manejo es la etapa más estresante de las estudiadas durante este transporte comercial.

*Key words:* stress, well-boat, cortisol, glucose, lactate.

*Palabras clave:* estrés, well-boat, cortisol, glucosa, lactato.

### INTRODUCTION

Animal welfare has become a subject of great importance to consumers and to authorities in some countries and it is also becoming an important issue in fish industry (Håstein 2004). In aquaculture, stressors can be multiple and of diverse origin; but handling and confinement are probably the most severe stressors seen in commercial aquaculture of salmon (Kestin 1994).

Harvest is an inevitable part of farm practices in aquaculture and places fish in an acute stress situation by reducing surrounding water volume and causing increased activity, as fish attempt to escape (Thomas *et al* 1999). Live transport in wellboats at high stocking densities and high stocking densities within the resting cages before slaughtering are stressful procedures (Skjervold *et al* 2001). Harvest is under-represented in investigations of welfare and stress, probably due to the endpoint (for the biologist) being the death of the fish (Erikson *et al* 1997,

Thomas *et al* 1999, Skjervold *et al* 2001). Harvest also entails a fasting period of one to three days, depending on the water temperature (Wall 2001). From a welfare point of view, fasting should be as short as possible (Ashley 2007, Stevenson 2007). Acute disturbances produced during harvest cause proportional physiological changes and these can be useful indicators of the degree of stress experienced by the fish (Barton 2000).

Stress in commercial salmon farming is also of great commercial importance as it may have severe detrimental effects on flesh quality, for example, meat firmness (Skjervold *et al* 2001), onset of rigor mortis and its duration, muscle pH, water holding capacity, increased soft texture, gaping and a shorter shelf life (Huss 1995, Thomas *et al* 1999, Skjervold *et al* 2001, Jittinandana *et al* 2005). From both the commercial and animal welfare point of view, it is important to understand the nature of the physiological changes that take place in fish metabolism and to implement appropriate measures to reduce their intensity (Kestin 1994, Pottinger 2001).

The real situation cannot be replicated on a small scale study so any research must take place in an industrial situation with the cooperation of a commercial operator. In Chile, commercial live transportation is carried out

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mainly by wellboats, and journeys can go from 1 hour up to 18 hours at the longest. Starvation periods also vary depending on the company involved, but they can come up to 5 or 7 days. The fish are loaded by means of pumps into the wellboat's chambers, where they are kept during the whole journey at stocking densities of up to 120 kg/m<sup>3</sup>. Once the wellboat arrives to the processing plants piers, unloading takes place usually into the resting cages where the fish are given the time to recover.

The purpose of the present study was to quantify the changes in blood concentration of glucose, lactate, cortisol, sodium, chlorides and osmolality as a measure of the response of salmon to the different stages involved with transport to slaughter by wellboat.

## MATERIAL AND METHODS

Three commercial wellboat journeys were studied during January 2006 in Chile. All three journeys started at a production farm in Garrao Island (Compañía Pesquera Camanchaca S.A., Archipiélago de las Guaitecas) and ended at the Yadrán Quellón S.A. processing plant in Quellón. At the farm salmon were kept in 30 m diameter and 25 m depth circular cages at a stocking density of 18 kg/m<sup>3</sup> and were deprived of food for 4 days before transport. The fish were loaded during approximately 2 hours from a crowding "pocket" made with a net in the cages, into the wellboat's tanks through a suction pump and then transported at a well density of 107.8 kg/m<sup>3</sup> with an average water temperature of 11.5 °C. The distance between the two sites was approximately 100 miles, and the whole journey took approximately 8 hours, with good weather conditions on each occasion.

A ship was used, which includes the latest technological devices required by the salmon industry and has a transport capacity of 780 m<sup>3</sup> with a pressurized tank. It also has a water recirculation system, an oxygen generation plant, oxygen and temperature monitoring system, an auxiliary oxygen diffusing system and underwater video cameras. Water oxygen conditions were monitored during the journeys and it was always kept above 6 mg/L by using an oxygen diffusing system. In average 18,700 fish were transported in each occasion.

