Effects of chronic and periodic exposures to ammonia on growth and eye health in juvenile Atlantic halibut (*Hippoglossus hippoglossus*)

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Abstract

The effect of chronic and periodic peaks of un-ionised ammonia (UIA-N) exposure on growth and eye health in juvenile Atlantic halibut, *Hippoglossus hippoglossus*, were examined. Fish with mean initial weight 51.7 g (SD, 13.2) were exposed to five treatments consisting of control group, three groups (ChronicLow, ChronicMedium and ChronicHigh,) chronically exposed with UIA-N ranging from 0.06, 0.12 to 0.17 mg l^{-1} respectivly for 62 days at 11.9ºC, pH 8.0 and salinity 34‰ and one group (HighPulse) exposed to the same high levels as above for a short daily period. The fish were reared under these treatments for 62 days. Growth was significantly reduced at UIA-N concentrations above 0.12 to 0.17 mg l^{-1}.

Mean weights and growth rates were significantly lower in groups exposed to chronically high ambient ammonia concentrations compared to corresponding control group throughout the period with ammonia treatments. Chronically low ammonia concentration did not have significant effect on weight and growth rate (NOEC= no observable effect concentration 0.06 mg l^{-1} UIA-N). The subsequent period of the experimental study (until day 100) no ammonia was added and water quality was normalized. However, weight differences were still present at day 100, although adaptive growth rates were observed in the previous chronic ammonia groups.

The simulated postprandial peak (HighPulse) did not have any significantly effects on either weight or growth rate in the present study, and the threshold limits for these peaks are yet to be determined. Minor differences were found in blood physiology between treatments.

No significant cataract formation was observed between the treatments. Previously unreported free amino acid composition in juvenile Atlantic halibut is presented in the study. Significantly difference in the imidiazole anserine was found in the group exposed to high ammonia (ChronicHigh). The eye histidine status differed significantly at day 62, and also osmotic differences (measured as NAH) were found in all group exposed to chronic levels of ammonia.

1 Introduction

The Atlantic halibut (*Hippoglossus hippoglossus,* L.) is an increasingly important and promising species in Norwegian aquaculture. In 2008 the sale of round weight of farmed Atlantic halibut was 1587 tons (Statistisk Sentralbyrå, 2009; Norwegian Directorate of Fisheries 2009). Development of successful juvenile production has been prolonged due to the highly specific environmental and nutritional requirements of halibut rearing (Shields, 2001). Most flatfish cultured in aquaculture are grown in land-based facilities and are stocked at high densities per unit of water volume or bottom area (Jeon et al., 1993; Björnsson, 1994; King et al., 1998; Irwin et al., 1999; Bengtson et al., 2003), and nearly all hatcheries producing Atlantic halibut juveniles are held in indoor facilities. Because of high construction and running cost of intensive rearing facilities, an effective utilization of water is required, especially in the juvenile phase where heated water is often used. In order to reduce the water spent, the fish may be kept at high densities and the water can be reused or recirculated. This is also being done in order to save energy. An important aspect of land-based production is thus to obtain strict water-quality control, which may be critical in order to maximize production as well as to maintain the well being of the fish. Poor welfare is known to cause many production related problems reducing both the capacity and efficiency of any production site. High stocking densities generally require high water exchange rates to supply the oxygen needs and to carry out the metabolic byproducts (Lawson, 1995). If oxygen needs can be satisfied by aeration or oxygenation devices, the next major water quality concerns are the nitrogenous metabolic byproducts excreted by the fish, especially ammonia (Colt and Armstrong, 1981; Handy and Poxton, 1993; Tanaka and Kadowaki, 1995).

1.1 Ammonia

Ammonia is one of the most critical water quality parameters for optimal performance in intensive raring facilities. In high density reuse or recirculation systems, the ammonia concentration may build up as a consequence of ammonia excretion, and exert toxic effects.

Total ammonia nitrogen (TA-N) excretion rates are directly related to dietary nitrogen and protein intake in fish (Liao and Mayo, 1974; Rychly, 1980; Beamish and Thomas, 1984; Handy and Poxton, 1993; Haskell, 1995; Wagner et al., 1995, Leung et al., 1999). In high density culture systems this may cause constant high levels or periodical peaks of ambient un-ionised ammonia depending on feeding regime and flow rate, resulting in periodical peaks or constant high levels of ambient un-ionized water ammonia which can be detrimental to the

fish. Also operational disturbances may periodically cause similar irregularities, e.g. from reduced efficiency in farms using recirculation systems, which may result in increased levels of ammonia in the system (Lyssenko and Wheaton, 2006). In addition to ammonia, fish can excrete metabolic nitrogen urea (Randall and Wright, 1987). Usually urea is reported to be non-toxic to fish (Dosdat et al., 1996), however it can be hydrolyzed rapidly to ammonia and carbon dioxide in culture systems if urea hydrolyzing bacteria are present, as often is the case in aquaculture systems (Colt and Armstrong, 1981; Pedersen et al., 1993).

Most teleost fish are ammoniotelic and are highly sensitive to ammonia toxicity (Haywood, 1983; Handy and Poxton, 1993). Due to a favourable blood to water concentration gradient, ammonia is rapidly excreted over the gills (Wilkie, 2002) and an internal build up is avoided. Ammonia is the main nitrogen excretion product of teleosts, formed primarily as a result of amino acid catabolism (Mommsen and Walsh, 1992). It can be extremely toxic to fish if allowed to accumulate in the body (Randall and Wrigth, 1987). In solution, ammonia exists in both ionized (IA, NH_4^+) and un-ionized (UIA, NH_3) forms. Total ammonia (TA, NH_3+NH_4^+) is the sum of these two. The two forms can be interconverted by the equilibrium equation:

$$NH_3 + nH_20 \leftrightarrow NH_4^+ + OH^- + (n\text{-}1)H_20 \qquad (pK \sim 9.8)$$

The toxicity of ammonia to fish and other aquatic organisms is primarily attributed to the un-ionized form because of its high ability to pass through biomembranes (Haywood, 1983). NH_3 is the most toxic form, among others, due to its high membrane permeability, and its toxicity increases with reduced temperature. In flow through systems, concentrations of TA-N is low (Rosten et al., 2007, Åtland et al., 2007) but can be significant in recirculation and closed transport systems. Most authors therefore present either UIA or UIA-N (NH_3-N, unionized ammonia-nitrogen) values when describing the ambient ammonia concentration. Ammonia excretion is directly related to protein intake and time after feeding (Handy and Poxton, 1993; Leung et al., 1999), and in intensive high-density systems or recirculation systems, the ammonia concentrations may build up as a consequence of ammonia excretion and increase to levels that can cause reduced growth or even death (Person-Le Ruyet et al., 1997). The proportions of the two forms in water are highly dependent on pH, but also on other factors, such as temperature and ionic strength. The amount of dissolved solids may influence the equilibrium constant (pK) and thereby influence the relative proportions (Bower and Bidwell,

1978). The pK-value of the system is used to calculate the fraction of UIA in the system (Khoo et al., 1977; see also the Materials and methods in this paper for more details).

Acute toxicity of ammonia has been investigated in a number of species (U.S EPA, 1984, 1989). Typical responses to short-term exposure to elevated ammonia in fish include increased gill ventilation, erratic movements, loss of equilibrium, and lack of foraging and even mortality (Meade, 1985; Russo and Thurston, 1991). Chronic exposure may typical result in gill hyperplasia (Thurston et al., 1981), changes in mucous production, muscle depolarization (Beaumont et al., 2000), reduced growth and stamina (Lang et al., 1987), but may also act directly on the central nervous system, causing hyperventilation (McKenzie et al., 1993), hyperexcitability, coma, convulsions and finally death (Ip et al., 2001). Chronic exposure of to sublethal concentrations of ammonia have been found to reduce growth and cause physiological disturbance in Atlantic cod (*Gadus morhua;* Foss et. al, 2004), turbot (*Scophthalmus maximus*; Person-Le Ruyet et al., 2003; Rasmussen and Korsgaard, 1998) and spotted wolffish (*Anarhichas minor*; Foss et al., 2003) juveniles. Concentrations of 25 µg to 300 µg NH_3 l^{-1} have been reported to cause mortality in salmonid fishes, and 10 µg NH_3 l^{-1} to cause negative gill interaction (Hjeltnes et al., 2008). In order to optimize rearing conditions, and thus prevent water quality from acting as a limiting factor for optimal growth and welfare of the fish, detailed knowledge on threshold levels for optimal growth is needed in order to exploit intensive rearing systems effectively.

Most cultured fish are presumably transported twice during their lifetime; once from the hatchery to the on-growing site and secondly from on-growing site to the slaughter house. Larval and juvenile stages of Atlantic halibut are often farmed on the same sites, whilst juveniles (30-350 g) are transported by truck, boat and plane (Iceland) to sea sites or marine tank facilities. Until now, slaughter fish have been killed and bled at site prior to transport and to packing and distribution facilities for markets and well boats are rarely used (Hjeltnes et al., 2008).

During closed transport, limiting factors for survival of the fish include maintaining optimum levels of oxygen, carbon dioxide and TA-N. 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 TA-N and pH, and eventually toxic concentrations of un-ionised ammonia. 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 limited 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 the future Atlantic halibut may be transported live to slaughter houses, and 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). The Atlantic halibut response to chronic exposure and periodic ammonia peaks is however currently unknown.

1.2 Eye health and cataract in fish

Fish growth and eye health clearly depend on water quality parameters (Waagbø et al., 2008). The eye health has been highlighted as a major issue in halibut production (Williams et al., 1995). Occurrence of cataract is usually a symptom of poor husbandry such as deteriorated water quality. The term cataract describes a condition with loss of transparency of the normal clear lens tissue leading to visual disturbance or blindness in farmed fish leading to decreased feed uptake. Cataracts have been commonly reported as a problem in salmon aquaculture (Bjerkås et. al 1996) and may cause severe production losses for the fish farmer (Williams et al., 1995). Development of cataracts is described as a production disease that may potentially affects any intensive farming of fish (Waagbø, 2008). Fish lens are sensitive to different types of stressors, which may result in reversible or irreversible opacification of the lens (Hargis 1991; Breck, 2004). This can be observed directly in the fish lens making it a useful diagnostic tool. Damage to cornea as a result of eye snatching, friction in high stocking density and handling may lead to cornea infection and cataract as a result of this, depending on the severity of the cornea damage (Breck et al., 2003). Halibut may be particularly exposed for this due to the protruding position of the eyes. In modern fish farming, cataract may be considered a multifunctional production-related disease, with several nutritional and environmental factors being able to induce the same final lesions (Treasurer et al., 2007). Cataract formation can be influenced by water temperature (Bjerkås et al., 2001), salinity changes (Bjerkås et al., 1998), nutritional imbalance (Hargis, 1991, Bjerkås et al., 2006),

exposures to UV light (Cullen et al., 1994), and toxins and parasites (Valtonen and Koskivaara, 1994).

Histidine deficiency as a nutritional factor have been reported as a causative factor in cataract formation for Atlantic salmon, probably due to removal of blood meal in the diet in the 1990s (Breck et al., 2003; Bjerkås and Sveier, 2006). The development of cataracts has also been related to rapid growth in salmon (Bjerkås et al., 1996; Waagbø et al., 1998) and lipid levels in the diet (Waagbø et al., 2003). Although these nutritional factors have been demonstrated in cataract formation, these can be interlinked with a range of husbandry and environmental factors (Bjerkås et al., 2006).

Histidine has been linked to several physiological processes in the fish lens. A diet with surplus histidine may reduce cataract development in almonds (Breck et al., 2005b). Histidine deficiency compromises lens homeostasis, leading to water uptake, swelling of the lens and finally rupture of the lens capsule (Treasurer et al., 2007). In salmon, free histidine in the lens is rapidly converted to N-acetyl histidine (NAH), which seems to have a possible osmoregulatory role in the fish lens (Breck et al., 2005a). This was demonstrated by a lower cataract score in salmon fed a diet with elevated histidine levels. The role of His and NAH in lens osmoregulation includes the ability of rapid osmolyte efflux to equalize any osmotic disturbances, since the lens easily endures in hyper- and hypo-osmotic environments by shrinking and swelling, respectively (Breck, 2004). Consequently, any physiological state that impact lens osmoregulation or put osmoregulatory stresses to the lens may be cataractogenous, while elevated dietary His may counteract such osmotic disturbances. Since His and His related compounds (imidazoles) also take part in the cellular integrated antioxidant system, muscle pH buffering system, and function as anti-glycating agents, the role of His in cataract prevention in salmon is not completely understood.

Histidine and histidine derivates may also act as important antioxidants (Wade and Tucker, 1998) and has been characterized as important buffers in muscular tissue of different fish species (Hiroshi and Murai, 1994, Munakata et al., 2000). Existing literature is mainly based studies with salmonids, and up to now no systematic experimental studies have been conducted on the eye status of Atlantic halibut in culture. The recording of the eye status is a selected indicator for stress. Analyses of the eye status as a welfare indicator includes inspection for cataract and cornea damage as well as analysis of lens histidine compounds.

1.3 Objectives

This experiment was carried out with Atlantic halibut juveniles in the size interval 13.4-104.8 g to determine the tolerance limits at which the fish experience physiological disturbance and reduced growth from being exposed to chronic high levels of ambient un-ionized ammonia. The experiment also included short daily peaks of un-ionized ammonia to determine if this could cause the halibut physiological disturbance and reduced growth.

Measurements of growth and blood physiology are presented. Monitoring blood physiological response was included to investigate if, and to what extent the hydromineral and acid base status (sodium, potassium, pH, CO_2, bicarbonate) of halibut were affected by different levels of un-ionized ammonia (chronic and short peaks). Water quality may have an influence on eye health and cataract in juvenile Atlantic halibut, and therefore random fish from each group were screened for cataract to see if ammonia could have an effect on cataract score. Samples were also taken to uncover possible differences in muscle buffering capacity (measured as free amino acids and histidine compounds) between treatment groups. To further analyse the ammonia potential affect on eye health; lenses were sampled for analysis of histidine status and the imidiazole NAH. The free amino acid compositions in juvenile Atlantic halibut muscle and lens tissues have previously not been reported.

The experiment was based on the following alternative hypotheses:

H_{A1}: Ammonia toxicity leads to reduced growth and altered physiology of juvenile Atlantic halibut at high concentrations.

H_{A2}: Periodic exposure to high ammonia levels has the same effect on juvenile Atlantic halibut as chronic high concentrations.

H_{A3}: Measured blood parameters will differ between the treatments.

H_{A4}: Muscle buffering capacity (measured as free amino acids) will differ between the treatments.

H_{A5}: Observed cataract formation will differ between treatments.

H_{A6}: The eye histidine status will be different between treatments.

H_{A7}: Osmotic differences (measured as N-acetyl histidine) in the lens will occur between treatments.

where **H_0** being that different exposures to unionized ammonia has no significant effect on the above mentioned parameters**.**

2 Materials and methods

2.1 Fish stock, rearing conditions and experimental facilities

The Atlantic halibut juveniles used in the present study were delivered by a commercial hatchery, Aga Marin AS, Norway. They originated from a common pooled egg batch. During and after first feeding the larvae and juveniles were reared under a natural light regime at an average temperature of around 11.5 °C. On the 10 of October 2008, 823 Atlantic halibut juveniles arrived at the Bergen High-Technology Centre Ltd. (BHTC, in Akvahall 2), and were placed in four holding tanks with a continuous light and temperature of 11.9±0.2 ºC. They were fed a commercial formulated feed from Skretting AS in excess by automatic feeders for 1 week prior to the start of the experiment (Skretting Topaz Respons 3mm extruded sinking pellet, Averøy, Norway AS). The diet contained 52 % protein from fishmeal, soy meal and fish protein concentrate and 18 % fat from fish oil. This diet was used throughout the whole experimental period. It also contained vitamin and mineral premix and is sold as a health diet for marine fish with Makroguard ®.

Table 2.1: Two samples of the diet were analyzed for total amino acids after acid hydrolysis according to an accredited HPLC method at NIFES. The data represents mean values and % deviation from mean. (Amino acid abbreviations are explained in Appendix II- Table I).

Feed analysis				
Amino acid (AA)	**Sample a**	**Sample b**	**Mean**	**% deviation**
Hypro	3.8	3.9	**3.9**	1.5
His	11.5	11.6	**11.6**	0.8
Tau	4.3	4.3	**4.3**	1.8
Ser	22.6	23.0	**22.8**	1.4
Arg	34.2	34.4	**34.3**	0.5
Gly	29.7	29.8	**29.8**	0.4
Asp	45.1	44.8	**44.9**	0.8
Glu	79.0	78.8	**78.9**	0.3
Thr	21.3	21.5	**21.4**	0.6
Ala	30.6	30.4	**30.5**	0.5
Pro	27.3	27.3	**27.3**	0.2
Lys	34.9	34.3	**34.6**	1.7
Tyr	15.5	15.5	**15.5**	0.4
Met	13.2	13.3	**13.2**	0.4
Val	26.0	25.6	**25.8**	1.5
Ile	21.4	21.0	**21.2**	1.9
Leu	38.2	38.1	**38.2**	0.3
Phe	22.3	22.4	**22.4**	0.7

On the 13 of October 299 juvenile halibut were anaesthetized with metacain (0.05 g l^{-1}) and tagged intraperitoneally with Trovan® Passive Integrated Transponder tags (Trovan Ltd) to

be able to observe growth performance of individual fish. On the 17 of October, two days before the experimental start-up, tagged and untagged fish (*n*=823) were distributed evenly and randomly into ten grey square fibreglass tanks with rounded corners (1 m x 1 m) and a rearing volume of 400 litres, with a bottom outlet and covered with a lid tanks. The tagged fish was reared together with additional untagged fish (~55 untagged and 30 tagged fish). The mean weight of tagged fish was 51.7 g ±13.2 (Mean ± SD) and did not differ significantly between tanks. A circular automatic feeder and a fluorescent spotlight (30 W) were built into the lid. The juveniles where exposed with continuous light, with a light intensity at 60 % of 30W on full light (tank centre: 6.5 μmol^{-1} m^2). Incoming seawater was pumped from 95 meters depth of the Bergen city fjord, run through particle- and UV filters, and aerated before entering the header tanks. Each header tank supplied two tanks with seawater, and thus five different treatments were possible in the ten tank facility. Initial seawater flow rate was 5 l min^{-1}, which was increased to 8 l min^{-1} at 13 of November 2008 to sustain adequate oxygen levels in the tanks (80 % saturation).

Table 2.2 Overview of the experimental conditions. Means of ammonia concentration (TA-N, calculated UIA-N), O_2 saturation, temperature (ºC) and pH ±SD are presented in the Table (see Table I-IV, Appendix III for further details on descriptive statistics). N is the number of fish used per treatment at start-up, and the density is the total mass of fish kg m^{-2} at the start (day 0) and at the end (day 100) of the experiment. Asterisk: values are presented in Table 2.3.

Treatment	N	TA-N (mg l^{-1})	UIA-N (mg l^{-1})	O_2 saturation (%)	Temp. (ºC)	pH	Salinity	Density (kg m^{-2})
	T0	Means±SD	Means±SD	Means±SD	Means±SD		(‰)	T0-T4
Control	167	0.2 ± 0.1	0.002 ± 0.001	81 ± 3.0	11.9 ± 0.1	8.0	34	4.39 - 8.64
ChronicLow	162	5.3 ± 0.70	0.06 ± 0.01	81 ± 2.5	11.9 ± 0.2	8.0	34	4.14 - 6.09
ChronicMedium	165	10.5 ± 1.43	0.12 ± 0.02	80 ± 2.2	11.9 ± 0.2	8.0	34	4.18 - 5.94
ChronicHigh	164	14.9 ± 1.43	0.17 ± 0.03	83 ± 3.2	11.9 ± 0.2	8.0	34	4.39 - 5.87
HighPulse	165	*	*	81 ± 3.0	11.9 ± 0.2	8.0	34	4.36 - 7.69
Total	823							

2.2 Experimental design

The experimental period took place between 21 October 2008 and 29 January 2009.

The experiment consisted of five different experimental conditions. The treatments consisted of a Control group, three groups (ChronicLow, ChronicMedium and ChronicHigh) chronically exposed to ammonia [TA-N (total ammonia nitrogen) levels of 5.0, 10.0 and 15.0 mg l^{-1}] an one group (HighPulse) exposed to ammonia for a short period (high TA-N level 2-3 h day^{-1} with a peak of 15 mg l^{-1}) simulating a postprandial increase of un-ionised ammonia in the tanks. All treatment groups were carried out in two replicate tanks. The experiment consisted of two parts: The first period (from day 0 to day 62) ammonia treatments were

performed, while the second period (from day 63 to day 100) the ammonia treatments were stopped. The last period was included in the present study in order to observe if any compensatory growth occurred in the groups experiencing the highest ammonia treatments.

The water temperature was set to approximately 12 ºC (11.9±0.2 ºC) and the pH and salinity of the incoming water were stable at 8.0 and 34 ‰, respectively (monitored by the technical staff at BHTC). The desired ammonia concentrations were obtained by pumping a solution of NH_4Cl (100 gram l^{-1} fresh water; VWR International AS, Oslo) by four electromagnetic metering dosage pumps into the header tanks (Iwaki electromagnetic metering pump, model EW-F10VC-20EPF2, Iwaki Co. Ltd, Tokyo, Japan) supplying the respective tanks with water. There where no ammonia added to the water in the control tanks. In the periodical exposed group, the dosage into the header tank was controlled manually. The pumps started daily at 09:00 and were active for half an hour.

TA-N in a 100 ml sample of water from the rearing tanks was measured once daily in the chronic treatment groups using an ammonia gas sensing combination electrode (Thermo Orion, Model 95-12) connected to an expandable ion analyzer (Thermo Orion, EA^{TM}920). A two-point calibration procedure was performed before measurements. Samples were continuously stirred while added 3 ml strong basic solution (ISA), to elevate the pH in order to convert all ammonia to the un-ionised, gaseous, form. Samples were analyzed immediately to avoid the gaseous NH_3 from escaping the samples before measurements. The ammonia electrode was kept in the sample until the meter presented a stable value of ammonia concentration. In the periodically exposed group TA-N levels were measured at 15 min after pump start-up and then after 30 min, 45 min 1 h, 1.5 h, 2 h and 3 h.

Percentage UIA-N (un-ionised ammonia nitrogen) was calculated using the equation of Johansson and Wedborg (1980), which gives the UIA-N/TA-N ratio as a function of pH, temperature and salinity. Corrections for pH measurements performed with low-ionic strength buffers e.g. conversion of the Hansson scale, where performed according to Whitfield (1974). The formulas are listed under ‘Calculation of UIA-N concentrations’ later in this chapter.
In total the experiment lasted 100 days and all the fish was slaughtered 1 week after the end of the experiment.

2.3 Daily measurements and feeding

Temperature and oxygen saturation were measured directly in the outflow using portable instruments; OxyGuard® and OxyGuard Handy Gamma® (OxyGuard International AS, Denmark), respectively. Feed was provided in excess from automatic feeders for 2 hours twice daily (from 8:00 to 10:00 and from 14:00 to 16:00) except in weekends (fed once from 8:00 to 10:00). The dry weight fed per day was noted for each tank. The tanks were flushed and the uneaten pellets were collected in a sieve in the outflow 30 min after feeding in the morning and afternoon and counted immediately in order to measure feed intake. The fish were not fed the day before or during sampling. The tanks where checked for dead fish daily.

Table 2.3 Experimental measurements of TAN and calculated UIA-N in the HighPulse group. Dosage pumps were on for 0.5 h starting from 9:00 to 9:30. Table showing levels at given times during the day with the highest concentration at 9:30.

Time span	TA-N (mg l^{-1}) Means±SD	UIA-N (mg l^{-1}) Means±SD
08:00	0.2 ± 0.1	0.002 ± 0.001
09:15	7.6 ± 1.25	0.09 ± 0.01
09:30	15.0 ± 1.32	0.18 ± 0.02
09:45	13.9 ± 1.06	0.16 ± 0.01
10:00	10.5 ± 1.38	0.12 ± 0.02
10:15	8.2 ± 0.58	0.10 ± 0.01
10:30	6.3 ± 0.64	0.07 ± 0.01
10:45	4.9 ± 0.61	0.06 ± 0.01
11:00	3.7 ± 0.56	0.04 ± 0.01
11:15	3.2 ± 0.55	0.04 ± 0.01
11:30	2.2 ± 0.43	0.03 ± 0.00
11:45	2.1 ± 0.34	0.02 ± 0.00
12:00	1.4 ± 0.18	0.02 ± 0.00
12:15	1.3 ± 0.19	0.01 ± 0.00
12:30	1.2 ± 0.13	0.01 ± 0.00

2.4 Sample procedures

Growth data and blood samples were collected five times: at experimental start up (21 October, T0), and approximately every third week during the experiment (T1, T2, T3 and T4). The fish were anesthetized (Metacain, 0.05 g l^{-1}) and weights and lengths of all tagged specimens were measured to the nearest 0.1 cm and 0.1 g at each sampling point, whereas only the weight was measured for the untagged fish.

Blood samples were collected at the start of the experiment (T0) and at the same days as the length and weight measurements, after a 24 h fasting period. Blood samples were collected from the caudal vessels of eight fish from each experimental group (4 per tank) and analyzed using an i-STAT Portable Clinical Analyzer (Emergo Europe, The Netherlands). The analyzer was used with $EC8^+$ disposable cartridges, measuring whole blood sodium and potassium concentrations, glucose, partial gas pressure of CO_2 (pCO_2), hematocrit and pH level, and displaying calculated values of blood bicarbonate, total carbon dioxide and haemoglobin concentrations. As the instrument is optimised for analyzing human blood at 37 ºC, temperature corrections of the measured blood pH and pCO_2 were needed (see Temperature corrections of blood analysis under Calculations).

In addition to the growth measurements, 4 random untagged fish were removed from each tank (40 fish per sampling) on 22 December (T3) and 29 January (T4) for blood samples, lens and muscle tissue samples. This was also done initially on 21 October (T0) with 20 random fish. Sampled fish were anaesthetized (metacain 0.05 g l^{-1}) and then killed with a blow to the head.

2.5 Cataract screening

Initially 20 fish were examined for lens opacities by use of a slit lamp (Kowa SL-14 with 16 x magnification, Kowa, Japan) under darkened conditions and after anaesthesia. Examined fish included 8 fish from each treatment, e.g. a total of 40 untagged fish on each of the sample days. Cataracteous changes per eye were scored on a scale from no changes (score 0) to complete cataracts (score 4), in accordance with the scoring method described by Wall and Bjerkås (1999). Cataract scores in individual fish are given as the sum score of both eyes (e.g. ranging from 0 to 8 per fish). Prevalence of fish with cataracteous changes and sum of scores of all examined individuals within the experimental groups are reported.

2.6 Muscle and lens sampling for analysis of free amino acid

Skin free muscle tissue was sampled from the random sampled fish (20 fish at T0, and 40 fish at T3 and T4). From the same individuals, both eye lenses were dissected by removing the cornea. Attached aqueous humour was removed by gently rolling the lenses on a clean filter paper. The samples and lenses were immediately frozen on dry ice and later stored at -80 ºC.
Muscle samples were analyzed for free amino acids by use of a Biochrom 20 Plus Amino Acid Analyser (Amersham, Cambridge, UK), according to a standardized procedure from the

manufacturer (Biochrom AAAFAQ08; Breck et al., 2005a). The muscle tissue was homogenized in 10 % sulfosalicylicacid and centrifuged for 15 min at 8000 rpm (g). The supernatant was thereafter mixed with running buffer (Lithium Citrate Loading Buffer, 80-2038-10, Biochrom Ltd., Cambridge, UK) and an internal standard was added. The samples were transferred using a syringe, and filtered through a membrane filter (0.45 µm, Millex® Syringe filter unit, Millipore Corp, USA) into vials. The amino acid concentration was then analyzed by use of a Biochrom 20 Plus Amino Acid Analyser (Biochrom Limited) based on low pressure ion-exchange chromatography. After post column ninhydrin derivatization, colorimetric detection was done at 570 nm and 440 nm (Waters 486, Waters Corporation) and the individual amino acid peaks compared to the external and internal standards.

Individual lenses (*n*=4 per tank) were analyzed for His an N-acetyl histidine (NAH) by the use of reversed-phase HPLC (High Performance Liquid Chromatography) according to the method by O'Dowd et al., (1990) with modifications by Breck et al., (2005b). The lenses were placed in 80 % ethanol and homogenized on a mill for 5 min at frequency 30 and later centrifuged at 3000 rpm for 30 min. The supernatants were concentrated to dryness by use of a Termaks incubator (40°C, normal atmosphere) (Termaks, Bergen, Norway) over night, dissolved in phosphate buffer (pH 2.0) and filtered through a membrane filter (0.45 µm, Millex® Syringe filter unit, Millipore Corp, USA). An isocratic reverse phase HPLC was performed, using a 4.6 mm ID x 250 mm column with as silica-based packing (ZORBAX SB-C18, Agilent Technologies AS, Norway) and a Waters 600 E pump (Waters Corporation, Milford, Massachusetts, USA). A 0.1 M Phosphate buffer (pH 2) was used as eluting solvent, with a flow rate of 0.6 ml min^{-1}. NAH and His were detected by UV absorbance (Waters 486 – Tuneable Absorbance Detector, Waters Corporation) at 210 nm, using external standards. A diet sample was analyzed by for total amino acids after acid hydrolysis according to an accredited HPLC method at NIFES (MET.NÆR.01-17, NIFES, Bergen, Norway).

2.7 Calculations

All growth estimates in the present study are based on individually tagged fish, whereas the feed conversion efficiency, feed consumption and daily feeding rate are based on the pooled biomass from each tank.

Specific growth rate (SGR)

The specific growth rate (% weight gain per day) was calculated by using the formula given by Houde and Schekter (1981):

$SGR = (e^{g}-1)*100$

The instantaneous growth coefficient g is:

$g= (\ln W_2-\ln W_1) (t_2-t_1)^{-1}$,

W_2 and W_1 are mean wet weights for individually tagged fish in g at days t_2 and t_1.

Temperature corrections of blood analyses

The i-STAT analyzer is optimized for analyzing human blood samples, holding 37 ºC. The blood collected from the fish held a temperature if approximately 12 ºC. To correct the present pH and pCO_2 values for temperature we used the formulas supplied by the i-STAT User Manual 2005:

$pH_{tp}= pH-0.0147(t-37)+0.0065(7.4-pH)(t-37)$

$pCO_{2tp}=pCO_2*10^{0.019(t-37)}$,

where the t is the actual blood temperature, and pH and pCO_2 are the values given by the i-STAT analyzer. Values corrected for temperature are denoted tp.

Calculation of UIA-N concentrations

The measured ammonia concentrations (TA-N, mg l^{-1} NH_3) where converted to total ammonia nitrogen concentrations (TA-N, mg l^{-1} N) by the conversion factor given by Haywood (1983):

NH_3 (mg l^{-1}) = 0.8224 NH_3-N (mg l^{-1})

In order to find the concentration of UIA-N, the pK of ammonia system was calculated using the equation given by Khoo et al., (1977):

$pK=0.09018+2727.9(T+273.1)+(0.1552+0.0003142T)I$,

where $pK= -\log(K_{eq})$, $K_{eq}=[NH_3][H^+]/[NH_4^+]$ (all species in mol kg^{-1}), T=temperature (ºC) , and I=ionic strength (M). The K_{eq} and pH (converted to the Hansson scale, according to Whitfied, 1974) was then used to find the fraction of UIA-N out of TA-N using the following expression (Khoo et al., 1977):

$UIA\text{-}N/TA\text{-}N= K_{eq}/10^{-pH}+ K_{eq}$

NAH (Na-Acetyl-L-Histidine) determination using HPLC:

Calculations of NAH and His levels in lenses were done by using an external standard method.

Standards: External standard method input in to Empower software (Waters Corporation, Milford, Massachusetts, USA) with the values:

0.25 mM NAH-0.25 mM His standard level 1 and

0.50 mM NAH-0.50 mM His standard level 2

Calibrate the standards against each other. Calculate samples after existing calibration curve.

Sample weight=*weight sample*200 µl volume sample/600 µl volume added*.

Muscle free amino acid determination - ninhydrin detection:

The method and the calculations are found in the NIFES quality assurance handbook. The method is considered robust (personal comment, Anita Birkenes, NIFES).

The concentrations of all the components in the standards are set as µmol/ml, and for the internal standard in sample are set as µmol/ml also. Dilution set as 0.6 ml. Sample weight is given as the measured amount g.

The results are calculated by using Empower software in the following way:

The areal under each top is measured both for standard and samples. The response is considering of the response of the internal standard.

$$R = \frac{\text{Areal (aa)} * C(is)}{\text{Areal (is)}}$$

With the help of linear regression the Empower programme calculates a standard curve for each amino acid.

$y=ax+b$, where

b=0, the curve is forced through, Origo

y=R

x=C2

a=response factor for each component in the standard.

To measure the content of amino acids in the sample the following equation is used:

$C1=(C2xV)\ w^{-1}$

Where:

R=corrected response for aa, mM

Areal (aa)= Respons for aa (areal in AU)

C(is)=concentration of internal standard (is), mg/ml

C1=concentration in the sample material, mg/g sample

C2=concentration of injected sample, mg/ml

V=dilution, ml

w=weighted amount, g

The concentration of the Internal standard in the sample is calculated this following way:

Continous samples:

$$C_{IS}=\frac{(2.5\ mmol\ l^{-1}\ x\ 131.2\ mg\ mol^{-1}\ x\ 150\ ml)}{(0.750\ ml\ x\ 1000\ ml\ l^{-1})}\ x\ 2.5^{1}=0.1640\ mg\ ml^{-1}$$

1=Dilution of the sample before adding IS

2.8 Statistical analyses

All statistical analyses were done in STATISTICA 8.0 (Statsoft, Inc., 2007) except for the Chi- square test for differences in mortality that was done manually by using Control as the internal control group (Zar, 1984). To assess normality of distributions a Kolmogorov-Smirnov test (Zar, 1984) was applied and homogenity of variance was evaluated by a Levene´s F test. Effects of ammonia on growth, blood parameters muscle free amino acids, and lens histidine and NAH were tested using a two way nested Analysis of Variance (ANOVA) (Zar, 1984). Replicates were nested in treatment factor in the analysis. Significant values for blood parameters were followed up by a two way Analysis of Covariance (ANCOVA) where weight was set as co-varying factor (Zar, 1984). Significant ANOVAs and ANCOVAs were followed by a multiple comparison test (Student-Newman-Keuls test; Zar, 1984). Student-Newman-Keuls test will be abbreviated SNK test in the following sections. A significance level of $\alpha=0.05$ was used if not otherwise stated.

