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[DEATH AS A CRITICAL TRANSITION]

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1. Introduction

1.1 Critical transition

Researchers have shown that in many complex systems there are certain points in which a minor perturbation can trigger a drastic change. These are so-called critical transitions.

To understand what critical transitions are, it is important to understand what dynamical systems are. Dynamical systems can be understood as a result of a balance of processes in which a stable state or equilibrium is created. A good example that concerns us all is physiological homeostasis. Homeostasis is the property of a system that regulates its internal environment and tends to maintain a stable, a constant condition. (e.g. temperature)
This means that the overall change of rate at equilibrium is zero. Such a stable state is also called an attractor.

Some systems however, can have several alternative attractors, which are mostly separated by unstable repelling points. Due to these repelling points a system can become so fragile that a minor perturbation can evoke a drastic change in which a system shifts from one attractor to another. These system shifts or transitions are irreversible and therefore called critical transitions.

Critical transitions have been found in many systems from financial systems to ecosystems.

Scheffer, *et al. (* 2009) describes that if a system has an alternative stable state, the curve that describes this system is typically folded like in figure 1.
The arrows in this graph indicate the direction in which the system moves if it is not on the curve. In this figure three states exist, however the middle dashed line is an unstable state.

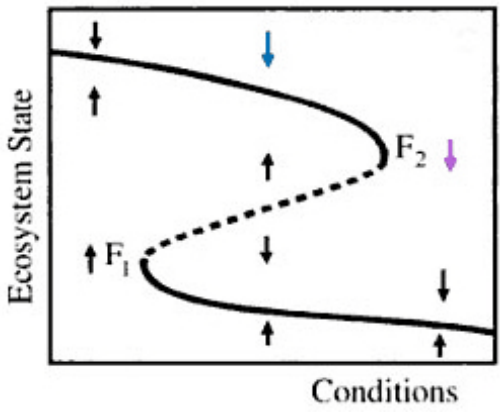


Figure 1. Folded curve. Modified from Scheffer, et al., (2009), box 1C.

Thus if the system changes from a stable state to the state that is near the blue arrow resilience will occur, which is the ability of a system to recover to the original state upon a disturbance. In this case that means that the system will move back to the stable equilibrium, the upper solid line. However if the system is, for example, in a state near F2, like the purple arrow, the system will shift forward and thus down, to the unstable state and move further to the stable equilibrium in the bottom branch. Because the bottom branch is a stable equilibrium it is difficult to reverse this shift. This is how a minor perturbation can trigger a drastic change, thus a critical transition. The point that marks a threshold at which the system changes from a stable state to another stable state and thus causes a critical transition is called a bifurcation point. In figure 1 for example, point F1 and F2 are the threshold bifurcation points at which a critical transition may occur. Research has yielded

important clues that have been suggested as indicators of whether a system is getting close to a critical threshold. These indications may give us more insight about the system itself and we may be able to avoid unwanted catastrophic critical transitions by predicting whether the system is near a critical threshold.
The most important indicator that occurs before bifurcations that cause a critical transition is known as critical slowing down. In systems like in figure 1, critical slowing down occurs, which means that the system approaches such critical points that the recovery rate from bifurcation points is close zero.
A consequence of slowing down is that the variation and the rate in fluctuation is increased. Because the recovery rate is slow, the fluctuations accumulate and cause an increase of variation. Also the asymmetry of fluctuations may increase before a catastrophic bifurcation.
So with respect to these indications we can try to predict whether a system is near a critical transition.

1.2 Senescence

Aging or senescence is defined as a decline in physiological functioning with age accompanied by a decrease in reproductive performance and an increase in mortality rate (Rose, M. R, 1991). Senescence is a complex process and affects most, if not all tissues and organs of the body. This phenomenon arises from the accumulation of damage resulting from a lack of capacity to protect, maintain and repair somatic tissues over time. The inability to maintain and repair somatic tissues over time leads to damage and consequently to the loss of function, which ultimately leads to death. Although the rate of senescence varies in species and individuals as a result of genetic, epigenetic and environmental variation as well as of stochastic events, (Murphy, M. P and Patridge, L., 2008) in many organisms mortality increases roughly exponentially over the main part of adulthood (Finch, C. E., 1990).
Recent research suggests that the senescence is associated with an accumulation of damage produced by reactive oxygen species (ROS). (Moe et al, 2009)
Senescence has also been associated with a weakened efficacy and/or availability of enzymatic antioxidants (Alonso-Alvarez et al., 2006; Beckman and Ames, 1998; Finkel and Holbrook, 2000; Torres and Velando, 2007; Wickens, 2001)
To counteract the negative effect of ROS, aerobic organisms have evolved a defense mechanisms like enzymatic catalase (CAT), peroxidases (GPX) and non-enzymatic scavengers. The non-enzymatic scavengers are mostly acquired with food. (carotenoids, vitamins E and C)

2. Aim

The aim of this thesis is to investigate whether death can be explained as a so-called critical transition by identifying objective criteria that would predict death in scientific literature.

If death can be explained as a critical transition it is expected that the indications that occur before a critical transition will also occur before death. Moreover, given that the mortality of organisms increases roughly exponentially with senescence, we hypothesize that the occurrence of these indications will also increase in occurrence with (accelerated) senescence.
So we expect that indications such as critical slowing down, a slow recovery rate and an increase of variation will occur before death and with accelerated senescence.

To compare the variance between age categories, the coefficient of variation has been calculated. This has been done by dividing the standard deviation by the mean.

(standard deviation = standard error of the mean $\times \sqrt{}$ n)
(coefficient of variation = $\frac{standard\ \ deviation}{mean}$)

Findings from research has been divided into three categories:

- Gene expression
- Physiological patterns
- Induced activation of the immune response.

3. Gene variation

An increase in variation is one of the indicators that predict a critical transition. Given that the mortality of organisms increases roughly exponentially with senescence, we hypothesize that the variation also increases with senescence. One possible factor in which variation can increase is gene expression. The deregulation of gene expression can cause DNA damage that leads to cellular damage and degeneration, the accumulation of somatic DNA damage has been implicated as a cause of ageing.

Bahar *et al.* (2006) investigated cell-to-cell variation in gene expression in ageing mouse heart. Heart cardiomyocytes were isolated from a similar area of ventricular heart tissue of 6months old and 27 months male mice. The genes that were selected were seven housekeeping genes, three heart-specific genes, two protease-encoding genes and three mitochondrial genes. The results of this study indicated a highly significant increase in cell-to-cell variation in the expression levels of all nuclear genes in the old animals as compared with the young animals.

Furthermore noise is inherent in the basic process of transcription, especially for genes expressed at low levels. Therefore they considered the possibility that alterations in the somatic genome, for example as induced by ROS, could contribute to increased stochasticity of gene expression.

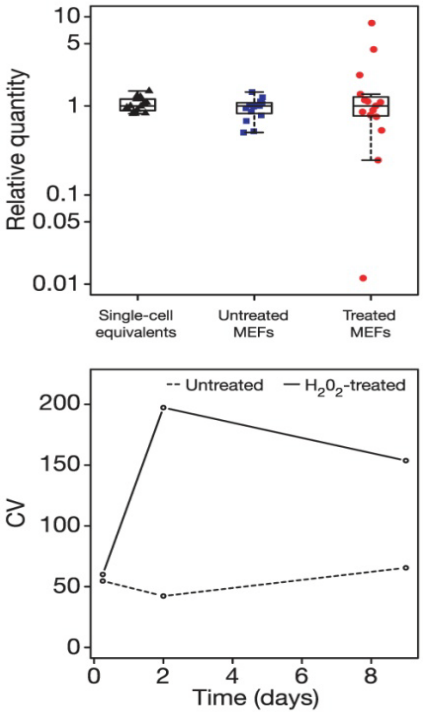


Figure 2. Source: Bahar, et al. (2006), Figure 3A and 3C.
relative quantity: cell to cell variation between treated and untreated mouse embryonic fibroblasts at 48 h after treatment H_2O_2. The variation among the treated cells is significantly greater than among the untreated cells (P = 0.0001)
CV: Coefficient of variation calculated for three housekeeping genes

To confirm that alterations in the somatic genome, induced by reactive oxygen species lead to increased stochasticity of gene expression in cells they treated cultured mouse embryonic fibroblasts with 0,1mM hydrogen peroxide (H_2O_2), which is a known generator of oxidative damage. As said before reactive oxygen species is a known contributor to accumulative damage in senescence, therefore H_2O_2 is a good replicate of senescence.

As compared with single-cell equivalents, untreated cells showed a trend (p = 0.0847) towards cell-to-cell variation in gene expression. At 48 hours after the treatment with H_2O_2 , cell-to-cell variation in gene expression greatly increased. In figure 2, the cell to cell variation in relative expression of Actb (housekeeping gene) is shown, 48 hours after the treatment with H_2O_2 in mice embryonic fibroblasts.

Also the coefficient of variation was calculated for three of the housekeeping genes. (see figure 2)
As shown the coefficient of variation is greatly increased in the samples treated with H_2O_2.