After wellboat transportation, into which the fish were unloaded into resting cages through the same pump with which they were loaded; this procedure took approximately 1 hour. The resting cages were located approximately 120 m from the shore opposite to the processing plant - and in turn, the latter was placed approximately 100 m inland. The cages were of the square type, with a capacity of approximately 4,000 m<sup>3</sup> and a depth of 30 m; the stocking density was 28.9 kg/m<sup>3</sup>. Water had 9.2 mg/L of dissolved oxygen with an 85% of saturation, the average water temperature in the resting cages was 14.1 °C with a visibility of 5 m. The fish were kept in the resting cages for 24 hours in each

journey and then delivered to the processing plant using a suction pipe of 222 m length.

## BLOOD SAMPLING PROCEDURES

A total of 180 individual Atlantic salmon (*Salmo salar*) of harvest size (4 to 5 kg) were randomly sampled at 6 different stages (from the production farm to the processing plant) during each of the 3 commercial journeys as detailed in table 1. At sampling the salmon were stunned with a blow to the head and bled by gill cut. The handling for blood collection lasted less than one minute in order to avoid changes in the variables induced by the manipulation during sampling. Two blood samples were taken from each salmon; the first into a 0.2% NaF/EDTA treated tube to obtain plasma by centrifugation for the determination of glucose and lactate concentrations; the second into a tube without anticoagulant, in order to obtain serum to determine concentrations of cortisol, sodium, chloride and osmolality. Both plasma and serum were frozen (-20 °C) for later analysis.

## BLOOD ANALYTICAL PROCEDURE

Glucose concentration was determined by the GOD-PAP method, without deproteinization, spectrophotometrically. The lactate concentration was also determined spectrophotometrically by means of an enzymatic-colorimetric method. Serum sodium concentration was determined with a Perkin-Elmer 238 atomic absorption spectrophotometer. Both urea and chlorides were determined colorimetrically with the GLDH enzymatic method and the tripyriditiazine (TPTZ) method respectively. Glucose,

**Table 1.** Number of fish sampled at each stage during transport and preslaughter handling from the production farm to the processing plant.

Número de peces muestreados en cada etapa del transporte y manejo *ante mortem* desde el centro de producción a la planta de proceso.

Sampling stages	Fish (n)
1: In the cages in the production farm, before loading the fish into the wellboat ( <b>farm</b> )	10
2: In the wellboat after loading the fish, before leaving the production farm ( <b>loading</b> )	10
3: In the wellboat at the end of the journey, before unloading the fish into the resting cages ( <b>transport</b> )	10
4: In the resting cages, after unloading the fish from the wellboat ( <b>unloading</b> )	10
5: After 24 hours in the resting cages ( <b>resting</b> )	10
6: Within the processing plant after pumping the fish from the resting cages ( <b>pumping</b> )	10
Total per wellboat journey	60

lactate, urea and chlorides were determined in a Cobas Mira Plus clinical chemical autoanalyzer (Roche®). Urea Nitrogen was determined in order to calculate the osmolality using the formula:  $Urea - N = \frac{urea}{2.14}$  Serum osmolality was obtained using the formula:

$$mosmol / kg = 1.86[Na] + \left(\frac{Glucose}{18}\right) + \left(\frac{Urea - N}{2.8}\right)$$

according to (Holmes 1962). Cortisol was determined by a RIA technique, using commercial kits (Cortisol Coat-A-Count, DPC, USA) validated for salmon.

## STATISTICAL ANALYSIS

Linear mixed effect model for repeated measures data formalise the sensible idea that an individual's pattern of responses is likely to depend on many characteristics of that individual, including some that are unobserved. Data were analysed with the MLwiN statistics package v 2.02 (Rasbash *et al* 2004) using a hierarchical model (Singer and Willett 2003). For each blood constituent, explicitly the model is:  $Y_{ij} = b_{0ij} + \beta_1 t_j + \beta_2 t_j + \dots + \beta_n t_j + \pi_{0i} + \mu_{0j} + \varepsilon_{0ij}$ . Where  $i$  = occasion during transportation and  $j$  = individual and  $t$  represents the observation made at time  $t_j$  on individual  $i$ . Assuming to have a bivariate normal distribution with zero means for both variables and variances  $\sigma_\mu^2$  and  $\sigma_\varepsilon^2$ . The variables considered in the analysis were: Stages (see table 1). Significance of each variable in each stage (P value) was expressed in accordance to the comparison with reference values of the first stage (farm).

