# Influence of Timing of Chemical Exposure on Growth of Atlantic Salmon Smolts: Multiple Comparisons of Time Series Models

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by

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## Abstract

The effects of timing of exposure of Atlantic salmon smolts to two putative endocrine disrupting chemicals was studied. Dependences of the growth characteristics (length and weight) are reported. Seven datasets obtained within the period of 10 months in 1999 were used. The first six sets contained data obtained after short term chemical exposure of smolts in freshwater (female hormone 17-beta-estradiol and pesticide chemical 4-Nonylphenol), whereas the seventh was used as the control set without chemical exposure. Starting dates of treatment were May 12, May 26, and June 9.
Applying mathematical models, we showed that absolute values of growth characteristics of salmon smolts exposed to these chemicals in seawater were statistically lower than growth characteristics without any exposure.
Moreover, we established that exposure of salmon during the latter stages of parr to smolt transformation (June 9) resulted in significantly lower growth when compared to salmon exposed in May 12.



Keywords: desirability function, female hormone 17-beta-estradiol, matrix, pesticide 4-Nonylphenol, uncertainty quantification, validation



## Résumé

Les effets du moment de l'exposition de jeunes saumons de l'Atlantique à deux perturbateurs endocriniens présumés ont été étudiés. Les dépendances des caractéristiques de la croissance (longueur et poids) ont été établies. Sept ensembles de données obtenus durant une période de 10 mois, en 1999, ont été utilisés. Les six premiers ensembles contenaient des données obtenues après une courte période d'exposition chimique de saumoneaux en eau douce (hormone femelle 17-béta-œstradiol et pesticide chimique 4-nonylphénol), tandis que le septième ensemble a été utilisé comme ensemble témoin et contenait des données qui n'ont pas été recueillies après une exposition chimique. Les dates de début du traitement étaient le 12 mai, le 26 mai et le 9 juin.

En appliquant des modèles mathématiques, nous avons démontré que les valeurs absolues des caractéristiques de la croissance des saumoneaux exposés à ces produits chimiques dans l'eau de mer étaient statistiquement plus faibles que les caractéristiques de la croissance sans exposition.

En outre, nous avons établi que l'exposition des saumons durant les derniers stades des tacons jusqu'à la transformation en saumoneaux (juin 9) s'est traduite par une croissance significativement inférieure par rapport aux saumons exposés le 12 mai.



Mots clés : fonction de désirabilité, hormone femelle 17-béta-œstradiol, matrice, pesticide 4-nonylphénol, quantification de l'incertitude, validation

## Introduction

Indigenous populations of Atlantic salmon have been decreasing throughout the northwest Atlantic area for a number of years (Fairchild *et al*. 1999). The cause of this decline is thought to be a result of failure of stocks to survive or thrive at sea (ICES 2009) but the factors responsible for poor at-sea survival remain unclear. Outward migrating salmon experience a number of man-made environmental challenges ranging from physical barriers to chemical contaminants (McCormick et al. 1998).

Carey and McCormick (1998) have shown that parr to smolt transformation (PST), the process by which anadromous species such as salmon physiologically adapt in order to survive life in seawater is a sensitive life stage as they show a greater biochemical stress response than juvenile fish exposed to the same stressor (handling and confinement). McCormick et al. (1998) also provide a good description of the optimum conditions for outward migration of Atlantic salmon to the sea and the potential consequences of delayed entry to seawater. In short, PST is triggered by day length, water temperature and internal cues. The period of time when the fish can make this switch is limited and if they don't go to seawater they end up staying in fresh water for another year, reverting to a freshwater physiological state. This is called desmoltification or smolt reversion. Alternatively, if timing and/or environmental conditions are not optimum and the smolts do go to sea, eventual return of salmon to their native rivers may be affected indicating poor survival at sea (McCormick et al. 1998). Smolts are also sensitive to chemical stressors (Lerner et al. 2007). In some cases chemicals elicit a typical stress response characterized by elevated levels of steroid hormones which help the fish either to escape the stressor or to mitigate the effects of the stressor; in other cases contaminant affects the fish's ability to mount this stress response which may lead to reduced survival, growth and resistance to disease or other stressors.

These challenges may affect the physiology of fish resulting in a reduced capacity to thrive at sea. Chemicals commonly referred to as endocrine disrupting compounds have been implicated in negative effects on aquatic species (Servos 1999, Kidd et al. 2007). Several authors have identified two compounds found throughout the world in municipal and industrial wastewater as capable of affecting at sea survival of Atlantic salmon (Fairchild et al. 1999, Arsenault et al. 2004, Lerner et al. 2007). These are the female hormone, estrogen (E2) and a surfactant 4- nonylphenol (4-NP).

Previous studies have described statistical methods for assessing fish growth (for example see, Newman 2000, Millar 2004 and Handeland et al.2008). These studies have focused on wild stocks or stocks of salmon being held and raised for aquaculture purposes. Other studies looked at problems of uncertainties in modeling bioaccumulation factors and in evaluating the growth of wild fish (Katsanevakis (2006), Hauck et al. (2011)).

In our earlier publications (Khots et al. 2010, 2011) we have described a statistical approach to investigating growth of Atlantic salmon (*Salmo salar* L.) during PST as well as the effects of E2 and 4-NP (Khots et al. 2011). We showed that salmon smolts exposed to these compounds in freshwater did not grow as well as untreated fish after transfer to seawater.

In the present study we report the effects of timing of exposure to E2 and 4-NP and subsequent seawater transfer on the response of Atlantic salmon.
Atlantic salmon usually move into bays and estuaries in May and migration can last into June depending on a number of factors including the particular fish stock and environmental conditions (McCormick et al. 1998). In addition, we herein describe statistical analyses that enable us to determine if timing of chemical exposure and transfer to seawater affects subsequent growth of Atlantic salmon smolts.

To statistically assess the effects of exposure of smolts, we had to determine some reasonable time characteristics of experiments. Our objective was to determine if exposing smolts to E2 or 4-NP at several time points late in PST resulted in different growth characteristics during subsequent holding in seawater. We hypothesize that the later the exposure period, after the onset of PST, the more likely that Atlantic salmon will be affected.

The main idea behind our approach is to create mathematical models expressing mean values of growth characteristics of smolts over time. With these models we can evaluate differences between conditions of growth not just at the specific days when measurements were taken but also for any days during the experiment. Our procedure allows us to split the whole time segment of experiment into several parts where differences between growth characteristics were statistically significant, not significant or uncertain.

To help assess the effects of chemical treatment on the growth of smolts, we used the results of our research Khots et al. 2010 describing the growth of fish in fresh and seawater without chemical treatment of salmon.

## Methods

Experimental Trials

Exposure of Atlantic salmon smolts to E2 and 4-NP has been described previously (Khots et al. 2010, 2011). Briefly, juvenile Atlantic salmon were brought to the St. Andrews Biological Station (SABS), implanted with passive integrated transponder (PIT) tags, 50 fish were placed in each 300L tank and held for several months prior to treatment. In order to determine if timing of exposure and subsequent transfer to seawater affected the response of Atlantic salmon smolts to contaminants, the fish were exposed to either E2 or 4 NP at one of three dates separated by two weeks and subsequently were transferred to seawater 2 weeks after exposure.

Each tank was treated with either water-borne 4-NP or E2 at one of three different times: May 12-16, May 26-30 and June 9-13, referred to as early, middle and late window of PST respectively. Two replicate tanks (with 50 fish in each) were treated with an environmentally-relevant concentration of 4-NP (20 μg/L) (Fairchild *et al*. 1999) and with E2 (100 ng/L), serving as a positive control. Six other tanks remained untreated to serve as controls, 2 tanks for each treatment.

As previously described (Khots et al. 2010, 2011) the test substances were dissolved in ethanol and diluted with water such that ethanol represented 10% of the delivery solution.

Control tanks received the 10% ethanol vehicle. 4-NP was delivered in two 24-hour pulses (day 1 and day 6) at a flow rate of 1 mL/min using a Mariott bottle system. E2 was delivered continuously throughout the treatment.

Beginning 12-14 days after the onset of the treatment at each time, fish were gradually acclimated to filtered seawater over a five-day period. The flow was maintained at approximately 5L/min. In June, July and October a sub-sample of each treatment group was sacrificed for biochemical analysis and the remaining fish were anaesthetized.
At the end of August 1999 the fish were moved to two large tanks and held until the final sampling in October.
Length and weight of smolts were recorded multiple times from June through October (see Table 1). At each sampling 8 fish were removed, euthanised and dissected for biochemical analysis (Arsenault et al. 2004) and length and weight of the remaining fish was recorded. As such, we treated experimental data as time series where each measurement corresponded to a certain point on the time scale. The accuracy of measurements is 0.1 centimeter (cm) for length, 0.1 gram (g) for weight and 1 day for time.
Figures 2A-C, 3A-C, 4A-C and 5A-C in Annex 2, show the change in length and weight of salmon under E2 and 4-NP treatments for different starting dates of exposure, from June through October. The data for smolts without chemical treatment was presented in (Khots et al. (2010).

The program packages STATA and MS EXCEL were used to perform the necessary calculations and to draw figures.

**Table 1**: Days since initial measurement (January 5, 6 and 7, 1999) and number of fish measured at each time point.

| Day | Number of Measurements | | | | | |
|---|---|---|---|---|---|---|
| | May 12 E2 | May 26 E2 | June 9 E2 | May 12 4-NP | May 26 4-NP | June 9 4-NP |
| 160 | | | 2 (0) | 8 (2) | 8 (2) | 4 (0) |
| 161 | 8 (2) | 8 (2) | 8 (2) | | 8 (2) | 16 (4) |
| 162 | 8 (2) | 8 (2) | | 8 (2) | | |
| 195 | | 8 (2) | | 8 (2) | 8 (2) | 16 (4) |
| 196 | 16 (4) | 26 (7) | 8 (2) | 8 (2) | 8 (2) | 33 (10) |
| 197 | 14 (4) | 15 (4) | | 16 (4) | | |
| 202 | | | | 17 (5) | 16 (4) | |
| 203 | 19 (5) | | 17 (5) | | 15 (4) | |
| 230 | | | | 19 (5) | 16 (4) | |
| 231 | 17 (4) | 19 (5) | 15 (4) | | 14 (4) | 37 (10) |
| 232 | 13 (3) | 12 (3) | | 18 (5) | | |
| 279 | | | | 8 (2) | 8 (2) | |
| 280 | 9 (2) | 9 (2) | 8 (2) | | 7 (2) | 17 (5) |
| 281 | 8 (2) | 8 (2) | | 9 (3) | | |
| 287 | | | | 11 (3) | 8 (2) | |
| 288 | 8 (2) | 10 (3) | 10 (3) | | 8 (2) | 17 (4) |
| 289 | 6 (2) | 3 (1) | | 6 (1) | | |

Statistical Method Development

The processing of data consisted of four parts.

Part 1. Preliminary processing of data (according to Khots et al. (2010))

Part 2. Development and validation of mathematical models for the study of dependences Time – Length and Time – Weight for each chemical and starting date of exposure (according to Khots et al. (2010), (2011))

Part 3. Comparison of growth characteristics models with and without chemicals (according to Khots et al. (2011))

Part 4. Matrix presentation and analysis of results is described below. This part contains two steps.

Step 1. Qualitative approach

Dependences Time – Length can be approximated by polynomial functions of the first order, and the dependences Time – Weight by polynomial functions of the second order (Khots et al. (2010), (2011)).

We denoted functions under consideration as $L_{ij}(t)$ for dependences Time – Length (1) and $W_{ij}(t)$ for dependences Time – Weight (2), where i is determined by the type of chemical treatment, specifically $i = 1$ corresponds to E2 treatment and $i = 2$ to 4-NP treatment; j is determined by the starting date of exposure, $j = 1$ corresponds to May 12, $j = 2$ to May 26, and $j = 3$ to June 9

$L_{ij}(t) = a_{0ij} + a_{1ij}*t$, $i= 1,2,\ j = 1,2,3$ **(1)**

$W_{ij}(t) = b_{0ij} + b_{1ij}*t + b_{2ij}*t^2$, $i= 1,2,\ j = 1,2,3$ **(2)**

$a_{0ij}, a_{1ij}, b_{0ij}, b_{1ij}$, and $b_{2ij}$ are constants.

To evaluate the rate of the weight change, we use the derivatives $W'_{ij}(t)$ and $Var(W'_{ij}(t))$, where $i = 1, 2$, $j = 1, 2, 3$.