Genes however do not work alone, but rather act within a functional group or pathway.
One of effect of senescence may be to diminish the coherence in expression of gene pathways. Research investigated if this is a possible effect of senescence by comparing co-expression network between young and old mice. (Southworth, L.K., Owen, A.B., Kim, S.K., 2009) In this study they used the gene expression data from 16- and 24-month old mice.

In addition to grouping genes based on co-expression they also grouped genes based on shared genetic functions. These are defined by Gene Ontology classification (GO). They used gene sets that shared the same GO molecular function, associated cellular component or biological process. Using GO categories is useful because they provide a functional grouping of related genes. Genes with similar function are often co-expressed. Among other findings, they found that the GO category selenium decreases with age. Selenium is a trace element that acts as a cofactor for reduction of antioxidant enzymes. Furthermore research suggested that low levels of selenium may be a risk factor for developing cancer in humans (Bjelakovic, G., et al., 2004)
They also investigated transcription factors, if transcription factors lose the ability to co-regulate a group of genes with age, a decline in correlation in expression between those genes and age is expected. Transcription factors contain DNA binding domains that attach to a specific sequence of DNA. They found that transcription factors containing binding sites for NF-kB and MEF-2 were significantly decreased with age.

NF-kB has many functions, it plays a role in ensuring the redox homeostasis. In addition, the age-associated increase of ROS production might be involved in many age-associated diseases such as cardiovascular, neurodegenerative and inflammatory diseases and cancer. (Gosselin, K. and Abbadie, C., 2003).
NF-kB is considered as the central regulator of innate and acquired immunity, inflammation and wound healing, it also plays an important role in apoptosis, which is the programmed death in cells. (Caamano, J. and Hunter, C. A., 2002; Pahl, 1999) Many research have shown that NF-kB has an anti-apoptotic effect. (Liu, Z. *et al*., 1996; Zong W. X., *et al*., 1998; Kothny-Wilkes, G., *et al*., 1999; Bernard, D., *et al*., 2001 a,b). The findings that the transcription factor containing binding sites for NF-kB were decreased with age most likely results in a decline in expression of NF-kB. This decrease in NF-kB might play an important role in age-associated diseases.
Furthermore MEF2 is a muscle-specific transcription factor that has been shown to increase binding affinity with oxidative stress in human primary skeletal muscle cells. (Al-Khalili, L., *et al.*, 2004)
They also found a cluster enriched for DNA-damage genes. The clustering distance were increased with age. This might be due to the increasing DNA-damage with age, it is possible that DNA-pathways are more frequently triggered in old age and therefore producing a more coordinated transcriptional response.

So not only is there an increase in the variability in expression levels in cell-to-cell expression levels associated with senescence, Southworth *et al.,* showed that there are also large-scale changes in gene co-expression. The decrease in cooperation in many genes and the inability to co-regulate a group of genes are associated with senescence and involved in many age-associated diseases. This can cause a delay in recovery from many diseases, a slower recovery rate from bifurcation points.

4. Physiological patterns

As said before one of the indicators that predict a critical transition is an increase of variation. This occurs because the rate of recovery slows down near a critical transition. If death can be explained as a critical transition, it is expected that the recovery rate from bifurcation points will decrease with age and that the variance increases with age.

4.1 Moribund salmon

Recently Hruska, K. A., *et al*. (2010) investigated the rapid-senescence phenomenon in terms of the physiological stress/cortisol hyper secretion and energy exhaustion hypotheses in salmon. This hypothesis predicts that fish will display elevated levels of stress metabolites and an imbalance of plasma ions when they become moribund. Furthermore, individuals with a higher level of physiological stress should die earlier after arrival at the spawning grounds.
To investigate this they assessed ions, stress metabolites, reproductive hormones and measured the energy from arrived salmon and moribund salmon. (see appendix 1)
From this table, the coefficient of variation has been calculated for females and males (see figure 3A and 3B respectively)

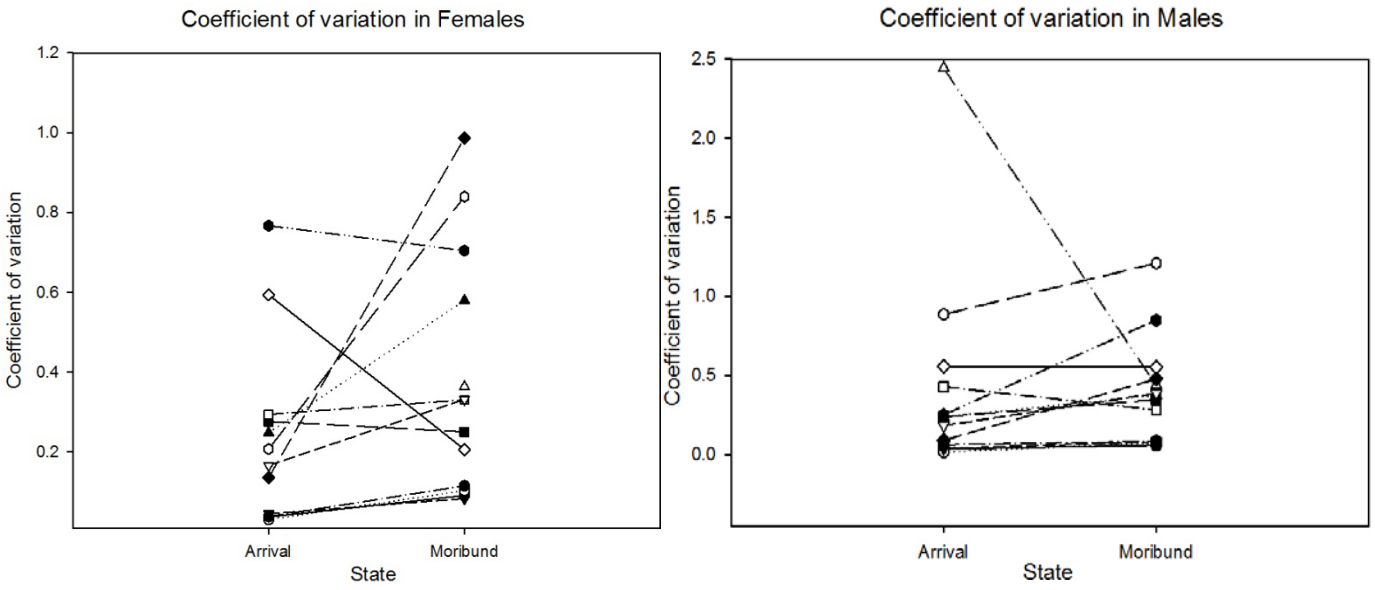


Figure 3A; The coefficient of variation in females; Calculated from Hruska, K. A., et al. (2010), Table 1.

For the calculations see appendix 1.

Figure 3B. The coefficient of variation in males; Calculated from Hruska, K. A., et al. (2010), Table 1.

For the calculations see appendix 1.

Figure 3A shows that for 8 out of 10 variables the coefficient of variation increases in female moribund salmon and figure 3B shows that for 9 out of 10 variables the coefficient of variation increases in male moribund salmon. The coefficient of variation of plasma osmolality, plasma concentrations of Cl^- and Na^+ increases as well as, glucose, testosterone, 11-ketotesterone and the energy in both sexes. The plasma concentrations of K^+ and lactate also increases in females contrast to males.
In males however, the gill Na^+ /K^+/ATPase activity increases as well as 17-20 β-progesterone in contrast to females. The cortisol however decreases in both sexes.

4.2 Iguanas

Romero, L. M and Wikelski, M. (2010) investigated iguanas on the Galapagos. During El Niño (a global climate event) marine iguanas are food-stressed and many individuals die of starvation. Romero and Wikelski hypothesized that corticosterone secretion helps individuals survive, as predicted by ecological theories of stress and biomedical theories of corticosterone physiology. To investigate this they assessed 4 aspects of corticosterone titers:

- the initial unstressed corticosterone titers (basic energy metabolism)
- each individuals response to a standardized stressor, which is capture and handling the iguanas
- the inter-renal maximal ability to secrete corticosterone, by injecting exogenous ACTH
- the ability of the iguana to regulate the termination of corticosterone response via negative feedback (DEX)

Dexamethasone (DEX) is a synthetic glucocorticoid that artificially stimulates negative feedback. Therefore if the feedback functions normally this would result in a decrease in circulating endogenous corticosterone titres.

Prior to release in early December 2002, they weighed each animal and measured their snout-to-vent length. They also assessed a body condition index. The body condition index was calculated by $\frac{Body\ mass}{snout-vent\ lengt\ h^3} \times 10^6$
All iguanas were also given a small identification brand on their flanks for later targeted recapturing and a unique brand on their bellies for individual identification.

El Niño struck the Galápagos in late December/early January of 2002/2003. They returned in July 2003 and captured every previously marked animal that could be found over the course of a week (77%).
They collected baseline and stress-induced samples (30 min) and injected 14 with DEX and the rest with ACTH. The iguanas that were previously marked but not found were classified as having died (23%). Given the strong site fidelity of the iguanas and that El Niños are the only source of mortality of adults on this island it is unlikely that many iguanas were misclassified.