For the final model, a likelihood ratio test was used to assess goodness-of-the-fit (-2\*loglikelihood, Iterated generalized least squares (IGLS) deviance) and the significance of individual parameter estimates were assessed

against a Normal distribution. In all models, higher order interactions were tested. The final models given above represent the most parsimonious summary of the data.

## RESULTS

The concentrations of each blood constituent at each sampling stage during transport and handling of Atlantic salmon are shown in table 2. Plasma glucose concentration was higher after transport in the wellboat showing an almost two-fold increase ( $P < 0.05$ ) compared with the initial value (farm), to return slowly to approximately the initial level during the rest of the process (table 2).

Compared with values on farm, plasma lactate concentrations increased slightly during loading and after transport (table 2). During the following 24 hours, in the resting cages, the lactate concentration tended to decrease but it was only after pumping the fish into the processing plant that the increase in plasma lactate concentration became significant ( $P < 0.05$ ). Cortisol concentration had no variation until after transport ( $P > 0.05$ ). After unloading and after the 24 hour resting period, cortisol reached its lowest level (table 2). The pumping into the processing plant caused the mayor increase ( $P < 0.05$ ) in cortisol concentration compared to the reference stage (farm, table 2). Serum sodium concentration remained constant during transport and handling (table 2) and only increased ( $P < 0.05$ ) after pumping from the resting cages to the processing plant. Serum chloride concentration showed a sustained increase from loading until after unloading (table 2), with the highest increase after pumping ( $P < 0.05$ ). No significant variations were observed in serum osmolality compared with on farm values until after unloading ( $P < 0.05$ , table 2). As with most variables, osmolality dropped to initial farm values following the 24 hour

**Table 2.** Blood concentrations (means  $\pm$  SD) of glucose, lactate, cortisol, Na, CL and, osmolality, at different sampling stages during the transport and preslaughter handling of salmon.

Concentraciones sanguíneas (media  $\pm$  DE) de glucosa, lactato, cortisol, Na, Cl y osmolalidad, en diferentes etapas del muestreo durante el transporte y manejo previo al beneficio de los salmones.

Sampling stages	Glucose mmol/L	Lactate mmol/L	Cortisol ng/ml	Na mmol/L	Cl mmol/L	Osmolality mosm/kg
Farm	1.8 $\pm$ 1.5	0.6 $\pm$ 0.3	205.7 $\pm$ 66.6	144.9 $\pm$ 6.1	119.0 $\pm$ 2.9	269.9 $\pm$ 11.2
Loading	2.9 $\pm$ 1.6	1.1 $\pm$ 1.3	226.2 $\pm$ 12.5	143.9 $\pm$ 7.0	120.8 $\pm$ 2.5	270.8 $\pm$ 12.0
Transport	3.4 $\pm$ 1.5*	1.0 $\pm$ 1.0	161.2 $\pm$ 85.5	147.6 $\pm$ 9.3	122.8 $\pm$ 3.6*	275.0 $\pm$ 18.2
Unloading	2.6 $\pm$ 1.7	1.4 $\pm$ 0.5	126.2 $\pm$ 51.6*	149.1 $\pm$ 8.8	123.4 $\pm$ 4.2*	282.4 $\pm$ 13.4*
Resting	2.3 $\pm$ 1.5	1.0 $\pm$ 0.6	103.2 $\pm$ 59.2*	143.1 $\pm$ 4.0	121.5 $\pm$ 2.8*	266.8 $\pm$ 7.4
Pumping	1.7 $\pm$ 1.1	3.8 $\pm$ 2.9*	326.0 $\pm$ 77.2*	156.9 $\pm$ 12.7*	126.1 $\pm$ 2.5)*	297.1 $\pm$ 20.4*

\* Statistical differences ( $P < 0.05$ ) between farm values with other sampling stages.