To determine the influence of chemicals on the change in length and weight over time, we used the equations for smolt growth without chemical treatment: Dependence Time - Length $L(t)$ with coefficients $a_0$ and $a_1$, Dependence Time – Weight $W(t)$ with coefficients $b_0$, $b_1$ and $b_2$, and their variances $Var(L(t))$ and $Var(W(t))$ (Khots et al. 2010).

The scaled variable t was defined by formula:

$t = (T-160)/124$ where variable T is broken down into two segments: the period of acclimation to laboratory conditions after PIT tagging (160 days from January until exposure began), and the remaining 124 days as the time segment under study. The time segment under study (124 days) occurred from the middle of June through the middle of October and corresponded to the period of transference of most smolts from fresh to seawater and the end of chemical treatment.

We studied separately three sets of models:

- Length models $\{L(t), L_{11}(t), L_{12}(t), L_{13}(t), L_{21}(t), L_{22}(t), L_{23}(t)\}$ **(3)**
- Weight models $\{W(t), W_{11}(t), W_{12}(t), W_{13}(t), W_{21}(t), W_{22}(t), W_{23}(t)\}$ **(4)**
- Derivatives of weight models $\{W'(t), W'_{11}(t), W'_{12}(t), W'_{13}(t), W'_{21}(t), W'_{22}(t), W'_{23}(t)\}$ **(5)**

Within each set (3), (4) and (5), we compared all possible pairs of models with each other. All pairs of compared models are displayed in Annex 1.

Our results indicated the time segment under study may be split into four parts:
V(-), V(0), V(+), and V(U) **(6)**
Where:
- V(-) first model is statistically less than the second model;
- V(0) first model is statistically not different from second model;
- V(+) first model is statistically more than second model;
- V(U) uncertain statistical relations between first and second model.

To present the results we used the square matrices of the seventh order:
- for length models **C**(-), **C**(0), **C**(+), and **C**(U) **(7)**
- for weight models **D**(-), **D**(0), **D**(+), and **D**(U) **(8)**
- for derivatives of weight models **E**(-), **E**(0), **E**(+), and **E**(U) **(9)**

where
**C**(-) contains segments $V_L$(-); similarly, **C**(0) has $V_L$(0), **C**(+) has $V_L$(+), and **C**(U) has $V_L$(U);
**D**(-) contains segments $V_W$(-); similarly, **D**(0) has $V_W$(0), **D**(+) has $V_W$(+), and **D**(U) has $V_W$(U);
**E**(-) contains segments $V_W$'(-); similarly, **E**(0) has $V_W$'(0), **E**(+) has $V_W$'(+) and **E**(U) has $V_W$'(U).
The letter index in segment V ($V_L$, $V_W$, and $V_W$') corresponds to comparison of length, weight, and the rate of weight change models.

Rows and columns in matrices of the seventh order (7), (8) and (9) are numbered as follows
1 - no chemical treatment;
2 - May 12 E2 exposure;
3 - May 26 E2 exposure;
4 - June 9 E2 exposure;
5 - May 12 4-NP exposure;
6 - May 26 4-NP exposure;
7 - June 9 4-NP exposure.

There are certain relations between elements of matrices (7), (8) and (9), see Annex 3.

Matrices (7), (8) and (9) allowed us to visualize the split of time segment under study into parts (6) for all pairs of length, weight, and rate of weight change models. However the qualitative approach contains certain limitations due to the difficulty of evaluating the results for all pairs simultaneously.

Step 2. Quantitative approach
We transformed matrices (7), (8) and (9) into matrices of real numbers, see Annex 3.
We denote the transformed matrices as:
- $\mathbf{C}_p$(-), $\mathbf{C}_p$(0), $\mathbf{C}_p$(+), and $\mathbf{C}_p$(U) **(10)**
- $\mathbf{D}_p$(-), $\mathbf{D}_p$(0), $\mathbf{D}_p$(+), and $\mathbf{D}_p$(U) **(11)**
- $\mathbf{E}_p$(-), $\mathbf{E}_p$(0), $\mathbf{E}_p$(+), and $\mathbf{E}_p$(U) **(12)**

Properties of matrices (10), (11) and (12) are described in Annex 3.

We call sums of matrices $\mathbf{C}_p(-) + \mathbf{C}_p(+)$, $\mathbf{D}_p(-) + \mathbf{D}_p(+)$, and $\mathbf{E}_p(-) + \mathbf{E}_p(+)$ as matrices of inequalities **(13)**

Although the form of presentation (10) - (12) does not provide a clear sense of the split of segment under study into parts (6), it is essentially simpler than (7) - (9). Moreover, using matrices (10), (11), and (12) we can study all pairs of models simultaneously because we can apply basic properties of matrices of real numbers (Householder, Alston S. 1975).

First, we used the elements of matrices of inequalities (13). The maximum of elements of these matrices defines the largest differences between growth characteristics for different pairs of models (i,j), where $i \neq j$.
Second, we used the elements of matrices of uncertainties $\mathbf{C}_p(U)$, $\mathbf{D}_p(U)$, and $\mathbf{E}_p(U)$ to quantify the uncertainties between growth characteristics for different pairs of models (i,j), where $i \neq j$.

Third, to evaluate summary differences for three growth characteristics at once, we combined them into one aggregate characteristic of differences. For this goal, we considered the transformed values of differences in length, weight, and rate of weight change (elements of matrices of inequalities (13)). Since these elements are scaled from 0 to 1 (see Annex 2), we could use them as partial desirability functions. Afterwards for summary evaluation of differences, we computed the cubical roots of products of corresponding elements of these matrices (overall multiplicative desirability functions). Such desirability functions are widely implemented for economic, industrial and scientific research (Harrington (1965), (Hendriks et al. (1992).

## Results and Discussion

Annex 2 and Annex 4 include figures and tables with supporting data. Figures 2 through 5 (Annex 2) show the growth of Atlantic salmon during the study. We see that smolts exposed to E2 or 4-NP grew to a maximum length of 35.2 cm and maximum weight of 502 g during the study. Meanwhile the range of the growth can vary widely. For example,

- For fish under E2 treatment with starting date of exposure May 12: [maximum of length minus minimum of length] divided by [average of length] varies from 0.13 to 0.44, and [maximum of weight minus minimum of weight] divided by [average of weight] varies from 0.48 to 1.03;
- For fish under 4-NP treatment with starting date of exposure June 9: [maximum of length minus minimum of length] divided by [average of length] varies from 0.10 to 0.39, and [maximum of weight minus minimum of weight] divided by [average of weight] varies from 0.38 to 1.49.

Looking at Figures 2-5, it would be difficult to evaluate the differences between growth characteristics. As such, we decided to apply a statistical procedure to assess whether the differences between growth characteristics were statistically significant, not significant or uncertain.

Qualitative Approach

Using experimental data, we successfully obtained and validated regression equations of growth characteristics. We applied the method of weighted least squares to approximate the dependences (1) and (2) and F-criterion to statistically test them (Draper and Smith. 1998). Details of calculations are presented in Tables 2 and 3 (Annex 4). Validation of models was realized in accordance with the Holdout method (Kriek *et al*. 2007). For the case under study we selected training and validation sets in the following way (see Table 1):

- Approximately a quarter of the observations were randomly selected as validation sets
- Training sets contained all other data.

Using these regression equations (Table 3, Annex 4) we studied the growth process of Atlantic salmon smolts in detail.

To compare the growth characteristics of smolts with and without chemical treatment, we solved the corresponding equations. See Table 4 (Annex 4) for equations and their roots for pairs of length models (3), Table 5 for pairs of weight models (4), and Table 6 (Annex 4) for pairs of rate of weight change models (5).

To present the split of time segment under study in calendar year dates (T), we used the inverse transformation of independent variable t into T: $T = 124*t + 160$.
Final results of calculations for pairs of length models are matrices **C**(-), **C**(0), **C**(+), and **C**(U) (Table 7); for pairs of weight models are matrices **D**(-), **D**(0), **D**(+), and **D**(U) (Table 8); for pairs of rate of weight change models are matrices **E**(-), **E**(0), **E**(+), and **E**(U) (Table 9).

Using matrices of Table 7, 8, and 9 (Annex 4), we can present the dynamics of relations between the growth characteristics in the time segment under study from June 15 to October 17. We provide three examples of comparisons below.

- 1st Example

Length models $L_{12}(t)$ [May 26 start] and $L_{13}(t)$ [June 9 start] under E2 treatment **(14)**
The time segment under study was split into three parts (see elements (3,4) of matrices in Table 7):
> [June 15, July 15], where $L_{12}(t)$ was statistically less than $L_{13}(t)$;
> [July 16, July 31], where differences between $L_{12}(t)$ and $L_{13}(t)$ were statistically uncertain;
> [August 1, October 17], where differences between $L_{12}(t)$ and $L_{13}(t)$ were not statistically significant.

- 2nd Example

Weight models $W_{13}(t)$ [June 9 start, E2 Treatment] and $W_{23}(t)$ [June 9 start, 4-NP treatment] **(15)**
The time segment under study was split into five parts (see elements (4,7) of matrices in Table 8):
> [June 15, July 4], where differences between $W_{13}(t)$ and $W_{23}(t)$ were not statistically significant;
> [July 5, July 28], where differences between $W_{13}(t)$ and $W_{23}(t)$ were statistically uncertain;

> [July 29, August 26], where $W_{13}(t)$ was statistically more than $W_{23}(t)$;
> [August 27, September 15], where differences between $W_{13}(t)$ and $W_{23}(t)$ were statistically uncertain;
> [September 16, October 17], where differences between $W_{13}(t)$ and $W_{23}(t)$ were not statistically significant.

- 3rd Example

Rate of weight change models $W'_{12}(t)$ [May 26 start, E2 treatment] and $W'_{22}(t)$ [May 26 start, 4-NP treatment] **(16)**

The time segment under study was split into five parts (see elements (3,6) of matrices in Table 9):
> [June 15, July 25], where $W'_{12}(t)$ was statistically less than $W'_{22}(t)$;
> [July 26, July 27], where differences between $W'_{12}(t)$ and $W'_{22}(t)$ were statistically uncertain;
> [July 28, September 10], where differences between $W'_{12}(t)$ and $W'_{22}(t)$ were not statistically significant;
> [September 11, September 22], where differences between $W'_{12}(t)$ and $W'_{22}(t)$ were statistically uncertain;
> September 23, October 17], where $W'_{12}(t)$ was statistically more than $W'_{22}(t)$.

As demonstrated in previous publication (Khots et al. 2011), we confirmed that exposure to E2 and 4-NP results in a smaller gain of length, weight and rate of weight change when compared with untreated fish.

Moreover, we established that time of exposure and subsequent transfer to seawater also affected the response.

- Statistical differences are observed when comparing length and weight measured on the May 12 starting date with the June 9 starting date. To evaluate differences of absolute values in length and weight, we can use Figures 5-8 where we presented dependences $L_{11}(t)$, $L_{13}(t)$, $L_{21}(t)$, $L_{23}(t)$, $W_{11}(t)$, $W_{13}(t)$, $W_{21}(t)$, $W_{23}(t)$ and their confidence bands.
For E2 treatment, in the beginning of the time segment under study, the length and the weight were greater for starting date June 9 than for starting date May 12. See elements (4,2) of matrix **C**(+) (Table 7, Annex 4) and of matrix **D**(+) (Table 8, Annex 4).
For 4-NP treatment, the situation is more complicated. In the beginning of the time segment under study, the length and the weight were also greater for starting date June 9 than for starting date May 12. However, by the end of the time segment, we observed that the length and the weight of smolts were less for starting date June 9 than for starting date May 12. See elements (5,7) and (7,5) of matrix **C**(+) (Table 7) and of matrix **D**(+) (Table 8, Annex 4).

- There are no significant differences in length gain with fish exposed to E2 or 4-NP using the same starting date (May 12 and May 26). See elements (2,5) and (3,6) of matrix **C**(0) (Table 7, Annex 4). Also, after 2-3 months there are no significant differences in weight gain with fish exposed to E2 or 4-NP using the same starting date (May 12, May 26 and June 9, ). See elements (2,5), (3,6) and (4,7) of matrix **D**(0) (Table 8, Annex 4).