They found that all animals were in excellent body condition prior to the El Niño and there was no difference between iguanas that ended up surviving or dying (t= 0.21, p-= 0.83).

There was also no difference in baseline corticosterone (t=1.50, d.f. = 24.49, p =0.15), corticosterone concentrations after 30 min of restraint or in the capacity to respond to a stressor as shown by the response to ACTH prior to El Niño .

Because the baseline corticosterone are similar in iguanas that are destined to die compared to iguanas that are destined to survive and they respond equally to we can assume that the termination of corticosterone via negative feedback is present in all of the iguanas.

Remarkably however, is that the iguanas that died had a reduced corticosterone concentrations in response to DEX injections compared with the uninjected controls, but a significant higher corticosterone concentrations compared to the iguanas that did survive. This means that the iguanas that did not survive had a weak response to regulate the termination of corticosterone via negative feedback. The iguanas that survived had a significantly strong response. (t = 2.40, d.f. = 37, p = 0.022) (See figure 4)

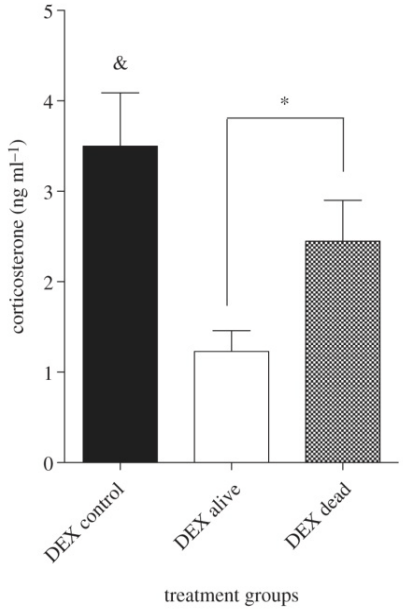


Figure 4. The corticosterone concentrations ng/ml. Iguanas that do not survive have a weak response to regulate the termination of corticosterone via negative feedback in contrast to iguanas that did survive.

Source: Romero, L. M and Wikelski, M. (2010), Figure 2.

Because the termination of corticosterone via negative feedback is present, it is expected that this elevated concentration in corticosterone in the iguanas that did not survive is due to a slower recovery rate in contrast to the iguanas that did survive. This is one of the indications that happen before a critical transition occurs.

4.3 Mice

Another recent study that has been done on identifying indicators that predict death has been done by Ray, M. A., *et al.* (2010), 'Identification of markers for Imminent Death in Mice used in Longevity and Aging Research'. Their goal of this study was to identify practical and objective criteria that could be used to predict imminent death in aged mice. Therefore they monitored mice until the time of spontaneous death.

Although their goal was to monitor mice until he time of spontaneous death, some mice underwent euthanasia based on their condition. (clinical signs that included hypothermia, inability or unwillingness to walk, lack of response to manipulation, severe dyspnea or cynanosis, and large, bleeding, or ulcerated tumors). Blood was collected from these aged mice (22±2 months of age) that underwent euthanasia. These sera was used for measurement of serum cytokines and adipokines and were compared with young mice (2/3 months old)

Furthermore all the mice were subcutaneously implanted with an identification chip for measurement of body temperature. The mice then were weighed and monitored regularly until spontaneous death occurred or until euthanasia was administered. Clinical signs that were monitored included body weight, temperature, general body condition, pattern of respiration, dehydration, posture, movement, response to manipulation, and condition of hair coat. Body weight, body temperature and respiratory effort were the three factors that were monitored as potential signs of imminent death.

From these data the coefficient of variation has been calculated (see figure 5 and appendix 3 for the calculations).

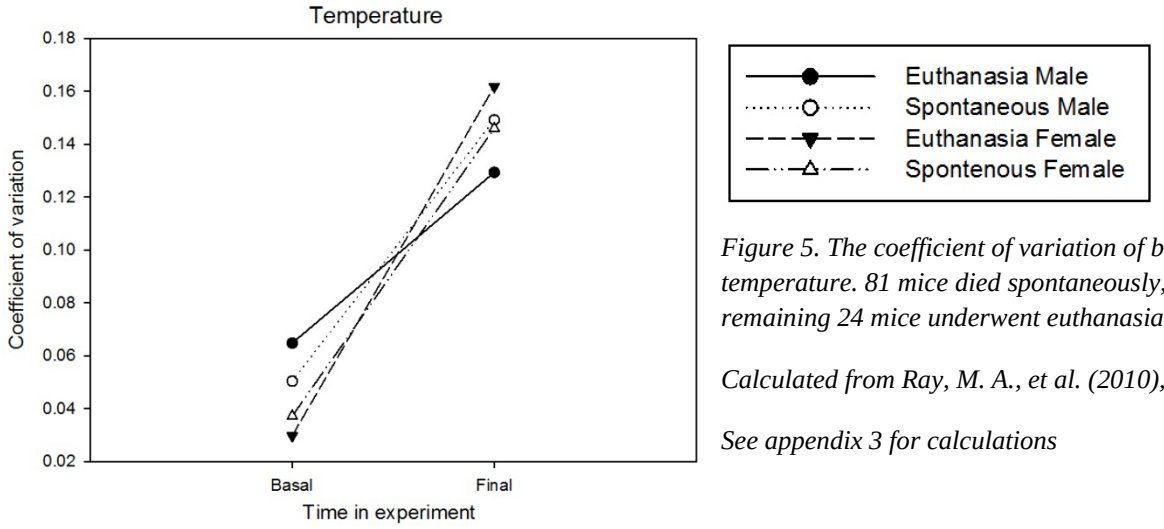


Figure 5. The coefficient of variation of body temperature. 81 mice died spontaneously, the remaining 24 mice underwent euthanasia.

Calculated from Ray, M. A., et al. (2010), Table 1,

See appendix 3 for calculations

As seen in figure 5, there is a larger coefficient of variation of body temperature in individuals that were near spontaneous death or in a moribund condition in comparison to the beginning values, when all the mice were eight months and free of known infections.

This was however, not the case when the coefficient of variation of body weight was calculated. (see figure 6 and appendix 3 for the calculations)

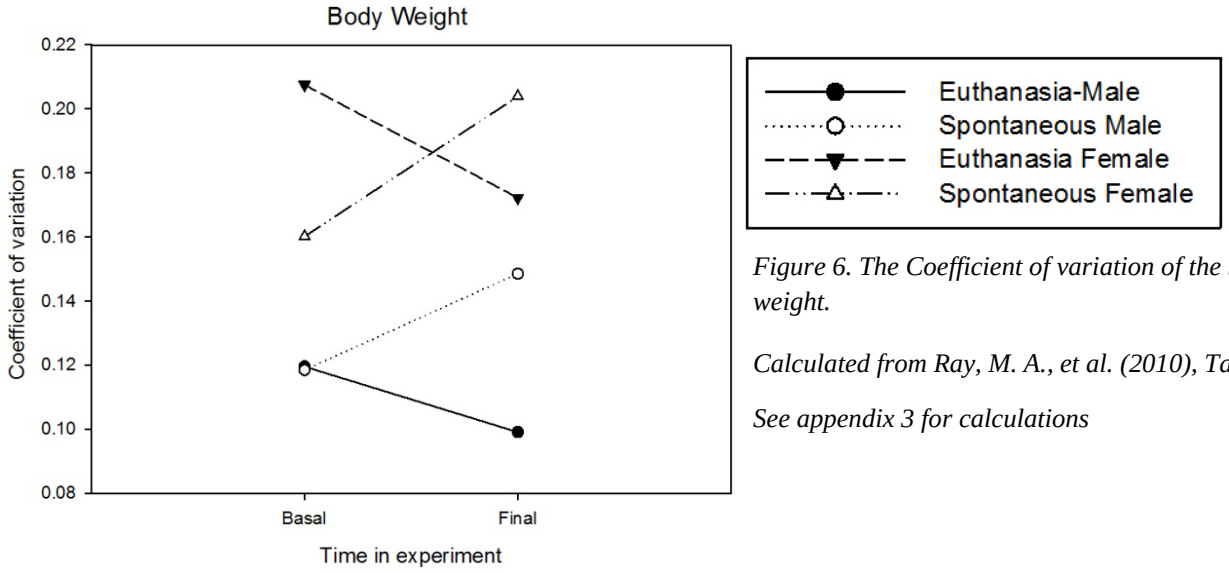


Figure 6. The Coefficient of variation of the body weight.