\* SD diferencia estadística ( $P < 0,05$ ) entre los valores iniciales (en el centro de cultivo) con los de otras etapas de muestreo.

rest and increased ( $P < 0.05$ ) again after pumping the fish into the processing plant.

## DISCUSSION

The importance of this study lies in the fact that it recognised the most stressful stages of transport, measured through blood variables and it took place during real conditions of commercial live transportation of fish; it was not an experimental set up. Results in table 2 reflect the natural variation of blood parameters related to stress during a routine transportation of live fish of the local salmon industry from the south of Chile.

At the start of the transport process the fish were sampled on farm, directly from a “pocket” made with the net inside the cages. The blood constituent values at this stage (farm) were considered as reference values for this study (table 2).

A primary response to stress is an increase in the levels of plasma cortisol (Barton and Iwama 1991, Barton 2002, Acerete *et al* 2004). High values of cortisol at the first sampling during the netting and loading are even higher than those observed in stressed Atlantic salmon in a previous study (Erikson *et al* 1999), therefore, we could assume that at this stage our fish were already stressed as a rapid elevation of this variable is seen after an exposure to acute stress (Wendelaar Bonga 1997). When the first sampling at the farm was done, the fish had already been crowded in the net for an average of 1 hour; this would explain the high initial values of cortisol as it was also seen in other studies (Skjervold *et al* 2001); hence, factors like handling before sampling and different farming conditions may have contributed to the high cortisol concentrations in fish at the first stage (farm). Compared with the initial on-farm mean, cortisol had no variation until unloading and after resting, when it reached its lowest concentration ( $P < 0.05$ ).

Plasma lactic acid concentration increases in animals subject to stress and with increased muscular activity. Once in the blood, lactic acid dissociates as protons and lactate ions (Thomas *et al* 1999). During the first three stages of transport on farm, after loading and after transport, although the stocking density was high (mean 107.8 kg/m<sup>3</sup>), the confinement probably did not lead to an increased muscular activity and, as expected, no differences in lactate concentration were observed. During heightened activity fish white muscle mainly generates energy by means of an anaerobic metabolism with increased production and accumulation of lactic acid, which must be transported to the liver for metabolization (Huss 1995).

Lactate concentration has been shown to increase significantly in several species following severe exercise or as a result of hypoxia (Acerete *et al* 2004). The concentration of lactate reflects the increased levels of physical activity as a response to the different stages of transport (Grutter and Pankhurst 2000). The low plasma lactate concentration seen in this study while the fish were in the wellboat (after loading and after transport), are in accordance with the

restriction of movement and thus muscular activity, due to the high stocking density.<sup>1</sup>

In our study, after loading the fish into the wellboat with the suction pump (stage 2), cortisol concentrations increased slightly whilst glucose levels peaked significantly ( $P < 0.05$ , table 2) later after the journey. In the case of a chronic situation such as overcrowding, cortisol concentrations stay elevated for long periods (Reddy and Leatherland 1998, Trenzado *et al* 2006). Significantly ( $P < 0.05$ ) low plasma cortisol concentration at the unloading stage is difficult to explain, as the fish exposed to loading and crowding did show an increase in plasma cortisol. A possible explanation is that the chronic stress of the confinement may have caused a negative feedback effect or downregulation of circulating cortisol, as described by Pickering (1992), resulting in a dampened response to subsequent stress.

A short-term intensive stress leads to a large increase in cortisol concentration followed by a slow decrease and the concentration of glucose has a similar course but with a certain delay (Svobodová *et al* 1999).

Significantly lower concentrations of cortisol concentrations ( $P < 0.05$ , table 2) were found after unloading. This could be attributable to the movement of the fish from the high stocking density in the well boat (107.8 kg/m<sup>3</sup>) to the much lower stocking density in the resting cages. Generally, all the blood variables measured indicated some recovery during the 24 hour period in the resting cages. In the resting cages cortisol reached the lowest levels within the whole journey (103.2 ng/ml), similar to those values found in previous studies (Skjervold *et al* 1999, 2001) for uncrowded salmon.