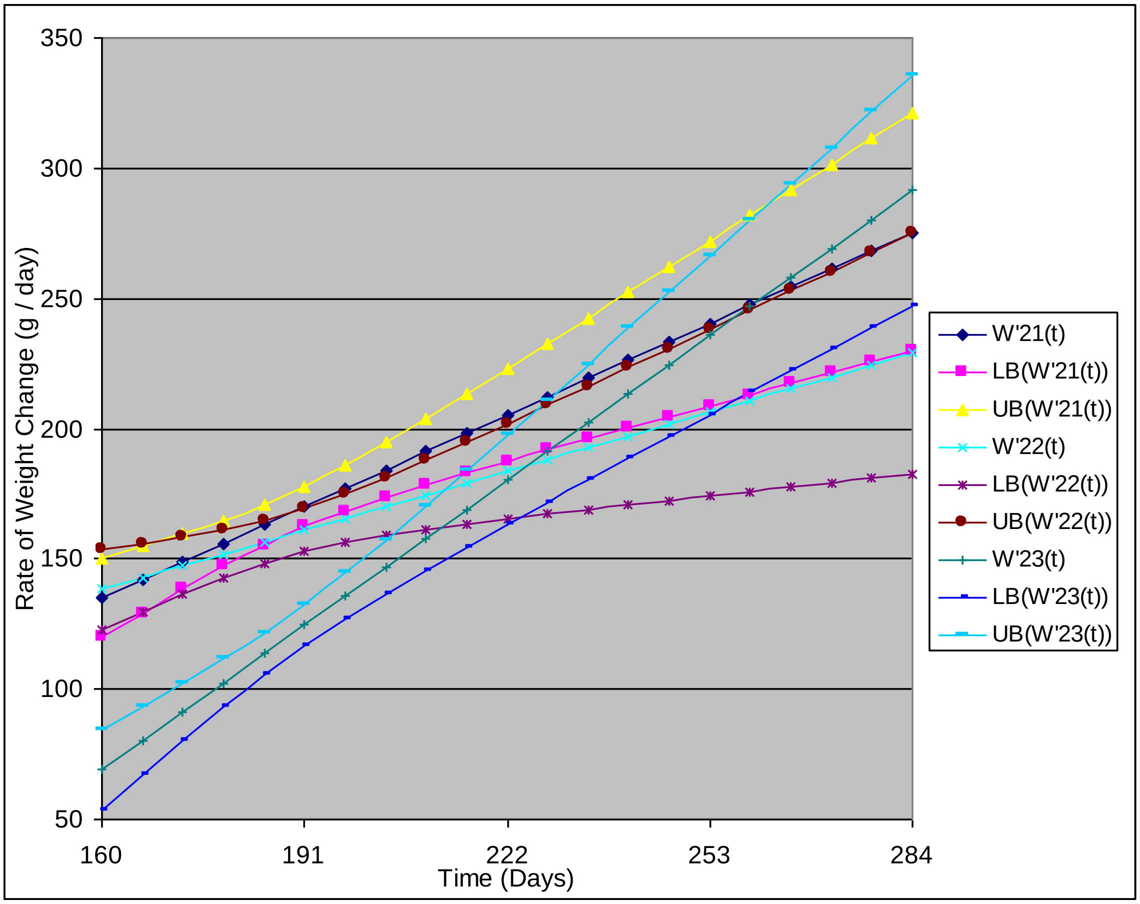
- After 2-3 months, for adjacent starting dates of exposure, the differences between length and weight for fish exposed to E2 or 4-NP are not statistically significant. See elements

(2,3), (3,4), (5,6) and (6,7) of matrix **C**(0) (Table 7) and of matrix **D**(0) (Table 8, Annex 4).

- There are no statistically significant differences between rates of weight change for starting dates of E2 exposure (see elements (2,3) and (3,4) of matrix **E**(0) (Table 9, Annex 4). On the other hand, there were statistically significant differences between rates of weight change for starting dates of 4-NP exposure in about one half of time segment under study (see elements (5,6), (5,7) and (6,7) of matrix **E**(-) and **E**(+) (Table 9, Annex 4). To evaluate differences of absolute values in rate of weight change, we can use Figure 1. where we presented dependences $W'_{21}(t)$, $W'_{22}(t)$, $W'_{23}(t)$ and their confidence bands.

Also, the durations of segments of uncertainty V(U) can last anywhere from several hours to one and a half month and can appear a few times in the time segment under study. Compare element (2,3) with (1,6) of matrix **C**(U) (Table 7, Annex 4), and see elements (2,4) and (2,5) of matrix **D**(U) (Table 8, Annex 4) and elements (2,3) and (3,6) of matrix **E**(U) (Table 9). For this research, we excluded several segments of uncertainty from consideration (for example, elements (5,6) of matrix **C**(U) and (2,5) of matrix **D**(U)) where the duration was less than half a day, since the accuracy of measurements for time is 1 day.

**Figure 1**. Relationship between rate of weight change and time after 2 x 24 hr 4-NP treatments (20 µg/L) with exposures starting on May 12 (W'21(t)), May 26 (W'22(t)) and June 9 (w'23(t)).




Quantitative Approach

On the basis of matrices (10), (11) and (12) we have the possibility to compare growth characteristics by means of four real numbers. We took (14), (15) and (16) and transformed this data in accordance with procedure described in Annex 3. The transformation results are presented below and can be used for a rough evaluation of relations between each pair of models.

➢ To compare length models (14), we consider elements (3,4) of matrices $\mathbf{C}_p(-)$, $\mathbf{C}_p(0)$, $\mathbf{C}_p(+)$, and $\mathbf{C}_p(U)$: 0.250, 0.620, 0, and 0.130 respectively.

➢ To compare weight models (15), we consider elements (4,7) of matrices $\mathbf{D}_p(-)$, $\mathbf{D}_p(0)$, $\mathbf{D}_p(+)$, and $\mathbf{D}_p(U)$: 0, 0.414, 0.235, and 0.351 respectively.

➢ To compare rate of weight change models (16), we consider elements (3,6) of matrices $\mathbf{E}_p(-)$, $\mathbf{E}_p(0)$, $\mathbf{E}_p(+)$, and $\mathbf{E}_p(U)$: 0.337, 0.366, 0.187 and 0.110 respectively.

Although this form of presentation does not give us the dynamics of the relations between each pair of models, it is very attractive since the results can be easily explained to users.

The quantitative approach in our study consisted of three steps.

1. Analysis of matrices of inequalities (Table 10, Annex 4)
First, judging by the highest values in all three matrices of inequalities, we concluded that the largest differences exist between untreated fish and fish exposed to E2 at starting date May 12, and between untreated fish and fish exposed to 4-NP at starting date June 9. See elements (1,2) and (1,7) of matrices $\mathbf{C}_p(-)+\mathbf{C}_p(+)$, $\mathbf{D}_p(-)+\mathbf{D}_p(+)$ and $\mathbf{E}_p(-)+\mathbf{E}_p(+)$.
Second, for E2 and 4-NP separately, we compared growth characteristics between the furthest starting dates of exposure (May 12 and June 9). For E2 treatment, we observed the most statistically significant difference in length, less statistically significant difference in weight and no statistically significant difference in rate of weight change. Compare elements (2,4) of matrices $\mathbf{C}_p(-)+\mathbf{C}_p(+)$, $\mathbf{D}_p(-)+\mathbf{D}_p(+)$ and $\mathbf{E}_p(-)+\mathbf{E}_p(+)$.
For 4-NP treatment, we observed the most statistically significant difference in weight, some statistically significant difference in rate of weight change and less statistically significant difference in length. Compare elements (5,7) of matrices $\mathbf{C}_p(-)+\mathbf{C}_p(+)$, $\mathbf{D}_p(-)+\mathbf{D}_p(+)$ and $\mathbf{E}_p(-)+\mathbf{E}_p(+)$.

2. Analysis of matrices of uncertainties (Table 11, Annex 4)
First, judging by the highest values in all three matrices $\mathbf{C}_p(U)$, $\mathbf{D}_p(U)$ and $\mathbf{E}_p(U)$, we concluded that the largest uncertainties:
- for length models exist between untreated fish and fish exposed to E2 at starting date June 9, and between untreated fish and fish exposed to 4-NP at starting date May 26. See elements (1,4) and (1,6) of matrix $\mathbf{C}_p(U)$;
- for weight models exist between untreated fish and fish exposed to E2 at starting date May 12, and between untreated fish and fish exposed to 4-NP at starting date May 12. See elements (1,2) and (1,5) of matrix $\mathbf{D}_p(U)$;
- for rate of weight change models exist between untreated fish and fish exposed to E2 at starting date June 9, and between untreated fish and fish exposed to 4-NP at starting date May 12. See elements (1,4) and (1,5) of matrix $\mathbf{E}_p(U)$.
Also significant statistical uncertainty exists between growth characteristics based on the type of treatment and on the starting date of exposure.
For Length models (3) – see elements (3,4), (4,5) and (4,7) of $\mathbf{C}_p(U)$:
- (May 26 E2 exposure, June 9 E2 exposure);
- (June 9 E2 exposure, May 12 4-NP exposure);
- (June 9 E2 exposure; June9 4-NP exposure).

For Weight models (4) – see elements (2,4) and (4,7) of matrix $\mathbf{D}_p(U)$:
- (May 12 E2 exposure, June 9 E2 exposure);
- (June 9 E2 exposure, June9 4-NP exposure).

For Rate of Weight Change models (5) – see element (3,6) of matrix $\mathbf{E}_p(U)$:
- (May 26 E2 exposure, May 26 4-NP exposure).

3. Afterwards, we evaluated summary differences for length, weight and rate of weight change at once by combining all three into an aggregate characteristic of differences and computing the overall multiplicative desirability function ($OMDF_{ij}$). These calculations for $OMDF_{ij}$ are presented in Table 12 (Annex 4). Using these results, we can see that the maxima of summary differences were obtained for the following scenarios:
- (Without Treatment, May 12 E2 exposure);
- (Without Treatment, May 26 E2 exposure);
- (Without Treatment, May 12 4-NP exposure);

- (Without Treatment, June 9 4-NP exposure).

See elements (1,2), (1,3), (1,5) and (1,7) of OMDF matrix in Table 12 (Annex 4).
Also, we observed some statistically significant summary differences for other scenarios:

- (Without Treatment, June 9 E2 exposure);
- (Without Treatment, May 26 4-NP exposure);
- (May 12 E2 exposure, May 26 4-NP exposure);
- (May 12 4-NP exposure, May 26 4-NP exposure);
- (May 12 4-NP exposure, June 9 4-NP exposure).

See elements (1,4), (1,6), (2,6), (5,6) and (5,7) of OMDF matrix in Table 12 (Annex 4).

## Conclusion

A procedure for the evaluation and comparison of influence of timing of chemical exposure on the growth of Atlantic salmon smolts has been proposed. The procedure includes development, validation, and comparison of one-dimensional mathematical models of growth characteristics for widespread types of chemicals. Application of this procedure allows for the determination of differences between growth characteristics (statistically significant, not significant or uncertain) not just at the specific days when measurements are taken but also for any days during the experiment. The summary changes of three growth characteristics (length, weight, and rate of weight change) of fish based on the type of treatment and on the starting date of exposure are estimated.
On the whole, our findings confirmed and made more accurate the results of independent biological observations.

A) Arsenault et al. (2004) previously showed that exposure to E2 and 4-NP affected growth of Atlantic salmon smolts after transfer to seawater. We confirmed these results using time series approach (Khots et al. 2011).

B) McCormick et al. (1998) reviewed smoltification and migration of Atlantic salmon. They speculated that exposure to pollutants could play a significant role in smolt survival and growth and suggested more research was appropriate. Our findings confirm their speculation with respect to growth.

C) In addition, McCormick et al. (1998), presented a simple model indicating that timing of entry to sea could affect subsequent returns of salmon to their native rivers. Applying our modified statistical approach we have now shown that the timing of exposure and of transfer to seawater affect the response of Atlantic salmon smolts to E2 and 4-NP. After calculations with mathematical models (Table 3) we can say that compared to untreated fish, length of treated fish is 1.1 to 3.9 percent less, weight is 9.0 to 14.3 percent less, and rate of weight change is 13.2 to 35.2 percent less. Therefore, if Atlantic salmon smolts are delayed in moving from their native streams by poor environmental conditions and experience anthropogenic stressors such as contaminants late in the smolt window, at sea growth, and possibly survival, could be affected. This data provides more evidence for the contention that freshwater exposure of anadromous fish to contaminants affects the fish’s ability to acclimate to seawater.

Such approach may be useful in other cases for evaluation of the influence of environmental factors on dynamic systems.