3 Results

3.1 Total mortality

Mortality occurred in all fish tanks during this experimental period and total mortality varied from 19.0-35.8 % (29-50 fish) between treatment groups (Table 3.1).

The day after the second sampling (T2=11 November) 27 fish were found dead in one of the tanks (ChronicLow replicate a). This was due to an experimental accident during sampling. In the first period (days 0-21) the ChronicLow group had a significantly lower total mortality than the Control group (χ^2 >5.44, p<0.05, Table 3.1) and the HighPulse group had significantly higher mortality than the Control group (χ^2 >5.44, p<0.05, Table 3.1).

In the second period (days 22-42) the ChronicMedium and ChronicHigh group displayed significantly higher mortality than the Control group (χ^2 >6.00, χ^2 >8.17 respectively, p<0.05, Table 3.1). In the third period (days 43-62) significantly higher mortalities observed in all groups compared to Control group (ChronicLow: χ^2 >208.33, p<0.05, ChronicMedium: χ^2 >16.33, p<0.05, ChronicHigh: χ^2 >12.00, p<0.05, HighPulse: χ^2 >8.33, p<0.05, Table 3.1).

During the last period (no treatment, days 63-100) only the HighPulse group had significantly higher mortality than the Control group (χ^2 >7.36, p<0.05, Table 3.1).

Table 3.1: Total mortality (N dead, number of dead fish) and percent dead of total number (% dead) of fish per treatment. ●=lower mortality than in control group, ■=higher mortality than in control group.

	Days 0-21		**Days 22-42**		**Days 43-62**		**Days 63-100**		**Total**	
	N dead	% dead	N dead	% dead	N dead	% dead	N dead	% dead	N Dead	% dead
Control	9	5.4	6	3.8	3	2.0	11	7.9	29	19.0
ChronicLow	2●	1.2	10	6.3	28■	19.2	10	9.0	50	35.8
ChronicMedium	9	5.4	12■	7.7	10■	6.9	14	11.4	45	31.4
ChronicHigh	4	2.4	13■	8.1	9■	6.1	20■	16.7	46	33.4
HighPulse	16■	9.6	10	6.8	8■	5.8	8	6.7	42	28.9

3.2 Effect of ammonia on growth

All growth information reported here is based on those individually tagged fish, and sampled at the same time as for the lens and muscle tissue samples (at T0, T3 and T4) for comparison. Average initial weight ranged from 51.4 - 54.1 g and there where no significant differences in initial weights between treatment groups (Figure 3.1; Table V; Two-way nested ANOVA p >0.35, Appendix III for details). The average weight ranged from 73.5-89.5 g after 62 days. At the end of the second growth period (day 100), the average weight ranged from 100.8 to 134.9 g. At day 62 (22 of December 2008) the ChronicHigh group displayed significantly lower mean weight (65.9 g) compared to Control, ChronicLow and HighPulse group (89.6,

80.0 and 88.5 g, Figure 3.1, Table CXI, SNK-test p<0.05; Table CCX, Appendix III) but not to the ChronicMedium group (73.6 g, Figure 3.1, Table CXI, SNK-test p<0.05; Table CCX, Appendix III). The ChronicMedium group showed no significant difference in mean weight when compared to ChronicLow and ChronicHigh groups, but to the HighPulse and Control it did.

At termination of the experiment (day 100), the ChronicHigh treatment group still displayed a significantly lower mean weight (100.0 g) than the Control and HighPulse group (138.4 and 128.1 g, Figure 3.1, Table CXII, SNK-test p<0.05; TABLE CCXI, Appendix III) but not to the ChronicMedium and no longer to the ChronicLow group (109.5 and 120.7 g respectively, Figure 3.1, Table CXII; SNK-test p<0.05; Table CCXI, Appendix III). The ChronicMedium group also had a lower significant mean weight than the Control group (Figure 3.1, Table CXII SNK-test p<0.05; Table CCXI, Appendix III).

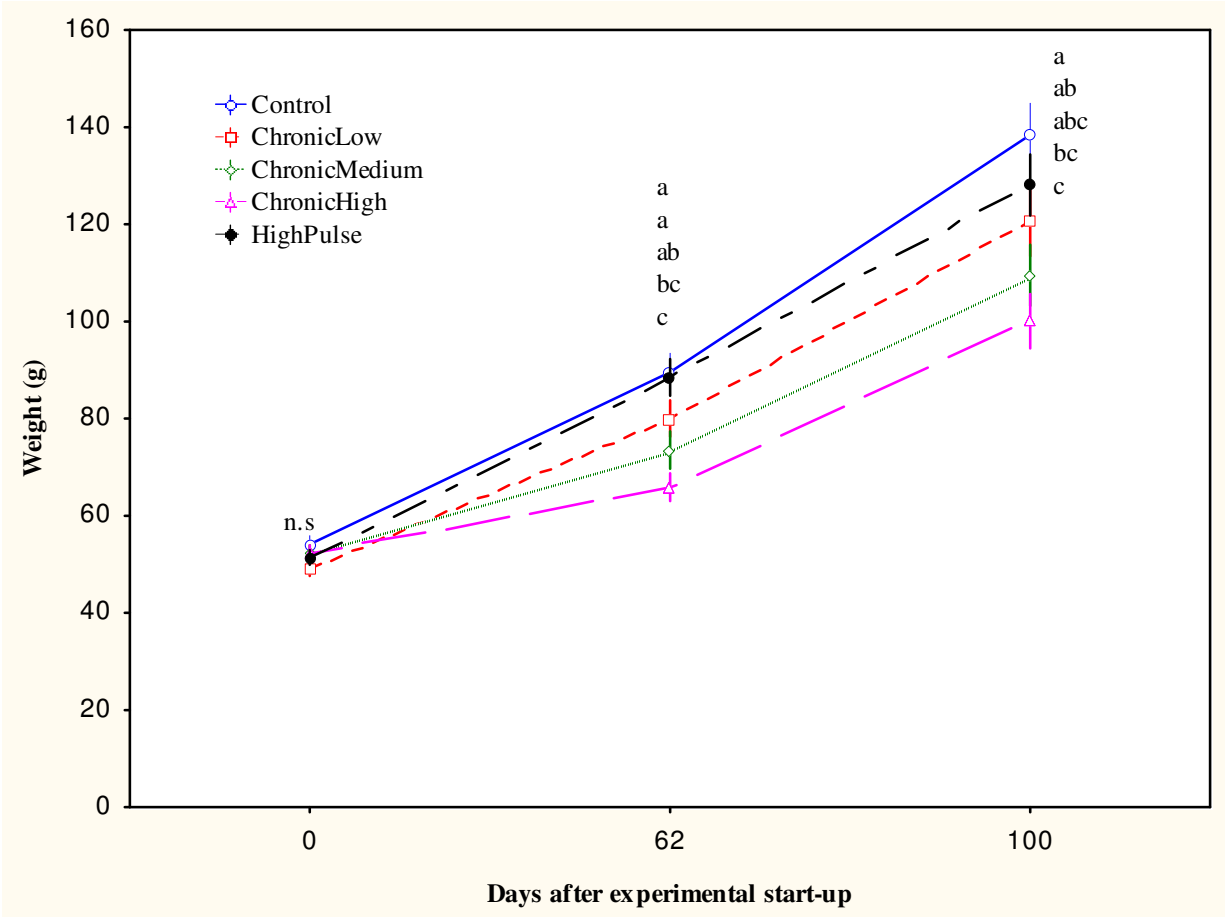

Figure 3.1: Weight data (group means ± SE, see Table V, Appendix III). The groups are separated by colour, line type and shape of marker: Control, ChronicLow, ChronicMedium, ChronicHigh and HighPulse. Means sharing a common letter are significantly different (SNK test, p<0.05, Table CCX to CCXI, Appendix III)

Mean individual specific growth rates (SGR) for the whole experimental period ranged from 0.28 to 1.00 % day^{-1} (Figure 3.2, Table VI, Appendix III). The first period presented here represent the whole period with the different ammonia treatments. The effect of the ammonia were significant and pronounced in the ChronicHigh group as this group had a significantly lower SGR compared to the other groups (Figure 3.2, SNK-test $p<0.001$, Table CXIII, Appendix III). The ChronicMedium group also had a significant lower SGR compared to Control, HighPulse and ChronicLow but also significantly different from ChronicHigh. In the last period there were no significant differences in SGR between any groups (Figure 3.2, SNK-test $p>0.001$,Table CXIV, Appendix III). For the calculated SGR Overall only the ChronicMedium and ChronicHigh groups had significantly lower SGR's compared to the other groups (SNK-test $p<0.001$, Table CXV).

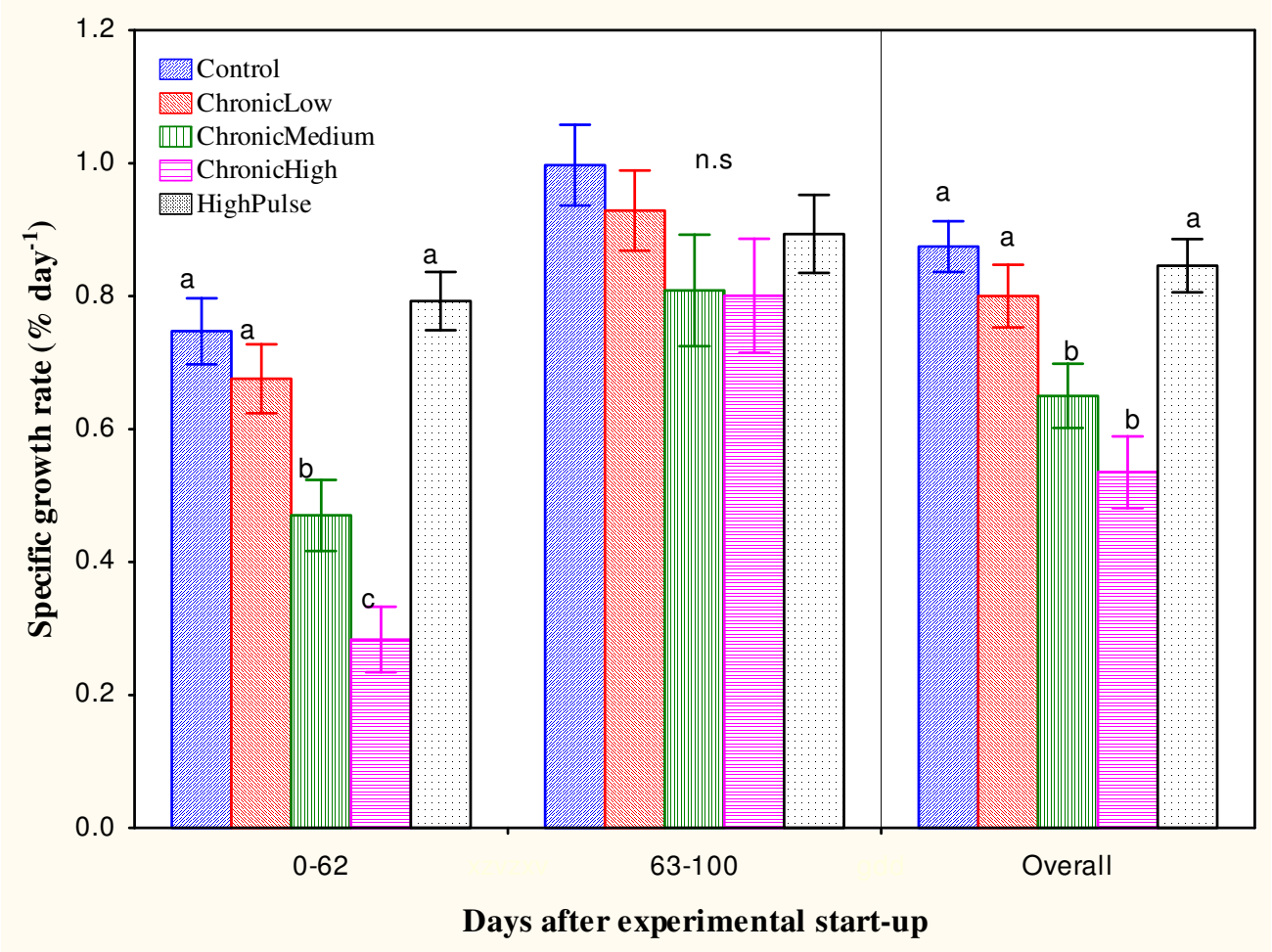

Figure 3.2: Mean individual specific growth rates (% day^{-1}, group means + SE, see TABLE VI, APPENDIX III). Colour pattern of the columns indicate the level of ammonia concentration. Means not sharing a common letter are significantly different (SNK test, $p<0.05$, Table CXIII-CXV, Appendix III).

3.3 Effects of ammonia on physiological status

Blood Na^+ concentration

Measured average Na^+ concentrations in blood samples were in the range 157-170 (mmol l^{-1}) during this experiment (Table 3.2). No significant differences were found between the groups at any times (Table 3.2, Two-way crossed ANCOVA $p>0.05$; Table CXCIX and CC, Appendix III).

Blood K^+ concentration

Mean measured K^+ concentrations ranged from 3.52 to 5.65 (mmol l^{-1}) in the blood samples from this experiment (Table 3.2). No significant differences in K^+ were found between treatments on day 62. However on day 100 there were a significant difference in the ChronicLow group compared to the other groups (Table 3.2, Two-way crossed ANCOVA $p<0.05$, Table CCII, SNK-test $p<0.05$, see Table CCXVII, Appendix III).

Blood pH

The average measured mean and temperature corrected pH was in the range of 7.20-7.34 (Table 3.2) in the blood samples taken in this experiment. The ammonia treatments had no significant effects on blood pH at day 62 and day 100 (Table CCXIX to CCXX, Appendix III).

Blood CO_2 partial pressure

The average values of blood CO_2 partial pressure (pCO_2) were in the range 4.33 – 6.67 mmHg (Table 3.2). On day 62 the ChronicHigh group a significantly lower pCO_2 mean compared to Control, ChronicLow, HighPulse group, but not significantly lower compared to ChronicMedium (SNK-test $p<0.05$, Table CCXXI, Appendix III). On day 100 the ChronicLow group had significantly higher pCO_2 compared to Control, ChronicHigh and HighPulse, but not compared to the ChronicMedium group (SNK-test $p<0.05$, Table CCXXII, Appendix III).

Blood HCO_3^-

Mean measured values of blood HCO_3^- concentrations ranged from 3.20-3.84 (mmol l^{-1}) during the experiment (Table 3.2). Significantly higher HCO_3^- concentrations were found for the ChronicHigh group at day 62 compared to the Control, but not significantly different from the other groups (SNK-test $p<0.05$, Table CCXXIII and CCXXIV, Appendix III)

.

Table 3.2: Blood parameters. Measured and temperature corrected (tp) blood parameters for juvenile Atlantic halibut exposed to five water ammonia treatments at three different sample dates. (Treatments being Control, ChronicLow, ChronicMedium, ChronicHigh and HighPulse, or Control, CL, CM, CH and HP respectively). Values are given as mean and standard deviation (SD). Means not sharing a common letter, at the same sampling date, are significantly different (SNK-test $p<0.05$).

Blood parameters																	
	Day 0	**Day 62**								**Day 100**							
	Control	**Control**		**CL**		**CM**		**CH**		**HP**		**Control**		**CL**		**CM**		**CH**		**HP**	
Na^+	157	168		170		169		168		167		170		171		168		170		169	
(mmol l^{-1})	(3.6)	(5.77)		(3.78)		(2.78)		(3.96)		(3.77)		(4.63)		(4.53)		(3.96)		(4.99)		(2.39)	
K^+	3.52	4.26		4.31		4.19		4.40		4.33		4.83	a	5.65	b	4.51	a	4.71	a	4.43	a
(mmol l^{-1})	(0.34)	(0.22)		(0.47)		(0.20)		(0.42)		(0.49)		(0.55)		(0.96)		(0.59)		(0.45)		(0.25)	
pH tp	7.34	7.31		7.29		7.29		7.33		7.31		7.25		7.20		7.23		7.27		7.26	
	(0.08)	(0.09)		(0.04)		(0.06)		(0.09)		(0.06)		(0.06)		(0.07)		(0.04)		(0.05)		(0.05)	
pCO_2 tp	4.77	5.79	a	5.25	a	4.92	ab	4.33	b	5.32	a	5.67	a	6.67	b	6.15	ab	5.55	a	5.64	a
(mmHg)	(0.97)	(0.44)		(0.39)		(0.64)		(1.02)		(0.39)		(0.49)		(0.93)		(0.73)		(0.40)		(0.31)	
HCO_3^-	3.56	4.14	a	3.61	ab	3.40	ab	3.20	b	3.79	ab	3.56		3.84		3.74		3.66		3.69	
(mmol l^{-1})	(0.76)	(0.95)		(0.51)		(0.44)		(0.84)		(0.28)		(0.47)		(0.87)		(0.59)		(0.57)		(0.53)	

3.4 Effect of ammonia on cataract

At start of the experiment, 10 % of mild cataract was found in the examined fish (Table 3.3), where no cataract were found in examined fish at the end of the first period (day 62) where the fish had been exposed to ammonia. After the following weeks with normalized water quality at the end of the experiment 10 % of mild cataract was observed. The number of fish affected had only a cataract score for one of the two eyes.

Table 3.3 Cataract development in groups of juvenile Atlantic halibut exposed to different ammonia regimes for approximately 9 weeks, and 6 week of no treatment (compensatory growth period).

						Total examined fish
Treatment	**Control**	**CL**	**CM**	**CH**	**HP**	**% affected**
Start						
Sum scores	1					20
# affected	1					10
day 62						
Sum scores	0	0	0	0	0	40
# affected	0	0	0	0	0	0
day 100						
Sum scores	1	1	1	1	0	40
# affected	1	1	1	1	0	10

Individual fish were examined by use of a slit-lamp biomicroscope and scored from 0 (no cataract) to 4 (complete cataract) per eye, e.g. score 0 to 8 per fish. Due to low prevalence of mild cataracts, data is given as sum of individual scores and (#) of affected fish observed per experimental treatment.

3.5 Effect of ammonia on muscle and lens free amino acids

Mean values of the free amino acids found in the white muscle tissue samples from all five experimental groups are given in Table 3.2. Muscle samples taken at day 62 only showed significant differences in Glu, Gln, Ala and Aaiba. The ChonicLow group had a significant higher level of Glu compared to the other groups (0.87 (μmol g^{-1}) versus 0.60 μmol g^{-1} in the Control group). The ChronicMedium and ChronicHigh groups had significantly higher levels of Gln compared to the others. Lower mean values of Ala where found in the ChronicHigh and HighPulse groups, and ChronicMedium group displayed lower levels of Aaiba compared to the other groups.

At the end of the experiment, after a 6 week period of normalized water quality (no ammonia added), differences were found in Urea, Asn, Glu, Gln, Aaba, Cysth2, Orn and Ans.

The ChronicHigh group had a significantly lower measured mean value of Urea compared to the other groups, with ChronicMedium and HighPulse having significantly higher value compared to ChronicHigh. Significantly higher values of Asn were found in the ChronicHigh group compared to the all the other groups and significantly higher value of Glu were found in ChronicLow compared to all the other groups. The ChronicMedium had a significantly higher Gln concentration than the other treatment groups. For the free amino acid Aaba, the HighPulse group had a significantly lower value compared to the rest, while the ChronicHigh group had the significantly higher value compared to the HighPulse group. A similar pattern was found for the free amino acid Cysth2. The ChronicLow group displayed significantly higher value of Orn compared to the other groups, except from the Control group. Ans were found at a significantly higher concentration in the HighPulse group compared to the other groups (SNK-tests $p<0.05$, see Tables CCXXVIII to CCXCVII, Appendix III).

Muscle levels of Urea and Amm ranged between 5.25 to 7.67 μmol g^{-1} and 3.24 to 3.96 μmol g^{-1}, respectively. No significant differences in muscle levels of Urea between treatments were found in this experiment, however some differences were found in muscle Amm as described above. Measured levels of the imidiazole His ranged between 0.37 to 0.97 μmol g^{-1} in the muscle tissue of juvenile Atlantic halibut in this experiment, although no significant differences were found between the treatment groups. The imidiazole Ans levels ranged between 0.37 to 0.97 μmol g^{-1}. The imidiazole Car were not detected in muscle tissue of juvenile Atlantic halibut.

Muscle levels of Tau ranged between 9.35 and 14.5 μmol g^{-1} in juvenile Atlantic halibut, and Tau was the free amino acid that was found in high levels. White fish normally have a higher content of Tau, and the muscle tissue samples had to be analyzed using a different method which was adapted to higher levels of Tau (different channel in the HPLC Empower software). Measured total free amino acid ranged between 40.41 to 47.34 μmol g^{-1}, and when Tau values were not included the values ranged between 31.0 to 35.4 μmol g^{-1}. There were no significant differences found in measured total free amino acid.

The results of measured free amino acids showed no systematic trend in response to the experimental treatments.

Table 3.4: Mean values (µmol g^{-1}) of free amino acids (FAA) found in juvenile Atlantic halibut muscle tissue exposed to five water ammonia treatments at three sampling dates. (Treatments being Control, ChronicLow, ChronicMedium, ChronicHigh and HighPulse, abbreviated Control, CL, CM, CH and HP respectively). Values are given as mean and standard deviation (SD). Means not sharing a common letter, at the same sampling date, are significantly different (SNK-test, $p<0.05$).

	T0		**T3**													**T4**											
			Control			**CL**			**CM**			**CH**			**HP**			**Control**			**CL**			**CM**			**CH**			**HP**		
Free Amino Acid	Mean	S.D	Mean	S.D		Mean	S.D		Mean	S.D		Mean	S.D		Mean	S.D		Mean	S.D		Mean	S.D		Mean	S.D		Mean	S.D		Mean	S.D	
Phser	0.01	0.00	0.01	0.00		0.01	0.00		0.01	0.00		0.01	0.01		0.01	0.01		0.01	0.00		0.01	0.00		0.01	0.00		0.01	0.00		0.01	0.00	
Tau	9.35	2.50	12.29	2.88		10.93	1.98		11.36	3.12		11.13	2.72		12.11	2.86		13.40	1.27		13.12	1.73		13.15	2.44		12.50	2.38		14.49	1.90	
Pea	0.09	0.03	0.08	0.01		0.08	0.01		0.08	0.01		0.07	0.02		0.07	0.02		0.08	0.01		0.09	0.02		0.09	0.02		0.08	0.01		0.08	0.02	
Urea	5.40	1.16	7.67	1.19		7.07	1.11		6.90	1.02		7.11	1.42		7.16	1.10		6.38	0.50	abc	6.29	1.02	abc	6.27	0.88	b	5.25	0.58	c	6.81	1.36	b
Asp	0.53	0.20	0.58	0.22		0.60	0.13		0.54	0.14		0.57	0.11		0.66	0.20		0.66	0.14		0.67	0.14		0.57	0.14		0.54	0.15		0.60	0.12	
Hypro	0.56	0.54	0.44	0.22		0.54	0.29		0.46	0.43		0.31	0.23		0.49	0.37		0.63	0.23		0.82	0.32		0.64	0.29		0.77	0.29		0.52	0.23	
Thr	1.13	0.36	1.46	0.39		1.35	0.43		1.19	0.49		1.03	0.29		1.31	0.48		1.62	0.52		1.73	0.34		1.50	0.25		1.95	0.23		1.48	0.28	
Ser	1.73	0.42	1.71	0.48		1.91	0.38		1.25	0.48		1.56	0.29		1.50	0.63		1.00	0.15		1.12	0.20		1.20	0.40		1.04	0.24		1.04	0.26	
Asn	0.64	0.58	0.67	0.54		0.33	0.14		0.94	0.76		0.71	0.29		0.49	0.31		0.71	0.90	a	0.45	0.34	a	1.19	0.64	a	1.82	0.62	b	0.52	0.39	a
Glu	0.69	0.25	0.60	0.17	a	0.87	0.19	b	0.64	0.14	a	0.59	0.07	a	0.67	0.07	a	0.65	0.19	ab	0.75	0.12	b	0.62	0.13	ab	0.49	0.10	a	0.61	0.10	ab
Gln	0.83	0.20	0.85	0.19	abc	0.80	0.16	ab	1.05	0.23	c	1.04	0.17	c	0.71	0.16	b	1.00	0.28	ab	0.93	0.20	ab	1.12	0.13	b	0.91	0.21	ab	0.73	0.23	a
Sarc	0.04	0.01	0.04	0.02		0.03	0.01		0.03	0.01		0.03	0.01		0.03	0.01		0.03	0.01		0.04	0.02		0.04	0.02		0.03	0.01		0.04	0.01	
Pro	0.87	0.96	1.32	0.91		1.76	1.09		1.53	1.48		0.93	0.65		1.28	0.76		2.30	0.97		1.76	1.09		1.71	0.91		2.46	0.88		0.96	0.30	
Gly	7.58	0.54	7.13	0.67		7.36	0.30		6.70	1.11		7.71	0.52		7.44	0.78		5.94	0.50		5.90	0.85		6.04	0.63		5.82	0.68		5.96	1.05	
Ala	4.28	0.49	4.06	0.53	abc	4.53	0.71	abc	4.88	0.89	b	3.69	0.51	bc	3.69	0.58	c	3.88	0.57		3.86	0.53		4.04	0.42		3.69	0.54		3.65	0.42	
Citr	0.05	0.04	0.08	0.07		0.08	0.07		0.06	0.06		0.05	0.04		0.12	0.09		0.14	0.08		0.16	0.07		0.13	0.05		0.09	0.04		0.11	0.04	
Aaba	0.06	0.02	0.06	0.01		0.06	0.02		0.06	0.03		0.05	0.02		0.06	0.02		0.07	0.02	abc	0.07	0.02	abc	0.07	0.02	abc	0.08	0.02	b	0.05	0.01	c
Val	0.09	0.03	0.07	0.03		0.07	0.03		0.09	0.03		0.09	0.03		0.08	0.04		0.05	0.01		0.05	0.02		0.05	0.01		0.05	0.02		0.06	0.02	
Met	0.05	0.02	0.04	0.01		0.04	0.01		0.05	0.02		0.05	0.02		0.05	0.03		0.04	0.01		0.03	0.01		0.03	0.01		0.03	0.01		0.04	0.01	
Cysth2	0.38	0.16	0.96	0.26		0.88	0.36		0.78	0.55		0.69	0.37		0.77	0.41		0.77	0.14	abc	0.80	0.28	abc	0.83	0.12	abc	0.93	0.17	b	0.60	0.16	c
Ile	0.06	0.02	0.04	0.02		0.04	0.02		0.05	0.02		0.06	0.02		0.05	0.03		0.03	0.01		0.03	0.01		0.02	0.01		0.02	0.01		0.03	0.01	
Leu	0.12	0.04	0.07	0.04		0.08	0.03		0.11	0.03		0.12	0.04		0.10	0.05		0.06	0.02		0.07	0.03		0.06	0.03		0.05	0.02		0.07	0.02	
Tyr	0.05	0.01	0.06	0.01		0.05	0.01		0.04	0.02		0.05	0.02		0.07	0.02		0.05	0.01		0.05	0.02		0.05	0.01		0.05	0.02		0.05	0.01	
Phe	0.04	0.01	0.04	0.01		0.03	0.01		0.03	0.01		0.04	0.02		0.04	0.01		0.04	0.01		0.04	0.01		0.03	0.01		0.03	0.01		0.04	0.00	
Aaiba	0.06	0.02	0.03	0.01	a	0.03	0.01	a	0.01	0.01	b	0.03	0.01	a	0.03	0.01	a	0.04	0.01		0.05	0.01		0.05	0.01		0.04	0.01		0.04	0.01	
Ethanolamine			0.02	0.01		0.04	0.01		0.03	0.00		0.03	0.01		0.03	0.01		0.02	0.01		0.01	0.00		0.02	0.01					0.03	0.02	
Amm	3.87	0.34	3.78	0.35		3.96	0.26		3.91	0.21		4.16	0.27		3.79	0.29		3.44	0.45		3.57	0.10		3.30	0.12		3.24	0.25		3.51	0.13	
Hylys1	0.05	0.05	0.07	0.03		0.06	0.02		0.06	0.03		0.05	0.02		0.05	0.02		0.13	0.03		0.12	0.04		0.13	0.07		0.14	0.04		0.10	0.04	
Hylys2	0.02	0.01	0.05	0.01		0.03	0.02		0.04	0.03		0.05	0.02		0.05	0.02		0.03	0.01		0.03	0.02		0.03	0.01		0.03	0.01		0.03	0.01	
Orn	0.02	0.01	0.03	0.02		0.02	0.01		0.02	0.01		0.01	0.01		0.03	0.02		0.03	0.01	ab	0.04	0.01	b	0.02	0.00	a	0.02	0.00	a	0.03	0.01	a
Lys	0.53	0.24	0.60	0.21		0.63	0.10		0.65	0.23		0.60	0.12		0.44	0.15		0.54	0.13		0.49	0.14		0.49	0.13		0.57	0.18		0.48	0.10	
His	0.84	0.58	1.44	0.58		1.24	0.55		1.07	0.68		1.07	0.60		1.32	0.66		1.66	0.16		1.72	0.13		1.50	0.38		1.72	0.23		1.62	0.26	
Ans	0.37	0.13	0.82	0.08		0.72	0.07		0.75	0.12		0.67	0.15		0.72	0.14		0.84	0.08	a	0.84	0.11	a	0.81	0.09	a	0.92	0.07	ab	0.97	0.08	b
Arg	0.09	0.05	0.15	0.07		0.12	0.04		0.11	0.03		0.11	0.03		0.11	0.04		0.15	0.05		0.14	0.03		0.14	0.06		0.17	0.09		0.12	0.03	
SUM Free AA(exl. Tau)	31.06	0.79	35.05	0.17		35.40	1.16		34.09	2.79		34.07	0.51		33.40	1.32		33.01	1.84		32.73	1.39		32.79	0.29		33.05	0.05		31.00	1.33	
SUM Free AA	**40.41**	**3.29**	**47.34**	**3.05**		**46.33**	**3.14**		**45.45**	**5.91**		**45.20**	**3.23**		**45.51**	**4.18**		**46.42**	**3.11**		**45.85**	**3.12**		**45.93**	**2.73**		**45.55**	**2.44**		**45.49**	**3.23**	

Mean lens His ranged from 0.95-1.44 µmol g^{-1} in samples taken during this experiment (Figure 3.8). At day 62 no significant differences were found between Control and the HighChronic and HighPulse groups, but significant lower lens His were found in the ChronicLow, and ChronicMedium groups than in the Control. Still there were no significant differences between ChronicMedium and ChronicHigh groups. At the end of the experiment after 6 weeks of no treatments (normal water quality) no significant differences were found between groups (see Tables CCXXV to CCXXVI, Appendix III).

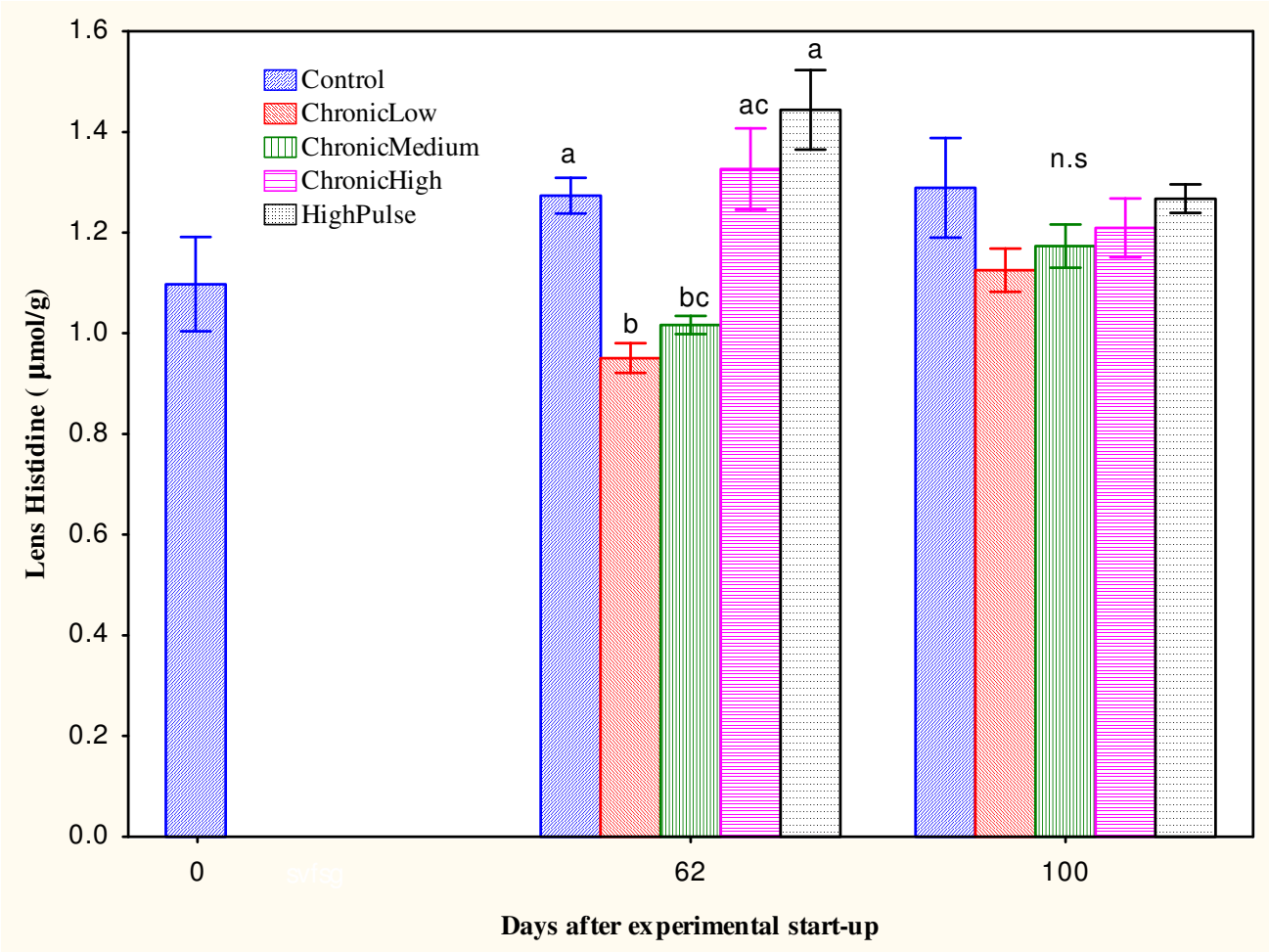

Figure 3.3: Histidine concentration in the lenses of Atlantic halibut (µmol g^{-1} sample, group means + SE, Table XIII, Appendix III). Colour and pattern indicate the water concentration of ammonia: Control, ChronicLow, ChronicMedium, ChronicHigh, and High Pulse. Means not sharing a common letter are significantly different (SNK test, $p<0.05$, see Table CCXXV-CCXXVI, Appendix III).