In a previous study Gatica *et al* (2005) found blood glucose to decrease after unloading, to increase slightly after resting and then significantly due to the acute stress produced by the suction pipe towards the processing plant. In the present study the average glucose concentration tended to decrease after pumping. However, taken together, glucose and cortisol are considered among the most useful and important stress indicators in fish (Svobodová *et al* 1999).

Rotllant and Tort (1997) suggest that chronic stress results in elevation of cortisol concentrations, but the levels reached are generally lower than those seen in acutely stressed fish. This was clearly seen in this study where the pipe suction to the processing plant, as an acute stress, produced the highest concentration of this hormone (table 2). These levels of cortisol were highly elevated (326 ng/ml) following suction pumping to the plant. Clearly, pumping to the plant turned out to be a more severe stressor in fish than any prior stage of the transportation process. The average transit time within the 222 m long pipe was 13 minutes. Thomas *et al* (1999) found significant increases of plasma lactate in two species of salmonids after lowering

<sup>1</sup> Mancera 2004. Personal communication. Curso: Osmorregulación en Peces Teleósteos. Instituto de Embriología. Universidad Austral de Chile, Chile y Departamento de Biología Animal, Facultad de Ciencias del Mar. Universidad de Cádiz, España. 9th-20th Agosto. Valdivia, Chile.

the water level to simulate the handling during harvest, by stimulating physical activity. Other previous studies have shown that the initial moments of the capture-loading process is a major driver of the stress response (Iversen *et al* 1998). However, even greater than this, appears to be the additional effect of crowding and then pumping fish for long distances (Gatica *et al* 2005).

Changes in the levels of blood  $\text{Na}^+$ ,  $\text{Cl}^-$  and osmolality are consistent with this mechanism and the changes in cortisol concentration found in this study. Earlier studies have also demonstrated that blood osmolality and electrolyte concentrations are similarly affected when fish are handled and transported (Redding and Schreck 1983, Urbinati *et al* 2004). Osmotic perturbations during stress are affected directly by changes in branchial permeability to water and electrolytes (Redding and Schreck 1983) and the magnitude and direction of stress-induced osmotic imbalance depends, at least partly, on water salinity. Fish stressed in seawater tend to gain electrolytes and lose water (Redding and Schreck 1983, Urbinati *et al* 2004).

In the present work, as transportation proceeded, the blood monovalent ion  $\text{Cl}^-$  was significantly higher ( $P < 0.05$ ) on each stage compared to the reference stage (farm, table 2). This has also been observed in previous studies; plasma chloride concentrations increase in seawater adapted fish in response to handling or confinement (Iversen *et al* 1998).

Additionally, the significant difference was also observed in  $\text{Na}^+$  and  $\text{Cl}^-$  ion concentrations after pumping suggests a net transepithelial flux of  $\text{Na}^+$  and  $\text{Cl}^-$  change in these euryhaline fish during stress (Redding and Schreck 1983).

In particular, stress caused during harvest, transport and slaughter has an important effect on fish meat quality (Sigholt *et al* 1997, Morzel *et al* 2002, Roth *et al* 2006). In fact, a considerable reduction in initial muscle pH and an associated increase in the rate of onset of rigor as handling during harvest and live transport proceeded was demonstrated in fish sampled in different stages during transport, both probably associated with considerable pre-slaughter stress (Gatica *et al* 2008). A rapid onset of rigor leaves a limited time in which to gut and process the fillets (Robb 2003)<sup>2</sup>, and particular importance appears to be the level of muscle activity at slaughter (Robb *et al* 2000). An increase in the rate of post-mortem muscle change has been previously reported in Atlantic salmon and other species in which the pre-mortem conditions involved extreme muscular activity (Warriss and Robb 1997, Robb 2001). This is extremely important to the fish industry as these changes adversely affect the ease of processing as well as meat quality. Ideally fish would move directly from the farm to slaughter and processing. The protracted type of handling observed in this study is common and a necessity in Chile due to the geographic conditions in

which the production farms and processors are located; however, it should be possible to improve the transport and pre-mortem handling conditions, once knowing in what way each stage and/or handling procedure affects the fish physiology and muscle biochemistry.