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## List of Abbreviations

1. $L_{ij,trng}(t)$ – mathematical models for dependences Time – Length constructed on the basis of training set (Table 2)
2. $a_{0ij,trng}$, $a_{1ij,trng}$ – coefficients of $L_{ij,trng}(t)$
3. $W_{ij,trng}(t)$ – mathematical models for dependences Time – Weight constructed on the basis of training set (Table 2)
4. $b_{0ij,trng}$, $b_{1ij,trng}$, $b_{2ij,trng}$ – coefficients of $W_{ij,trng}(t)$
5. $F_{obs,trng}$ – observed values of F-criterion calculated for mathematical models on training set (Table 2)
6. $F_{obs,val}(L_{ij,trng}(t)$ – observed values of F-criterion calculated for $L_{ij,trng}(t)$ on validation set
7. $F_{obs,val}(W_{ij,trng}(t))$ – observed values of F-criterion calculated for $W_{ij,trng}(t)$ on validation set
8. $F_{obs,val}$ – observed values of F-criterion calculated for mathematical models on validation set (Table 2)
9. $F_{obs}$ – observed values of F-criterion calculated for mathematical models on the united set of observations (Table 3)
10. $F_{crit}(0.95, m, \infty)$, $F_{crit}(0.95, s, \infty)$ – values which were copied from F-distribution table
11. Date abbreviations (Tables 7-9). For example [Jun15, Jul3] is the segment beginning June 15 and ending July 3.

## Annex 1: Change of length and weight with time.

**Table 1.** Matrix presentation of pairs of Length, Weight and Rate of Weight Change models.

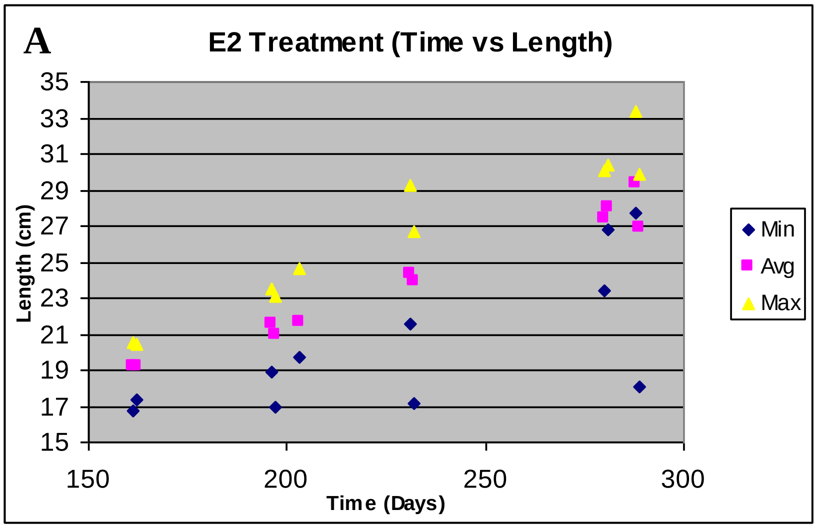
| **Length** | | **W/o treatment** | **May 12 E2** | **May 26 E2** | **June 9 E2** | **May 12 4-NP** | **May 26 4-NP** | **June 9 4-NP** |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| **W/o treatment** | 1 | $(L(t), L(t))$ | $(L(t), L_{11}(t))$ | $(L(t), L_{12}(t))$ | $(L(t), L_{13}(t))$ | $(L(t), L_{21}(t))$ | $(L(t), L_{22}(t))$ | $(L(t), L_{23}(t))$ |
| **May 12 E2** | 2 | | $(L_{11}(t), L_{11}(t))$ | $(L_{11}(t), L_{12}(t))$ | $(L_{11}(t), L_{13}(t))$ | $(L_{11}(t), L_{21}(t))$ | $(L_{11}(t), L_{22}(t))$ | $(L_{11}(t), L_{23}(t))$ |
| **May 26 E2** | 3 | | | $(L_{12}(t), L_{12}(t))$ | $(L_{12}(t), L_{13}(t))$ | $(L_{12}(t), L_{21}(t))$ | $(L_{12}(t), L_{22}(t))$ | $(L_{12}(t), L_{23}(t))$ |
| **June 9 E2** | 4 | | | | $(L_{13}(t), L_{13}(t))$ | $(L_{13}(t), L_{21}(t))$ | $(L_{13}(t), L_{22}(t))$ | $(L_{13}(t), L_{23}(t))$ |
| **May 12 4-NP** | 5 | | | | | $(L_{21}(t), L_{21}(t))$ | $(L_{21}(t), L_{22}(t))$ | $(L_{21}(t), L_{23}(t))$ |
| **May 26 4-NP** | 6 | | | | | | $(L_{22}(t), L_{22}(t))$ | $(L_{22}(t), L_{23}(t))$ |
| **June 9 4-NP** | 7 | | | | | | | $(L_{23}(t), L_{23}(t))$ |

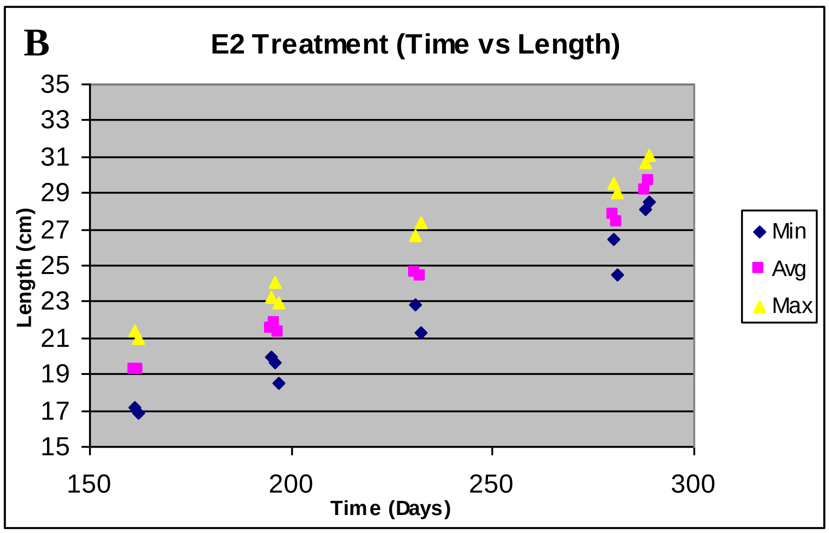
| **Weight** | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|---|
| **W/o treatment** | 1 | $(W(t), W(t))$ | $(W(t), W_{11}(t))$ | $(W(t), W_{12}(t))$ | $(W(t), W_{13}(t))$ | $(W(t), W_{21}(t))$ | $(W(t), W_{22}(t))$ | $(W(t), W_{23}(t))$ |
| **May 12 E2** | 2 | | $(W_{11}(t), W_{11}(t))$ | $(W_{11}(t), W_{12}(t))$ | $(W_{11}(t), W_{13}(t))$ | $(W_{11}(t), W_{21}(t))$ | $(W_{11}(t), W_{22}(t))$ | $(W_{11}(t), W_{23}(t))$ |
| **May 26 E2** | 3 | | | $(W_{12}(t), W_{12}(t))$ | $(W_{12}(t), W_{13}(t))$ | $(W_{12}(t), W_{21}(t))$ | $(W_{12}(t), W_{22}(t))$ | $(W_{12}(t), W_{23}(t))$ |
| **June 9 E2** | 4 | | | | $(W_{13}(t), W_{13}(t))$ | $(W_{13}(t), W_{21}(t))$ | $(W_{13}(t), W_{22}(t))$ | $(W_{13}(t), W_{23}(t))$ |
| **May 12 4-NP** | 5 | | | | | $(W_{21}(t), W_{21}(t))$ | $(W_{21}(t), W_{22}(t))$ | $(W_{21}(t), W_{23}(t))$ |
| **May 26 4-NP** | 6 | | | | | | $(W_{22}(t), W_{22}(t))$ | $(W_{22}(t), W_{23}(t))$ |
| **June 9 4-NP** | 7 | | | | | | | $(W_{23}(t), W_{23}(t))$ |

| **Rate of Weight Change** | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|---|
| **W/o treatment** | 1 | $(W'(t), W'(t))$ | $(W'(t), W'_{11}(t))$ | $(W'(t), W'_{12}(t))$ | $(W'(t), W'_{13}(t))$ | $(W'(t), W'_{21}(t))$ | $(W'(t), W'_{22}(t))$ | $(W'(t), W'_{23}(t))$ |
| **May 12 E2** | 2 | | $(W'_{11}(t), W'_{11}(t))$ | $(W'_{11}(t), W'_{12}(t))$ | $(W'_{11}(t), W'_{13}(t))$ | $(W'_{11}(t), W'_{21}(t))$ | $(W'_{11}(t), W'_{22}(t))$ | $(W'_{11}(t), W'_{23}(t))$ |
| **May 26 E2** | 3 | | | $(W'_{12}(t), W'_{12}(t))$ | $(W'_{12}(t), W'_{13}(t))$ | $(W'_{12}(t), W'_{21}(t))$ | $(W'_{12}(t), W'_{22}(t))$ | $(W'_{12}(t), W'_{23}(t))$ |
| **June 9 E2** | 4 | | | | $(W'_{13}(t), W'_{13}(t))$ | $(W'_{13}(t), W'_{21}(t))$ | $(W'_{13}(t), W'_{22}(t))$ | $(W'_{13}(t), W'_{23}(t))$ |
| **May 12 4-NP** | 5 | | | | | $(W'_{21}(t), W'_{21}(t))$ | $(W'_{21}(t), W'_{22}(t))$ | $(W'_{21}(t), W'_{23}(t))$ |
| **May 26 4-NP** | 6 | | | | | | $(W'_{22}(t), W'_{22}(t))$ | $(W'_{22}(t), W'_{23}(t))$ |
| **June 9 4-NP** | 7 | | | | | | | $(W'_{23}(t), W'_{23}(t))$ |

## Annex 2. Figures showing the relationship between growth, treatment and starting date

**Figure 2.** Growth (length) with time after 6 day exposure to E2 (100 ng/L). Exposures started on: A) May 12; B) May 26; C) June 9



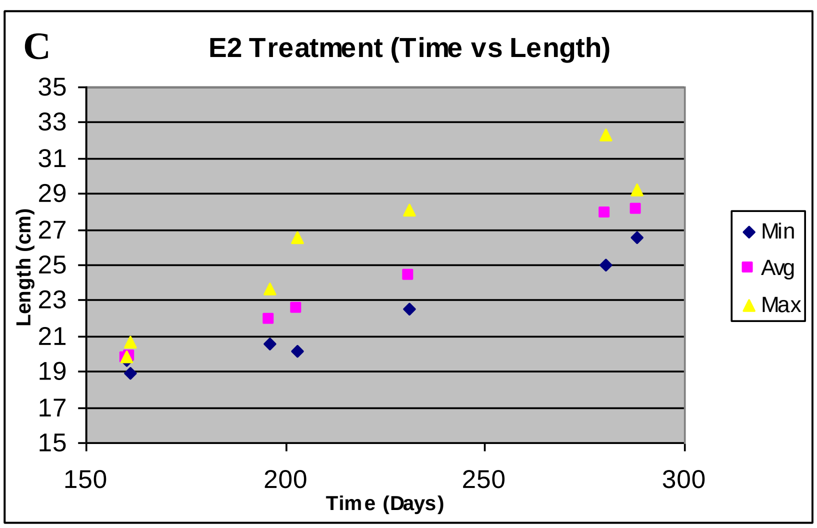



**Figure 3**. Growth (weight) with time after 6 day exposure to E2 (100 ng/L). Exposures started on: A) May 12; B) May 26; C) June 9