During this experiment the mean value of NAH ranged from 7.30-12.8 µmol g^{-1} in the lens samples taken in this experiment (Figure 3.9). Significantly lower values were found after the 9 week exposure to the different ammonia treatments in ChronicLow, ChronicMedium and ChronicHigh groups compared to the Control and HighPulse groups. Sample taken at the end of this experiment showed no significant differences (See Tables CCXXVII to CCXXVIII, Appendix III).

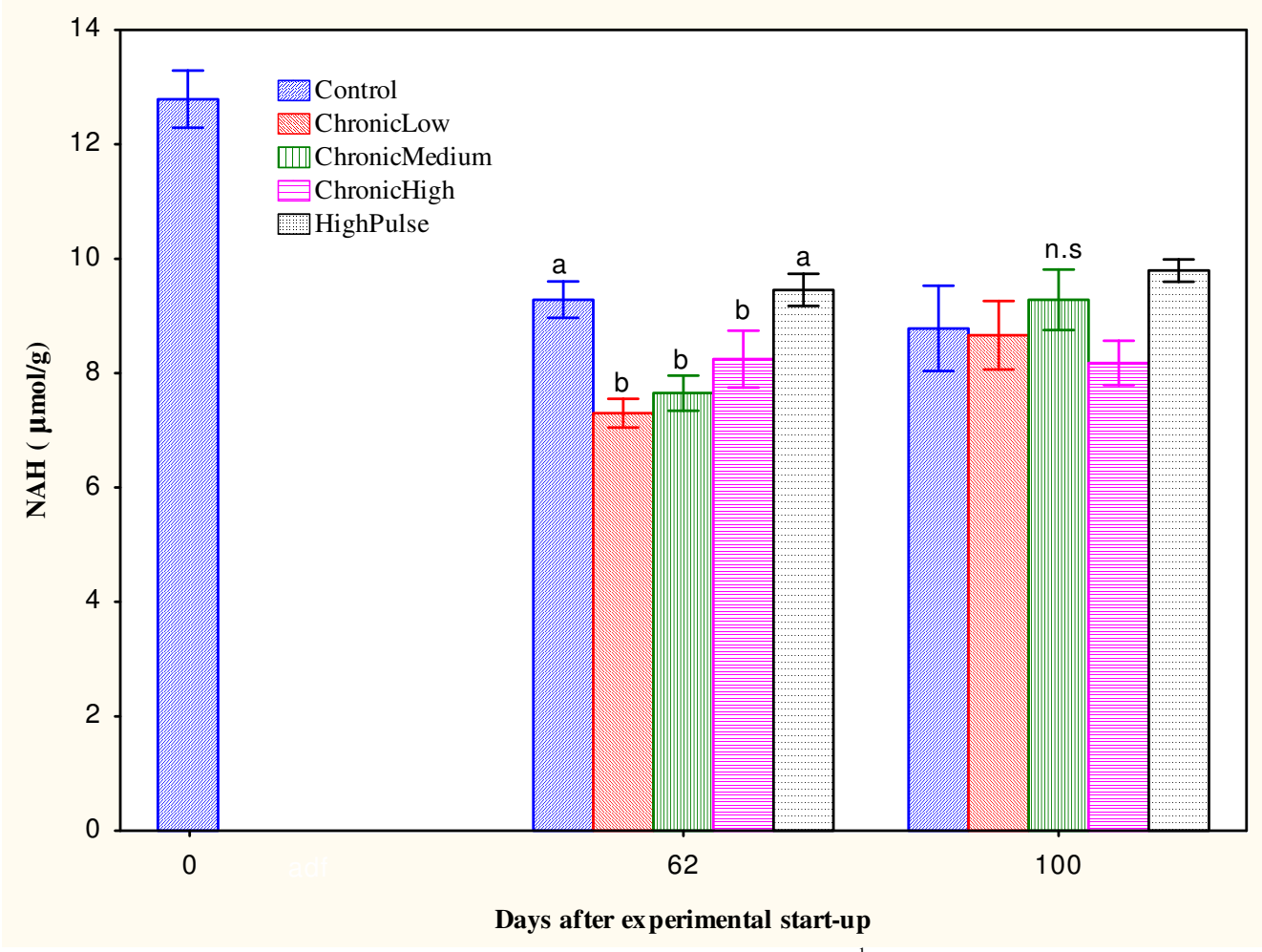

Figure 3.4: NAH concentration in the lenses of Atlantic halibut juveniles (µmol g^{-1} sample, group means + SE, see Table XIV, APPENDIX III). Colour and pattern of the columns indicate the level of ammonia concentration: Control, ChronicLow, ChronicMedium, ChronicHigh and HighPulse. Means not sharing a common letter are significantly different (SNK test, $p<0.05$, see Table CCXXVII and CCXXVIII, Appendix III).

4 Discussion

4.1 Effect of ammonia on growth

In the present experiment, juvenile Atlantic halibut were exposed to ambient UIA concentrations ranging from 0.06-0.17 mg l^{-1} for 62 days. In the subsequent period (days 63 - 100) ammonia treatment was terminated and water quality normalized to examine if the groups previously exposed to high levels experienced any compensatory growth. Condition factor (CF), feed conversion efficiency (FCE), daily feeding rate (F %) calculations are not presented in the results, as they were part of another study (Paust, 2010).

Mean weight and growth rates for the juvenile Atlantic halibut were significantly lower in groups exposed to chronically high ambient ammonia concentrations compared to corresponding control group, throughout the period with ammonia treatments. Chronically low ammonia concentrations did not have significant effect on growth and SGR.

In the present study significant differences in mean weights between the experimental groups were seen at day 62, and these differences were still present at day 100. In contrast to what seen during the ammonia treatment no significant differences were found in growth rates between groups during this period. The groups previously exposed to high levels of ammonia were not able to catch up with the control group during a relatively fast growing period. If there were any indications of compensatory growth the previously exposed groups would have grown remarkably during these weeks and better than growth in control groups, and this did not happen. Compensatory growth (CG) in fish is commonly described as a phase of unusually rapid growth following a period of reduced feeding (Ali et al., 2003), or suboptimal growing conditions (Foss and Imsland, 2002). Application of this growth spurt mechanism has been suggested (Quinton and Blake, 1990; Jobling et al., 1994) as appropriate exploitation may result in increased growth and higher food conversion efficiency (Wang et al., 2000; Foss and Imsland, 2002). This has been applied with cyclic starvation/re-feeding regimes to induce CG. Short term starvation (2-5 weeks) and subsequent recommencement of feeding in juvenile (200-400g) Atlantic halibut resulted in partial growth compensation, and a tendency of increased feed conversion efficiency (Heide et al., 2006).

Studies aiming at determining the effect of both acute and chronic ammonia exposure and identifying threshold levels, have been performed for several marine fish species such as Atlantic cod (Foss et al., 2004; Remen et al., 2008), Dover sole (*Solea solea*) (Alderson, 1979), European seabass (*Dicentrarchus labrax*) (Dosdat et al., 2003; Lemarié et al., 2004) and giltheaded seabream (*Sparus aurata*) (Wasjbrot et al., 1993). The threshold limit for reduced growth (NOEC=no observable effect) observed in the current study with halibut juvenile (0.06 mg UIA-N l^{-1}) is similar compared to what has been juvenile Atlantic cod (Foss et al., 2004) and the same low levels were found in juvenile Dover sole (0.066 mg UIA-N l^{-1}) (Alderson, 1979). Threshold limits have found to be higher for turbot juveniles (11 – 0.18 mg UIA-N l^{-1}) (Rasmussen and Korsgaard, 1996; Person-Le Ruyet et al., 1997) for juvenile giltheaded seabream (0.27 mg UIA-N l^{-1}) (Wajsbrot et al., 1993) and Lemarié et al., (2004) identified a safe limit of 0.26 mg UIA-N l^{-1} for optimal growth of juvenile seabass.

Most previous studies have investigated chronic exposure of ammonia, but as pointed out by Colt (2006), determining threshold levels by chronic exposure will rarely or ever reflect a true culture situation, whereas postprandial peaks is a reality under culture conditions for halibut as well as for other fish species. Results from studies with chronic exposure levels are thus not directly applicable and more realistic trials are necessary, at least if the aquaculture industry shall benefit from the research done. Postprandial ammonia peaks have been reported to occur 4 to 12 h after feeding in the Japanese flounder (*Paralichthys olivaceus*), the giltheaded seabream, striped seabream (*Lithognathus mormyrus*), aerolated grouper (*Epinephelus areolatus*) and Mangrove snapper (*Lutjanus argentimaculatus*), with peak rates of exretion being several times higher than fasting rates (Klumpp and von Westerhagen, 1986; Cockcroft and Du-Preez, 1990; Kikuchi, et al., 1991; Leung et al., 1999).Surprisingly, the short daily peaks resulted in no equivalent growth reductions as seen in the chronically high ammonia exposed groups. In our experiment the high ammonia peak group showed no significant difference in mean weights or SGR compared to the Control group. The high ammonia peak group (HighPulse) was used as an experimental condition which intended to mimic postprandial ammonia peaks. In a study by Yigit et al., (2005) ammonia exretion rates was measured in Black Sea turbot (*Scophthalmus maeoticus*) at 12 ºC under natural light conditions. They observed that the rates were 2-3 times higher immidiately after feeding than in starved fish, reaching a peak 3-6 hours after feeding and declining afterwards. The effect of short daily peaks on growth performance has not been previously investigated in juvenile Atlantic halibut, however this experiment did not meet the threshold ammonia peak to

observe the same affect that chronic high levels had on growth performance. Studies done on turbot (Foss et al., 2007; Foss et al., 2009) demonstrated that chronic exposure to increased TA-N levels (6.5 and 12.3 mg l^{-1}) resulted in reduced growth, and short exposure (3-4 hours a day) may result in almost the same growth reduction (~15 % compared to the control) as chronic levels. With this in knowledge it will be of outmost importance to make sure that the threshold levels determined are not exceeded at any time of the day. Measurements of TA-N levels in the rearing systems should be performed at various times during the day, and especially following the time of day where feed intake are expected to be at its highest. Foss et al., (2009) demonstrated that short daily ammonia peaks may result in negative effects on growth, equivalent to that found under chronic exposure in juvenile turbot. Comparing measured TA-N and calculated UIA-N levels for the experimental group HighPulse with levels used in Foss et al., 2009 it is clear that levels in the HighPulse group is ~LowPulse levels in the Foss et al., 2009 trial. Although a 5 % growth reduction was found in their LowPulse group when compared to their Control.

Table 4.1: Measured total ammonia nitrogen TA-N (mg l^{-1}) and calculated values of un-ionised ammonia (UIA-N) in the experimental group HighPulse compared to values from experimental groups LowPulse and HighPulse in Foss et al., 2009.

Treatment	TA-N				UIA-N				
	$T_{0.5}$ h	T_1h	$T_{1.5}$	T_3 h	$T_{0.5}$ h	T_1h	$T_{1.5}$	T_3 h	
LowPulse	5.26	6.41	5.1	1.3	0.11	0.13	0.1	0.03	Foss et al., 2009
HighPulse	10.63	13.06	9.72	2.24	0.22	0.27	0.2	0.05	Foss et al., 2009
HighPulse	15	6.3	3.7	1.4	0.18	0.07	0.04	0.02	

In summary, chronic high levels of 0.17 mg l^{-1} UIA-N resulted in significantly reduced overall growth in juvenile Atlantic halibut in this experiment and threshold limits of UIA-N for growth reduction is between 0.12-0.17 mg l^{-1} UIA-N (SGR between 0.06% day^{-1} and 0.12 % day^{-1}). Further trials need to be conducted in relation of identifying the threshold limits of postprandial peaks of ammonia. When considering the various threshold limits that exist for different species and also the different life stages of species, it becomes clear that comprehensive studies are necessary in order to assist safe rearing practises in intensive aquaculture facilities. Studies like these provides the farmer with information to which TA-N levels that should be avoided generally by increasing water flow, reducing biomass, changing feeeding regime or increasing biofilter capacity (if rearing in recirculation systems). It has also been demonstrated that ammonia interacts with several other environmental factors, e.g. dissolved oxygen, (Wajsbrot et al., 1991; Foss et al., 2003), pH (Thurston et al., 1981), salinity (Alabaster et al., 1979; Sampaio et al., 2002) and carbon dioxide (Randall and Wright,

1989). Tolerance in variations in such parameters is species and age specific and needs to be identified in order to balance the use of water with water quality requirements of the species and life-stage in question. Significant efforts is still needed in mapping the species tolerance to important water quality parameters using a multifactorial approach, that might reflect "true" culture situation, whereas several parameters interact to affect fish performance simultaneously.

4.2 Effects of ammonia on physiological status

Measured parameters were mostly within the range of what has previously been reported from studies on halibut and other marine species (Jonassen et al., 1999; Staurnes, 2001; Imsland et al., 2008a, 2008b; Magnussen et al., 2008). With few exceptions blood parameters were not affected by either chronic levels or periodic peaks. Measurement of blood parameters did not indicate any major physiological disturbances induced by our treatments, but some values indicated physiological disturbances of the juvenile Atlantic halibut. At day 62 differences were found in pCO_2 and HCO_3^- in ChronicHigh group, and at day 100 differences were found in K^+ and pCO_2 in the ChronicLow group. Minor variations in blood Na^+ and K^+ levels were seen throughout the experiment, indicating that the hydromineral balance was maintained. Blood pCO_2 and HCO_3^- showed significant difference in the ChronicHigh compared to the Control group. According to the Henderson Hasselbach equation of acid-base regulation, a higher concentration of bicarbonate as a proportion of CO_2 will result in higher pH (Claiborne, 1998). No significant differences were found in blood pH values between treatments in samples taken during the experiment, which according to the Henderson Hasselbach equation means that the levels are equivalent which is in agreement with the present data (ChronicHigh vs. Control). Person-Le Ruyet et al (2003) found an increase in electrolytes and total Ca^+ concentrations in blood in juvenile turbot reared above 0.34 mg l^{-1} UIA-N so it is possible that the ammonia levels in the present study (max 0.17 mg l^{-1} UIA-N) were too low to cause disruptions in homeostasis in juvenile Atlantic halibut.

All together, measurements of blood parameters do not give any clear evidence that the halibut exposed to the different chronic and periodic exposure of UIA-N in this study were subjected to any form of physiological stress.

4.3 Effect of ammonia on cataract

During sampling the anesthetized fish were screened for cataract using a slit-lamp biomicroscope. The slit-lamp biomicroscope has proven to be useful for clinical characterisation and classifiction of cataracts and is more applicable under practical field conditions , together with a scoring system for the extension of changes in the lens (Wall et al., 1999; Bjerkås et al., 2006) The changes are graded as seen straight trough the pupil. Each eye is scored seperately as follows: 0: normal lens, 1: opacity affecting lens less than 10 % of the lens, 2: 10-15 % opacity, 3: 50-75 % opacity, and 4: complete cataract (>75 %). The results from the eyescreening in the present study showed that observed cataract did not differ significantly between treatments. At day 62 no cataract was observed, and cataracts graded as 1, were found in four fishes (one eye) in four out of the five treatments (Table 3.3) at day 100. This result should however be regarded with caution because of the small number of fish per treatment in this experiment (n=8).

Pankhurst and Montgomery (1994) found that growth of the eye is maintained at the expense of low somatic growth during suboprimal rearing conditions. A diet sample was analyzed by for total amino acids after acid hydrolysis and found a mean His level to be 11.6 ± 0.8 mg g^{-1} sample (mean±SD, Table 2.1). Breck et al (2005b), found that Atlantic salmon lens under rich supply of dietary His accumulated high levels of NAH (five times free His concentrations) and that high lens NAH concentration was positively correlated with low cataract scores. Further they found that in fish fed lower His levels, cataract scores were higher in fish that have been exposed to fluctuation in water salinity and elevation of water temperature than in fish maintained in stable environment. The diet used in this study was sold and marketed as a health diet with added His to reduce stress during rearing e.g. handling of fish. By using this diet in our experiment we most likely masked some of the effect our UIA-N treatments had on free amino acid composition in muscle and lenses. Although the same food was given to all experimental groups it would have been interesting to see if a more “normal” halibut diet would have given a different result than those presented in the present experiment.

4.4 Effect of ammonia on muscle and lens free amino acids

In most fish skeletal muscle constitutes of more than 50 % of the whole body mass. Muscles concetrates of the largest pools of free amino acids (Smutna et al., 2002).
No significant differences were found in the total free amino acid content in sampled juvenile Atlantic halibut between different ammonia treatments. Total free amino acids content in juvenile Atlantic halibut ranged between 40.4 to 47.4 μmol g^{-1} in this experiment.
Sum of free amino acid found in salmon fed either control og high His diets ranged from 24.1 to 28.3 μmol g^{-1} (Breck et al., 2005a) and compared to the results found in this experiment the Atlantic halibut muscle tissue contains almost double levels of free amino acids.

Muscle Urea ranged from 5.25 to 7.67 μmol g^{-1} in this experiment and differences were found at day 100 with significant lower levels of Urea found in the ChronighHigh group. Muscle Amm ranged from 3.24 to 3.96 μmol g^{-1}, though no differences were found.

White fish often contain higher levels of taurine (Tau). And reported levels of Tau in this experiment ranged from 9.4 to 14.5 μmol g^{-1}, and was the free amino acid reported in largest quanta in the total amount of free amino acids. Tau is synthesized from methionine (Met) via cysteine (Cys) and is known to play a physiological role in osmoregulation in fish an other animals (Schaffer et al., 2000; Buentello and Gatlin 2002). The physiological role of Tau is not completely clear within species or across species. Tissue Tau levels have been demonstrated to be responsive to osmotic pressure, and Tau is thought to serve some antioxidiative capacity. Japanese flounder have been reported to require Tau in the diet, at least at the juvenile stages, to maximise growth rates (Takeuchi, 2001)
Breck et al, 2005a reported levels of Tau in salmon fed different His diets at range of 0.93 to 2.25 μmol g^{-1}.

Van Waarde (1988) classified of imidiazole-related compounds (histidine and anserine) in skeletal muscle of fish and reported that very low levels (< 1 μmol g^{-1}) of imidiazole related compounds are found in the families of Pleuronectidae (flounders).
No significant differences were found in muscle His (0.84 to 1.72 μmol g^{-1}) in muscle tissue of the fish in the present experiment. As a His dipeptide, muscle anserine (Ans) constitute a major marker of His status in salmonids, probably important for its strong buffering capacity at physiological pH (Hiroshi and Murai, 1994). Metabolic stress due to the ammonia treatment could eventually lead to increased susceptibility for cataract development. Elevated

muscle Ans were only observed in the ChronicHigh group. The reduction in muscle non-essential amino acids following increased levels of imidiazole compounds has been described in masu salmon (*Oncorhynchus masou*) as well as in yellowtail (*Seriola quinqueradiata*) Ogata et al., (1998) suggested a physiological mechanism by white muscle selectivity accumulates imidiazole compounds and maintains the total amino acid pool by down-regulating the level of non-essential amino acids. Both Ans and NAH is believed to keep His "trapped" in the respective tissues and prevent this essential amino acid from being catabolised or used in protein synthesis. Breck et al., (2005a) found Ans levels in salmon fed different His diets to be 9.73-16-07 μmol g^{-1}, and the levels found in this experiment 0.33-1.82 μmol g^{-1}. The imidiazole carnosine (Car) was not detected in muscle tissue of juvenile Atlantic halibut. The imidazole-related compounds have postulated to have numerous biological roles such as H^+ buffer (Abe and Okuma, 1991; Sewell et al., 1992), a divalent ion regulator (Baran, 2000), a neurotransmitter (Petroff et al., 2001), a non-enzymatic free radical scavenger (Kohen et al., 1988; Guitto et al., 2005), an antioxidant (Boldyrev et al., 2004) and a blood glucose regulator (Nagai et al., 2003; Sauerhofer et al., 2007).

In the present study lens His was found in levels within the range of 0.95 to 1.44 μmol g^{-1}. Mean lens His was found at different levels between the five treatments at day 62. Lower levels of lens His were found in the ChronicLow and ChronicMedium groups. Samples taken at the end of this experiment had no significant differences in lens His levels.

Another natural imidiazole-related compound N-α-acetylhistidine (NAH), is found in very high concentrations ubiquitously in the central nervous system as well as in the lens and retina and occasionally in the hart (Baslow, 1965; Erspramer et al.,1965, Yamada and Furuchi, 1990). NAH is considered an "imidazole dipeptide", whose α-amino group C-terminal amino acid (histidine) is bound to a carboxylic group of acetate in place of β-alanine or γ-aminobutyric acid. Yamada et., al (2009) suggested that NAH may play some exlusive role as an emergency reservoir for histidine (an essential amino acid) against a long period of food deprivation, in skeletal muscle of fish species possessing high levels of muscle NAH. In the present study NAH mean values ranged from 7.30-12.97 μmol g^{-1} in the lens of juvenile halibut which is within the critical low levels of imidiazoles <1.0 and 9.1 μmol NAH g^{-1} lens found in Atlantic salmon (Breck, 2004). Significant differences lower levels of measured NAH were found in all the chronic exposed groups compared to the Control and HighPulse groups. Baslow (1998) suggested that together with other histidine containing derivates;

carnosine, anserine and homocarnosine, NAH serves as a molecular water pump regulating hydration of lens tissue. A decrease in lens NAH level has been associated with cataract development in Atlantic salmon undergoing parr-smolt transformation (Breck et al., 2005b). NAH may be important for water homeostasis in lens (Breck, 2004), as well as a central nervous tissue (Baslow, 1998). NAH is synthesized from L-His and acetyltransferase, in the brain and lens (Baslow, 1966; Yamada et al., 1995), and hydrolyzed to histidine by the NAH-degrading enzyme, anserinase in these tissues (Baslow and Lenney, 1967; Lenney et el., 1978; Yamada et al., 1991; Yamada et al., 1993).

Table 4.2: N-α-acetylhistidine (NAH) in ocular lens of several fish species.

	NAH (µmol g-1)	
Species	lens	Reference
Perch (*Lateolabrax japonicus*)	4.7	Abe, 1995
White croaker (*Argyrosomus argentatus*)	2.83	Abe, 1995
Bigeye Tuna (*Thunnus obesus*)	11.4	Abe and Okuma, 1992
Japanese Barracuda (*Sphyraena japonica*)	3.44	Abe, 1995
Coho salmon (*Oncorhynhus kiutch*)	1.68	Abe and Okuma, 1992
Rainbow trout (*Oncorhynhus mykiss*)	3.15	Abe and Okuma, 1992
Japanese charr (*Salvelinus leucomaenis*)	4.85	Abe and Okuma, 1992
Japanese eel (*Anguilla japonica*)	1.98	Espramer et al., 1965
Conger eel (*Astroconger myriaster*)	1.17	Hanson, 1966
Skipjack tuna (*Katsuwonus pelamis*)	22.6	Togashi et al., 1998
Atlantic salmon (*Salmo salar*) smolt	1.3-12.9[1]	Breck et al., 2005b
Atlantic halibut (*Hippoglossus hippoglossus*) juveniles	7.3-13[2]	Author

[1]Range depending on dietary level of histidine

[2]Range depending on exposed levels of UIA-N

This is the first report on free amino acids in muscle and lens tissue of juvenile Atlantic halibut. Most of the work in this field (Waagbø et al., 1998; Breck et al,2005a,b,; Bjerkås and Sveier,2006) is conducted on salmonids and may not be fully comparable with findings in Atlantic halibut due to salmonids being anadromous fish and Atlantic halibut being merely a marine species. Diffences in values of free amino acid when compared to those in salmonids are discussed. More systematic trials and research is required to further investigate factors that leads to cataract in Atlantic halibut.

5 Summary and conclusions

Chronic high levels of ammonia led to lowered mean weights and lower growth rates and growth was significantly reduced in ChronicHigh and ChronicMedium groups compared to the Control group. Thus $\mathbf{H_{A1}}$ can be accepted.

High periodic peaks was used as an experimental condition to mimic postprandial peaks often found in culture systems. In this study the periodic peaks did not have the same effect on growth as chronically high exposure had, and no significant effect on weight or growth rates were found, when compared to the Control group. Accordingly $\mathbf{H_{A2}}$ cannot be accpeted

Measurements of blood parameters did not give any clear evidence that the halibut exposed to the different chronic and periodic exposure of UIA-N in this study was subjected to any form of physiological stress. Minor differences were found in ChronicHigh group measured pCO_2 and HCO_3^- at day 62, and diffences in K^+ and pCO_2 in the ChronicLow group at day 100 $\mathbf{H_{A3}}$ can be accepted.

Minor differences were found in measured free amino acids (FAA) used to explain bufering capacity. At day 62 differences were found in Glu, Gln and Aaiba and at day 100 after a period of normalised water quality conditions differences were found in more free amino acids (Urea, Asn, Glu, Gln, Aaba, Cysth2, Orn and Ans). Although no systematic trend was found $\mathbf{H_{A4}}$ is accepted.

The sampled fish was screened for cataract and indications of catract formation was found, although the results showed no clear evidence that the treatments contributed to differences and $\mathbf{H_{05}}$ cannot be rejected.

Differences in eye histidine status were found on samples taken on day 62, with lower levels found in ChronicLow and ChronicMedium group. $\mathbf{H_{A6}}$ can be accepted. .

Osmotic differences (measured as NAH) in the lens was found at day 62 in all the chronically ammonia exposed groups. The groups had lower levels of NAH compaired to Control group and HighPulse group. Accordingly, $\mathbf{H_{A7}}$ can be accepted

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7 APPENDIX I

7.1 Discussion of materials and methods

7.1.1 Total mortality

Significantly higher total mortality was observed in all treatment groups in the third period of the experiment (ChronicLow, ChronicMedium, ChronicHigh and HighPulse) compared to Control. The ChronicLow group had massive mortality in this period, and was caused by an accident in connection with the sampling or they were sensitive to the sampling procedure. From just the one tank 27 fish were found dead the day after the sampling day. The necessity to perform a test for bacterial infection was considered, although not committed because there were no mortalities in the replicate tank. A reason for the high mortality is most likely due to the anaesthesia and wake-up period. During sampling the fish were anesthetised with Metacain, and after length and weight measurement the fish were placed in a wake-up tub with added oxygen. It is probably a shorter duration of time in the wake-up tub that might have caused this to happen. Since Halibut is a flatfish located on the bottom floor of the tank, and sometimes stacked on-top of each other, the effect of many fish dying at the same time may be substantial. The dead fish will lie upon the live fish and mucus and slime formation could suffocate the others and following more fish died. The colour of the water in the tank was greyish, and when removing dead fish a lot of mucus was observed.

The number of fish varied substantially between periods in all groups, the significant differences between treatment groups and control group can be explained by coincidence rather than treatment effect. Some of these fish also showed signs of physical damage (injuries eyes, pectoral fins and tails) and they may have been victims to aggressive behaviour by dominant individuals during e.g. feeding as proposed by Greaves and Tuene (2001). Growth coefficient (Zar, 1984) was calculated to determine if the dead fish generally were smaller than the remaining fish (Table I, Appendix IV), and the growth coefficient were high in four treatment groups, but not in the Control. Communication errors lead to all dead fish from the last period to be thrown away without weight descriptions. The scanner was unavailable the last period and therefore the fish was supposed to be temporarily stored in the freezer until the scanner came back, and during this time the fish was thrown away due to renovations in the storage freezer. This made comparing weights from dead fish with remaining fish impossible.

7.1.2 Experimental design

The experiment consisted of 823 juvenile halibuts at start up and to be able to observe growth performance of individual fish, 299 fish were tagged. The tagged fish was randomly chosen. The untagged fish was used for blood samples, weight and growth measurements, sampled for histidine analysis and chosen randomly. Twenty fish was sampled at start-up and 40 fish (n=2 per tank, n=4 per treatment) sampled on each sample day. All the fish was weighted at the sample days to be able to calculate growth data (specific growth rate) and feed conversion ratio.

The fish used in this experiment was poorly sorted by size ranging from 13g to approximately 104 g. This probably resulted in aggressive behaviour in the tanks when small fish was pooled together with larger more dominant fish (see discussion above). An aggressive behaviour also observed was eye snatching. Several fish lost one or both eyes, or lost the one good eye (Pers. Observation). This is expected to have an influence on the death rate. There will be problematic to state that the ammonia treatment cause high death rates if the death rate is the same between the groups during or post exposure of ammonia. We found altogether 212 dead fish and some of these were even tagged fish. A few of these tagged fish could have died because of buccal infections post tagging or by eye snatching. Also noted that the gills of some dead fish were slimy and this could be caused by ammonia toxicity.

Some fish exhibited altered behaviour e.g. very active and would not settle at the bottom. This was even noticed to be occurring in one control tank. These could well in fact be losers selected because they were easy to catch by the farmer. The muscle tissue samples were sampled from fish with poor pigmentation. It is shown that albinism causes lower activity of some enzymes.

The formulated feed that was used in this experiment was a health diet, with extra added histidine, that was supposed to reduce the stress in the juveniles when handling. According to the manufacturer this is given to fish normally for a two week period prior to stressful handling. (e.g. transportation, vaccination etc). AGA Marin recommended this diet since they achieved higher survival by providing this diet to the juvenile halibut.

The sampled lenses at the last sample day were sampled in Eppendorf tubes. These were supposed to be sampled in Brand tubes as the others since the Eppedorf tubes cracks during centrifugation in the laboratory. The lenses had to be transferred over to Brandt tubes and we may have lost some of the material that was already weighed in with the Eppondorf tubes. They were transferred while frozen at -80 ºC and knocked over to the Brand tube. When they started melting the lenses got sticky and where harder to transfer. This could probably bias the results and the calculations when comparing relative lens size.

A daily measurement of pH was not included in the experimental design as the buffer capacity of seawater is high and the density of fish in the tank was low.
Daily measurement of pH would therefore have given a more precise basis for the calculation of UIA.

The i-STAT Analyzer is made for analyzing human blood and the blood sample in the i-STAT cartridge is heated to 37 ºC when inserted to the analyzer. pH and pCO_2 are temperature-dependent measures, and as the body temperature of the Atlantic halibut juveniles was approximately 12 ºC, a temperature correction was made in order to obtain the actual pH and pCO_2 in the blood samples. We used the temperature correction formulas given in the i-STAT Analyzer Manual, although these formulas are more suitable for temperature corrections in human blood samples close to 37 ºC than for fish blood holding 12 ºC. As the purpose of this study was not to determine the exact pH and pCO_2 values, but rather to investigate any possible differences in pH/pCO_2 in relation to the treatments, this matter was not pursued, and calculated values were presented.

Any factors in the room that could affect the fish are considered small, as all tanks were covered, with equal lightning, flow and temperature and where subject to the same experimental procedures.

7.1.3 Statistical methods

Random sampling and independence among observations are requirements needed to be fulfilled for statistical testing. In this study fish were randomly distributed into 8 tanks, where each tank can be regarded as independent since they were isolated with no contact between fish in the different tanks. As the predictor variables (ammonia and replicates) were fixed, categorical factors, and the response variables continuous factors, we used ANOVA to

distinguish treatment effects from variability due to random error (Zar, 1984). This procedure may be defended because of the fish were randomly distributed in the tanks, fish were randomly selected for blood, lens and muscle sampling and because there were equal numbers of fish in the groups used for analyses (Zar, 1984). The fulfilment of the underlying assumptions for ANOVA testing, namely a homogeneity of variances and normally distributed sample populations (Zar, 1984), is discussed below.

For growth, feed and blood parameters the Kolmogorov-Smironv test revealed only few deviations from normality (Appendix III). The Levene´s F-test for homogenity revealed minor deviation from homogenity of measured growth, but some inhomogenity ($p<0.05$) for a few blood parameters, and measured muscle and lens amino acids. However, analysis of variance is robust, operating well despite considerable heterogeneity of variances as long as the number of observations is equal or approximately equal (Zar, 1984). Hence, the requirements were fulfilled and a parametric approach was applied on all measured growth, feed and blood parameters. Weight was included as a covarying factor in the ANCOVA (analysis of covariance) because ANCOVA neutralizes any size effect, ensuring that any experimental effect found are in fact real. All significant ANOVA and ANCOVA´s were followed by a Student-Newman-Keuls multiple comparison test.

8 APPENDIX II

Table I: Abbreviation of free amino acids explained and included in the TABLE.