Calculated from Ray, M. A., et al. (2010), Table 1

See appendix 3 for calculations

There is an increase in coefficient of variation in the individuals that spontaneously died, in males as well as females. The individuals that underwent euthanasia however, had a decline in the coefficient of variation of body weight.
From the animals that underwent euthanasia cytokines and plasma adipokines were assessed, from these values the coefficient of variation has been calculated. (see figure 7 and appendix 3 for the calculations)

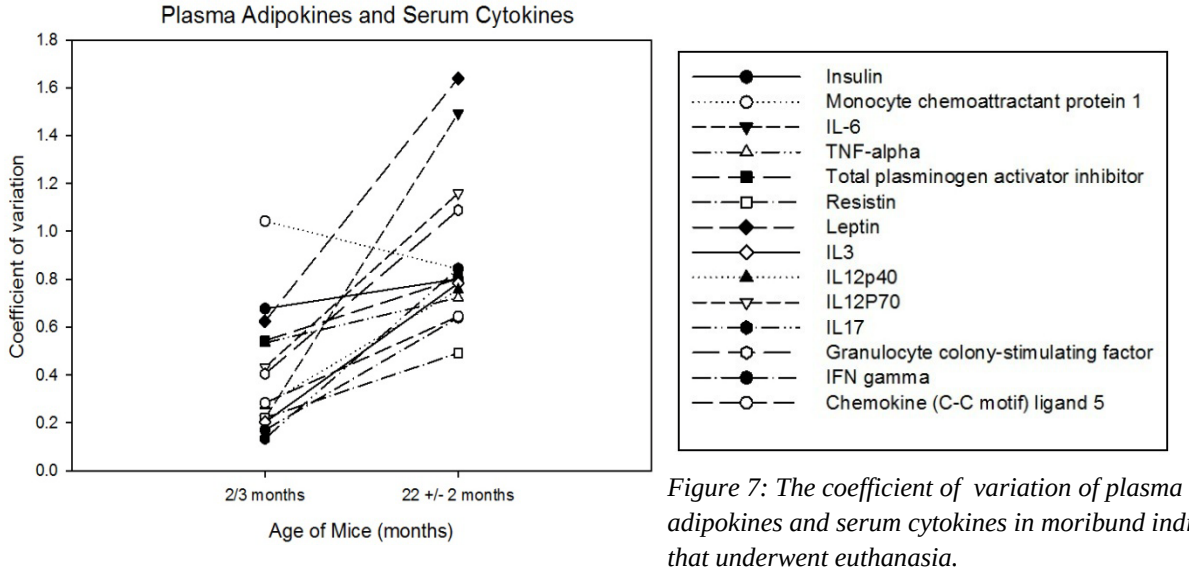


Figure 7: The coefficient of variation of plasma adipokines and serum cytokines in moribund individuals that underwent euthanasia.

Calculated from Ray, M. A., et al. (2010), Table 2
See appendix 3 for calculations.

In 13 of the 14 variables the coefficient of variation increased in aged mice that were 22±2 months old in comparison to the young mice that were 2/3 months old. Monocyte Chemoattractant Protein 1 had a decline in coefficient of variation from 1.04 to 0.8.

4.4 Human

Mendoza-Núñez, V. M., *et al.* (2007) investigated the age-related oxidative stress in healthy humans. In this study they analyzed the relationship between aging and some markers of oxidative stress.

For this study they used 249 healthy , without arterial hypertension, diabetes mellitus or cancer man and woman categorized in 6 groups;

- 25-29 years (n=22)
- 30-39 years (n=24)
- 40-49 years (n=30)
- 50-59 years (n=48)
- 60-69 years (n=60)
- ≥ 70 years (n=65)

None of the subjects studied had been taking antioxidant supplementation, none smoked or had acute or chronic diseases.

They assessed the body mass index, lipoperoxides, total plasma antioxidant status, glutathione peroxidase, erythrocyte superoxide dismutase, urate concentrations, albumin concentrations and cholesterol concentrations.

From these results the coefficient of variations have been calculated for males and females, see figure 8A and 8B respectively. (See appendix 4)

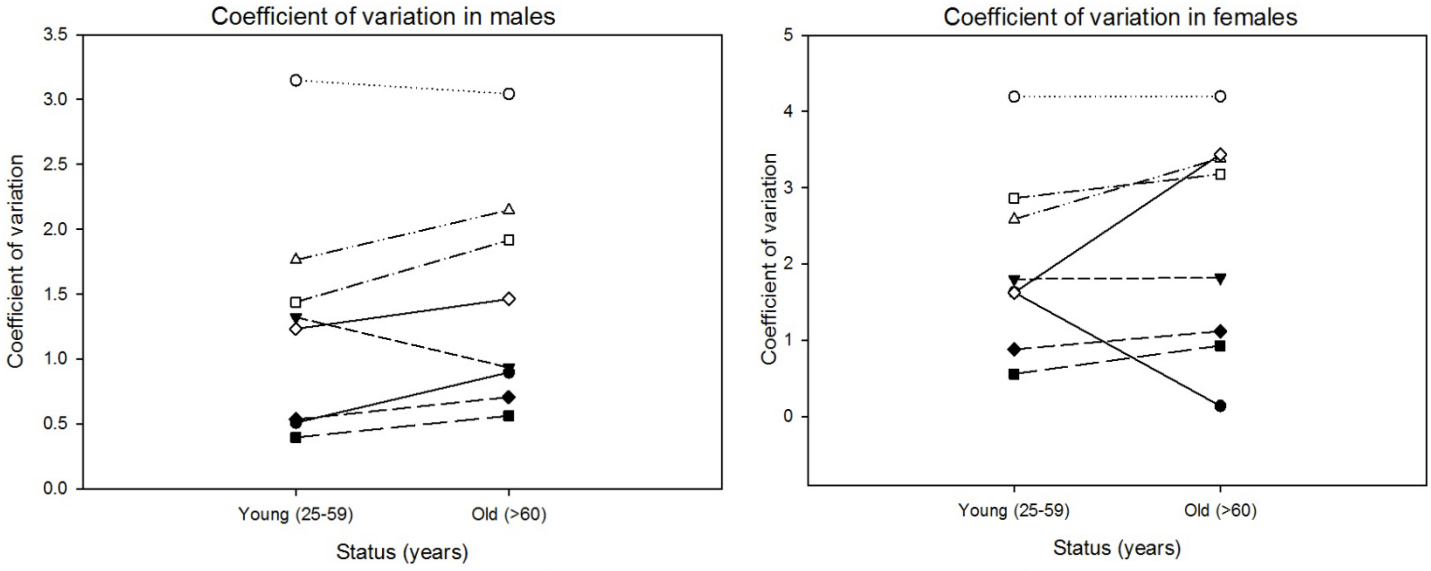


Figure 8A. The coefficient of variation in males. The coefficient of variation increases in all variables but Lipoperoxides.

Calculated from Mendoza-Núñez, V. M., et al. (2007), Table 1
See appendix 4 for calculations.

Figure 8B. The coefficient of variation in females. The coefficient of variation increases in all variables but Body Mass Index.

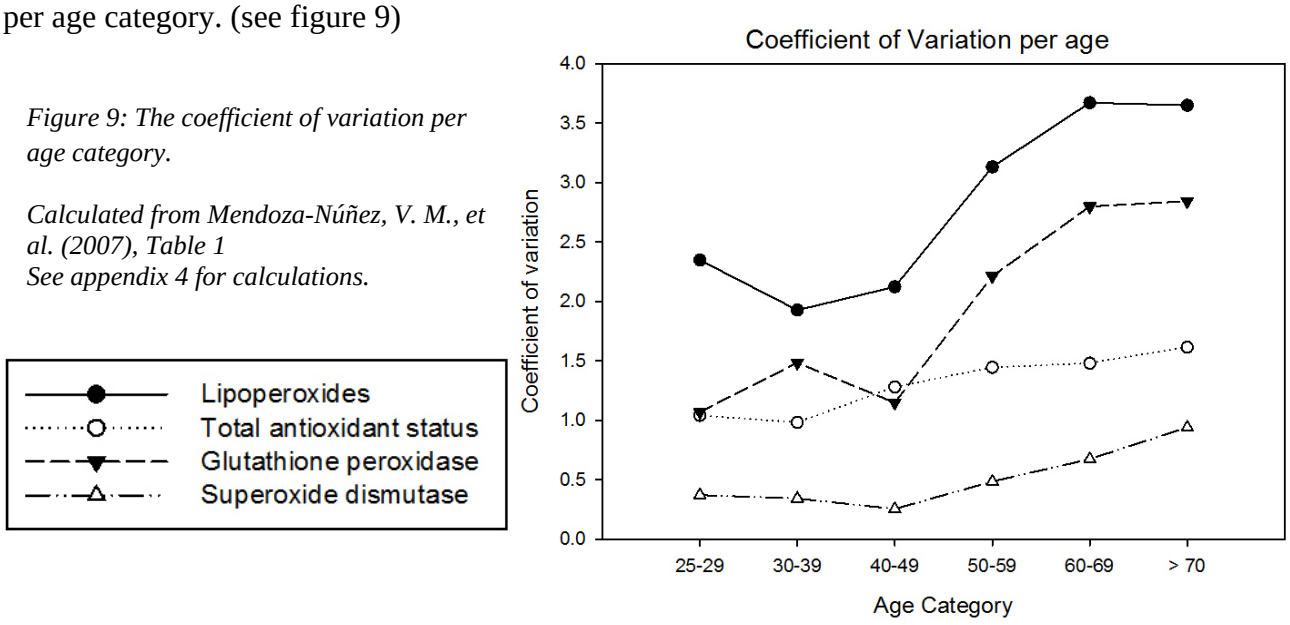
Calculated from Mendoza-Núñez, V. M., et al. (2007), Table 1
See appendix 4 for calculations.