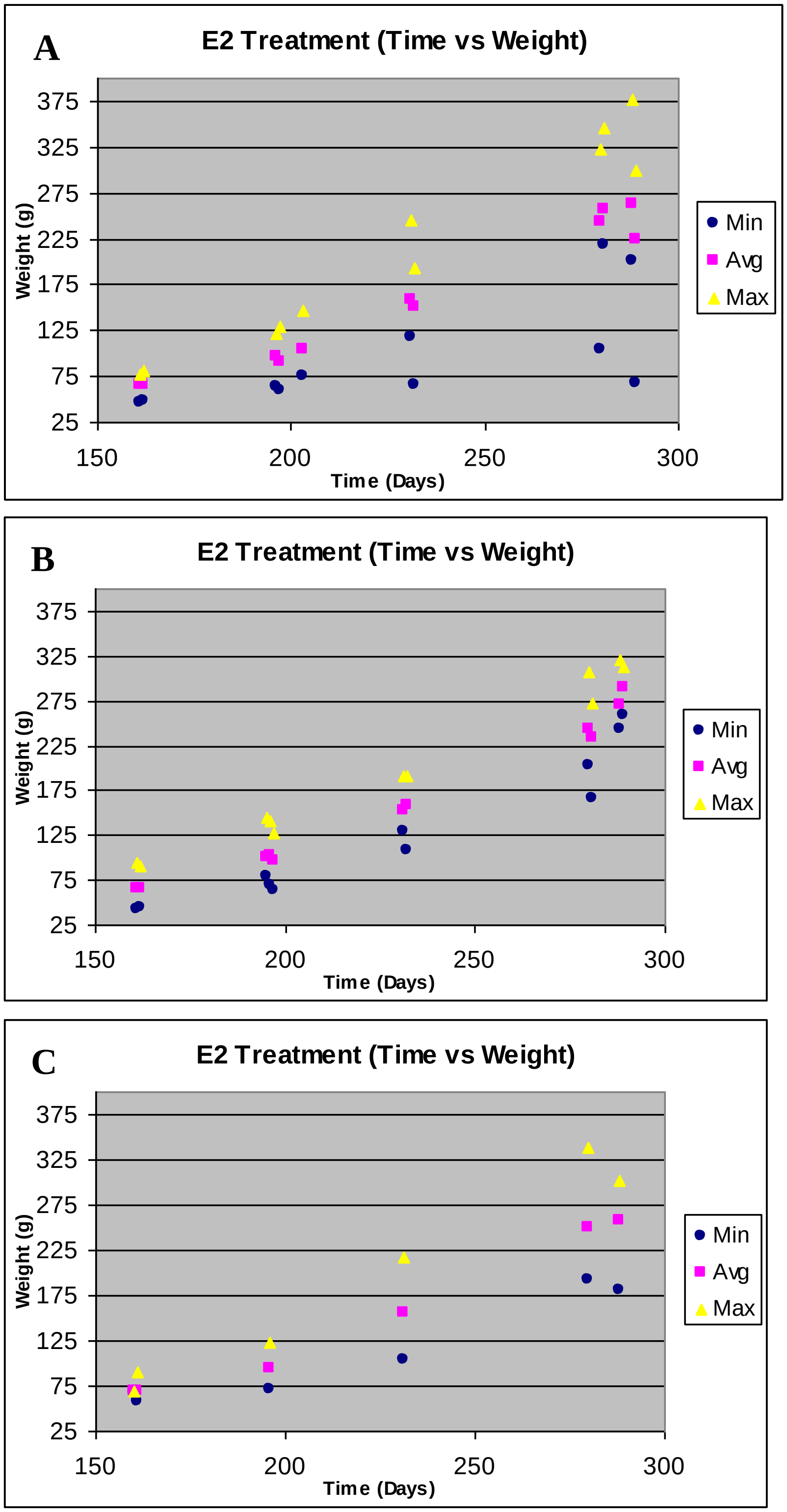


**Figure 4.** Growth (length) with time after 2 x 24 hour exposures to 4-NP (20 µg/L). The exposures were separated by 5 days and started on: A) May 12; B) May 26; C) June 9

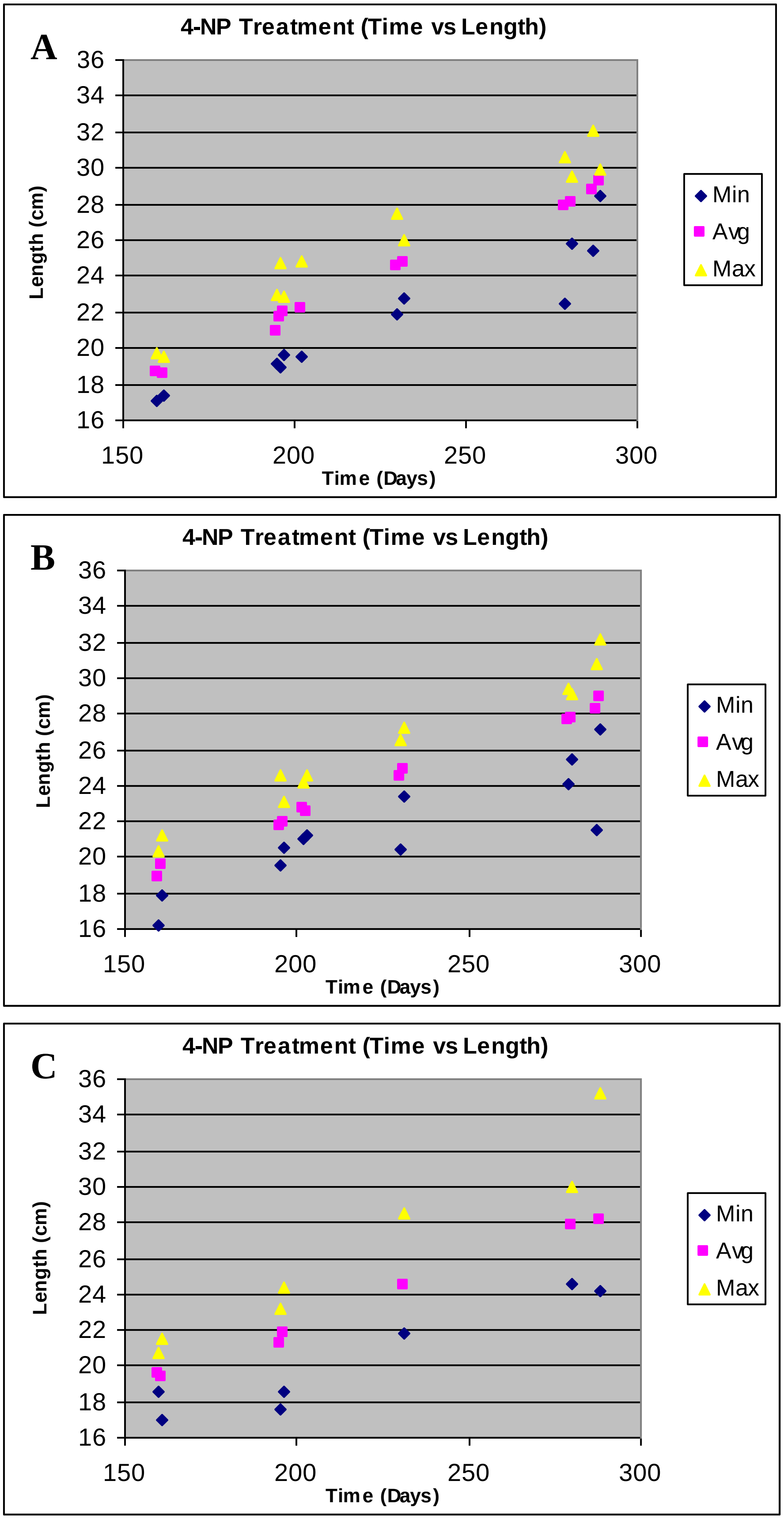
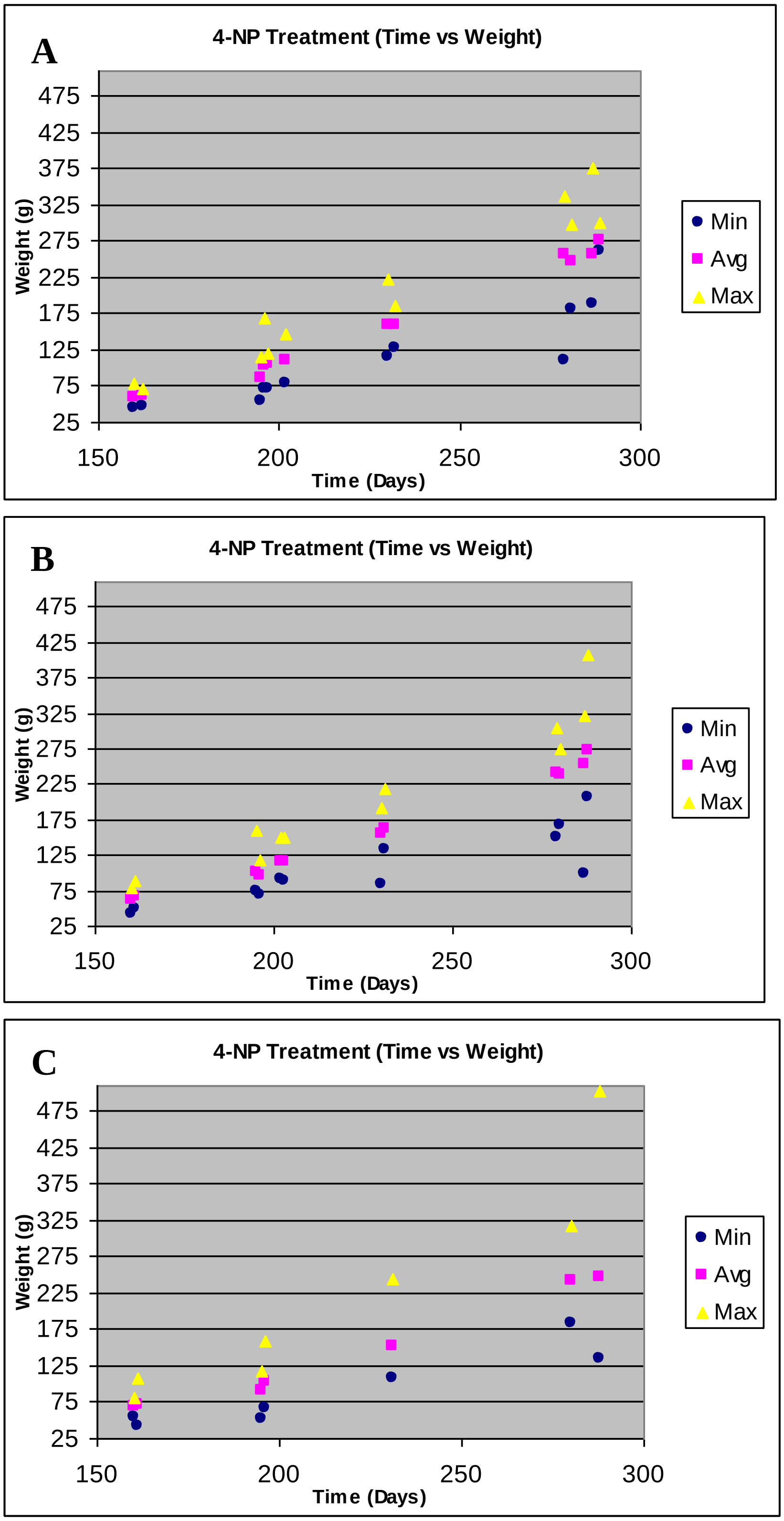


**Figure 5**. Growth (weight) with time after 2 x 24 hour exposures to 4-NP (20 µg/L). The exposures were separated by 5 days and started on: A) May 12; B) May 26; C) June 9.



## ANNEX 3. Properties of matrices for qualitative and quantitative approaches

*Relations between elements of matrices (7), (8) and (9) used in qualitative approach*

> **C**(0), **D**(0) and **E**(0) are symmetric matrices where the diagonal elements are equal to the time segment under study;

> **C**(U), **D**(U) and **E**(U) are symmetric matrices where the diagonal elements are equal to the empty set;

> $\mathbf{C}(-) = \mathbf{C}^T(+)$, $\mathbf{D}(-) = \mathbf{D}^T(+)$, $\mathbf{E}(-) = \mathbf{E}^T(+)$, where $^T$ means transposition

*Procedure of transformation of matrices (7), (8) and (9) into matrices of real numbers*

First, we calculate the sums of durations of segments for each element of matrices (7), (8) and (9). Then, we divide the sums by the duration of the time segment under study.

*Relations between elements of matrices (10), (11) and (12) used in quantitative approach*

> All elements of matrices (10), (11) and (12) are real numbers ranging from 0 to 1.

> When we compare two models on the entire time segment under study, the boundary points 0 and 1 signify the following statistical relations:

- For matrices of inequalities: 0 means no difference between the two models, whereas 1 means that the two models are different.

- For matrices of uncertainties: 0 means no uncertainty exists between the two models, whereas 1 means there is uncertainty between the two models.