Abbreviations		
		fw
phser	O-Phospho-L-serine	185.1
tau	Taurine	125.1
pea	O-Phosphoethanolamine	141.1
urea	Urea	60.1
asp	L-Aspartic acid	133.1
hypro	Hydroxy-L-proline	131.1
thr	L-Threonine	119.1
ser	L-Serine	105.1
asn	L-Aspargine	132.1
glu	L-Glutamic acid	147.1
gln	L-Glutamine	146.1
sarc	L-Sarcosine	89.1
aaaa	L-alfa-Aminoadipic Acid	161.2
pro	L-Proline	115.1
gly	L-glycine	75.1
ala	L-Alanine	89.1
citr	L-Citrulline	175.2
aaba	L-alfa-Amino-n-butyric Acid	103.1
val	L-Valine	117.1
cystine	L-Cystine	240.2
met	L-Methionine	149.2
cysth1	Cystathionin1	222.2
cysth2	Cystathionin2	222.2
ile	L-Isoleucine	131.2
leu	L-Leucine	131.2
nor	L-Norleucine	131.2
tyr	L-Tyrosine	181.2
b-ala	b-Alanine	89.1
phe	L-Phenylalanine	165.2
aaiba	DL-beta-Aminoisobutyric Acid	103.1
gaba	Gamma-Amino-n-butyric Acid	103.1
ethanolamin	Ethanolamine	61.08
amm	Ammoniumklorid	18
hylys1	Hydroxylysin1	162.6
hylys2	Hydroxylysin2	162.6
orn	L-Ornithine	132.2
lys	L-Lysine	146.2
1-mhis	1-Methyl-L-histidine	169.2
his	L-Histidine	155.2
trp	L-Trypthofan	204.2
3-mhis	3-metylhistidin	169.2
ans	anserine	240.2
car	carnosin	226.2
arg	arginine	174.2

8.1 Applied chemicals

8.1.1 Ammonia measurements

Two NH_4Cl standards were used in the calibration of the Thermo Orion 720A meter. These were made by adding 100 ml distilled water to 0.3 and 3 ml of 0.1 M NH_4Cl (5.348 g NH4Cl per liter; VWR International, Oslo), respectively.

In order to measure the concentrations of total ammonia in water samples, the pH in the samples was elevated by adding 3 ml of a strong basic solution (ISA). ISA was made of 100ml methanol, 18.612 g EDTA, 200 g NaOH and enough destilled H_20 to make 1 l solution. 100 ml methanol was added to 0.8 l destilled H_20 in a glass flask (1 l) where the solution was continuously stirred. Then 18.612 g EDTA was added, and then NaOH was added a little at a time, to avoid overheating. During the addition of NaOH the glass flask was placed in a sink with iced slurry to cool down. Enough distilled water was added to get 1 l of ISA.

8.1.2 Muscle and lens and free amino acids

Physiological amino acid determination, Ninhydrin detection (MET.NÆR.01-31, NIFES, Bergen, Norway)

Chemicals:

Sulfosalicylic acid ($C_7H_6O_6S*2H_2O$) Sigma, Cat. No. S-0640

Lithium Citrate Loading buffer, pH 2.2 Biochrom Cat. No. 80-2038-10

Hydrochloric acid, 37% (HCl) Merck, Cat. No. 1.00317

Norleucin, DL, Sigma, Cat. No. N-1398

Fysiological aa standard A/N, Sigma, Cat. No. A-6407

Fysiological aa. Standard B, Sigma, Cat. No. A-6282

Glutamin, Sigma, Cat. No. G-3126

Running buffers:

Lithium buffer A, Biochrom, Cat. No. 80-2038-15

Lithium buffer B. Biochrom, Cat. No. 80-2038-16

Lithium buffer C II, Biochrom, Cat. No. 80-2099-83

Lithium buffer D II, Biochrom, Cat. No. 80-2038-18

Lithiumhydroxide F, Biochrom, Cat. No. 80-20338-20

Lithium buffer pH 3.55, Biochrom, Cat. No. 80-2037-69

Ultrasolve, Biochrom, Cat. No. 80-2110-75

MilliQ-water

Solutions and standards:

10% Sulfursalicylic acid

Wighted 10 g of Sulfosalicylic acid and transferred it to a graduated flask, and diluted up to the mark with water.

Hydrochloric acid, 6M

Added 500 ml of 37 % hydrochloric acid (HCl) to water in a 1000 ml graduated flask. When the solution was chilled enough more water was added up to the mark.

Internal standard I (2.5mM Nor)

Exactly 0.3280 g of Norleucin was transferred to a 1000 ml graduated flask and dissolved with 17 ml 6 M hydrochloric acid, and further diluted with water until the mark.

2.5 mM glutamine

Exactly 0.0365 g of Glutamin was transferred to a 100 ml graduated cylinder and diluted with water up to mark.

Workstandard, 0.625 mM

Pipetted out accurately and transferred to 4 ml vial 500 µl standard A/N, 500 µl standard B, 500 µl standard I and 500 µl Loading buffer. Then the solution was mixed well with a whirlmixer. Stored at -20 ºC.

External standard 0.5 mM

200 µl of the workstandard was accurately pipetted out and 50 µl glutamine was added and mixed well using a whirlmixer.

Ninhydrinereagent

The Ninhydrine solution was placed in an ultrasound bath for 10 min. Ultrasolve was transferred to a lightfiltered flask and placed for stirring using a magnetic stirrer with addition of Nitrogen gas for 10 min. (Some of the Ultrasolve was kept to rinse out the flask containing Ninhydrine solution). The sonycated Ninhydrine solution was transferred to the Ultrasolve and continued stirring with additton of Nitrogengas for another 15 min.

Equipment:

Weigh boat

Flask 1000ml (Blue cork)

Reservoarflask with lightfilter 2000 ml (blue cork)

Graduated cylinders: 250-500-1000 ml

Graduated flasks: 100-1000-2000 ml

Chromacol samplevials: 2SV without insert

Chromacol samplevials: 2SV with insert

Watch glass

Brand eppendorftube, 2.0 ml rounded tip: 780550

Eppendorftube, 1.5 ml

Micropipette 20-200 µl, 200 µl, 200 µl, 1000 µl and 1000-5000 µl

Pipette tips, 200-1000-5000 µl microtips

Cork for Chromacol samplevials: 2SV without insert: 8 mm 8-SC-8RT1

Cork for Chromacol samplevialss: 2SV with insert: 9 mm 9-SC-8RT1

Skalpel

Balls for grinding in the mill

Mill: Retch MM301

Analytical balance, 4 decimals

Centrifuge: Eppendorf centrifuge 54 1

Filter: Syringefilter 4 mm-0.22 µm, Millex-GV

Whirlmixer

Biochrom 20 Plus

Coloumn, Biochrom Physological coloumn 200 mm Cat. No. 80-2038-20

Midas coolray-injector, Spark

BusSAT/IN Waters

Integratingsystem, Empower Build 1154

Nitrogen gas

Experimental:

Flow: 25 ml h^{-1} for buffer, 20 ml h^{-1} for Ninhydrin

Wavelength: 570 nm for Channel 1, 440 nm for Channel 2

Injection volume: 20 µl

Midas carusell: 8 ºC

Chemical used in Determination of NAH (Na-Acetyl-l.Histidene) using HPLC (MET.NÆR.01-32, NIFES, Bergen, Norway).

Chemicals:

1. di-Sodiumhydrogen phophate-dihydrate ($Na_2HPO_4*2H_20$), p.a, Merck, Cat. No. 6580.
2. Ortho-Phosphoracid , 85 % (H_3PO_4),p.a Merck, Cat. No. 0573
3. Ethanol, (Et-OH), rectified, 96 %., Arcus
4. Na-acetyl-l-Histidine (NAH), F.W.215.21, Aldrich 85,754-8
5. L-Histidine (HIS), $C_6H_9N_3O_2$, Sigma grade, Cat. No. H-8776
6. Methanol, Merck, Cat. No. 6018.
7. Distilled water, MilliQ, Millipore.

Solutions and standards:

80 % Et-OH:

Measured up 80.0 ml of 96 % Et-OH in a 100ml measuring cylinder using a 3.3 ml automatic pipette and diluted with distilled water to the mark.

Eluent I (0.1 M Sodiumphospatebuffer, pH 2.0):

Weighted 17.8 g $Na_2HPO_4*2H_2O$ and diluted to the mark with distilled water in a 1000 ml graduated flask. Then 13.5 ml was pipetted out and diluted further with distilled water to 2000 ml. Took out approximately 350 ml of the Na_2HPO_4-solution and transferred it to a 2000 ml beaker and then added the H_3PO_4-solution to the sodiumphosphatebuffer until the pH measuren 2.0.

0.5 mM NAH- 0.5 mM HIS standard:

These were prepared by the technical staff at NIFES.

0.0538 g NAH was weighted and added 0.0388 HIS in their own weigh boats. Both were transferred into a 100 ml graduated flask. To dissolve them a little sodiumphosphatebuffer was added, and diluted with the buffer to the mark. 5.0 ml was taken out and transferred to a 25 ml graduated flask and diluted with buffer. Small portions of this solution were put in the freezer, and can be stored for 3 years. Shake them well before use.

0.25 mM NAH-0.25 mM HIS standard:

Mixed 0.5 mM NAH and 0.5mM HIS standard with sodiumphosphatebuffer in a 1:1 relation. Mix well.

Eluent II: (Methanol and water):

Mixed Methanol and distilled water 1:1.

Instruments and equipment:

Analytical balance, 4 decimals

Vortex Genie2, Scientific instrument

pH-meter

Homogenisator, Retsh Mill

Eppendorftubes, 2 ml Brand

Graduated cylinders/Erlenmeyer flasks (25, 100, 500, 1000 and 2000 ml)

Beaker, 2000 ml

Graduated cylinder, 100 ml

Chromacol sampletubes, 4 ml, springs and inserts

Pastaurpipette

Micropipette with tips, 200-1000 µl

Heating cabinet

Centrifuge, Eppendorf 5415C

Vibramixer

Planatery balls

Syringe filter, 4 mm-22 µl, Millex-GV

HPLC-system-Waters

Autosampler: Waters 717^{+}

System control/pump: Waters 600E

Detector: Waters 468 Tunable absorbance detector

Experimental:

HPLC-system:

Waters 600 Controller/pump module

Waters 468 Absorbance detector, wavelength 210 nm

Waters 717 Autosampler

Coloumn: Zorbax SB250x4.6 mm id, Reversed-phase C18, 5 µm

Flow: 0.6 ml min^{-1} (Eluent I) for 10 min, wash, 1.0 ml (Eluent II) for 10 min, condition coloumn with Eluent I for 25 min.

Integrating system: Waters Empower

Computer

9 APPENDIX III

9.1 Descriptive statistics

Experimental conditions

Table I: Descriptive statistics based on daily temperature (ºC) measurements in treatment groups. Means, total number of observations (N), standard deviation (SD), and standard error (SE), minimum and maximum are included in the TABLE.

Temperature ºC				
Treatment	**N**	**Mean**	**SD**	**SE**
Control	118	11.87	0.14	0.01
ChronicLow	120	11.94	0.17	0.02
ChronicMedium	118	11.91	0.18	0.02
ChronicHigh	115	11.89	0.16	0.01
HighPulse	119	11.90	0.16	0.01

Table II: Descriptive statistics based on daily temperature (ºC) measurements in all tanks. Means, total number of observations (N), standard deviation (SD), and standard error (SE), minimum and maximum are included in the TABLE.

Temperature ºC							
Treatment	**Replicate**	**N**	**Mean**	**SD**	**SE**	**Min**	**Max**
Control	a	59	11.9	0.13	0.02	11.6	12.2
Control	b	59	11.9	0.14	0.02	11.6	12.2
ChronicLow	a	60	12.0	0.17	0.02	11.5	12.3
ChronicLow	b	60	11.9	0.18	0.02	11.4	12.3
ChronicMedium	a	59	11.9	0.18	0.02	11.5	12.2
ChronicMedium	b	59	11.9	0.18	0.02	11.5	12.2
ChronicHigh	a	57	11.9	0.16	0.02	11.5	12.2
ChronicHigh	b	58	11.9	0.16	0.02	11.5	12.3
HighPulse	a	60	11.9	0.17	0.02	11.5	12.3
HighPulse	b	59	11.9	0.16	0.02	11.5	12.3

Table III: Descriptive statistics based on daily oxygen measurements in treatment groups, represented here in % saturation. Means, total number of observations (N), standard deviation (SD), and standard error (SE), minimum and maximum are included in the TABLE.

Oksygen %				
Treatment	**N**	**Mean**	**SD**	**SE**
Control	94	80.81	3.04	0.31
ChronicLow	100	80.70	2.52	0.25
ChronicMedium	97	80.22	2.21	0.22
ChronicHigh	98	83.02	3.16	0.32
HighPulse	99	80.89	2.97	0.30

Table IV: Descriptive statistics based on daily oxygen measurements in all tanks, presented in % saturation. Means, total number of observations (N), standard deviation (SD), and standard error (SE), minimum and maximum are included in the TABLE.

Oksygen %							
Treatment	**Replicate**	**N**	**Mean**	**SD**	**SE**	**Min**	**Max**
Control	a	44	80.5	1.99	0.30	77.0	87.1
Control	b	50	81.1	3.72	0.53	73.0	89.0
ChronicLow	a	51	80.4	2.38	0.33	74.0	86.9
ChronicLow	b	49	81.1	2.63	0.38	75.0	86.8
ChronicMedium	a	48	80.3	1.98	0.29	74.0	84.8
ChronicMedium	b	49	80.1	2.42	0.35	76.0	85.2
ChronicHigh	a	49	83.8	3.27	0.47	76.0	90.1
ChronicHigh	b	49	82.2	2.85	0.41	74.0	88.0
HighPulse	a	50	81.4	2.81	0.40	74.0	88.1
HighPulse	b	49	80.4	3.06	0.44	73.0	87.6

9.2 Response variables

Table V: Descriptive statistics base don measurements of weights at T0 (day 0), T3 (day 62) and T4(day 100). Means, total number of observations (N), standars deviation (SD) and standard error (SE) for each group are included in the TABLE **.**

	Weight T0				**Weight T3**				**Weight T4**			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	54.1	59	14.5	1.89	89.6	56	29.7	3.97	138.4	52	47.4	6.58
ChronicLow	49.1	60	11.7	1.51	80.0	45	25.1	3.74	120.7	42	47.1	7.27
ChronicMedium	52.1	60	14.4	1.86	73.6	54	28.3	3.85	109.5	48	43.8	6.32
ChronicHigh	52.2	60	13.3	1.71	65.9	52	20.5	2.84	100.0	40	35.6	5.62
HighPulse	51.4	60	11.7	1.51	88.5	47	25.9	3.78	128.1	41	40.3	6.30

Table VI: Descriptive statistics base don calculated SGR from T0-T3 (day 0-62) and T3-T4 (day 62-100). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	SGR1 (T0-T3)				**SGR2 (T3-T4)**				**SGR Overall**			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	0.75	56	0.4	0.05	1.00	52	0.4	0.06	0.87	52	0.3	0.04
ChronicLow	0.68	45	0.3	0.05	0.93	42	0.4	0.06	0.80	42	0.3	0.05
ChronicMedium	0.47	52	0.4	0.05	0.81	48	0.6	0.08	0.65	48	0.3	0.05
ChronicHigh	0.28	52	0.4	0.05	0.80	40	0.5	0.09	0.54	40	0.3	0.05
HighPulse	0.79	47	0.3	0.04	0.89	41	0.4	0.06	0.85	41	0.3	0.04

Table VII: Descriptive statistics based on measurements of blood Na^+ at three sampling dates (T0/day 0, T3/day 62, T4/day 100). Means, total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE.

	Blood Na^+ T0				**Blood Na^+ T3**				**Blood Na^+ T4**			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	156.5	19	3.60	0.82	167.9	8	5.77	2.04	170.4	8	4.63	1.64
ChronicLow					170.4	8	3.78	1.34	171.4	8	4.53	1.60
ChronicMedium					168.5	8	2.78	0.98	168.4	8	3.96	1.40
ChronicHigh					168.3	8	3.96	1.40	170.0	8	4.99	1.76
HighPulse					166.8	8	3.77	1.33	168.5	8	2.39	0.85

Table VIII: Descriptive statistics based on measurements of blood K^+ at three sampling dates (T0/day 0, T3/day 62, T4/day 100). Means, total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE.

	Blood K^+ T0				Blood K^+ T3				Blood K^+ T4			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	3.52	19	0.34	0.08	4.26	7	0.22	0.08	4.83	8	0.55	0.20
ChronicLow					4.31	8	0.47	0.17	5.65	8	0.96	0.34
ChronicMedium					4.19	8	0.20	0.07	4.51	8	0.59	0.21
ChronicHigh					4.40	8	0.42	0.15	4.71	7	0.45	0.17
HighPulse					4.33	8	0.49	0.17	4.43	8	0.25	0.09

Table IX: Descriptive statistics based on measurements of blood pH at three sampling dates (T0/day 0, T3/day 62, T4/day 100). Means, total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE.

	Blood pHtp T0				Blood pHtp T3				Blood pHtp T4			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	7.34	19	0.08	0.02	7.31	7	0.09	0.04	7.25	8	0.06	0.02
ChronicLow					7.29	8	0.04	0.02	7.20	8	0.07	0.02
ChronicMedium					7.29	8	0.06	0.02	7.23	8	0.04	0.01
ChronicHigh					7.33	8	0.09	0.03	7.27	8	0.05	0.02
HighPulse					7.31	8	0.06	0.02	7.26	8	0.05	0.02

Table X: Descriptive statistics based on measurements of blood pCO_2 at three sampling dates (T0/day 0, T3/day 62, T4/day 100). Means, total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE.

	Blood pCO_2 tp T0				Blood pCO_2 tp T3				Blood pCO_2 tp T4			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	4.77	19	0.97	0.22	5.79	7	0.44	0.17	5.67	8	0.49	0.17
ChronicLow					5.25	8	0.39	0.14	6.67	8	0.93	0.33
ChronicMedium					4.92	8	0.64	0.23	6.15	8	0.73	0.26
ChronicHigh					4.33	8	1.02	0.36	5.55	8	0.40	0.14
HighPulse					5.32	8	0.39	0.14	5.64	8	0.31	0.11

Table XI: Descriptive statistics based on measurements of blood HCO_3^- at three sampling dates (T0/day 0, T3/day 62, T4/day 100). Means, total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE.

	Blood HCO_3^- T0				Blood HCO_3^- T3				Blood HCO_3^- T4			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	3.56	19	0.76	0.18	4.14	7	0.95	0.36	3.56	8	0.47	0.16
ChronicLow					3.61	8	0.51	0.18	3.84	8	0.87	0.31
ChronicMedium					3.40	8	0.44	0.16	3.74	8	0.59	0.21
ChronicHigh					3.20	8	0.84	0.30	3.66	8	0.57	0.20
HighPulse					3.79	8	0.28	0.10	3.69	8	0.53	0.19

Table XII: Descriptive statistics based on analysis of Histidine in the lens at three sampling dates (T0/day 0, T3/day 62, T4/day 100). Means, total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE.

	Lens His T0				Lens His T3				Lens His T4			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	1.10	20	0.42	0.09	1.27	7	0.09	0.04	1.29	8	0.279	0.10
ChronicLow					0.95	8	0.08	0.03	1.13	8	0.12	0.04
ChronicMedium					1.02	8	0.05	0.02	1.17	8	0.12	0.04
ChronicHigh					1.44	8	0.22	0.08	1.27	8	0.08	0.03
HighPulse					1.33	8	0.23	0.08	1.21	8	0.17	0.06

Table XIII: Descriptive statistics based on analysis of NAH in the lens at three sampling dates (T0/day 0, T3/day 62, T4/day 100). Means, total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE.

	Lens NAH T0				Lens NAH T3				Lens NAH T4			
Treatments	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
Control	12.79	20	2.23	0.50	9.28	7	0.845	0.32	8.78	8	2.111	0.75
ChronicLow					7.30	8	0.71	0.25	8.66	8	1.69	0.60
ChronicMedium					7.65	8	0.87	0.31	9.28	8	1.50	0.53
ChronicHigh					8.25	8	1.41	0.50	8.18	8	1.10	0.39
HighPulse					9.45	8	0.79	0.28	9.79	8	0.55	0.19

Table XIV : Descriptive statistics based on analysis of free amino acids (FAA) in the muscle tissue samples at T0 (day 0). Means (μmol g^{-1}), total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE. Abbriviations explained in Table I, APPENDIX II.

Free amino acid	**T0**			
	N	**Mean**	**SD**	**SE**
Phser	20	0.01	0.00	0.00
Tau	20	9.35	2.50	0.56
Pea	20	0.09	0.03	0.01
Urea	20	5.40	1.16	0.26
Asp	20	0.53	0.20	0.05
Hypro	20	0.56	0.54	0.12
Thr	20	1.13	0.36	0.08
Ser	20	1.73	0.42	0.09
Asn	20	0.64	0.58	0.13
Glu	20	0.69	0.25	0.06
Gln	20	0.83	0.20	0.05
Sarc	5	0.04	0.01	0.00
Aaaa	0	n.d		
Pro	20	0.87	0.96	0.21
Gly	20	7.58	0.54	0.12
Ala	20	4.28	0.49	0.11
Citr	20	0.05	0.04	0.01
Aaba	20	0.06	0.02	0.00
Val	20	0.09	0.03	0.01
Cystine	0	n.d		
Met	20	0.05	0.02	0.00
Cysth1	0	n.d		
Cysth2	20	0.38	0.16	0.04
Ile	20	0.06	0.02	0.00
Leu	20	0.12	0.04	0.01
Tyr	20	0.05	0.01	0.00
B-ala	0	n.d		
Phe	20	0.04	0.01	0.00
Aaiba	20	0.06	0.02	0.00
Gaba	0	n.d		
Ethanolamine	0	n.d		
Amm	20	3.87	0.34	0.08
Hylys1	10	0.05	0.05	0.01
Hylys2	20	0.02	0.01	0.00
Orn	20	0.02	0.01	0.00
Lys	20	0.53	0.24	0.05
1-mhis	0	n.d		
His	20	0.84	0.58	0.13
Trp	0	n.d		
3-mhis	0	n.d		
Ans	20	0.37	0.13	0.03
Car	0	n.d		
Arg	20	0.09	0.05	0.01
Sum free AA	**20**	**40.41**	**3.29**	**0.74**

Table XV : Descriptive statistics based on analysis of free amino acids (FAA) in the muscle tissue samples at T3 (day 62). Means (μmol g^{-1}), total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE. Abbriviations explained in Table I, APPENDIX II.

T3

	Control				CL				CM				CH				HP			
Free Amino Acid	N	Mean	S.D	S.E	N	Mean	S.D	S.E	N	Mean	S.D	S.E	N	Mean	S.D	S.E	N	Mean	S.D	S.E
Phser	8	0.01	0.00	0.00	8	0.01	0.00	0.00	8	0.01	0.00	0.00	8	0.01	0.01	0.00	8	0.01	0.01	0.00
Tau	8	12.29	2.88	1.02	8	10.93	1.98	0.70	8	11.36	3.12	1.10	8	11.13	2.72	0.96	8	12.11	2.86	1.01
Pea	8	0.08	0.01	0.00	8	0.08	0.01	0.00	8	0.08	0.01	0.00	8	0.07	0.02	0.01	8	0.07	0.02	0.01
Urea	8	7.67	1.19	0.42	8	7.07	1.11	0.39	8	6.90	1.02	0.36	8	7.11	1.42	0.50	8	7.16	1.10	0.39
Asp	8	0.58	0.22	0.08	8	0.60	0.13	0.04	8	0.54	0.14	0.05	8	0.57	0.11	0.04	8	0.66	0.20	0.07
Hypro	8	0.44	0.22	0.08	8	0.54	0.29	0.10	8	0.46	0.43	0.15	8	0.31	0.23	0.08	8	0.49	0.37	0.13
Thr	8	1.46	0.39	0.14	8	1.35	0.43	0.15	8	1.19	0.49	0.17	8	1.03	0.29	0.10	8	1.31	0.48	0.17
Ser	8	1.71	0.48	0.17	8	1.91	0.38	0.13	8	1.25	0.48	0.17	8	1.56	0.29	0.10	8	1.50	0.63	0.22
Asn	8	0.67	0.54	0.19	8	0.33	0.14	0.05	8	0.94	0.76	0.27	8	0.71	0.29	0.10	8	0.49	0.31	0.11
Glu	8	0.60	0.17	0.06	8	0.87	0.19	0.07	8	0.64	0.14	0.05	8	0.59	0.07	0.03	8	0.67	0.07	0.02
Gln	8	0.85	0.19	0.07	8	0.80	0.16	0.06	8	1.05	0.23	0.08	8	1.04	0.17	0.06	8	0.71	0.16	0.06
Sarc	8	0.04	0.02	0.01	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00
Aaaa	0	n.d			0	n.d			0	n.d			0	n.d			0			
Pro	8	1.32	0.91	0.32	8	1.76	1.09	0.39	8	1.53	1.48	0.52	8	0.93	0.65	0.23	8	1.28	0.76	0.27
Gly	8	7.13	0.67	0.24	8	7.36	0.30	0.11	8	6.70	1.11	0.39	8	7.71	0.52	0.18	8	7.44	0.78	0.28
Ala	8	4.06	0.53	0.19	8	4.53	0.71	0.25	8	4.88	0.89	0.31	8	4.44	0.51	0.18	8	3.69	0.58	0.21
Citr	8	0.08	0.07	0.02	8	0.08	0.07	0.02	8	0.06	0.06	0.02	8	0.05	0.04	0.01	8	0.12	0.09	0.03
Aaba	8	0.06	0.01	0.00	8	0.06	0.02	0.01	8	0.06	0.03	0.01	8	0.05	0.02	0.01	8	0.06	0.02	0.01
Val	8	0.07	0.03	0.01	8	0.07	0.03	0.01	8	0.09	0.03	0.01	8	0.09	0.03	0.01	8	0.08	0.04	0.01
Cystine	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Met	8	0.04	0.01	0.00	8	0.04	0.01	0.00	8	0.05	0.02	0.01	8	0.05	0.02	0.01	8	0.05	0.03	0.01
Cysth1	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Cysth2	8	0.96	0.26	0.09	8	0.88	0.36	0.13	8	0.78	0.55	0.19	8	0.69	0.37	0.13	8	0.77	0.41	0.15
Ile	8	0.04	0.02	0.01	8	0.04	0.02	0.01	8	0.05	0.02	0.01	8	0.06	0.02	0.01	8	0.05	0.03	0.01
Leu	8	0.07	0.04	0.01	8	0.08	0.03	0.01	8	0.11	0.03	0.01	8	0.12	0.04	0.02	8	0.10	0.05	0.02
Tyr	8	0.06	0.01	0.00	8	0.05	0.01	0.00	8	0.04	0.02	0.01	8	0.05	0.02	0.01	8	0.07	0.02	0.01
B-ala	1	0.03			1	0.01			2	0.02	0.00	0.00	0	n.d			1	0.02		
Phe	8	0.04	0.01	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.04	0.02	0.01	8	0.04	0.01	0.01
Aaiba	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.01	0.01	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00
Gaba	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Ethanolamine	7	0.02	0.01	0.00	3	0.04	0.01	0.01	3	0.03	0.00	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00
Amm	8	3.78	0.35	0.12	8	3.96	0.26	0.09	8	3.91	0.21	0.07	8	4.16	0.27	0.10	8	3.79	0.29	0.10
Hylys1	7	0.07	0.03	0.01	6	0.06	0.02	0.01	6	0.06	0.03	0.01	5	0.05	0.02	0.01	6	0.05	0.02	0.01
Hylys2	8	0.05	0.01	0.00	8	0.03	0.02	0.01	6	0.04	0.03	0.01	6	0.05	0.02	0.01	6	0.05	0.02	0.01
Orn	8	0.03	0.02	0.01	8	0.02	0.01	0.00	8	0.02	0.01	0.00	8	0.01	0.01	0.00	8	0.03	0.02	0.01
Lys	8	0.60	0.21	0.07	8	0.63	0.10	0.03	8	0.65	0.23	0.08	8	0.60	0.12	0.04	8	0.44	0.15	0.05
1-mhis	0	n.d			0	n.d			0	n.d			0				0	n.d		
His	8	1.44	0.58	0.21	8	1.24	0.55	0.19	8	1.07	0.68	0.24	8	1.07	0.60	0.21	8	1.32	0.66	0.23
Trp	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
3-mhis	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Ans	8	0.82	0.08	0.03	8	0.72	0.07	0.02	8	0.75	0.12	0.04	8	0.67	0.15	0.05	8	0.72	0.14	0.05
Car	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Arg	8	0.15	0.07	0.02	8	0.12	0.04	0.01	8	0.11	0.03	0.01	8	0.11	0.03	0.01	8	0.11	0.04	0.01
SUM Free AA	8	47.34	3.05	1.08	8	46.33	3.14	1.11	8	45.45	5.91	2.09	8	45.20	3.23	1.14	8	45.51	4.18	1.48

Table XVI : Descriptive statistics based on analysis of free amino acids (FAA) in the muscle tissue samples at T4 (day 100). Means (μmol g^{-1}), total number of observations (N), standard deviation (SD) and standard error (SE) are included in the TABLE. Abbriviations explained in Table I, APPENDIX II.