It can be seen that the coefficient of variation increases in 5 variables in both sexes in old individuals. (glutathione peroxidase, superoxide dismutase, urate, albumin and cholesterol) However the coefficient of variation in old males declined for lipoperoxides in comparison to the young males as well as the total antioxidant status. In females only the coefficient of variation in body mass index decrease in old males in comparison to young females.

The lipoperoxides, total antioxidant status, gluthioneperoxidase and superoxide dismutase were also assessed per age category. (see appendix 4). To see whether the coefficient of variation increases gradually or spontaneously near death, the coefficient of variation was also calculated per age category. (see figure 9)

Figure 9: The coefficient of variation per age category.

Calculated from Mendoza-Núñez, V. M., et al. (2007), Table 1
See appendix 4 for calculations.



In this graph it can be seen that the coefficient of variation in all variables increases with age despite some minor decreases. Thus the coefficient of variation increases gradually. In this graph it can also be seen that the lipoperoxides increases with age if the age category >60 is compared with the age category > 25-29 in contrast to the graph in males.

5. Induced immune response

As we can see in the iguanas, older individuals recover more slowly from a bifurcation point in comparison to a young individual. This is according to one of the indications that predict a critical transition. Cote, J., *et al.* (2010) and Cichoń, M., *et al.* (2003) investigated the immune response of old and young individuals.

Cote, J., *et al.* (2010) investigated the age-related allocation of carotenoids to sexual signal versus antioxidant defenses in zebra finches in "Age-dependent allocation of carotenoids to coloration versus antioxidant defenses"

In this study they injected half of the birds with a mixture of antigens. This treatment triggered the recognition of different pathogen-associated molecular patterns by the immune system of the host, and then stimulates different signaling pathways and branches of the inflammatory process (Akira et al., 2006; Janeway and Medzhitov, 2002). Therefore, it mimics infection with a large array of pathogens. The remaining birds were injected with the same volume (0.1ml) of PBS (phosphate-buffered saline) as a control. Because antioxidants are necessary to stimulate the immune response, they measured the total antioxidant activity (TAA).
Figure 10 shows the initial antioxidant activity in male and female zebra finches.
As seen here older males have a lower antioxidant activity than young males (P=0.02), in females however, there is no correlation between antioxidant activity and age. This might be because males invest more in sexual signals rather than their own protection against oxidative damage.
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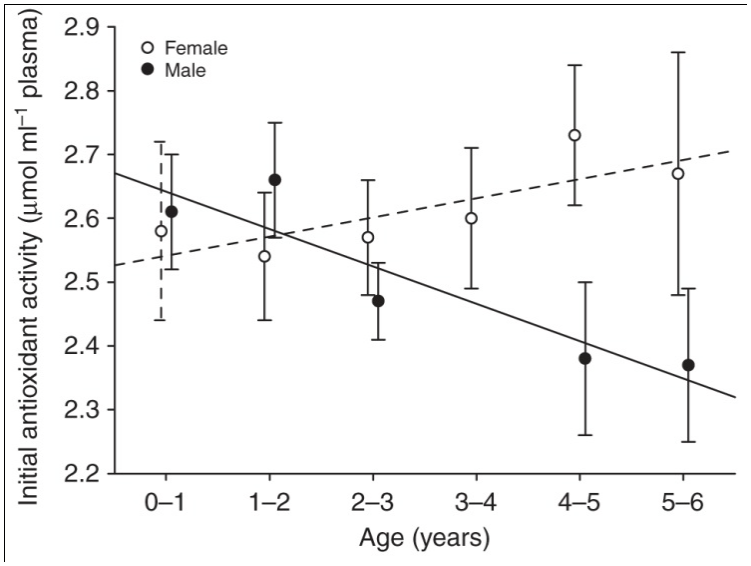

Figure 10. The initial antioxidant activity µmol/ml plasma

Source: Cote, J., et al. (2010). Figure 1.

From the initial antioxidant activity, the coefficient of variation is calculated. (See figure 11)

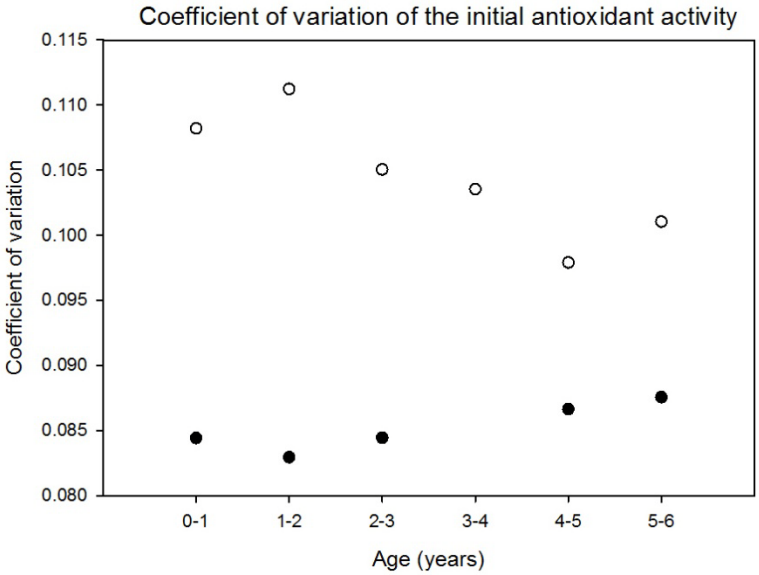

Figure 11. The coefficient of variation of the initial antioxidant activity.

Calculated from Cote, J., et al. (2010), Figure 1.
See appendix 5 for calculations.

In males it can be seen that the coefficient increases with age, which is expected although the differences in coefficient of variation between age changes are very small. (From 0.0844 to 0.0875 in year 0-1 to year 5-6 respectively) Since there were no male zebra finches of the age 3-4, the coefficient of variation could not be calculated for this group.
Unexpected however is the coefficient of variation of the female zebra finches. In figure 11 it is shown that the coefficient of variation decreases with age. (From 0.108 to 0.101 in year 0-1 to year 5-6 respectively) The changes in coefficient of variation in females are also very small and in disagreement with our hypothesis.

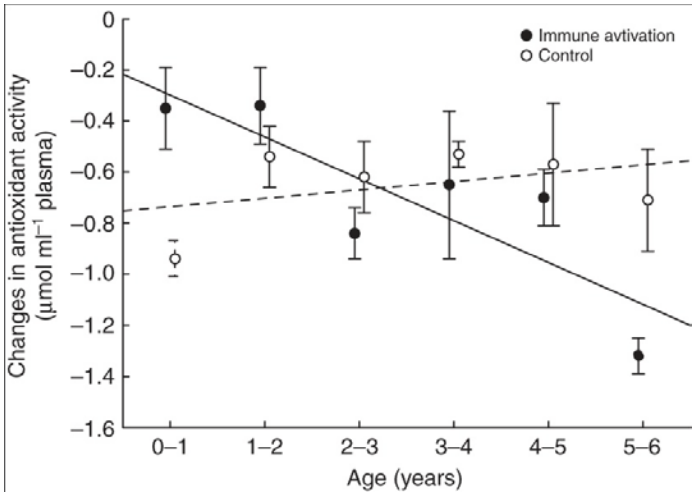

Figure 12. Mean change in antioxidant activity µmol/ml plasma. (mean ± s.e.m) in relation to age and immune treatment.
Source: Cote, J., et al. (2010), Figure 3.

When Cote, J., *et al*. measured the changes in immune response 24 hours after injecting either PBS or a mixture of antigens, they found that the total antioxidant activity in older birds decreased in immune-activated birds whereas the total antioxidant activity was decreased in young birds that were injected with PBS (see figure 12). The fact that the total antioxidant activity decreased more in older individuals reinforces the idea that senescent individuals are more susceptible to inflammation, which might be due to the slower recovery of antioxidant activity.