> $\mathbf{C}_p(0)$, $\mathbf{D}_p(0)$ and $\mathbf{E}_p(0)$ are symmetric matrices where the diagonal elements are equal to 1;

> $\mathbf{C}_p(U)$, $\mathbf{D}_p(U)$ and $\mathbf{E}_p(U)$ are symmetric matrices where the diagonal elements are equal to 0;

> $\mathbf{C}_p(-) = \mathbf{C}_p{}^T(+)$, $\mathbf{D}_p(-) = \mathbf{D}_p{}^T(+)$, $\mathbf{E}_p(-) = \mathbf{E}_p{}^T(+)$, where $^T$ means transposition

> Sums of matrices $\mathbf{C}_p(-) + \mathbf{C}_p(+)$, $\mathbf{D}_p(-) + \mathbf{D}_p(+)$, $\mathbf{E}_p(-) + \mathbf{E}_p(+)$ are symmetric matrices where the diagonal elements are equal to 0;

> All elements of sums of matrices $\mathbf{C}_p(-) + \mathbf{C}_p(0) + \mathbf{C}_p(+) + \mathbf{C}_p(U)$, $\mathbf{D}_p(-) + \mathbf{D}_p(0) + \mathbf{D}_p(-) + \mathbf{D}_p(U)$, and $\mathbf{E}_p(-) + \mathbf{E}_p(0) + \mathbf{E}_p(+) + \mathbf{E}_p(U)$ are equal to 1.

*Construction of overall multiplicative desirability function (OMDF)*

We considered the elements of three matrices of inequalities (13) as partial desirability functions since they satisfied the following requirement: they vary from 0 to 1. We computed OMDF with the following formula:

$\mathrm{OMDF}_{ij} = ((\mathbf{C}_p(-)_{ij}+\mathbf{C}_p(+)_{ij})*(\mathbf{D}_p(-)_{ij}+\mathbf{D}_p(+)_{ij})*(\mathbf{E}_p(-)_{ij}+\mathbf{E}_p(+)_{ij}))\wedge(1/3)$, where $1 \le i < j \le 7$

To compute the OMDF, we assigned equal weight to all elements of matrices of inequalities (13) for length, weight, and rate of weight change.

## ANNEX 4. Supporting Tables

**Table 2**: Validation of Length and Weight models

| **Length Models** | $a_{0ij,trng}$ | $a_{1ij,trng}$ | | $F_{obs,trng}$ | $F_{crit}(0.95,m,\infty)$ | $F_{obs,val}$ | $F_{crit}(0.95,s,\infty)$ |
|---|---|---|---|---|---|---|---|
| $L_{11,trng}(t)$ | 18.61 | 9.54 | | 2.46 | 3.04 (m=2) | 0.75 | 1.55 (s=30) |
| $L_{12,trng}(t)$ | 19.14 | 9.23 | | 0.87 | | 1.08 | 1.55 (s=31) |
| $L_{13,trng}(t)$ | 19.37 | 8.26 | | 0.48 | | 1.11 | 1.74 (s=14) |
| $L_{21,trng}(t)$ | 18.64 | 10.18 | | 0.84 | | 0.53 | 1.52 (s=34) |
| $L_{22,trng}(t)$ | 19.7 | 8.16 | | 1.79 | | 1.13 | 1.55 (s=30) |
| $L_{23,trng}(t)$ | 18.92 | 9.22 | | 1.23 | | 0.87 | 1.52 (s=34) |
| | | | | | | | |
| **Weight Models** | $b_{0ij,trng}$ | $b_{1ij,trng}$ | $b_{2ij,trng}$ | $F_{obs,trng}$ | $F_{crit}(0.95,m,\infty)$ | $F_{obs,val}$ | $F_{crit}(0.95,s,\infty)$ |
| $W_{11,trng}(t)$ | 64.2 | 74.9 | 112.4 | 3.33 | 3.88 (m=1) | 1.08 | 1.56 (s=29) |
| $W_{12,trng}(t)$ | 66.4 | 96.6 | 95.5 | 0.88 | | 1.06 | 1.55 (s=30) |
| $W_{13,trng}(t)$ | 69.3 | 59.8 | 125.8 | 1.2 | | 1.11 | 1.77 (s=13) |
| $W_{21,trng}(t)$ | 57.4 | 128.8 | 79.2 | 0.73 | | 0.52 | 1.53 (s=33) |
| $W_{22,trng}(t)$ | 66.9 | 131.4 | 39 | 1.5 | | 1.01 | 1.56 (s=29) |
| $W_{23,trng}(t)$ | 65.3 | 76.3 | 111.7 | 1.99 | | 1.12 | 1.54 (s=31) |

**Table 3**: Equations and statistical characteristics of Length and Weight models

| Length Models | $a_{0ij}$ | $a_{1ij}$ | | $F_{obs}$ | $F_{crit}(0.95,m,\infty)$ | $Var(a_{0ij})$ | $Var(a_{1ij})$ | | $2Cov(a_{0ij},a_{1ij})$ |
|---|---|---|---|---|---|---|---|---|---|
| $L_{11}(t)$ | 18.46 | 9.97 | | 0.65 | 3.04 (m=2) | 0.0498 | 0.1016 | | -0.0556 |
| $L_{12}(t)$ | 18.99 | 9.13 | | 1.59 | | 0.0508 | 0.1015 | | -0.0594 |
| $L_{13}(t)$ | 19.73 | 8.26 | | 0.03 | | 0.0806 | 0.1622 | | -0.0807 |
| $L_{21}(t)$ | 18.55 | 10.23 | | 1.55 | | 0.0485 | 0.0915 | | -0.0556 |
| $L_{22}(t)$ | 19.35 | 9.11 | | 1.44 | | 0.0477 | 0.0977 | | -0.055 |
| $L_{23}(t)$ | 19.06 | 9.03 | | 1.51 | | 0.0396 | 0.0787 | | -0.0398 |
| $L(t)$ | 19.32 | 9.79 | | 1.9 | | 0.0171 | 0.0268 | | -0.0187 |

| Weight Models | $b_{0ij}$ | $b_{1ij}$ | $b_{2ij}$ | $F_{obs}$ | $F_{crit}(0.95,m,\infty)$ | $Var(b_{0ij})$ | $Var(b_{1ij})$ | $Var(b_{2ij})$ | $2Cov(b_{0ij},b_{2ij})$ | $2Cov(b_{1ij},b_{2ij})$ | $2Cov(b_{0ij},b_{1ij})$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $W_{11}(t)$ | 62.5 | 84 | 111.4 | 1.46 | 3.88 (m=1) | 8.48 | 53.33 | 181.63 | 3.98 | -167.65 | -2.83 |
| $W_{12}(t)$ | 64 | 94.9 | 93.8 | 1.46 | | 9.88 | 56.06 | 177.71 | 4.84 | -169.96 | -3.62 |
| $W_{13}(t)$ | 68.6 | 98.5 | 87.8 | 0.14 | | 14.92 | 105.36 | 320.47 | 6.56 | -307.89 | -4.85 |
| $W_{21}(t)$ | 55.3 | 134.9 | 70.3 | 0.57 | | 5.64 | 60.5 | 222.35 | 2.95 | -201.1 | -1.8 |
| $W_{22}(t)$ | 61.6 | 138.3 | 45.3 | 1.17 | | 7.19 | 62.05 | 225.03 | 3.23 | -201.08 | -2.1 |
| $W_{23}(t)$ | 67.6 | 69 | 111.2 | 2.73 | | 6.16 | 61.64 | 209.08 | 3.09 | -194.92 | -2 |
| $W(t)$ | 67.6 | 83.9 | 134.7 | 2.02 | | 3.19 | 23.09 | 87.55 | 1.86 | -79.17 | -1.17 |

**Table 4**: Comparison of Length models

| Pairs of length models | Equations | Roots |
|---|---|---|
| $(L(t), L_{12}(t))$ | $L(t)-UB(L_{12}(t))=0$ | 0.123 |
| $(L(t), L_{13}(t))$ | $L(t)-UB(L_{13}(t))=0$ | 0.666 |
| | $LB(L(t))-L_{13}(t)=0$ | 0.416 |
| | $UB(L(t))-L_{13}(t)=0$ | 0.106 |
| $(L(t), L_{21}(t))$ | $L(t)-UB(L_{21}(t))=0$ | 0.788 |
| $(L(t), L_{22}(t))$ | $L(t)-UB(L_{22}(t))=0$ | 0.75 |
| | $LB(L(t))-L_{22}(t)=0$ | 0.38 |
| $(L(t), L_{23}(t))$ | $L(t)-UB(L_{23}(t))=0$ | 0.148 |
| | $LB(L(t))-L_{23}(t)=0$ | 0.001 |
| $(L_{11}(t), L_{12}(t))$ | $L_{11}(t)-LB(L_{12}(t))=0$ | 0.142 |
| | $UB(L_{11}(t))-L_{12}(t)=0$ | 0.148 |
| $(L_{11}(t), L_{13}(t))$ | $L_{11}(t)-LB(L_{13}(t))=0$ | 0.427 |
| | $UB(L_{11}(t))-L_{13}(t)=0$ | 0.497 |
| $(L_{11}(t), L_{22}(t))$ | $L_{11}(t)-LB(L_{22}(t))=0$ | 0.545 |
| | $UB(L_{11}(t))-L_{22}(t)=0$ | 0.538 |
| $(L_{11}(t), L_{23}(t))$ | $L_{11}(t)-LB(L_{23}(t))=0$ | 0.259 |
| | $UB(L_{11}(t))-L_{23}(t)=0$ | 0.219 |
| $(L_{12}(t), L_{13}(t))$ | $L_{12}(t)-LB(L_{13}(t))=0$ | 0.25 |
| | $UB(L_{12}(t))-L_{13}(t)=0$ | 0.38 |
| $(L_{12}(t), L_{21}(t))$ | $L_{12}(t)-LB(L_{21}(t))=0$ | 0.868 |
| | $L_{12}(t)-UB(L_{21}(t))=0$ | 0.001 |
| | $UB(L_{12}(t))-L_{21}(t)=0$ | 0.9 |
| $(L_{13}(t), L_{21}(t))$ | $L_{13}(t)-LB(L_{21}(t))=0$ | 0.85 |
| | $L_{13}(t)-UB(L_{21}(t))=0$ | 0.405 |
| | $LB(L_{13}(t))-L_{21}(t)=0$ | 0.33 |
| | $UB(L_{13}(t))-L_{21}(t)=0$ | 0.995 |
| $(L_{13}(t), L_{23}(t))$ | $L_{13}(t)-UB(L_{23}(t))=0$ | 0.378 |
| | $UB(L_{13}(t))-L_{23}(t)=0$ | 0.18 |
| $(L_{21}(t), L_{22}(t))$ | $L_{21}(t)-LB(L_{22}(t))=0$ | 0.36 |
| | $UB(L_{21}(t))-L_{22}(t)=0$ | 0.361 |
| $(L_{21}(t), L_{23}(t))$ | $L_{21}(t)-LB(L_{23}(t))=0$ | 0.118 |
| | $L_{21}(t)-UB(L_{23}(t))=0$ | 0.83 |
| | $LB(L_{21}(t))-L_{23}(t)=0$ | 0.85 |
| | $UB(L_{21}(t))-L_{23}(t)=0$ | 0.084 |