T4

	Control				CL				CM				CH				HP			
	N	Mean	S.D	S.E	N	Mean	S.D	S.E	N	Mean	S.D	S.E	N	Mean	S.D	S.E	N	Mean	S.D	S.E
Phser	8	0.01	0.00	0.00	8	0.01	0.00	0.00	8	0.01	0.00	0.00	8	0.01	0.00	0.00	8	0.01	0.00	0.00
Tau	8	13.40	1.27	0.45	8	13.12	1.73	0.61	8	13.15	2.44	0.86	8	12.50	2.38	0.84	8	14.49	1.90	0.67
Pea	8	0.08	0.01	0.00	8	0.09	0.02	0.01	8	0.09	0.02	0.01	8	0.08	0.01	0.01	8	0.08	0.02	0.01
Urea	8	6.38	0.50	0.18	8	6.29	1.02	0.36	8	6.27	0.88	0.31	8	5.25	0.58	0.20	8	6.81	1.36	0.48
Asp	8	0.66	0.14	0.05	8	0.67	0.14	0.05	8	0.57	0.14	0.05	8	0.54	0.15	0.05	8	0.60	0.12	0.04
Hypro	8	0.63	0.23	0.08	8	0.82	0.32	0.11	8	0.64	0.29	0.10	8	0.77	0.29	0.10	8	0.52	0.23	0.08
Thr	8	1.62	0.52	0.18	8	1.73	0.34	0.12	8	1.50	0.25	0.09	8	1.95	0.23	0.08	8	1.48	0.28	0.10
Ser	8	1.00	0.15	0.05	8	1.12	0.20	0.07	8	1.20	0.40	0.14	8	1.04	0.24	0.08	8	1.04	0.26	0.09
Asn	8	0.71	0.90	0.32	8	0.45	0.34	0.12	8	1.19	0.64	0.23	8	1.82	0.62	0.22	8	0.52	0.39	0.14
Glu	8	0.65	0.19	0.07	8	0.75	0.12	0.04	8	0.62	0.13	0.05	8	0.49	0.10	0.03	8	0.61	0.10	0.04
Gln	8	1.00	0.28	0.10	8	0.93	0.20	0.07	8	1.12	0.13	0.05	8	0.91	0.21	0.07	8	0.73	0.23	0.08
Sarc	8	0.03	0.01	0.00	8	0.04	0.02	0.01	8	0.04	0.02	0.01	7	0.03	0.01	0.00	8	0.04	0.01	0.00
Aaaa	3	0.01	0.00	0.00	5	0.02	0.00	0.00	2	0.02	0.00	0.00	0	n.d			0	n.d		
Pro	8	2.30	0.97	0.34	8	1.76	1.09	0.39	8	1.71	0.91	0.32	8	2.46	0.88	0.31	8	0.96	0.30	0.11
Gly	8	5.94	0.50	0.18	8	5.90	0.85	0.30	8	6.04	0.63	0.22	8	5.82	0.68	0.24	8	5.96	1.05	0.37
Ala	8	3.88	0.57	0.20	8	3.86	0.53	0.19	8	4.04	0.42	0.15	8	3.69	0.54	0.19	8	3.65	0.42	0.15
Citr	8	0.14	0.08	0.03	8	0.16	0.07	0.02	8	0.13	0.05	0.02	8	0.09	0.04	0.01	8	0.11	0.04	0.01
Aaba	8	0.07	0.02	0.01	8	0.07	0.02	0.01	8	0.07	0.02	0.01	8	0.08	0.02	0.01	8	0.05	0.01	0.00
Val	8	0.05	0.01	0.00	8	0.05	0.02	0.01	8	0.05	0.01	0.00	8	0.05	0.02	0.01	8	0.06	0.02	0.01
Cystine	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Met	8	0.04	0.01	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.04	0.01	0.00
Cysth1	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Cysth2	8	0.77	0.14	0.05	8	0.80	0.28	0.10	8	0.83	0.12	0.04	8	0.93	0.17	0.06	8	0.60	0.16	0.06
Ile	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.02	0.01	0.00	8	0.02	0.01	0.00	8	0.03	0.01	0.00
Leu	8	0.06	0.02	0.01	8	0.07	0.03	0.01	8	0.06	0.03	0.01	8	0.05	0.02	0.01	8	0.07	0.02	0.01
Tyr	8	0.05	0.01	0.00	8	0.05	0.02	0.01	8	0.05	0.01	0.00	8	0.05	0.02	0.01	8	0.05	0.01	0.00
B-ala	0	n.d			0	n.d			1	0.04			0	n.d			0	n.d		
Phe	8	0.04	0.01	0.00	8	0.04	0.01	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.04	0.00	0.00
Aaiba	8	0.04	0.01	0.00	8	0.05	0.01	0.00	8	0.05	0.01	0.00	8	0.04	0.01	0.00	8	0.04	0.01	0.00
Gaba	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Ethanolamine	8	0.02	0.01	0.00	6	0.01	0.00	0.00	7	0.02	0.01	0.00	0	n.d			7	0.03	0.02	0.01
Amm	8	3.44	0.45	0.16	8	3.57	0.10	0.04	8	3.30	0.12	0.04	8	3.24	0.25	0.09	8	3.51	0.13	0.05
Hylys1	8	0.13	0.03	0.01	8	0.12	0.04	0.01	8	0.13	0.07	0.02	8	0.14	0.04	0.02	8	0.10	0.04	0.01
Hylys2	8	0.03	0.01	0.00	8	0.03	0.02	0.01	8	0.03	0.01	0.00	8	0.03	0.01	0.00	8	0.03	0.01	0.00
Orn	8	0.03	0.01	0.00	8	0.04	0.01	0.00	8	0.02	0.00	0.00	8	0.02	0.00	0.00	8	0.03	0.01	0.00
Lys	8	0.54	0.13	0.04	8	0.49	0.14	0.05	8	0.49	0.13	0.04	8	0.57	0.18	0.06	8	0.48	0.10	0.03
1-mhis	0	n.d			1	0.01			0	n.d			0	n.d			0	n.d		
His	8	1.66	0.16	0.06	8	1.72	0.13	0.04	8	1.50	0.38	0.14	8	1.72	0.23	0.08	8	1.62	0.26	0.09
Trp	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
3-mhis	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Ans	8	0.84	0.08	0.03	8	0.84	0.11	0.04	8	0.81	0.09	0.03	8	0.92	0.07	0.03	8	0.97	0.08	0.03
Car	0	n.d			0	n.d			0	n.d			0	n.d			0	n.d		
Arg	8	0.15	0.05	0.02	8	0.14	0.03	0.01	8	0.14	0.06	0.02	8	0.17	0.09	0.03	8	0.12	0.03	0.01
SUM Free AA	8	46.42	3.11	1.10	8	45.85	3.12	1.10	8	45.93	2.73	0.96	8	45.55	2.44	0.86	8	45.49	3.23	1.14

9.3 ANOVA results

One-way ANOVAs

Table XVII: Test results from one-way ANOVA on measured weight data from T0 (day 0)

Weight T0					
	One way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	801561.7	1	801561.7	4630.764	<0.001
Treatment	766.1	4	191.5	1.106	0.354
Error	50889.9	294	173.1		

Table XVIII: Test results from one-way ANOVA on measured weight data from T3 (day 62)

Weight T3					
	One way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	1594675	1	1594675	2318.804	<0.001
Treatment	21056	4	5264	7.654	<0.001
Error	171241	249	688		

Table XIX: Test results from one-way ANOVA on measured weight data from T4 (day 63-100)

Weight T4					
	One way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	3144319	1	3144319	1670.417	<0.001
Treatment	41538	4	10385	5.517	<0.001
Error	410353	218	1882		

Table XX: Test results from one-way ANOVA on calculated SGR data from T0-T3 (day 0-62)

SGR1 (T0-T3)					
	One way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	88.96808	1	88.96808	698.9173	<0.001
Treatment	9.30697	4	2.32674	18.2785	<0.001
Error	31.69624	249	0.12729		

Table XXI: Test results from one-way ANOVA on calculated SGR data from T3-T4 (day 63-100)

SGR2 (T3-T4)					
	One way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	173.1746	1	173.1746	770.7624	<0.001
Treatment	1.2956	4	0.3239	1.4416	0.221
Error	48.9802	218	0.2247		

Table XXII: Test results from one-way ANOVA on calculated SGR data from T0-T4 (day 0-100)

SGR Overall					
	One way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	121.2266	1	121.2266	1314.003	<0.001
Treatment	3.6087	4	0.9022	9.779	<0.001
Error	20.1121	218	0.0923		

Table XXIII: Test results from one-way ANOVA on measured blood Na+ data from T3 (day 62)

Blood Na^+ T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	1133669	1	1133669	66602.45	<0.001
Treatment	55	4	14	0.81	0.526
Error	596	35	17		

Table XXIV: Test results from one-way ANOVA on measured blood Na+ data from T4 (day 100)

Blood Na^+ T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	1152263	1	1152263	65297.24	<0.001
Treatment	52	4	13	0.74	0.570
Error	618	35	18		

Table XXV: Test results from one-way ANOVA on measured blood K^+ data from T3 (day 62)

Blood K^+ T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	717.8616	1	717.8616	4852.689	<0.001
Treatment	0.2001	4	0.0500	0.338	0.850
Error	5.0296	34	0.1479		

Table XXVI: Test results from one-way ANOVA on measured blood K^+ data from T4 (day 100)

Blood K^+ T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	905.4917	1	905.4917	2418.947	<0.001
Treatment	7.5917	4	1.8979	5.070	0.003
Error	12.7273	34	0.3743		

Table XXVII: Test results from one-way ANOVA on measured Blood pH temperature corrected data from T3 (day 62)

Blood pHtp T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	2076.603	1	2076.603	400800.5	<0.001
Treatment	0.009	4	0.002	0.4	0.791
Error	0.176	34	0.005		

Table XXVIII: Test results from one-way ANOVA on measured Blood pH temperature corrected data from T4 (day 100)

Blood pHtp T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	2096.548	1	2096.548	686964.9	<0.001
Treatment	0.026	4	0.007	2.1	0.096
Error	0.107	35	0.003		

Table XXIX: Test results from one-way ANOVA on measured Blood pCO_2 temperature corrected data from T3 (day 62)

Blood pCO_2 tp T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	1019.999	1	1019.999	2573.852	<0.001
Treatment	8.784	4	2.196	5.541	0.002
Error	13.474	34	0.396		

Table XXX: Test results from one-way ANOVA on measured Blood pCO_2 temperature corrected data from T4 (day 100)

Blood pCO_2 tp T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	1408.850	1	1408.850	3737.935	<0.001
Treatment	7.108	4	1.777	4.714	0.004
Error	13.192	35	0.377		

Table XXXI: Test results from one-way ANOVA on measured HCO_3^- data from T3 (day 62)

Blood HCO_3^- T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	512.0317	1	512.0317	1233.406	<0.001
Treatment	3.9361	4	0.9840	2.370	0.072
Error	14.1146	34	0.4151		

Table XXXII: Test results from one-way ANOVA on measured HCO_3^- data from T4 (day 100)

Blood HCO_3^- T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	546.8603	1	546.8603	1425.839	<0.001
Treatment	0.3260	4	0.0815	0.212	0.930
Error	13.4238	35	0.3835		

Table XXXIII: Test results from one-way ANOVA on measured Lens His data from T3 (day 62)

Lens His T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	56.19951	1	56.19951	2294.480	<0.001
Treatment	1.40793	4	0.35198	14.371	<0.001
Error	0.83277	34	0.02449		

Table XXXIV: Test results from one-way ANOVA on measured Lens His data from T4 (day 100)

Lens His T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	58.83535	1	58.83535	2080.523	<0.001
Treatment	0.14426	4	0.03606	1.275	0.298
Error	0.98977	35	0.02828		

Table XXXV: Test results from one-way ANOVA on measured NAH data from T3 (day 62)

NAH T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	2734.696	1	2734.696	2962.472	<0.001
Treatment	28.708	4	7.177	7.775	<0.001
Error	31.386	34	0.923		

Table XXXVI: Test results from one-way ANOVA on measured NAH data from T4 (day 100)

NAH T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	3195.353	1	3195.353	1443.388	<0.001
Treatment	12.202	4	3.050	1.378	0.262
Error	77.483	35	2.214		

Table XXXVII: Test results from one-way ANOVA on measured Phser data from T3 (day 62)

Phser T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.006917	1	0.006917	327.7042	<0.001
Treatment	0.000040	4	0.000010	0.4779	0.752
Error	0.000739	35	0.000021		

Table XXXVIII: Test results from one-way ANOVA on measured Phser data from T4 (day 100)

Phser T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.006838	1	0.006838	659.5601	<0.001
Treatment	0.000002	4	0.000000	0.0458	0.996
Error	0.000363	35	0.000010		

Table XXXIX: Test results from one-way ANOVA on measured Tau data from T3 (day 62)

Tau T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	5346.893	1	5346.893	712.1014	<0.001
Treatment	11.672	4	2.918	0.3886	0.815
Error	262.801	35	7.509		

Table XL: Test results from one-way ANOVA on measured Tau data from T4 (day 100)

Tau T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	7108.836	1	7108.836	1793.387	<0.001
Treatment	17.034	4	4.259	1.074	0.384
Error	138.737	35	3.964		

Table XLI: Test results from one-way ANOVA on measured Pea data from T3 (day 62)

Pea T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.248535	1	0.248535	1222.741	<0.001
Treatment	0.001040	4	0.000260	1.279	0.297
Error	0.007114	35	0.000203		

Table XLII: Test results from one-way ANOVA on measured Pea data from T4 (day 100)

Pea T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.297218	1	0.297218	1041.173	<0.001
Treatment	0.001357	4	0.000339	1.189	0.333
Error	0.009991	35	0.000285		

Table XLIII: Test results from one-way ANOVA on measured Urea data from T3 (day 62)

Urea T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	2063.604	1	2063.604	1496.623	<0.001
Treatment	2.714	4	0.678	0.492	0.742
Error	48.259	35	1.379		

Table XLIV: Test results from one-way ANOVA on measured Urea data from T4 (day 100)

Urea T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	1536.323	1	1536.323	1800.656	<0.001
Treatment	10.473	4	2.618	3.069	0.029
Error	29.862	35	0.853		

Table XLV: Test results from one-way ANOVA on measured Asp data from T3 (day 62)

Asp T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	13.88509	1	13.88509	510.6066	<0.001
Treatment	0.05937	4	0.01484	0.5458	0.703
Error	0.95177	35	0.02719		

Table XLVI: Test results from one-way ANOVA on measured Asp data from T4 (day 100)

Asp T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	14.79142	1	14.79142	749.2163	<0.001
Treatment	0.09903	4	0.02476	1.2540	0.306
Error	0.69099	35	0.01974		

Table XLVII: Test results from one-way ANOVA on measured Hypro data from T3 (day 62)

Hypro T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	7.965563	1	7.965563	79.04611	<0.001
Treatment	0.236277	4	0.059069	0.58617	0.675
Error	3.526988	35	0.100771		

Table XLVIII: Test results from one-way ANOVA on measured Hypro data from T4 (day 100)

Hypro T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	18.35077	1	18.35077	244.4606	<0.001
Treatment	0.46530	4	0.11632	1.5496	0.209
Error	2.62732	35	0.07507		

Table XLIX: Test results from one-way ANOVA on measured Thr data from T3 (day 62)

Thr T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	64.19889	1	64.19889	359.7979	<0.001
Treatment	0.87320	4	0.21830	1.2234	0.319
Error	6.24506	35	0.17843		

Table L: Test results from one-way ANOVA on measured Thr data from T4 (day 100)

Thr T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	109.4485	1	109.4485	954.9980	<0.001
Treatment	1.1689	4	0.2922	2.5498	0.056
Error	4.0112	35	0.1146		

Table LI: Test results from one-way ANOVA on measured Ser data from T3 (day 62)

Ser T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	100.7682	1	100.7682	463.5998	<0.001
Treatment	1.9159	4	0.4790	2.2036	0.089
Error	7.6076	35	0.2174		

Table LII: Test results from one-way ANOVA on measured Ser data from T4 (day 100)

Ser T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	46.52002	1	46.52002	670.0178	<0.001
Treatment	0.19750	4	0.04938	0.7112	0.590
Error	2.43009	35	0.06943		

Table LIII: Test results from one-way ANOVA on measured Asn data from T3 (day 62)

Asn T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	15.80175	1	15.80175	74.12781	<0.001
Treatment	1.67823	4	0.41956	1.96819	0.121
Error	7.46091	35	0.21317		

Table LIV: Test results from one-way ANOVA on measured Asn data from T4 (day 100)

Asn T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	35.29514	1	35.29514	94.51336	<0.001
Treatment	10.39468	4	2.59867	6.95872	<0.001
Error	13.07043	35	0.37344		

Table LV: Test results from one-way ANOVA on measured Glu data from T3 (day 62)

Glu T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	18.18048	1	18.18048	981.1229	<0.001
Treatment	0.42648	4	0.10662	5.7538	0.001
Error	0.64856	35	0.01853		

Table LVI: Test results from one-way ANOVA on measured Glu data from T4 (day 100)

Glu T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	15.68381	1	15.68381	880.9492	<0.001
Treatment	0.28967	4	0.07242	4.0676	0.008
Error	0.62312	35	0.01780		

Table LVII: Test results from one-way ANOVA on measured Gln data from T3 (day 62)

Gln T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	31.72317	1	31.72317	939.1252	<0.001
Treatment	0.70969	4	0.17742	5.2524	0.002
Error	1.18228	35	0.03378		

Table LVIII: Test results from one-way ANOVA on measured Gln data from T4 (day 100)

Gln T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	35.14875	1	35.14875	743.3242	<0.001
Treatment	0.65429	4	0.16357	3.4592	0.018
Error	1.65501	35	0.04729		

Table LIX: Test results from one-way ANOVA on measured Sarc data from T3 (day 62)

Sarc T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.042055	1	0.042055	227.9107	<0.001
Treatment	0.000857	4	0.000214	1.1616	0.344
Error	0.006458	35	0.000185		

Table LX: Test results from one-way ANOVA on measured Sarc data from T4 (day 100)

Sarc T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.048764	1	0.048764	264.5185	<0.001
Treatment	0.000726	4	0.000181	0.9844	0.429
Error	0.006268	34	0.000184		

Table LXI: Test results from one-way ANOVA on measured Pro data from T3 (day 62)

Pro T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	135.1298	1	135.1298	177.2607	<0.001
Treatment	11.0587	4	2.7647	3.6266	0.014
Error	26.6813	35	0.7623		

Table LXII: Test results from one-way ANOVA on measured Pro data from T4 (day 100)

Pro T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	135.1298	1	135.1298	177.2607	<0.001
Treatment	11.0587	4	2.7647	3.6266	0.014
Error	26.6813	35	0.7623		

Table LXIII: Test results from one-way ANOVA on measured Gly data from T3 (day 62)

Gly T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	2112.691	1	2112.691	3967.977	<0.001
Treatment	4.583	4	1.146	2.152	0.095
Error	18.635	35	0.532		

Table LXIV: Test results from one-way ANOVA on measured Gly data from T4 (day 100)

Gly T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	1407.486	1	1407.486	2396.511	<0.001
Treatment	0.200	4	0.050	0.085	0.987
Error	20.556	35	0.587		

Table LXV: Test results from one-way ANOVA on measured Ala data from T3 (day 62)

Ala T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	746.1159	1	746.1159	1724.095	<0.001
Treatment	6.7262	4	1.6815	3.886	0.010
Error	15.1465	35	0.4328		

Table LXVI: Test results from one-way ANOVA on measured Ala data from T4 (day 100)

Ala T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	583.9864	1	583.9864	2313.189	<0.001
Treatment	0.7902	4	0.1975	0.782	0.544
Error	8.8361	35	0.2525		

Table LXVII: Test results from one-way ANOVA on measured Citr data from T3 (day 62)

Citr T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.251857	1	0.251857	57.03696	<0.001
Treatment	0.021428	4	0.005357	1.21320	0.323
Error	0.154549	35	0.004416		

Table LXVIII: Test results from one-way ANOVA on measured Citr data from T4 (day 100)

Citr T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.633026	1	0.633026	193.2047	<0.001
Treatment	0.026047	4	0.006512	1.9874	0.118
Error	0.114676	35	0.003276		

Table LXIX: Test results from one-way ANOVA on measured Aaba data from T3 (day 62)

Aaba T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.132365	1	0.132365	349.1132	<0.001
Treatment	0.000516	4	0.000129	0.3401	0.849
Error	0.013270	35	0.000379		

Table LXX: Test results from one-way ANOVA on measured Aaba data from T4 (day 100)

Aaba T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.180096	1	0.180096	619.8617	<0.001
Treatment	0.002971	4	0.000743	2.5561	0.056
Error	0.010169	35	0.000291		

Table LXXI: Test results from one-way ANOVA on measured Val data from T3 (day 62)

Val T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.257282	1	0.257282	233.1339	<0.001
Treatment	0.002759	4	0.000690	0.6250	0.648
Error	0.038625	35	0.001104		

Table LXXII: Test results from one-way ANOVA on measured Val data from T4 (day 100)

Val T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.111725	1	0.111725	383.2852	<0.001
Treatment	0.000741	4	0.000185	0.6354	0.641
Error	0.010202	35	0.000291		

Table LXXIII: Test results from one-way ANOVA on measured Met data from T3 (day 62)

Met T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.084548	1	0.084548	243.6317	<0.001
Treatment	0.000441	4	0.000110	0.3176	0.864
Error	0.012146	35	0.000347		

Table LXXIV: Test results from one-way ANOVA on measured Met data from T4 (day 100)

Met T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.046104	1	0.046104	671.1629	<0.001
Treatment	0.000526	4	0.000131	1.9130	0.130
Error	0.002404	35	0.000069		

Table LXXV: Test results from one-way ANOVA on measured Cysth2 data from T3 (day 62)

Cysth2 T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	26.78951	1	26.78951	166.1815	<0.001
Treatment	0.35475	4	0.08869	0.5502	0.700
Error	5.64222	35	0.16121		

Table LXXVI: Test results from one-way ANOVA on measured Cysth2 data from T4 (day 100)

Cysth2 T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	24.70555	1	24.70555	750.8695	<0.001
Treatment	0.47360	4	0.11840	3.5985	0.015
Error	1.15159	35	0.03290		

Table LXXVII: Test results from one-way ANOVA on measured Ile data from T3 (day 62)

Ile T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.097713	1	0.097713	228.2924	<0.001
Treatment	0.002701	4	0.000675	1.5777	0.202
Error	0.014981	35	0.000428		

Table LXXVIII: Test results from one-way ANOVA on measured Ile data from T4 (day 100)

Ile T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.029322	1	0.029322	212.4634	<0.001
Treatment	0.000744	4	0.000186	1.3484	0.272
Error	0.004830	35	0.000138		

Table LXXIX: Test results from one-way ANOVA on measured Leu data from T3 (day 62)

Leu T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.372683	1	0.372683	235.4002	<0.001
Treatment	0.011086	4	0.002772	1.7506	0.161
Error	0.055412	35	0.001583		

Table LXXX: Test results from one-way ANOVA on measured Leu data from T4 (day 100)

Leu T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.145926	1	0.145926	264.0144	<0.001
Treatment	0.003020	4	0.000755	1.3661	0.266
Error	0.019345	35	0.000553		

Table LXXXI: Test results from one-way ANOVA on measured Tyr data from T3 (day 62)

Tyr T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.125776	1	0.125776	381.3506	<0.001
Treatment	0.003163	4	0.000791	2.3976	0.069
Error	0.011544	35	0.000330		

Table LXXXII: Test results from one-way ANOVA on measured Tyr data from T4 (day 100)

Tyr T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.109830	1	0.109830	486.6674	<0.001
Treatment	0.000137	4	0.000034	0.1516	0.961
Error	0.007899	35	0.000226		

Table LXXXIII: Test results from one-way ANOVA on measured Phe data from T3 (day 62)

Phe T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.053876	1	0.053876	395.4380	<0.001
Treatment	0.000664	4	0.000166	1.2182	0.321
Error	0.004769	35	0.000136		

Table LXXXIV: Test results from one-way ANOVA on measured Phe data from T4 (day 100)

Phe T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.050980	1	0.050980	705.2514	<0.001
Treatment	0.000234	4	0.000059	0.8107	0.527
Error	0.002530	35	0.000072		

Table LXXXV: Test results from one-way ANOVA on measured Aaiba data from T4 (day 100)

Aaiba T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.029322	1	0.029322	487.9776	<0.001
Treatment	0.001676	4	0.000419	6.9715	<0.001
Error	0.002103	35	0.000060		

Table LXXXVI: Test results from one-way ANOVA on measured Aaiba data from T4 (day 100)

Aaiba T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.078854	1	0.078854	1123.625	<0.001
Treatment	0.000503	4	0.000126	1.793	0.152
Error	0.002456	35	0.000070		

Table LXXXVII: Test results from one-way ANOVA on measured Ethanolamine data from T3 (day 62)

Ethanolamine T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.022611	1	0.022611	205.9627	<0.001
Treatment	0.000810	4	0.000203	1.8446	0.153
Error	0.002635	24	0.000110		

Table LXXXVIII: Test results from one-way ANOVA on measured Amm data from T3 (day 62)

Amm T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	614.4208	1	614.4208	7903.417	<0.001
Treatment	0.7834	4	0.1958	2.519	0.059
Error	2.7209	35	0.0777		

Table LXXXIX: Test results from one-way ANOVA on measured Amm data from T4 (day 100)

Amm T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	465.8336	1	465.8336	7604.719	<0.001
Treatment	0.5984	4	0.1496	2.442	0.065
Error	2.1440	35	0.0613		

Table XC: Test results from one-way ANOVA on measured Hylys1 data from T3 (day 62)

Hylys1 T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.094772	1	0.094772	136.0083	<0.001
Treatment	0.001300	4	0.000325	0.4664	0.760
Error	0.017420	25	0.000697		

Table XCI: Test results from one-way ANOVA on measured Hylys1 data from T4 (day 100)

Hylys1 T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.606144	1	0.606144	302.0399	<0.001
Treatment	0.006554	4	0.001639	0.8165	0.523
Error	0.070239	35	0.002007		

Table XCII: Test results from one-way ANOVA on measured Hylys2 data from T3 (day 62)

Hylys2 T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.063341	1	0.063341	154.4727	<0.001
Treatment	0.001541	4	0.000385	0.9397	0.455
Error	0.011891	29	0.000410		

Table XCIII: Test results from one-way ANOVA on measured Hylys2 data from T4 (day 100)

Hylys2 T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.033074	1	0.033074	259.1812	<0.001
Treatment	0.000201	4	0.000050	0.3936	0.812
Error	0.004466	35	0.000128		

Table XCIV: Test results from one-way ANOVA on measured Orn data from T3 (day 62)

Orn T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.021344	1	0.021344	149.0754	<0.001
Treatment	0.001934	4	0.000484	3.3775	0.019
Error	0.005011	35	0.000143		

Table XCV: Test results from one-way ANOVA on measured Orn data from T4 (day 100)

Orn T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.035046	1	0.035046	592.9300	<0.001
Treatment	0.001251	4	0.000313	5.2906	0.002
Error	0.002069	35	0.000059		

Table XCVI: Test results from one-way ANOVA on measured Lys data from T3 (day 62)

Lys T3					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	13.62239	1	13.62239	479.0907	<0.001
Treatment	0.22374	4	0.05593	1.9672	0.121
Error	0.99518	35	0.02843		

Table XCVII: Test results from one-way ANOVA on measured Lys data from T4 (day 100)

Lys T4					
	One-way ANOVA				
	SS	Degr. of	MS	F	p
Intercept	10.47655	1	10.47655	573.5021	<0.001
Treatment	0.05135	4	0.01284	0.7028	0.595
Error	0.63937	35	0.01827		

Table XCVIII: Test results from one-way ANOVA on measured Muscle His data from T3 (day 62)

Muscle His T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	60.31690	1	60.31690	158.8677	<0.001
Treatment	0.83607	4	0.20902	0.5505	0.700
Error	13.28836	35	0.37967		

Table XCIX: Test results from one-way ANOVA on measured Muscle His data from T4 (day 100)

Muscle His T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	108.3068	1	108.3068	1759.993	<0.001
Treatment	0.2547	4	0.0637	1.035	0.403
Error	2.1538	35	0.0615		

Table C: Test results from one-way ANOVA on measured Ans data from T3 (day 62)

Ans T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	21.60165	1	21.60165	1640.033	<0.001
Treatment	0.09574	4	0.02393	1.817	0.148
Error	0.46100	35	0.01317		

Table CI: Test results from one-way ANOVA on measured Ans data from T4 (day 100)

Ans T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	30.56378	1	30.56378	4102.857	<0.001
Treatment	0.14615	4	0.03654	4.905	0.003
Error	0.26073	35	0.00745		

Table CII: Test results from one-way ANOVA on measured Arg data from T3 (day 62)

Arg T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.581774	1	0.581774	320.8500	<0.001
Treatment	0.009409	4	0.002352	1.2972	0.290
Error	0.063463	35	0.001813		

Table CIII: Test results from one-way ANOVA on measured Arg data from T4 (day 100)

Arg T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.824551	1	0.824551	271.7815	<0.001
Treatment	0.009806	4	0.002452	0.8081	0.528
Error	0.106186	35	0.003034		

Table CIV: Test results from one-way ANOVA on measured SUM free amino acids (FAA) data from T3 (day 62)

SUM FAA T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	84509.50	1	84509.50	5148.388	<0.001
Treatment	24.67	4	6.17	0.376	0.824
Error	574.52	35	16.41		

Table CV: Test results from one-way ANOVA on measured SUM free amino acids (FAA) data from T4 (day 100)

SUM FAA T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	84083.23	1	84083.23	9723.884	<0.001
Treatment	4.35	4	1.09	0.126	0.972
Error	302.65	35	8.65		

Table CVI: Test results from one-way ANOVA on measured Sampled Fish Weight (g) data from T3 (day 62)

Sampled fish weight (g) T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	314849.5	1	314849.5	466.9436	<0.001
Treatment	2537.3	4	634.3	0.9408	0.452
Error	23599.7	35	674.3		

Table CVII: Test results from one-way ANOVA on measured Sampled Fish Weight (g) data from T4 (day 100)

Sampled fish weight (g) T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	819390.6	1	819390.6	338.2744	<0.001
Treatment	2274.1	4	568.5	0.2347	0.917
Error	84779.3	35	2422.3		

Table CVIII : Test results from one-way ANOVA on measured Lens Weight (g) data from T3 (day 62)

Lens weight (g) T3					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.085441	1	0.085441	1542.336	<0.001
Treatment	0.001024	4	0.000256	4.620	0.004
Error	0.001884	34	0.000055		

Table CIX : Test results from one-way ANOVA on measured Lens Weight (g) data from T4 (day 100)

Lens weight (g) T4					
One-way ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.154990	1	0.154990	2418.517	<0.001
Treatment	0.000026	4	0.000007	0.103	0.981
Error	0.002243	35	0.000064		

Two-way Nested ANOVAs

Table CX : Test results from two-way nested ANOVA on measured Weight data from T0 (day 0). Replicate was nested in treatment.

Weight T0					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	801664.0	1	801664.0	4611.648	<0.001
Treatment	772.7	4	193.2	1.111	0.351
Replicate(Treatment)	651.7	5	130.3	0.750	0.587
Error	50238.2	289	173.8		

Table CXI : Test results from two-way nested ANOVA on measured Weight data from T3 (day 62). Replicate was nested in treatment.

Weight T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	1570721	1	1570721	2335.376	<0.001
Treatment	21443	4	5361	7.970	<0.001
Replicate(Treatment)	7132	5	1426	2.121	0.064
Error	164109	244	673		

Table CXII : Test results from two-way nested ANOVA on measured Weight data from T4 (day 100). Replicate was nested in treatment.

Weight T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	3083466	1	3083466	1642.524	<0.001
Treatment	40332	4	10083	5.371	<0.001
Replicate(Treatment)	10494	5	2099	1.118	0.352
Error	399859	213	1877		

Table CXIII : Test results from two-way nested ANOVA on calculated SGR data from T0-T3 (day 0-62). Replicate was nested in treatment.

SGR1 (T0-T3)					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	88.13842	1	88.13842	730.8090	<0.001
Treatment	9.40153	4	2.35038	19.4884	<0.001
Replicate(Treatment)	2.26889	5	0.45378	3.7625	0.003
Error	29.42735	244	0.12060		

Table CXIV : Test results from two-way nested ANOVA on calculated SGR data from T3-T4 (day 62-100). Replicate was nested in treatment.

SGR2 (T3-T4)					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	170.9606	1	170.9606	778.0334	<0.001
Treatment	1.2426	4	0.3106	1.4137	0.230
Replicate(Treatment)	2.1767	5	0.4353	1.9813	0.083
Error	46.8034	213	0.2197		

Table CXV : Test results from two-way nested ANOVA on calculated SGR data from T0-T4 (day 0-100). Replicate was nested in treatment.

SGR Overall					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	119.0980	1	119.0980	1289.242	<0.001
Treatment	3.5698	4	0.8924	9.661	<0.001
Replicate(Treatment)	0.4355	5	0.0871	0.943	0.454
Error	19.6766	213	0.0924		

Table CXVI : Test results from two-way nested ANOVA on measured Blood Na^+ data from T3 (day 62). Replicate was nested in treatment.

Blood Na^+ T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	1133669	1	1133669	75915.33	<0.001
Treatment	55	4	14	0.93	0.462
Replicate(Treatment)	148	5	30	1.98	0.111
Error	448	30	15		

Table CXVII : Test results from two-way nested ANOVA on measured Blood Na^+ data from T4 (day 100). Replicate was nested in treatment.

Blood Na^+ T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	1152263	1	1152263	76015.15	<0.001
Treatment	52	4	13	0.86	0.497
Replicate(Treatment)	163	5	33	2.15	0.087
Error	455	30	15		

Table CXVIII : Test results from two-way nested ANOVA on measured Blood K^+ data from T3 (day 62). Replicate was nested in treatment.

Blood K^+ T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	2066.069	1	2066.069	451648.4	<0.001
Treatment	0.009	4	0.002	0.5	0.725
Replicate(Treatment)	0.043	5	0.009	1.9	0.125
Error	0.133	29	0.005		

Table CXIX : Test results from two-way nested ANOVA on measured Blood K^+ data from T4 (day 100). Replicate was nested in treatment.

Blood K^+ T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	903.0542	1	903.0542	2408.145	<0.001
Treatment	7.5564	4	1.8891	5.038	0.003
Replicate(Treatment)	1.8523	5	0.3705	0.988	0.442
Error	10.8750	29	0.3750		

Table CXX : Test results from two-way nested ANOVA on blood pH temperature corrected from T3 (day 62). Replicate was nested in treatment.

Blood pHtp T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	2066.069	1	2066.069	451648.4	<0.001
Treatment	0.009	4	0.002	0.5	0.725
Replicate(Treatment)	0.043	5	0.009	1.9	0.125
Error	0.133	29	0.005		

Table CXXI : Test results from two-way nested ANOVA on blood pH temperature corrected from T4 (day 100). Replicate was nested in treatment.

Blood pHtp T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	2096.548	1	2096.548	694778.6	<0.001
Treatment	0.026	4	0.007	2.2	0.097
Replicate(Treatment)	0.016	5	0.003	1.1	0.391
Error	0.091	30	0.003		

Table CXXII : Test results from two-way nested ANOVA on blood pCO_2 temperature corrected from T3 (day 62). Replicate was nested in treatment.

Blood pCO_2 tp T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	1015.726	1	1015.726	2273.210	<0.001
Treatment	8.768	4	2.192	4.906	0.004
Replicate(Treatment)	0.516	5	0.103	0.231	0.946
Error	12.958	29	0.447		

Table CXXIII : Test results from two-way nested ANOVA on blood pCO_2 temperature corrected from T4 (day 100). Replicate was nested in treatment.

Blood pCO_2 tp T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	1408.850	1	1408.850	3343.542	<0.001
Treatment	7.108	4	1.777	4.217	0.008
Replicate(Treatment)	0.551	5	0.110	0.261	0.931
Error	12.641	30	0.421		

Table CXXIV : Test results from two-way nested ANOVA on measured HCO_3^- data from T3 (day 62). Replicate was nested in treatment.

Blood HCO_3^- T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	505.8668	1	505.8668	1342.804	<0.001
Treatment	3.4296	4	0.8574	2.276	0.085
Replicate(Treatment)	3.1896	5	0.6379	1.693	0.168
Error	10.9250	29	0.3767		

Table CXXV : Test results from two-way nested ANOVA on measured HCO_3^- data from T4 (day 100). Replicate was nested in treatment.

Blood HCO_3^- T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	546.8602	1	546.8602	1290.018	<0.001
Treatment	0.3260	4	0.0815	0.192	0.941
Replicate(Treatment)	0.7062	5	0.1413	0.333	0.889
Error	12.7175	30	0.4239		

Table CXXVI : Test results from two-way nested ANOVA on measured Lens His data from T3 (day 62). Replicate was nested in treatment.

Lens His T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	55.98297	1	55.98297	1976.219	<0.001
Treatment	1.40949	4	0.35237	12.439	<0.001
Replicate(Treatment)	0.01125	5	0.00225	0.079	0.995
Error	0.82152	29	0.02833		

Table CXXVII : Test results from two-way nested ANOVA on measured Lens His data from T4 (day 100). Replicate was nested in treatment.

Lens His T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	58.83535	1	58.83535	3486.013	<0.001
Treatment	0.14426	4	0.03606	2.137	0.101
Replicate(Treatment)	0.48344	5	0.09669	5.729	0.001
Error	0.50633	30	0.01688		

Table CXXVIII : Test results from two-way nested ANOVA on measured NAH data from T3 (day 62). Replicate was nested in treatment.

NAH T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	2716.164	1	2716.164	2805.171	<0.001
Treatment	28.019	4	7.005	7.234	<0.001
Replicate(Treatment)	3.306	5	0.661	0.683	0.640
Error	28.080	29	0.968		

Table CXXIX : Test results from two-way nested ANOVA on measured NAH data from T4 (day 100). Replicate was nested in treatment.

NAH T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	3195.353	1	3195.353	1679.457	<0.001
Treatment	12.202	4	3.050	1.603	0.199
Replicate(Treatment)	20.404	5	4.081	2.145	0.087
Error	57.078	30	1.903		

Table CXXX : Test results from two-way nested ANOVA on measured Phser data from T3 (day 62). Replicate was nested in treatment.

Phser T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.006917	1	0.006917	301.8284	<0.001
Treatment	0.000040	4	0.000010	0.4402	0.779
Replicate(Treatment)	0.000051	5	0.000010	0.4473	0.812
Error	0.000688	30	0.000023		

Table CXXXI : Test results from two-way nested ANOVA on measured Phser data from T4 (day 100). Replicate was nested in treatment.

Phser T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.006838	1	0.006838	912.7775	<0.001
Treatment	0.000002	4	0.000000	0.0634	0.992
Replicate(Treatment)	0.000138	5	0.000028	3.6874	0.010
Error	0.000225	30	0.000007		

Table CXXXII : Test results from two-way nested ANOVA on measured Tau data from T3 (day 62). Replicate was nested in treatment.