The other study done on immune response in birds was done by Cichoń, M., Sendecka J. and Gustafsson, L. (2003) studied the age-related ability to mount a humoral immune response evoked by a nonpathogenic antigen in female Collared Flycatchers from a free-living population. Three groups of females were involved in this study, young (1 year old), mid-age (3-year old) and old female birds (5-6 year old)

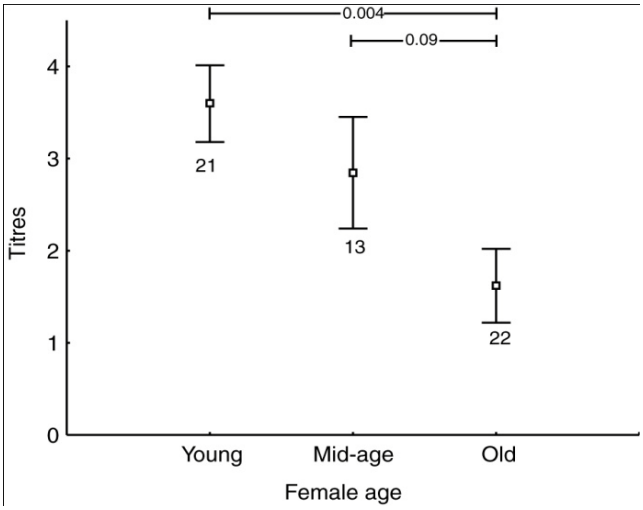

Figure 13; Immune responses to sheep red blood cells of females from different age categories. (mean ± s.e.m)

Source: Cichoń, M., Sendecka J. and Gustafsson, L. (2003), Figure 1.

In order to assess whether immune function differs between these age classes a humoral immune response was evoked with sheep red blood cells, a nonpathogenic antigen.

In figure 13 it is shown that there is a significant difference in the strength of the humoral immune response between age. The old females have a reduced immune response in comparison to the young female birds.

Individuals that have a weakened immune response will most likely have a slower recovery from diseases.

From these results the coefficient of variation was also calculated. (see figure 14, and appendix 6 for calculations. As shown in figure 14, the coefficient of variation increases gradually with age.

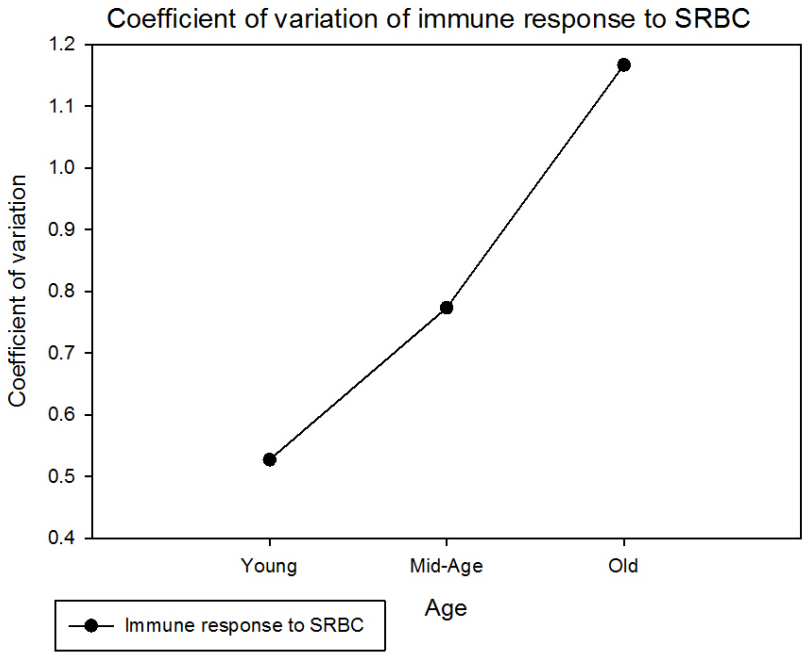

Figure 14: The coefficient of variation of the immune response.

Cichoń, M., Sendecka J. and Gustafsson, L. (2003), Figure 1.

See figure 13 and Appendix 6 for calculations.

6. Conclusion & Discussion

The aim of this study is to investigate whether death can be explained as a so-called critical transition by identifying objective criteria that would predict death in scientific literature. We hypothesized that indications that occur before a critical transition will also occur before death and with accelerated senescence, indications such as critical slowing down, a slow recovery rate and an increase of variation will occur before death and with accelerated senescence.

6.1 Gene expression:

Bahar, R., *et al.* (2006) investigated the increased cell-to-cell variation in gene expression in ageing mouse heart. The results of this study showed a significant increase in cell-to-cell variation in the expression levels of all nuclear genes in the old animals compared with the young animals. Moreover to confirm that alterations in the somatic genome, induced by reactive oxygen species lead to increased stochasticity of gene expression in cells they treated cultured mouse embryonic fibroblasts with H_2O_2, which is a known generator of oxidative damage, this is likely a causal factor in senescence. The results showed an increase of cell-to-cell variation in gene expression. It can be seen from figure 2, that the variation in gene expression increases after 48 hours after treatment with H_2O_2. Also the coefficient of variation in embryonic mice fibroblasts was greatly increased in the samples treated with H_2O_2 in contrast to the untreated cells. However the cell-to-cell variation in the relative expression level was not increased 6 hours after the treatment. This result strongly suggests that the increased transcriptional noise induced by H_2O_2 is not due to direct chemical damage of DNA, proteins or lipids. Meaning that the variance might not entirely be due to H_2O_2 which is a good replicate of senescence. It is also remarkable that the coefficient of variation decreases after 48 hours, it is not unthinkable that the coefficient might decrease back to the starting value. Furthermore it cannot be excluded that the change in variations were from other cellular alterations.

Nonetheless, this study give promising indications that there is an increase in variability in expression levels in cell-to-cell expression with senescence. These findings support our hypothesis, in which we expect that the variation increases before critical transitions. The findings of Southworth *et al.* also support our hypothesis. They showed that there are large-scale changes in gene-co expression. Many of those changes are in the decrease in cooperation and the inability to co-regulate a group of genes that are involved in senescence and play an important role in many age-associated diseases. These changes can cause a delay in the recovery from many diseases, which can lead to a progressive disease due to accumulation of damage. The slower recovery rate from bifurcation points in senescence, in this case due to changes in gene-co expression, is what we expect before critical transitions.

6.2 Physiological patterns

As said in the introduction, the most important indicator is known as critical slowing down, meaning that the recovery rate from bifurcation points is very slow. A consequence of slowing down is that the variance in fluctuation is increased. To be able to compare the several studies I calculated the coefficient of variation by dividing the standard deviation by the mean.

Figure 3A and 3B, show that the calculated coefficient of variation for most variables in the blood physiology of individual sockeye salmon increases in moribund salmon in comparison to salmon in the spawning-ground arrival. This is consistent with our hypothesis, if death can be explained as a critical transition, it is expected that individuals that are near death, thus in a moribund state show the indications that happen near a critical transition. An increased variation is one of the indications. However, not all of the variables were increased, the plasma concentrations of K^+ and lactate were only increased in females. Moreover, the gill Na^+ /K^+/ATPase activity and 17-20 β-progesterone were only increased in males. Additionally the coefficient of variation of cortisol showed no elevation at all, and even showed an decrease.

The coefficient of variation that has been calculated from the results of the research done by Ray *et al.* (2010) were also for the greater part consistent with our hypothesis. The coefficient of variation of temperature in individuals that were near spontaneous death or in a moribund condition were elevated in comparison to when the individuals were only eight months and free of known infections. Again an increase in variation is expected if death can be explained as a critical transition.
However the coefficient in body weight were not all increased in the mice that were near spontaneous death or in a moribund state. An explanation might be that body weight gradually decreases as age increases, however when an individual becomes sick and/or moribund the decrease in body weight might become sudden and less gradually which results in a large variation.
The coefficient of variation in plasma adipokines and serum cytokines increased in all variables except Monocyte Chemoattractant Protein 1 (MCP-1). The concentration of mcp-1 is 19±7 pg/ml (mean+ s.e.m) in 2-3 months old mice. In mice that are 22±2 months, the concentrations increased to 78±24. Research found that MCP-1 is up regulated in brains of patients that suffer from Alzheimer Disease which is an age associated disease. (Nishimura, M., *et al., 2003)* Despite that the coefficient of variations decreased in MCP-1, most of the variables did increase in coefficient of variation which support our hypothesis.

The coefficient in most of the variables calculated from the study done by Mendoza-Núñez, V. M., *et al.* (2007) were also increased in old individuals in comparison to young individuals. This might be the most interesting study since they investigated the relationship between aging and markers of oxidative stress in humans. Although the coefficient of variation of lipoperoxides did not increase in old males, when the coefficient of variation was calculated per age class lipoperoxides did show an increase in elderly in comparison to young people. Additionally the body mass index decreased in coefficient of variation in old females in comparison to young females . (Body mass index is obtained by $\frac{\text{weight}}{(\text{length})^2}$) This was also found in another recent study

done by Thinggaard, M., *et al.*, 2010. The large variation in young females might be due to the different lifestyles. In elderly, a low BMI is most likely a sign of terminal phase (Thinggaard, M., *et al.*, 2010).
Although the coefficient of variation of the body mass index decreased, almost all variables increased which is promising for our hypothesis, in which we expect that the coefficient of variation increases with senescence and/or before death.