**Table 5**: Comparison of Weight models

| Pairs of Weight models | Equations | Roots |
|---|---|---|
| $(W(t), W_{11}(t))$ | $W(t)-UB(W_{11}(t))=0$ | 0.195 |
| $(W(t), W_{12}(t))$ | $W(t)-UB(W_{12}(t))=0$ | 0.45 |
| | $LB(W(t))-W_{12}(t)=0$ | 0.007; 0.284 |
| $(W(t), W_{13}(t))$ | $W(t)-UB(L_{13}(t))=0$ | 0.708 |
| | $LB(W(t))-W_{13}(t)=0$ | 0.523 |
| $(W(t), W_{21}(t))$ | $W(t)-UB(W_{21}(t))=0$ | 0.188; 0.730 |
| | $LB(W(t))-W_{21}(t)=0$ | 0.244; 0.590 |
| $(W(t), W_{22}(t))$ | $W(t)-UB(W_{22}(t))=0$ | 0.013; 0.650 |
| | $LB(W(t))-W_{22}(t)=0$ | 0.05; 0.582 |
| $(W(t), W_{23}(t))$ | $W(t)-UB(W_{23}(t))=0$ | 0.26 |
| | $LB(W(t))-W_{23}(t)=0$ | 0.19 |
| $(W_{11}(t), W_{13}(t))$ | $W_{11}(t)-LB(W_{13}(t))=0$ | 0.178; 0.302 |
| | $UB(W_{11}(t))-W_{13}(t)=0$ | 0.54 |
| $(W_{11}(t), W_{21}(t))$ | $W_{11}(t)-LB(W_{21}(t))=0$ | 0.341; 0.682 |
| | $W_{11}(t)-UB(W_{21}(t))=0$ | 0.053 |
| | $LB(W_{11}(t))-W_{21}(t)=0$ | 0.032 |
| | $UB(W_{11}(t))-W_{21}(t)=0$ | 0.384; 0.68 |
| $(W_{11}(t), W_{22}(t))$ | $W_{11}(t)-LB(W_{22}(t))=0$ | 0.14; 0.607 |
| | $UB(W_{11}(t))-W_{22}(t)=0$ | 0.151; 0.610 |
| $(W_{11}(t), W_{23}(t))$ | $W_{11}(t)-LB(W_{23}(t))=0$ | 0.018 |
| $(W_{12}(t), W_{21}(t))$ | $W_{12}(t)-UB(W_{21}(t))=0$ | 0.106 |
| | $LB(W_{12}(t))-W_{21}(t)=0$ | 0.067 |
| $(W_{12}(t), W_{22}(t))$ | $W_{12}(t)-LB(W_{22}(t))=0$ | 0.267; 0.536 |
| | $UB(W_{12}(t))-W_{22}(t)=0$ | 0.325; 0.505 |
| $(W_{13}(t), W_{21}(t))$ | $W_{13}(t)-UB(W_{21}(t))=0$ | 0.255 |
| | $LB(W_{13}(t))-W_{12}(t)=0$ | 0.163 |
| $(W_{13}(t), W_{22}(t))$ | $W_{13}(t)-UB(W_{22}(t))=0$ | 0.044 |
| $(W_{13}(t), W_{23}(t))$ | $W_{13}(t)-UB(W_{23}(t))=0$ | 0.164; 0.750 |
| | $LB(W_{13}(t))-W_{23}(t)=0$ | 0.358; 0.593 |
| $(W_{21}(t), W_{22}(t))$ | $W_{21}(t)-LB(W_{22}(t))=0$ | 0.245 |
| | $UB(L_{21}(t))-W_{22}(t)=0$ | 0.29 |
| $(W_{21}(t), W_{23}(t))$ | $W_{21}(t)-LB(W_{23}(t))=0$ | 0.12 |
| | $W_{21}(t)-UB(W_{23}(t))=0$ | 0.348; 0.892 |
| | $LB(W_{21}(t))-W_{23}(t)=0$ | 0.340; 0.884 |
| | $UB(W_{21}(t))-W_{23}(t)=0$ | 0.124 |
| $(W_{22}(t), W_{23}(t))$ | $W_{22}(t)-LB(W_{23}(t))=0$ | 0.017 |
| | $W_{22}(t)-UB(W_{23}(t))=0$ | 0.200; 0.730 |
| | $LB(W_{22}(t))-W_{23}(t)=0$ | 0.210; 0.718 |
| | $UB(W_{22}(t))-W_{23}(t)=0$ | 0.01 |

**Table 6:** Comparison of Rate of Weight Change models

| Pairs of Rate of Weight Change models | Equations | Roots |
|---|---|---|
| $(W'(t), W'_{11}(t))$ | $W'(t)-UB(W'_{11}(t))=0$ | 0.175 |
| | $LB(W'(t))-W_{11}(t)=0$ | 0.126 |
| $(W'(t), W'_{12}(t))$ | $W'(t)-UB(W'_{12}(t))=0$ | 0.23 |
| | $LB(W'(t))-W'_{12}(t)=0$ | 0.192 |
| | $UB(W'(t))-W'_{12}(t)=0$ | 0.033 |
| $(W'(t), W'_{13}(t))$ | $W'(t)-UB(W'_{13}(t))=0$ | 0.275 |
| | $LB(W'(t))-W'_{13}(t)=0$ | 0.204 |
| | $UB(W'(t))-W'_{13}(t))=0$ | 0.082 |
| $(W'(t), W'_{21}(t))$ | $W'(t)-LB(W'_{21}(t))=0$ | 0.323 |
| | $W'(t)-UB(W'_{21}(t))=0$ | 0.558 |
| | $LB(W'(t))-W'_{21}(t)=0$ | 0.475 |
| | $UB(W'(t))-W'_{21}(t)=0$ | 0.346 |
| $(W'(t), W'_{22}(t))$ | $W'(t)-LB(W'_{22}(t))=0$ | 0.257 |
| | $W'(t)-UB(W'_{22}(t))=0$ | 0.37 |
| | $LB(W'(t))-W'_{22}(t)=0$ | 0.337 |
| | $UB(W'(t))-W'_{22}(t)=0$ | 0.277 |
| $(W'(t), W'_{23}(t))$ | $W'(t)-UB(W'_{23}(t))=0$ | 0.006 |
| $(W'_{11}(t), W'_{13}(t))$ | $UB(W'_{11}(t))-W'_{13}(t)=0$ | 0.049 |
| $(W'_{11}(t), W'_{21}(t))$ | $W'_{11}(t)-LB(W'_{21}(t))=0$ | 0.441 |
| | $UB(W'_{11}(t))-W'_{21}(t)=0$ | 0.451 |
| $(W'_{11}(t), W'_{22}(t))$ | $W'_{11}(t)-LB(W'_{22}(t))=0$ | 0.333 |
| | $W'_{11}(t)-UB(W'_{22}(t))=0$ | 0.581 |
| | $LB(W'_{11}(t))-W'_{22}(t)=0$ | 0.55 |
| | $UB(W'_{11}(t))-W'_{22}(t)=0$ | 0.34 |
| $(W'_{11}(t), W'_{23}(t))$ | $W'_{11}(t)-UB(W'_{23}(t))=0$ | 0.01; 0.460 |
| | $LB(W'_{11}(t))-W'_{23}(t)=0$ | 0.48 |
| $(W'_{12}(t), W'_{21}(t))$ | $W'_{12}(t)-LB(W'_{21}(t))=0$ | 0.487 |
| | $UB(W'_{12}(t))-W'_{21}(t)=0$ | 0.508 |
| $(W'_{12}(t), W'_{22}(t))$ | $W'_{12}(t)-LB(W'_{22}(t))=0$ | 0.337 |
| | $W'_{12}(t)-UB(W'_{22}(t))=0$ | 0.813 |
| | $LB(W'_{12}(t))-W'_{22}(t)=0$ | 0.714 |
| | $UB(W'_{21}(t))-W'_{22}(t)=0$ | 0.348 |
| $(W'_{12}(t), W'_{23}(t))$ | $W'_{12}(t)-UB(W'_{23}(t))=0$ | 0.396 |
| | $LB(W'_{12}(t))-W'_{23}(t)=0$ | 0.408 |
| $(W'_{13}(t), W'_{21}(t))$ | $W'_{13}(t)-LB(W'_{21}(t))=0$ | 0.514 |
| | $UB(W'_{13}(t))-W'_{21}(t)=0$ | 0.475 |
| $(W'_{13}(t), W'_{22}(t))$ | $W'_{13}(t)-LB(W'_{22}(t))=0$ | 0.342 |
| | $UB(W'_{13}(t))-W'_{22}(t)=0$ | 0.322 |
| $(W'_{13}(t), W'_{23}(t))$ | $W'_{13}(t)-UB(W'_{23}(t))=0$ | 0.382 |
| | $LB(W'_{13}(t))-W_{23}(t)=0$ | 0.346 |
| $(W'_{21}(t), W'_{22}(t))$ | $W'_{21}(t)-UB(W'_{22}(t))=0$ | 0.23 |
| | $LB(W'_{21}(t))-W'_{22}(t)=0$ | 0.22 |
| $(W'_{21}(t), W'_{23}(t))$ | $W'_{21}(t)-UB(W'_{23}(t))=0$ | 0.56 |
| | $LB(W'_{21}(t))-W'_{23}(t)=0$ | 0.554 |
| $(W'_{22}(t), W'_{23}(t))$ | $W'_{22}(t)-LB(W'_{23}(t))=0$ | 0.76 |
| | $W'_{22}(t)-UB(W'_{23}(t))=0$ | 0.424 |
| | $LB(W'_{22}(t))-W'_{23}(t)=0$ | 0.419 |
| | $UB(W'_{22}(t))-W'_{23}(t)=0$ | 0.783 |

**Table 7**: Matrix presentation of time segment under study for pairs of Length models

| C(0) | | W/o treatment | May 12 E2 | May 26 E2 | June 9 E2 | May 12 4-NP | May 26 4-NP | June 9 4-NP |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| W/o treatment | 1 | [Jun15, Oct17] | Ø | Ø | [Jun28, Aug4] | Ø | [Jun15, Jul31] | Ø |
| May 12 E2 | 2 | | [Jun15, Oct17] | [Jul3, Oct17] | [Aug15, Oct17] | [Jun15, Oct17] | [Aug21, Oct17] | [Jul17, Oct17] |
| May 26 E2 | 3 | | | [Jun15, Oct17] | [Aug1, Oct17] | [Jun15, Sep29] | [Jun15, Oct17] | [Jun15, Oct17] |
| June 9 E2 | 4 | | | | [Jun15, Oct17] | [Aug4, Sep27] | [Jun15, Oct17] | [Jul31, Oct17] |
| May 12 4-NP | 5 | | | | | [Jun15, Oct17] | [Jul29, Oct17] | [Jun29, Sep24] |
| May 26 4-NP | 6 | | | | | | [Jun15, Oct17] | [Jun15, Oct17] |
| June 9 4-NP | 7 | | | | | | | [Jun15, Oct17] |

| C(-) | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| W/o treatment | 1 | Ø | Ø | Ø | Ø | Ø | Ø | Ø |
| May 12 E2 | 2 | [Jun15, Oct17] | Ø | [Jun15, Jul1] | [Jun15, Aug5] | Ø | [Jun15, Aug19] | [Jun15, Jul11] |
| May 26 E2 | 3 | [Jun30, Oct17] | Ø | Ø | [Jun15, Jul15] | [Oct4, Oct17] | Ø | Ø |
| June 9 E2 | 4 | [Sept5, Oct17] | Ø | Ø | Ø | [Oct17] | Ø | Ø |
| May 12 4-NP | 5 | [Jun15, Sep19] | Ø | Ø | [Jun15, Jul24] | Ø | [Jun15, Jul28] | [Jun15, Jun24] |
| May 26 4-NP | 6 | [Sep16, Oct17] | Ø | Ø | Ø | Ø | Ø | Ø |
| June 9 4-NP | 7 | [Jul3, Oct17] | Ø | Ø | [Jun15, Jul6] | [Sep28, Oct17] | Ø | Ø |

| C(U) | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| W/o treatment | 1 | Ø | Ø | [Jun15, Jun29] | [Jun15,Jun27] U [Aug5,Sep4] | [Sep20, Oct17] | [Aug1, Sep15] | [Jun15, Jul2] |
| May 12 E2 | 2 | | Ø | [Jul2] | [Aug6, Aug14] | Ø | [Aug20] | [Jul12, Jul16] |
| May 26 E2 | 3 | | | Ø | [Jul16, Jul31] | [Sep30, Oct3] | Ø | Ø |
| June 9 E2 | 4 | | | | Ø | [Jul25, Aug3] U [Sep28,Oct16] | Ø | [Jul7, Jul30] |
| May 12 4-NP | 5 | | | | | Ø | Ø | [Jun25, Jun28] U [Sep25,Sep27] |
| May 26 4-NP | 6 | | | | | | Ø | Ø |
| June 9 4-NP | 7 | | | | | | | Ø |