Tau T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	5346.893	1	5346.893	686.7664	<0.001
Treatment	11.672	4	2.918	0.3748	0.825
Replicate(Treatment)	29.233	5	5.847	0.7510	0.592
Error	233.568	30	7.786		

Table CXXXIII : Test results from two-way nested ANOVA on measured Tau data from T4 (day 100). Replicate was nested in treatment.

Tau T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	7108.836	1	7108.836	1938.166	<0.001
Treatment	17.034	4	4.259	1.161	0.348
Replicate(Treatment)	28.703	5	5.741	1.565	0.200
Error	110.034	30	3.668		

Table CXXXIV : Test results from two-way nested ANOVA on measured Pea data from T3 (day 62). Replicate was nested in treatment.

Pea T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.248535	1	0.248535	1373.692	<0.001
Treatment	0.001040	4	0.000260	1.437	0.246
Replicate(Treatment)	0.001686	5	0.000337	1.864	0.130
Error	0.005428	30	0.000181		

Table CXXXV : Test results from two-way nested ANOVA on measured Pea data from T4 (day 100). Replicate was nested in treatment.

Pea T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.297218	1	0.297218	1105.652	<0.001
Treatment	0.001357	4	0.000339	1.262	0.307
Replicate(Treatment)	0.001927	5	0.000385	1.434	0.241
Error	0.008065	30	0.000269		

Table CXXXVI : Test results from two-way nested ANOVA on measured Urea data from T3 (day 62). Replicate was nested in treatment.

Urea T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	2063.604	1	2063.604	1740.104	<0.001
Treatment	2.714	4	0.678	0.572	0.685
Replicate(Treatment)	12.682	5	2.536	2.139	0.088
Error	35.577	30	1.186		

Table CXXXVII : Test results from two-way nested ANOVA on measured Urea data from T4 (day 100). Replicate was nested in treatment.

Urea T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	1536.323	1	1536.323	1616.785	<0.001
Treatment	10.473	4	2.618	2.755	0.046
Replicate(Treatment)	1.355	5	0.271	0.285	0.917
Error	28.507	30	0.950		

Table CXXXVIII : Test results from two-way nested ANOVA on measured Asp data from T3 (day 62). Replicate was nested in treatment.

Asp T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	13.88509	1	13.88509	689.8779	<0.001
Treatment	0.05937	4	0.01484	0.7374	0.574
Replicate(Treatment)	0.34796	5	0.06959	3.4577	0.014
Error	0.60381	30	0.02013		

Table CXXXIX : Test results from two-way nested ANOVA on measured Asp data from T4 (day 100). Replicate was nested in treatment.

Asp T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	14.79142	1	14.79142	803.5630	<0.001
Treatment	0.09903	4	0.02476	1.3449	0.276
Replicate(Treatment)	0.13877	5	0.02775	1.5078	0.217
Error	0.55222	30	0.01841		

Table CXL : Test results from two-way nested ANOVA on measured Hypro data from T3 (day 62). Replicate was nested in treatment.

Hypro T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	7.965563	1	7.965563	83.49775	<0.001
Treatment	0.236277	4	0.059069	0.61918	0.652
Replicate(Treatment)	0.665032	5	0.133006	1.39422	0.255
Error	2.861956	30	0.095399		

Table CXLI : Test results from two-way nested ANOVA on measured Hypro data from T4 (day 100). Replicate was nested in treatment.

Hypro T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	18.35077	1	18.35077	259.0982	<0.001
Treatment	0.46530	4	0.11632	1.6424	0.189
Replicate(Treatment)	0.50256	5	0.10051	1.4191	0.246
Error	2.12477	30	0.07083		

Table CXLII : Test results from two-way nested ANOVA on measured Thr data from T3 (day 62). Replicate was nested in treatment.

Thr T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	64.19889	1	64.19889	425.0631	<0.001
Treatment	0.87320	4	0.21830	1.4454	0.243
Replicate(Treatment)	1.71405	5	0.34281	2.2698	0.073
Error	4.53101	30	0.15103		

Table CXLIII : Test results from two-way nested ANOVA on measured Thr data from T4 (day 100). Replicate was nested in treatment.

Thr T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	109.4485	1	109.4485	952.5573	<0.001
Treatment	1.1689	4	0.2922	2.5433	0.060
Replicate(Treatment)	0.5642	5	0.1128	0.9821	0.445
Error	3.4470	30	0.1149		

Table CXLIV : Test results from two-way nested ANOVA on measured Ser data from T3 (day 62). Replicate was nested in treatment.

Ser T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	100.7682	1	100.7682	501.2716	<0.001
Treatment	1.9159	4	0.4790	2.3827	0.074
Replicate(Treatment)	1.5769	5	0.3154	1.5688	0.199
Error	6.0308	30	0.2010		

Table CXLV : Test results from two-way nested ANOVA on measured Ser data from T4 (day 100). Replicate was nested in treatment.

Ser T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	46.52002	1	46.52002	634.8569	<0.001
Treatment	0.19750	4	0.04938	0.6738	0.615
Replicate(Treatment)	0.23179	5	0.04636	0.6327	0.676
Error	2.19829	30	0.07328		

Table CXLVI : Test results from two-way nested ANOVA on measured Asn data from T3 (day 62). Replicate was nested in treatment.

Asn T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	15.80175	1	15.80175	165.7214	<0.001
Treatment	1.67823	4	0.41956	4.4001	0.006
Replicate(Treatment)	4.60037	5	0.92007	9.6493	<0.001
Error	2.86054	30	0.09535		

Table CXLVII : Test results from two-way nested ANOVA on measured Asn data from T4 (day 100). Replicate was nested in treatment.

Asn T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	35.29514	1	35.29514	109.1296	<0.001
Treatment	10.39468	4	2.59867	8.0349	<0.001
Replicate(Treatment)	3.36770	5	0.67354	2.0825	0.095
Error	9.70272	30	0.32342		

Table CXLVIII : Test results from two-way nested ANOVA on measured Glu data from T3 (day 62). Replicate was nested in treatment.

Glu T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	18.18048	1	18.18048	1237.535	<0.001
Treatment	0.42648	4	0.10662	7.258	<0.001
Replicate(Treatment)	0.20783	5	0.04157	2.829	0.033
Error	0.44073	30	0.01469		

Table CXLIX : Test results from two-way nested ANOVA on measured Glu data from T4 (day 100). Replicate was nested in treatment.

Glu T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	15.68381	1	15.68381	1123.983	<0.001
Treatment	0.28967	4	0.07242	5.190	0.003
Replicate(Treatment)	0.20450	5	0.04090	2.931	0.029
Error	0.41861	30	0.01395		

Table CL : Test results from two-way nested ANOVA on measured Gln data from T3 (day 62). Replicate was nested in treatment.

Gln T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	31.72317	1	31.72317	1202.116	<0.001
Treatment	0.70969	4	0.17742	6.723	0.001
Replicate(Treatment)	0.39060	5	0.07812	2.960	0.027
Error	0.79168	30	0.02639		

Table CLI : Test results from two-way nested ANOVA on measured Gln data from T4 (day 100). Replicate was nested in treatment.

Gln T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	35.14875	1	35.14875	930.3993	<0.001
Treatment	0.65429	4	0.16357	4.3298	0.007
Replicate(Treatment)	0.52166	5	0.10433	2.7617	0.036
Error	1.13334	30	0.03778		

Table CLII : Test results from two-way nested ANOVA on measured Sarc data from T3 (day 62). Replicate was nested in treatment.

Sarc T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.042055	1	0.042055	244.0460	<0.001
Treatment	0.000857	4	0.000214	1.2439	0.314
Replicate(Treatment)	0.001289	5	0.000258	1.4956	0.221
Error	0.005170	30	0.000172		

Table CLIII : Test results from two-way nested ANOVA on measured Sarc data from T4 (day 100). Replicate was nested in treatment.

Sarc T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.048372	1	0.048372	228.4136	<0.001
Treatment	0.000734	4	0.000184	0.8668	0.496
Replicate(Treatment)	0.000126	5	0.000025	0.1193	0.987
Error	0.006142	29	0.000212		

Table CLIV : Test results from two-way nested ANOVA on measured Pro data from T3 (day 62). Replicate was nested in treatment.

Pro T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	74.67649	1	74.67649	74.66790	<0.001
Treatment	3.04299	4	0.76075	0.76066	0.559
Replicate(Treatment)	6.59388	5	1.31878	1.31862	0.283
Error	30.00345	30	1.00012		

Table CLV : Test results from two-way nested ANOVA on measured Pro data from T4 (day 100). Replicate was nested in treatment.

Pro T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	135.1298	1	135.1298	240.2374	<0.001
Treatment	11.0587	4	2.7647	4.9151	0.004
Replicate(Treatment)	9.8067	5	1.9613	3.4869	0.013
Error	16.8745	30	0.5625		

Table CLVI : Test results from two-way nested ANOVA on measured Gly data from T3 (day 62). Replicate was nested in treatment.

Gly T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	2112.691	1	2112.691	4199.269	<0.001
Treatment	4.583	4	1.146	2.277	0.084
Replicate(Treatment)	3.542	5	0.708	1.408	0.250
Error	15.093	30	0.503		

Table CLVII : Test results from two-way nested ANOVA on measured Gly data from T4 (day 100). Replicate was nested in treatment.

Gly T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	1407.486	1	1407.486	2125.968	<0.001
Treatment	0.200	4	0.050	0.075	0.989
Replicate(Treatment)	0.694	5	0.139	0.210	0.956
Error	19.861	30	0.662		

Table CLVIII : Test results from two-way nested ANOVA on measured Ala data from T3 (day 62). Replicate was nested in treatment.

Ala T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	746.1159	1	746.1159	1564.274	<0.001
Treatment	6.7262	4	1.6815	3.525	0.018
Replicate(Treatment)	0.8373	5	0.1675	0.351	0.877
Error	14.3092	30	0.4770		

Table CLIX : Test results from two-way nested ANOVA on measured Ala data from T4 (day 100). Replicate was nested in treatment.

Ala T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	583.9864	1	583.9864	2143.122	<0.001
Treatment	0.7902	4	0.1975	0.725	0.582
Replicate(Treatment)	0.6613	5	0.1323	0.485	0.784
Error	8.1748	30	0.2725		

Table CLX : Test results from two-way nested ANOVA on measured Citr data from T3 (day 62). Replicate was nested in treatment.

Citr T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.251857	1	0.251857	60.61222	<0.001
Treatment	0.021428	4	0.005357	1.28924	0.296
Replicate(Treatment)	0.029892	5	0.005978	1.43878	0.239
Error	0.124657	30	0.004155		

Table CLXI : Test results from two-way nested ANOVA on measured Citr data from T4 (day 100). Replicate was nested in treatment.

Citr T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.633026	1	0.633026	199.3290	<0.001
Treatment	0.026047	4	0.006512	2.0504	0.112
Replicate(Treatment)	0.019402	5	0.003880	1.2219	0.323
Error	0.095274	30	0.003176		

Table CLXII : Test results from two-way nested ANOVA on measured Aaba data from T3 (day 62). Replicate was nested in treatment.

Aaba T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.132365	1	0.132365	380.9705	<0.001
Treatment	0.000516	4	0.000129	0.3712	0.827
Replicate(Treatment)	0.002847	5	0.000569	1.6388	0.180
Error	0.010423	30	0.000347		

Table CLXIII : Test results from two-way nested ANOVA on measured Aaba data from T4 (day 100). Replicate was nested in treatment.

Aaba T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.180096	1	0.180096	725.8049	<0.001
Treatment	0.002971	4	0.000743	2.9929	0.034
Replicate(Treatment)	0.002725	5	0.000545	2.1964	0.081
Error	0.007444	30	0.000248		

Table CLXIV : Test results from two-way nested ANOVA on measured Val data from T3 (day 62). Replicate was nested in treatment.

Val T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.257282	1	0.257282	253.0929	<0.001
Treatment	0.002759	4	0.000690	0.6786	0.612
Replicate(Treatment)	0.008129	5	0.001626	1.5993	0.191
Error	0.030497	30	0.001017		

Table CLXV : Test results from two-way nested ANOVA on measured Val data from T4 (day 100). Replicate was nested in treatment.

Val T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.111725	1	0.111725	493.3756	<0.001
Treatment	0.000741	4	0.000185	0.8179	0.524
Replicate(Treatment)	0.003409	5	0.000682	3.0106	0.026
Error	0.006794	30	0.000226		

Table CLXVI : Test results from two-way nested ANOVA on measured Met data from T3 (day 62). Replicate was nested in treatment.

Met T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.084548	1	0.084548	279.6594	<0.001
Treatment	0.000441	4	0.000110	0.3645	0.832
Replicate(Treatment)	0.003076	5	0.000615	2.0351	0.102
Error	0.009070	30	0.000302		

Table CLXVII : Test results from two-way nested ANOVA on measured Met data from T4 (day 100). Replicate was nested in treatment.

Met T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.046104	1	0.046104	744.0145	<0.001
Treatment	0.000526	4	0.000131	2.1207	0.103
Replicate(Treatment)	0.000545	5	0.000109	1.7598	0.152
Error	0.001859	30	0.000062		

Table CLXVIII : Test results from two-way nested ANOVA on measured Cysth2 data from T3 (day 62). Replicate was nested in treatment.

Cysth2 T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	26.78951	1	26.78951	171.3955	<0.001
Treatment	0.35475	4	0.08869	0.5674	0.688
Replicate(Treatment)	0.95315	5	0.19063	1.2196	0.324
Error	4.68907	30	0.15630		

Table CLXIX : Test results from two-way nested ANOVA on measured Cysth2 data from T4 (day 100). Replicate was nested in treatment.

Cysth2 T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	24.70555	1	24.70555	786.5330	<0.001
Treatment	0.47360	4	0.11840	3.7694	0.013
Replicate(Treatment)	0.20927	5	0.04185	1.3325	0.277
Error	0.94232	30	0.03141		

Table CLXX : Test results from two-way nested ANOVA on measured Ile data from T3 (day 62). Replicate was nested in treatment.

Ile T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.097713	1	0.097713	243.9223	<0.001
Treatment	0.002701	4	0.000675	1.6857	0.179
Replicate(Treatment)	0.002963	5	0.000593	1.4792	0.226
Error	0.012018	30	0.000401		

Table CLXXI : Test results from two-way nested ANOVA on measured Ile data from T4 (day 100). Replicate was nested in treatment.

Ile T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.029322	1	0.029322	281.2009	<0.001
Treatment	0.000744	4	0.000186	1.7847	0.158
Replicate(Treatment)	0.001702	5	0.000340	3.2647	0.018
Error	0.003128	30	0.000104		

Table CLXXII : Test results from two-way nested ANOVA on measured Leu data from T3 (day 62). Replicate was nested in treatment.

Leu T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.372683	1	0.372683	243.3623	<0.001
Treatment	0.011086	4	0.002772	1.8098	0.153
Replicate(Treatment)	0.009470	5	0.001894	1.2368	0.317
Error	0.045942	30	0.001531		

Table CLXXIII : Test results from two-way nested ANOVA on measured Leu data from T4 (day 100). Replicate was nested in treatment.

Leu T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.145926	1	0.145926	302.2085	<0.001
Treatment	0.003020	4	0.000755	1.5638	0.209
Replicate(Treatment)	0.004859	5	0.000972	2.0127	0.105
Error	0.014486	30	0.000483		

Table CLXXIV : Test results from two-way nested ANOVA on measured Tyr data from T3 (day 62). Replicate was nested in treatment.

Tyr T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.125776	1	0.125776	462.6536	<0.001
Treatment	0.003163	4	0.000791	2.9088	0.038
Replicate(Treatment)	0.003388	5	0.000678	2.4924	0.053
Error	0.008156	30	0.000272		

Table CLXXV : Test results from two-way nested ANOVA on measured Tyr data from T4 (day 100). Replicate was nested in treatment.

Tyr T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.109830	1	0.109830	546.5105	<0.001
Treatment	0.000137	4	0.000034	0.1702	0.952
Replicate(Treatment)	0.001870	5	0.000374	1.8608	0.131
Error	0.006029	30	0.000201		

Table CLXXVI : Test results from two-way nested ANOVA on measured Phe data from T3 (day 62). Replicate was nested in treatment.

Phe T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.053876	1	0.053876	445.2529	<0.001
Treatment	0.000664	4	0.000166	1.3717	0.267
Replicate(Treatment)	0.001139	5	0.000228	1.8818	0.127
Error	0.003630	30	0.000121		

Table CLXXVII : Test results from two-way nested ANOVA on measured Phe data from T4 (day 100). Replicate was nested in treatment.

Phe T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.050980	1	0.050980	681.5455	<0.001
Treatment	0.000234	4	0.000059	0.7834	0.545
Replicate(Treatment)	0.000286	5	0.000057	0.7647	0.582
Error	0.002244	30	0.000075		

Table CLXXVIII : Test results from two-way nested ANOVA on measured Aaiba data from T3 (day 62). Replicate was nested in treatment.

Aaiba T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.029322	1	0.029322	554.9073	<0.001
Treatment	0.001676	4	0.000419	7.9277	<0.001
Replicate(Treatment)	0.000518	5	0.000104	1.9601	0.114
Error	0.001585	30	0.000053		

Table CLXXIX : Test results from two-way nested ANOVA on measured Aaiba data from T4 (day 100). Replicate was nested in treatment.

Aaiba T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.078854	1	0.078854	1137.323	<0.001
Treatment	0.000503	4	0.000126	1.815	0.152
Replicate(Treatment)	0.000376	5	0.000075	1.085	0.389
Error	0.002080	30	0.000069		

Table CLXXX : Test results from two-way nested ANOVA on measured Ethanolamine data from T3 (day 62). Replicate was nested in treatment.

Ethanolamine T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.020319	1	0.020319	194.9596	<0.001
Treatment	0.000656	4	0.000164	1.5742	0.222
Replicate(Treatment)	0.000655	5	0.000131	1.2562	0.323
Error	0.001980	19	0.000104		

Table CLXXXI : Test results from two-way nested ANOVA on measured Amm data from T3 (day 62). Replicate was nested in treatment.

Amm T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	614.4208	1	614.4208	10048.28	<0.001
Treatment	0.7834	4	0.1958	3.20	0.026
Replicate(Treatment)	0.8865	5	0.1773	2.90	0.030
Error	1.8344	30	0.0611		

Table CLXXXII : Test results from two-way nested ANOVA on measured Amm data from T4 (day 100). Replicate was nested in treatment.

Amm T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	465.8336	1	465.8336	10073.28	<0.001
Treatment	0.5984	4	0.1496	3.23	0.025
Replicate(Treatment)	0.7566	5	0.1513	3.27	0.018
Error	1.3873	30	0.0462		

Table CLXXXIII : Test results from two-way nested ANOVA on measured Hylys1 data from T3 (day 62). Replicate was nested in treatment.

Hylys1 T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.076767	1	0.076767	150.0595	<0.001
Treatment	0.001308	4	0.000327	0.6393	0.641
Replicate(Treatment)	0.007189	5	0.001438	2.8104	0.044
Error	0.010232	20	0.000512		

Table CLXXXIV : Test results from two-way nested ANOVA on measured Hylys1 data from T4 (day 100). Replicate was nested in treatment.

Hylys1 T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.606144	1	0.606144	295.2457	<0.001
Treatment	0.006554	4	0.001639	0.7981	0.536
Replicate(Treatment)	0.008649	5	0.001730	0.8425	0.530
Error	0.061591	30	0.002053		

Table CLXXXV : Test results from two-way nested ANOVA on measured Hylys2 data from T3 (day 62). Replicate was nested in treatment.

Hylys2 T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.059272	1	0.059272	148.8033	<0.001
Treatment	0.001498	4	0.000375	0.9405	0.458
Replicate(Treatment)	0.002332	5	0.000466	1.1707	0.352
Error	0.009560	24	0.000398		

Table CLXXXVI : Test results from two-way nested ANOVA on measured Hylys2 data from T4 (day 100). Replicate was nested in treatment.

Hylys2 T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.033074	1	0.033074	243.7749	<0.001
Treatment	0.000201	4	0.000050	0.3702	0.828
Replicate(Treatment)	0.000396	5	0.000079	0.5839	0.712
Error	0.004070	30	0.000136		

Table CLXXXVII : Test results from two-way nested ANOVA on measured Orn data from T3 (day 62). Replicate was nested in treatment.

Orn T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.021344	1	0.021344	181.9383	<0.001
Treatment	0.001934	4	0.000484	4.1221	0.009
Replicate(Treatment)	0.001492	5	0.000298	2.5431	0.049
Error	0.003520	30	0.000117		

Table CLXXXVIII : Test results from two-way nested ANOVA on measured Orn data from T4 (day 100). Replicate was nested in treatment.

Orn T4					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	0.035046	1	0.035046	578.8010	<0.001
Treatment	0.001251	4	0.000313	5.1645	0.003
Replicate(Treatment)	0.000252	5	0.000050	0.8332	0.536
Error	0.001817	30	0.000061		

Table CLXXXIX : Test results from two-way nested ANOVA on measured Lys data from T3 (day 62). Replicate was nested in treatment.

Lys T3					
Nested ANOVA					
	SS	Degr. of	MS	F	p
Intercept	13.62239	1	13.62239	429.7432	<0.001
Treatment	0.22374	4	0.05593	1.7645	0.162
Replicate(Treatment)	0.04422	5	0.00884	0.2790	0.921
Error	0.95097	30	0.03170		

Table CXC : Test results from two-way nested ANOVA on measured Lys data from T4 (day 100). Replicate was nested in treatment.

Lys T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	10.47655	1	10.47655	548.0017	<0.001
Treatment	0.05135	4	0.01284	0.6715	0.617
Replicate(Treatment)	0.06584	5	0.01317	0.6887	0.636
Error	0.57353	30	0.01912		

Table CXCI : Test results from two-way nested ANOVA on measured Muscle His data from T3 (day 62). Replicate was nested in treatment.

Muscle His T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	60.31690	1	60.31690	193.7861	<0.001
Treatment	0.83607	4	0.20902	0.6715	0.617
Replicate(Treatment)	3.95071	5	0.79014	2.5386	0.050
Error	9.33765	30	0.31126		

Table CXCII : Test results from two-way nested ANOVA on measured Muscle His data from T4 (day 100). Replicate was nested in treatment.

Muscle His T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	108.3068	1	108.3068	1899.081	<0.001
Treatment	0.2547	4	0.0637	1.116	0.367
Replicate(Treatment)	0.4429	5	0.0886	1.553	0.203
Error	1.7109	30	0.0570		

Table CXCIII : Test results from two-way nested ANOVA on measured Ans data from T3 (day 62). Replicate was nested in treatment.

Ans T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	21.60165	1	21.60165	1635.575	<0.001
Treatment	0.09574	4	0.02393	1.812	0.153
Replicate(Treatment)	0.06478	5	0.01296	0.981	0.445
Error	0.39622	30	0.01321		

Table CXCIV : Test results from two-way nested ANOVA on measured Ans data from T4 (day 100). Replicate was nested in treatment.

Ans T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	30.56378	1	30.56378	5467.720	<0.001
Treatment	0.14615	4	0.03654	6.536	0.001
Replicate(Treatment)	0.09303	5	0.01861	3.329	0.016
Error	0.16770	30	0.00559		

Table CXCV : Test results from two-way nested ANOVA on measured Arg data from T3 (day 62). Replicate was nested in treatment.

Arg T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.581774	1	0.581774	305.7572	<0.001
Treatment	0.009409	4	0.002352	1.2362	0.317
Replicate(Treatment)	0.006381	5	0.001276	0.6707	0.649
Error	0.057082	30	0.001903		

Table CXCVI : Test results from two-way nested ANOVA on measured Arg data from T4 (day 100). Replicate was nested in treatment.

Arg T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	0.824551	1	0.824551	258.6658	<0.001
Treatment	0.009806	4	0.002452	0.7691	0.554
Replicate(Treatment)	0.010554	5	0.002111	0.6622	0.655
Error	0.095631	30	0.003188		

Table CXCVII : Test results from two-way nested ANOVA on measured SUM free amino acids (FAA) data from T3 (day 62). Replicate was nested in treatment.

SUM FAA T3					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	84509.50	1	84509.50	4974.870	<0.001
Treatment	24.67	4	6.17	0.363	0.833
Replicate(Treatment)	64.90	5	12.98	0.764	0.583
Error	509.62	30	16.99		

Table CXCVIII : Test results from two-way nested ANOVA on measured SUM free amino acids (FAA) data from T4 (day 100). Replicate was nested in treatment.

SUM FAA T4					
	Nested ANOVA				
	SS	Degr. of	MS	F	p
Intercept	84083.23	1	84083.23	10717.74	<0.001
Treatment	4.35	4	1.09	0.14	0.967
Replicate(Treatment)	67.29	5	13.46	1.72	0.161
Error	235.36	30	7.85		

Two-way crossed ANCOVA

Table CXCIX : : Test results from two-way crossed ANCOVA on measured Blood Na^+ data from T3 (day 62). Weight was included as a co-varying factor.

Blood Na^+ T3					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	56203.69	1	56203.69	3708.251	<0.001
W (g)	8.46	1	8.46	0.558	0.461
Treatment	62.64	4	15.66	1.033	0.407
Replicate	88.05	1	88.05	5.810	0.023
Treatment*Replicate	41.88	4	10.47	0.691	0.604
Error	439.54	29	15.16		

Table CC : Test results from two-way crossed ANCOVA on measured Blood Na^+ data from T4 (day 100). Weight was included as a co-varying factor.

Blood Na^+ T4					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	80532.20	1	80532.20	5256.612	<0.001
W (g)	10.46	1	10.46	0.683	0.415
Treatment	53.53	4	13.38	0.873	0.492
Replicate	33.02	1	33.02	2.156	0.153
Treatment*Replicate	104.45	4	26.11	1.704	0.176
Error	444.29	29	15.32		

Table CCI : Test results from two-way crossed ANCOVA on measured Blood K^+ data from T3 (day 62). Weight was included as a co-varying factor.

Blood K^+ T3					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	33.23574	1	33.23574	213.8201	<0.001
W (g)	0.06691	1	0.06691	0.4304	0.517
Treatment	0.19953	4	0.04988	0.3209	0.862
Replicate	0.21461	1	0.21461	1.3807	0.250
Treatment*Replicate	0.24157	4	0.06039	0.3885	0.815
Error	4.35226	28	0.15544		

Table CCII : Test results from two-way crossed ANCOVA on measured Blood K^+ data from T4 (day 100). Weight was included as a co-varying factor.

Blood K^+ T4					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	78.44827	1	78.44827	214.6431	<0.001
	0.64149	1	0.64149	1.7552	0.196
Treatment	7.49989	4	1.87497	5.1301	0.003
Replicate	0.32634	1	0.32634	0.8929	0.353
Treatment*Replicate	1.43205	4	0.35801	0.9796	0.435
Error	10.23351	28	0.36548		

Table CCIII : Test results from two-way crossed ANCOVA on measured Blood pH temperature corrected data from T3 (day 62). Weight was included as a co-varying factor.

Blood pHtp T3					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	100.6086	1	100.6086	24286.34	<0.001
W (g)	0.0167	1	0.0167	4.02	0.055
Treatment	0.0118	4	0.0030	0.71	0.590
Replicate	0.0200	1	0.0200	4.82	0.037
Treatment*Replicate	0.0142	4	0.0036	0.86	0.502
Error	0.1160	28	0.0041		

Table CCIV : Test results from two-way crossed ANCOVA on measured Blood pH temperature corrected data from T4 (day 100). Weight was included as a co-varying factor.

Blood pHtp T4					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	152.2478	1	152.2478	55507.39	<0.001
W (g)	0.0110	1	0.0110	4.00	0.055
Treatment	0.0269	4	0.0067	2.45	0.069
Replicate	0.0009	1	0.0009	0.33	0.572
Treatment*Replicate	0.0149	4	0.0037	1.36	0.273
Error	0.0795	29	0.0027		

Table CCV : Test results from two-way crossed ANCOVA on measured Blood pCO_2 temperature corrected data from T3 (day 62). Weight was included as a co-varying factor.

Blood pCO_2 tp T3					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	41.76044	1	41.76044	91.97107	<0.001
W (g)	0.24421	1	0.24421	0.53784	0.469
Treatment	8.22666	4	2.05667	4.52950	0.006
Replicate	0.01062	1	0.01062	0.02340	0.880
Treatment*Replicate	0.69246	4	0.17312	0.38126	0.820
Error	12.71370	28	0.45406		

Table CCVI : Test results from two-way crossed ANCOVA on measured Blood pCO_2 temperature corrected data from T4 (day 100). Weight was included as a co-varying factor.

Blood pCO_2 tp T4					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	113.6737	1	113.6737	269.9038	<0.001
W (g)	0.4272	1	0.4272	1.0143	0.322
Treatment	7.0665	4	1.7666	4.1946	0.008
Replicate	0.0214	1	0.0214	0.0508	0.823
Treatment*Replicate	0.3871	4	0.0968	0.2298	0.919
Error	12.2137	29	0.4212		

Table CCVII : Test results from two-way crossed ANCOVA on measured HCO_3^- data from T3 (day 62). Weight was included as a co-varying factor.

Blood HCO_3^- T3					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	30.23268	1	30.23268	80.23742	<0.001
W (g)	0.37487	1	0.37487	0.99491	0.327
Treatment	3.73905	4	0.93476	2.48086	0.067
Replicate	1.41547	1	1.41547	3.75666	0.063
Treatment*Replicate	1.33414	4	0.33354	0.88520	0.486
Error	10.55013	28	0.37679		

Table CCVIII : Test results from two-way crossed ANCOVA on measured HCO_3^- data from T4 (day 100). Weight was included as a co-varying factor.

Blood HCO_3^- T4					
Two-way crossed ANCOVA					
	SS	Degr. of	MS	F	p
Intercept	54.99264	1	54.99264	141.7018	<0.001
W (g)	1.46297	1	1.46297	3.7697	0.062
Treatment	0.34268	4	0.08567	0.2208	0.925
Replicate	0.18412	1	0.18412	0.4744	0.496
Treatment*Replicate	0.33855	4	0.08464	0.2181	0.926
Error	11.25453	29	0.38809		

9.4 Student-Newman-Keuls test

Table CCIX : p-values from SNK test, testing for differences in Weight at T0 (day 0) between treatments.

Weight T0						
	Treatment	{1}	{2}	{3}	{4}	{5}
		54.108	49.127	52.067	52.190	51.397
1	Control		0.233	0.673	0.425	0.673
2	ChronicLow	0.233		0.440	0.580	0.345
3	ChronicMedium	0.673	0.440		0.959	0.781
4	ChronicHigh	0.425	0.580	0.959		0.942
5	HighPulse	0.673	0.345	0.781	0.942	

Table CCX : p-values from SNK test, testing for differences in Weight at T3 (day 62) between treatments.

Weight T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
		89.575	80.020	73.559	65.883	88.506
1	Control		0.160	0.012	<.0.001	0.838
2	ChronicLow	0.160		0.216	0.019	0.104
3	ChronicMedium	0.012	0.216		0.142	0.012
4	ChronicHigh	<.0.001	0.019	0.142		<.0.001
5	HighPulse	0.838	0.104	0.012	0.000	

Table CCXI : p-values from SNK test, testing for differences in Weight at T4 (day 100) between treatments.

Weight T4						
	Treatment	{1} 138.41	{2} 120.69	{3} 109.53	{4} 100.04	{5} 128.10
1	Control		0.133	0.010	<.0.001	0.264
2	ChronicLow	0.133		0.227	0.065	0.423
3	ChronicMedium	0.010	0.227		0.304	0.110
4	ChronicHigh	<.0.001	0.065	0.304		0.013
5	HighPulse	0.264	0.423	0.110	0.013	

Table CCXII : p-values from SNK test, testing for differences in SGR at T0-T3 (day 0-62) between treatments.

SGR1 (T0-T3)						
	Treatment	{1} .74735	{2} .67575	{3} .47022	{4} .28347	{5} .79259
1	Control		0.313	<0.001	<0.001	0.524
2	ChronicLow	0.313		0.004	<0.001	0.227
3	ChronicMedium	<0.001	0.004		0.009	<0.001
4	ChronicHigh	<0.001	<0.001	0.009		<0.001
5	HighPulse	0.524	0.227	<0.001	<0.001	

Table CCXIII : p-values from SNK test, testing for differences in SGR at T3-T4 (day 62-100) between treatments.

SGR2 (T3-T4)						
	Treatment	{1} .99702	{2} .92877	{3} .80871	{4} .80067	{5} .89364
1	Control		0.499	0.243	0.293	0.561
2	ChronicLow	0.499		0.459	0.582	0.728
3	ChronicMedium	0.243	0.459		0.937	0.400
4	ChronicHigh	0.293	0.582	0.937		0.627
5	HighPulse	0.561	0.728	0.400	0.627	

Table CCXIV : p-values from SNK test, testing for differences in SGR at T0-T4 (day 0-100) between treatments.

SGR Overall						
	Treatment	{1} .87440	{2} .80007	{3} .65009	{4} .53513	{5} .84580
1	Control		0.483	0.003	0.000	0.658
2	ChronicLow	0.483		0.020	0.000	0.479
3	ChronicMedium	0.003	0.020		0.075	0.007
4	ChronicHigh	<0.001	<0.001	0.075		<0.001
5	HighPulse	0.658	0.479	0.007	<0.001	

Table CCXV: p-values from SNK test, testing for differences in Blood Na^+ at T3 (day 62) between treatments.

Blood Na^+ T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
		167.88	170.38	168.50	168.25	166.75
1	Control		0.624	0.951	0.857	0.589
2	ChronicLow	0.624		0.370	0.563	0.414
3	ChronicMedium	0.951	0.370		0.904	0.831
4	ChronicHigh	0.857	0.563	0.904		0.749
5	HighPulse	0.589	0.414	0.831	0.749	

Table CCXVI: p-values from SNK test, testing for differences in Blood Na^+ at T4 (day 100) between treatments.