Furthermore, the study done by Romero, L. M and Wikelski, M. (2010) on iguanas had promising results regarding our hypothesis. Figure 5 showed an elevated corticosterone concentration in iguanas that do not survive El Niño in comparison to iguanas that do survive. The termination of corticosterone via negative feedback was clearly present, and it could be well possible that the corticosterone concentration of the iguanas that did not survive would terminate back to the corticosterone concentrations of the iguanas that did survive with a longer time interval than 240 min. The elevation in iguanas that did not survive is therefore most likely due to a slower recovery rate which according to the hypothesis occurs before the critical transition, death.

6.3 Induced immune response

Cote, J., *et al.* (2010) and Cichoń, M., *et al.* (2003) investigated the immune response of old and young individuals. Cote, J., *et al*. found that the total antioxidant activity decreased in immune-activated birds with age. This is in agreement with de findings from Cichorí *et al.*, they found that old females birds had a weakened humoral immune response.
The fact that both immune responses are weakened reinforces the idea that senescent individuals will have a slower recovery from bifurcation points like inflammation or diseases.
However when the coefficient of variation was calculated from the initial antioxidant activity there was an increase in males but a decrease in females with age. The decrease in coefficient in variation in females is in contrast to our hypothesis. The changes are however very small, the largest coefficient of variation is 0.111 while the smallest is 0.097 which results in a small change of 0.013.
The coefficient of variation calculated from the humoral immune response in Collared Flycatchers was increased in variation in aged individuals compared to young individuals. This supports our hypothesis that the variation increases with (accelerated) senescence.

6.4 Death as a critical transition

The results of this study showed that there are promising indications that death can be explained as a so-called critical transition. In most results there was an increase of coefficient of variation before death and accelerated senescence and/or a slow recovery rate from bifurcation points. However the functions of these variables that have been calculated were not taken in account. This could explain why a coefficient decreases or increases and whether this is associated with senescence and death.

Additionally the study done by Mendoza-Núñez, V. M., *et al.* (2007) investigated the age-related oxidative stress in age classes *between* healthy humans. The differences in variables done by Hruska, K. A., *et al*. (2010), Romero, L. M and Wikelski, M. (2010) , Ray, M. A., *et al.* (2010),

Cote, J., *et al.* (2010) and Cichoń, M., Sendecka J. and Gustafsson, L. (2003) were done *within* the individuals themselves. This is preferred because differences and variables between individuals are excluded.

Furthermore I calculated the coefficient of variation. The coefficient of variation is calculated by $\frac{standard\ \ deviation}{mean}$ and the standard deviation was calculated by taking the standard error of the mean $\times \sqrt{n}$
This means that the coefficient of variation is a combination of the variation *within* individuals (by comparing the age) as well as *between* individuals (since the sample number included in the calculation).

Our hypothesis however refers only to the variation *within* individuals. To investigate whether death can be explained as a critical transition, the differences in variation must be compared between the different stages *within* the individual.

Additionally the variation between individuals might influence the results.
I expect that the variation of individuals *between* individuals is normally distributed with age. (See figure 15). The variation between young individuals are probably small because they are most likely healthy and fit, which makes their physiological patterns practically similar. Due to the variation of the speed of senescence between individuals I expect that the variation between mid-aged individuals is increased, additionally when the individuals become very old I expect that all the individuals are in a moribund state, therefore I expect that the physiological patterns in old individuals become similar again.

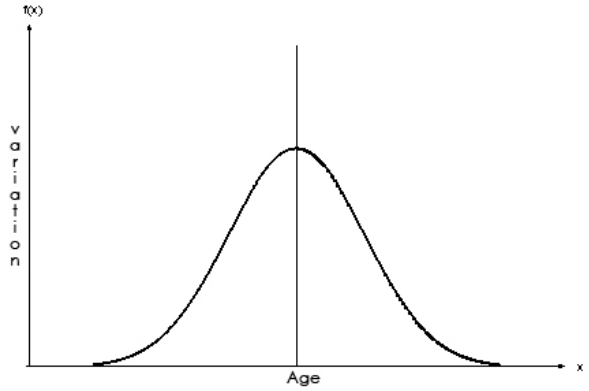

Figure 15: Normal distribution.

Thus the fact that the coefficient of variation is a combination *within* and *between* might have influenced the results.
However I expect that the variation between individuals that are young are small as well as individuals in a moribund state. Even in research where these individuals were compared with each other the coefficient of variation increased. The results also show that the coefficient of variation increases gradually. So it might be that the variation between individuals is relatively small and therefore do not chance the outcome. But further experimentation is needed to confirm this.

So all in all the results of this study show that there are promising indications that death can be explained as a critical transition. However more data and research is needed for conformation.

7. Acknowledgement

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9. Appendices

Appendix 1

Calculated from Hruska, K. A., et al. (2010), Table 1

Category		**Female**			
Ions:		*Arrival*			
	N	mean	s.e.m	st_dev = s.e.m*√n	Coefficient of variation (st_dev/mean)
Osmolality (mmol/L)	13	291.8	3.0	10.82	0.04
Cl (mmol/L)	13	129.8	1.1	3.97	0.03
Na+ (mmol/L)	13	151.7	1.9	6.85	0.05
K+ (mmol/L)	13	2.01	0.19	0.69	0.34
Gill Na+/K+ ATPase micromol ADP/mg/protein h	11	2.28	0.19	0.63	0.28
Stress metabolites					
Lactate (mmol/L)	12	1.53	0.13	0.45	0.29
Glucose (mmol/L)	13	5.58	0.21	0.76	0.14
Cortisol (ng/mL)	12	350	60	207.85	0.59
Testosterone (ng/mL)	13	39.3	2.7	9.73	0.25
Reproductive hormones					
11-KT (ng/mL)	12	1.922	0.092	0.32	0.17
17,20 Beta-P (ng/mL)	13	832	177	638.18	0.77
	13	2.61	0.15	0.54	0.21
Other					
Energy (MJ/kg)	13	4.74	0.05	0.18	0.04

Category		**Female**			
Ions:		*Moribund*			
	N	mean	s.e.m	st_dev = s.e.m*√n	Coefficient of variation (st_dev/mean)
Osmolality (mmol/L)	13	242	6.1	21.99	0.09
Cl (mmol/L)	13	80.7	2.3	8.29	0.10
Na+ (mmol/L)	13	108.6	2.5	9.01	0.08
K+ (mmol/L)	13	2.78	0.28	1.01	0.36
Gill Na+/K+ ATPase micromol ADP/mg/protein h	13	2.16	0.15	0.54	0.25
Stress metabolites					
Lactate (mmol/L)	13	12.66	1.16	4.18	0.33
Glucose (mmol/L)	13	5.08	1.39	5.01	0.99
Cortisol (ng/mL)	10	1,287	84	265.63	0.21
Testosterone (ng/mL)	13	5.6	0.9	3.24	0.58
Reproductive hormones					
11-KT (ng/mL)	13	0.424	0.039	0.14	0.33
17,20 Beta-P (ng/mL)	13	174	34	122.59	0.70
	13	0.73	0.17	0.61	0.84
Other					
Energy (MJ/kg)	13	3.45	0.11	0.40	0.11

Category		**Male**			
Ions:		*Arrival*			
	N	mean	s.e.m	st_dev = s.e.m*√n	Coefficient of variation (st_dev/mean)
Osmolality (mmol/L)	10	284.7	3.1	9.80	0.03
Cl (mmol/L)	10	124.1	0.6	1.90	0.02
Na+ (mmol/L)	10	151.4	1.8	5.69	0.04
K+ (mmol/L)	10	2.59	2.0	6.32	2.44
Gill Na+/K+ ATPase micromol ADP/mg/protein h	7	2.44	0.22	0.58	0.24
Stress metabolites					
Lactate (mmol/L)	10	1.26	0.17	0.54	0.43
Glucose (mmol/L)	10	4.59	0.13	0.41	0.09
Cortisol (ng/mL)	10	91	16.0	50.60	0.56
Testosterone (ng/mL)	10	21.3	1.6	5.06	0.24
Reproductive hormones					
11-KT (ng/mL)	10	11.494	0.665	2.10	0.18
17,20 Beta-P (ng/mL)	10	63	5	15.81	0.25
	9	0.61	0.18	0.54	0.89
Other					
Energy (MJ/kg)	10	4.63	0.09	0.28	0.06

Category		**Male**			
Ions:		*Moribund*			
	N	mean	s.e.m	st_dev = s.e.m*√n	Coefficient of variation (st_dev/mean)
Osmolality (mmol/L)	10	268.5	4.9	15.50	0.06
Cl (mmol/L)	10	95.9	2.2	6.96	0.07
Na+ (mmol/L)	10	129.1	3.3	10.44	0.08
K+ (mmol/L)	10	4.34	0.6	1.90	0.44
Gill Na+/K+ ATPase micromol ADP/mg/protein h	10	2.37	0.26	0.82	0.35
Stress metabolites					
Lactate (mmol/L)	10	13.55	1.21	3.83	0.28
Glucose (mmol/L)	10	4.91	0.74	2.34	0.48
Cortisol (ng/mL)	9	737	136	408.00	0.55
Testosterone (ng/mL)	10	9.3	1.1	3.48	0.37
Reproductive hormones					
11-KT (ng/mL)	10	6.180	0.755	2.39	0.39
17,20 Beta-P (ng/mL)	10	149	40	126.49	0.85
	10	0.34	0.13	0.41	1.21
Other					
Energy (MJ/kg)	10	3.78	0.1	0.32	0.08

Appendix 2

Calculated from Romero, L. M and Wikelski, M. (2010), Figure 1 & 2.