Transport of live Atlantic salmon to the processing plant by wellboat caused changes in several blood constituents related to stress. Their concentrations allowed a differentiation of the degree of stress associated with the various stages in the handling procedures the fish were subject to. The initial moment of the capture-loading process has been put forward as a critical time during transport (Iversen *et al* 1998). However, the present study has shown that during harvest, although the loading process and transport does have a measurable effect, long distance pumping of the fish as the final stage of transport had by far the greatest effect on the fish. This was seen in all of the blood variables measured except glucose, which was highest after transport, and which may have been depleted by the severity of the handling process prior to pumping. A handling process that elicits a response of the magnitude seen in the present study is likely to be associated with poor welfare and, additionally, will have an important impact on meat quality (Gatica *et al* 2008) and so should be avoided.

Recovery between successive stressful stages of handling and transport is recommended since their effects can be additive (Rotllant and Tort 1997, Reddy and Leatherland 1998). The changes seen in the blood variables following 24 hours in the resting cages suggest that this period was beneficial in allowing some recovery before the final stages of transport. In fact, as seen in a previous work with these same fish (Gatica *et al* 2008), a low muscle pH and an increased rate of onset of rigor mortis was seen as transport proceeded and specially after the pumping, but some recovery was seen after resting in cages. When moving fish, the use of fish pumps and transfer pipes appears to be preferable in terms of welfare and they should be as short as possible (Ashley 2007). According to Benson (2004)<sup>3</sup>, any delay over a maximum of 2 minutes can have an adverse effect and increase stress levels. In this case, the fish were pumped through long pipes (222 m) and the procedure lasted 13 minutes, hence, results in terms of increases of most blood constituents related to stress are not surprising. The results as well as the effects observed on muscle pH and rigor mortis point it out (Gatica *et al* 2008). Adequate infrastructure, as well as better handling conditions for the fish during transport and antemortem handling are essential requirements to achieve an acceptable product in terms of welfare as well as meat quality in the Chilean or any other country in the salmon industry.

In conclusion we can say that glucose had its peak after transport and the rest of the blood constituents increased until after unloading. The concentration of all variables studied dropped following a 24 hour rest in the cages and

<sup>2</sup> How harvest can affect quality, Canadian Edition. EWOS. Available via DIALOG. Pp. 9-12. [http://www.ewos.com/ca/ewos/ewebea2.nsf/077d2e128aace4411288256d050082be88/\\$FILE/How%20Harvest%20Can%20Effect%20Quality.pdf](http://www.ewos.com/ca/ewos/ewebea2.nsf/077d2e128aace4411288256d050082be88/$FILE/How%20Harvest%20Can%20Effect%20Quality.pdf) Accessed 25 July 2006.

<sup>3</sup> Advancing Aquaculture: Fish welfare at slaughter. Available via DIALOG. <http://seafood.ucdavis.edu/pubs/fishwelfare.pdf> Accessed 19 March 2006.

most of them, with the exception of glucose, were increased after the fish were pumped through the pipes from the resting cages to the processing plant. This confirms that the last handling procedure is the most stressful of the stages studied during this commercial transport.

## SUMMARY

The effects of commercial harvest, transport in wellboat and *ante mortem* handling on stress related blood constituents in salmon (*Salmo salar*) were evaluated. Ten fish were sampled at each of six stages: on farm; after loading; after transport in wellboat; after unloading; after resting and after pumping to the processing plant. Blood concentrations of cortisol, glucose, lactate, sodium, chloride and osmolality were determined. The results of this study indicate that one of the most stressful stages was during pumping from the resting cages to the processing plant. The concentration of all variables studied dropped following a 24 hour rest in the cages and most of them, with the exception of glucose, were increased after the fish were pumped through the pipes from the resting cages to the processing plant. This confirms that the last handling procedure is the most stressful of the stages studied during this commercial transport.

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