Blood Na^+ T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
		170.38	171.38	168.38	170.00	168.50
1	Control		0.637	0.777	0.859	0.649
2	ChronicLow	0.637		0.614	0.791	0.527
3	ChronicMedium	0.777	0.614		0.722	0.953
4	ChronicHigh	0.859	0.791	0.722		0.480
5	HighPulse	0.649	0.527	0.953	0.480	

Table CCXVII : p-values from SNK test, testing for differences in blood K^+ at T3 (day 62) between treatments.

Blood K^+ T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
		4.2571	4.3125	4.1875	4.4000	4.3250
1	Control		0.778	0.723	0.883	0.936
2	ChronicLow	0.778		0.799	0.895	0.949
3	ChronicMedium	0.723	0.799		0.811	0.894
4	ChronicHigh	0.883	0.895	0.811		0.703
5	HighPulse	0.936	0.949	0.894	0.703	

Table CCXVIII : p-values from SNK test, testing for differences in blood K^+ at T4 (day 100) between treatments.

Blood K^+ T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
		4.8250	5.6500	4.5125	4.7143	4.4250
1	Control		0.012	0.578	0.724	0.576
2	ChronicLow	0.012		0.005	0.013	0.003
3	ChronicMedium	0.578	0.005		0.520	0.780
4	ChronicHigh	0.724	0.013	0.520		0.624
5	HighPulse	0.576	0.003	0.780	0.624	

Table CCXIX : p-values from SNK test, testing for differences in Blood pH temperature corrected at T3 (day 62) between treatments.

Blood pHtp T3						
	Treatment	{1} 7.3054	{2} 7.2934	{3} 7.2936	{4} 7.3337	{5} 7.3109
1	Control		0.942	0.749	0.720	0.881
2	ChronicLow	0.942		0.994	0.802	0.963
3	ChronicMedium	0.749	0.994		0.693	0.884
4	ChronicHigh	0.720	0.802	0.693		0.536
5	HighPulse	0.881	0.963	0.884	0.536	

Table CCXX : p-values from SNK test, testing for differences in Blood pH temperature corrected at T4 (day 100) between treatments.

Blood pHtp T4						
	Treatment	{1} 7.2451	{2} 7.1977	{3} 7.2259	{4} 7.2673	{5} 7.2625
1	Control		0.214	0.492	0.703	0.532
2	ChronicLow	0.214		0.315	0.109	0.107
3	ChronicMedium	0.492	0.315		0.449	0.391
4	ChronicHigh	0.703	0.109	0.449		0.863
5	HighPulse	0.532	0.107	0.391	0.863	

Table CCXXI : p-values from SNK test, testing for differences in Blood pCO_2 temperature corrected at T3 (day 62) between treatments.

Blood pCO_2 tp T3						
	Treatment	{1} 5.7853	{2} 5.2464	{3} 4.9240	{4} 4.3336	{5} 5.3176
1	Control		0.224	0.050	0.001	0.152
2	ChronicLow	0.224		0.320	0.019	0.825
3	ChronicMedium	0.050	0.320		0.073	0.442
4	ChronicHigh	0.001	0.019	0.073		0.020
5	HighPulse	0.152	0.825	0.442	0.020	

Table CCXXII : p-values from SNK test, testing for differences in Blood pCO_2 temperature corrected at T4 (day 100) between treatments.

Blood pCO_2 tp T4						
	Treatment	{1} 5.6735	{2} 6.6658	{3} 6.1508	{4} 5.5479	{5} 5.6358
1	Control		0.007	0.129	0.912	0.903
2	ChronicLow	0.007		0.102	0.007	0.010
3	ChronicMedium	0.129	0.102		0.221	0.228
4	ChronicHigh	0.912	0.007	0.221		0.776
5	HighPulse	0.903	0.010	0.228	0.776	

Table CCXXIII : p-values from SNK test, testing for differences in HCO_3^- at T3 (day 62) between treatments.

Blood HCO_3^- T3						
	Treatment	{1} 4.1429	{2} 3.6125	{3} 3.4000	{4} 3.2000	{5} 3.7875
1	Control		0.250	0.125	0.049	0.285
2	ChronicLow	0.250		0.520	0.426	0.596
3	ChronicMedium	0.125	0.520		0.545	0.470
4	ChronicHigh	0.049	0.426	0.545		0.292
5	HighPulse	0.285	0.596	0.470	0.292	

Table CCXXIV : p-values from SNK test, testing for differences in HCO_3^- at T4 (day 100) between treatments.

Blood HCO_3^- T4						
	Treatment	{1} 3.5625	{2} 3.8375	{3} 3.7375	{4} 3.6625	{5} 3.6875
1	Control		0.899	0.942	0.749	0.914
2	ChronicLow	0.899		0.749	0.942	0.879
3	ChronicMedium	0.942	0.749		0.968	0.873
4	ChronicHigh	0.749	0.942	0.968		0.936
5	HighPulse	0.914	0.879	0.873	0.936	

Table CCXXV : p-values from SNK test, testing for differences in Lens His at T3 (day 62) between treatments.

Lens His T3						
	Treatment	{1} 1.2734	{2} .95062	{3} 1.0165	{4} 1.3263	{5} 1.4439
1	Control		0.001	0.003	0.510	0.095
2	ChronicLow	0.001		0.412	<0.001	<0.001
3	ChronicMedium	0.003	0.412		0.001	<0.001
4	ChronicHigh	0.510	<0.001	0.001		0.148
5	HighPulse	0.095	<0.001	<0.001	0.148	

Table CCXXVI : p-values from SNK test, testing for differences in Lens His at T4 (day 100) between treatments.

Lens His T4						
	Treatment	{1} 1.2888	{2} 1.1251	{3} 1.1731	{4} 1.2095	{5} 1.2675
1	Control		0.313	0.523	0.618	0.802
2	ChronicLow	0.313		0.572	0.580	0.342
3	ChronicMedium	0.523	0.572		0.668	0.507
4	ChronicHigh	0.618	0.580	0.668		0.495
5	HighPulse	0.802	0.342	0.507	0.495	

Table CCXXVII : p-values from SNK test, testing for differences in Lens NAH at T3 (day 62) between treatments.

Lens NAH T3						
	Treatment	{1} 9.2829	{2} 7.2959	{3} 7.6513	{4} 8.2454	{5} 9.4534
1	Control		0.002	0.006	0.041	0.729
2	ChronicLow	0.002		0.471	0.141	0.001
3	ChronicMedium	0.006	0.471		0.231	0.004
4	ChronicHigh	0.041	0.141	0.231		0.047
5	HighPulse	0.729	0.001	0.004	0.047	

Table CCXXVIII : p-values from SNK test, testing for differences in Lens NAH at T4 (day 100) between treatments.

Lens NAH T4						
	Treatment	{1} 8.7799	{2} 8.6625	{3} 9.2792	{4} 8.1762	{5} 9.7910
1	Control		0.876	0.507	0.699	0.373
2	ChronicLow	0.876		0.688	0.518	0.439
3	ChronicMedium	0.507	0.688		0.459	0.496
4	ChronicHigh	0.699	0.518	0.459		0.215
5	HighPulse	0.373	0.439	0.496	0.215	

Table CCXXIX : p-values from SNK test, testing for differences in Phser at T3 (day 62) between treatments.

Phser T3						
	Treatment	{1} .01275	{2} .01313	{3} .01150	{4} .01425	{5} .01412
1	Control		0.871	0.590	0.914	0.822
2	ChronicLow	0.871		0.761	0.877	0.666
3	ChronicMedium	0.590	0.761		0.753	0.666
4	ChronicHigh	0.914	0.877	0.753		0.957
5	HighPulse	0.822	0.666	0.666	0.957	

Table CCXXX : p-values from SNK test, testing for differences in Phser at T4 (day 100) between treatments.

Phser T4						
	Treatment	{1} .01300	{2} .01300	{3} .01350	{4} .01288	{5} .01300
1	Control		1.000	0.758	1.000	1.000
2	ChronicLow	1.000		0.948	0.997	1.000
3	ChronicMedium	0.758	0.948		0.995	0.990
4	ChronicHigh	1.000	0.997	0.995		0.939
5	HighPulse	1.000	1.000	0.990	0.939	

Table CCXXXI : p-values from SNK test, testing for differences in Tau at T3 (day 62) between treatments.

Tau T3						
	Treatment	{1} 12.286	{2} 10.927	{3} 11.360	{4} 11.125	{5} 12.110
1	Control		0.857	0.779	0.832	0.899
2	ChronicLow	0.857		0.947	0.886	0.824
3	ChronicMedium	0.779	0.947		0.865	0.588
4	ChronicHigh	0.832	0.886	0.865		0.754
5	HighPulse	0.899	0.824	0.588	0.754	

Table CCXXXII : p-values from SNK test, testing for differences in Tau at T4 (day 100) between treatments.

Tau T4						
	Treatment	{1} 13.402	{2} 13.118	{3} 13.145	{4} 12.497	{5} 14.493
1	Control		0.956	0.798	0.800	0.281
2	ChronicLow	0.956		0.979	0.537	0.520
3	ChronicMedium	0.798	0.979		0.793	0.376
4	ChronicHigh	0.800	0.537	0.793		0.285
5	HighPulse	0.281	0.520	0.376	0.285	

Table CCXXXIII : p-values from SNK test, testing for differences in Pea at T3 (day 62) between treatments.

Pea T3						
	Treatment	{1} .08362	{2} .08487	{3} .07950	{4} .07175	{5} .07437
1	Control		0.862	0.567	0.357	0.406
2	ChronicLow	0.862		0.733	0.367	0.464
3	ChronicMedium	0.567	0.733		0.528	0.477
4	ChronicHigh	0.357	0.367	0.528		0.715
5	HighPulse	0.406	0.464	0.477	0.715	

Table CCXXXIV : p-values from SNK test, testing for differences in Pea at T4 (day 100) between treatments.

Pea T4						
	Treatment	{1} .08150	{2} .09450	{3} .09187	{4} .08025	{5} .08288
1	Control		0.426	0.445	0.883	0.872
2	ChronicLow	0.426		0.758	0.455	0.364
3	ChronicMedium	0.445	0.758		0.522	0.294
4	ChronicHigh	0.883	0.455	0.522		0.948
5	HighPulse	0.872	0.364	0.294	0.948	

Table CCXXXV : p-values from SNK test, testing for differences in Urea at T3 (day 62) between treatments.

Urea T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
1	Control		0.736	0.682	0.607	0.385
2	ChronicLow	0.736		0.772	0.947	0.989
3	ChronicMedium	0.682	0.772		0.932	0.972
4	ChronicHigh	0.607	0.947	0.932		0.938
5	HighPulse	0.385	0.989	0.972	0.938	

Table CCXXXVI : p-values from SNK test, testing for differences in Urea at T4 (day 100) between treatments.

Urea T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
		6.3755	6.2877	6.2670	5.2515	6.8054
1	Control		0.851	0.970	0.089	0.358
2	ChronicLow	0.851		0.965	0.078	0.508
3	ChronicMedium	0.970	0.965		0.035	0.652
4	ChronicHigh	0.089	0.078	0.035		0.015
5	HighPulse	0.358	0.508	0.652	0.015	

Table CCXXXVII : p-values from SNK test, testing for differences in Asp at T3 (day 62) between treatments.

Asp T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
		.57650	.60438	.54288	.56675	.65538
1	Control		0.737	0.913	0.907	0.609
2	ChronicLow	0.737		0.878	0.892	0.540
3	ChronicMedium	0.913	0.878		0.774	0.654
4	ChronicHigh	0.907	0.892	0.774		0.707
5	HighPulse	0.609	0.540	0.654	0.707	

Table CCXXXVIII : p-values from SNK test, testing for differences in Asp at T4 (day 100) between treatments.

Asp T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
		.66112	.66738	.56900	.54113	.60187
1	Control		0.930	0.399	0.335	0.405
2	ChronicLow	0.930		0.508	0.391	0.624
3	ChronicMedium	0.399	0.508		0.694	0.643
4	ChronicHigh	0.335	0.391	0.694		0.666
5	HighPulse	0.405	0.624	0.643	0.666	

Table CCXXXIX : p-values from SNK test, testing for differences in Hypro at T3 (day 62) between treatments.

Hypro T3						
	Treatment	{1} .43913	{2} .53538	{3} .45750	{4} .30725	{5} .49200
1	Control		0.929	0.909	0.412	0.941
2	ChronicLow	0.929		0.876	0.609	0.786
3	ChronicMedium	0.909	0.876		0.615	0.829
4	ChronicHigh	0.412	0.609	0.615		0.653
5	HighPulse	0.941	0.786	0.829	0.653	

Table CCXL : p-values from SNK test, testing for differences in Hypro at T4 (day 100) between treatments.

Hypro T4						
	Treatment	{1} .63038	{2} .82450	{3} .64125	{4} .76962	{5} .52088
1	Control		0.498	0.937	0.572	0.430
2	ChronicLow	0.498		0.384	0.691	0.198
3	ChronicMedium	0.937	0.384		0.355	0.657
4	ChronicHigh	0.572	0.691	0.355		0.283
5	HighPulse	0.430	0.198	0.657	0.283	

Table CCXLI : p-values from SNK test, testing for differences in Thr at T3 (day 62) between treatments.

Thr T3						
	Treatment	{1} 1.4575	{2} 1.3544	{3} 1.1915	{4} 1.0260	{5} 1.3050
1	Control		0.629	0.594	0.268	0.752
2	ChronicLow	0.629		0.723	0.417	0.817
3	ChronicMedium	0.594	0.723		0.439	0.595
4	ChronicHigh	0.268	0.417	0.439		0.393
5	HighPulse	0.752	0.817	0.595	0.393	

Table CCXLII : p-values from SNK test, testing for differences in Thr at T4 (day 100) between treatments.

Thr T4						
	Treatment	{1} 1.6176	{2} 1.7265	{3} 1.4974	{4} 1.9466	{5} 1.4826
1	Control		0.524	0.482	0.142	0.707
2	ChronicLow	0.524		0.376	0.202	0.483
3	ChronicMedium	0.482	0.376		0.055	0.931
4	ChronicHigh	0.142	0.202	0.055		0.068
5	HighPulse	0.707	0.483	0.931	0.068	

Table CCXLIII : p-values from SNK test, testing for differences in Ser at T3 (day 62) between treatments.

Ser T3						
	Treatment	{1} 1.7137	{2} 1.9093	{3} 1.2540	{4} 1.5618	{5} 1.4973
1	Control		0.408	0.218	0.519	0.626
2	ChronicLow	0.408		0.058	0.308	0.306
3	ChronicMedium	0.218	0.058		0.394	0.304
4	ChronicHigh	0.519	0.308	0.394		0.784
5	HighPulse	0.626	0.306	0.304	0.784	

Table CCXLIV : p-values from SNK test, testing for differences in Ser at T4 (day 100) between treatments.

Ser T4						
	Treatment	{1} .99838	{2} 1.1181	{3} 1.1956	{4} 1.0373	{5} 1.0428
1	Control		0.800	0.571	0.770	0.940
2	ChronicLow	0.800		0.560	0.814	0.571
3	ChronicMedium	0.571	0.560		0.630	0.484
4	ChronicHigh	0.770	0.814	0.630		0.967
5	HighPulse	0.940	0.571	0.484	0.967	

Table CCXLV : p-values from SNK test, testing for differences in Asn at T3 (day 62) between treatments.

Asn T3						
	Treatment	{1} .67462	{2} .33063	{3} .93600	{4} .70750	{5} .49388
1	Control		0.308	0.501	0.888	0.439
2	ChronicLow	0.308		0.088	0.374	0.484
3	ChronicMedium	0.501	0.088		0.329	0.240
4	ChronicHigh	0.888	0.374	0.329		0.628
5	HighPulse	0.439	0.484	0.240	0.628	

Table CCXLVI : p-values from SNK test, testing for differences in Asn at T4 (day 100) between treatments.

Asn T4						
	Treatment	{1} .71162	{2} .45150	{3} 1.1916	{4} 1.8185	{5} .52350
1	Control		0.674	0.125	0.003	0.542
2	ChronicLow	0.674		0.091	0.001	0.815
3	ChronicMedium	0.125	0.091		0.048	0.088
4	ChronicHigh	0.003	0.001	0.048		0.001
5	HighPulse	0.542	0.815	0.088	0.001	

Table CCXLVII : p-values from SNK test, testing for differences in Glu at T3 (day 62) between treatments.

Glu T3						
	Treatment	{1} .59625	{2} .87313	{3} .64337	{4} .59275	{5} .66538
1	Control		0.002	0.493	0.959	0.572
2	ChronicLow	0.002		0.005	0.002	0.004
3	ChronicMedium	0.493	0.005		0.739	0.749
4	ChronicHigh	0.959	0.002	0.739		0.712
5	HighPulse	0.572	0.004	0.749	0.712	

Table CCXLVIII : p-values from SNK test, testing for differences in Glu at T4 (day 100) between treatments.

Glu T4						
	Treatment	{1} .65387	{2} .75275	{3} .62262	{4} .48738	{5} .61425
1	Control		0.147	0.643	0.078	0.824
2	ChronicLow	0.147		0.140	0.003	0.181
3	ChronicMedium	0.643	0.140		0.121	0.901
4	ChronicHigh	0.078	0.003	0.121		0.066
5	HighPulse	0.824	0.181	0.901	0.066	

Table CCXLIX : p-values from SNK test, testing for differences in Gln at T3 (day 62) between treatments.

Gln T3						
	Treatment	{1} .85350	{2} .79575	{3} 1.0489	{4} 1.0400	{5} .71462
1	Control		0.534	0.099	0.050	0.298
2	ChronicLow	0.534		0.044	0.031	0.384
3	ChronicMedium	0.099	0.044		0.924	0.007
4	ChronicHigh	0.050	0.031	0.924		0.006
5	HighPulse	0.298	0.384	0.007	0.006	

Table CCL : p-values from SNK test, testing for differences in Gln at T4 (day 100) between treatments.

Gln T4						
	Treatment	{1} 1.0010	{2} .93063	{3} 1.1178	{4} .91113	{5} .72650
1	Control		0.522	0.290	0.689	0.073
2	ChronicLow	0.522		0.212	0.859	0.160
3	ChronicMedium	0.290	0.212		0.246	0.008
4	ChronicHigh	0.689	0.859	0.246		0.099
5	HighPulse	0.073	0.160	0.008	0.099	

Table CCLI : p-values from SNK test, testing for differences in Sarc at T3 (day 62) between treatments.

Sarc T3						
	Treatment	{1} .04100	{2} .02988	{3} .02950	{4} .02825	{5} .03350
1	Control		0.244	0.343	0.348	0.277
2	ChronicLow	0.244		0.956	0.969	0.597
3	ChronicMedium	0.343	0.956		0.855	0.827
4	ChronicHigh	0.348	0.969	0.855		0.866
5	HighPulse	0.277	0.597	0.827	0.866	

Table CCLII : p-values from SNK test, testing for differences in Sarc at T4 (day 100) between treatments.

Sarc T4						
	Treatment	{1} .02875	{2} .03812	{3} .03537	{4} .03343	{5} .04138
1	Control		0.531	0.605	0.502	0.372
2	ChronicLow	0.531		0.692	0.776	0.640
3	ChronicMedium	0.605	0.692		0.779	0.662
4	ChronicHigh	0.502	0.776	0.779		0.659
5	HighPulse	0.372	0.640	0.662	0.659	

Table CCLIII : p-values from SNK test, testing for differences in Pro at T3 (day 62) between treatments.

Pro T3						
	Treatment	{1} 1.3169	{2} 1.7616	{3} 1.5345	{4} .93425	{5} 1.2845
1	Control		0.663	0.673	0.737	0.950
2	ChronicLow	0.663		0.660	0.496	0.787
3	ChronicMedium	0.673	0.660		0.647	0.877
4	ChronicHigh	0.737	0.496	0.647		0.498
5	HighPulse	0.950	0.787	0.877	0.498	

Table CCLIV : p-values from SNK test, testing for differences in Pro at T4 (day 100) between treatments.

Pro T4						
	Treatment	{1} 2.2963	{2} 1.7634	{3} 1.7135	{4} 2.4560	{5} .96088
1	Control		0.230	0.386	0.717	0.021
2	ChronicLow	0.230		0.910	0.265	0.172
3	ChronicMedium	0.386	0.910		0.339	0.094
4	ChronicHigh	0.717	0.265	0.339		0.013
5	HighPulse	0.021	0.172	0.094	0.013	

Table CCLV : p-values from SNK test, testing for differences in Gly at T3 (day 62) between treatments.

Gly T3						
	Treatment	{1} 7.1339	{2} 7.3550	{3} 6.6997	{4} 7.7095	{5} 7.4396
1	Control		0.549	0.242	0.404	0.682
2	ChronicLow	0.549		0.186	0.599	0.818
3	ChronicMedium	0.242	0.186		0.064	0.197
4	ChronicHigh	0.404	0.599	0.064		0.465
5	HighPulse	0.682	0.818	0.197	0.465	

Table CCLVI : p-values from SNK test, testing for differences in Gly at T4 (day 100) between treatments.

Gly T4						
	Treatment	{1} 5.9399	{2} 5.8955	{3} 6.0371	{4} 5.8244	{5} 5.9625
1	Control		0.909	0.965	0.951	0.953
2	ChronicLow	0.909		0.983	0.854	0.983
3	ChronicMedium	0.965	0.983		0.981	0.847
4	ChronicHigh	0.951	0.854	0.981		0.984
5	HighPulse	0.953	0.983	0.847	0.984	

Table CCLVII : p-values from SNK test, testing for differences in Ala at T3 (day 62) between treatments.

Ala T3						
	Treatment	{1} 4.0570	{2} 4.5297	{3} 4.8790	{4} 4.4415	{5} 3.6873
1	Control		0.333	0.078	0.250	0.269
2	ChronicLow	0.333		0.296	0.790	0.068
3	ChronicMedium	0.078	0.296		0.388	0.008
4	ChronicHigh	0.250	0.790	0.388		0.070
5	HighPulse	0.269	0.068	0.008	0.070	

Table CCLVIII : p-values from SNK test, testing for differences in Ala at T4 (day 100) between treatments.

Ala T4						
	Treatment	{1} 3.8754	{2} 3.8558	{3} 4.0374	{4} 3.6910	{5} 3.6452
1	Control		0.938	0.523	0.745	0.797
2	ChronicLow	0.938		0.752	0.516	0.682
3	ChronicMedium	0.523	0.752		0.521	0.532
4	ChronicHigh	0.745	0.516	0.521		0.857
5	HighPulse	0.797	0.682	0.532	0.857	

Table CCLIX : p-values from SNK test, testing for differences in Citr at T3 (day 62) between treatments.

Citr T3						
	Treatment	{1} .08325	{2} .07888	{3} .06175	{4} .05287	{5} .12000
1	Control		0.896	0.795	0.797	0.276
2	ChronicLow	0.896		0.610	0.716	0.439
3	ChronicMedium	0.795	0.610		0.791	0.313
4	ChronicHigh	0.797	0.716	0.791		0.278
5	HighPulse	0.276	0.439	0.313	0.278	

Table CCLX : p-values from SNK test, testing for differences in Citr at T4 (day 100) between treatments.

Citr T4						
	Treatment	{1} .13925	{2} .16412	{3} .12750	{4} .08975	{5} .10838
1	Control		0.391	0.684	0.324	0.533
2	ChronicLow	0.391		0.416	0.093	0.227
3	ChronicMedium	0.684	0.416		0.394	0.509
4	ChronicHigh	0.324	0.093	0.394		0.520
5	HighPulse	0.533	0.227	0.509	0.520	

Table CCLXI : p-values from SNK test, testing for differences in Aaba at T3 (day 62) between treatments.

Aaba T3						
	Treatment	{1} .06388	{2} .05775	{3} .05713	{4} .05287	{5} .05600
1	Control		0.533	0.769	0.790	0.850
2	ChronicLow	0.533		0.949	0.958	0.982
3	ChronicMedium	0.769	0.949		0.901	0.909
4	ChronicHigh	0.790	0.958	0.901		0.750
5	HighPulse	0.850	0.982	0.909	0.750	

Table CCLXII : p-values from SNK test, testing for differences in Aaba at T4 (day 100) between treatments.

Aaba T4						
	Treatment	{1} .06813	{2} .06687	{3} .06875	{4} .07938	{5} .05238
1	Control		0.884	0.942	0.394	0.169
2	ChronicLow	0.884		0.974	0.468	0.098
3	ChronicMedium	0.942	0.974		0.221	0.238
4	ChronicHigh	0.394	0.468	0.221		0.025
5	HighPulse	0.169	0.098	0.238	0.025	

Table CCLXIII : p-values from SNK test, testing for differences in Val at T3 (day 62) between treatments.

Val T3						
	Treatment	{1} .07212	{2} .06900	{3} .08875	{4} .08900	{5} .08213
1	Control		0.852	0.581	0.741	0.551
2	ChronicLow	0.852		0.638	0.749	0.712
3	ChronicMedium	0.581	0.638		0.988	0.693
4	ChronicHigh	0.741	0.749	0.988		0.910
5	HighPulse	0.551	0.712	0.693	0.910	

Table CCLXIV : p-values from SNK test, testing for differences in Val at T4 (day 100) between treatments.

Val T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
	Treatment	.05450	.05375	.04938	.04713	.05950
1	Control		0.931	0.821	0.823	0.562
2	ChronicLow	0.931		0.612	0.720	0.780
3	ChronicMedium	0.821	0.612		0.794	0.640
4	ChronicHigh	0.823	0.720	0.794		0.601
5	HighPulse	0.562	0.780	0.640	0.601	

Table CCLXV : p-values from SNK test, testing for differences in Met at T3 (day 62) between treatments.

Met T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
		.04312	.04137	.04625	.04963	.04950
1	Control		0.852	0.739	0.897	0.774
2	ChronicLow	0.852		0.861	0.900	0.819
3	ChronicMedium	0.739	0.861		0.930	0.729
4	ChronicHigh	0.897	0.900	0.930		0.989
5	HighPulse	0.774	0.819	0.729	0.989	

Table CCLXVI : p-values from SNK test, testing for differences in Met at T4 (day 100) between treatments.

Met T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
		.03700	.03338	.03025	.03000	.03913
1	Control		0.388	0.247	0.345	0.611
2	ChronicLow	0.388		0.456	0.697	0.358
3	ChronicMedium	0.247	0.456		0.952	0.160
4	ChronicHigh	0.345	0.697	0.952		0.203
5	HighPulse	0.611	0.358	0.160	0.203	

Table CCLXVII : p-values from SNK test, testing for differences in Cysth2 at T3 (day 62) between treatments.

Cysth2 T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
		.96338	.88187	.78200	.69263	.77200
1	Control		0.687	0.642	0.663	0.776
2	ChronicLow	0.687		0.622	0.782	0.849
3	ChronicMedium	0.642	0.622		0.897	0.961
4	ChronicHigh	0.663	0.782	0.897		0.695
5	HighPulse	0.776	0.849	0.961	0.695	

Table CCLXVIII : p-values from SNK test, testing for differences in Cysth2 at T4 (day 100) between treatments.

Cysth2 T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
		.77287	.79600	.83050	.93250	.59762
1	Control		0.800	0.802	0.309	0.062
2	ChronicLow	0.800		0.706	0.301	0.088
3	ChronicMedium	0.802	0.706		0.269	0.067
4	ChronicHigh	0.309	0.301	0.269		0.006
5	HighPulse	0.062	0.088	0.067	0.006	

Table CCLXIX : p-values from SNK test, testing for differences in Ile at T3 (day 62) between treatments.

Ile T3						
	Treatment	{1} .03925	{2} .04025	{3} .05462	{4} .05987	{5} .05312
1	Control		0.924	0.456	0.290	0.382
2	ChronicLow	0.924		0.357	0.248	0.222
3	ChronicMedium	0.456	0.357		0.615	0.886
4	ChronicHigh	0.290	0.248	0.615		0.792
5	HighPulse	0.382	0.222	0.886	0.792	

Table CCLXX : p-values from SNK test, testing for differences in Ile at T4 (day 100) between treatments.

Ile T4						
	Treatment	{1} .02800	{2} .02800	{3} .02425	{4} .02112	{5} .03400
1	Control		1.000	0.800	0.649	0.314
2	ChronicLow	1.000		0.527	0.478	0.569
3	ChronicMedium	0.800	0.527		0.598	0.360
4	ChronicHigh	0.649	0.478	0.598		0.207
5	HighPulse	0.314	0.569	0.360	0.207	

Table CCLXXI : p-values from SNK test, testing for differences in Leu at T3 (day 62) between treatments.

Leu T3						
	Treatment	{1} .07287	{2} .08300	{3} .10888	{4} .11850	{5} .09937
1	Control		0.614	0.286	0.171	0.387
2	ChronicLow	0.614		0.404	0.298	0.416
3	ChronicMedium	0.286	0.404		0.632	0.636
4	ChronicHigh	0.171	0.298	0.632		0.606
5	HighPulse	0.387	0.416	0.636	0.606	

Table CCLXXII : p-values from SNK test, testing for differences in Leu at T4 (day 100) between treatments.

Leu T4						
	Treatment	{1} .06187	{2} .06600	{3} .05525	{4} .04688	{5} .07200
1	Control		0.728	0.577	0.418	0.668
2	ChronicLow	0.728		0.635	0.377	0.613
3	ChronicMedium	0.577	0.635		0.481	0.493
4	ChronicHigh	0.418	0.377	0.481		0.228
5	HighPulse	0.668	0.613	0.493	0.228	

Table CCLXXIII : p-values from SNK test, testing for differences in Tyr at T3 (day 62) between treatments.

Tyr T3						
	Treatment	{1} .05875	{2} .05475	{3} .04213	{4} .05488	{5} .06987
1	Control		0.899	0.276	0.672	0.229
2	ChronicLow	0.899		0.173	0.989	0.357
3	ChronicMedium	0.276	0.173		0.350	0.033
4	ChronicHigh	0.672	0.989	0.350		0.238
5	HighPulse	0.229	0.357	0.033	0.238	

Table CCLXXIV : p-values from SNK test, testing for differences in Tyr at T4 (day 100) between treatments.

Tyr T4						
	Treatment	{1} .05425	{2} .05300	{3} .04913	{4} .05388	{5} .05175
1	Control		0.985	0.959	0.961	0.987
2	ChronicLow	0.985		0.864	0.908	0.869
3	ChronicMedium	0.959	0.864		0.921	0.729
4	ChronicHigh	0.961	0.908	0.921		0.957
5	HighPulse	0.987	0.869	0.729	0.957	

Table CCLXXV : p-values from SNK test, testing for differences in Phe at T3 (day 62) between treatments.

Phe T3						
	Treatment	{1} .03563	{2} .03487	{3} .03062	{4} .04038	{5} .04200
1	Control		0.899	0.671	0.421	0.525
2	ChronicLow	0.899		0.471	0.618	0.618
3	ChronicMedium	0.671	0.471		0.354	0.312
4	ChronicHigh	0.421	0.618	0.354		0.782
5	HighPulse	0.525	0.618	0.312	0.782	

Table CCLXXVI : p-values from SNK test, testing for differences in Phe at T4 (day 100) between treatments.

Phe T4						
	Treatment	{1} .03712	{2} .03737	{3} .03388	{4} .03187	{5} .03825
1	Control		0.954	0.450	0.441	0.962
2	ChronicLow	0.954		0.691	0.573	0.838
3	ChronicMedium	0.450	0.691		0.641	0.734
4	ChronicHigh	0.441	0.573	0.641		0.570
5	HighPulse	0.962	0.838	0.734	0.570	

Table CCLXXVII : p-values from SNK test, testing for differences in Aaiba at T3 (day 62) between treatments.

Aaiba T3						
	Treatment	{1} .03112	{2} .02663	{3} .01487	{4} .02950	{5} .03325
1	Control		0.484	0.001	0.678	0.587
2	ChronicLow	0.484		0.005	0.463	0.334
3	ChronicMedium	0.001	0.005		0.002	<.0.001
4	ChronicHigh	0.678	0.463	0.002		0.602
5	HighPulse	0.587	0.334	<.0.001	0.602	

Table CCLXXVIII : p-values from SNK test, testing for differences in Aaiba at T4 (day 100) between treatments.

Aaiba T4						
	Treatment	{1} .03975	{2} .05038	{3} .04537	{4} .04425	{5} .04225
1	Control		0.106	0.543	0.536	0.555
2	ChronicLow	0.106		0.241	0.321	0.231
3	ChronicMedium	0.543	0.241		0.790	0.738
4	ChronicHigh	0.536	0.321	0.790		0.636
5	HighPulse	0.555	0.231	0.738	0.636	

Table CCLXXIX : p-values from SNK test, testing for differences in Ethanolamine at T3 (day 62) between treatments.

Ethanolamine T3						
	Treatment	{1} .02257	{2} .04133	{3} .02900	{4} .02975	{5} .03213
1	Control		0.076	0.355	0.552	0.511
2	ChronicLow	0.076		0.294	0.227	0.190
3	ChronicMedium	0.355	0.294		0.913	0.891
4	ChronicHigh	0.552	0.227	0.913		0.731
5	HighPulse	0.511	0.190	0.891	0.731	

Table CCLXXX : p-values from SNK test, testing for differences in Amm at T3 (day 62) between treatments.

Amm T3						
	Treatment	{1} 3.7805	{2} 3.9579	{3} 3.9092	{4} 4.1627	{5} 3.7859
1	Control		0.586	0.629	0.068	0.970
2	ChronicLow	0.586		0.729	0.151	0.442
3	ChronicMedium	0.629	0.729		0.179	0.382
4	ChronicHigh	0.068	0.151	0.179		0.049
5	HighPulse	0.970	0.442	0.382	0.049	

Table CCLXXXI : p-values from SNK test, testing for differences in Amm at T4 (day 100) between treatments.

Amm T4						
	Treatment	{1} 3.4411	{2} 3.5698	{3} 3.3021	{4} 3.2441	{5} 3.5059
1	Control		0.558	0.269	0.263	0.604
2	ChronicLow	0.558		0.154	0.086	0.609
3	ChronicMedium	0.269	0.154		0.642	0.240
4	ChronicHigh	0.263	0.086	0.642		0.168
5	HighPulse	0.604	0.609	0.240	0.168	

Table CCLXXXII : p-values from SNK test, testing for differences in Hylys1 at T3 (day 62) between treatments.