Status	N	mean	s.e.m	st_dev = s.e.m*√n	Coefficient of variation (st_dev/mean)
Baseline alive	73	2.94	0.42	3.59	1.22
Baseline dead	23	4.92	0.75	3.60	0.73
Stressed alive	74	5.28	0.54	4.64	0.88
Stressed dead	22	6.93	1.02	4.78	0.69
ACTH alive	37	16.76	1.95	11.86	0.71
ACTH dead	12	18.35	3.36	11.63	0.63

Status	N	mean	s.e.m	st_dev = s.e.m*√n	Coefficient of variation (st_dev/mean)
Dex control (uninjected)	8	3.499299	0.59	1.67	0.48
DEX alive	31	1.227209	0.23	1.29	1.05
DEX dead	7	2.440393	0.46	1.21	0.49

Appendix 3

Calculated from Ray, M. A., et al. (2010), Table 1,

Temperature												
Type of death	Sexe	n	age at death (months) mean	1.9	basal mean	basal s.e.m	basal st_dev	coef.var basal	final mean	final s.e.m	final st_dev	coef.var final
Euthanasia	Male	11	25.5	1.7	35.9	0.7	2.32	0.06	30.8	1.2	3.98	0.13
Euthanasia	Female	13	24.2	0.8	36.5	0.3	1.08	0.03	29	1.3	4.69	0.16
Spontaneous	Male	36	27.1	0.8	35.8	0.3	1.80	0.05	32.2	0.8	4.80	0.15
Spontaneous	Female	45	23.1	1.9	36.2	0.2	1.34	0.04	32.2	0.7	4.70	0.15

Body Weight												
Type of death	Sexe	n	age at death (months) mean	1.9	basal mean	basal s.e.m	basal st_dev	coef.var basal	final mean	final s.e.m	final st_dev	coef.var final
Euthanasia	Male	11	25.5	1.7	44.4	1.6	5.31	0.12	40.2	1.2	3.98	0.10
Euthanasia	Female	13	24.2	0.8	41.7	2.4	8.65	0.21	33.5	1.6	5.77	0.17
Spontaneous	Male	36	27.1	0.8	45.6	0.9	5.40	0.12	40.4	1	6.00	0.15
Spontaneous	Female	45	23.1	1.9	41.9	1	6.71	0.16	36.2	1.1	7.38	0.20

2-3 Months old Mice (n=8)	Mean	S.e.m	St_dev	Coefficient of variation
Plasma adipokines				
Insulin	439.0	105.0	296.98	0.68
Monocyte chemoattractant protein 1	19.0	7.0	19.80	1.04
IL 6	2.6	0.2	0.57	0.22
TNF alfa	5.3	1.0	2.83	0.53
Total plasminogen activator inhibitor 1	6,429.0	1,234.0	3,490.28	0.54
Resistin	2,232.0	174.0	492.15	0.22
Leptin	789.0	174.0	492.15	0.62
Serum cytokines				
IL3	7.0	0.5	1.41	0.20
IL12p40	559.0	54.0	152.74	0.27
IL12P70	118.0	18.0	50.91	0.43
IL17	275.0	13.0	36.77	0.13
Granulocyte colony-stimulating factor	63.0	9.0	25.46	0.40
IFN gamma	217.0	13.0	36.77	0.17
Chemokine (C-C motif) ligand 5	100.0	10.0	28.28	0.28

22 ±2 Months old Mice (n=7.5)	Mean	S.e.m	St_dev	Coefficient of variation
Plasma adipokines				
Insulin	253	74	202.66	0.80
Monocyte chemoattractant protein 1	78	24	65.73	0.84
IL 6	178	97	265.65	1.49
TNF alfa	18.2	4.8	13.15	0.72
Total plasminogen activator inhibitor 1	14503	4250	11,639.10	0.80
Resistin	1528	274	750.38	0.49
Leptin	284	170	465.56	1.64
Serum cytokines				
IL3	3.5	1	2.74	0.78
IL12p40	214	59	161.58	0.76
IL12P70	52	22	60.25	1.16
IL17	120	37	101.33	0.84
Granulocyte colony-stimulating factor	302	120	328.63	1.09
IFN gamma	150	35	95.85	0.64
Chemokine (C-C motif) ligand 5	34	8	21.91	0.64

Appendix 4

Calculated from Mendoza-Núñez, V. M., et al. (2007), Table 1

Young adults (25-59 years)						Older adults ≥ 60 years)				
Males	n	mean	s.e.m	st_dev	Coef.var	n	mean	s.e.m	st_dev	coef.var
Body mass index (BMI)	47	26.2	1.94	13.30	0.51	38	26.5	3.85	23.73	0.90
Lipoperoxides (LPO µmol/l)	47	0.305	0.14	0.96	3.15	38	0.385	0.19	1.17	3.04
Total antioxidant status (TAS mmol/l)	47	1.35	0.26	1.78	1.32	38	1.19	0.18	1.11	0.93
Glutathione peroxidase (GPx UI/l)	47	7.661	1.97	13.51	1.76	38	6.217	2.165	13.35	2.15
Superoxide dismutase (SOD UI/l)	47	174	10	68.56	0.39	38	176	16	98.63	0.56
Urate (mg/dl)	47	6.2	1.3	8.91	1.44	38	5.8	1.8	11.10	1.91
albumin (g/dl)	47	4.5	0.35	2.40	0.53	38	4.2	0.48	2.96	0.70
Cholesterol (mg/dl)	47	206	37	253.66	1.23	38	211	50	308.22	1.46

	25-29 (n =22)				30-39 (= 24)				40-49 (n=30)			
	mean	s.e.m	St_dev	Coeff_var	Mean	S.e.m	St_dev	Coeff_var	mean	S.e.m	St_dev	coeff.var
Lipoperoxides (LPO µmol/l)	0.22	0.11	0.52	2.35	0.28	0.11	0.54	1.92	0.31	0.12	0.66	2.12
Total antioxidant status (TAS mmol/l)	1.40	0.31	1.45	1.04	1.30	0.26	1.27	0.98	1.2	0.28	1.53	1.28
Glutathione peroxidase (GPx UI/l)	7,966	1,813	8,503.72	1.07	7,660	2,316	11,346.04	1.48	7,827	1,637	8,966.22	1.15
Superoxide dismutase (SOD UI/l)	178	14	65.67	0.37	173	12	58.79	0.34	174	8	43.82	0.25

	50-59 (n =48)				60-69 (= 60)				>70 (n=65)			
	mean	s.e.m	St_dev	Coeff_var	Mean	S.e.m	St_dev	Coeff_var	mean	S.e.m	St_dev	coeff.var
Lipoperoxides (LPO µmol/l)	0.31	0.14	0.97	3.13	0.38	0.18	1.39	3.67	0.42	0.19	1.53	3.65
Total antioxidant status (TAS mmol/l)	1.20	0.25	1.73	1.44	1.10	0.21	1.63	1.48	1.10	0.22	1.77	1.61
Glutathione peroxidase (GPx UI/l)	6,981	2,226	15,422.18	2.21	6,193	2,235	17,312.24	2.80	6,547	2,307	18,599.63	2.84
Superoxide dismutase (SOD UI/l)	172	12	83.14	0.48	173	15	116.19	0.67	172	20	161.25	0.94

Appendix 5

Initial antioxidant activity micromol/ml plasma in **females**					
Age	N	Mean	s.e.m	St_dev	Coefficient of variation
0-1	4	2.58	0.14	0.28	0.11
1-2	8	2.54	0.10	0.28	0.11
2-3	9	2.57	0.09	0.27	0.11
3-4	6	2.60	0.11	0.27	0.10
4-5	6	2.73	0.11	0.27	0.10
5-6	2	2.67	0.19	0.27	0.10

Initial antioxidant activity micromol/ml plasma in **males**					
Age	N	Mean	s.e.m	St_dev	Coefficient of variation.
0-1	6	2.61	0.09	0.22	0.08
1-2	6	2.66	0.09	0.22	0.08
2-3	12	2.47	0.06	0.21	0.08
3-4					
4-5	3	2.38	0.12	0.21	0.09
5-6	3	2.37	0.12	0.21	0.09

Appendix 6

Humoral response to Sheep Red Blood Cells of female Collared Flycatchers					
Age	N	Mean	s.e.m	Standard deviation	Coefficient of variation
Young (1 year old)	21	3.60	0.41	1.90	0.53
Mid-Aged (3 year old)	13	2.83	0.61	2.17	0.77
Old (5-6 year old)	22	1.61	0.40	1.89	1.17