**Table 8:** Matrix presentation of time segment under study for pairs of Weight models

| **D(0)** | | **W/o treatment** | **May 12 E2** | **May 26 E2** | **June 9 E2** | **May 12 4-NP** | **May 26 4-NP** | **June 9 4-NP** |
|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **W/o treatment** | **1** | [Jun15, Oct17] | Ø | [Jun16, Jul19] | [Jun15, Aug17] | [Jul15, Aug26] | [Jun24, Aug25] | [Jun15, Jul7] |
| **May 12 E2** | **2** | | [Jun15, Oct17] | [Jun15, Oct17] | [Aug20, Oct17] | [Jun21, Jul26] U [Sep7, Oct17] | [Jun15, Jul1] U [Aug29, Oct17] | [Jun17, Oct17] |
| **May 26 E2** | **3** | | | [Jun15, Oct17] | [Jun15, Oct17] | [Jun28, Oct17] | [Jun15, Jul17] U [Aug20, Oct17] | [Jun15, Oct17] |
| **June 9 E2** | **4** | | | | [Jun15, Oct17] | [Jul16, Oct17] | [Jun20, Oct17] | [Jun15, Jul4] U [Sep16, Oct17] |
| **May 12 4-NP** | **5** | | | | | [Jun15, Oct17] | [Jul20, Oct17] | [Jun30, Jul26] U [Oct4, Oct17] |
| **May 26 4-NP** | **6** | | | | | | [Jun15, Oct17] | [Jun17, Jul8] U [Sep13, Oct17] |
| **June 9 4-NP** | **7** | | | | | | | [Jun15, Oct17] |

| **D(-)** | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|---|
| **W/o treatment** | **1** | Ø | Ø | Ø | Ø | Ø | Ø | Ø |
| **May 12 E2** | **2** | [Jul9, Oct17] | Ø | Ø | [Jul7, Jul21] | [Aug1, Sep6] | [Jul3, Aug28] | Ø |
| **May 26 E2** | **3** | [Aug9, Oct17] | Ø | Ø | Ø | [Jun15, Jun22] | [Jul25, Aug15] | Ø |
| **June 9 E2** | **4** | [Sep10, Oct17] | Ø | Ø | Ø | Ø | Ø | Ø |
| **May 12 4-NP** | **5** | [Jun15, Jul7] U [Sep13, Oct17] | [Jun15, Jun17] | Ø | [Jun15, Jul4] | Ø | [Jun15, Jul14] | [Jun15, Jun28] |
| **May 26 4-NP** | **6** | [Jun15] U [Sep3, Oct17] | Ø | Ø | Ø | Ø | Ø | [Jun15] |
| **June 9 4-NP** | **7** | [Jul17, Oct17] | Ø | Ø | [Jul29, Aug26] | [Jul28, Oct1] | [Jul11, Sep11] | Ø |

| D(U) | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| **W/o treatment** | **1** | Ø | [Jun15, Jul8] | [Jun15] U [Jul20, Aug8] | [Aug18, Sep9] | [Jul8, Jul14] U [Aug27, Sep12] | [Jun16, Jun20] U [Aug26, Sep2] | [Jul8, Jul16] |
| **May 12 E2** | **2** | | Ø | Ø | [Jun15, Jul6] U [Jul22, Aug19] | [Jun18, Jun20] U [Jul27, Jul31] | [Jul2] | [Jun15, Jun16] |
| **May 26 E2** | **3** | | | Ø | Ø | [Jun23, Jun27] | [Jul18, Jul24] U [Aug16, Aug19] | Ø |
| **June 9 E2** | **4** | | | | Ø | [Jul5, Jul15] | [Jun15, Jun19] | [Jul5, Jul28] U [Aug27, Sep15] |
| **May 12 4-NP** | **5** | | | | | Ø | [Jul15, Jul19] | [Jun29] U [Jul27] U [Oct2] |
| **May 26 4-NP** | **6** | | | | | | Ø | [Jun16] U [Jul9, Jul10] U [Sep12] |
| **June 9 4-NP** | **7** | | | | | | | Ø |

**Table 9**: Matrix presentation of time segment under study for pairs of Rate of Weight Change models

| E(0) | | W/o treatment | May 12 E2 | May 26 E2 | June 9 E2 | May 12 4-NP | May 26 4-NP | June 9 4-NP |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| W/o treatment | 1 | [Jun15, Oct17] | [Jun15, Jun29] | [Jun19, Jul7] | [Jun25, Jul9 | [Jul27, Aug11] | [Jul19, Jul25] | Ø |
| May 12 E2 | 2 | | [Jun15, Oct17] | [Jun15, Oct17] | [Jun21, Oct17] | [Aug9, Oct17] | [Jul27, Aug21] | [Aug13, Oct17] |
| May 26 E2 | 3 | | | [Jun15, Oct17] | [Jun15, Oct17] | [Aug16, Oct17] | [Jul28, Sep10] | [Aug4, Oct17] |
| June 9 E2 | 4 | | | | [Jun15, Oct17] | [Aug17, Oct17] | [Jul27, Oct17] | [Aug1, Oct17] |
| May 12 4-NP | 5 | | | | | [Jun15, Oct17] | [Jun15, Jul11] | [Aug23, Oct17] |
| May 26 4-NP | 6 | | | | | | [Jun15, Oct17] | [Aug6, Sep16] |
| June 9 4-NP | 7 | | | | | | | [Jun15, Oct17] |

| E(-) | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| W/o treatment | 1 | Ø | Ø | Ø | Ø | [Jun15, Jul24] | [Jun15, Jul15] | Ø |
| May 12 E2 | 2 | [Jul6, Oct17] | Ø | Ø | Ø | [Jun15, Aug7] | [Jun15, Jul25] | Ø |
| May 26 E2 | 3 | [Jul13, Oct17] | Ø | Ø | Ø | [Jun15, Aug13] | [Jun15, Jul25] | Ø |
| June 9 E2 | 4 | [Jul19, Oct17] | Ø | Ø | Ø | [Jun15, Aug11] | [Jun15, Jul23] | Ø |
| May 12 4-NP | 5 | [Aug23, Oct17 | Ø | Ø | Ø | Ø | Ø | Ø |
| May 26 4-NP | 6 | [Jul30, Oct17] | [Aug26, Oct17] | [Sep23, Oct17] | Ø | [Jul13, Oct17] | Ø | [Sep20, Oct17] |
| June 9 4-NP | 7 | [Jun16, Oct17] | [Jun16, Aug10] | [Jun15, Aug2] | [Jun15, Jul26] | [Jun15, Aug21] | [Jun15, Aug4] | Ø |

| E(U) | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| W/o treatment | 1 | Ø | [Jun30, Jul5] | [Jun15, Jun18] U [Jul8, Jul12] | [Jun15, Jun24] U [Jul10, Jul18] | [Jul25, Jul26] U [Aug12, Aug22] | [Jul16, Jul18] U [Jul26, Jul29] | [Jun15] |
| May 12 E2 | 2 | | Ø | Ø | [Jun15, Jun20] | [Aug8] | [Jul26] U [Aug22, Aug25] | [Jun15] U [Aug11, Aug12] |
| May 26 E2 | 3 | | | Ø | Ø | [Aug14, Aug15] | [Jul26, Jul27] U [Sep11, Sep22] | [Aug3] |
| June 9 E2 | 4 | | | | Ø | [Aug12, Aug16] | [Jul24, Jul26] | [Jul27, Jul31] |
| May 12 4-NP | 5 | | | | | Ø | [Jul12] | [Aug22] |
| May 26 4-NP | 6 | | | | | | Ø | [Aug5] U [Sep17, Sep19] |
| June 9 4-NP | 7 | | | | | | | Ø |

**Table 10**: Matrices of inequalities

| $C_p(-) + C_p(+)$ | | W/o treatment | May 12 E2 | May 26 E2 | June 9 E2 | May 12 4-NP | May 26 4-NP | June 9 4-NP |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| W/o treatment | 1 | 0 | 1 | 0.877 | 0.334 | 0.788 | 0.25 | 0.852 |
| May 12 E2 | 2 | | 0 | 0.142 | 0.427 | 0 | 0.538 | 0.219 |
| May 26 E2 | 3 | | | 0 | 0.25 | 0.1 | 0 | 0 |
| June 9 E2 | 4 | | | | 0 | 0.335 | 0 | 0.18 |
| May 12 4-NP | 5 | | | | | 0 | 0.36 | 0.234 |
| May 26 4-NP | 6 | | | | | | 0 | 0 |
| June 9 4-NP | 7 | | | | | | | 0 |

| $D_p(-) + D_p(+)$ | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| W/o treatment | 1 | 0 | 0.805 | 0.55 | 0.292 | 0.458 | 0.363 | 0.74 |
| May 12 E2 | 2 | | 0 | 0 | 0.13 | 0.328 | 0.456 | 0 |
| May 26 E2 | 3 | | | 0 | 0 | 0.067 | 0.18 | 0 |
| June 9 E2 | 4 | | | | 0 | 0.163 | 0 | 0.235 |
| May 12 4-NP | 5 | | | | | 0 | 0.245 | 0.656 |
| May 26 4-NP | 6 | | | | | | 0 | 0.518 |
| June 9 4-NP | 7 | | | | | | | 0 |

| $E_p(-) + E_p(+)$ | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| W/o treatment | 1 | 0 | 0.825 | 0.77 | 0.725 | 0.765 | 0.887 | 0.994 |
| May 12 E2 | 2 | | 0 | 0 | 0 | 0.441 | 0.752 | 0.45 |
| May 26 E2 | 3 | | | 0 | 0 | 0.487 | 0.524 | 0.396 |
| June 9 E2 | 4 | | | | 0 | 0.475 | 0.322 | 0.346 |
| May 12 4-NP | 5 | | | | | 0 | 0.77 | 0.554 |
| May 26 4-NP | 6 | | | | | | 0 | 0.636 |
| June 9 4-NP | 7 | | | | | | | 0 |

**Table 11**: Matrices of uncertainties

| $C_p(U)$ | | Control | May 12 E2 | May 26 E2 | June 9 E2 | May 12 4-NP | May 26 4-NP | June 9 4-NP |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| **W/o treatment** | **1** | 0 | 0 | 0.123 | 0.356 | 0.212 | 0.37 | 0.147 |
| **May 12 E2** | **2** | | 0 | 0.006 | 0.07 | 0 | 0.007 | 0.04 |
| **May 26 E2** | **3** | | | 0 | 0.13 | 0.033 | 0 | 0 |
| **June 9 E2** | **4** | | | | 0 | 0.22 | 0 | 0.198 |
| **May 12 4-NP** | **5** | | | | | 0 | 0.001 | 0.054 |
| **May 26 4-NP** | **6** | | | | | | 0 | 0 |
| **June 9 4-NP** | **7** | | | | | | | 0 |

| $D_p(U)$ | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| **W/o treatment** | **1** | 0 | 0.195 | 0.173 | 0.185 | 0.196 | 0.105 | 0.07 |
| **May 12 E2** | **2** | | 0 | 0 | 0.41 | 0.066 | 0.014 | 0.018 |
| **May 26 E2** | **3** | | | 0 | 0 | 0.039 | 0.089 | 0 |
| **June 9 E2** | **4** | | | | 0 | 0.092 | 0.044 | 0.351 |
| **May 12 4-NP** | **5** | | | | | 0 | 0.045 | 0.02 |
| **May 26 4-NP** | **6** | | | | | | 0 | 0.029 |
| **June 9 4-NP** | **7** | | | | | | | 0 |

| $E_p(U)$ | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| **W/o treatment** | **1** | 0 | 0.049 | 0.071 | 0.153 | 0.106 | 0.053 | 0.006 |
| **May 12 E2** | **2** | | 0 | 0 | 0.049 | 0.01 | 0.038 | 0.03 |
| **May 26 E2** | **3** | | | 0 | 0 | 0.021 | 0.11 | 0.012 |
| **June 9 E2** | **4** | | | | 0 | 0.039 | 0.02 | 0.036 |
| **May 12 4-NP** | **5** | | | | | 0 | 0.01 | 0.006 |
| **May 26 4-NP** | **6** | | | | | | 0 | 0.028 |
| **June 9 4-NP** | **7** | | | | | | | 0 |

**Table 12**: Overall multiplicative desirability function

| OMDF | | Control | May 12 E2 | May 26 E2 | June 9 E2 | May 12 4-NP | May 26 4-NP | June 9 4-NP |
|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **W/o treatment** | **1** | | 0.87 | 0.72 | 0.41 | 0.65 | 0.43 | 0.86 |
| **May 12 E2** | **2** | | | 0 | 0 | 0 | 0.57 | 0 |
| **May 26 E2** | **3** | | | | 0 | 0.15 | 0 | 0 |
| **June 9 E2** | **4** | | | | | 0.3 | 0 | 0.24 |
| **May 12 4-NP** | **5** | | | | | | 0.41 | 0.44 |
| **May 26 4-NP** | **6** | | | | | | | 0 |
| **June 9 4-NP** | **7** | | | | | | | |