Hylys1 T3						
	Treatment	{1} .06743	{2} .05883	{3} .05567	{4} .04820	{5} .05250
1	Control		0.580	0.726	0.720	0.765
2	ChronicLow	0.580		0.838	0.899	0.911
3	ChronicMedium	0.726	0.838		0.878	0.838
4	ChronicHigh	0.720	0.899	0.878		0.781
5	HighPulse	0.765	0.911	0.838	0.781	

Table CCLXXXIII : p-values from SNK test, testing for differences in Hylys1 at T4 (day 100) between treatments.

Hylys1 T4						
	Treatment	{1} .12550	{2} .12463	{3} .12700	{4} .13875	{5} .09962
1	Control		0.969	0.947	0.826	0.487
2	ChronicLow	0.969		0.994	0.922	0.272
3	ChronicMedium	0.947	0.994		0.603	0.617
4	ChronicHigh	0.826	0.922	0.603		0.420
5	HighPulse	0.487	0.272	0.617	0.420	

Table CCLXXXIV : p-values from SNK test, testing for differences in Hylys1 at T3 (day 62) between treatments.

Hylys2 T3						
	Treatment	{1} .04975	{2} .03188	{3} .04267	{4} .04583	{5} .04783
1	Control		0.502	0.919	0.934	0.864
2	ChronicLow	0.502		0.339	0.430	0.486
3	ChronicMedium	0.919	0.339		0.777	0.888
4	ChronicHigh	0.934	0.430	0.777		0.858
5	HighPulse	0.864	0.486	0.888	0.858	

Table CCLXXXV : p-values from SNK test, testing for differences in Hylys2 at T4 (day 100) between treatments.

Hylys2 T4						
	Treatment	{1} .02712	{2} .02925	{3} .02775	{4} .03290	{5} .02675
1	Control		0.925	0.913	0.738	0.948
2	ChronicLow	0.925		0.792	0.522	0.971
3	ChronicMedium	0.913	0.792		0.637	0.983
4	ChronicHigh	0.738	0.522	0.637		0.811
5	HighPulse	0.948	0.971	0.983	0.811	

Table CCLXXXVI : p-values from SNK test, testing for differences in Orn at T3 (day 62) between treatments.

Orn T3						
	Treatment	{1} .03025	{2} .02375	{3} .01575	{4} .01462	{5} .03113
1	Control		0.285	0.053	0.061	0.885
2	ChronicLow	0.285		0.190	0.292	0.442
3	ChronicMedium	0.053	0.190		0.852	0.067
4	ChronicHigh	0.061	0.292	0.852		0.065
5	HighPulse	0.885	0.442	0.067	0.065	

Table CCLXXXVII : p-values from SNK test, testing for differences in Orn at T4 (day 100) between treatments.

Orn T4						
	Treatment	{1} .03200	{2} .03913	{3} .02438	{4} .02400	{5} .02850
1	Control		0.072	0.131	0.179	0.369
2	ChronicLow	0.072		0.003	0.003	0.024
3	ChronicMedium	0.131	0.003		0.923	0.291
4	ChronicHigh	0.179	0.003	0.923		0.478
5	HighPulse	0.369	0.024	0.291	0.478	

Table CCLXXXVIII : p-values from SNK test, testing for differences in Lys at T3 (day 62) between treatments.

Lys T3						
	Treatment	{1} .60325	{2} .62825	{3} .65037	{4} .59713	{5} .43888
1	Control		0.769	0.843	0.943	0.140
2	ChronicLow	0.769		0.795	0.928	0.131
3	ChronicMedium	0.843	0.795		0.921	0.112
4	ChronicHigh	0.943	0.928	0.921		0.069
5	HighPulse	0.140	0.131	0.112	0.069	

Table CCLXXXIX : p-values from SNK test, testing for differences in Lys at T4 (day 100) between treatments.

Lys T4						
	Treatment	{1} .53587	{2} .48738	{3} .48563	{4} .57087	{5} .47913
1	Control		0.478	0.740	0.608	0.835
2	ChronicLow	0.478		0.980	0.441	0.992
3	ChronicMedium	0.740	0.980		0.593	0.924
4	ChronicHigh	0.608	0.441	0.593		0.658
5	HighPulse	0.835	0.992	0.924	0.658	

Table CCXC : p-values from SNK test, testing for differences in Muscle His at T3 (day 62) between treatments.

Muscle His T3						
	Treatment	{1} 1.4449	{2} 1.2395	{3} 1.0690	{4} 1.0715	{5} 1.3150
1	Control		0.784	0.740	0.624	0.676
2	ChronicLow	0.784		0.845	0.589	0.808
3	ChronicMedium	0.740	0.845		0.994	0.855
4	ChronicHigh	0.624	0.589	0.994		0.711
5	HighPulse	0.676	0.808	0.855	0.711	

Table CCXCI : p-values from SNK test, testing for differences in Muscle His at T4 (day 100) between treatments.

Muscle His T4						
	Treatment	{1} 1.6645	{2} 1.7154	{3} 1.5041	{4} 1.7229	{5} 1.6206
1	Control		0.684	0.409	0.886	0.726
2	ChronicLow	0.684		0.337	0.952	0.727
3	ChronicMedium	0.409	0.337		0.410	0.354
4	ChronicHigh	0.886	0.952	0.410		0.843
5	HighPulse	0.726	0.727	0.354	0.843	

Table CCXCII : p-values from SNK test, testing for differences in Ans at T3 (day 62) between treatments.

Ans T3						
	Treatment	{1} .81950	{2} .71650	{3} .74687	{4} .67063	{5} .72088
1	Control		0.293	0.214	0.093	0.213
2	ChronicLow	0.293		0.858	0.430	0.940
3	ChronicMedium	0.214	0.858		0.551	0.653
4	ChronicHigh	0.093	0.430	0.551		0.659
5	HighPulse	0.213	0.940	0.653	0.659	

Table CCXCIII : p-values from SNK test, testing for differences in Ans at T4 (day 100) between treatments.

Ans T4						
	Treatment	{1} .84000	{2} .83613	{3} .80688	{4} .91712	{5} .97050
1	Control		0.929	0.725	0.083	0.013
2	ChronicLow	0.929		0.503	0.161	0.018
3	ChronicMedium	0.725	0.503		0.069	0.005
4	ChronicHigh	0.083	0.161	0.069		0.225
5	HighPulse	0.013	0.018	0.005	0.225	

Table CCXCIV : p-values from SNK test, testing for differences in Arg at T3 (day 62) between treatments.

Arg T3						
	Treatment	{1} .14913	{2} .12338	{3} .11425	{4} .10688	{5} .10937
1	Control		0.235	0.244	0.295	0.261
2	ChronicLow	0.235		0.671	0.865	0.789
3	ChronicMedium	0.244	0.671		0.936	0.820
4	ChronicHigh	0.295	0.865	0.936		0.907
5	HighPulse	0.261	0.789	0.820	0.907	

Table CCXCV : p-values from SNK test, testing for differences in Arg at T4 (day 100) between treatments.

Arg T4						
	Treatment	{1} .14775	{2} .13863	{3} .13775	{4} .17062	{5} .12313
1	Control		0.742	0.930	0.412	0.808
2	ChronicLow	0.742		0.975	0.483	0.841
3	ChronicMedium	0.930	0.975		0.635	0.599
4	ChronicHigh	0.412	0.483	0.635		0.433
5	HighPulse	0.808	0.841	0.599	0.433	

Table CCXCVI : p-values from SNK test, testing for differences in SUM free amino acids (FAA) at T3 (day 62) between treatments.

SUM FAA T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
		47.340	46.326	45.450	45.195	45.512
1	Control		0.620	0.787	0.826	0.643
2	ChronicLow	0.620		0.902	0.944	0.690
3	ChronicMedium	0.787	0.902		0.901	0.976
4	ChronicHigh	0.826	0.944	0.901		0.987
5	HighPulse	0.643	0.690	0.976	0.987	

Table CCXCVII : p-values from SNK test, testing for differences in SUM free amino acids (FAA) at T4 (day 100) between treatments.

SUM FAA T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
		46.415	45.852	45.933	45.552	45.490
1	Control		0.923	0.745	0.935	0.969
2	ChronicLow	0.923		0.957	0.840	0.967
3	ChronicMedium	0.745	0.957		0.964	0.990
4	ChronicHigh	0.935	0.840	0.964		0.967
5	HighPulse	0.969	0.967	0.990	0.967	

Table CCXCVIII : p-values from SNK test, testing for differences in Sampled Fish Weight at T3 (day 62) between treatments.

Sampled Fish weight T3						
	Treatment	{1}	{2}	{3}	{4}	{5}
		90.375	86.625	82.650	80.750	103.20
1	Control		0.775	0.824	0.880	0.330
2	ChronicLow	0.775		0.761	0.894	0.418
3	ChronicMedium	0.824	0.761		0.885	0.401
4	ChronicHigh	0.880	0.894	0.885		0.430
5	HighPulse	0.330	0.418	0.401	0.430	

Table CCXCIX : p-values from SNK test, testing for differences in Sampled Fish Weight at T4 (day 100) between treatments.

Sampled Fish weight T4						
	Treatment	{1}	{2}	{3}	{4}	{5}
		146.75	144.15	136.97	133.13	154.63
1	Control		0.917	0.917	0.945	0.751
2	ChronicLow	0.917		0.772	0.896	0.905
3	ChronicMedium	0.917	0.772		0.877	0.890
4	ChronicHigh	0.945	0.896	0.877		0.905
5	HighPulse	0.751	0.905	0.890	0.905	

Table CCC : p-values from SNK test, testing for differences in Lens Weight at T3 (day 62) between treatments.

Lens weight T3						
	Treatment	{1} .04501	{2} .05349	{3} .04737	{4} .03845	{5} .05004
1	Control		0.132	0.536	0.091	0.388
2	ChronicLow	0.132		0.251	0.003	0.367
3	ChronicMedium	0.536	0.251		0.060	0.485
4	ChronicHigh	0.091	0.003	0.060		0.021
5	HighPulse	0.388	0.367	0.485	0.021	

Table CCCI : p-values from SNK test, testing for differences in Lens Weight at T4 (day 100) between treatments.

Lens weight T4						
	Treatment	{1} .06146	{2} .06205	{3} .06284	{4} .06139	{5} .06350
1	Control		0.884	0.937	0.985	0.956
2	ChronicLow	0.884		0.845	0.985	0.930
3	ChronicMedium	0.937	0.845		0.984	0.870
4	ChronicHigh	0.985	0.985	0.984		0.984
5	HighPulse	0.956	0.930	0.870	0.984	

9.5 Kolmorogov smirnof test for normality

Table CCCII : Test result from Kolmogorov-Smirnov test for normality. The distributions of measured Weight were analyzed at T0 (day 0), T3 (day 62) and T4 (day 100).

Treatment	Weight T0			Weight T3			Wieght T4		
	N	d	P	N	d	P	N	d	P
Control	59	0.118	n.s	56	0.172	<0.10	52	0.209	<0.05
ChronicLow	60	0.195	<0.05	45	0.124	n.s	42	0.107	n.s
ChronicMedium	60	0.114	n.s	54	0.218	<0.05	48	0.257	<0.01
ChronicHigh	60	0.086	n.s	52	0.258	<0.01	40	0.283	<0.05
HighPulse	60	0.091	n.s	47	0.194	<0.10	41	0.128	n.s

Table CCCIII : Test result from Kolmogorov-Smirnov test for normality. The distributions of measured SGR were analyzed at T0-T3 (day 0-62), T3-T4 (day 62-100) and T0-T4 (day 0-100).

Treatment	SGR1			SGR2			SGR Overall		
	N	d	P	N	d	P	N	d	P
Control	56	0.216	<0.05	52	0.215	<0.05	52	0.281	<0.01
ChronicLow	45	0.164	<0.20	42	0.133	n.s	42	0.174	<0.20
ChronicMedium	54	0.147	<0.20	48	0.140	n.s	48	0.144	n.s
ChronicHigh	52	0.340	<0.01	40	0.122	n.s	40	0.256	<0.05
HighPulse	47	0.363	<0.01	41	0.147	n.s	41	0.269	<0.01

Table CCCIV : Test result from Kolmogorov-Smirnov test for normality. The distributions of measured blood parameters were analyzed at T0 (day 0), T3 (day 62) and T4 (day 100).

Variable	T0			T3			T4		
	N	d	P	N	d	P	N	d	P
Blood Na^+	19	0.132	n.s	40	0.109	n.s	40	0.169	<0.20
Blood K^+	19	0.156	n.s	39	0.118	n.s	39	0.185	<0.15
Blood pHtp	19	0.103	n.s	39	0.131	n.s	40	0.091	n.s
Blood pCO_2 tp	19	0.243	<0.20	39	0.142	n.s	40	0.173	<0.20
Blood HCO_3^-	19	0.097	n.s	39	0.160	n.s	40	0.140	n.s
Sampled fish weight	20	0.162	n.s	40	0.091	n.s	40	0.113	n.s
Lens weight	20	0.266	<0.10	39	0.221	<0.05	40	0.177	<0.20
Lense His	20	0.222	n.s	39	0.118	n.s	40	0.114	n.s
NAH	20	0.124	n.s	39	0.070	n.s	40	0.100	n.s
Phser	20	0.109	n.s	40	0.263	<0.01	40	0.126	n.s
Tau	20	0.183	n.s	40	0.106	n.s	40	0.078	n.s
Pea	20	0.190	n.s	40	0.075	n.s	40	0.185	<0.15
Urea	20	0.166	n.s	40	0.098	n.s	40	0.069	n.s
Asp	20	0.138	n.s	40	0.120	n.s	40	0.111	n.s
Hypro	20	0.171	n.s	40	0.825	n.s	40	0.112	n.s
Thr	20	0.139	n.s	40	0.094	n.s	40	0.099	n.s
Ser	20	0.170	n.s	40	0.087	n.s	40	0.102	n.s
Asn	20	0.177	n.s	40	0.141	n.s	40	0.177	<0.20
Glu	20	0.234	<0.20	40	0.136	n.s	40	0.140	n.s
Gln	20	0.142	n.s	40	0.735	n.s	40	0.108	n.s
Sarc	5	0.240	n.s	40	0.142	n.s	39	0.120	n.s
Pro	20	0.331	<0.05	40	0.140	n.s	40	0.124	n.s
Gly	20	0.102	n.s	40	0.085	n.s	40	0.086	n.s
Ala	20	0.130	n.s	40	0.086	n.s	40	0.065	n.s
Citr	20	0.234	<.020	40	0.177	<0.20	40	0.116	n.s
Aaba	20	0.084	n.s	40	0.098	n.s	40	0.149	n.s
Val	20	0.168	n.s	40	0.177	<0.20	40	0.126	n.s
Met	20	0.238	<0.20	40	0.205	<0.10	40	0.120	n.s
Cysth2	20	0.151	n.s	40	0.082	n.s	40	0.086	n.s
Ile	20	0.133	n.s	40	0.171	<0.20	40	0.089	n.s
Leu	20	0.153	n.s	40	0.175	<0.20	40	0.090	n.s
Tyr	20	0.165	n.s	40	0.152	n.s	40	0.061	n.s
B-ala				5	0.183	n.s			
Phe	20	0.134	n.s	40	0.206	<0.10	40	0.086	n.s
Aaiba	20	0.141	n.s	40	0.097	n.s	40	0.102	n.s
Ethanolamine				29	0.147	n.s	28	0.284	<0.05
Amm	20	0.216	n.s	40	0.157	n.s	40	0.113	n.s
Hylys1	10	0.256	n.s	30	0.115	n.s	40	0.108	n.s
Hylys2	20	0.128	n.s	34	0.096	n.s	40	0.011	n.s
Orn	20	0.191	n.s	40	0.108	n.s	40	0.144	n.s
Lys	20	0.198	n.s	40	0.060	n.s	40	0.104	n.s
Muscle His	20	0.175	n.s	40	0.074	n.s	40	0.110	n.s
Ans	20	0.158	n.s	40	0.114	n.s	40	0.094	n.s
Arg	20	0.245	<0.20	40	0.113	n.s	40	0.154	n.s
SUM FAA	20	0.118	n.s	40	0.098	n.s	40	0.105	n.s

9.6 Levene's test

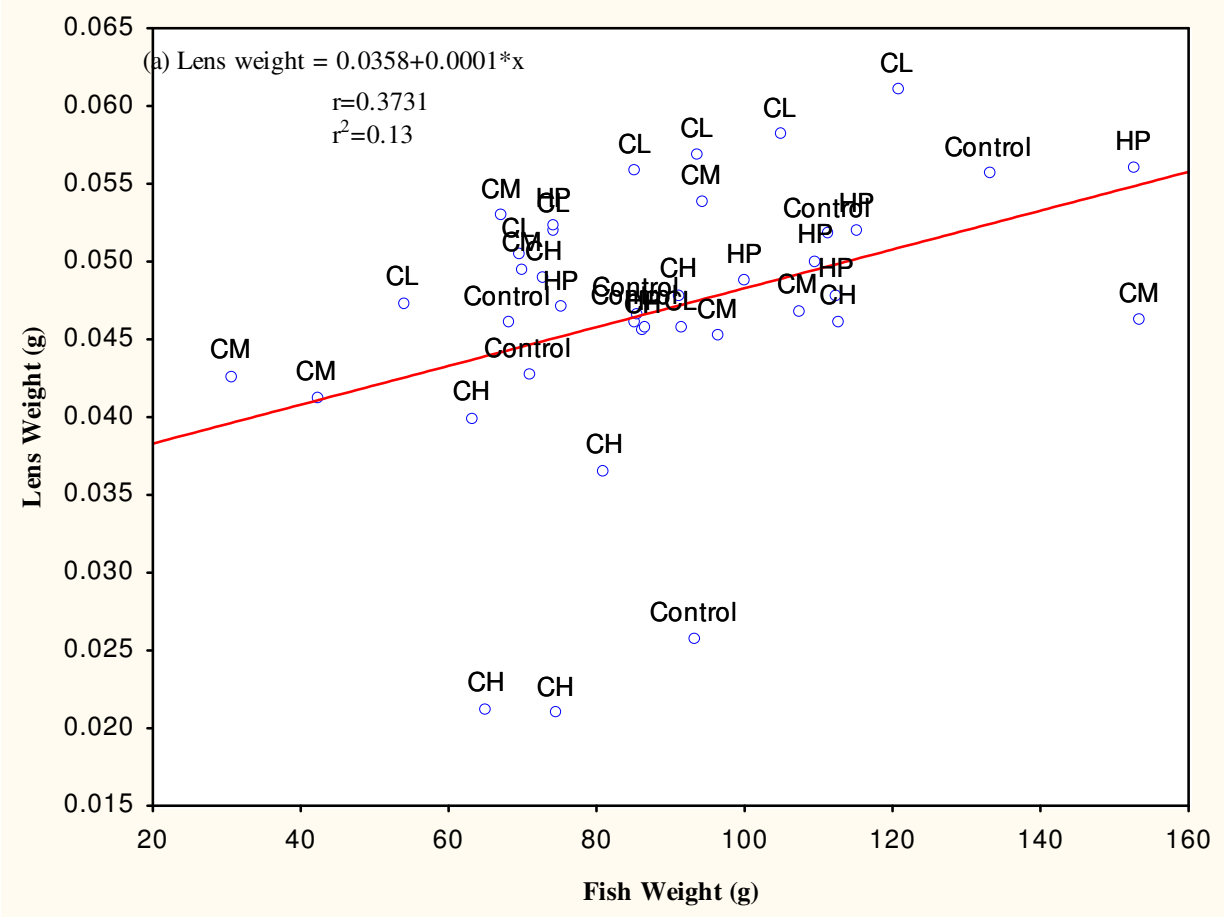
Table CCCV : Test results from Levene's test performed on observations of all response variables, for each sampling date, or period.

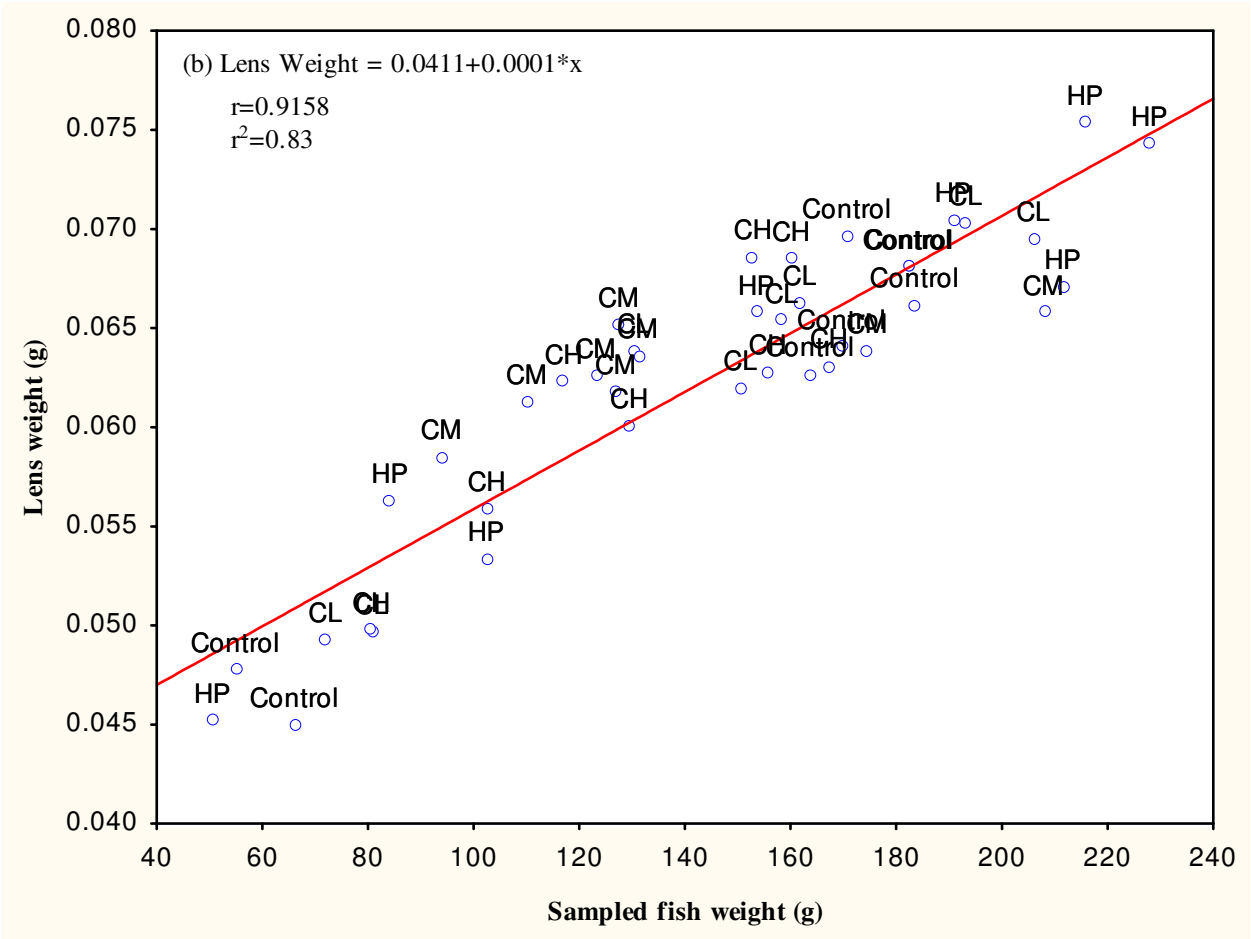
Levene's test for homogenity of variance				
Variable	MS Effect	MS Error	F	p
Weight T0	116.352	61.900	1.880	0.114
Weight T3	261.016	263.198	0.992	0.413
Weight T4	913.722	642.660	1.422	0.228
SGR1	0.062	0.045	1.386	0.239
SGR2	0.257	0.080	3.224	0.013
SGR Overall	0.042	0.032	1.314	0.266
Blood Na^+ T3	3.733	7.025	0.531	0.713
Blood Na^+ T4	5.162	6.250	0.826	0.518
Blood K^+ T3	0.054	0.064	0.841	0.509
Blood K^+ T4	0.341	0.112	3.043	0.030
Blood pHtp T3	0.002	0.002	1.170	0.341
Blood pHtp T4	0.000	0.001	0.656	0.627
Blood pCO_2 tp T3	0.257	0.129	1.991	0.118
Blood pCO_2 tp T4	0.359	0.107	3.357	0.020
Blood HCO_3^- T3	0.298	0.172	1.726	0.167
Blood HCO_3^- T4	0.047	0.136	0.347	0.844
Sampled Fish Weight T3	377.809	228.080	1.656	0.182
Sampled Fish Weight T4	1302.547	618.772	2.105	0.101
Sampled Lens Weight T3	<0.001	<0.001	2.753	0.044
Sampled Lens Weight T4	<0.001	<0.001	3.299	0.021
Lens His T3	0.034	0.008	4.440	0.005
Lens His T4	0.038	0.008	4.741	0.004
NAH T3	0.358	0.325	1.100	0.372
NAH T4	1.458	0.738	1.974	0.121

Table CCCVI: Test results from Levene's test performed on observations of all response variables, for each sampling date, or period.

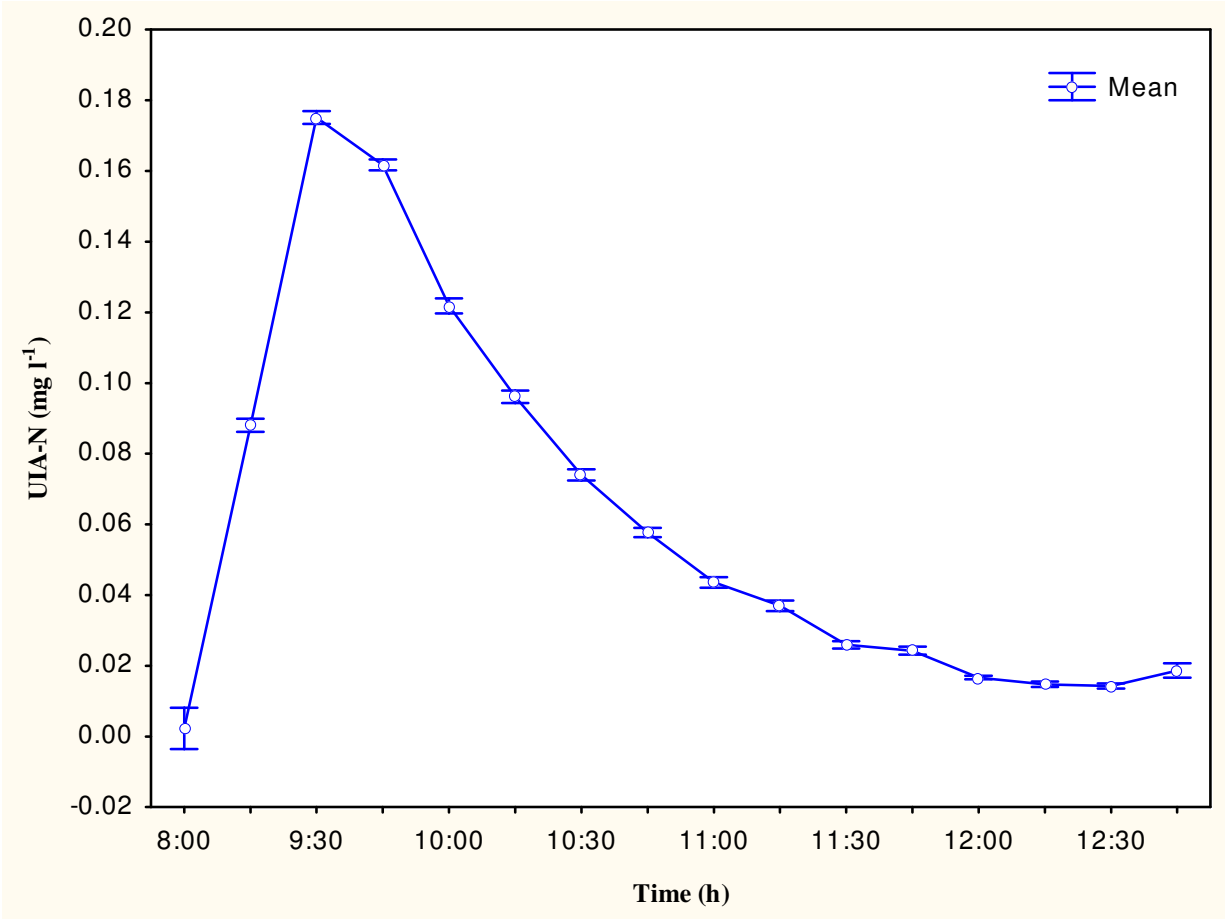
Levene's test for homogenity of variance				
Variable	MS Effect	MS Error	F	p
Phser T3	<0.001	<0.001	2.620	0.051
Phser T4	<0.001	<0.001	0.823	0.520
Tau T3	0.927	1.919	0.483	0.748
Tau T4	1.602	1.309	1.224	0.319
Pea T3	<0.001	<0.001	1.179	0.337
Pea T4	<0.001	<0.001	0.858	0.499
Urea T3	0.135	0.332	0.407	0.802
Urea T4	0.644	0.247	2.609	0.053
Asp T3	0.011	0.008	1.512	0.220
Asp T4	0.000	0.007	0.039	0.997
Hypro T3	0.041	0.024	1.749	0.161
Hypro T4	0.010	0.029	0.338	0.850
Thr T3	0.037	0.050	0.729	0.578
Thr T4	0.089	0.038	2.366	0.072
Ser T3	0.087	0.060	1.461	0.235
Ser T4	0.032	0.027	1.207	0.326
Asn T3	0.296	0.055	5.431	0.002
Asn T4	0.241	0.112	2.159	0.095
Glu T3	0.014	0.007	2.187	0.091
Glu T4	0.009	0.005	1.742	0.164
Gln T3	0.004	0.011	0.377	0.823
Gln T4	0.015	0.016	0.914	0.467
Sarc T3	<0.001	<0.001	0.877	0.487
Sarc T4	<0.001	<0.001	0.941	0.452
Pro T3	0.458	0.290	1.579	0.202
Pro T4	0.550	0.185	2.966	0.033
Gly T3	0.502	0.149	3.360	0.020
Gly T4	0.179	0.196	0.916	0.466
Ala T3	0.219	0.116	1.889	0.134
Ala T4	0.027	0.089	0.305	0.873
Citr T3	0.002	0.002	1.092	0.376
Citr T4	0.001	0.001	0.782	0.544
Aaba T3	<0.001	<0.001	1.234	0.314
Aaba T4	<0.001	<0.001	1.684	0.176
Val T3	<0.001	<0.001	0.118	0.975
Val T4	<0.001	<0.001	1.377	0.262
Met T3	<0.001	<0.001	1.131	0.358
Met T4	<0.001	<0.001	0.747	0.567
Cysth2 T3	0.081	0.052	1.556	0.208
Cysth2 T4	0.013	0.009	1.476	0.230
Ile T3	<0.001	<0.001	0.233	0.918
Ile T4	<0.001	<0.001	0.345	0.846
Leu T3	<0.001	0.001	0.201	0.936
Leu T4	<0.001	<0.001	0.261	0.901
Tyr T3	<0.001	<0.001	1.513	0.220
Tyr T4	<0.001	<0.001	0.582	0.678
Phe T3	<0.001	<0.001	0.796	0.536
Phe T4	<0.001	<0.001	1.497	0.224
Aaiba T3	<0.001	<0.001	1.738	0.164
Aaiba T4	<0.001	<0.001	0.287	0.884
Ethanolamine T	<0.001	<0.001	1.500	0.255
Amm T3	0.035	0.017	2.127	0.131
Amm T4	0.086	0.026	3.353	0.020
Hylys1 T3	<0.001	<0.001	1.213	0.349
Hylys1 T4	0.001	0.001	1.833	0.145
Hylys2 T3	<0.001	<0.001	2.139	0.130
Hylys2 T4	<0.001	<0.001	0.743	0.569
Orn T3	<0.001	<0.001	1.107	0.392
Orn T4	<0.001	<0.001	3.269	0.022
Lys T3	0.004	0.004	1.071	0.407
Lys T4	0.003	0.007	0.385	0.818
His T3	0.014	0.090	0.158	0.958
His T4	0.048	0.021	2.276	0.081
Ans T3	0.008	0.003	2.771	0.042
Ans T4	0.001	0.003	0.336	0.852
Arg T3	0.001	0.001	2.660	0.049
Arg T4	0.001	0.002	0.978	0.432
SUM FAA T3	4.143	6.378	0.650	0.631
SUM FAA T4	0.425	2.241	0.190	0.942

10 APPENDIX IV


(a) Lens weight = 0.0358+0.0001*x
r=0.3731
r²=0.13
Lens Weight (g)
Fish Weight (g)
0.065
0.060
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
20
40
60
80
100
120
140
160
CL
CM
HP
CH
Control



Figur I: Lens weight mean regression for each treatment. Each datapoint from individually sampled fish n=8 per treatment. (a) day 62: Lens Weight=0.038+0.0001*x, p=0.0193, (b) Lens Weight=0.411+0.0001*x, p=0.0000.



Figur II: Measured mean values of UIA—N (mg l^{-1}) in HighPulse group during the experimental days.

Table I: Results calculated growth coefficient V=S.D/Mean (Zar, 1984) for each treatment and sampling date. Calculated from all fish, tagged and untagged, between all treatment groups.

Growth coefficient V=S.D/Mean					
Treatment	W 21/10	W 11/11	W 2/12	W 22/12	W 29/01
Control	0	0	0	0	0
ChronicLow	0.3236	0.3330	0.3315	0.3300	0.3662
ChronicMedium	0.33475	0.32701	0.36071	0.38175	0.39154
ChronicHigh	0.318959	0.340282	0.354139	0.353037	0.398113
HighPulse	0.329559438	0.324211443	0.321384285	0.333219813	0.349176696